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DATA EVALUATION RECORD

REVIEWED BY: Anthony Q. Armstrong, and Patricia H. Reno, Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract number DE-AC05-00OR22725

EPA REVIEWER: Robyn Rose, Biopesticides and Pollution Prevention Division (7511C) Robyn Rose

STUDY TYPE: NonTarget Insect Testing, Tier I (OPPTS 885.4340)

MRID NO: 455423-15

TEST MATERIAL: Cry1F protein and Cry1Ac protein

CITATION:
Authors: John R. Porch and Henry O. Krueger
Title: Cry1F (synpro) Delta Endotoxin and Cry1Ac (synpro) Delta Endotoxin: A Dietary Toxicity Study with the Ladybird Beetle
Study Completion Date: October 3, 2001
Laboratory: Wildlife International LTD, 8598 Commerce Dr., Easton, MD 21601
Sponsor: Dow AgroSciences, LLC, Indianapolis, IN 46268
Laboratory Report ID: 379-118
EPA Reg. No: 68467-G

CLASSIFICATION: Acceptable

QUALITY ASSURANCE STATEMENT: Acceptable

GLP COMPLIANCE STATEMENT: Acceptable

STUDY PARAMETERS:
Test Organism: Adult Lady Beetles (*Hippodamia convergens*)
Definitive Study Duration: August 10, 2001 - October 2, 2001

SUBMISSION PURPOSE: To evaluate the potential hazards of *Bacillus thuringiensis* endotoxins Cry1F and Cry1Ac to the nontarget insect lady beetle (*Hippodamia convergens*). OPPTS 885.4340 recommends testing of the microbial agent on three species of nontarget insects.

METHODS: This study was based upon procedures outlined in Series 885 of US EPA's, Office of Prevention, Pesticides and Toxic Substances Microbial Pesticide Test Guidelines (OPPTS Number 885.4340, Nontarget insect testing Tier I, February 1996). The test substances were

Bacillus thuringiensis microbial proteins Cry1F (lot # 1650-85) and full-length Cry1Ac (lot # 1757-66) supplied by the sponsor as powder. According to Dow AgroSciences, the test substance they provided to the testing facility contained 15% Cry1F protein/mg powder and 14% Cry1Ac protein/mg powder.

The test organisms, adult lady beetle (*Hippodamia convergens*), were randomly placed in test chambers. The test chambers were disposable one-pint rolled paper containers approximately 9 cm in diameter and 9 cm high and equipped with a 20-mL glass vial containing deionized water and covered with a ~10 cm disposable petri dish. Each test group received four replicate test chambers that contained twenty-five beetles for a total of 100 test organisms per treatment. A cotton swab containing the appropriate diet was inserted through the side of each chamber. The chambers were maintained in an incubator where temperature averaged 26.8 ± 0.4°C and relative humidity averaged 85.5% ± 2.1% for the test period. The photoperiod during the test was 12 hours of light and was controlled with an automatic timer.

Treatments included a negative control as well as Cry1F and Cry1Ac treatments administered alone and combined for a total of three treatments and a control. The test diets were prepared weekly at concentrations of 300 µg a.i./mL of Cry1F, 22.5 µg a.i./mL of Cry1Ac and a combined dose of 300 µg a.i./mL of Cry1F plus 22.5 µg a.i./mL of Cry1Ac as a mixture with sugar water. Two mg of Cry1F and 0.161 mg of Cry1Ac were added to each mL of sugar water to account for the purity of the proteins. According to Dow AgroSciences, these diet concentrations represent approximately 50x the expression of the endotoxins in young cotton leaf tissue. The negative control consisted of sugar water only. Fresh diets were supplied at least twice weekly and beetles allowed *ad libitum* access to test diets and fresh water. Beetles were observed periodically in order to evaluate mortality and the number of individuals exhibiting clinical signs of toxicity or abnormal behavior. Observations were made within 2 hours after test initiation and continued daily for 15 days at which time mortality in the negative control exceeded 20%.

An estimation of the LC_{50} was made by visual inspection of the mortality data and the NOEC was determined by visually inspecting the mortality and clinical observation data. A PROC GLM was run by SAS Version 8 to compare mortality in the treatment and control groups with a Dunnett's t-test ($\alpha = 0.05$).

REPORTED RESULTS: Data from observations of lady beetles for mortality and other signs of toxicity indicated that mortality in the negative control group reached 21% on day 15 resulting in test termination. After 15 days of exposure, mortality was 29% in the 300 µg a.i./mL of Cry1F and in the 22.5 µg a.i./mL of Cry1Ac treatment groups (Table 1). The combined dose of 300 µg a.i./mL of Cry1F plus 22.5 µg a.i./mL of Cry1Ac exhibited a mortality of 11%. Results of statistical analysis of mean mortality indicated there were no significant differences ($p>0.05$) in mean mortality between the treatment and control groups.

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TABLE 1. Cumulative mortality of lady beetles exposed to Cry1F and Cry1Ac microbial proteins

Treatment Group (µg a.i./mL)	*Day*									
	0	1	2	3	4	5	6	7	8	9
Negative Control	0/100	0/100	3/100	4/100	5/100	7/100	7/100	8/100	13/100	13/100
300 (Cry1F)	0/100	1/100	3/100	3/100	8/100	9/100	9/100	11/100	11/100	11/100
22.5 (Cry1Ac)	0/100	2/100	2/100	8/100	9/100	9/100	10/100	10/100	12/100	13/100
300 + 22.5 (Cry1F + (Cry1Ac)	0/100	0/100	1/100	1/100	2/100	4/100	5/100	8/100	8/100	8/100

Treatment Group (µg a.i./mL)	*Day*						
	10	11	12	13	14	15	% Mortality
Negative Control	15/100	17/100	17/100	17/100	18/100	21/100	21
300 (Cry1F)	14/100	18/100	20/100*	22/100	25/100*	29/100	29
22.5 (Cry1Ac)	19/100	19/100	20/100*	22/100	25/100*	29/100	29
300 + 22.5 (Cry1F + (Cry1Ac)	9/100	9/100	9/100	9/100	11/100	11/100	11

Mortality data are presented as cumulative number of dead beetles/number exposed.
* Beetles exhibited signs of toxicity, i.e., lethargic.
Data taken from Table 1, pp.15-18 of the study report.

STUDY AUTHORS CONCLUSIONS: The dietary LC_{50} for adult lady beetles (*Hippodamia convergens*) exposed to the *Bacillus thuringiensis* Cry1F and Cry1Ac proteins was greater than 300 µg a.i./mL for Cry1F, greater than 22.5 µg a.i./mL for Cry1Ac and greater than the combined dose of 300 µg a.i./mL for Cry1F plus 22.5 µg a.i./mL for Cry1Ac. Likewise, the no-observed-effect-concentrations were 300 µg a.i./mL for Cry1F, 22.5 µg a.i./mL for Cry1Ac and 300 µg a.i./mL for Cry1F plus 22.5 µg a.i./mL for Cry1A.

REVIEWER'S COMMENTS:
This study was performed according to established protocols and guidelines (OPPTS 885.4340) making it acceptable for fulfilling FIFRA Guideline 153A-23. Both summarized and raw data were included in the study report (Table 1, pp.15-18 of MRID No. 455423-15). No deviations were noted in the study report or identified in this data evaluation record.

Lady beetles (*Hippodamia convergens*) were exposed to either a single dietary dose of 300 µg a.i./mL of Cry1F, a single dose of 22.5 µg a.i./mL of Cry1Ac or a combined dose of 300 µg a.i./mL of Cry1F plus 22.5 µg a.i./mL of Cry1Ac as a mixture with sugar water. Four replicates of 25 beetles each were used for treatment and control groups which were observed for mortality and clinical changes until the negative control mortality exceeded 20% on day 15 of the test. Cumulative mortality and signs of toxicity observed in the treatment groups were used to calculate the dietary LC_{50}. The dietary LC_{50} was greater than 300 µg a.i./mL for Cry1F, greater than 22.5 µg a.i./mL for Cry1Ac and greater than the combined dose of 300 µg a.i./mL for Cry1F plus 22.5 µg a.i./mL for Cry1Ac.

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