6-4-91

MRID No. 417771-01

DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** Propanil Technical; Code # BLUE; 98 ±2% active ingredient (a.i.); a blue-gray crystal.

3. **STUDY TYPE:** Mollusc 96-Hour, Flow-Through Shell Deposition Study. Species Tested: Eastern Oyster (Crassostrea virginica).

4. **CITATION:** Dionne, E. 1990. (Propanil) - Acute Toxicity to Eastern Oyster (Crassostrea virginica) Under Flow-Through Conditions. SLI Report No. 89-10-3184. Prepared by Springborn Laboratories, Inc., Wareham, Massachusetts. Submitted by The Propanil Task Force, Liberty, Missouri. EPA MRID No. 417771-01.

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

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

Signature:
Date:

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for a 96-hour flow-through mollusc shell deposition acute toxicity test. The 96-hour EC_{50} value for eastern oysters exposed to Propanil was 4.96 mg a.i./L, based on mean measured concentrations. Therefore, Propanil is classified as moderately toxic to Crassostrea virginica. The NOEC could not be confirmed or determined, since replicate data were not presented in this report.

8. **RECOMMENDATIONS:** The registrant should submit the raw shell deposition data.

OK

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9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Eastern oysters (Crassostrea virginica) were obtained from Aquacultural Research Corporation (ARC), Dennis, Massachusetts. The supplier held the oysters in natural flowing seawater with a temperature range of 13-18°C, a pH range of 7.8-8.0, and a salinity range of 30-31 parts per thousand (ppt). The oysters were continuously fed a combination of marine algae (25 X 10^3 cells/mL). Oysters were transported (1.5 h) to the testing laboratory outside of water. No mortality was noted during transportation. Once received in the laboratory the animals were held in wooden epoxy-painted trays with flowing seawater and examined for suitability in the test. The oysters were of similar age and size and had a mean valve height was 36 ±3 mm.

Seventy-two hours prior to test initiation, the oysters were held in water at a temperature of 21-22°C, a salinity of 31-32 ppt, a pH of 7.5-7.9, and a dissolved oxygen level of 85-105% of saturation. No mortality was noted during the holding period at the testing laboratory.

B. **Test System:** The test was conducted in a continuous flow serial diluter system. This system supplied 75 mL of test solution per minute to each aquarium, providing six volume replacements every 24 hours. The glass test aquaria were each equipped with a standpipe to regulate solution volume at 18 L. Circulation was provided within each aquarium to provide even an distribution of algae and test solution. The combined flow-through volume and recirculated volume of test solution equalled 7.5 L/oyster/hour.

Fourteen aquaria (two aquaria per test concentration and the controls) were randomly positioned in a temperature-controlled water bath (20 ±2°C). Each aquarium was stocked with 20 oysters. The oysters were placed equidistance from one another.

The test was conducted under fluorescent lighting on a 16-hour light and 8-hour dark photoperiod.

The dilution water was natural unfiltered seawater collected from Cape Cod Canal, Massachusetts. The pH range was 7.8-8.0. Salinity was 32-33 ppt throughout the test period. Temperature was maintained at 20°C throughout the test.

C. **Dosage:** 96-hour flow-through acute test. Five nominal test concentrations (1.3, 2.2, 3.6, 6.0, and 10.0 mg a.i./L), a dilution water control, and a solvent control were used.

D. **Design:** Twenty-four hours prior to test initiation, approximately 3-5 mm of the new peripheral shell growth of each oyster was removed by grinding the shell to a blunt edge. Immediately prior to test initiation the outer shell edge were buffed to remove any new shell deposition. The test was initiated by impartially selecting and positioning 20 oysters in each test aquarium (40 per treatment).

During exposure 180 mL of concentrated algae (10^7 cells/mL) were added to each aquarium three times daily. The resulting algal density in the test aquaria was approximately 10^5 cells/mL.

Every 24 hours the oysters were observed for mortality and visible abnormalities. The oysters were removed from the test containers after 96 hours of continuous exposure and new shell growth of each oyster was measured to the nearest 0.1 mm using a calibrated micrometer reticle in the eyepiece of a microscope.

The temperature, salinity, pH, and dissolved oxygen concentration were measured daily in each aquarium. The temperature was also continuously monitored in the control with a minimum/maximum thermometer. Analytical determination of Propanil was performed on the control, solvent control, and each test concentration on days 0 and 4 using liquid chromatography.

E. **Statistics:** "The mean shell growth measurement of 40 individual oysters for each of the five exposure concentrations were expressed as a percentage of the control oyster growth."

EC_{50} values (95 percent confidence limits), based on mean measured concentrations, were determined by fitting untransformed and transformed data to a best fit linear regression curve based on least squares.

Four linear regression curves were computed and the best fit of the untransformed or transformed data was selected based on the highest associated coefficient of determination (i.e., r^2). This regression equation was then applied to calculate the EC_{50} (95% confidence limits), using the method of inverse prediction (Sokal and Rohlf, 1969). An SLI computer program was used to assist in the calculation. If no concentration caused a reduction of shell growth $\geq$ 50%, the EC_{50} was empirically estimated to be greater that the highest concentration tested (Figure 1).

Williams' (1972) test method was used to determine the NOEC. There was no significant difference between the control and solvent control, therefore the control data were pooled for analysis.

12. **REPORTED RESULTS:** Mean measured concentrations were 1.4, 2.0, 3.4, 5.7, and 11 mg a.i./L, based on analyses of test solutions at test initiation and test termination (Table 2, attached). The mean measured concentrations ranged from 91% to 110% of the nominal concentrations.

"After 24 hours of exposure, no feeding and no fecal matter production were observed among oysters exposed to the highest concentration tested (11 mg a.i./L Propanil). No toxicant-related sublethal effects or abnormal behavior were observed among organisms exposed to the remaining mean measured concentrations ($\leq$ 5.7 mg a.i./L)." The NOEC was <1.4 mg a.i./L, based on mean measured concentrations.

"Following 96 hours of exposure, shell growth at treatment level 11, 5.7, 3.4, 2.0, and 1.4 mg a.i./L was reduced by 91, 47, 31, 22, and 16%, respectively (Table 3 [attached]). Shell growth at these exposure levels ranged from 0.33 to 2.7 mm and was significantly ($p \leq 0.05$) reduced as compared to that of the control organisms (pooled; 3.2 mm)." The EC_{50} (95% confidence interval) was 5.8 (5.1-6.5) mg a.i./L, based on mean measured concentrations.

During the test period, the pH was 7.8-8.1, the DO was 7.1-8.6 mg/L, the temperature was 20°C, and the salinity was 32-33 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Based on these results and criteria established by the U.S. EPA (1985), Propanil is classified as moderately toxic to eastern oysters (Crassostrea virginica).

A Good Laboratory Practice Statement was included in the report, indicating that the study was conducted in accordance with the FIFRA Good Laboratory Practice Standards, with this exception: "the stability, characterization and verification of the test substance identity and maintenance of records on the test substance is the responsibility of the test Sponsor." This statement was signed by representatives of the performing laboratory and the Propanil Task Force.

A Quality Assurance Statement was also included and was signed by a quality assurance representative of the performing laboratory.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP and ASTM as follows:

In this study the flow rate of the "recirculating" test solution was 7.5 L/oyster/hour. According to protocols recommended by the SEP (APHA, 1981 and Anonymous, 1976) each oyster should receive a minimum of 5 L of "once-through" flow through test solution per hour. However, this test is considered acceptable because a supplemental diet was added.

The SEP recommends a 16-hour light and an 8-hour dark photoperiod with 15 to 30 minute transition periods between light and dark. The report did not indicate whether a 15-30 minute transition period was employed.

The SEP recommends that oysters are introduced into test system, then the toxicant is injected into the system. The report indicates that the toxicant was present in the system and then the oysters were introduced.

The concentration of acetone in the solvent control was not reported. The SEP recommends that the solvent concentration not exceed 0.5 mL/L.

The text indicates that the data for individual oyster measurements are included. The data was not in the report reviewed. Therefore, an NOEC could not be determined.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to calculate the 96-hour EC_{50} value (attached printout). The EC_{50} was 4.96 mg a.i./L with a confidence interval of 4.4-5.6 mg a.i./L, mean measured concentration. The reviewer's EC_{50} value were lower than and the confidence interval narrower than that of the author, therefore, the reviewer's value should be used for purpose of risk assessment.

The NOEC could not be determined since individual measurements were not presented in the report.

C. **Discussion/Results:** The deviations from test protocol (Section 14A) probably did not alter the test results.

The study is scientifically sound and but does not meet the guideline requirements for a 96-hour mollusc shell deposition acute toxicity test, since the report does not include the raw shell deposition data.

The 96-hour EC_{50} value, based on mean measured concentrations of Propanil, was 4.96 mg a.i./L. Therefore, Propanil is classified as moderately toxic to Crassostrea virginica. The NOEC could not be determined.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental

(2) **Rationale:** Raw shell deposition data was not presented in the report, therefore the reviewer was unable to validate the author's NOEC value.

(3) **Repairability:** The registrant should submit the missing data.

15. **COMPLETION OF ONE-LINER:** Yes, June 4, 1991.

RIN 1876-95

PROPANIL EEB REVIEWS

Page_____ is not included in this copy.

Pages 8 through 10 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.

Rosemary Graham Mora Propanil Crassostrea virginica 04-17-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
11	100	91	91	0
5.7	100	47	47	0
3.4	100	31	31	0
2	100	22	22	0
1.4	100	16	16	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 5.925554

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	3.243228E-02	4.960189	4.423765	5.598388

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.438541	5.468196	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 = 2.354126

95 PERCENT CONFIDENCE LIMITS = .7951668 AND 3.913086

LC50 = 4.593221

95 PERCENT CONFIDENCE LIMITS = 2.784877 AND 10.11758

LC10 = 1.326292

95 PERCENT CONFIDENCE LIMITS = .1451809 AND 2.324194

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Shaughnessey # 028201 Chemical Name Propanil (3,4-Dichloropropionanilide) Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
48-Hour EC_{50}	_____	EC_{50} - pp 95% C.L. ( ) Control Mortality (%) - Solvent Control Mortality (%) - Slope - # Animals/Level - Temperature - 48-Hour Dose Level pp /(% Effect) ( ), ( ), ( ), ( ), ( ) Comments:	_____	_____
96-Hour LC_{50} Species: Crassostrea virginica Lab: Springborn Lab MRID # 417771-01	98±2%	EC_{50} - 4.96 a.i.* ppm 95% C.L. moving average (4.4, 5.6) Control Mortality (%) - ~~NA~~ 0 Solvent Control Mortality (%) - 0 Slope - NA # Animals/Level - 40 Temperature - 20 96-Hour Dose Level pp a.i.* ppm /(% ~~Mortality~~ Effect) 1.4 (16), 2 (22), 3.4 (31), 5.7 (47), 11 (91) Comments: *Based on mean measured concentrations and shell deposition. Raw shell deposition data was not included in the report.	RGH 6/4/91	Supplemental