DATA EVALUATION REPORT

1. Chemical: Foil - Bacillus thuringiensis var. kurstaki (Btk) Strain EG2424

2. Test Material: Technical

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

4. Study Identification: A Dietary Pathogenicity and Toxicity Study with the Honey Bee. By K. A. Hoxter and S. P. Lynn. Prepared By Wildlife International LTD, November 1992. Project No. 235-125B. Submitted By Ecogen, Inc. Langhorne, EPA Acc. No. 421939-01.

5. Reviewed By: David C. Bays
Microbiologist
EFED/EEB
Signature: D. C. Bays
Date: 4/1/92

Les W. Touart
Head, Section 1
EFED/EEB
Signature: L. T.
Date: 4/6/92

6. Conclusions: The study is scientifically sound and demonstrated an $LC_{50} > 10^8$ cfu (781 ug technical powder)/g diet. This indicates that Foil is practically nontoxic to Honey Bee.

7. Recommendations: N/A

8. Background: This study was submitted to support the request for the registration of the Btk product Foil.

10. Materials and Methods:

A. Test Organisms: The test bees were obtained from the Wildlife International Ltd. hives located in Easton, Maryland and two froames were purchased from Chesapeake Apiaries. One frame of pupae was taken from the hives (3 days before test initiation) and placed in a Marsh Roll-X automatic incubator for 3 days to allow the adult bees to emerge. The bees used in the test were 1 to 2 days old and were healthy in appearance.

B. Dosage Form: The test diets were prepared by mixing together a calculated amount of Mycostop (specific activity of 9.8×10^8 cfu/g diet) and honey. The nominal concentrations used were 240, 276 and 2400 ppm with no adjustment for purity of the test substance. The attenuated control was prepared by autoclaving a portion of the test substance (highest concentration tested) at 121C for 30 minutes.

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 each of 3 treatment levels (10^4, 10^6 and 10^8 cfu/g) along with the attenuated and negative (untreated honey) controls. The bees were immobilized with nitrogen at the start of the study and the test diets were placed atop the test chambers. 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 study was terminated when the negative control mortality exceeded 20%. The environmental conditions were as follows: 8 hours of light/day, a temperature of 22-23C, and an mean relative humidity of 63%.

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 associated with the test substance found in this study.

12. Reported Results:

Dosage	cfu/g diet	Replicate	Number Dead/Number Exposed (At 4 Days After Dosing)
Negative control	0	A	6/25
		B	7/25
Attenuated control		A	9/25
		B	1/25
Treatment	10^4	A	4/25
		B	4/25
	10^6	A	2/25
		B	3/25
	10^8	A	3/25
		B	3/25

$LC_{50} > 10^8$ cfu/g diet

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Mortalities occurred in both of the control groups (negative and attenuated) and in all 3 of the treatment groups. The mortalities in the negative and attenuated control groups were 26% and 20%, respectively, while those in the 10^4, 10^6, and 10^8 cfu/g diet concentrations averaged 16%, 10% and 12%, respectively. The pattern of mortality was found not to be dose responsive and did not appear to be treatment related. The LC_{50} was determined to be greater than 10^8 cfu/g diet and the no effects concentration was 10^8 cfu/g diet, which was the highest concentration tested.

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

$LC_{50} > 10^8$ cfu/g diet

"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 with the following exception: Samples of test diets were taken for confirmation of test dietary concentrations but were not analyzed." 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 LC_{50} value.

C. Discussion/Results: An $LC_{50} > 10^8$ cfu/g diet indicates that Foil is practically non-toxic to Honey Bee.

D. Adequacy of the Study:

1. Validation Category: Core

2. Rationale: Scientifically sound study

15. Completion of the One-liner:

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Bacillus thuringiensis var. kurstaki

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