

DATA EVALUATION RECORD

1. **CHEMICAL:** Fipronil and derivatives (M&B 46030).
Shaughnessey No. 129121.
2. **TEST MATERIAL:** M&B 45950; Batch No. JJW2126/79, RM1502;
100% active ingredient; a yellow powder.
3. **STUDY TYPE:** 72-4. Freshwater Invertebrate Life-Cycle Test.
Species Tested: *Daphnia magna*.
4. **CITATION:** McNamara, P.C. 1990. (M&B 45950) - Chronic
Toxicity to Daphnids (*Daphnia magna*) Under Flow-Through
Conditions. SLI Report No. 90-4-3292. Study conducted by
Springborn Laboratories, Inc., Wareham, MA. Submitted by
Rhone-Poulenc Ag Company, Research Triangle Park, NC. EPA
MRID No. 429186-70.
5. **REVIEWED BY:**

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

Signature: *Rosemary Graham Mora*
Date: *2/18/94* *3/3/94*
6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *P. Kosalwat*
Date: *2/18/94*

James J. Goodyear, Ph.D.
Project Officer, EEB/EFED
USEPA

Signature: *James J. Goodyear*
Date: *3/2/94*
7. **CONCLUSIONS:** This study is not scientifically sound and
does not meet the guideline requirements for a daphnid life-
cycle test. Measured concentrations were highly
inconsistent. Two of the test concentrations decreased to
below the detection limit by test termination. Therefore,
the actual concentrations to which the test organisms were
exposed are unknown. Based on results presented by the
author, the MATC of *Daphnia magna* exposed to M&B 45950 was
>9.6 and <19.5 $\mu\text{g ai/l}$. The geometric mean MATC was 13.7 $\mu\text{g ai/l}$.
The 21-day EC_{50} was 22.5 $\mu\text{g ai/l}$.
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 in fortified well water at $20 \pm 2^\circ\text{C}$. The daphnids were fed daily a combination of green alga (*Ankistrodesmus falcatus*) and a trout food suspension.

B. Test System: The test system was a 200-ml proportional diluter with a 50% dilution factor. The test vessels were 1.6-l glass battery jars with a solution volume of 1.4 l. Test solutions drained through two 2-cm holes near the top edge of each jar. The drains were covered with Nitex® 40-mesh screen. The diluter delivered test solution to each vessel at an approximate rate of 6 volume replacements per day. The 90% replacement time was approximately 9 hours.

The dilution water, which was the same as the culture water, was fortified well water. The dilution water had a pH range of 7.9-8.3, a specific conductivity range of 400-600 $\mu\text{mhos/cm}$, and 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 prior to use.

Sixteen hours of light at an intensity of 60-100 footcandles were provided each day. Sudden transitions from light to dark and vice versa were avoided. Test temperature was maintained at approximately $20 \pm 2^\circ\text{C}$ by an air-temperature controlled room.

A diluter stock solution (3.0 mg ai/ml) was prepared by diluting 0.15 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 (3.1, 6.3, 13, 25, and 50 $\mu\text{g ai/l}$) were selected for this study. A dilution water control and a solvent control were also included. The solvent control contained 17 $\mu\text{l/l}$ 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 test 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 green alga (*Ankistrodesmus falcatus*; 4×10^7 cells/ml) 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 twice weekly.

The number of immobilized daphnids was determined on days 1, 2, 4, 7, 9, 10, 13, 14, 17, 20, and 21. Following day 7, the offspring produced were counted and discarded at least three times weekly. The number of immobilized offspring and the time to first brood were also recorded. 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 one replicate vessel of each group. Temperature was measured daily in one replicate of each group and monitored continuously with a max/min thermometer in one vessel of the 25 µg ai/l (nominal) group. Total hardness, alkalinity, specific conductivity, and pH were measured weekly in one replicate vessel of each group.

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. These samples were analyzed for M&B 45950 using high pressure liquid chromatography.

- E. **Statistics:** The percentage survival data were arcsine square-root transformed before analysis. A one-way single clarification analysis of variance demonstrated that the control responses for reproduction and survival were statistically similar; therefore, the pooled control data were used to assess significant treatment effects for these parameters. Growth data in the dilution water control and the solvent control were shown to be different from one another; therefore, the treatment growth data were compared to the solvent control data.

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 test which was $p \leq 0.01$.

The 21-day EC_{50} was calculated using a computer program by C.E. Stephan (1977, 1982).

12. **REPORTED RESULTS:** Throughout the 21 day exposure period, no undissolved test material (e.g., precipitate, film on the surface of the test solutions) was observed in any of the closed exposure vessels. "Analysis of the exposure solutions on day 21 resulted in measured concentrations that were inconsistent between replicates and sampling intervals." Therefore, day 21 analysis data were excluded from calculation of the mean measured concentrations. Based on day 0, 7, and 14 analytical data, mean measured concentrations were 4.0, 7.0, 13, 22, and 45 $\mu\text{g ai/l}$ (Table 2, attached). The average coefficient of variation was 21%.

Survival and reproductive rates for the control groups exceeded the minimum EPA guideline requirements of 70% survival and 40 offspring/female. Survival in the highest test concentration was significantly reduced when compared to that of the pooled control (Table 3, attached). Sublethal effects observed during the test are presented in Table 4 (attached). The 21-day EC_{50} (95% confidence interval) for immobilization was 27 (22-45) $\mu\text{g ai/l}$. The number of offspring produced per female at 22 $\mu\text{g ai/l}$ was statistically reduced when compared to that of the pooled control data (Table 7, attached). Mean total body length of daphnids at 22 $\mu\text{g ai/l}$ was significantly reduced when compared to the solvent control data (Table 8, attached).

During the study, the test solutions had a pH of 7.9-8.4, a specific conductance of 500 $\mu\text{mhos/cm}$, a mean DO range of 7.4-8.5 mg/l, a temperature of 19-22°C, and a mean total hardness and alkalinity of 170 and 120 mg/l as CaCO_3 , respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Based on the observed effect of M&B 45950 on daphnid reproduction and growth, the maximum acceptable toxicant concentration (MATC) of this test material to *Daphnia magna* was estimated to be >13 and <22 $\mu\text{g ai/l}$ (geometric mean MATC = 17 $\mu\text{g ai/l}$).

A GLP compliance statement and a quality assurance statement were 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. Study weaknesses were as follows:

Measured concentrations were highly variable (Table 2, attached). The highest measured concentration in each treatment was more than 2.0 times the lowest measured concentration at the same level. Two of the five treatment concentrations decreased to below the detection limit by test termination.

Individual daphnid weight was not measured at test termination as required by EPA.

Raw data for survival, length, and water quality were not included in the report. All raw data must be presented with the report.

The author evaluated the effects of the test material on reproduction using average number of young produced per female. Since there was more than one female per test chamber, and reproduction was not monitored on a daily basis, the appropriate endpoint for reproduction is the number of young per female reproductive day, rather than number of young per female.

The author states that the survival and reproduction rates of control daphnids met "the standard criteria established by the U.S. EPA (1985) under FIFRA guidelines." The literature cited shows only a reference to the SEP for acute toxicity test for freshwater fish (1985). This is a discrepancy in the report.

- B. Statistical Analysis:** Raw survival data were not included in the report; therefore, the reviewer was unable to determine the number of young per female reproductive day. In addition, raw length data were not included in the report. Consequently, the reviewer

was not able to verify the author's results for survival, length, or reproduction.

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 highest treatment which showed effects on survival. Length data for this treatment level should have been included in the analysis since it was part of the experiment and could have contributed to the experimental error in the ANOVA. Furthermore, excluding these data from statistical analysis would make it appear as if only survival was affected at this treatment level.

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

- C. Discussion/Results: The reviewer calculated mean measured concentrations based on all analytical data (i.e., days 0, 7, 14, and 21). Values below the detection limit were entered into the calculation as one-half the detection limit. The actual mean measured concentrations were 3.4, 5.3, 9.6, 19.5, and 40 $\mu\text{g ai/l}$.

This study is not scientifically sound and does not meet the guideline requirements for a daphnid life-cycle test. Substantial variability in measured concentrations at all test levels affects the validity of this study. In addition, daphnid weight was not measured. Based on the results presented by the author and mean measured concentrations calculated by the reviewer, the MATC of *Daphnia magna* exposed to M&B 45950 was >9.6 and $<19.5 \mu\text{g ai/l}$. The geometric mean MATC was $13.7 \mu\text{g ai/l}$. The 21-day EC_{50} was $22.5 \mu\text{g ai/l}$.

- D. Adequacy of the Study:

- (1) **Classification:** Invalid.
- (2) **Rationale:** The actual concentrations to which the test organisms were exposed are unknown.

(3) Repairability: No.

15. COMPLETION OF ONE-LINER: Yes; 2 February 1994.

Fifteen reviewed

Page _____ is not included in this copy.

Pages 8 through 12 are not included in this copy.

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.
