DATA EVALUATION REPORT

1. Chemical: Mycostop - Streptomyces griseoviridis

2. Test Material: Dried spores and mycelia of Streptomyces griseoviridis with an activity of 9.8×10^8 CFU/g of mycelia.

3. Study/Action Type: Daphnia magna EC_{50} (154A-20)

4. Study Identification: A 21-day Static Renewal Toxicity and Pathogenicity Evaluation on the effects of Streptomyces griseoviridis to Daphnia magna, By Dorothy England, Biologist II. Prepared By Analytical Bio-Chemistry (ABC) Laboratories, Inc., November 26, 1990. Project ID. #38679. Submitted By Kemira Oy, Helsinki, Finland. EPA Acc. No. 418211-22.

5. Reviewed By: David C. Bays
Microbiologist
EFED/EEB
Signature:
Date: 9/8/92

Les W. Touart
Head, Section 1
EFED/EEB
Signature:
Date: 9/10/92

6. Conclusions:

The study is scientifically sound and demonstrated an EC_{50} = 190 ppm ($1.9X10^5$ cfu/ml). This indicates that Streptomyces griseovirdis 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 used in this study were obtained from an in-house daphid culture which had been maintained 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, dried spores and mycelia, was found to have an activity of 9.8×10^8 colony-forming units per gram of mycelia. A preliminary range-finding test was conducted using the concentrations 0.01, 0.1 and 1.0 and from these results, a maximum hazard concentration of 1×10^6 cfu/ml was selected with a medium dose of 1×10^5 and a low dose of 1×10^4 cfu/ml. A heat-killed treatment, which consisted of 1×10^6 spores/ml which had been killed by autoclaving at 15 psi/250C for one hour, was also included in the test design.

C. Referenced Protocol: The test was initiated when all daphnids (5 first instars/replicate chamber) were randomly distributed to the test and control chambers, 1 liter glass test vessels containing 500 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 $CaCO_3$. The test daphnids were uniformly fed an equal volume per test chamber of an algal suspension twice daily and supplemented with a suspension of trout chow, cereal leaves and yeast. All test vessels were aerated because of the presence of an oxygen demand due to the nature of the test material (fungal spores and mycelia).

The test was initiated on a Friday and all solutions were renewed (by transferring the daphnids from the old to new solutions using a mechanical pipetter) 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 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:

Nominal Conc. mg/ml	Rep.	Number Killed/Number Exposed (At 21 Days After Dosing)
Control	A	0/5
	B	0/5
	C	0/5
	D	0/5
Low Level ($1x10^4$)	A	0/5
	B	0/5
	C	0/5
	D	0/5
Middle Level ($1x10^5$)	A	5/5
	B	0/5
	C	0/5
	D	0/5
High Level ($1x10^6$)	A	5/5
	B	5/5
	C	5/5
	D	5/5
Killed Spores ($1x10^6$)	A	5/5
	B	5/5
	C	5/5
	D	5/5

EC_{50} > 190 mg/l (ppm)

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 100% for the control and low level concentration, 75% for the middle concentration, and 0% for the high concentration and killed spore treatment. Mortality from killed spore treatments have been reported from previous studies, and is thought to be caused by creating a toxic environment through the autoclaving process. At day 21 EC_{50} was calculated to be approximately 190 mg/l. Daphnid growth was found to be significantly affected at the middle concentration ($1x10^5$). The NOEC was found to be 10 mg/l (ppm), the LOEC was 100mg/l (ppm) and the MATC was found to be 32 mg/l (ppm).

The mean temperatures of the test solutions were 20.6±0.5C for the old and 20.5±0.5C for the new solutions. The pH values for the renewed solutions ranged from 7.8 to 8.5 (mean=8.3±0.2) and for the expired solutions ranged from 4.8 to 8.8 (mean=8.2±0.9). Dissolved oxygen concentrations in the renewed

solutions ranged from 8.0 to 8.5 mg/l (94% to 100% saturation) at 21C (mean=8.3±0.2 mg/l) and in the expired solutions ranged from 0.7 to 9.5 mg/l (8% to 112% saturation) at 21 C (mean=7.1±2.4 mg/l).

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

EC_{50} = 190 mg/l (ppm)

"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, Dorothy C. England

14. 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 procedures used were acceptable for the type of data analyzed and were used appropriately in supporting the conclusions presented in this study.

C. Discussion/Results: An EC_{50} = 190 mg/l indicates that Streptomyces griseoviridis is practically non-toxic to Daphnia magna.

D. Adequacy of the Study:

1. Validation Category: Core

2. Rationale: Meets EPA Guideline requirements

15. Completion of the One-Liner: