3/21/94

MRID No. 429186-24

DATA EVALUATION RECORD

FILE COPY

1. **CHEMICAL:** MB 46030 (Fipronil).
Shaughnessey No. 129121.

2. **TEST MATERIAL:** M&B 46030; Lot No. PGS963; 100% active ingredient; an off-white powder.

3. **STUDY TYPE:** 72-1. Freshwater Fish Acute Flow-Through Toxicity Test. Species Tested: Bluegill Sunfish (*Lepomis macrochirus*).

4. **CITATION:** Ward, G.S. 1991. M&B 46030: Acute Toxicity to Bluegill, *Lepomis macrochirus*, Under Flow-Through Test Conditions. Laboratory Project No. J9005012b. Prepared by Toxikon Environmental Sciences, Jupiter, FL. Submitted by Rhone-Poulenc Ag Company, Research Triangle Park, NC. EPA MRID No. 429186-24.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Mark A. Mossler
Date: 1/13/94

6. **APPROVED BY:**

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

Signature: Rosemary Graham Mora
Date: 1/13/94 2/17/94

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

Signature: James J. Goodyear 3/10/94
Date: 3 21 94

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a flow-through acute toxicity test using bluegill sunfish. Based on mean measured concentrations, the 96-hour LC_{50} of 83 µg ai/l classifies MB 46030 as very highly toxic to bluegill sunfish. The NOEC was 43 µg ai/l.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Juvenile bluegill sunfish (*Lepomis macrochirus*) were obtained from a commercial supplier in New Jersey. The fish were maintained in test dilution water for 21 days prior to test initiation. The fish were kept in five part per thousand saltwater for the first two days of acclimation. The temperature during the week prior to test initiation was 19.7-22.4°C. Commercial feed and newly hatched brine shrimp were offered daily during holding. The fish were not fed for 48 hours prior to test initiation and no mortality was observed in the population during this period. Mean wet weight and standard length of the fish were 0.22 (0.12-0.44) g and 20 (17-23) mm, respectively.

B. **Test System:** A proportional diluter was used to deliver the solutions to the test vessels. The test chambers were 24-l glass tanks containing 15 l of test solution. The test solution depth was 13 cm. The number of volume replacements was approximately 8.1 per day.

The test chambers were randomly positioned in a water bath under a 16-hour light photoperiod with a light intensity of 325-433 lux. Fifteen-minute dawn and dusk simulations were used.

The test dilution water was carbon-treated city water. The water was vigorously aerated prior to use. During the test, the hardness was 56 mg/l as $CaCO_3$ and the alkalinity was 20-24 mg/l as $CaCO_3$. The specific conductivity was 361-372 µmhos/cm.

A stock solution of 10 mg active ingredient (ai)/ml was prepared in dimethylformamide (DMF). Approximately 0.642 mg of test material was pumped into the chemical mixing chamber with each diluter cycle (3.213 l of dilution water) providing a nominal concentration of 200 µg ai/l. This solution was proportionally diluted to provide the remaining nominal treatment solutions.

C. **Dosage:** Ninety-six-hour flow-through test. Based on a preliminary test, five nominal concentrations (26, 43, 72, 120, and 200 µg ai/l) were selected for testing. A dilution water and solvent (0.02 ml DMF/l) control were also prepared.

D. **Design:** Twenty bluegill were impartially distributed (by twos) to each aquarium. One aquarium was used per treatment or control. Fish were not fed during the study. Observations of mortality and sublethal responses were made every 24 hours. Dead fish were removed from the containers. The temperature was measured hourly using a data logger. The temperature of the water bath was monitored with a minimum/maximum thermometer. The dissolved oxygen concentration and pH were measured in all test solutions (containing surviving fish) daily.

The concentration of test material in samples collected at test initiation and termination was measured using gas chromatography with electron capture detection.

E. **Statistics:** The median lethal concentration (LC_{50}) and associated 95% confidence interval (C.I.) for each 24-hour interval were calculated using a computer program which employed multiple analysis methods (i.e., moving average angle, probit analysis, and binomial probability).

12. **REPORTED RESULTS:** The mean measured concentrations were 27.1, 43.2, 67.4, 134, and 217 µg ai/l (Table 1, attached) and ranged between 94 and 112% of nominal.

Responses of the fish and mortalities are presented in Table 2 (attached). The 96-hour LC_{50}, based on mean measured concentrations, was 85.2 µg ai/l (95% C.I.= 74.2-99.0 µg ai/l) using the moving average angle method. The slope of the probit curve was 8.3. The no-observed-effect concentration (NOEC) was 43.2 µg ai/l, based on the lack of mortality or sublethal responses at this level.

During the test, dissolved oxygen concentration remained at or above 83% of saturation and the pH values ranged from 7.1 to 8.0. Based on the results of the hourly monitoring, the temperature was 19.9-23.1°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The author presented no conclusions.

Quality Assurance and Good Laboratory Practice statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practices Regulations set forth under FIFRA.

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:

The dilution water was dechlorinated city water. Use of this type water is discouraged; however, since no mortality or sublethal effects were noted in the controls, the use of this water probably did not influence the results of the study.

The test temperature (19.9-23.1°C) was occasionally lower than recommended (22 ±1°C).

The biomass loading rate was not reported.

B. Statistical Analysis: The reviewer used EPA's Toxanal program to calculate the 96-hour LC_{50} value and obtained similar results (see attached printout). Since the results of the probit analysis were slightly more conservative, they will be reported. Based on mean measured concentrations, the 96-hour LC_{50} for bluegill sunfish exposed to MB 46030 was 83 µg ai/l (95% C.I.= 72-98 µg ai/l).

C. Discussion/Results: The author stated that all treatment solutions contained the same amount of solvent (10 µl DMF/l). However, the reviewer does not know how this is possible when a proportional diluter was used to deliver the treatment solutions. Future reports should clarify this issue.

Although the biomass loading rate was not reported, the reviewer determined that this value was 0.036 g/l/day, which is within guideline specifications.

This study is scientifically sound and meets the guideline requirements for a flow-through acute toxicity test using bluegill sunfish. Based on mean measured concentrations, the 96-hour LC_{50} of 83 µg ai/l classifies MB 46030 as very highly toxic to bluegill sunfish. The NOEC was 43 µg ai/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, 1-10-94.

MOSSLER MB 46030 LEPOMIS MACROCHIRUS 1-10-94

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
217	20	20	100	9.536742E-05
134	20	19	95	2.002716E-03
67.4	20	5	25	2.069473
43.2	20	0	0	9.536742E-05
27.1	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 67.4 AND 134 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 84.20984

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	3.650065E-02	87.10031	75.41683	102.1735

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.1515336	1	.9618218

SLOPE = 8.315393

95 PERCENT CONFIDENCE LIMITS = 5.078433 AND 11.55235

LC50 = 83.03442

95 PERCENT CONFIDENCE LIMITS = 72.21255 AND 97.80446

LC10 = 58.41566

95 PERCENT CONFIDENCE LIMITS = 45.56665 AND 67.657

****

Fipronil Revised

Page 7 is not included in this copy.

Pages 8 through 10 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.