

US EPA ARCHIVE DOCUMENT

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** The diatom used in the test, Skeletonema costat originally came from the Culture Centre of Algae and Freshwater Biological Association, The Ferry House, Ambleside, UK. The culture has been kept under axenic conditions since Stock cultures were maintained in synthetic nutrient medium Alexander, 1980) at a temperature of $20 \pm 1^\circ\text{C}$ with orbital sha rpm. Cool white light provided 4340 lux illumination on a photoperiod. Cultures that were growing logarithmically we inoculum for the test.

B. **Test System:** Test vessels used were glass 250 ml conical flas with foam stoppers. The test medium was the same as that culturing, with a pH of 8.2.

The test vessels were kept in an incubator with environmental like those employed in culturing.

C. **Dosage:** Nominal rates of 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0 control, and a blank (no algal inoculum) were used for the def

D. **Test Design:** The highest nominal rate of 64 mg ai/l was prepa direct addition of the test material to sterile culture media stock were added to sterile culture medium to obtain the lo concentrations. All solutions were clear and colorless. milliliters of the test solution were placed in each of three flasks (3 per treatment level). The control flasks were repli

An inoculum volume of 0.945 ml was used to provide 1.0×10^4 cells/ml per flask. Cell counts were performed ever for 4 days using an electronic particle counter. The randomized by rows within the incubator.

At the start of the test, samples were taken of each test solu excess remaining after filling the test vessels, and were an concentration of test substance by gas chromatography

(GC). At the end of the test, each blank solution was analyzed in the same manner.

The pH of the test solutions were measured at test initiation. Light intensity was measured once during the experiment. Temperature was monitored continuously.

E. Statistics: For each nominal concentration, the mean of the measured concentrations from the blanks on day 0 and day 4 was calculated. The mean measured concentrations were then used as the basis for analysis. Area under the growth curve and growth rate were expressed as a function of time. Probit and Dunnett's analysis ($p \leq 0.05$) were used on both of these parameters at day 4.

12. REPORTED RESULTS: Algal cell densities for the control and the experimental concentrations throughout the test are given in Table 2 (attached).

Measured concentrations on day 0 were 94% to 106% of nominal while measured concentrations were between 84% and 98%. The means of measured concentrations were 0.94, 2.0, 3.9, 8.2, 16.0, 30.0, and 50.0 mg ai/l.

Increasing concentrations of molinate had increasingly inhibitory effects on growth and reproduction of Skeletonema costatum.

By day 4, the effect of the test material on the area under the growth curve relative to the control, ranged between 7% and 101% inhibition (Table 4, attached). The EC_{50} was 4.3 mg ai/l with confidence limits of 1.8 and 10.0 mg ai/l.

By day 4, the effect of the test material on the growth rate, ranged between -7% (stimulation) and 118% inhibition (Table 4, attached). The EC_{50} was 10.0 mg ai/l with confidence limits of 2.5 and 43.0 mg ai/l.

Results from Dunnett's analysis indicated that the areas under the growth curve on day 4 at the six highest concentrations were significantly different from controls. The NOEC was determined to be 0.94 mg ai/l. The results of the growth rate data demonstrated that the three highest concentrations were significantly less than the controls. The NOEC was reported as 0.94 mg ai/l.

The pH in the control and the exposure concentrations were 9.1 and 9.2 respectively, by test termination. The hourly temperatures range from 20.5°C to 23.5°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

No conclusions were made by the authors.

Good laboratory practice and Quality Assurance Unit statements were included in the report indicating compliance with EPA Good Laboratory Practice under the Federal Insecticide, Fungicide, and Rodenticide Act.

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 dissolved oxygen and conductivity of the test solution were not measured.

Studies were conducted for 4 days. All algal studies should have been conducted for 5 days.

The age of the organisms used for inoculum was not stated.

The light intensity was 4340 lux. The recommended intensity is 10,000 lux.

The cell inoculum was 6400 cells/ml rather than 10,000 cells/ml.

Although the raw data were submitted for cell density data, the raw data for area under the growth curve and growth rate were not submitted.

No subtoxic (EC₂₅) values were reported.

- B. **Statistical Analysis:** The reviewer used a computer program to perform a statistical analysis (attached) of the 4 day cell density data to determine the NOEC. The area under the growth curve data were used for the determination of EC values. Probit and Dunnett's tests were used to determine the EC and NOEC values, respectively. The results of the Dunnett's analysis were in agreement with the authors' values determined from area under the growth curve data. However, the NOEC derived from the growth rate data was more conservative (i.e., 0.94 mg ai/l). The reviewer's NOEC will therefore be taken to be 0.94 mg ai/l. The reviewer's EC values that were slightly higher than the authors'. Since the EC₅₀ value of 4.3 mg ai/l is more conservative, and will be used for non-target plants, it will be taken to be the correct EC value.

- C. **Discussion/Results:** Although the dosages were not adjusted for purity of the test material, the reviewer reported rates in because of the purity of the test material (99%).

This study is scientifically sound but does not meet the requirements for a Tier 2 non-target aquatic plant study. It was not conducted for the recommended length of time (5 days). Skeletonema costatum was increasingly inhibited by increasing the concentration of molinate.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** The study was not conducted for the correct length of time (i.e., 5 days).

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, 6/4/91.