

MRID Numbers 417766-01 and 421456-02

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-grey-colored crystal.
- 3. **STUDY TYPE:** 72-4. Freshwater Invertebrate Life-Cycle Test. Species Tested: *Daphnia magna*.
- 4. **CITATION:** McNamara, P.C. 1991. (Propanil Technical) - Chronic Toxicity to Daphnids (*Daphnia magna*) Under Flow-Through Conditions. SLI Report No. 90-6-3361. SLI Study No. 12177.1189.6105.130. Study conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by Propanil Task Force, Liberty, Missouri. EPA MRID Numbers 417766-01 (report) and 421456-02 (raw data).

5. **REVIEWED BY:**

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Date: *5 May 1993*

6. **APPROVED BY:**

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7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a daphnid life-cycle test. Based on the effects of the test material to daphnid reproduction, the MATC of Propanil Technical for *Daphnia magna* was >86 µg a.i./l and <160 µg a.i./l mean measured concentrations. The geometric mean MATC was 117 µg a.i./l. The 21-day EC₅₀ was 430 µg a.i./l.

8. **RECOMMENDATIONS:**

9. **BACKGROUND:**

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

11. MATERIALS AND METHODS:

- A. Test Animals: *Daphnia magna* were obtained from populations cultured at the testing facility. The cultures were maintained under static-renewal conditions at $20 \pm 2^\circ\text{C}$. The daphnids were fed a combination of green alga (*Ankistrodesmus falcatus*) and a trout food daily.
- B. Test System: The flow-through test system was a 200-ml proportional diluter with a 0.5 dilution factor. Test vessels were 1.6-l glass battery jars. Each jar drained through a 1.9 cm hole about 15 cm from the bottom of the test vessel. Each drain was covered with a Nitex[®] 40-mesh screen. The volume of test solutions was maintained at 1.4 l. The diluter delivered test solution to each vessel at an average rate of approximately 6 volume replacements per day. The 90% replacement time was approximately 9 hours.

The culture water, which was also used as the dilution water, was prepared with well water fortified to total hardness and alkalinity ranges of 160-180 and 110-130 mg/l as CaCO_3 , respectively. The fortified water was filtered through a resin column and a carbon filter. The dilution water had a pH range of 7.9-8.3 and a specific conductivity range of 400-600 $\mu\text{mhos/cm}$.

Sixteen hours of light at an intensity of 90-130 footcandles were provided each day. Test temperature was maintained at approximately $20 \pm 1^\circ\text{C}$ by an air-temperature controlled room.

A diluter stock solution (37 mg a.i./ml) was prepared by mixing 1.895 g of test material with acetone to a final volume of 50 ml.

- C. Dosage: Twenty-one-day, flow-through test. Based on the results of preliminary testing, five nominal test concentrations (50, 100, 200, 400, and 800 $\mu\text{g a.i./l}$) were selected for this study. A dilution water control and a solvent control were also included. The solvent control contained 0.022 ml/l of acetone, the highest solvent concentration used in any exposure solution.
- D. Design: Ten daphnids (≤ 24 hours old) were impartially selected and distributed to each of four exposure vessels per treatment (i.e., 40 daphnids/treatment).

The daphnids were fed 2.0 ml of trout food (5 mg/ml), 3.0 ml of (4×10^7 cells/ml) green alga (*Ankistrodesmus falcatus*) suspension, and 0.5 ml of Selco® (0.6 mg/ml) two to three times daily. The jars were brushed and the solutions filtered through fine-mesh nets at least twice weekly.

Adult survival was determined on days 1, 2, 4, 7, 14, and 21. Offspring production was noted on days 8, 11, 14, 15, 18, and 21. The offspring were discarded after counting. At test termination, total body length of each surviving adult was recorded.

Dissolved oxygen concentration (DO), pH, and temperature were measured once a week in every test vessel. The DO was also measured every weekday in alternate replicate vessels of all groups. Temperature was continuously monitored with a max/min thermometer in one vessel of the 800 μg a.i./l (nominal) group. Total hardness and alkalinity, specific conductance, and pH were measured weekly in alternate replicate vessels of all groups.

Water samples were collected from the midpoint of two of the four replicate vessels of all groups on test days 0, 7, 14, and 21 for determination of Propanil technical and 3,4-dichloroaniline (DCA), a major metabolite of Propanil.

- E. **Statistics:** The percentage survival data were arcsine square-root transformed before analysis. A Student t-test demonstrated that the acetone control response was not significantly different from the negative control response; therefore, the control data were pooled to assess significant Propanil effects.

Survival, reproduction, and length data were normally distributed (Chi-square goodness of fit test); therefore, Williams' test was used to assess exposure-level effects. If daphnid survival in any treatment level was significantly affected, growth and reproduction data for that level were excluded from further statistical analysis.

All analyses were performed using the mean organism response in each replicate vessel rather than individual responses. The level of significance was set at $p \leq 0.05$ for all analyses except the Chi-square goodness of fit test which was $p \leq 0.01$.

12. **REPORTED RESULTS:** "Throughout the 21 day exposure period, a small amount of precipitate formed on the end of the syringe tubing in the mixing chamber. This was removed when deemed necessary. No undissolved Propanil Technical (e.g., precipitate, film on the surface of the test solutions) was observed in any of the test solutions." Mean measured concentrations were 52, 86, 160, 330, and 620 $\mu\text{g a.i./l}$, with an average coefficient of variation of 9.4% (Table 3, attached). Levels of DCA were detected in the three highest concentrations, averaging 2% of the mean measured concentrations of Propanil (Table 4, attached).

A summary of the biological results are presented in Tables 5, 7, and 8 (attached). The survival and reproductive rates for both control groups exceeded the minimum EPA guideline requirements of 70% survival and 40 offspring/female. Survival in the highest concentration was significantly different from that of the pooled controls. The number of offspring produced per female in the 160 and 180 $\mu\text{g a.i./l}$ groups was significantly reduced when compared to that of the pooled control. No young were produced by adult daphnids in the highest concentration. Mean total body length for each exposure group was not significantly affected when compared to the controls. The highest test concentration was not statistically analyzed, due to the obvious effect of this concentration on survival.

The EC_{50} (95% confidence interval) for immobilization was 430 (330-620) $\mu\text{g a.i./l}$ at test termination.

During the study, the test solutions had a pH of 7.9-8.5, a total hardness and alkalinity of 160-180 and 120 mg/l as CaCO_3 , respectively, a specific conductance of 500 $\mu\text{mhos/cm}$, a DO range of 6.2-9.7 mg/l, and a temperature of 19-22°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Based on the observed adverse effect of Propanil Technical on daphnid reproduction in treatment levels $\geq 160 \mu\text{g a.i./l}$ the Maximum Acceptable Toxicant Concentration (MATC) of this test material to *Daphnia magna* was estimated to be $\geq 86 \mu\text{g a.i./l}$ (NOEC) and $\leq 160 \mu\text{g a.i./l}$ (LOEC). The Geometric mean (MATC) established for this study was 120 $\mu\text{g a.i./l}$."

A GLP compliance statement and a quality assurance statement 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 (40 CFR Part 160) except in the case of stability, characterization and verification of test substance identity.

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

- A. Test Procedure: An SEP for *Daphnia* chronic flow-through studies is not available at this time; therefore, the SEP for the *Daphnia magna* static-renewal test was used as a general guidance.

By test termination, there were nine surviving daphnids in replicate B of the solvent control (page 83 of the raw data, attached); yet only eight length values for this replicate are presented in the raw data (page 138 of the raw data, attached). There is no explanation for this discrepancy.

- B. Statistical Analysis: The author evaluated the effects of the test material on reproduction using average number of young produced per female. Since reproduction of all females did not start on the same day, the appropriate endpoint is the number of young per female reproductive day, rather than number of young per female.

The reviewer used a two-way ANOVA and Bonferroni's test to analyze the length data. Reproduction (number of young produced per female reproductive day) data were analyzed using Steels Many-One Rank test since the data did not pass a homogeneity test. Survival data were analyzed as proportional survival and were arcsine squareroot transformed prior to analysis. The survival data were analyzed using Williams' test. The results of the length analyses were more conservative than those of the author (page 5 of printout, attached). The results of the reproduction and survival analyses were the same as those of the author (pages 14 and 16 of printout, attached).

The geometric mean MATC reported by the author (120 μg a.i./l) is incorrect. The correct value is 117 μg a.i./l.

The reviewer used EPA's Toxanal computer program to verify the author's 21-day EC_{50} value and obtained less conservative results (page 17 of printout, attached).

- C. Discussion/Results: The length data were individually measured; however, the data from this parameter were statistically analyzed using the mean value of each replicate. When mean values are used, the variation that exists within each replicate is ignored.

Individual measurements of length (i.e., raw data) should have been used.

The author excluded from statistical analysis the growth data for the highest level (620 $\mu\text{g a.i./l}$) that showed effects on survival. Length data from this treatment level should have been included in the analysis since they were part of the experiment and could have contributed to the experimental error in the ANOVA. Furthermore, excluding this data from statistical analysis would make it appear as if only survival was affected at this treatment level.

Based on the effects of the test material to daphnid reproduction, the MATC of Propanil Technical for *Daphnia magna* was $>86 \mu\text{g a.i./l}$ and $<160 \mu\text{g a.i./l}$ mean measured concentrations. The geometric mean MATC was $117 \mu\text{g a.i./l}$. The 21-day EC_{50} was $430 \mu\text{g a.i./l}$.

D. Adequacy of the Study:

- (1) **Classification:** Core.
- (2) **Rationale:** N/A.
- (3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes, April 26, 1993.