DATA EVALUATION REPORT

1. Chemical: Mycostop™ (Streptomyces griseoviridis)

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

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

4. Study Identification: A 30-day Static Renewal Toxicity and Pathogenicity Evaluation on the Effects of Streptomyces griseoviridis to Rainbow Trout (Oncorhynchus mykiss), By Dorthy England, Biologist II. Prepared By Analytical Bio-Chemistry (ABC) Laboratories, Inc., May 21, 1990. Project ID. #38678. Submitted By Kemira Oy, Helsinki, Finland. EPA Acc. No. 418211-21.

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

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

6. Conclusions:

The study showed sporatic fish mortality and failed to demonstrate a definitive LC_{50} value due to the absence of a dose-response trend with the observed mortality. The study will be considered invalid and the results are not adequate for making a risk assessment.

7. Recommendations:

The study will need to be repeated so that a definitive LC_{50} value can be calculated.

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 80 rainbow trout (lot #2190) used in this study were obtained from Mt. Lassen Trout Farm in Red Bluff, CA. The fish were reared and maintained at ABC Laboratories in well water. They were fed daily with newly hatched brine shrimp and/or a commercially available fish food. Three days before test

initiation, the fish were placed in a temperature acclimation unit and a once a day feeding schedule was initiated.

B. Dosage Form: The test material, dried fungal spores and mycelia, was found to have an activity of 9.8×10^8 colony-forming units per gram of mycelia. The maximum hazard dose (1×10^6 cfu/ml), which conforms to the Subdivision M guidelines, was found to cause 100% mortality after 7 days. A range of concentrations, based on a range finding test, were then tested to determine the LC_{50} value. The first test, using the concentrations 6.5×10^2, 1.2×10^3, 2.5×10^3, 5.0×10^3, and 1.0×10^4 cfu/ml plus a killed spore treatment (1.0×10^4 cfu/ml) and a dilution water control, was discontinued at day 26 due to excessive mortality in the control chamber. A third test was run with slightly higher concentrations (2.5×10^3, 5.0×10^3, 1.0×10^4, 2.0×10^4, and 4.0×10^4 cfu/ml plus a killed spores treatment of 4×10^4 cfu/ml) and the test was successfully completed.

C. Referenced Protocol: The five gallon glass jars, containing 15 liters, were placed in temperature controlled water baths (12C), were dosed by dispersing the test material in about 10 ml of dilution water (total hardness-40 to 48 mg/l as $CaCO_3$). The spores were allowed to suspend for 1 hour and then added to the test vessels and stirred with a glass rod to enhance mixing. Food was dosed with fungal mycelium to achieve a concentration of 10% of the water exposure, and was provided to the fish daily at a rate of 3.0% their body weight per day. The control fish were fed an equivalent weight of undosed food.

Ten fish were impartially distributed to each test chamber (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).

Temperature, pH, and dissolved oxygen were measured at the time of solution renewal for each chamber. Supplemental aeration was used because of an oxygen demand created by test material.

D. Statistical Analysis: Since trout mortality did not follow a dose-response, 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	0/10
$2.5x10^3$	0/10
$5.0x10^3$	0/10
$1.0x10^4$	0/10
$2.0x10^4$	9/10
$4.0x10^4$	0/10
Killed spores ($4.0x10^4$)	10/10

LC_{50} > 10.2 mg/l

One hundred percent mortality occurred in the killed spore group at day 16, with 70% and 90% mortality in the $2.0x10^4$ group at day 25 and 26 respectively. Due to sporadic mortality, an LC_{50} could not be calculated, but was estimated to be at least greater than 10.2 mg/l. A no effects concentration was determined to be $5.0x10^3$ cfu/ml and there was no observed infectivity after 30 days. However, the possibility of toxicity was indicated by the killed spore and the $2.0x10^4$ test concentration results, but a lack of toxicity at the $4.0x10^4$ test concentration made this interpretation questionable.

Problems with nonspecific toxicity when testing fungal preparations have been observed in the past and have been related to the the autoclaving process used to kill the fungal spores for the killed spore control. All trout were normal in appearance and behavior throughout the test period. The dissolved oxygen ranged from 8.0 to 10.2 mg/l in the renewed solutions and 4.8 to 10.6 mg/l in the expired solutions, pH ranged from 7.6 to 8.4 in the renewed solutions and 7.1 to 8.3 in the expired solutions, and the temperature ranged from 11 to 13C in both solutions.

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

LC_{50} > 10.2 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 sporadic mortality, an LC_{50} was not able to be calculated and a statistical analysis of the data was not done.

C. Discussion/Results: An LC_{50} > 10.2 mg/l indicating that Streptomyces griseoviridis is slightly toxic to rainbow trout was estimated from the data, but a definitive value could not be determined because of sporadic mortalities observed in this study.

D. Adequacy of the Study:

1. Validation Category: Invalid

2. Rationale: Due to the absence of a dose-response trend with the observed fish mortality.

15. Completion of the One-Liner: