

D183211

2-10-93

MRID No. 424753-01

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Propanil.
Shaughnessey No. 028201.
- 2. **TEST MATERIAL:** 1) Nonradiolabeled Propanil (3,4-dichloropropionanilide); Batch 01; two samples: Aliquot 12 and 14; 98 and 98.2% active ingredient, respectively; light brown powder. 2) Radiolabeled Propanil; Lot Nos. 010H9242 and 051H9216; >98% active ingredient; 18.2 and 32.4 mCi/mmol specific activity, respectively; off-white powder.
- 3. **STUDY TYPE:** 72-5. Fish Life-Cycle Toxicity Test. Species Tested: Fathead Minnow (*Pimephales promelas*).
- 4. **CITATION:** Dionne, E. 1992. (Propanil Technical) - The Chronic Toxicity to the Fathead Minnow (*Pimephales promelas*) During a Full Life-Cycle Exposure. SLI Report No. 92-1-4085. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by Propanil Task Force, c/o John M. Wise, Liberty, MO. EPA MRID No. 424753-01.

5. **REVIEWED BY:**

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6. **APPROVED BY:**

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- 7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a life-cycle chronic toxicity test using freshwater fish. The maximum acceptable toxicant concentration was >9.1 and <21 µg a.i./l mean measured concentrations (geometric mean MATC = 13.8 µg a.i./l).
- 8. **RECOMMENDATIONS:** N/A.
- 9. **BACKGROUND:**

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.11. MATERIALS AND METHODS:

- A. Test Animals: Fathead minnow (*Pimephales promelas*) embryos were obtained from in-house cultures. The culture water used was from the same source and of similar quality as the test dilution water. Embryos from 15 separate spawns were obtained for the test. The embryos were less than 24 hours old at test initiation.
- B. Test System: An intermittent flow proportional diluter system with a 50% dilution factor was used. During the pre-spawning phase of the study, the flow rate provided 8 volume turnovers per day. During the spawning phase, flow was reduced to 5 volume turnovers per day. The exposure system was two-tiered consisting of an upper and lower level water bath, each containing 14 aquaria. The glass aquaria measured 60 x 30 x 30 cm and were randomly positioned in the water baths. A 15-cm high end-drain maintained a solution volume of 27 l. A 7.5 x 16 x 7.5 cm incubation chamber was positioned at the inflow end of each aquarium and contained two embryo incubation cups. Test solution flowed into and passed through the incubation chamber by means of a self-starting cap-siphon. The embryo incubation cups were 5-cm diameter glass jars with nylon screen bottoms (40 mesh). Two larval growth chambers measuring 30 x 13 x 25 cm were placed in each aquarium on the upper level of the diluter system. Additional F₁ larval growth chambers measuring 28 cm high and 10 cm in diameter were constructed of petri dishes and 40-mesh nylon screening and used to rear extra fish for residue analysis at the highest concentration.

The aquaria on the lower level were separated into two spawning compartments using a nylon mesh screen divider. Spawning tiles constructed of halved 10-cm lengths of PVC pipe (4-cm diameter) were placed in each compartment.

The test temperature was 25 ±1°C. The test system was maintained under a graduated photoperiod depending on the developmental stages (Benoit, 1981). Light intensity varied from 20 to 100 footcandles. The entire test system was enclosed in black plastic curtains to prevent disturbance and minimize the influence of laboratory lighting on the intended photoperiod.

The dilution water was well water supplemented with Town of Wareham well water. The water had a hardness range of 20-40 mg/l as CaCO₃, a pH of 6.9-7.5, and a conductivity of 80-150 μ mhos/cm.

During the exposure, several radiolabeled stock solutions (26.92 mg a.i./ml) were prepared. The stocks were prepared by dissolving an appropriate amount of nonradiolabeled test material and an appropriate amount of ¹⁴C-Propanil superstock solution in acetone. "Stock solutions prepared using the first shipment of radiolabeled test material (Lot #010H9242) had an approximate Propanil Technical/Propanil-Ring-UL-¹⁴C ratio of 78.9%/21.1% with a specific activity of 39090 dpm/ μ g. Stock solutions prepared using the second shipment of radiolabeled test material (Lot #051H9216) had an approximate Propanil Technical/Propanil-Ring-UL-¹⁴C ratio of 87.9%/12.1% with a specific activity of 39905 dpm/ μ g." With each diluter cycle, 0.00575 ml of the radiolabeled stock solution was pumped into the diluter mixing chamber. The stock and dilution water were mixed using a magnetic stirrer and a Teflon-coated stir bar. The concentration in the mixing chamber was equivalent to the highest test concentration (40 μ g a.i./l) and was subsequently diluted to provide the four remaining treatment levels.

- C. Dosage: Two-hundred and sixty-three-day, flow-through, life-cycle toxicity test. Based on the results of an early-life stage test, five nominal concentrations (2.5, 5.0, 10, 20, and 40 μ g a.i./l), a solvent control (0.0015 ml acetone/l), and a dilution water control were used. The solvent control contained the maximum amount of solvent used in any test concentration.
- D. Design: Each treatment level and control were replicated two times. Fathead minnow embryos were randomly assigned, five at a time, to each of the 28 incubation cups until each cup contained 50 embryos. Two cups were placed in each replicate in the upper level of the test system.

Each day until hatching began (day 3), embryos in each cup were removed and counted. Dead embryos were removed. On day 3, live embryos and hatched larvae were observed and counted but not removed from the incubation cups. When hatching was complete (day 4), percentage hatching success was calculated.

Newly-hatched fry were fed live brine shrimp nauplii with small amounts of Tetramin flakes three times daily (twice on weekends) for the first 30 days. Juvenile and adult fish were fed previously-frozen brine shrimp once daily and Tetramin flakes once daily.

Twenty-five newly-hatched larvae were impartially selected from each embryo incubation group and placed into a larval growth chamber in the corresponding exposure aquarium providing four groups of larvae per treatment level (two groups per replicate). The growth chambers were examined daily for dead larvae. After 30 and 62 days of exposure, each group was photographed over a grid for length determinations. Percentage survival was also determined at these intervals. On day 62, the two larval groups in each aquarium were combined and randomly thinned to 25 larvae per aquarium. Discontinued fish were blotted and weighed. Four 62-day old larvae from the highest treatment level were radiometrically analyzed for whole-body ^{14}C residues.

After approximately 152 days of exposure, two spawning groups consisting of 1 male and 2 females each were transferred to the corresponding lower level spawning aquarium. During spawning activity, dead males were replaced. Dead females were not replaced since reproduction was assessed on a per female basis. Spawning substrates were checked daily for the presence of eggs. For each spawning group, the number of eggs spawned and incubated was recorded. Fifty embryos from the first 10 spawns consisting of greater than 50 eggs in each aquarium were incubated and the percentage hatch determined. Subsequently, every third spawn of ≥ 50 eggs in each aquarium was incubated for percentage hatch determination. "Eggs not used for F_1 exposure were collected from the highest treatment level with surviving and reproducing F_0 adults and were incubated to produce two additional life stages of fathead minnows for tissue analysis of total ^{14}C content."

As F_1 embryo groups hatched, groups of 25 newly-hatched fry were established in each aquarium. Two larval groups from separate spawning groups could be reared in each aquarium at any one time. After 30 days post-hatch, each larval group was terminated and individual total length and wet weight were determined. Percentage survival was also determined for each group. The whole body of several F_1 larvae exposed to the highest test level was analyzed for total ^{14}C content.

Exposure of F₀ parental spawners was terminated when no spawning had occurred in any aquarium for 14 consecutive days (day 263). At test termination, each fish was individually measured and weighed and internally examined to verify sex and gonadal condition. Two mature male and female fish from the highest concentration were sampled to determine ¹⁴C tissue content.

The test aquaria were scraped and siphoned at least three times weekly. All diluter cells were brushed and siphoned weekly and the glass delivery tubes were brushed monthly. The calibration of the diluter was checked weekly.

The dissolved oxygen concentration (DO), temperature, and pH were measured in an alternating replicate of each treatment and controls daily. In addition, the temperature of one replicate aquarium on each level of the system was recorded continuously using minimum/maximum thermometers. Hardness, alkalinity, and conductivity were measured weekly in one control aquarium and one treatment aquarium on a rotating basis.

Prior to the initiation of spawning, the concentration of Propanil in each replicate was determined at least weekly using radiometric analysis. After the initiation of spawning, solution samples were taken weekly from an alternating replicate of each test level in each level of the test system. Exposure solutions were analyzed every third week for Propanil and 3,4-dichloroaniline (DCA; degradation product) using high pressure liquid chromatography (HPLC) with radiometric analysis.

- E. **Statistics:** Endpoints statistically analyzed were listed in Table 1 (attached). Proportional data were arcsine square root transformed prior to analysis. Hatch and survival data for the dilution water control and solvent control were compared using Fisher's Exact test. The responses of the treatments were compared to the controls using the Cochran-Armitage Trend test. Length, weight, and reproduction data for the control and solvent control were compared using Student's t-test. All data were tested for homogeneity of variance using Bartlett's test. The responses of the exposed fish were compared to the control using William's test. Test levels which were significantly different from the controls for survival were excluded from subsequent

analyses. For all parameters, statistical comparisons of exposure concentrations were made to the pooled controls.

12. **REPORTED RESULTS:** No undissolved test material was observed in the test system or exposure solutions during the test. The mean measured concentrations for the exposure period using radiometric analysis were 2.1, 5.0, 9.1, 21, and 41 μg a.i./l (Table 5, attached). Measured concentrations were generally consistent between replicate exposure vessels and sampling intervals. Recoveries of quality control samples averaged 99.9% of nominal fortified levels.

The results of HPLC analyses were presented in Table 7 (attached) and compared favorably to the radiometric results. The presence of DCA generally increased over time and was highest during the period of the most biological activity (day 176-218).

Mean hatching success (%) of F_0 embryos exposed to 41 and 21 μg a.i./l was significantly different from the pooled control (Table 8, attached). However, since the responses at these two levels did not follow a well defined concentration-response, the differences in percentage hatch were not considered biologically significant. Statistical comparison of F_0 larval survival and total length after 30-days post-hatch determined that at 41 μg a.i./l, both parameters were significantly lower than those of the pooled control (Table 8, attached). The author considered survival at 41 μg a.i./l to be within the normal range for fathead minnows and therefore did not consider the statistical difference to be biologically significant.

Following 62 days of exposure, larval survival at the 21 and 41 μg a.i./l test levels was significantly lower than the pooled controls (Table 8, attached). Length and weight of fish in the three lowest concentrations (where survival was not significantly affected) were statistically similar to the controls.

At test termination, survival among fish exposed to the 41 μg a.i./l concentration was 69% and was significantly reduced compared to the pooled control data (Table 9, attached). Analysis of male and female growth established a significant decrease in male length and weight at 21 μg a.i./l when compared to the pooled control data for males. Female growth at concentrations ≤ 21 μg a.i./l was similar to that of the pooled control.

The reproduction data were summarized in Table 10 (attached). No significant differences were established for any of the reproduction parameters analyzed. No eggs were produced by F₀ adults exposed in replicate A of the 41 µg a.i./l treatment level. "The variability in the number of eggs/female observed between replicate treatment levels was considered a typical response."

Hatching success of F₁ embryos was evaluated on an average of 10 groups of 50 eggs per replicate for each treatment and control. Hatching success was unaffected by exposure to the test material (Table 11, attached). Following 30 days of post-hatch exposure, larval survival in the exposure groups was similar to the controls. Length and weight of F₁ larvae from the 21 µg a.i./l exposure group were significantly lower than those of the pooled control (Table 11, attached). Since there were no larvae in replicate A of the 41 µg a.i./l group, this group was excluded from the statistical analysis. "Larval survival and growth (total length and wet weight) were based on the performance of an average of four larval groups per treatment level (2 groups per replicate exposure aquarium). The larval groups in each replicate aquarium were the progeny of two different spawning groups."

The results of analyses for total ¹⁴C-residues in tissue samples were presented in Table 12 (attached). Three-day-old F₁ embryos had the lowest bioconcentration factor (BCF) and 14-day-old F₁ larvae had the highest BCF.

Mean water quality values and ranges for each concentration were presented in Tables 3a and 3b (attached). Continuous temperature monitoring established the temperature range as 24 to 27°C. Dissolved oxygen concentrations were ≥5.0 mg/l (60% of saturation) and pH ranged from 6.7 to 7.8 during the study. The conductivity was 150-200 µmhos/cm. The alkalinity and hardness were 14-26 mg/l as CaCO₃ and 28-40 mg/l as CaCO₃, respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

Based on the adverse effects observed at the 21 µg a.i./l test concentration, the maximum acceptable toxicant concentration (MATC) for Propanil Technical to fathead minnow was estimated to be >9.1 and <21 µg a.i./l. The geometric mean MATC was 14 µg a.i./l.

Quality Assurance and Good Laboratory Practice Compliance Statements were included in the report, indicating that the study was conducted in accordance with USEPA Good Laboratory Practice Standards (40 CFR Part 160). The dates and types

of quality assurance audits performed were also included in the report.

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

- A. Test Procedure: The test procedures were generally in accordance with the SEP except for the following:

Raw water quality data were not included in the report. However, for each parameter measured, the range of measured values was reported and indicated that adequate water quality was maintained.

The SEP states that 50 embryos should be distributed to each of four replicate larval growth chambers at test initiation. In this test, two egg cups were used per aquarium (two aquaria per concentration) and therefore constitutes a nested design rather than individual replicates.

The hardness of the dilution water (28-40 mg/l as CaCO₃) was less than recommended (40-48 mg/l).

The light intensity used during the test (215-2152 lux) was greater than recommended by the SEP (10-100 lux).

- B. Statistical Analysis: The reviewer used several computer programs (Toxstat version 3.3, Crunch version 3, and Systat 5.0), depending on the type of data, to analyze embryo, juvenile, and adult survival, and juvenile and adult growth. Proportional data were arcsine square root transformed prior to analysis. Two-way ANOVA was used when appropriate.

The reproduction results (Table 10, attached) were reviewed but not statistically analyzed. For all parameters measured, the reproduction at 41 µg a.i./l appeared significantly lower than responses in the dilution water control. In one replicate of the 41 µg a.i./l exposure, no reproduction occurred.

For all analyzed parameters, the reviewer obtained results similar to those of the author (see Table A1 and attached printouts 1 through 20).

The analysis of F₁ larval weights determined that levels 1, 2, and 4 (2.1, 5.0, and 21 µg a.i./l, respectively) were significantly lower than solvent control weights (printouts 17 and 18, attached). Only the 21 µg a.i./l level was significantly lower than the

dilution water control. Data for the highest test level were not included in the analysis due to lack of replication. Mean weights were 0.2093, 0.1930, 0.1799, 0.1800, 0.1996, and 0.1451 g for the solvent control, dilution water control and the four lowest treatment levels, respectively. Since the data include larvae from several different batches spawned over a period of approximately 100 days, the reviewer believes the differences between means represent natural variability and only the mean at 21 $\mu\text{g a.i./l}$ is biologically significantly lower than the solvent control data. The results for F_1 lengths were similar to those for weight (printouts 19 and 20, attached). The reviewer proposes the same explanation for these results.

The results of the reviewer's verification of the results gives the MATC as >9.1 and $<21 \mu\text{g a.i./l}$ mean measured concentrations, the same as the author's.

- C. Discussion/Results: The analytical results indicate that the concentration of the test material was somewhat variable during the study. However, relative standard deviations by concentration ranged from 13.2 to 19.6% and are considered acceptable for a study of this length. Concentrations determined by HPLC were in agreement with the radiometric analyses but tended to be slightly lower.

The reviewer chose not to analyze reproduction statistically because, compared to the dilution water control data, no clear dose-response relationship was evident for any of the parameters measured (Table 10, attached). Reproduction at 41 $\mu\text{g a.i./l}$ was strongly reduced compared to the dilution water control, but statistical analysis was impractical since the 41 $\mu\text{g a.i./l}$ had only one replicate. The concentration of solvent appeared to affect reproduction. The two test levels with similar solvent concentrations, the solvent control and highest test level, had depressed reproduction. The remaining levels had similar reproduction.

This study is scientifically sound and meets the guideline requirements for a life-cycle chronic toxicity test using freshwater fish. The maximum acceptable toxicant concentration was >9.1 and $<21 \mu\text{g a.i./l}$ mean measured concentrations (geometric mean MATC = 13.8 $\mu\text{g a.i./l}$).

- D. Adequacy of the Study:

(1) **Classification:** Core.

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

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

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 11-20-92.

Table A1. Results of independent statistical analysis in mean measured concentrations ($\mu\text{g a.i./l}$) of Propanil Technical by radiometric analysis.

| <u>Parental Generation</u> | | | |
|--|-------------|-------------|---------------------|
| <u>Parameter</u> | <u>NOEC</u> | <u>LOEC</u> | <u>Method</u> |
| embryo survival | 41 | --- | --- |
| 30-day survival | 41 | --- | --- |
| 30-day length | 21 | 41 | Bonferroni |
| 62-day survival | 41 | --- | --- |
| 62-day length | 41 | --- | --- |
| 62-day weight | 41 | --- | --- |
| adult survival at termination | 21 | 41 | Dunnett |
| 263-day length - females | 41 | --- | --- |
| 263-day length - males | 9.1 | 21 | Bonferroni's t-test |
| 263-day weight - females | 41 | --- | --- |
| 263-day weight - males | 21 | 41 | Bonferroni's t-test |
| <u>Progeny Generation, All Spawning Trials</u> | | | |
| embryo survival | 21 | * | --- |
| 30-day survival | 21 | * | --- |
| 30-day weight | 9.1 | 21 | Bonferroni |
| 30-day length | 9.1 | 21 | Bonferroni |

n.d. = no data available

*no statistically derived LOEC available, the highest test concentration ($41 \mu\text{g a.i./l}$, mean measured) had insufficient data to be included in the analysis