


US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD

1. **CHEMICAL:** MB 46030 (Fipronil).
Shaughnessey No. 129121.
2. **TEST MATERIAL:** M&B 46136; Reference No. 42; 99.2% active ingredient; a white powder.
3. **STUDY TYPE:** 72-1. Freshwater Fish Acute Flow-Through Toxicity Test. Species Tested: Rainbow Trout (*Oncorhynchus mykiss*).
4. **CITATION:** Bettencourt, M.J. 1992. (M&B 46136) - Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions. SLI Report No. 91-7-3831. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by Rhone-Poulenc Ag Company, Research Triangle Park, NC. EPA MRID No. 429186-73.

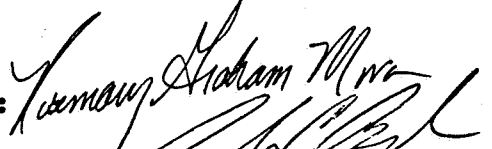
5. **REVIEWED BY:**

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

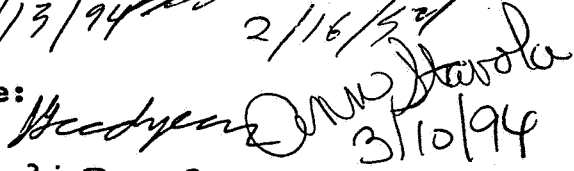
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Date: 1/13/94

6. **APPROVED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
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Signature: 
Date: 1/13/94 2/16/94

James J. Goodyear, Ph.D.
Project Officer, EEB/EFED
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Signature: 
Date: 3-21-94 3/10/94

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 LC₅₀ value of 39 µg ai/l classifies M&B 46136 as very highly toxic to rainbow trout. The NOEC was 18 µg ai/l.

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

9. **BACKGROUND:**

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

11. MATERIALS AND METHODS:

- A. **Test Animals:** Rainbow trout (*Oncorhynchus mykiss*) were obtained from a commercial supplier in California. The fish were maintained in flowing well water (11.5-12.3 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 29-32 mg/l as CaCO₃, an alkalinity of 21-23 mg/l as CaCO₃, a conductivity of 130-140 μ mhos/cm, a pH of 6.7-7.2, a dissolved oxygen (DO) concentration of 74-85% of saturation, and a temperature of 12°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 0.55 (0.32-0.95) g and 39 (33-48) mm, respectively. There was no 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 (6.4 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 12 \pm 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 24-80 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 [11 mg active ingredient (ai)/ml] was prepared by dissolving 0.5486 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 (13, 22, 36, 60, and 100 $\mu\text{g ai/l}$) were selected for testing. A solvent and dilution water control were also prepared.
- D. **Design:** Twenty trout were impartially selected and distributed, two at a time, to two aquaria (10 per aquarium) for each treatment and control. The biomass loading was 0.057 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 dilution water control.

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 median lethal concentration (LC_{50}) and associated 95% confidence interval (C.I.) for each 24 hour period 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:** An electrical circuit failed and the diluter system did not function for four hours between days 3 and 4 of the study. However, treatment solutions were stable during this period. No precipitate was noted in the diluter system or treatment solutions. The mean measured concentrations were 11, 18, 29, 47, and 79 $\mu\text{g ai/l}$ (Table 3, attached). Measured concentrations between sampling days were generally consistent.

The responses of the rainbow trout are given in Table 4 (attached). The 96-hour LC_{50} was determined to be 39 μg

ai/l with a 95% C.I. of 35-43 μg ai/l. The slope of the probit curve was 8.4. The NOEC was 18 μg ai/l.

Dissolved oxygen ranged from 9.0 to 9.8 mg/l or 83 to 91% of saturation. The pH ranged from 7.0 to 7.4. The temperature was 12-13°C throughout the test. Hardness ranged from 30 to 34 mg/l as CaCO_3 , alkalinity ranged from 22 to 24 mg/l as CaCO_3 , and conductivity ranged from 130 to 140 $\mu\text{mhos/cm}$.

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

The author concluded that the test material would be classified as very highly toxic to rainbow trout.

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 the SEP, but deviated as follows:

The age of the test organisms was not reported.

The hardness of the water (30-34 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 the same results as the author (see attached printout). However, since the slope obtained by the reviewer (12.8) is greater than that of the author, it will be reported.

C. Discussion/Results: The amount of acetone in the solvent control was reported to be 100 $\mu\text{l/l}$. However, the maximum amount of acetone in any treatment solution was 9 $\mu\text{l/l}$. However, mortality or sublethal effects were not noted in either control group.

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 39 μg ai/l

classifies M&B 46136 as very highly toxic to rainbow trout. The NOEC, based on the lack of mortality and sublethal effects, was 18 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, 1-11-94.

Final Review

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