D176782

EEB file. 12-10-93

MRID Numbers 417765-01 and 422596-01

DATA EVALUATION RECORD

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

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

3. **STUDY TYPE:** 72-4. 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 Numbers 417765-01 (report) and 422596-01 (raw data).

5. **REVIEWED BY:**

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Signature: Rosemary Graham Mora
Date: 4 May 1993

6. **APPROVED BY:**

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Signature: P. Kosalwat
Date: 5/4/93

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7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a fish early life-stage test. Based on the effects on survival, the MATC of Propanil Technical for *Pimephales promelas* was >9.3 and <19 µg a.i./l mean measured concentrations (geometric mean MATC = 13.3 µg a.i./l).

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

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

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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. 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 a pH range of 6.8-7.2, a specific conductivity range of 110-140 µmhos/cm, and total hardness and alkalinity ranges of 24-35 and 20-27 mg/l as $CaCO_3$, respectively.

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, flow-through test. Based on preliminary testing, five nominal test concentrations (5, 10, 20, 40, and 80 µg a.i./l) were selected for this study. A solvent control (18 µl acetone/l) and a dilution water control were also included in the test. The stock solution was prepared in acetone.

D. **Design:** Two replicate aquaria were used for each treatment. Sixty embryos (≤24 hours old) were impartially distributed to each of 14 incubation cups. One incubation 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

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(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 weekdays and twice daily on weekend days and holidays. The fish were not fed during the 19 hours before test termination. 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, alkalinity, 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 (ANOVA) indicated that the acetone control response was not different from the negative control response; therefore, the control data were pooled.

The hatchability, survival, and growth 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. Mean measured concentrations of Propanil 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, the test solutions had a pH of 6.9-7.4, a mean total hardness and alkalinity of 20-40 and 16-24 mg/l as $CaCO_3$, respectively, and a specific conductivity of 130-150 µmhos/cm. The temperature ranged from 24 to 26°C and levels of dissolved oxygen concentration 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)."

"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. 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. <u>STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES</u>: "Based on significantly reduced larval 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.

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 day-31 measured concentration of replicate A for the highest test level was presented in the report (Table 2, attached) as 50 µg a.i./l; the corresponding value from the raw data (Table 1, attached) was 46 µg a.i./l. This is a discrepancy in the report.

The chemical analytical data for day-35 samples were not presented in the supplemental report (raw data).

Page 156 (attached) of the raw data appears to be the raw survival data collected during the course of the study. Page 159 (attached) of the raw data appears to be a calculation sheet for the arcsine squareroot

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transformation of the survival data. The number of survivors on page 159 (which corresponds to the number of survivors suggested by the length and weight data) for replicate A of the 20 µg/l nominal concentration, and replicates B of the control, 5 and 10 µg/l nominal concentrations do not agree with the number of survivors on page 156 in the same replicates. The author used survival values on page 159 in the analysis of percentage survival. No explanation for this discrepancy was presented.

B. **Statistical Analysis:** Individual length and weight data were analyzed using a 2-way ANOVA coupled with Bonferroni's test for treatment comparisons (pages 8 and 9 of printout, attached). The analyses indicated a statistically significant reduction in length and weight only at the 60 µg a.i./l test level. These results are less conservative than those of the author.

Survival and hatchability data were analyzed using Toxstat® (Version 3.3). The hatchability data did not pass a homogeneity test, therefore, the Kruskal-Wallis test was used to analyze the data. The reviewer obtained the same results as the author. The reviewer analyzed percentage survival based on survival data suggested by the growth data (page 159 of the raw data). The survival data met the assumptions of normality (Shapiro Wilks test) and of homogeneity of variance (Bartletts test). Therefore, the survival data were analyzed using Williams test (page 17 of printout, attached). Survival was significantly reduced at the three highest test concentrations (19, 34, and 60 µg a.i./l) when compared to that of the dilution water control. The reviewer's conclusions are more conservative than the author's.

C. **Discussion/Results:** The length and weight data were individually measured but only the mean values of each replicate were used in the statistical analyses. 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 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

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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.

This study is scientifically sound and meets the guideline requirements for a fish early life-stage test. Based on the effects on survival, the MATC of Propanil Technical for fathead minnow was determined to be >9.3 and <19 µg a.i./l mean measured concentrations (geometric mean MATC = 13.3 µg a.i./l).

D. Adequacy of the Study:

(1) **Classification:** Core.

(2) **Rationale:** N/A.

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

15. COMPLETION OF ONE-LINER: Yes, 23 April 1993.

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