6-23-94

DATA EVALUATION REPORT

1. Chemical: *Bacillus thuringiensis* subsp. *tenebrionis* expressed in Potato

2. Test Material: Technical

3. Study/Action Type: Nontarget Honey Bee (*Aphis mellifera*) Testing (154A-24)

4. Study Identification: Evaluation of the Dietary Effect(s) of Purified *Btt* Protein on Honey Bee Adults. By Victor L. Maggi. Prepared By California Agricultural Research, Inc., August 1993. Project No. CAR 189-92. Submitted By Monsanto Company. St. Louis, MO. EPA Acc. No. 429322-10.

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 not scientifically sound and could not be used as part of a risk assessment. Excessive bee mortality (negative control exceeded 20% after only three days) made the study invalid.

7. Recommendations: The current honey bee protocol (154-24) is not scientifically sound because of the harsh environment that the newly emerged adult honey bees are subjected to during the study. This leads to excessive mortality in the control bees and consequently very little usable information. The registrant will be required to submit a scientifically valid honey bee study but not using this protocol. Therefore, this study should not be repeated.

8. Background: This study was submitted to support the request for the registration of transgenic potato containing *Btt* even though the registrant was told that this study would not be necessary or if done, produce any usable information.

10. Materials and Methods:

A. Test Organisms: The test bees were obtained in hives from a professional beekeeper. All hives were strong and in good condition with no noticeable disease observed.

B. Dosage Form: The test diets were prepared by mixing together a carefully measured amount of *Btt* (687 mg *Btt* protein powder dissolved in 125 ml of 0.1 M-Na_2CO_3/$NaHCO_3$, pH

10.5) and honey:water. The nominal concentration used was 100 ppm.

C. Referenced Protocol: The test insects were placed in disposable one pint rolled paper containers (110 mm top and 85 bottom diameter/160 mm high) that were covered with snug fitting circles of 3.2 mesh/cm metal hardware cloth. The test diet (available ad libitum) was placed in a 6 ml glass shell vial which contained a cotton wick, and then inserted into a hole in each container's bottom.

Three replicates, containing at least 40 bees each, were randomly assigned to the 100 ppm treatment along with the attenuated and negative (untreated honey:water) controls. The test insects were observed for mortality and signs of toxicity on the day the experiment started (first observation four hours following the introduction of the test diets) and once a day thereafter until the end of the study. The study was terminated when the negative control mortality exceeded 20%. The environmental conditions were as follows: temperature of 25-27C, and a mean relative humidity of 37%-55%.

D. Statistical Analysis: After study completion, Analysis of Variance (ANOVA) and Duncan's Multiple Range Test were carried out using Pesticide Research Manager (PRM) software, version 4.06 (Gyllings Data Management, Inc., Brookings, SD). A calculation of the LD^{50} value was not necessary because a maximum hazard dose study was conducted.

12. Reported Results:

Dosage	ppm	Replicate	Number Dead/Number Exposed (At 3 Days After Dosing)
Negative control	0	A	9/42
		B	14/42
		C	14/40
Attenuated control	100	A	9/39
		B	14/41
		C	22/42
Treatment	100	A	10/39
		B	11/40
		C	14/64

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 37%, respectively, while those in the 100 ppm diet

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concentrations was 25%.

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

"This study was conducted in accordance with Good Laboratory Practice Standards, as published by the Environmental Protection Agency in 40 CFR 160, dated August 17, 1989, with the exception of the stability, characterization,and verification of the test substance identity and maintenance of records on the test substance being the responsibility of the study sponsor and provides a true and accurate representation of the raw data." Signed by study director, Victor L. Maggi.

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: ANOVA and Duncan's Multiple Range Test.

C. Discussion/Results: An LD_{50} > 100 ppm indicates that *Btt* expressed in potato is practically non-toxic to Honey Bee. Results are questionable based on excessive mortality in the controls

D. Adequacy of the Study:

1. Validation Category: Invalid

2. Rationale: Excessive mortality in the controls.

15. Completion of the One-liner: