

DATA EVALUATION REPORT

1. Chemical: *Bacillus thuringiensis* subsp. *tenebrionis* (Btt) protein
2. Test Material: Technical Powder
3. Study/Action Type: Nontarget Insect-Ladybird Beetles (*Hippodamia convergens*) (154A-23)
4. Study Identification: BTT PROTEIN: A Dietary Toxicity Study with Ladybird Beetles. By Kimberly A. Hoxter and Gregory J. Smith. Prepared By Wildlife International LTD, March 1993. Project No. 139-348. Submitted By Monsanto Agricultural Company, St. Louis, Missouri. EPA Acc. No. 429322-12.
5. Reviewed By: David C. Bays, PhD. Microbiologist EFED/EEB
 Signature: *David C. Bays*
 Date: 6/16/94
 Robert I. Rose, PhD. Entomologist EFED/EEB
 Signature: *Robert I. Rose*
 Date: 6/23/94
6. Conclusions: The study is scientifically sound and demonstrated an LC₅₀ > 100ppm Btt protein. This indicates that Btt protein is practically nontoxic to Ladybird Beetles. The study fulfills EPA Guideline requirements for a nontarget insect pathogenicity/toxicity test.
7. Recommendations: N/A
8. Background: This study was submitted to support the request for the registration of *Bacillus thuringiensis* subsp. *tenebrionis* protein produced by potato plants.
10. Materials and Methods:
 - A. Test Organisms: Apparently healthy, Ladybird Beetles (*Hippodamia convergens*) were used in the study and were obtained from the Rincon-Vitova Insectaries, Inc. located in Oakview, California.
 - B. Dosage Form: The test material (687 mg Btt protein powder dissolved in 125 ml of 0.1 M-Na₂CO₃/NaHCO₃, pH 10.5) was received from the registrant as a milky liquid. The test diets were prepared by measuring a calculated amount of the test substance (100ppm-nominal concentration) and then adding water and honey.
 - C. Referenced Protocol: The test insects were placed in disposable one pint rolled paper containers (87 mm in

diameter/85 mm high) that were covered with a disposable plastic petri dish (90 mm in diameter). The test diet (available ad libitum) was placed in a 20 ml glass vial which was covered with cheese cloth, and then inserted into the container's cover. A moist sponge, which was misted daily, was placed on the top of each container to increase humidity within the test chamber.

Insects were randomly assigned to the Btt protein treatment, the attenuated control and negative control (12.5% sucrose mixture). Fresh diet was given to the beetles and the average feed consumption for each test concentration and control group was determined on a weekly basis. The test insects were observed for mortality and signs of toxicity twice on the day the experiment started (first observation immediately following the introduction of the test diets) and once a day thereafter until the end of the study. The environmental conditions were as follows: the test beetles were given a photoperiod of 8 hours of light per day, kept at a temperature of 18.7-23.2 C with an average relative humidity of 49±19%(SD) .

- D. Statistical Analysis: After study completion, an estimation of the LC⁵⁰ value was made by visual inspection of the mortality data. A calculation of the LC⁵⁰ value was not necessary because of the lack of mortalities found in this study.

12. Reported Results:

<u>Dosage</u>	<u>ppm</u>	<u>Replicate</u>	<u>Number Dead/Number Exposed</u> <u>(At 10 Days After Dosing)</u>
Negative control	0	A	4/25
		B	7/25
Attenuated control	100	A	5/25
		B	5/25
Treatment	100	A	7/25
		B	7/25
		C	11/25
		D	1/25
		E	8/25
		F	10/25

LC₅₀ > 100ppm of Btt protein

Mortalities occurred in both of the control groups (negative and attenuated) and in all of the treatment groups.

The mortalities in the negative and attenuated control groups were 20% and 20%, respectively, while those in the treatment groups were 28%, 28%, 44%, 4%, 32% and 40%, respectively. The mortality in the treatment groups did not appear to be treatment related. No additional signs of toxicity were observed during the test.

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

LC₅₀ > 100ppm Btt protein

"This study was conducted so as to conform with Good Laboratory Practices as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160, 17 August 1989; OECD, ISBN 92-84-12367-9, Paris 1982; and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August, with the following exception: Samples of the test diets were sent to Monsanto Agricultural Company for analysis. The results were not reported to or audited by Wildlife International Ltd. for compliance with Good Laboratory Practice Standards. Characterization of the test substance was the responsibility of Sponsor." Signed by study director, Kimberly A. Hoxter.

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

A. Test Procedures: The procedures used follow those recommended by EPA in the 1989 Pesticide Testing Guidelines for Microbial and Biochemical Pest Control Agents, Subdivision M.

B. Statistical Analysis: None was needed since the pattern of mortality did not facilitate the calculation of an LC₅₀ value.

C. Discussion/Results: An LC₅₀ > 100ppm indicates that is Btt protein is practically non-toxic to Ladybird Beetles.

D. Adequacy of the Study:

1. Validation Category: Core

2. Rationale: Meets EPA Guideline requirements

5. Completion of the One-liner: