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

2. **TEST MATERIAL:** M&B 46030; Lot No. JWZ2092/1/94; 100% active ingredient; a white crystal.


4. **CITATION:** McNamara, P.C. 1990. (M & B 46030) - Acute Toxicity to Daphnids (Daphnia magna) During a 48-Hour Flow-Through Exposure. Laboratory Report No. 89-11-3161.  
   Prepared by Springborn Laboratories, Inc., Wareham, MA.  
   Submitted by Rhone-Poulenc Ag Company, Research Triangle Park, NC. EPA MRID No. 429186-25.

5. **REVIEWED BY:**  
   Andrew C. Bryceland, Fishery Biologist  
   Review Section 5  
   Ecological Effects Branch  
   Environmental Fate and Effects Division (7507C)  
   Date: 3/10/94

6. **APPROVED BY:**  
   Ann Stavola, Supervisory Biologist  
   Review Section 5  
   Ecological Effects Branch  
   Environmental Fate and Effects Division (7507C)  
   Date: 5/6/94

7. **CONCLUSIONS:** This study is not scientifically sound and does not fulfill the guideline requirements. Solvent control mortality (10%) was greater than allowed (5%).

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

   A. **Test Animals:** Daphnia magna (524 hours old) were obtained from in-house cultures maintained under a 16-hour light photoperiod at 20 ±2°C. The culture water was fortified well water filtered through a resin column (Amberlite XAD-7) and a carbon filter. The
**DATA EVALUATION RECORD**

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

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

3. **STUDY TYPE:** 72-2. Freshwater Invertebrate Acute Flow-Through Toxicity Test. Species Tested: *Daphnia magna*.


5. **REVIEWED BY:**
   Mark A. Mossier, M.S.
   Associate Scientist
   KBN Engineering and Applied Sciences, Inc.
   Signature: 
   Date: 1/13/94

6. **APPROVED BY:**
   Rosemary Graham Mora, M.S.
   Associate Scientist
   KBN Engineering and Applied Sciences, Inc.
   Signature: 
   Date: 1/13/94
   James J. Goodyear, Ph.D.
   Project Officer, EEB/EFED USEPA
   Signature: 
   Date: 2/13/94
   3/10/94

7. **CONCLUSIONS:** This study is not scientifically sound and does not fulfill the guideline requirements. Solvent control mortality (10%) was greater than allowed (5%). Based on mean measured concentrations, the 48-hour EC50 for daphnids exposed to M&B 46030 was 190 µg ai/l. Therefore, M&B 46030 is classified as highly toxic to *Daphnia magna*. The NOEC was 52 µg ai/l.

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

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.
cultures were fed *Ankistrodesmus falcatus* and a trout food preparation once daily.

**B. Test System:** An intermittent-flow proportional diluter was used to deliver the test solutions. The test vessels were made of glass and contained a constant solution volume of 1.8 l. The test solution depth was approximately 14 cm. The flow of test solution from the mixing/splitting chambers into the test chambers was restricted using glass capillary tubes to minimize turbulence in the chambers. Test solutions were delivered to each vessel at an approximate rate of six volume replacements per day.

The test area was controlled to maintain the solution temperature at 20 ±1°C. The area was illuminated at an intensity of 40-70 footcandles using fluorescent tubes on a 16-hour light/8-hour dark photoperiod.

The dilution water was from the same source as that used in culturing. The water had a total hardness of 170 mg/l as CaCO₃, an alkalinity of 120 mg/l as CaCO₃, a pH of 8.2-8.3, and a specific conductivity of 500 μmhos/cm.

A 4.0 mg active ingredient (ai)/ml stock solution was prepared by dissolving 0.199 g of the test material to a final volume of 50 ml in acetone. The test material was injected into the diluter chemical mixing chamber resulting in a high nominal test concentration of 360 g ai/l. This solution was subsequently diluted to provide the remaining treatment solutions. The diluter was pre-conditioned with the test material for one day prior to test initiation.

**C. Dosage:** Forty-eight-hour, flow-through test. Based on preliminary testing, five nominal concentrations (47, 78, 130, 220, and 360 g ai/l) were selected for the test. A dilution water and solvent (0.09 ml acetone/l) control were also prepared.

**D. Design:** Two chambers were used for each treatment or control with ten impartially-selected daphnids per chamber. The number of immobilized daphnids was recorded daily. Observations of sublethal effects and of the physical characteristics of the test solutions were made at test initiation and every 24 hours thereafter. The daphnids were not fed during the test.
Dissolved oxygen concentration (DO), pH, and temperature were measured once daily in all replicates. At test initiation, hardness, alkalinity, and conductivity were determined in replicate vessel of each treatment and control group. The temperature of one test vessel was monitored continuously.

Water samples from both replicates of each treatment and control group were taken at test initiation and termination. The concentration of test material was determined using high pressure liquid chromatography.

E. **Statistics:** The median effective concentration (EC$_{50}$) and associated 95% confidence interval (C.I.) were calculated using a computer program that employed three methods of analysis. The probit, moving average angle, and binomial probability methods were examined to determine the best-fitting model. The no-observed-effect concentration (NOEC) was defined as the highest concentration tested at and below which there were no toxicant-related mortalities or sublethal effects.

12. **REPORTED RESULTS:** No undissolved test material was noted in the exposure aquaria. The mean measured concentrations were 34, 52, 110, 160, and 280 g ai/l and averaged 74% of nominal concentrations (Table 3, attached). Measured concentrations between sampling days were generally consistent. Quality control samples averaged 103% of nominal fortification levels.

The response of the daphnids is given in Table 4 (attached). The 48-hour EC$_{50}$ was 190 g ai/l with a 95% C.I. of 110-280 g ai/l. The NOEC was 52 g ai/l.

Dissolved oxygen ranged from 7.7 to 8.7 mg/l. The pH values ranged from 8.2 to 8.3. The temperature was 18-21°C. Hardness and alkalinity were 160-170 and 120 mg/l as CaCO$_3$, respectively. Specific conductance was 500 mhos/cm.

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

Quality Assurance and Good Laboratory Practice Statements were included in the report, indicating that the study was conducted in accordance with all pertinent EPA Good Laboratory Practice Regulations (40 CFR, Part 160). However, the stability, characterization, and verification of the test substance is the responsibility of the study sponsor.
14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures deviated from the SEP as follows:

Observations of the daphnid cultures such as adult mortality, stress, and the presence of ephippia were not reported.

First instar *Daphnia magna* used in tests should be from the fourth or later broods of a given parent. The author did not indicate which brood was the source of the test animals.

Mortality in the solvent control (10%) was higher than allowed for a flow-through study (no more than 5%).

B. Statistical Analysis: The reviewer used EPA's Toxanal program to calculate the EC₅₀ value and obtained similar results (see attached printout).

C. Discussion/Results: This study is not scientifically sound and does not fulfill the guideline requirements.

D. Adequacy of the Study:

(1) Classification: Invalid.

(2) Rationale: Solvent control mortality was greater than allowed.

(3) Repairability: No.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 1-12-94.
Page ___ is not included in this copy.
Pages 1 through 7 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.
NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE
OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY.
 THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

MOSSLER MB 46030 DAPHNIA MAGNA 1-12-94
******************************************************************************
CONC. NUMBER NUMBER PERCENT BINOMIAL
EXPOSED DEAD DEAD PROB.(PERCENT)
280 20 17 85 .1288414
160 20 7 35 13.1588
110 20 0 0 9.536742E-05
52 20 1 5 2.002716E-03
34 20 2 10 2.012253E-02

THE BINOMIAL TEST SHOWS THAT 110 AND 280 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 187.557

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
SPAN G LC50 95 PERCENT CONFIDENCE LIMITS
2 8.643136E-02 191.7032 170.1911 221.5025

RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS G H GOODNESS OF FIT PROBABILITY
5 2.69841 6.049063 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 = 2.80836
95 PERCENT CONFIDENCE LIMITS =-1.804887 AND 7.421607

LC50 = 195.2257
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 68.91532
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY
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