3-3-92

MRID No. 417767-01 72-4B

DATA EVALUATION RECORD

1. **CHEMICAL:** Propanil (3,4-Dichloropropionanilide). Shaughnessey Number: 028201.

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

3. **STUDY TYPE:** 123-2 Growth and Reproduction of Aquatic Plants - Tier II. Species Tested: Anabaena flos-aquae.

4. **CITATION:** Giddings, J.M., M.C.R. Bayne, J. Mao, and S.P. Shepherd. 1990. Propanil - Toxicity to the Freshwater Blue-Green Alga Anabaena flos-aquae. SLI Study No. 90-3-3273. Prepared by Springborn Laboratories, Inc., Wareham, Massachusetts. Submitted by The Propanil Task Force, Liberty, Missouri. EPA MRID No. 417767-01.

5. **REVIEWED BY:**

Michael W. Davy
Agronomist
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EEB/EFED/OPP/EPA

Signature: Michael Davy ✓
Date: 2/10/92

6. **APPROVED BY:**

Daniel Rieder
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Ecological Effects Branch
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Signature: Daniel Rieder
Date: 3-3-92

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for a Tier II growth and reproduction of a non-target algal test since light intensity is 40 percent lower than recommended by SEP.

Based on total cell density, the 5-day EC_{50} value of Propanil for Anabaena flos-aquae was 0.111 mg a.i./L (95 percent confidence limits are 0.082 and 0.146). The 5-day NOEC was 0.025 mg a.i./L (mean measured concentration).

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:** This study is in support of reregistration of propanil.

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

11. MATERIALS AND METHODS:

A. Test Species: Anabaena flos-aquae used in this test were obtained from laboratory stock cultures at the testing facility. The original culture was obtained from Carolina Biological Supply Company, Burlington, North Carolina. Stock cultures were transferred weekly or twice weekly into fresh Marine Biological Laboratory (MBL) medium. The test inoculum was taken from stock cultures which were four days old.

B. Test System: The phytotoxicity test was conducted in an environmental chamber which was maintained at a temperature of 23-24°C. The test vessels were 125-ml sterile flasks containing 50 ml of test solution with steel caps to permit gas exchange. Three replicates were used for each control and test treatment. Flasks were impartially placed on an orbital shaker and continuously shaken at 100 revolutions/minute. A light intensity of 1200-1500 lux was provided continuously throughout the test period. The test medium used was the same as that used in culturing, excluding Na_2EDTA (Table 1, attached).

C. Dosage: Five-day growth and reproduction test. The nominal test concentrations of Propanil are based on the active ingredient. Six nominal concentrations (0.031, 0.063, 0.13, 0.25, 0.50, and 1.0 mg/L), based upon results of a preliminary test, were used. A medium control and a solvent control (0.1 ml acetone/L) were also used.

D. Design: A primary stock solution (10 mg/ml) was prepared by mixing 0.5103 g of Propanil with acetone to a final volume of 50 ml. The primary stock was diluted further with acetone to create secondary stock solutions. Equal volumes (0.05 ml) of secondary stock were diluted with MBL medium to create nominal concentrations.

Each concentration and control was replicated three times. An inoculum volume of 740 µl was introduced (within 30 minutes of test solution addition) into each test vessel, resulting in initial cell densities of 3,000 cells/ml.

The Ph and conductivity of the test solutions were measured and recorded at test initiation and termination. Test temperature was measured

continuously. Light intensity was recorded at test initiation and thereafter at 24-hour intervals.

Each replicate chamber was monitored daily for growth using a hemocytometer and microscope (cells/ml). One sample was taken from each flask and sonicated for 90 seconds before counting.

The concentration of Propanil was determined by chemical analysis at test initiation and test termination.

E. **Statistics:** The EC_{10}, EC_{50}, and EC_{90} values and confidence limits for 72-, 96-, and 120-hours exposure were calculated. If a significant difference was determined between the controls and solvent controls, the solvent control was used for EC calculations. Calculations were "determined by linear regression of response (percent reduction of cell density as compared to the controls) vs. mean measured exposure over the range of test concentrations where a clear exposure-response relationship was observed." Four linear regressions were estimated based different transformations, and the one which best fitted the data was selected based on the highest coefficient of determination (r^2). From this regression, the EC values and their 95% confidence limits, were estimated using the method of inverse prediction (Sokal and Rohlf, 1981). An SLI computer program was used to assist in these computations.

12. **REPORTED RESULTS:** The mean measured concentrations for the definitive study were 0.025, 0.066, 0.13, 0.23, 0.48, and 0.89 mg a.i./L (Table 3, attached). The concentrations were fairly consistent between observations. The mean total cell density (x 10^4 cells/ml) in relation to mean measured concentration are shown in Table 4 (attached). The 5-day EC_{50} value (corresponding 95% confidence intervals) using cell density was 0.12 (0.041-0.33) mg a.i./L, based on mean measured concentrations (Table 5, attached).

Cell densities increased at all concentrations following 96 hours exposure, however increasing with decreasing concentration of Propanil. Control and solvent control densities averaged 32-33 (x 10^4 cells/ml) at test termination, respectively. No significant difference was demonstrated between the cell densities of the control and solvent control, and therefore data were combined for EC calculations.

During the test period conductivity ranged from 100 to 130 μmhos/cm. The Ph ranged from 7.2 to 7.6. Temperatures ranged from 23 to 24°C during the study.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were presented in the report.

Quality Assurance Unit and Good Laboratory Practice compliance Statements were included in the report, indicating that the study was conducted in accordance with the FIFRA Good Laboratory Practice Standards set forth in 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 light intensity ranged from 1200-1500 lux. The guidelines recommend a continuous light intensity of 2000 lux.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to calculate the 5-day EC_{50} value using percent inhibition and mean measured concentrations. Percent inhibition (I) of growth compared to the solvent control was calculated for cell count according to the following formula:

$$\% I = \frac{C - X}{C} \times 100$$

where: C = mean growth in the solvent control,
X = mean growth in test concentration.

The 5-day EC_{50} value using cell density was 0.111 mg a.i./L with a 95 percent confidence interval of 0.082-0.146 mg a.i./L, based on mean measured concentrations (Printout 1, attached).

This EC_{50} value is similar to that presented by the authors. However, since the confidence interval of the reviewer's values are narrower, the reviewer's values should be used for the purpose of hazard assessment.

The reviewer used Toxstat Version 3.3 to determine the NOEC for this study. A square root transformation was applied to the cell density data to obtain homogeneity and normal distribution. Once the data was

transformed, Bonferroni's t-test was applied. This analysis indicate the NOEC for the study was 0.025 mg a.i./L, based on mean measured concentrations (Printout 2, attached). The authors did not present an NOEC value in the report.

C. **Discussion/Results:** The study appears to be scientifically sound but does not meet the requirements for growth and reproduction study of aquatic plants-Tier II based on light intensity being 40 percent lower than recommended in SEP. This lower light intensity could possibly affect the algae in that it could be an additional inhibitory factor as well as the chemical.

Based on cell density, the 5-day EC_{50} value of Propanil for Anabaena flos-aquae was determined to be 0.111 mg a.i./L (95 percent confidence limits are 0.082 and 0.146). The 5-day NOEC was 0.025 mg a.i./L, based on mean measured concentrations.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** Lower light intensity than recommended by SEP.

(3) **Repairability:** Not Repairable.

15. COMPLETION OF ONE-LINER: Yes.

DATA EVALUATION RECORD

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3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants - Tier II. Species Tested: Anabaena flos-aquae.

4. **CITATION:** Giddings, J.M., M.C.R. Bayne, J. Mao, and S.P. Shepherd. 1990. Propanil - Toxicity to the Freshwater Blue-Green Alga Anabaena flos-aquae. SLI Study No. 90-3-3273. Prepared by Springboro Laboratories, Inc., Wareham, Massachusetts. Submitted by The Propanil Task Force, Liberty, Missouri. EPA MRID No. 417767-01.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
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KBN Engineering and
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Signature: Rosemary Graham Mora
Date: 6/4/91

Michael 2/13/92
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6. **APPROVED BY:**

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7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for a Tier II growth and reproduction of a non-target algal test, since the maximum label rate was not included in the report. Based on total cell density, the 5-day EC_{50} value of Propanil for Anabaena flos-aquae was 0.011 mg a.i./L (mean measured concentration). The 5-day NOEC was 0.025 mg a.i./L (mean measured concentration).

0.110?

8. **RECOMMENDATIONS:** The registrant should submit the maximum label rate for this chemical.

9. **BACKGROUND:**

Page_____ is not included in this copy.

Pages 1 through 11 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

__✓__ Information about a pending registration action.

__✓__ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

__✓__ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

PRINTOUT 1

LEWIS PROPANIL ANABEANA

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.89	100	97	97	0
.48	100	93	93	0
.23	100	87	87	0
.13	100	61	61	0
.066	100	23	23	0
.025	100	4	4	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .1075118

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	3.012153E-02	.1021432	8.735768E-02	.1178665

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	9.831815E-02	2.654884	3.118932E-02

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.580148

95 PERCENT CONFIDENCE LIMITS = 1.771124 AND 3.389173

LC50 = .1109452

95 PERCENT CONFIDENCE LIMITS = 8.162521E-02 AND .1460247

LC10 = 3.571814E-02

95 PERCENT CONFIDENCE LIMITS = .0182932 AND 5.274475E-02

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