DATA EVALUATION REPORT

1. Chemical: Mycoleptodiscus terrestris

2. Test Material: Mycelia of Mycoleptodiscus terrestris, a tan-brown fungus, with an activity of 1.2×10^{9} CFU/g of mycelia.

3. Study/Action Type: Freshwater Fish LC_{50} (154A-19)

4. Study Identification: A 30-day Static Renewal Toxicity and Pathogenicity Evaluation on the Effects of Mycoleptodiscus terrestris to Bluegill (Lepomis macrochirus), By James Swigert, Supervisor, Aquatic Toxicology. Prepared By Analytical Bio-Chemistry (ABC) Laboratories, Inc., May 21, 1990. Project ID. #38178. Submitted By EcoScience Laboratories, Inc., Amherst, Massachusetts. EPA Acc. No. 418335-08.

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

Signature: DCB
Date: 7/15/91

Les W. Touart
Head, Section 1
EFED/EEB

Signature: LWT
Date: 7.16.91

6. Conclusions:

The study is scientifically sound and demonstrated an $LC_{50} > 100$ mg/l. This indicates that Mycoleptodiscus terrestris is practically non-toxic to bluegill. The study fulfills EPA Guideline requirements for an acute toxicity test for freshwater fish.

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 60 bluegill (lot #389) used in this study were obtained from Osage Catfisheries in Osage Beach, Missouri. The fish were reared and maintained at ABC Laboratories in soft blended water. They were fed daily with newly hatched brine shrimp and/or a commercially available fish food. Seventy-two hours before test initiation, the fish were placed in a temperature acclimation unit (22C) and held without food.

B. Dosage Form: The test material, tan/brown fungal mycelia, was found to have an activity of 1.2×10^3 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 (8.0 mg/l for old control and test solutions; 8.3 mg/l for new control and test solutions).

C. Referenced Protocol: The 15 liter test vessels, which were placed in temperature controlled water baths, were dosed by removing 0.5 liter of the diluent water (total hardness-40 to 48 mg/l as $CaCO_3$). To this 0.5 liter, 1.5 g of test material was added and mixed by using a magnetic teflon stir bar to stir the solution for 30 minutes. The mycelial mixture was then added back to the original test vessel from which it was removed. Food was dosed with fungal mycelium to achieve a concentration of 1.0×10^8 CFU/g, and was provided to the fish daily at a rate of 3.0% their body weight per day which provided a dietary dose of 7.9×10^5 CFU/fish/day. The control fish were fed an equivalent weight of undosed food.

The test was initiated on a Friday and all solutions were renewed (as previously described) every Monday, Wednesday and Friday throughout the 30-day exposure period. Observations for mortality, behavioral/sublethal effects, or any gross pathogenic or toxic responses were made on a daily basis. The pH of the control and test solutions were also measured throughout the study. At the end of the study all living fish were measured for standard length and body weight, and examined for infectivity and any microbe related effects. In addition, six control and six test fish were sacrificed for detailed evaluation (2 for each replicate).

D. Statistical Analysis: Since no trout mortality occurred, an LC_{50} value could not be calculated and a statistical analysis of the data was not possible. Therefore, an estimation of the LC_{50} value was made by a visual inspection of the mortality data. Fish weights and lengths were compared by a t-test procedure to detect any differences with the control group. Means and ranges were reported for the water chemistry data.

12. Reported Results:

	Number Dead/Number Exposed (At 30 Days After Dosing)
Control A	0/10
Control B	1/10
Control C	0/10
Test A	1/10
Test B	3/10
Test C	1/10

LC_{50} > 100.0 mg/l

Ten percent mortalities occurred in one of the control replicates and in two of the treated replicates at the dosage level tested. Replicate B of the treated group experienced a 30% mortality over the 30 day test period. However, the observed pattern of mortality was not considered to be an effect of the treatment, because of the similarities of fish weights before and after testing. This indicated that the fish were not eating and starved to death instead of dying due to a toxic or pathogenic response caused by the test substance. The rest of the bluegill were normal in appearance and behavior throughout the test period.

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

LC_{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, PhD.

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 bluegill mortalities attributable to the test substance, a statistical analysis of the data was not necessary.

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