



6-29-94

DATA EVALUATION REPORT

1. Chemical: *Bacillus thuringiensis* subsp. *tenebrionis* (Btt) protein
2. Test Material: Technical
3. Study/Action Type: Nontarget Insect-Green Lacewing Larvae (*Chrysopa carnea*) (154A-23)
4. Study Identification: BTT PROTEIN: A Dietary Toxicity Study with Green Lacewing Larvae (*Chrysopa carnea*). By K. A. Hoxter and Gregory J. Smith. Prepared By Wildlife International LTD, April 1993. Project No. 139-347. Submitted By Monsanto Agricultural Company, St. Louis, Missouri. EPA Acc. No. 429322-13.
5. Reviewed By: David C. Bays, PhD.
Microbiologist
EFED/EEB
Signature: 
Date: 6/29/94

Robert I. Rose, PhD.
Entomologist
EFED/EEB
Signature: 
Date: 06/29/94
6. Conclusions: The study is scientifically sound and demonstrated an $LC_{50} > 10^8$ cfu (781 ug technical powder)/g feed. This indicates that Btt in potato is practically nontoxic to Green Lacewing Larvae. 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 Btt potato.
10. Materials and Methods:
 - A. Test Organisms: Apparently healthy, Green Lacewing Larvae (*Chrysopa carnea*) were used in the study and were obtained from the Rincon-Vitova Insectaries, Inc. located in Oakview, California.

- B. Dosage Form: The test diets were prepared by weighing a calculated amount of the test substance (687 mg Btt protein powder dissolved in 125 ml of 0.1 M- $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$, pH 10.5) and diluting with a calculated amount honey and water for the desired concentration. The nominal concentration for the test diet and attenuated control diet was 100 ppm.
- C. Referenced Protocol: The test insects were placed in one ounce semi-transparent plastic cups with semi-transparent lids (1 replicate-individual/cup). The test diet was prepared by mixing together weighed amounts of test substance, pollen substitute and distilled water, if necessary.

Thirty larvae were randomly assigned to each of 3 treatment levels (10^4 , 2×10^6 , 2×10^8 cfu/g of feed) along with the attenuated (equal to highest test concentration used) and negative (did not receive viable or attenuated test substance) controls. Each test group of 30 larvae was divided into 3 subgroups of 10 individuals to facilitate record keeping.

For the first 5 days of the study, the larvae were fed the test diets. From Day 5 until the end of the study, the test diets were replaced with untreated eggs of the Angoumois grain moth (Sitotroga cerealella) as a food source. The test larvae were observed for mortality and signs of toxicity immediately following introduction of the test diets and continued twice a day until the end of the study. The environmental conditions were as follows: 8 hours of light/day, a temperature of 24-25C and an average relative humidity of 44%.

- D. Statistical Analysis: After study completion, an estimation of the LC^{50} value was made by visual inspection of the mortality data. A calculation of the LC^{50} value was not necessary because of the lack of mortalities found in this study.

12. Reported Results:

<u>Dosage</u>	<u>cfu/g</u>	<u>Replicate</u>	<u>Number Dead/Number Exposed</u> <u>(At 6 Days After Dosing)</u>
Negative control	0	A	0/10
		B	0/10
		C	0/10
Attenuated control	0	A	0/10
		B	0/10
		C	0/10

Treatment			
	10 ⁴	A	0/10
		B	0/10
		C	0/10
	10 ⁶	A	0/10
		B	0/10
		C	0/10
	10 ⁸	A	0/10
		B	0/10
		C	2/10

LC₅₀ > 10⁸ cfu/g feed

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

LC₅₀ > 10⁸ cfu/g feed

"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." 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 > 10⁸ indicates that Foil is practically non-toxic to Green Lacewing Larvae.

D. Adequacy of the Study:

1. Validation Category: Core

2. Rationale: Meets EPA Guideline requirements

15. Completion of the One-liner: