6-23-94

006405

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 Using Larvae.

4. Study Identification: Evaluation of the Dietary Effect(s) of Purified *Btt* Protein on Honey Bee Larvae. By Victor L. Maggi. Prepared By California Agricultural Research, Inc., August 1993. Project No. CAR 188-92. Submitted By Monsanto Company. St. Louis, MO. EPA Acc. No. 429322-09.

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:
Date: 06/23/94

6. Conclusions: The study is scientifically sound and demonstrated that *Btt* protein in potato was practically non-toxic to honeybee larvae. However, the study needs to be validated with a positive control to ensure that the test system is working properly. The protocol itself is reasonable but without a positive control it's not possible to determine if the negative results are due to a lack of effect by the *Btt* or an inability of the test system to demonstrate adverse effects. The registrant should validate the test using a substance that will elucidate a positve response (ie. B-exotoxin). Also, it would improve the test if the registrant used 1-2 day larvae instead of 3 day larvae because the younger larvae would feed more actively on the test substance and would get a larger exposure to the test substance.

7. Recommendations: The larval honey bee protocol is scientifically sound and the study itself was well-run, but the test protocol will need to be validated, especially since this is a new protocol. The registrant will need to test a positive control, such as B-exotoxin, to ensure that the test is working properly. The registrant should also use 1-2 day larvae to maximize exposure to the test substance. It will be necessary to re-test the *Btt* including a positive control to sucessfully validate the test.

8. Background: This study was submitted to support the request for the registration of transgenic cotton containing *Btt*. This is a new test protocol that was requested because of the potential production of *Btt* in cotton pollen.

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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 bee hive frames were observed for egg laying activity. When at least 50 eggs were laid, the frame was moved into a queen-excluded hive box (ensures no more eggs are laid in the test frames). Treatment doseages were delivered with an electonic micro-applicator, calibrated to deliver 5 microliters of test material to each larval cell.

Four replicates of four treatments, containing at least 50 bees/treatment, were used. The treatments were as follows: untreated contol; diluent only (distilled water); *Btt* protein (100 ppm); and heat attenuated *Btt* protein (100 ppm). Once bees began to emerge, all frames were checked and evlauated daily. The frames were transferred to the lab and all emerged bees were counted, including the ones that had died and moved from the emergence cages to adult holding cages. The environmental conditions were as follows: temperature of 26-30C in the larval growth chamber, temperature of 22-27C in the emerged adult growth chamber and a mean relative humidity of 38%-70%.

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:

Table I. Larval Survival from Dosage to Emergence

Dosage	ppm	Replicate	Number Emerged /Number Exposed	Percent Survival
Untreated (Diet Only)	0	1	42/50	84
		2	46/50	92
		3	50/50	100

		4	45/50	90
		Mean	**45.8**	**91.5**
Distilled water	0	1	47/50	94
		2	41/50	82
		3	39/50	78
		4	38/50	76
		Mean	**41.3**	**82.5**
Btt protein	100	1	45/50	90
		2	50/50	100
		3	29/50	58
		4	48/50	96
		Mean	**43.0**	**86.0**
Heat atten.	100	1	42/50	84
		2	49/50	98
		3	44/50	88
		4	30/50	60
		Mean	**41.3**	**82.5**

Table II. Adult Survival from Emergence through Trial Termination

Dosage	ppm	Replicate	Number Dead /Number Emerged	Percent Survival
Untreated (Diet Only)	0	1	18/42	57.1
		2	20/46	56.5
		3	5/50	90.0
		4	21/45	53.3
		Mean	**16.0/45.8**	**64.2**
Distilled water	0	1	24/47	48.9
		2	20/41	51.2
		3	12/39	69.2
		4	18/38	52.6
		Mean	**18.5/41.3**	**55.5**
Btt protein	100	1	44/45	2.2
		2	25/50	50.0
		3	8/29	72.4
		4	8/48	83.3
		Mean	**21.3/43.0**	**52.0**
Heat atten.	100	1	40/42	4.8
		2	21/49	57.1
		3	23/44	47.7
		4	4/30	86.7
		Mean	**22.0/41.3**	**49.1**

Mortalities occurred in the control groups (diet only, and attenuated) and in the treatment group with

both the larvae and adult bees (Table I). The average survival of bees in the distilled water and heat attenuated control groups in the larval portion of the test was 82.5% and 91.5% for the untreated control, and 86% in the 100 ppm diet concentration.

In the adult portion of the test, the untreated, distilled water, and attenuated controls had a mean survival of 64.2%, 55.5%, and 49.1%, respectively. The 100 ppm treatment had a mean survival of 52.0%. Two treatments demonstrated an unusually high mortality (rep. #1- heat atten. treatment and rep. #1-*Btt* protein), but this was attributed to the emergence cage being pressed too tightly over one end of the treated area which led to dehydration due to entrapment. No significant differences were observed among treatment means using ANOVA.

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 were developed by the testing company and registrant following recommendations by EPA.

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 larvae. The study was scientifically sound, but the protocol needs to be validated.

D. Adequacy of the Study:

1. Validation Category: Supplemental

2. Rationale: The protocol, since it is new, needs to be validated using a positve control.

15. Completion of the One-liner: