3-4-92

MRID No. 417774-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Propanil (3,4-dichloropropionlide). 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: Skeletonema costatum.

4. **CITATION:** Giddings, J.M., M.C.R. Bayne, J. Mao, and Shepherd, S.P. 1990. Propanil- Toxicity to the Marine Diatom Skeletonema costatum. SLI Report No. 90-3-3255. Performed by Springborn Laboratories, Inc., Wareham, Massachusetts. Submitted by The Propanil Task Force, Liberty, Missouri. EPA MRID No. 417774-01.

5. **REVIEWED BY:**

Michael 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 marine diatom test.

Based on cell density, the 5-day EC_{50} value of Propanil was 0.030 mg a.i./L (mean measured concentration). The 5-day NOEC was 0.013 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:** Skeletonema costatum used in this test were obtained from laboratory stock cultures at the testing facility. The original culture was obtained from Bigelow Marine Laboratory, Boothbay Harbor, Maine. Stock cultures were transferred weekly or twice weekly into fresh Marine Algal Medium. The test inoculum was taken from stock cultures which were six days old.

B. **Test System:** The phytotoxicity test was conducted in an environmental chamber which was maintained at a temperature of 18-21°C. The test vessels were 125-ml sterile flasks (with steel caps) containing 50 ml of test solution. Three replicates were used for each of the controls and test treatments. Flasks were continuously shaken at 60 revolutions/minute and a 16 hour light/8 hour dark 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).

C. **Dosage:** Five-day growth and reproduction test. Based on preliminary test, five nominal test concentrations (0.012, 0.025, 0.052, 0.10, and 0.20 mg/L, based on mean measured concentrations) of Propanil were used. The concentrations were adjusted to reflect the amount of active ingredient. A medium control and solvent control (0.1 ml acetone/L) were also used.

D. **Design:** About 105 minutes after test concentrations were introduced into the flasks, the flasks were inoculated with cell densities of 10,000 cells/ml. The inoculum volume was 840 µL per flask.

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 per flask was collected for counting.

E. **Statistics:** The EC_{10}, EC_{50}, and EC_{90} values and confidence limits after 48-, 72-, 96-, and 120-hours of exposure were calculated. If a significant difference was determined between the controls and solvent controls, the solvent control was used for EC

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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 on (a) untransformed data, (b) untransformed response vs. logarithm-transformed concentration, (c) probit-transformed response vs. untransformed concentration, and (d) probit-transformed response vs. logarithm-transformed concentration. The regression that best fitted the data was selected based on the highest coefficient of determination (r^2). This regression equation was then applied to estimate EC values and their 95% confidence limits, using the method of inverse prediction (Sokal and Rohlf, 1981). A computer program developed and validated at SLI was used to assist in these computations."

12. **REPORTED RESULTS:** The mean measured concentrations were fairly consistent between observations (Table 3, attached).

Cell densities increased at all concentrations ≤0.078 mg a.i./L during the exposure period (Table 4, attached). Control and solvent control densities averaged 199-200 (x 10^4 cells/ml) at test termination, respectively. The controls (medium and solvent) were not significantly different from one another. The 5-day EC_{50} value (95% confidence interval) using cell density as the growth endpoint was 0.032 (0.018-0.057) mg a.i./L (Table 5, attached).

"At test termination, pH ranged from 8.0 to 9.3 and decreased with increasing test concentration. 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 18-21°C during the study." The conductivity was 39,000-46,000 μmhos/cm during the test period.

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 during the study (4.0-5.0 klux) was higher than recommended (4.0 klux).

B. Statistical Analysis: The reviewer used the 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 solvent control,
X = mean growth in test concentration.

Percent inhibition determined at test termination was 0, 43, 82, 97, and 99% for the 0.013, 0.024, 0.049, 0.078, and 0.16 mg a.i./L mean measured concentrations, respectively. The 5-day EC_{50} value using cell density, based on mean measured concentration, was 0.030 mg a.i./L with a 95 percent confidence interval of 0.017-0.050 mg a.i./L, based on mean measured concentration (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 0.013 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 and meets the requirements for a growth and reproduction study of aquatic plants -Tier II.

The 5-day EC_{50} value of Propanil for Skeletonema costatum was determined to be 0.030 mg a.i./L (mean measured concentration). The 5-day NOEC was 0.013 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

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DATA EVALUATION RECORD

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3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants - Tier II. Species Tested: Skeletonema costatum.

4. **CITATION:** Giddings, J.M., M.C.R. Bayne, J. Mao, and Shepherd, S.P. 1990. Propanil- Toxicity to the Marine Diatom Skeletonema costatum. SLI Report No. 90-3-3255. Performed by Springborn Laboratories, Inc., Wareham, Massachusetts. Submitted by The Propanil Task Force, Liberty, Missouri. EPA MRID No. 417774-01.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Rosemary Graham Mora
Date: 6/4/91

6. **APPROVED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Rifici 6/10/92
Date: 6/4/91

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

Signature: Henry T. Craven
Date: 3/24/92

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 the maximum label rate for Propanil was not presented in the report. Based on cell density, the 5-day EC_{50} value of Propanil was 0.031 mg a.i./L (mean measured concentration). The 5-day NOEC was 0.013 mg a.i./L (mean measured concentration).

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

9. **BACKGROUND:**

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RIN 1876-95

PROPANIL-EEB REVIEW

Page_____ is not included in this copy.

Pages 7 through 12 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 skeltonema

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.16	100	99	99	0
.078	100	97	97	0
.049	100	82	82	0
.024	100	43	43	0
.013	100	0	0	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 .0270669

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	1.041091E-02		3.078666E-02	
2.866535E-02		3.299717E-02		

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.4791773	8.332662	

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 = 4.463327

95 PERCENT CONFIDENCE LIMITS = 1.373694 AND 7.552959

LC50 = 2.998744E-02

95 PERCENT CONFIDENCE LIMITS = 1.659182E-02 AND .0497123

LC10 = 1.557393E-02

95 PERCENT CONFIDENCE LIMITS = 2.770037E-03 AND 2.405536E-02

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PRINTOUT 2

propanil skeletonema
File: prop Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	134091.908	26818.382	62.888
Within (Error)	12	5117.343	426.445	
Total	17	139209.251		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

propanil skeletonema
File: prop 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	0	199.500	199.500		
2	.013	210.057	210.057	-0.626	
3	.024	114.083	114.083	5.066	*
4	.049	35.250	35.250	9.741	*
5	.078	6.583	6.583	11.442	*
6	.16	1.250	1.250	11.758	*

Bonferroni T table value = 2.68 (1 Tailed Value, P=0.05, df=12,5)

propanil skeletonema
File: prop 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	0	3			
2	.013	3	45.205	22.7	-10.557
3	.024	3	45.205	22.7	85.417
4	.049	3	45.205	22.7	164.250
5	.078	3	45.205	22.7	192.917
6	.16	3	45.205	22.7	198.250

PRINTOUT 2 (CONT)

TITLE: propanil skeletonema
FILE: prop
TRANSFORM: NO TRANSFORM NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	0	1	192.0000	192.0000
1	0	2	234.5000	234.5000
1	0	3	172.0000	172.0000
2	.013	1	254.5000	254.5000
2	.013	2	192.0000	192.0000
2	.013	3	183.6700	183.6700
3	.024	1	112.7500	112.7500
3	.024	2	112.0000	112.0000
3	.024	3	117.5000	117.5000
4	.049	1	29.5000	29.5000
4	.049	2	39.5000	39.5000
4	.049	3	36.7500	36.7500
5	.078	1	9.0000	9.0000
5	.078	2	5.5000	5.5000
5	.078	3	5.2500	5.2500
6	.16	1	2.0000	2.0000
6	.16	2	1.7500	1.7500
6	.16	3	0.0000	0.0000

propanil skeletonema
File: prop Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	0	3	172.000	234.500	199.500
2	.013	3	183.670	254.500	210.057
3	.024	3	112.000	117.500	114.083
4	.049	3	29.500	39.500	35.250
5	.078	3	5.250	9.000	6.583
6	.16	3	0.000	2.000	1.250

propanil skeletonema
File: prop Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	0	1018.750	31.918	18.428
2	.013	1498.755	38.714	22.351
3	.024	8.896	2.983	1.722
4	.049	26.688	5.166	2.983
5	.078	4.396	2.097	1.210
6	.16	1.188	1.090	0.629

PRINTOUT 2 (CONT.)

propanil skeletonema
File: prop Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	134091.908	26818.382	62.888
Within (Error)	12	5117.343	426.445	
Total	17	139209.251		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

propanil skeletonema
File: prop 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	0	199.500	199.500		
2	.013	210.057	210.057	-0.626	
3	.024	114.083	114.083	5.066	*
4	.049	35.250	35.250	9.741	*
5	.078	6.583	6.583	11.442	*
6	.16	1.250	1.250	11.758	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

propanil skeletonema
File: prop 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	0	3			
2	.013	3	42.153	21.1	-10.557
3	.024	3	42.153	21.1	85.417
4	.049	3	42.153	21.1	164.250
5	.078	3	42.153	21.1	192.917
6	.16	3	42.153	21.1	198.250