RETURN TO EEB file 9-5-91

MRID No. 417765-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Propanil.
Shaughnessey No. 028201.

2. **TEST MATERIAL:** Propanil Technical; 3,4-dichloropropionanilide; Code No. BLUE; Batch No. 01; Aliquot No. 14; 98% active ingredient; a blue-gray-colored crystal.

3. **STUDY TYPE:** Freshwater Fish Early Life-Stage Test. Species Tested: Fathead minnow (Pimephales promelas).

4. **CITATION:** Sousa, J.V. 1991. (Propanil Technical) - Toxicity Test with Fathead Minnow (Pimephales promelas) Embryos and Larvae. SLI Report No. 90-6-3357. Study conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by Propanil Technical Task Force, Liberty, Missouri. EPA MRID No. 417765-01.

5. **REVIEWED BY:**

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6. **APPROVED BY:**

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Henry T. Craven, M.S. **Signature:** Dan Rieff
Supervisor, EEB/HED
USEPA **Date:** 9-5-91

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a fish early life-stage test. Raw data were not presented in the report. According to the author's report, the MATC of Propanil Technical for Pimephales promelas was >19 and <34 µg a.i./L mean measured concentrations (geometric mean MATC=25 µg a.i./L).

8. **RECOMMENDATIONS:** The registrant must submit all applicable raw data for validation.

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20 hours

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9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Fertilized eggs were obtained from the fathead minnow (Pimephales promelas) culture unit at the testing facility.

B. **Test System:** The test system was a modified proportional flow dilution apparatus with a 50% dilution factor. The diluter delivered five nominal concentrations of test material, a solvent control, and a dilution water control to duplicate test aquaria. Each glass test aquarium measured 39 x 20 x 25 cm with a 19.5-cm high screen-covered overflow drain that maintained a constant solution volume of 15 L.

The diluter delivered 0.5 L of solution per cycle to each aquarium at an average rate of approximately 5.9 volume replacements per day. Embryo incubation cups were glass jars (5 cm O.D., 8 cm high) with 40-mesh Nitex® screen bottoms. A rocker arm apparatus was used to gently oscillate the incubation cup in each test aquarium.

The dilution water was obtained from an aerated epoxy-coated concrete reservoir that was filled from an on-site well and supplemented with Town of Wareham untreated well water. The water, characterized weekly, had the total hardness and alkalinity ranges of 24-35 and 20-27 mg/L as $CaCO_3$, respectively; the pH range of 6.8-7.2; and the specific conductivity range of 110-140 µmhos/cm.

Sixteen hours of light at an intensity of 50-140 footcandles at the water surface were provided each day. Test temperature was maintained at 25 ± 1°C by a water bath containing circulating water.

C. **Dosage:** Thirty-five-day embryo-larval, flow-through test. Nominal test concentrations selected based on results of a range-finding study were 5, 10, 20, 40, and 80 µg a.i./L. The stock solution was prepared in acetone.

D. **Design:** Two replicates of five Propanil concentrations, a solvent control (18 µL acetone/L), and a dilution water control were included in the test.

Embryos were impartially distributed to the 14 incubation cups (60 embryos/cup). Each cup was then suspended in each test aquarium. A rocker arm apparatus gently oscillated the cups in the test solutions. Dead embryos were counted and removed daily. Hatching was considered complete (test day 4) when no more than 10% unhatched viable embryos remained in any incubation cup.

On test day 4, 40 larvae were impartially selected from each incubation cup and placed into their respective aquarium. Larvae were fed live brine shrimp (Artemia salina) nauplii three times daily on week days and twice daily on weekend days and holidays. The aquaria were brushed and siphoned when necessary (generally several times a week). The exposure period lasted until day 31 post-hatch (i.e., test day 35).

Behavior and appearance of larvae were observed and recorded daily. Larval survival was estimated twice weekly. At test termination (test day 35), the larvae were counted, and individually measured and weighed.

Dissolved oxygen concentration (DO), pH, and temperature were measured daily in every aquarium. Total hardness and alkalinity as $CaCO_3$, and specific conductance were measured on day 0 and then weekly in alternating replicates of the highest and lowest exposure groups and both controls.

Water samples were collected from the midpoint of each aquarium on test days 0, 4, 11, 18, 24, 31, and 35 for determination of Propanil concentrations using HPLC. In addition, a second set of water samples from each sampling interval was analyzed for 3,4-dichloroaniline (DCA), a degradation product of Propanil.

E. **Statistics:** The percentage survival data were transformed (arcsine square-root percentage) before analysis. A one-way analysis of variance indicated that the acetone control response was not different from the negative control response; therefore, the control data were pooled.

The data were normally distributed (Chi-square goodness of fit test) with homogeneous variance (Bartlett's test); therefore, Williams' test was used to assess exposure-level effects. Treatment levels that caused significant survival effects were excluded from the analysis of growth data. All analyses were performed using the mean organism response in each replicate aquarium.

12. **REPORTED RESULTS:** Throughout the exposure period, no signs of undissolved Propanil were observed in the exposure aquaria. The mean measured concentrations of Propanil established in the test solutions were 6.3, 9.3, 19.0, 34.0, and 60.0 µg a.i./L (Table 2, attached). The highest average level of DCA detected was 25 µg/L found in the 80 µg a.i./L nominal concentration (Table 3, attached).

During the study, pH was 6.9-7.4; mean total hardness and alkalinity were 20-40 and 16-24 mg/L as $CaCO_3$, respectively; specific conductance was 130-150 µmhos/cm; and the temperature was 24-26°C. The levels of dissolved oxygen concentrations ranged from 5.6 to 8.7 mg/L.

A summary of the biological results is presented in Table 4 (attached). "Fathead minnow survival at the completion of the hatching period (day 4) in all test concentrations of Propanil Technical (60-6.3 µg a.i./L) ranged from 87 to 94% and was statistically comparable to the survival of the control organisms (pooled control and solvent control data, 90%) (Figure 2.)" (attached).

"At the end of the post-hatch exposure period (31 days), 30% and 75% survival was observed in the two highest treatment levels (60 and 34 µg a.i./L, respectively) which was significantly different ($p \leq 0.05$) when compared to the pooled control organisms (95%). Survival of larvae in the remaining treatment levels of Propanil Technical (19-6.3 µg a.i./L) ranged from 89 to 95% and was comparable to the survival of the pooled control larvae (Figure 3, attached). Due to the adverse effect on larval survival at the 60 and 34 µg a.i./L treatment levels, analysis of the growth data for these two concentrations was not performed."

The mean wet weight and mean total length of surviving larvae in the 19, 9.3, and 6.3 µg a.i./L groups were comparable to those in the controls (Table 4, attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Based on significantly reduced embryo survival of fathead minnow exposed to Propanil Technical, the Lowest Observed Effect Concentration (LOEC) was determined to be 34 µg a.i./L. The No Observed Effect Concentration (NOEC) established for this study was 19 µg a.i./L as Propanil Technical. Based on these results, the Maximum Acceptable Toxicant Concentration (MATC) for this material and fathead minnows is estimated to be >19 µg a.i./L and <34 µg a.i./L (geometric mean MATC = 25 µg a.i./L)."

A GLP compliance statement, signed by the study director and a representative of the sponsor company, was included in the report indicating that the data and report prepared for this study were produced and compiled in accordance with all pertinent EPA Good Laboratory Practice Regulations except in the case of stability, characterization and verification of test substance identity. The report also included a quality assurance statement which was signed by a representative of the laboratory's quality assurance unit.

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

A. **Test Procedure:** The test procedure is generally in accordance with the SEP and ASTM guidelines, except for the following deviations:

The author reported that the DO in the two highest test concentrations fell below 75% of saturation for more than 24 hours; DO must be maintained above 75% of saturation at all times.

The total hardness of dilution water (20-40 mg/L as $CaCO_3$ during the exposure period) used in the test was slightly lower than the recommended hardness of 40-48 mg/L as $CaCO_3$.

The light intensity employed in this study was 538-1506 Lux (reported as 50-140 footcandles) at water surface. The SEP recommends the intensity of 400-800 Lux.

There was no information on how fertilized eggs were obtained from the culture. In addition, the age of the eggs was not reported.

Time to swim-up was not reported.

Embryos were "impartially" selected; the SEP recommends random selection.

Two replicate incubation cups with 60 embryos in each cup were used per treatment level and control. The SEP recommends a minimum of 20 embryos per replicate cup, with four replicates per concentration (80 embryos total).

The report did not indicate that whether food was withheld from the fish for at least 24 hours prior to test termination. The SEP recommends terminating feeding at least 24 hours prior to test termination.

B. **Statistical Analysis:** Since raw data were not submitted with the report, statistical analyses could not be verified.

C. **Discussion/Results:** Raw data were not submitted by the registrant. All raw data for each biological endpoint and for physical parameters measured during the test must always be submitted.

The length and weight data were reported as being individually measured; however, the data from these two parameters were statistically analyzed using the mean values of each replicate. When mean values were used, the variation that existed within each replicate was ignored. An experimental design which consists of only two replicates such as this one, using only mean values of replicates in statistical analysis may lead to the wrong conclusion. Individual measurements (i.e., raw data) of these two growth parameters should have been used.

The author excluded from statistical analysis the highest levels for growth data that showed effects on survival. Growth data from those two treatment levels should have been included in the analysis since they are part of the experiment and could have contributed to the experimental error in the ANOVA. Furthermore, excluding these growth data from statistical analysis would make it appear as if only survival was affected at these two treatment levels.

According to the author's report, the MATC of Propanil Technical for fathead minnow was determined to be >19 and <34 µg a.i./L mean measured concentrations (geometric mean MATC = 25 µg a.i./L).

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D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** 1) Raw data (biological and physical) were not submitted with the report. 2) Statistical analyses could not be verified due to the lack of raw data.

(3) **Repairability:** Pending the reviewer's evaluation of the above raw data.

15. **COMPLETION OF ONE-LINER:** Yes, April 23, 1991.

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RIN 1876-95

PROPANIL EEB REVIEW

Page_____ is not included in this copy.

Pages 9 through 13 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.

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____ 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.

Shaughnessey # 417765-01 Chemical Name Propanil Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
Chronic Fish Species: Pimephales promelas Lab: Springborn Labs. MRID # 417765-01	98%	Concentrations Tested (ppb a.i.) - 6.3, 9.3, 19.0, 34.0, 60.0 MATC - > 19 < 34 ppb. Author's results Effected Parameters - survival, weight, length Control Mortality (%) - 9% Solvent Control Mortality (%) - 10% Comments: based on mean measured concentrations Raw data not available, therefore reviewers could not verify author's results	RMM 4/23/91	Supplemental
Chronic Invertebrate Species: Lab: MRID #	_____	Concentrations Tested (pp___) - _____ MATC - >_____<_____ pp___. Effected Parameters - _____ Control Mortality (%) - Solvent Control Mortality (%) - Comments:	_____	_____

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