6-23-94

DATA EVALUATION REPORT

1. Chemical: *Bacillus thuringiensis* subsp. *tenebrionis* endotoxin protein produced in potato.

2. Test Material: *Btt* protein

3. Study/Action Type: Nontarget Insect-Parasitic Hymenopteran (*Nasonia vitripennis*) (154A-23)

4. Study Identification: Btt PROTEIN: A Dietary Toxicity Study with Parasitic Hymenoptera (*Nasonia vitripennis*) By Kimberly A. Hoxter and Gregory J. Smith. Prepared By Wildlife International LTD, May 1993. Project No. 139-349B. Submitted By Monsanto Agricultural Company, St. Louis, Missouri. EPA Acc. No. 429322-11.

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: 06/23/94

6. Conclusions: The study is scientifically sound and demonstrated an LC_{50} > 100 ppm. This indicates that this product is practically nontoxic to parasitic wasps (hymenopteran). The study fulfills EPA Guideline requirements for a nontarget insect path./toxicity test.

7. Recommendations: N/A

8. Background: This study was submitted to support the request for the registration of transgenic potato which expresses the *Bacillus thuringiensis* subsp. *tenebrionis* endotoxin protein.

10. Materials and Methods:

A. Test Organisms: Parasitic hymenoptera (*Nasonia vitripennis*) were used in the study and were obtained from Carolina Biological Supply Company, Burlington, North Carolina.

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-Na_2CO_3/$NaHCO_3$, 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 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.

Two replicates, containing 25 insects each, were randomly assigned to the treatment and the attenuated and negative controls. Fresh diet was given to the wasps every three days. All wasps were immobilized with nitrogen at the start of the study. The test diet was introduced by placing it on top of the container. The negative controls were treated identically to the treated wasps with the exception of not receiving the viable or attenuated test substance. Observations were made at the time of diet introduction, twice on the first day of the study, and daily until test termination. The environmental conditions were as follows: the test wasps were given a photoperiod of 8 hours of light per day, kept at a temperature of 25.5-27.0 C with an average relative humidity of 34±12%.

D. Statistical Analysis: The mortality data were transformed to percentages and analyzed to detect differences among groups using analysis of variance with the Duncan's multiple range test. Percentage data were subjected to an arcsine transformation prior to analysis of variance.

12. Reported Results:

Dosage	ppm	Replicate	Number Dead/Number Exposed (At 9 Days After Dosing)
Negative control	0	A	7/25
		B	8/25
Attenuated control		A	2/25
		B	4/25
Treatment	100	A	4/25
		B	10/25

LC_{50} > 100 ppm

The test was terminated on day 9 when the control mortality exceeded 20%. Mortalities occurred in both of the control groups (negative and attenuated) and in the treatment group. The mortalities in the negative and attenuated control groups were 30% and 12%, respectively, while those in the 100 ppm diet concentration averaged 28%. The mortality in the treatment group was not statistically significantly different from the

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controls and was not considered to be treatment related. The mortalities were considered to be attributable to the life expectancy of the insects under test conditions.

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

LC_{50} > 100 ppm

"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 1984, with the following items noted: 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 the Sponsor." Signed by study director, Kimberly A. Hoxter, Senior Research Biologist

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: Analysis of Variance with the Duncan's Multiple Range Test.

C. Discussion/Results: An LC_{50} > 100 ppm indicates that this product is practically non-toxic to parasitic wasps.

D. Adequacy of the Study:

1. Validation Category: Core

2. Rationale: Meets EPA Guideline requirements

15. Completion of the One-liner: