

1. **CHEMICAL:** Molinate
Shaughnessey No. 041402
2. **TEST MATERIAL:** Molinate technical; S-ethyl hexahydro-1H-azepine-1-carbothioate;
substance No. T256; 99% w/w active ingredient; a straw-colored liquid.
3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants -- Tier 2. Species T
Selenastrum capricornutum.
4. **CITATION:** Smyth, D.V., J.F. Tapp, S.A. Sankey and P.A. Johnson. 1990. Molin
Determination of Toxicity to the Green Alga Selenastrum capricornutum. Labo
T256/F. Conducted by Imperial Chemical Industries PLC, Brixham, Devon, UK. Sub
Agrochemicals, Fernhurst, Surrey, UK. EPA MRID No. 416136-12.
5. **REVIEWED BY:**

Mark A. Mossler, M.S. Signature:
Associate Scientist II
KBN Engineering and Date:
Applied Sciences, Inc.
6. **APPROVED BY:**

Louis M. Rifici, M.S. Signature:
Associate Scientist II
KBN Engineering and Date:
Applied Sciences, Inc.

Henry T. Craven, M.S. Signature:
Supervisor, EEB/HED
USEPA Date:
7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline
for a Tier 2 non-target plant growth and reproduction test. The study was cond
rather than the recommended 5 days. The 4-day EC₂₅ and EC₅₀ values of molinate f
capricornutum were 0.15 and 0.22 mg ai/l, respectively. The NOEC was determine
mg ai/l.
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 Printz,
laboratory stock cultures kept under axenic conditions. Stock cultures we
synthetic nutrient medium (Miller et al., 1978) at a temperature of 24 ±
shaking at 100 rpm. Cool white tubes provided a light intensity of 3570 l
Cultures that were growing logarithmically were used as inoculum for the te

B. **Test System:** Test vessels used were glass 250 ml conical flasks fitted wit
stoppers. The test medium was the same as that used for culturing, with a

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

- C. **Dosage:** Nominal rates of 0.056, 0.10, 0.18, 0.32, 0.56, 1.0, 1.8 mg ai/l, control and a blank (no algal inoculum) were used for the definitive test.
- D. **Test Design:** A stock solution of 72 mg ai/l was prepared by direct addition of material to sterile water. Aliquots of the stock were added to sterile water to obtain the nominal concentrations. All solutions were clear and colorless. Two milliliters of the test solution were placed in each of three replicate flasks (2 treatment level). The control flasks were replicated six times.

An inoculum volume of 0.125 ml was used to provide 0.3×10^4 cells/ml per flask. Cell counts were performed every 24 hours for an electronic particle counter. The flasks were randomized by rows within the incubator.

At the start of the test, samples were taken of each test solution, subsamples remaining after filling the test vessels, and were analyzed for the concentration of the substance by gas chromatography (GC). At the end of the test each blank was sampled and analyzed in the same manner.

The pH of the test solutions were measured at test initiation and terminal pH was measured once during the experiment and temperature was monitored continuously.

- E. **Statistics:** For each nominal concentration, the mean of the measured concentration from the blanks on day 0 and day 4 was calculated. The mean measured concentration were then used as the basis for the data analysis. The area under the growth curve were examined as a function of time. Probit and Dunnett's analyses were conducted on both of these parameters at day 4.

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

Measured concentrations on day 0 were 94% to 102% of nominal while day 4 concentrations were between 94% and 105%. The means of the measured concentrations were 0.055, 0.10, 0.17, 0.32, 0.58, 1.0, and 1.7 mg ai/l.

Increasing concentrations of molinate had increasingly inhibitory effects upon reproduction of Selenastrum capricornutum.

By day 4, the effect of the test material on the area under the growth curve, ranged between 7% and 100% inhibition (Table 3, attached). The EC_{50} was 0.22 mg ai/l with confidence intervals of 0.15 and 0.32 mg ai/l.

By day 4, the effect of the test material on the growth rate, relative to the control, ranged between 1% and 95% inhibition (Table 4, attached). The EC_{50} was 0.50 mg ai/l with confidence intervals of 0.36 and 0.69 mg ai/l.

Results from Dunnett's analysis indicated that the areas under the growth curve for the four highest concentrations were significantly less than the control. The NOEC was determined to be 0.17 mg ai/l. The results from the growth rate data were similar to the area under the curve results. The NOEC was again reported as 0.17 mg ai/l.

The pH in the control and the exposure concentrations were 8.0 to 8.1 and respectively, by test termination. The hourly temperatures ranged from 24.4 to 2

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 indicating compliance with EPA Good Laboratory Practice Standards under the 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 accord with the SEP and Subdivision J guidelines, except for the following deviations:

The dissolved oxygen and conductivity of the test solutions were not measured.

The study was conducted for 4 days. All algal studies should be conducted

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

The light intensity was 3570 lux. The recommended intensity is 4000 lux.

Although the raw data were submitted for cell densities, the converted rates under the growth curve and growth rate were not.

No subtoxic (EC₂₅) values were reported.

- B. Statistical Analysis: The reviewer used a computer program to perform statistical analysis (attached) of the 4 day cell density data to determine the NOEC. growth curve data were used for the determination of EC values. This procedure is better than growth rate. Probit and Dunnett's tests were used to determine EC and NOEC values, respectively. The results from Dunnett's analysis were compared with the authors'. The reviewer obtained EC values that were slightly higher than the authors'. Since the authors' EC₅₀ value of 0.22 mg ai/l is more conservative, and will better protect non-target plants, it will be taken as the value.

- C. Discussion/Results: Although the dosages were not adjusted for percent pure test material, the reviewer reported rates in terms of mg ai/l because of the high purity of test material (99%).

This study is scientifically sound but does not meet the guideline requiring 2 non-target aquatic plant studies. The study was not conducted for the amount of time (i.e., 5 days). The NOEC and EC₅₀ of molinate for S. capricornutum were 0.17 and 0.22 mg ai/l. Growth of Selenastrum capricornutum was increasingly inhibited by increasing amounts of molinate.

- D. Adequacy of the Study:

(1) Classification: Supplemental.

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

(3) Repairability: No.

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