9-5-91

MRID No. 413602-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Propanil-3,4-Dichloropropionilide. Shaughnessey No. 028201.

2. **TEST MATERIAL:** Propanil-4 Formulation; 44% active ingredient (as determined by performing laboratory); a dark liquid.

3. **STUDY TYPE:** Freshwater Fish Acute Flow-Through Toxicity Test. Species Tested: Oncorhynchus mykiss.

4. **CITATION:** Richie, P. and W. McAllister. 1989. Acute 96-Hour Flow-Through Toxicity of Propanil-4 Formulation to Rainbow Trout (Oncorhynchus mykiss). ABC Report No. 37769. Performed by Analytical Bio-Chemistry Laboratories, Inc. Columbia, Missouri. Submitted by Propanil Task Force, Inc., c/o John M. Wise Associates, Liberty, Missouri. EPA MRID No. 413602-01.

5. **REVIEWED BY:**

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

Signature:

Date:

6. **APPROVED BY:**

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

Signature:

Date:

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

Signature: Dan Ballhoff

Date: 9-5-91

7. **CONCLUSIONS:** This study appears scientifically sound and fulfills guideline requirements for a freshwater fish acute toxicity test of Propanil-4 Formulation to rainbow trout. The LC50 value was 12.8 mg/L, based on mean measured formulated test substance (5.6 mg/L ai). The NOEC was 3.8 mg/L of formulated test substance (1.7 mg/L ai).

However, these results should be viewed with caution because a precipitate was reported in the test vessels and test solution samples taken for analysis were not filtered before they were measured for test material concentrations. Therefore, the portion of the measured Propanil-4

Formulation in the test solution that was actually available to the test organisms is in question.

8. RECOMMENDATIONS: N/A.

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Oncorhynchus mykiss (as green eggs and sperm) were obtained from Mt. Lassen Trout Farms of Red Bluff, California. The fish were maintained in ABC well water and were fed brine shrimp or commercial food daily. The photoperiod was 16 hours of light. Seventy-two hours prior to the test, approximately 200 fish were removed from the holding tank and placed in a temperature acclimation unit. Feeding was discontinued during acclimation.

A representative group of fish used during this study had a mean weight of 1.6 (±0.35) g and a mean standard length of 48 (±3.8) mm.

B. **Test System:** The flow-through test system was a proportional diluter apparatus. The test vessels were 30-L aquaria.

A syringe pump was used to deliver stock solution to the diluter system. The diluter delivered seven nominal concentrations of test material, a solvent control, and a dilution water control to each test vessel. Test solutions were delivered to each vessel at an average rate of 7.4 volume replacements/day.

The diluent, ABC soft blended water, had a total hardness of approximately 40 mg/L as $CaCO_3$.

C. **Dosage:** Ninety-six hour acute flow-through test. Seven nominal test concentrations (1.0, 2.0, 4.0, 8.0, 16.0, 32.0, and 64.0 mg/L) were selected for this study, based on the results of range-finding study .

The diluter stock solution was prepared by mixing 635.2 g of formulated test substance up to 1 liter with acetone, creating a concentration of 635,200 mg/L. The diluter delivered 400 µL of the stock solution to 3.97 L of dilution volume.

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D. Design: Twenty rainbow trout were impartially assigned and distributed to each exposure vessel (one vessel per concentration). The biomass loading rate was 0.14 g/L/day.

Observations of survival and abnormal effects were made every 24 hours. Dead fish were removed at each observation. Temperature, dissolved oxygen concentration (DO), and pH were measured in the controls, high, and low concentrations containing live fish at 0- and 96-hours of testing. The temperature in a control vessel was monitored continuously.

The concentration of Propanil-4 Formulation present in solution was determined by high pressure liquid chromatography (HPLC). Measured concentrations were adjusted to reflect the quantity of the total product.

E. Statistics: The LC_{50} (with 95% confidence limits) was determined using the computer program by Stephan (1978). For this study only the binomial method generated in an LC_{50} value.

12. REPORTED RESULTS: "A light brown precipitate was present in the mixing box, concentration cells and the two highest test levels. This precipitate persisted throughout the study."

Results of HPLC analyses indicated mean measured concentrations were 1.8, 2.3, 3.8, 10, 15, 31, and 68 mg/L of formulated test substance (Table 3, attached). Measured concentrations at 0 and 96 hours were 100-210% and 58-140% of nominal concentrations, respectively.

The 96-hour LC_{50} (95% confidence interval) was 14 (10-15) mg/L, based on mean measured concentrations (Table 5, attached). During the study, behavioral/sublethal effects were noted at the 10 and 15 mg/L test concentrations. No sublethal effects were noted in the 1.8, 2.3, and 3.8 mg/L concentrations, therefore the no-effect concentration of Propanil-4 Formulation to rainbow trout was determined to be 3.8 mg/L (Table 6, attached).

During the study, the DO concentrations were maintained at 9.2-9.6 mg/L. The pH range was 8.0-8.1. The temperature was maintained at 11.8-12.4°C.

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

A GLP compliance statement was included in the report indicating that this study was conducted in accordance with EPA Good Laboratory Practice Standards. This statement was signed by the Study Director and other representatives.

A Quality Assurance Statement was included and signed by a representative of the Quality Assurance Unit of the preforming 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 or Subdivision E as follows:

During the study, the concentration of Propanil-4 Formulation decreased by as much as 56% of initial (0 hour) measured concentration. The concentration of test substance in solution was not consistent within concentrations.

The report did not indicate aging or aeration of the control water prior to test initiation. A seven day aging period and intense aeration is recommended.

A period of acclimation of the test organism to test conditions was not indicated in the report. A two-week acclimation period to test conditions is recommended.

The test concentrations selected for this study were not 60% of the next highest concentrations as recommended in the SEP.

The report did not indicate the depth of test solution maintained in test vessel, the construction material, or dimensions of the test aquaria.

Impartial selection was used to distribute the test organisms to the test vessels. Random selection should have been employed as recommended in the guidelines.

Water quality parameters were obtained only from the high and low test concentrations and the control. Parameters in the medium concentration should have been recorded.

Age of the test organisms was not given in the text of the report.

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The report did not indicate the manner in which the test temperature was maintained.

The report stated the photoperiod used during the pre-test acclimation period, but did not indicate the photoperiod used during the study.

The manner in which the test solutions were prepared for each concentration was not clear (Section 11B).

B. **Statistical Analysis:** The reviewer used EPA's Toxanal to verify the authors' LC_{50} value and 95% confidence interval (printout, attached). The reviewer's results were the same as the authors.

C. **Discussion/Results:** Given the results of the controls, it is probable that most of the above mentioned deviations did not affect the results of the study. However, the concentration of Propanil-4 Formulation decreased by as much as 56% of initial measured concentration during the test period. The report states that precipitates were visible in the diluter system and in the test vessels of the two highest test concentrations. The method used to quantify the Propanil-4 Formulation concentration does not include a filtration step. The precipitate may have been put into solution prior to analysis, resulting in a false description of the amount of test material in the solution during exposure.

The LC_{50} was 12.8 mg/L (95% confidence interval 10 to 15 mg/L), which indicates that Propanil-4 Formulation is moderately toxic to Oncorhynchus mykiss. The NOEC was 3.8 mg/L.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** However, these results should be viewed with caution because a precipitate was reported in the test vessels and test solution samples taken for analysis were not filtered before they were measured for test material concentrations. Therefore, the portion of the measured Propanil-4 Formulation in the test solution that was actually available to the test organisms is in question.

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

15. **COMPLETION OF ONE-LINER:** Yes, May 31, 1991.

RIN 1876-95

FEB PROPANIL REVIEWS

Page_____ is not included in this copy.

Pages 7 through 9 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-4 FORMULATION ONOCOHYNCHUS MYKISS 05-14-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
68	20	20	100	9.536742E-05
31	20	20	100	9.536742E-05
15	20	18	90	2.012253E-02
10	20	0	0	9.536742E-05
3.8	20	0	0	9.536742E-05
2.3	20	0	0	9.536742E-05
1.8	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 10 AND 15 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 12.79774

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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Shaughnessey # 028201 Chemical Name Propanil-4 Formulation 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 (%) =		
Species:		Slope = # Animals/Level = Temperature =		
Lab:		48-Hour Dose Level pp /(% Effect) ( ), ( ), ( ), ( ), ( )		
MRID #		Comments:		

96-Hour LC_{50}	44%	LC_{50} = 12.8 *ppm (95% C.L. Binomial 10, 15) Control Mortality (%) = 0 Solvent Control Mortality (%) = 0	RON 5/31/91	Invalid
Species:	Oncorhynchus mykiss	Slope = N/A # Animals/Level = 20 Temperature = ~12°C		
Lab:	Analytical Bio-Chemistry Labs.	96-Hour Dose Level *ppm /(% Mortality) 1.8 (0), 2.3 (0), 3.8 (0), 10 (0), 15 (0), 31 (100), 68 (100)		
MRID #	413602-01	Comments: * Based on mean measured concentrations. The measured concentrations decreased substantially during the exposure period.		