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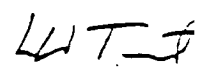
## DATA EVALUATION REPORT

1. Chemical: Mycoleptodiscus terrestris
2. Test Material: Mycelia of Mycoleptodiscus terrestris, a tan-brown fungus, with an activity of  $1.2 \times 10^7$  CFU/g of mycelia.
3. Study/Action Type: Daphnia magna EC<sub>50</sub> (154A-20)
4. Study Identification: A 21-day Prolonged Static Renewal Toxicity of Mycoleptodiscus terrestris to Daphnia magna, By Bruce Young, Biologist II. Prepared By Analytical Bio-Chemistry (ABC) Laboratories, Inc., May 21, 1990. Project ID. #38180. Submitted By EcoScience Laboratories, Inc., Amherst, Massachusetts. EPA Acc. No. 418335-09.

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

Signature:   
Date: 7/10/91

Les W. Touart  
Head, Section 1  
EFED/EEB

Signature:   
Date: 7/6/91

### 6. Conclusions:

The study is scientifically sound and demonstrated an EC<sub>50</sub> > 100 mg/l. This indicates that Mycoleptodiscus terrestris is practically non-toxic to Daphnia magna. 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 daphnids (ABC Lot#89-A9) used in this study were obtained from an in-house culture which had been maintained by the registrant since 1977. All daphnids were cultured in a temperature (20±2) and light (40-80 footcandles on a 16-hour day) controlled area and were fed a suspension of algae (Selenastrum capricornutum) supplemented with a Tetramin, cereal leaves and yeast suspension. Only first-instar daphnids (<24 hours old) were used in the test.

- B. Dosage Form: The test material, tan/brown fungal mycelia, was found to have an activity of  $1.2 \times 10^6$  colony-forming units per gram of mycelia. The recommended dosage ( $1 \times 10^6$  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 (7.1-8.5 mg/l for old control and test solutions; 7.7-8.8 mg/l for new control and test solutions). The nominal concentrations to be tested were 25, 50 and 100 mg/l and were prepared by weighing 0.05, 0.10 and 0.20 grams, respectively, of Mycoleptodiscus terrestris into 2 liter volumetric flasks. The flasks were brought to volume with test water and stirred for 20-25 minutes. The daphnids were transferred to the new solutions within 30 minutes.
- C. Referenced Protocol: The test was initiated when all daphnids (10 first instars/replicate chamber) were randomly distributed to the test and control chambers, 1 liter glass test vessels containing 400 ml of solution. The dilution water used in this test was Daphnia test water prepared to a total hardness of between 160 to 180 mg/l as  $\text{CaCO}_3$ . The test daphnids were uniformly fed an equal volume per test chamber of an algal suspension twice daily.
- 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 survival, abnormal effects, and observance of first brood of the organisms were made on a daily basis. Reproduction success was also determined by counting and discarding the offspring produced in each test concentration every Monday, Wednesday and Friday. The pH of the control and test solutions were also measured throughout the study. At the end of the study surviving adult daphnids were measured for standard length.
- D. Statistical Analysis: The study was a nested design and all statistical data were analyzed using a Northgate PC/AT computer using either SYSTAT (Version 4.0) and/or Dunnett's multiple mean comparison test (as modified for use at ABC). Survival and reproduction data were analyzed by analysis of variance and the Dunnett's multiple means comparison test to determine which exposure levels differed from the control values. Daphnia growth data were assessed by analysis of variance techniques for nested design experiments. If statistically significant effects due to concentration were determined by ANOVA calculations, Tukey's HSD multiple mean comparison test was used to determine those treatment levels

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having responses significantly different from the control. The 21 day  $EC_{50}$  and its 95 percent confidence limits using the binomial, the moving average, and the probit analysis.

12. Reported Results:

<u>Nominal Conc. mg/ml</u>	<u>Rep.</u>	<u>Number Survived/Number Exposed (At 21 Days After Dosing)</u>
Control	A	10/10
	B	10/10
	C	10/10
	D	9/10
25	A	9/10
	B	10/10
	C	10/10
	D	10/10
50	A	10/10
	B	10/10
	C	10/10
	D	10/10
100	A	10/10
	B	10/10
	C	10/10
	D	10/10

$EC_{50} > 100.0$  mg/l

No significant differences ( $P > 0.05$ ) were found between the control and the exposure levels for reproduction and time to first brood. All of the offspring produced during the study appeared normal. Mean survival was 98% for the control and 25 mg/l concentration and 100% for the 50 and 100 mg/l concentrations. A day 21  $EC_{50}$  was estimated to be  $>100$  mg/l based on the absence of movement with gentle prodding. Daphnid lengths were found to be significantly different between the control and the 100 mg/l treatment, but was not considered to be a deleterious effect and was attributed to experimental variability. The NOEL was found to be 100 mg/l and since no effects were observed, the MATC was estimated to be  $>100$  mg/l. The results indicate that Daphnia survival, reproduction, time to first brood, and length of surviving adults does not appear to be affected by Mycoleptodiscus terrestris concentrations up to 100 mg/l.

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

EC<sub>50</sub> > 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, Bruce M. Young.

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: Due to an absence of daphnid mortalities attributable to the test substance, a statistical analysis of the data was not necessary.

C. Discussion/Results: An EC<sub>50</sub> > 100.0 mg/l indicates that Mycoleptodiscus terrestris is practically non-toxic to Daphnia magna, but a definitive value could not be determined because of a lack of mortalities caused by the fungus in this study.

D. Adequacy of the Study:

1. Validation Category: Core

2. Rationale: Meets EPA Guideline requirements

15. Completion of the One-Liner: