1-14-94

MRID No. 417773-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Propanil.
Shaughnessey No. 028201.

2. **TEST MATERIAL:** Propanil technical; Batch No. 01; Aliquot No. 14; 98 ±2% active ingredient; a blue-gray crystalline solid.

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

4. **CITATION:** Giddings, J.M. 1990. Propanil - Toxicity to the Freshwater Green Alga *Selenastrum capricornutum*. Report No. 90-3-3253. Conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by The Propanil Task Force, c/o John M. Wise, Liberty, MO. EPA MRID No. 417773-01.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: [signature]

Date: 6/23/92

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat

Date: 6/23/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: [signature]

Date: 1-14-94

7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for a Tier 2 aquatic plant growth and reproduction study. Based on mean measured concentrations, the NOEC, LOEC, and EC_{50} for *S. capricornutum* exposed to propanil were 0.006, 0.011, and 0.029 mg ai/l, respectively.

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 Company, Burlington, NC. Stock cultures were maintained in Marine Biological Laboratory (MBL) medium (Table 1, attached) under test conditions. The cultures were transferred to fresh medium once or twice a week to maintain active growth. The culture used as the inoculum for the test was transferred to fresh medium three days before test initiation.

B. **Test System:** Test vessels used were sterile 125-ml flasks fitted with stainless steel caps which permitted gas exchange. The test medium was the same as that used for culturing (excluding EDTA) with the pH adjusted to 7.5. Test vessels were maintained on an orbital shaker (shaking rate of 100 rpm) under continuous illumination (4-5 klux at the surface of the media) in a growth chamber. The temperature in the growth chamber was maintained at 23-24°C.

C. **Dosage:** Five-day growth and reproduction test. Based on the results of a preliminary test, seven nominal propanil concentrations of 0.0064, 0.012, 0.025, 0.052, 0.10, 0.20, and 0.40 mg/l were selected for the definitive test.

A 4 g active ingredient (ai)/l primary stock solution was prepared by dissolving 0.4084 g of the test material in 100 ml of acetone. Secondary stocks were prepared in acetone and a solvent control (0.1 ml acetone/l of nutrient medium) was prepared.

Appropriate volumes of the secondary stocks were diluted with sterile MBL medium to prepare the test solutions.

D. **Test Design:** Three replicate flasks (3 per treatment level and the controls) were conditioned by rinsing with the appropriate test solution. Fifty ml of the appropriate test or control solution were then placed into each flask.

An inoculum of *Selenastrum capricornutum* cells calculated to provide 3,000 cells/ml was aseptically introduced into each flask within ninety minutes of solution preparation. The inoculum volume was 950 µl/flask. At each 24-hour interval, cell counts were

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conducted on each replicate vessel using a hemacytometer and compound microscope.

The pH and conductivity were measured at test initiation and pH was measured at termination. Temperature was recorded continuously with a minimum/maximum thermometer. The shaking rate of the orbital shaker was recorded daily. The light intensity was measured at the beginning of the test and every 24-hour interval of the exposure period.

At test initiation and termination, samples were removed from each test solution and the control for analysis by high performance liquid chromatography. Three quality control samples were prepared and remained with the set of exposure solution samples throughout the analysis.

E. **Statistics:** If the negative and solvent control data were not significantly different, data were pooled. If the data were significantly different, data from the solvent control were used for EC determination. For each observation period, the EC_{10}, EC_{50}, and EC_{90} values and their 95% confidence interval (C.I.) were determined by linear regression of response (percent reduction of cell density as compared with the control) vs. mean measured exposure concentration over the range of test concentrations where a clear dose-response relationship was observed. Various mathematical manipulations (logarithm and probit transformations) were used on the concentration and response data to get the linear regression with the highest coefficient of determination (R^2).

12. **REPORTED RESULTS:** The measured concentrations ranged from 95% of nominal at test initiation to 85% of nominal at termination. The mean measured concentrations were 0.006, 0.011, 0.023, 0.045, 0.089, 0.177, and 0.358 mg/l (Table 3, attached).

Cell densities determined at each observation time are presented in Table 4 (attached). In comparison to the pooled control data, the 120-hour EC_{50} was calculated to be 0.041 mg/l with a 95% C.I. of 0.024-0.068 mg/l. Cells exposed to 0.18 mg/l propanil appeared pale when observed at 48 hours. No other abnormalities were observed.

Conductivity ranged from 250 to 390 µmhos/cm. The pH was between 7.4 and 7.5 in all test solutions and the controls

at test initiation and between 7.0 and 7.9 at termination. The temperature ranged from 23 to 24°C during the study.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions other than those stated above were made by the study author.

Good Laboratory Practice and Quality Assurance Unit statements were included in the report indicating compliance to EPA Good Laboratory Practice Regulations (40 CFR Part 160). The GLP statement indicated that the maintenance of records on the test substance stability, characterization, and verification were the responsibility of the test sponsor.

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:

Three-day old cultures were used to initiate the test. Six to eight-day old cultures are recommended.

The daily light intensity measurements were not reported.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to determine the EC_{50} and analysis of variance (ANOVA) coupled with Dunnett's test to determine the lowest-observed-effect concentration (LOEC) and the no-observed-effect concentration (NOEC). The results obtained by the reviewer for the EC_{50} are more conservative than those obtained by the author. Using the moving average angle method, the EC_{50} and 95% C.I. were 0.029 mg ai/l and 0.025-0.035 mg ai/l, respectively. The NOEC and LOEC were determined to be 0.006 and 0.011 mg ai/l, respectively.

C. **Discussion/Results:** There appears to be a typographical error in the methods section. The author stated that the chemical analyses were conducted using gas chromatography. However, the analytical appendix indicated that high performance liquid chromatography was employed for quantification.

This study is scientifically sound and meets the requirements for a Tier 2 aquatic plant growth and reproduction study. Based on mean measured

concentrations, the NOEC, LOEC, and EC_{50} for *S. capricornutum* exposed to propanil were 0.006, 0.011, and 0.029 mg ai/l, respectively.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 6-1-92.