STUDY TITLE

Thermolability of Cry1F(truncated) Delta-Endotoxin

DATA REQUIREMENTS

None

AUTHOR(S)

R. A. Herman (317) 337-3551
[raherman@dowagro.com]

STUDY COMPLETED ON

November 17, 2000

PERFORMING LABORATORY

Global Environmental Chemistry Laboratory—Indianapolis Lab
Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, Indiana 46268-1054

LABORATORY STUDY ID

GH-C 5144

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: Microbial Cry1F(truncated) Delta-Endotoxin

Title: Thermolability of Cry1F(truncated) Delta-Endotoxin

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d)(1)(A)(B), or (C).*

Company: Dow AgroSciences LLC

Company Agent: [redacted]

Title: Regulatory Manager

Signature: [redacted]

Date: 11/13/00

*In the United States, the above statement supersedes all other statements of confidentiality that may occur elsewhere in this report.

THIS DATA MAY BE CONSIDERED CONFIDENTIAL IN COUNTRIES OUTSIDE THE UNITED STATES.

STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Title: Thermolability of Cry1F(truncated) Delta-Endotoxin

Study Initiation Date: October 9, 2000 Study Completion Date: November 17, 2000
Experimental Start Date: October 19, 2000 Experiment Termination Date: October 25, 2000

This report represents data generated after the effective date of the EPA FIFRA Good Laboratory Practice Standards.

United States Environmental Protection Agency
Title 40 Code of Federal Regulations Part 160
FEDERAL REGISTER, August 17, 1989

Organisation for Economic Co-Operation and Development
ISBN 92-64-12367-9, Paris 1982

This study was not conducted in accordance with GLP and was not monitored by the quality assurance unit.

[redacted] 11/3/00
Sponsor Date
Dow AgroSciences LLC

[redacted] 11/3/00
Submitter Date
Dow AgroSciences LLC

[redacted] 11/17/00
Study Director Date
Dow AgroSciences LLC

QUALITY ASSURANCE STATEMENT

Compound: Microbial Cry1F(truncated) Delta-Endotoxin

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NON-GLP STUDY

SIGNATURE PAGE

[redacted] 11/17/00
Author Date
Dow AgroSciences LLC

[redacted] 11/6/00
Global ECL Group Leader Date
Dow AgroSciences LLC

[redacted] 11/3/00
Peer Reviewer Date
Dow AgroSciences LLC

[redacted] 11/6/00
Peer Reviewer Date
Dow AgroSciences LLC

[redacted]
Date
ECL Molecule Leader
Dow AgroSciences LLC

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Thermolability of Cry1F(truncated) Delta-Endotoxin

ABSTRACT

Maize plants have been modified through the insertion of a gene that produces an insect-active, truncated Cry1F delta-endotoxin. This study was conducted to measure the lability of the Cry1F protein when exposed to heat. After incubating aqueous formulations of the Cry1F protein at various temperatures for 30 minutes, neonate tobacco budworm (TBW), *Heliothis virescens*, were exposed to artificial dietary substrates that had been treated with the Cry1F protein. Insect mortality and weight were measured after 6 days of exposure to the treated diet.

Bioassay results indicate that the Cry1F protein is deactivated after exposure to 75 °C or 90 °C for 30 minutes.

INTRODUCTION

Maize plants have been modified through the insertion of a gene that produces an insect-active, truncated Cry1F delta-endotoxin. This study was conducted to measure the lability of the Cry1F protein when exposed to heat. After incubating aqueous formulations of the Cry1F protein at various temperatures for 30 minutes, neonate tobacco budworm (TBW), *Heliothis virescens*, were exposed to artificial dietary substrates that had been treated with the Cry1F protein. Insect mortality and weight were measured after 6 days of exposure to the treated diet.

LABORATORY PHASE

Test Substances

A powder containing 11.4%, truncated Cry1F delta-endotoxin, isolated from recombinant *Pseudomonas fluorescens*, was used in this study.

Material	Reference Number	% AI (w/w)	Reference
Cry1F Powder	TSN 101788	11.4%	MYCO98-001 (1)

Heat Treatment and Bioassay

The test system for the TBW consisted of treating the surface of solid agar-based diets with an aqueous formulation of the Cry1F powder, allowing the treated diets to dry, and infesting the treated diets with neonate larvae. Mortality and insect weight data were collected after 6 days.

The formulation for the test substance consisted of 10 mM potassium phosphate buffer (pH 7.5). A single formulation of the Cry1F protein was prepared on the test date, and was targeted to produce a bioassay concentration of 100 ng active ingredient (AI)/cm^2 of diet. The formulation

of the test substance was divided into 4 aliquots. Each vial containing one of the aliquots was placed in a water bath of the appropriate temperature (60°, 75° or 90 °C) for the heat treatments, or in a refrigerator for the 4 °C, positive-control treatment. After 30 minutes the vials were removed from the water baths and placed on ice. The negative control treatment consisted of 10 mM potassium phosphate buffer (pH 7.5) only.

Formulations were applied to the surface of artificial insect diet (Multiple species insect diet, Southland Products, Lake Village, AR) in 128-well bioassay trays (C-D International, Pitman, NJ). Approximately 500 µL of diet was in each well with an approximate surface area of 1.5 cm^2. Fifty microliters of the protein formulation from each treatment was applied to each of 16 wells. The surface of the diet was allowed to dry and then each well was infested with a single neonate larva. The wells were covered using vented lids provided with the bioassay trays. The bioassay trays were labeled with the test date, insect, and protocol number. The tray sections were labeled with a treatment number. A grading sheet identifying the treatments accompanied the test setup. Additional test system specifications are listed in Table 2.

Data Analysis

Percent mortality was calculated for each treatment from the number of dead insects and the total number of insects. Percent growth inhibition was calculated based on the reduction in the total weight of live insects in the treatments as compared to the negative control treatment (10 mM potassium phosphate buffer, pH 7.5).

RESULTS AND DISCUSSION

Bioassay Results

Mortality and insect weight data are presented in Table 1. There was no mortality in the negative control treatment. Percent mortality and percent growth inhibition for the Cry1F treatments were as follows:

TBW Results

Treatment	% Mortality	% Growth Inhibition
Cry1F @ 4 °C*	31 %	96 %
Cry1F @ 60 °C	25 %	93 %
Cry1F @ 75 °C	0 %	8 %
Cry1F @ 90 °C	0 %	3 %

* Positive control

CONCLUSIONS

Bioassay results indicate that the Cry1F protein is deactivated after exposure to 75 °C or 90 °C for 30 minutes.

REFERENCES

1. Evans, S. L., 1998. Equivalency of microbial and maize expressed Cry1F protein: characterization of test substances for biochemical and toxicological studies. Project ID MYCO98-001.

Table 1. Results of Tobacco Budworm Bioassay of Cry1F Protein Heat-Treated at 60°, 75° and 90 °C for 30 Minutes

Treatment	Dead	Total	Total Weight (mg)
Cry1F @ 4 °C[a]	5	16	24.9
Cry1F @ 60 °C	4	16	45.8
Cry1F @ 75 °C	0	16	647.2
Cry1F @ 90 °C	0	16	680.0
buffer control[b]	0	16	702.9

[a] Positive control
[b] Negative control
Note: Raw data in DAS notebook E 0670.

Table 2. Insect Test System Specifications

Insect common name: tobacco budworm
Insect species: *Heliothis virescens*
Insect supplier: NCSU Entomology Insectary, Raleigh, NC.
Insect test stage: neonate larvae
Insect pre-test holding condition: Eggs hatched at between 15 °C and 33 °C.
Test holding conditions: Tests held at approximately 26 °C.