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REVIEW NO.

EEB REVIEW

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CASE # : 193777 REREG CASE #:
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SRRD/RD **ACTION** CODE/TYPE OF REVIEW 575

MRID #(S) 419583-01

DP TYPE 001 Submission Related Data Package

PRODUCT MANAGER, NO. Phil Hutton (18)

PRODUCT NAME(S) Bacillus thuringiensis (Berliner)

TYPE PRODUCT F R I N H D biological

COMPANY NAME Ecogen Inc.

SUBMISSION PURPOSE data review

INCLUDE USE(S)

COMMON CHEMICAL NAME Foil OF

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Review of a Dietary Pathogenicity and Toxicity Study with Ladybird Beetles (Hippodamia convergens) for the Bacillus thuringiensis (Bt) Product Foil.

TO: Phil Hutton (PM18)
Registration Division (H7505C)

FROM: D. Urban, Acting Chief
Ecological Effects Branch
Environmental Fate and Effects Division
(H7507C)

Douglas J. Urban 3/9/92

EBB has reviewed a Ladybird Beetle study (154A-23) submitted by Ecogen, Inc. to support the registration of the Bt insecticide Foil. The study was found to be scientifically sound and demonstrated an $LC_{50}>10^8$ cfu/ml. This indicates that Foil is practically nontoxic to Ladybird Beetles. This study fulfills EPA guideline requirements for a nontarget insect pathogenicity /toxicity test.

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DATA EVALUATION REPORT

1. Chemical: Foil - Bacillus thuringiensis (Berliner)

2. Test Material: Technical Powder

3. Study/Action Type: Nontarget Insect-Ladybird Beetles (Hippodamia convergens) (154A-23)

4. Study Identification: Foil[R] Technical Powder: A Dietary Pathogenicity and Toxicity Study with Ladybird Beetles. By Kimberly A. Hoxter, Gregory J. Smith and Steven P. Lynn. Prepared By Wildlife International LTD, May 1991. Project No. 235-126. Submitted By Ecogen, Inc., Langhorne, Pennsylvania. EPA Acc. No. 419583-01.

5. Reviewed By: David C. Bays
Microbiologist
EFED/EEB
Signature: D. C. Bays
Date: 2/21/92

Les W. Touart
Head, Section 1
EFED/EEB
Signature: L W Touart
Date: 3-3-92

6. Conclusions: The study is scientifically sound and demonstrated an $LC_{50} > 10^8$ cfu/ml. This indicates that Foil is practically nontoxic to Ladybird Beetles. 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 the Bacillus thuringiensis (Bt) product Foil.

10. Materials and Methods:

A. Test Organisms: Apparently healthy, Ladybird Beetles (Hippodamia convergens) 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 the registrant and received as a cloudy liquid in the following different concentrations: 10^4, 10^6, and 10^8 cfu/ml diet attenuated (equal to the highest concentration administered to beetles) and a negative control.

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, 10^7, 10^8 cfu/ml of diet) along with the attenuated (equal to highest test concentration used) and negative (12.5% sucrose mixture) controls. Fresh diet was given to the beetles and the average feed consumption for each test concentration and control group was determined on a weekly basis. The beetles were immobilized with nitrogen at the start of the study and when the test diet was introduced. 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 environmental conditions were as follows: the test beetles were given a photoperiod of 8 hours of light per day, kept at a temperature of 19-23C with an average relative humidity of 34%.

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:

Dosage	cfu/ml	Replicate	Number Dead/Number Exposed (At 28 Days After Dosing)
Negative control	0	A	6/25
		B	5/25
Attenuated control	10^9	A	8/25
		B	2/25
Treatment			
	10^4	A	2/25
		B	4/25
	10^6	A	5/25
		B	6/25
	10^8	A	5/25
		B	5/25

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

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 22% and 20%, respectively, while those in the 10^4, 10^6, and 10^8 cfu/ml diet concentrations averaged 12%, 22% and 20%, respectively. The mortality in the treatment groups was found to be less than, or equal to the control mortality and did not appear to be treatment related. No additional signs of toxicity were observed during the test.

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

$LC_{50} > 10^9$ cfu/ml 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, 17 August 1989; OECD, ISBN 92-84-12367-9, Paris 1982; and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August, with the following exception: Samples of the test diets were taken for confirmation of dietary concentrations but were not analyzed." Signed by study director, Steven P. Lynn.

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 $LC_{50} > 10^8$ indicates that Foil is practically non-toxic to Ladybird Beetles.

D. Adequacy of the Study:

1. Validation Category: Core

2. Rationale: Meets EPA Guideline requirements

15. Completion of the One-liner:

chamber only

MEMORANDUM

SUBJECT: EPA Reg. No./File Symbol 55638-10
Foil OF Flowable Bioinsecticide

FROM: William S. Woodrow WSW 3-11-92.
Precautionary Review Section
Registration Support Branch
Registration Division (H75-05C)
E 3/12/92

TO: R. Hutton / Mike Mendelsohn (PM 18)
Insecticide - Rodenticide Branch
Registration Division (H75-05C)

APPLICANT: Ecogen, Inc.
2005 Cabot Blvd. West
Langhorne, PA 19047-1810

FORMULATION FROM LABEL:

Active Ingredient(s):	% by wt.
Bacillus thuringensis kurstaki strain EG 2424	
Lepidopteran active toxin	5.25
Coleopteran active toxin	2.25
Inert Ingredient(s):	92.50
Total	100.0%

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