

3-21-94

MRID No. 429186-56

DATA EVALUATION RECORD

1. **CHEMICAL:** MB 46030 (Fipronil).
Shaughnessey No. 129121.
2. **TEST MATERIAL:** MB 46030 (fipronil); CAS No. 120068-37-3;
Batch No. 6ADM93; 96.1% active ingredient; a grey powder.
3. **STUDY TYPE:** 122-2. Growth and Reproduction of Aquatic
Plants - Tier 1. Species Tested: Duckweed (*Lemna gibba*).
4. **CITATION:** Hoberg, J.R. 1993. MB 46030 - Toxicity to
~~Duckweed, *Lemna gibba*.~~ SLI Report No. 93-5-4794. Conducted
by Springborn Laboratories, Inc., Wareham, MA. Submitted by
Rhone-Poulenc Ag Company, Research Triangle Park, NC. EPA
MRID No. 429186-56.
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*

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

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

3/10/94
7. **CONCLUSIONS:** This study is scientifically sound but does
not meet the requirements for a Tier 1 aquatic plant growth
and reproduction study. The mean measured concentration was
less than the required concentration. Based on the mean
measured concentration, the growth and reproduction of *L.*
gibba were not detrimentally affected by the presence of
0.10 mg ai/l of fipronil.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

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11. MATERIALS AND METHODS:

A. **Test Species:** *Lemna gibba* G3 used in the test came from laboratory stock cultures originally obtained from the University of California, Los Angeles. Stock cultures were maintained in Hoagland's medium (with pH adjusted to 5.0) under continuous 3.8-5.4 klux illumination and a temperature of 25 \pm 2°C. Transfers were made into fresh medium once weekly. The plants used in the test were taken from a seven-day old stock culture.

B. **Test System:** Sterile, covered 270-ml crystallizing dishes were conditioned by rinsing with the appropriate solution. One-hundred ml of the appropriate test solution were placed into each dish.

The test was performed in a growth chamber with conditions similar to those used in culturing. Light was provided continuously at an intensity of 4.0-4.6 klux.

A 20 mg ai/ml primary stock solution was prepared by diluting 0.5203 g (0.5 g ai) of test material to the final volume of 25 ml in acetone. One ml of the primary stock was brought to the final volume of 10 ml with acetone to create a secondary stock solution (2 mg ai/ml). An appropriate volume (50 μ l) of the secondary stock solution was brought to the final volume of 500 ml with sterile medium to create the treatment solution. A medium and solvent (0.1 ml acetone/l) control were also prepared.

C. **Dosage:** Fourteen-day growth and reproduction test. Based on the results of a range-finding test, one nominal concentration of 0.20 mg active ingredient (ai)/l was selected for the definitive test. The maximum application rate of the test material was reported to be 0.20 lb active ingredient/acre, which is equivalent to a concentration of 0.15 mg ai/l if applied to a 15-cm water column.

D. **Test Design:** The test consisted of 3 replicate dishes per treatment level and control. *Lemna gibba* (5 plants with 3 fronds each) was aseptically introduced into each dish within 15 minutes of solution addition. On test days 3, 6, 9, 12, and 14, fronds were counted and observations were made. At initiation and after each counting, the dishes were positioned in the assigned random location in the growth chamber. After terminal

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counting, the fronds were dried at 100°C for three days to determine dry weight per replicate.

The pH was measured at test initiation and termination. Temperature was recorded continuously with a minimum/maximum thermometer in a flask of water in the environmental chamber.

At test initiation and termination, samples were removed from each treatment and control solution for analysis by high performance liquid chromatography. A set of three quality control (QC) samples were prepared at test initiation and termination to monitor the precision and quality control during analysis.

E. **Statistics:** Negative and solvent control data were pooled. A t-test was used to determine if a significant reduction in frond number or biomass had occurred in the treatment solution in comparison to the pooled control data.

12. **REPORTED RESULTS:** Initial measured concentrations averaged 79% of nominal (Table 3, attached). Terminal measured concentrations averaged 21% of nominal. The results are based on initial measured concentrations. Recoveries of the 0- and 14-day QC samples ranged between 76 and 110% of nominal.

Frond counts for the control and the exposure groups after 14 days are given in Table 4 (attached). Plants exposed to fipronil were slightly chlorotic in comparison to the control plants. Frond number was significantly reduced (7.7% inhibition) in the treatment group in comparison to the pooled control. Based on frond number data, the 14-day EC_{50} and NOEC were determined to be >0.16 and <0.16 mg ai/l, respectively.

Frond biomass was not significantly reduced (8.8% stimulation) in the treatment group in comparison to the pooled control (Table 5, attached). The 14-day EC_{50} and NOEC were determined to be >0.16 and 0.16 mg ai/l, respectively.

During the test, pH was 5.0-5.1 in all treatment and control solutions at test initiation and 6.1-6.2 at test termination. The temperature ranged from 24 to 25°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
No conclusions were made by the study author.

The study director confirmed that this study was conducted in compliance with EPA Good Laboratory Practice (GLP) regulations (40 CFR Part 160) with the exception that maintenance of records on the test substance (stability, characterization, verification) is the responsibility of the sponsor. Additionally, routine water analyses were conducted at an independent laboratory that did not collect data in accordance with GLP procedures. A Quality Assurance statement was included in the report.

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

- A. Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines with the exception that the light intensity (4.0-4.6 klux) was lower than recommended (5 klux).
- B. Statistical Analysis:** Results of a t-test (attached) between the solvent control and treatment group demonstrated that a significant reduction in frond number did not exist. Since the dry biomass in the treatment group was greater than that of the solvent control, it is apparent that the presence of the test material at a mean measured concentration of 0.10 mg ai/l did not adversely impact the biomass accumulation of *L. gibba*.
- C. Discussion/Results:** The mean measured concentration (0.10 mg ai/l) was only 67% of the required concentration of 0.15 mg ai/l. Renewals should have been conducted at three to five day intervals to insure that the mean measured concentration would be equal to or greater than the required concentration. Therefore, this study is scientifically sound but does not meet the requirements for a Tier 1 aquatic plant growth and reproduction study. Based on the mean measured concentration, the growth and reproduction of *L. gibba* were not detrimentally affected by the presence of 0.10 mg ai/l of fipronil.
- D. Adequacy of the Study:**
- (1) **Classification:** Supplemental.
 - (2) **Rationale:** The mean measured concentration was less than the required concentration.
 - (3) **Repairability:** No.

15. COMPLETION OF ONE-LINER: Yes, 1-7-94.

Excluded Review

Page _____ is not included in this copy.

Pages 5 through 7 are not included in this copy.

The material not included contains the following type of information:

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Eiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiii»
° STUDENT'S T-TEST (two-tailed) °
Eiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiii¼

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Enter the name of the DATAFILE you wish to analyze: lem
(Press RETURN if you wish to skip directly to T evaluation)

```

What are the SAMPLE NUMBERS of the 2 variables you want to compare?

```
1 'solcont'
```

2 'trt'

Means = 402.3334

382

Variances = 561.3336

229.0001

Are these INDEPENDENT or PAIRED samples? (I or P) i

T = 1.252754

$$df = 4$$

$p = .2785354$

~~The MEANS of these 2 samples are NOT significantly different.~~

The confidence limits on the DIFFERENCE between the means of these samples can be calculated as:

20.33334 +/- T(4) * 16.23092

Do you want another T-TEST using this datafile?