DATA EVALUATION REPORT

1. Chemical: Mycoleptodiscus terrestris

2. Test Material: Mycelia of Mycoleptodiscus terrestris, a tan-brown fungus, with an activity of 4.3×10^7 CFU/g of mycelia.

3. Study/Action Type: Midge LC_{50} (154A-20)

4. Study Identification: A 21-day Static Renewal Toxicity/Pathogenicity Test of Fungal Mycelium of Mycoleptodiscus terrestris to Midge (Chironomus tentans), By James Swigert, Ph.D. Supervisor, Aquatic Toxicology. Prepared By Analytical Bio-Chemistry (ABC) Laboratories, Inc., May 21, 1990. Project ID. #38179. Submitted By EcoScience Laboratories, Inc., Amherst, Massachusetts. EPA Acc. No. 418335-10.

5. Reviewed By: David C. Bays
Microbiologist
EFED/EEB

Signature: [signature]
Date: 7/10/91

Les W. Touart
Head, Section 1
EFED/EEB

Signature: [signature]
Date: 7.16.91

6. Conclusions:

The study is scientifically sound and demonstrated an LC_{50} (IC_{50}) > 100 mg/l. This indicates that Mycoleptodiscus terrestris is practically non-toxic to Midge. The study fulfills EPA Guideline requirements for an acute toxicity test for an aquatic invertebrate.

7. Recommendations: N/A

8. Background:

This study was submitted to meet the requirements for the registration of this microbial pesticide.

10. Materials and Methods:

A. Test Organisms: The test midge used in this study were obtained from an in-house culture maintained by the registrant. All midge were cultured at ambient room temperature and lighting was provided by cool white-fluorescent lights (16 hour day length). Midge were fed a suspension of Tetramin and cereal leaves. Midge larvae 15 days old (from egg deposition or 12-13 days from hatching) were used for the testing.

B. Dosage Form: The test material, tan/brown fungal mycelia, was found to have an activity of 4.3×10^7 colony-forming units per gram of mycelia. The recommended dosage (1×10^6 CFU/ml) which conforms to the Subdivision M guidelines was found to be so large as to cloud the test solutions with mycelial mats and to create a critical oxygen demand. These conditions would be incompatible with the survival of the test species. Therefore, the exposure concentration was reduced to 100 mg/l (as per EEB recommendation) and supplemental aeration was provided (7.4-6.9 mg/l for old control and test solutions; 7.5-7.6 mg/l for new control and test solutions).

C. Referenced Protocol: The test was initiated when the midge larvae (10 midge/replicate chamber) were randomly distributed to the test and control chambers, 600 ml glass beakers. The dilution water used in this test was Midge test water prepared to a total hardness of between 40 to 48 mg/l as $CaCO_3$. The test midge were fed 10 drops per test chamber of a suspension of Tetramin and cereal leaves in water at initiation and at every renewal date.

Test solutions were prepared by placing 50 g of sand (to act as a habitat for the midge larvae) in the bottom of a 600 ml beaker then adding 500 ml of fresh dilution water (total hardness 40-48 mg/l). Some of these solutions were left as controls, while the test solutions were dosed by transferring water from their beakers to scintillation vials in which 0.05 g of Mycoleptodiscus terrestris mycelia had been weighed. The vials were shaken and poured back into their respective beakers. This rinsing procedure was repeated 3 times, with a final rinse of 1-2 ml of dilution water being used to ensure all mycelia has been transferred. Each of the test beakers was stirred with a glass rod to ensure all of the mycelia was evenly distributed.

The test was initiated on a Friday and all solutions were renewed (as previously described) every Monday, Wednesday and Friday throughout the 21-day exposure period. Observations for mortality, immobility, and any unusual behavioral attributes were conducted on each renewal day. The pH of the control and test solutions were also measured throughout the study. At the end of the study surviving midge larvae were measured for total length.

D. Statistical Analysis: Biological data were evaluated in light of whether or not the LC_{50} (or IC_{50}) was greater than or less than the 100 mg/l exposure level. Any statistically significant mortality was based on a comparison of control and test group mortality with a t-test.

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12. Reported Results:

	Percent Mortality (After 21 Days)	
Replicate	Control	100 mg/l
1	17	50
2	70	42
3	15	23
4	18	23
5	9	36
Mean	26	35

LC_{50} (IC_{50}) > 100.0 mg/l

No significant differences (t-test using 95% confidence level) were found between the control and the exposure levels for midge mortality. No unusual behavioral or sublethal effects that could be attributable to a symptom of infectivity were observed. A day 21 LC_{50} and IC_{50} was estimated to be >100 mg/l because midge mortality was <50% at this dose level. The fungal mycelia did not seem to interfere with normal midge development.

13. Study Author's Conclusions/Quality Assurance Measures:

LC_{50} (IC_{50}) > 100.0 mg/l

"In accordance with ABC Laboratories' intent that all aquatic toxicity tests conducted by our facility follow good laboratory practices, ABC's study director for the above test herein confirms that the study was conducted in compliance with the U.S. E.P.A. Good Laboratory Practices Standards; Pesticides Programs (40 CFR 160)." Signed by study director, James P. Swigert, Ph.D.

14. Reviewer's Discussion and Interpretation of the Study:

A. Test Procedures: The procedures used followed those recommended by EPA in Section 158.170 of the EPA Registration Guidelines (Pesticide Testing Guidelines, Subdivision M, Microbial and Biochemical Control Agents).

B. Statistical Analysis: The mortality data was analyzed using a t-test with a 95% confidence level.

C. Discussion/Results: An LC_{50} (IC_{50}) > 100.0 mg/l indicates that Mycoleptodiscus terrestris is practically non-toxic to midge.

D. Adequacy of the Study:

1. Validation Category: Core

2. Rationale: Meets EPA Guideline requirements

15. Completion of the One-Liner: