US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD 3/21/94

1. **CHEMICAL:** MB 46030 (Fipronil).
Shaughnessey No. 129121.

2. **TEST MATERIAL:** MB 46030 (fipronil); CAS No. 120068-37-3; Batch No. 6ADM93; 96.1% active ingredient; a grey powder.

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

4. **CITATION:** Hoberg, J.R. 1993. MB 46030 - Toxicity to the Freshwater Green Alga, *Selenastrum capricornutum*. Report No. 93-5-4789. Conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by Rhone-Poulenc Ag Company, Research Triangle Park, NC. EPA MRID No. 429186-60.

5. **REVIEWED BY:**

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6. **APPROVED BY:**

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7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for a Tier 1 aquatic plant growth and reproduction study. Based on the mean measured concentration, the growth and reproduction of *S. capricornutum* were not detrimentally affected by the presence of 0.14 mg ai/l of fipronil.

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

9. **BACKGROUND:**

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

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11. MATERIALS AND METHODS:

A. Test Species: The alga used in the test, *Selenastrum capricornutum*, came from laboratory stock cultures originally obtained from Carolina Biological Supply, Burlington, NC. Stock cultures were maintained in sterile Algal Assay Procedure (AAP) medium under test conditions. Transfers were made to fresh medium approximately twice a week. The culture used as the inoculum for the test was transferred to fresh medium two days before test initiation.

B. Test System: Test vessels were sterile 125-ml flasks fitted with stainless steel caps which permitted gas exchange. The vessels were conditioned by rinsing with appropriate test solution and 50 ml of the treatment or control solutions were placed into each flask. The test medium was the same as that used for culturing with a pH of 7.5. Test vessels were randomly placed and maintained on an orbital shaker (shaking rate of 100 rpm) under continuous illumination (3.2-4.3 klux at the surface of the media) in an environmental chamber. The temperature in the chamber was maintained at 24-25°C.

A 20 mg ai/ml primary stock solution was prepared by diluting 0.5203 g (0.5 g ai) of test material to the final volume of 25 ml in acetone. One ml of the primary stock was brought to the final volume of 10 ml with acetone to create a secondary stock solution (2 mg ai/ml). An appropriate volume (50 µl) of the secondary stock solution was brought to the final volume of 500 ml with sterile AAP medium to create the treatment solution. A medium and solvent (0.1 ml acetone/l) control were also prepared.

C. Dosage: Five-day growth and reproduction test. Based on the results of a range-finding test, one nominal concentration of 0.20 mg active ingredient (ai)/l was selected for the definitive test. The maximum application rate of the test material was reported to be 0.20 lb active ingredient/acre, which is equivalent to a concentration of 0.15 mg ai/l if applied to a 15-cm water column.

D. Test Design: The test consisted of 3 replicate flasks for each treatment and control group. An inoculum of *S. capricornutum* cells calculated to provide 3,000 cells/ml was aseptically introduced into each flask within five minutes of solution addition. The inoculum

volume was 0.32 ml per flask. At each 24-hour interval, observations of cellular health were made and cell counts were conducted on each replicate vessel using a hemacytometer and compound microscope.

The conductivity and pH were measured at test initiation and termination. Temperature in a flask of water adjacent to the test flasks was recorded continuously with a minimum/maximum thermometer. The shaking rate of the orbit shaker was recorded daily.

At test initiation and termination, samples were removed from each treatment and control solution for analysis by high performance liquid chromatography. Terminal samples were centrifuged at 2000 rpm for 15 minutes to remove algal cells. Three quality control (QC) samples were prepared at test initiation and termination to judge the precision of the analyses.

E. **Statistics:** Negative and solvent control data were pooled. A t-test was used to determine if a significant reduction in cell density had occurred in the treatment solution in comparison to the pooled control data.

12. **REPORTED RESULTS:** Initial and terminal concentrations of fipronil demonstrated a slight decrease in the concentration of test material over the study and averaged 71% of nominal. The mean measured concentration was determined to be 0.14 mg ai/l (Table 3, attached). Recoveries of the QC samples ranged between 87 and 109% of nominal.

Cell densities determined at each observation time are presented in Table 4 (attached). At test termination, mean cell densities in the negative and solvent control were 147 $x10^4$ and 141 $x10^4$ cells/ml, respectively. The mean cell density for the treatment group was 148 $x10^4$ cells/ml (2.8% stimulation). Based on these results, the 120-hour EC_{50} was determined to be >0.14 mg ai/l and the 120-hour no-observed-effect concentration was 0.14 mg ai/l.

Conductivity ranged between 70 and 110 µmhos/cm during the test. The pH was 7.4-7.5 in the treatment and control solutions at test initiation and 8.7-10.0 at test termination.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The author concluded that further testing at higher concentrations was not considered necessary, since the Tier

1 test was conducted at a concentration which approximated the maximum required concentration of 0.15 mg ai/l.

The study director confirmed that this study was conducted in compliance with Good Laboratory Practice (GLP) regulations (40 CFR Part 160) with the exception that stability, characterization, and verification of the test substance are the responsibility of the test sponsor. Additionally, routine water analyses were conducted by an independent laboratory that did not collect data in accordance to GLPs. A Quality Assurance statement was enclosed in the report.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures deviated from the SEP and Subdivision J guidelines in the following manner:

The light intensity (3.2-4.3 klux) was occasionally lower or higher than recommended (4 klux).

B. Statistical Analysis: Since the cellular growth in the treatment group was greater than that of either control, it is apparent that the presence of the test material at a mean measured concentration of 0.14 mg ai/l did not adversely impact the growth and survival of *S. capricornutum*.

C. Discussion/Results: Although the mean measured concentration of 0.14 mg ai/l was slightly less than the required concentration of 0.15 mg ai/l, the reviewer believes that an additional 0.01 mg ai/l of the test material would probably not reduce the growth of the alga significantly. Therefore, this study is scientifically sound and meets the requirements for a Tier 1 aquatic plant growth and reproduction study. Based on the mean measured concentration, the growth and reproduction of *S. capricornutum* were not detrimentally affected by the presence of 0.14 mg ai/l of fipronil.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

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15. **COMPLETION OF ONE-LINER:** Yes, 1-6-94.

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