3-1-92

MRID No. 417775-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Propanil (3,4-dichloropropionilide). Shaugnessey 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: Navicula pelliculosa.

4. **CITATION:** Giddings, J.M., M.C.R. Bayne, and J. Mao. 1990. Propanil - Toxicity to the Freshwater Diatom Navicula pelliculosa. SLI Report No. 90-3-3254. Performed by Springborn Laboratories, Inc., Wareham, Massachusetts. Submitted by The Propanil Task Force, Liberty, Missouri. EPA MRID No. 417775-01.

5. **REVIEWED BY:**

Michael W. Davy
Agronomist
Ecological Effects Branch
EEB/EFED/OPP/EPA

Signature: Michael Davy
Date: 2/10/92

6. **APPROVED BY:**

Daniel Rieder
Section Head
Ecological Effects Branch
EEB/EFED/OPP/EPA

Signature: Daniel Rieder
Date: 3.4.92

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier II growth and reproduction of a non-target freshwater diatom test.

Based on total cell density, the 5-day EC_{50} value of Propanil was 0.016 mg a.i./L (95 percent confidence limit is 0.006 and 0.036). The 5-day NOEC was 0.0063 mg a.i./L, based on mean measured concentrations.

8. **RECOMMENDATIONS:** N/A

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

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

11. MATERIALS AND METHODS:

A. Test Species: Navicula pelliculosa 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 Algal Assay Procedure (AAP) 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 23-24°C. The test vessels were 125-ml sterile flasks fitted with stainless steel caps to permit gas exchange. Each vessel contained 50 ml of test solution. Three replicates were used for each control and test treatment. Inoculum was introduced into the test vessels thirty minutes after the test solution. Flasks were continuously shaken at 100 revolutions/minute and a continuous photoperiod at an intensity of 4000-5000 lux was provided. The test medium used was the same as that used in culturing, excluding Na_2EDTA (Table 1, attached) and was adjusted to a pH of 7.5 ±0.1.

C. Dosage: Five-day growth and reproduction test. The nominal test concentrations of Propanil are based on the active ingredient. Based on results of a preliminary test, six nominal concentrations were used (0.0075, 0.016, 0.033, 0.063, 0.13, and 0.25 mg/L). A medium control and a solvent control (0.1 ml acetone/L) were also used.

D. Design: A primary stock solution (2.5 g/L) was prepared by mixing 0.1277 g of Propanil with acetone to a final volume of 50 ml. The primary stock was diluted with acetone to create secondary stock solutions. Equal volumes (0.05 ml) of the secondary stock solution were diluted with AAP medium to create nominal concentrations.

Within 30 minutes of test solution addition, an inoculum volume of 890 µl per flask was introduced to each test vessel resulting in a cell densities of 3000 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). Concentrations of Propanil were verified 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 0.0063, 0.0115, 0.0285, 0.0529, 0.1138, and 0.2366 mg a.i./L (Table 3, attached). The measured concentrations were fairly consistent between sampling days. The mean total cell density (x 10^4 cells/ml) in relation to mean measured concentration are shown in Table 4 (attached). Cell densities increased over time at all concentrations $\leq$0.053 mg a.i./L. Control and solvent control densities averaged 68-81 (x 10^4 cells/ml) at test termination, respectively, and were not significantly different from one another. The 5-day EC_{50} value (corresponding 95% confidence interval) based on cell density was 0.019 (0.0094-0.038) mg a.i./L (mean measured concentration) (Table 5, attached).

"... At test termination, pH ranged from 7.0 to 7.6 and decreased with increasing test concentrations. These pH changes during the test period reflect photosynthesis and respiration of algae and are consistent with the observed growth of the cultures at the different treatment levels.

Temperatures ranged from 23-24°C during the study." The conductivity was 170-200 µmhos.

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 used in the test (4.0-5.0 klux) was occasionally higher or lower than recommended (4.3 klux).

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 control was calculated for cell count according to the following formula:

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

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

The 5-day EC_{50} value using cell density as the growth endpoint was 0.016 mg a.i./L with a 95 percent confidence interval of 0.006-0.036 mg a.i./L based on mean measured concentrations (Printout 1, attached).

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 were transformed, Bonferroni's t-test was applied. The NOEC for the study was 0.0063 mg a.i./L (mean measured concentration) (Printout 2, attached). The authors did not present an NOEC value in the report.

C. **Discussion/Results:** The study appears to be scientifically sound and meets the requirements for a growth and reproduction study of aquatic plants -Tier II.

The 5-day EC_{50} value of Propanil for Navicula pelliculosa was determined to be 0.016 mg a.i./L (95 percent confidence limits are 0.006 and 0.036) based on cell density. The 5-day NOEC was 0.0063 mg a.i./L (mean measured concentration).

D. **Adequacy of the Study:**

(1) **Classification:** Core

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

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

15. **COMPLETION OF ONE-LINER:** Yes

RIN 1876-95

PROPANIL EEB REVIEW

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PRINTOUT 1

DAVY PROPANIL NAVICULA

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.2554	100	99	99	0
.1138	100	99	99	0
.0529	100	94	94	0
.0285	100	82	82	0
.0115	100	35	35	0
.0063	100	7	7	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 1.517128E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	1.537241E-02		1.601263E-02	
1.441189E-02		1.772221E-02		

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.5652951	13.6327	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

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

SLOPE = 2.96535
95 PERCENT CONFIDENCE LIMITS = .7358189 AND 5.194881

LC50 = 1.617161E-02
95 PERCENT CONFIDENCE LIMITS = 5.772503E-03 AND 3.589413E-02

LC10 = 6.032167E-03
95 PERCENT CONFIDENCE LIMITS = 1.88498E-04 AND 1.176633E-02

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PRINTOUT 6

Navicula in Propanil
File: propanil.nav Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	23327.000	3887.833	52.017
Within (Error)	14	1046.375	74.741	
Total	20	24373.375		

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

Navicula in Propanil
File: propanil.nav Transform: NO TRANSFORM

TUKEY method of multiple comparisons

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 6	0 7	0 5	0 4	0 3	0 2	0 1
6		0.1138	0.750	\						
7		0.2554	0.750	.	\					
5		0.0529	4.917	.	.	\				
4		0.0285	14.500	.	.	.	\			
3		0.0115	53.000	*	*	*	*	\		
2		0.0063	75.667	*	*	*	*	.	\	
1	control solvent		81.417	*	*	*	*	*	.	\

* = significant difference (p=0.05) . = no significant difference
Tukey value (7,14) = 4.83 s = 74.741

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Navicula in Propanil
File: propanil.nav Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	23327.000	3887.833	52.017
Within (Error)	14	1046.375	74.741	
Total	20	24373.375		

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

Navicula in Propanil
File: propanil.nav Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control solvent	81.417	81.417		
2	0.0063	75.667	75.667	0.815	
3	0.0115	53.000	53.000	4.026	*
4	0.0285	14.500	14.500	9.480	*
5	0.0529	4.917	4.917	10.837	*
6	0.1138	0.750	0.750	11.428	*
7	0.2554	0.750	0.750	11.428	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

Navicula in Propanil
File: propanil.nav Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control solvent	3			
2	0.0063	3	17.859	21.9	5.750
3	0.0115	3	17.859	21.9	28.417
4	0.0285	3	17.859	21.9	66.917
5	0.0529	3	17.859	21.9	76.500
6	0.1138	3	17.859	21.9	80.667
7	0.2554	3	17.859	21.9	80.667

icula in Propanil
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	23327.000	3887.833	52.017
Within (Error)	14	1046.375	74.741	
Total	20	24373.375		

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

Navicula in Propanil
File: propanil.nav Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control solvent	81.417	81.417		
2	0.0063	75.667	75.667	0.815	
3	0.0115	53.000	53.000	4.026	*
4	0.0285	14.500	14.500	9.480	*
5	0.0529	4.917	4.917	10.837	*
6	0.1138	0.750	0.750	11.428	*
7	0.2554	0.750	0.750	11.428	*

Bonferroni T table value = 2.72 (1 Tailed Value, P=0.05, df=14,6)

Navicula in Propanil
File: propanil.nav Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control solvent	3			
2	0.0063	3	19.186	23.6	5.750
3	0.0115	3	19.186	23.6	28.417
4	0.0285	3	19.186	23.6	66.917
5	0.0529	3	19.186	23.6	76.500
6	0.1138	3	19.186	23.6	80.667
7	0.2554	3	19.186	23.6	80.667