3-3-92

MRID No. 417772-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Propanil (3,4-dichloropropionanilide)
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: Lemna gibba.

4. **CITATION:** Giddings, J.M., C. DeCosta, J. Mao, and S.P. Shepherd. 1990. Propanil - Toxicity to Duckweed (Lemna gibba G3). SLI Report No. 90-4-3294. Performed by Springborn Laboratories, Inc., Wareham, Massachusetts. Submitted by The Propanil Task Force, Liberty, Missouri. EPA MRID No. 417772-01.

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 3 92

7. **CONCLUSIONS:** This study appears to be scientifically sound but does not meet the guideline requirements for a Tier II - growth and reproduction of a non-target area aquatic plant study, since test procedures deviated from SEP recommendations in light intensity, temperature and algae contamination.

Based on number of fronds, the 14-day EC_{50} value of Propanil for Lemna gibba was 0.110 mg a.i./L (0.074 - 0.161). The NOEC value was 0.02 mg a.i./L (mean measured concentration).

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:** Lemna gibba G3 used in this test were originally obtained from Charles F. Cleland, of the U.S. Department of Agriculture, Washington, D.C. and maintained in stock cultures at the laboratory. The culture vessels were 270-ml crystallizing dishes containing 100 Ml of nutrient medium. Stock cultures were acclimated to test conditions for 7 days at a temperature of 25 ±2°C. A continuous photoperiod at a light intensity of 300-500 footcandles (approximately 3230-5382 lux) was provided. Stock cultures were transferred to fresh medium at least weekly.

The M-type Hoagland's medium was used for the cultures (Table 1, attached). The medium was autoclaved and adjusted to a Ph of 5.1 prior to use.

B. **Test System:** The test vessels and test medium were the same as those used in culturing. The phytotoxicity test was conducted in an incubator at a temperature of 25-28°C. A continuous photoperiod at an intensity of 150-500 footcandles (approximately 1615-5382 lux) was provided.

C. **Dosage:** Fourteen-day growth and reproduction test. The nominal test concentrations, based on active ingredient of Propanil and a preliminary test, were 0.026, 0.052, 0.10, 0.20, 0.40, and 0.80 mg/L. A control and solvent control (0.1 Ml acetone/L) were also used.

D. **Design:** Each concentration and control was replicated three times. Thirty minutes after the solutions were added to each of the dishes, 15 fronds (5 plants containing 3 fronds each) were added to each dish. Test solutions were renewed on Days 3, 6, 9, and 12. Observations and the number of fronds were recorded at renewal on Days 3, 6, 9, 12, and 14. Temperature was measured continuously; light intensity was measured daily. The Ph of old and new test solutions were measured at each renewal and at test termination.

The concentration of Propanil was analyzed from samples collected at 0 and 72 hours.

E. **Statistics:** A comparison of the control and solvent control was made using Student's t-test. The 14-day EC value for the frond count was calculated using linear regression of response analysis. "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 the 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."

The NOEC was calculated using a one-way analysis of variance (ANOVA) (Sokal and Rohlf, 1981) and Dunnett's procedure (Dunnett 1955, 1964).

12. **REPORTED RESULTS:** The mean measured concentrations were 0.02, 0.039, 0.087, 0.17, 0.34, and 0.70 mg a.i./L (Table 3, attached). The concentrations were consistent between sampling observations with the exception of 0.052 mg/L nominal concentration which measured 0.052 mg a.i./L at test initiation and 0.026 mg a.i./L at test termination.

Growth and reproduction data are presented in Table 4 (attached). "Control frond production averaged 306 per replicate at test termination while solvent control frond production averaged 293 per replicate. Statistical comparison demonstrated there was no significant difference ($p = 0.05$) between frond production of the control and solvent control. Therefore, all further comparisons were based on a pooled control response. Average frond production in the exposure concentrations ranged from 265 to 29 in the 0.020 and 0.70 mg a.i./L mean measured concentrations, respectively and clearly followed the concentration gradient established.

"The 14-day EC_{10}, EC_{50}, and EC_{90} values based on response (untransformed data) vs. log concentration (transformed data) were 0.019 (0.0063-0.051), 0.11 (0.039-0.29) and 0.60 (0.22-1.8) mg a.i./L as Propanil, respectively. Statistical analyses using Dunnett's Procedure established a No Observed Effect Concentration (NOEC) for the 14-day study of 0.020 mg a.i./L Propanil" (Table 5, attached).

During the test period, the pH ranged from 4.9-5.5, the light intensity was 150-500 footcandles, and the temperature was 25-28°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors made no conclusions.

A Good Laboratory Practice Compliance Statement was included in the report, indicating that the study was conducted in accordance with the Good Laboratory Practice Standards set forth in 40 CFR Part 160, with the exception of stability, characterization, and verification of test substance identity. A Quality Assurance Unit Statement was also included in the report. These statements were signed by representatives of the performing laboratory and/or the Propanil Task Force.

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 range during the test was 150-500 footcandles (1615-5382 lux). The SEP recommends continuous light at 5000 lux.

Temperature during the test was 25-28°C. The SEP recommends 25°C.

There appear to be algae contamination in the test.

The ASTM document E1415-91 recommends that Lemna gibba cultures should be held under synthetic dilution water, light and temperature testing conditions for at least 8 weeks prior to start of test. It is unclear as to how long the plants were under these conditions.

B. **Statistical Analysis:** The reviewer used the EPA's Toxanal computer program to calculate the 14-day EC_{50} values using percent inhibition of the number of fronds and mean measured concentrations. Percent inhibition (I) of growth compared to controls was calculated for the number of fronds according to the following formula:

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

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

An ANOVA was performed to compare the number of fronds at each treatment level to those of the solvent control (printout, attached).

The EC and NOEC values presented by the author are similar to the results obtained by the reviewer.

C. **Discussion/Results:** The study appears to be scientifically sound but does not meet the requirements for a growth and reproduction study of aquatic plants - Tier II. The light intensity was usually below the recommended SEP guidelines by more than 35% and on day 4, the light intensity was down to 68% below the guidelines. Algae contamination was mentioned as a possible explanation for the decline of propanil concentration at 72 hours measurement. Therefore it appears that algae contamination may be a factor in the test.

Based on the number of fronds, the 14-day EC_{50} value of propanil for Lemna gibba was 0.110 mg a.i./L (mean measured concentration). The 14-day NOEC was 0.02 mg a.i./L (mean measured concentration).

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** Light intensity below guidelines and appearance of possible algae contamination.

(3) **Repairability:** Not Repairable

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

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5. **REVIEWED BY:**

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KBN Engineering and
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Signature: Rosemary Graham Mora
Date: 6/4/91

6. **APPROVED BY:**

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Date: 6/4/91

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

Signature: Henry T. Craven
Date: 3/04/92

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a Tier II - growth and reproduction of a non-target area aquatic plant study, since the maximum label rate was not included in the report. Based on number of fronds, the 14-day EC_{50} value of Propanil for Lemna gibba was 0.10 mg a.i./L (mean measured concentration). The NOEC value was 0.02 mg a.i./L (mean measured concentration).

8. **RECOMMENDATIONS:** The registrant should submit maximum label rate information.

9. **BACKGROUND:**

RIN 1876-95

PROPANIL EEB REVIEW

Page_____ is not included in this copy.

Pages 7 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.

LEWIS PROPANIL (LEMNA)

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.7	100	90	90	0
.34	100	82	82	0
.17	100	66	66	0
.087	100	31	31	0
.039	100	30	30	0
.02	100	10	10	0

PRINTOUT 1

THE BINOMIAL TEST SHOWS THAT .087 AND .17 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .1253132

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
5	.0247117	.1087102	9.198314E-02 .1279193

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
2	.1101547	2.596653	3.439528E-02

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

SLOPE = 1.657497

95 PERCENT CONFIDENCE LIMITS = 1.107381 AND 2.207613

LC50 = .1099874

95 PERCENT CONFIDENCE LIMITS = 7.452354E-02 AND .1605494

LC10 = 1.884201E-02

95 PERCENT CONFIDENCE LIMITS = 6.932491E-03 AND 3.272509E-02

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PRINTOUT 2

lemna
File: lemna.stat Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	194203.905	32367.317	41.578
Within (Error)	14	10898.667	778.476	
Total	20	205102.571		

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

lemna
File: lemna.stat Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	292.667	292.667		
2	.02	265.333	265.333	1.200	
3	.039	205.000	205.000	3.848	*
4	.087	202.000	202.000	3.980	*
5	.17	101.333	101.333	8.399	*
6	.34	52.000	52.000	10.564	*
7	.70	28.667	28.667	11.588	*

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

lemna
File: lemna.stat Transform: NO TRANSFORMATION

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	.02	3	57.636	19.7	27.333
3	.039	3	57.636	19.7	87.667
4	.087	3	57.636	19.7	90.667
5	.17	3	57.636	19.7	191.333
6	.34	3	57.636	19.7	240.667
7	.70	3	57.636	19.7	264.000