DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** M&B 46136; Reference No. 42; Batch No. WAB 487; 99.2% active ingredient; a white powder.

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

4. **CITATION:** Bettencourt, M.J. 1992. (M&B 46136) - Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Conditions. SLI Report No. 92-3-4185. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by Rhone-Poulenc Ag Company, Research Triangle Park, NC. EPA MRID No. 429186-74.

5. **REVIEWED BY:**

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

6. **APPROVED BY:**

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2/16/94

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7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using freshwater fish. Based on mean measured concentrations, the 96-hour LC_{50} value of 25 µg ai/l classifies M&B 46136 as very highly toxic to bluegill sunfish. The NOEC was 6.7 µg ai/l.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Bluegill sunfish (*Lepomis macrochirus*) were obtained from a commercial supplier in Connecticut. The fish were maintained in flowing well water (6.9-8.3 tank volume replacements/day) and fed a commercially available pelleted fish food, *ad libitum*, daily. Water quality characteristics of the well water were: a total hardness of 28-30 mg/l as $CaCO_3$, an alkalinity of 24-30 mg/l as $CaCO_3$, a conductivity of 110-130 µmhos/cm, a pH of 6.3-6.8, a dissolved oxygen (DO) concentration of 63-77% of saturation, and a temperature of 22-23°C. The laboratory was maintained on a 16-hour daylight photoperiod.

The fish were acclimated to the laboratory for a minimum of two weeks. Feeding was discontinued 48 hours before the test. The fish were from the same year class and mean weight and length of a representative group were 1.5 (0.73-2.4) g and 45 (34-57) mm, respectively. There was 0.005% mortality in the population in the 48 hours before test initiation.

B. **Test System:** The system was an intermittent-flow proportional diluter. Test vessels were glass aquaria (39 x 20 x 25 cm), each containing approximately 15 l of test solution for a solution depth of 19.5 cm. The diluter delivered 500 ml/cycle (6.5 volume replacements per day) of treatment or control solution to the individual aquaria. The test aquaria were impartially placed in a circulating water bath set to maintain 22 ±1°C.

The dilution water was the same as that used in holding. A 16-hour light/8-hour dark photoperiod with a light intensity of 22-56 footcandles at the solution surface was used and sudden transitions between light and dark were avoided. The diluter was calibrated before test initiation and at test termination and was checked twice daily during the test.

A stock solution [5.1 mg active ingredient (ai)/ml] was prepared by dissolving 0.2541 g ai of test material and diluting with acetone to a final volume of 50 ml. The stock solution was delivered to the mixing chamber via a syringe pump to create the highest concentration treatment solution. This solution was proportionally diluted to prepare the remaining treatment solutions.

C. **Dosage:** Ninety-six-hour flow-through test. Based on preliminary testing, five nominal concentrations (9.7, 16, 27, 45, and 75 µg ai/l) were selected for testing. A solvent (0.015 ml acetone/l) and dilution water control were also prepared.

D. **Design:** Twenty sunfish were impartially selected and distributed, two at a time, to two aquaria (10 per aquarium) for each treatment and control. The biomass loading was 0.153 g/l/day. Observations of mortality and test solution characteristics were made every 24 hours. Dead fish were removed at each observation.

The temperature, DO, and pH were measured once daily in each replicate of the exposure concentrations and the controls. Hardness, alkalinity, and conductivity were measured in one replicate of each group at test initiation. The temperature was also monitored continuously in replicate A of the lowest concentration solution.

The concentration of M&B 46136 in samples collected at test initiation and termination in each replicate aquarium was determined using high pressure liquid chromatography.

E. **Statistics:** The 48-, 72-, and 96-hour median lethal concentration (LC_{50}) and associated 95% confidence interval (C.I.) were calculated using a computer program that employed probit analysis, moving average angle analysis, and binomial probability. Mean measured concentrations and mortality data were used to determine the LC value. The no-observed-effect concentration (NOEC) was defined as the highest concentration tested at and below which there were no toxicant-related mortalities or physical and behavioral abnormalities.

12. **REPORTED RESULTS:** Although precipitate was noted in the diluter system mixing chamber, no undissolved material was noted in the treatment solutions. The mean measured concentrations were 6.7, 10, 17, 26, and 51 µg ai/l (Table 3, attached), and averaged 64% of nominal concentrations.

The responses of the sunfish are given in Table 4 (attached). The 96-hour LC_{50} was determined to be 25 µg ai/l with a 95% C.I. of 21-30 µg ai/l. The slope of the probit curve was 5.1. The NOEC was 6.7 µg ai/l.

Dissolved oxygen ranged from 7.8 to 9.2 mg/l or 87 to 103% of saturation. The pH ranged from 6.7 to 6.9. The temperature was 21-23°C throughout the test. Hardness ranged from 26 to 34 mg/l as $CaCO_3$, alkalinity ranged from 18 to 20 mg/l as $CaCO_3$, and conductivity was 120 µmhos/cm.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The author concluded that the test material would be classified as very highly toxic to bluegill sunfish.

Quality Assurance and Good Laboratory Practice (GLP) Regulation Statements were included in the report, indicating that the study was conducted in accordance with EPA GLP Regulations (40 CFR Part 160). The GLP statement also indicated that maintenance of records on the stability, characterization, and verification was the responsibility of the sponsor and that routine water and food analyses were conducted at a laboratory that did not collect the data under GLPs.

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

A. **Test Procedure:** The test procedures were generally in accordance with protocols recommended by the guidelines, but deviated as follows:

The age of the test organisms was not reported.

The hardness of the water (26-34 mg/l) was less than recommended (40-200 mg/l).

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the LC_{50} value and obtained similar results as the author (see attached printout).

C. **Discussion/Results:** This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using freshwater fish. Based on mean measured concentrations, the 96-hour LC_{50} value of 25 µg ai/l classifies M&B 46136 as very highly toxic to bluegill sunfish. The NOEC, based on the lack of mortality and sublethal effects, was 6.7 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, 1-12-94.

Final Revised

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