3/21/94

MRID No. 429186-26

DATA EVALUATION RECORD

FILE COPY

1. **CHEMICAL:** Fipronil and derivatives (M&B 46030). Shaughnessey No. 129121.

2. **TEST MATERIAL:** M&B 46030; Lot No. JJW2092/1/94; tested as 100% active ingredient; a white powder.

3. **STUDY TYPE:** 72-4. Freshwater Invertebrate Life-Cycle Test. Species Tested: *Daphnia magna*.

4. **CITATION:** McNamara, P.C. 1990. The Chronic Toxicity of M&B 46030 to *Daphnia magna* Under Flow-Through Conditions. SLI Report No. 90-01-3210. Study conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by Rhone-Poulenc Ag Company, Research Triangle Park, NC. EPA MRID No. 429186-26.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
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Signature: Rosemary Graham Mora
Date: 2/9/94

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
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Signature: P. Kosalwat
Date: 2/9/94

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

Signature: 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. Several problems with this test weaken its validity, including high control mortality (50%) and variable measured concentrations. ~~In addition, daphnid weight was not measured.~~ ~~Based on the results presented~~ by the author, the MATC of *Daphnia magna* exposed to M&B 46030 was >9.8 and <20 µg ai/l mean measured concentrations. The geometric mean MATC was 14 µg ai/l. The 21-day EC_{50} was 39 µg 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 under static-renewal conditions at 20 ±2°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 0.5 dilution factor. The test vessels were 1.4-l glass battery jars. Test solutions drained through two 2-cm holes at the top edge of each jar. Each drain was 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 µmhos/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 40-90 footcandles were provided each day. Test temperature was maintained at approximately 20 ±2°C by an air-temperature controlled room.

Diluter stock solutions (5.9 mg ai/ml) were prepared by diluting 0.147 g of test material with acetone to a final volume of 25 ml.

C. **Dosage:** Twenty-one-day, flow-through test. Based on the results of preliminary testing, five nominal test concentrations (6.3, 13, 25, 50, and 100 µg ai/l) were selected for this study. A dilution water control and a solvent control were also included. The solvent control contained 17 µl/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 x 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.

Adult survival and offspring production were determined on days 1, 2, and 4, and three times weekly thereafter. 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 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 50 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 46030 using gas chromatography.

E. **Statistics:** The percentage survival data were arcsine square-root transformed before analysis. A Student t-test demonstrated that the solvent control response was significantly different from the dilution water control response; therefore, the solvent control data were used to assess significant treatment effects.

Survival, reproduction, and length data were normally distributed (Chi-Square Goodness of Fit test); therefore, Williams' or Dunnett's 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 determined using mean measured concentrations and a computer program by C.E. Stephan.

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 test solutions. Mean measured concentrations were 5.0, 9.8, 20, 34, and 79 µg ai/l and averaged 76% of nominal concentrations (Table 2, attached). The average coefficient of variation was 22%.

The survival and reproductive rates for the solvent control group exceeded the minimum EPA guideline requirements of 70% survival and 40 offspring/female. Survival in the dilution water control averaged 50% at test termination. "Since 98% of the solvent control organisms survived the 21 day exposure and no significant mortality occurred in the treatment level solutions between days 14 and 21, the unexpected decrease in the survival of the dilution water control daphnids was determined to be due to a condition isolated to the control vessels and not representative of the overall conditions maintained during the chronic test." Reproduction in the dilution water control averaged 100 offspring per female.

Survival in the two highest test concentrations was significantly reduced when compared to that of the solvent control (Table 4, attached). Sublethal effects observed during the test are presented in Table 5 (attached). The number of offspring produced per female at concentrations ≤20 µg ai/l was statistically similar when compared to that of the solvent control (Table 8, attached). Mean total body length of daphnids at 20 µg ai/l was significantly reduced when compared to the solvent control (Table 9, attached).

The 21-day EC_{50} (95% confidence interval) for immobilization was 39 (34-79) µg ai/l.

During the study, the test solutions had a pH of 7.7-8.4, a specific conductance of 500 µmhos/cm, a mean DO range of 7.0-8.4 mg/l, a temperature of 20-22°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 46030 on daphnid growth, the Maximum Acceptable Toxicant Concentration (MATC) of this test material to *Daphnia magna* was estimated to be ≥9.8 µg ai/l and ≤20 µg ai/l (geometric mean MATC = 14 µg 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:

By test termination, there was 50% mortality in the dilution water control. This level of mortality is unacceptable.

Measured concentrations were highly variable at all exposure levels (Table 2, attached). The highest measured concentration in each treatment was more than 1.5 times the lowest measured concentration at the same level.

Qs 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 stated that the test material was tested as 100% active ingredient; the exact purity of the test material was not reported.

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 and 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.

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.

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

C. **Discussion/Results:** 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 those treatments which showed effects on survival. Length data for these treatment levels 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 these treatment levels.

This study is not scientifically sound and does not meet the guideline requirements for a daphnid life-cycle test. Several problems with this test weaken its validity, including high control mortality (50%) and variable measured concentrations. ~~In addition, daphnid weight was not measured.~~ Based on the results presented by the author, the MATC of *Daphnia magna* exposed to M&B 46030 was >9.8 and <20 µg ai/l mean measured concentrations. The geometric mean MATC was 14 µg ai/l. The 21-day EC_{50} was 39 µg ai/l.

D. **Adequacy of the Study:** based on growth as

(1) **Classification:** Invalid.

(2) **Rationale:** 1) Dilution water control mortality was extremely high. 2) Measured concentrations were highly variable.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes; 6 January 1994.

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE
OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY,
THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

Rosemary Graham Mora M&B 46030 Daphnia

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
79	39	39	100	0
34	39	14	35.8974	0
20	39	1	2.5641	0
9.8	39	3	7.6923	0
5	39	3	7.6923	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT
CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE
UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 39.06904

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	2.880671E-02	38.41065	34.70571	42.62187

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	2.643807	15.42326	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.863583
95 PERCENT CONFIDENCE LIMITS =-1.792542 AND 7.519708

LC50 = 35.84522
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 12.91014
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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Ecological Effects Branch One-Liner Data Entry Form

Chemical Fipronil and derivatives EUP (M&B 46030) Shaughnessy No. 129121 Pesticide Use _

INVERTEBRATE ACUTE TOXICITY	% AI	EC_{50} (95%CL) SLOPE	HRS/ TYPE	NOEC	STUDY/ REVIEW DATES	MRID/ CATEGORY	LAB	RC
1.								
2.								
3.								
4.								
5.								
CHRONIC TOX.	**% AI**	**MATC LC_{50}**	**DAYS**	**AFFECTED PARA.**	**STUDY/ REVIEW DATES**	**MRID/ CATEGORY**	**LAB**	**RC**
1. *Daphnia magna*	tested as 100% ai	>9.8 <20 µg ai/l (geometric mean MATC of 14 µg ai/l) 39 µg ai/l	21 d flow through	length	1990/ 1994	429186-26 Invalid	SLI	RGM
2.								
3.								

COMMENTS: Results based on mean measured concentrations. SLI=Springborn Laboratories, Inc.

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Page _____ is not included in this copy.

Pages 9 through 11 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.
