CRY 1F *BACILLUS THURINGIENSIS* VAR. *AIZAWAI* DELTA ENDOTOXIN: A DIETARY TOXICITY STUDY WITH GREEN LACEWING LARVAE

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 354-115A

U.S. Environmental Protection Agency
Series 885 Microbial Pesticide Test Guidelines
OPPTS Number 885.4340

AUTHORS:

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STUDY INITIATION DATE: March 16, 1999

STUDY COMPLETION DATE: December 8, 1999

SUBMITTED TO:

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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: Mycogen c/o Dow AgroSciences (Typed Name)

Company Agent: Diane Shanahan (Typed Name)

Title: Registration Manager

Signature: Diane Shanahan Date: 12/10/99

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: Mycogen Corporation

TITLE: Cry 1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin: A Dietary Toxicity Study with Green Lacewing Larvae

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 354-115A

STUDY COMPLETION DATE: December 8, 1999

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Parts 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice, ENV/MC/CHEM (98)17, Paris 1998; and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984, with the following exceptions:

Verification of the test concentrations, stability and homogeneity of the test substance in the diet were not determined.

The stability of the test substance under the conditions of storage at the test site was not conducted in accordance with Good Laboratory Practice Standards.

STUDY DIRECTOR:

_____ DATE 8 Dec 99
John R. Porch
Senior Biologist

SPONSOR:

Mycogen c/o
Dow AgroSciences DATE 12/10/99
Sponsor

Diane Shanahan DATE 12/10/99
Applicant/Submitter

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Parts 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice, ENV/MC/CHEM (98)17, Paris 1998; and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984. The dates of all inspections and audits, and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

		DATE REPORTED TO:	
ACTIVITY	DATE CONDUCTED	STUDY DIRECTOR	MANAGEMENT
Protocol	March 12, 1999	March 12, 1999	March 18, 1999
Initial Trial 354-115			
Test Substance Preparation and Test Initiation	March 22, 1999	March 22, 1999	March 22, 1999
Definitive Trial 354-115A			
Observations	April 8, 1999	April 9, 1999	April 9, 1999
Data and Draft Report	July 6, 1999	July 6, 1999	July 9, 1999
Final Report	December 8, 1999	December 8, 1999	December 8, 1999

DATE 12/8/99

Timothy A. Springer
Manager, Technical and Regulatory Support

REPORT APPROVAL

SPONSOR: Dow AgroSciences LLC/Mycogen Corporation

TITLE: Cry 1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin: A Dietary Toxicity Study with Green Lacewing Larvae

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 354-115A

STUDY DIRECTOR:

DATE 8 Dec 99

John R. Porch
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MANAGEMENT:

DATE 12/8/99

Mark Jaber
Wildlife Toxicologist

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TABLES AND APPENDICES

SUMMARY

SPONSOR:	Dow AgroSciences LLC/Mycogen Corporation
SPONSOR'S REPRESENTATIVE:	Ms. Diane Shanahan
LOCATION OF STUDY, RAW DATA AND A COPY OF FINAL REPORT:	Wildlife International Ltd. 8598 Commerce Drive Easton, Maryland 21601

WILDLIFE INTERNATIONAL LTD. PROJECT NO.:	354-115A
TEST SUBSTANCE:	Cry 1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin
STUDY:	Cry 1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin: A Dietary Toxicity Study with Green Lacewing Larvae
NOMINAL TEST CONCENTRATIONS:	Negative Control and 480 ppm
TEST DATES:	Experimental Start (OECD) - March 22, 1999 Experimental Start (EPA) - April 5, 1999 Experimental Termination - April 19, 1999
LENGTH OF EXPOSURE:	13 Days

TEST ORGANISM:	Green Lacewing (*Chrysoperla carnea*)
SOURCE OF TEST ORGANISMS:	Rincon-Vitova Insectaries, Inc. P.O. Box 1555 Ventura, California 93002
AGE OF TEST ORGANISMS:	Larval Stage

DIETARY LC50:	> 480 ppm a.i.
NO OBSERVED EFFECT CONCENTRATION:	480 ppm a.i.

INTRODUCTION

The study was conducted by Wildlife International Ltd. for Dow AgroSciences LLC/Mycogen Corporation at the Wildlife International Ltd. toxicology facility in Easton, Maryland. The test was repeated once due to high control mortality in the first trial. The definitive test was conducted from April 5, 1999 to April 18, 1999. Raw data generated at Wildlife International Ltd. and a copy of the final report are filed under Project Number 354-115A in the archives located on the Wildlife International Ltd. site.

OBJECTIVE

The objective of the study was to evaluate the dietary toxicity of Cry 1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin, administered to green lacewing larvae (*Chrysoperla carnea*) in the diet.

EXPERIMENTAL DESIGN

Green lacewing larvae were exposed to one limit test concentration of Cry 1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin in a moth egg diet. The test substance concentration represented up to 30X the expression of Cry 1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin present in pollen. A negative control group was maintained concurrently. Thirty individual test chambers were maintained in the treatment and control groups, with one larva in each test chamber. Observations of mortality, pupation and other clinical signs were made once within the first four hours of test initiation, and then continued daily through Day 13 of the test. The test was terminated on Day 13 after mortality exceeded 20% in the negative control. The cumulative mortality percentage observed in the treatment group was used to determine the LC50 value. The no observed effect concentration was determined by examination of the mortality, pupation and clinical observation data.

Selection of the limit test concentration was based upon information supplied by the Sponsor. The nominal test concentration to which the green lacewing larvae were exposed was 480 ppm of Cry 1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin (ppm a.i.).

MATERIALS AND METHODS

The study was conducted based upon the procedures outlined in the protocol, "Bt Cry 1F delta-endotoxin: A Dietary Toxicity Study with Green Lacewing Larvae". The protocol was based upon procedures outlined in Series 885 of The U.S. Environmental Protection Agency's microbial pesticide registration guidelines, OPPTS Number 885.4340 (1).

Test Substance

The test substance was received from Mycogen on March 9, 1999 and was assigned the Wildlife International Ltd. identification number 4807. The test substance was an off white powder, identified as: Cry 1F microbial (truncated); Lot no. 1599-45. The reported purity of the test substance was 11.4% active ingredient. The test substance was stored refrigerated. A summary of the GLP characterization of the test substance is presented in Appendix I.

Test Organism

The green lacewing larva is useful in evaluating the potential hazards of agricultural chemicals and microbiological pest control agents to nontarget insects and is an important predator of a great variety of agricultural pests. Lacewing larvae used in the test were obtained as eggs from Rincon-Vitova Insectaries, Inc., Ventura, California. Upon receipt, the eggs were placed in plastic petri dishes in an incubator, set to maintain a temperature of approximately 26-28°C, to allow the eggs to hatch. Newly hatched larvae in the incubator were fed moth eggs (*Sitotroga* sp.) (Rincon-Vitova Insectaries, Inc., Ventura, California).

Test Chambers

The test chambers were disposable one ounce semitransparent plastic cups (Solo No. P125) with semitransparent plastic lids (Solo No. 601P). Each test chamber housed one lacewing larva. Feed was presented to the larvae in the form of a moth egg and water meal, placed on the bottom of each test chamber. The larvae were allowed *ad libitum* access to the diets throughout the test period. The test chambers were identified by dosage group and replicate, with each group of larvae identified by study number.

Preparation of Diets

The test diet was prepared weekly at a nominal concentration of 480 ppm a.i. (Appendix II). A stock suspension was prepared by suspending a calculated amount of the test substance in deionized water. An aliquot of the stock suspension then was mixed with a calculated amount of moth eggs (*Sitotroga* sp.) to form a moist, grainy meal. The negative control diet consisted of the moth egg meal, without the addition of any test substance.

Diet Administration

Immediately prior to the initiation of the test, the appropriate treated or control diet was placed on the bottom of each test chamber. To initiate the test, the newly hatched lacewing larvae were removed from the petri dishes and impartially placed in the test chambers, so that each chamber housed one larva. Fresh diets were presented to the larvae weekly by carefully transferring each surviving or unpupated larva into a new test chamber containing fresh diet at the appropriate concentration.

Environmental Conditions

During the test, the lacewing larvae were placed in an incubator set to maintain a temperature range of approximately 20 to 22°C, with relative humidity above approximately 40%. Temperature and relative humidity were measured in the incubator twice daily. During the test the temperature in the incubator averaged 21.1 ± 0.1°C, with a range of 20.9 to 21.3°C, while average relative humidity was 86 ± 7%, with a range of 54 to 90%. The photoperiod during the test was 12 hours of light and was controlled with an automatic timer. Overhead fluorescent lighting was used during testing.

Observations

The lacewing larvae were observed periodically in order to evaluate the numbers of mortalities and pupated larvae, and the numbers of individuals exhibiting clinical signs of toxicity or abnormal behavior. Observations were made approximately 2½ hours after test initiation and then continued daily throughout the remainder of the test period. The test was terminated after mortality exceeded 20% in the negative control on Day 13 of the test.

Data Analysis

Because this was a limit test, the LC50 value could not be statistically defined. Therefore, an estimation of the value was made by a visual inspection of the mortality data. The no observed effect concentration was determined by examination of the mortality, pupation and clinical observation data.

RESULTS AND DISCUSSION

Observations and Measurements

The data from observations of the larvae for mortality, pupation and other clinical signs are presented in Table 1. The test terminated on Day 13 after mortality exceeded 20% in the negative control group. At test termination, mortality in the negative control group was 24% (7 of 29) with 14% pupation (4 of 29). One larva was eliminated from the test when it apparently escaped from the test chamber. All surviving control larvae appeared normal during the test.

At test termination, percent mortality in the 480 ppm a.i. treatment group was 30% (9 of 30) with 17% (5 of 30) pupation, respectively. No overt signs of toxicity were noted among the larvae in the treatment group. Mortality in the treatment group was comparable to the negative control, therefore, it was not considered to be treatment-related. The pupation rate in the 480 ppm a.i. treatment group was also comparable to the negative control group.

CONCLUSIONS

Green lacewing larvae (*Chrysoperla carnea*) exposed to a single test concentration of Cry 1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin in the diet showed no mortality and no signs of toxicity over the 13 day test period. The test substance concentration, which represented up to 30X the expression of Cry 1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin present in pollen, was therefore determined to be a no-observed-effect-concentration, and the LC50 value was estimated to be greater than the test concentration of 480 ppm a.i..

REFERENCES

1 U.S. Environmental Protection Agency. 1996. Series 885 Microbial Pesticide Test Guidelines, OPPTS Number 885.4340; Nontarget Insect Testing, Tier 1.

TABLE 1

Cumulative Mortality and Pupation of Green Lacewing Larvae
Exposed to Cry 1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin

Concentration (ppm a.i.)		Day of Test														Percent at Termination
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	
Negative Control	Mortality[1]	0	0	0	0	0	0	0	1	1	1[2]	2	2	4	7	24
	Pupation[1]	0	0	0	0	0	0	0	0	0	0	0	0	1	4	14
480	Mortality	0	0	0	1	1	1	1	2	2	2	3	6	8	9	30
	Pupation	0	0	0	0	0	0	0	0	0	0	0	1	2	5	17

[1] Mortality and pupation data are presented as the cumulative number dead or pupated per number exposed (30).
[2] One larva observed to be missing from test chamber and presumed escaped. Mortality and pupation values subsequently based on 29 larvae rather than 30 in the control group.

APPENDIX I

Test Substance Characterization

SUMMARY

(In accordance with 40 CFR part 152, this summary is available for public release after registration)

STUDY TITLE

Characterization of Expressed Cry1F Protein in Maize Tissues (Pollen, Grain, Grain-Containing Feed, and Purified Maize-Expressed Cry1F Protein) and Microbial Expressed Cry1F Delta Endotoxin by Biological and Biochemical Procedures

DATA REQUIREMENTS

Not Applicable

AUTHORS

D. L. Young, R. A. Herman

STUDY COMPLETED ON

November 18, 1999

PERFORMING LABORATORIES

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Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, Indiana 46268-1054

Pioneer Hi-Bred International
7300 NW 62nd Ave.
Johnston, Iowa 50131

LABORATORY STUDY ID

990027

Characterization of Expressed Cry1F Protein in Maize Tissues (Pollen, Grain, Grain-Containing Feed, and Purified Maize-Expressed Cry1F Protein) and Microbial Expressed Cry1F Delta Endotoxin by Biological and Biochemical Procedures

SUMMARY

This report contains characterization information of maize lines that have been modified to express the Cry1F protein to support regulatory submissions including equivalency and toxicological studies. Maize tissues expressing Cry1F protein (pollen, grain, grain-containing feed and purified maize-expressed Cry1F protein) and microbial expressed Cry1F protein were evaluated and characterized by biological and biochemical analysis. The biological analysis results confirmed the biological activity of the pollen, grain, purified maize-expressed Cry1F protein and bacterially derived Cry1F protein when tested with susceptible insect species, either European corn borer or tobacco budworm. The biochemical analysis was performed to quantify and characterize the extractable Cry1F protein of the pollen, grain, purified maize-expressed Cry1F protein and bacterially derived Cry1F protein. The biochemical analysis of the tissues included ELISA and SDS-PAGE followed by Western Blotting. Biochemical analysis data demonstrated the test materials contained immunoreactive Cry1F protein at the expected molecular weight.

STUDY TITLE

Characterization of Expressed Cry1F Protein in Maize Tissues (Pollen, Grain, Grain-Containing Feed, and Purified Maize-Expressed Cry1F Protein) and Microbial Expressed Cry1F Delta Endotoxin by Biological and Biochemical Procedures

DATA REQUIREMENTS

Not Applicable

AUTHORS

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R. A. Herman

STUDY COMPLETED ON

November 18, 1999

PERFORMING LABORATORY

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Indianapolis, Indiana 46268-1054

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7300 NW 62nd Ave.
Johnston, Iowa 50131

LABORATORY STUDY ID

990027

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: Cry1F Delta Endotoxin Protein

Title: Characterization of Expressed Cry1F Protein in Maize Tissues (Pollen, Grain, Grain-Containing Feed, and Purified Maize-Expressed Cry1F Protein) *and Microbial* Expressed *Cry1F* Delta Endotoxin by Biological and Biochemical Procedures

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: D. M. Shanahan

Title: Regulatory Manager

Signature: [signature]

Date: 11/17/99

*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: Characterization of Expressed Cry1F Protein in Maize Tissues (Pollen, Grain, Grain-Containing Feed, and Purified Maize-Expressed Cry1F Protein) *and Microbial* Expressed *Cry1F* Delta Endotoxin by Biological and Biochemical Procedures

Study Initiation Date: August 4, 1998 Study Completion Date: November 18, 1999

Experimental Start Date: August 4, 1998 Experiment Termination Date: September 24, 1999

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

At Pioneer Hi-Bred, during the first three biological experiments (8/98, 9/98, and 2/99) the laboratory was working towards being GLP compliant; therefore, several GLP-required elements were not yet in place. GLP training and personnel record information was instituted for scientists performing bioassay tests during the course of this study. Protocols and SOPs had been approved, and Quality Assurance conducted in-phase inspections but in some instances SOPs were not present or available during the conduct of the study. On several occasions data were not recorded or corrected exactly as required by GLPs. Maintenance logs were not in place for some equipment used in the study, some reagents were not properly labeled and calibrations were not always performed. The GLP required documentation of the two reference substances used in the biochemical study was not performed (the bacterially derived Cry1F protein and the BioRad BSA protein).

At Dow AgroSciences, management-approved SOPs specific to the insect bioassay were not in place. The GLP required documentation for reference standards were not met.

D. M. Shanahan, Sponsor
Dow AgroSciences LLC — 11/17/99 Date

D. M. Shanahan, Submitter
Dow AgroSciences LLC — 11/17/99 Date

D. L. Young, Study Director/Author
Dow AgroSciences LLC — 11/18/99 Date

Dow AgroSciences Quality Assurance Unit
Good Laboratory Practice Statement Page

Compound: Cry 1F Protein **Study ID:** 990027

Title: Characterization of Expressed Cry1F Protein in Maize Tissues (Pollen, Grain, Grain Containing Feed, and Purified Maize-Expressed Cry1F Protein) and Microbial Expressed Cry1F Delta Endotoxin by Biological and Biochemical Procedures

Study Initiation Date: 8/4/98 **Study Completion Date:** 11/18/99

GLP Quality Assurance Inspections

Date of GLP Inspection(s)	Date Reported to the Study Director and to Management	Phases of the Study which received a GLP Inspection by the Quality Assurance Unit
8/4/98	8/12/98	Elisa, extraction, Bradford assay, Bioassay of pollen (PHI)
2/23/99	3/1/99	Bioassay of microbial tox lot
6/17/99	6/18/99	Bioassays of pollen, microbial protein (PHI)
8/11/99	8/12/99	Bioassay for Amendment 8 – Test/Control substance preparation, dilution, application, test system placement
8/19/99	8/25/99	Sample prep for Elisa assay of corn grain, quail and fish feed
9/22/99	9/23/99	Raw data and draft report (PHI)
9/22-24/99	9/24/99	Raw data and draft report (PHI)
11/1-4/99	11/16/99	Raw data and draft report

QUALITY ASSURANCE STATEMENT:

The Quality Assurance Unit has reviewed the final study report and has determined that the report reflects the raw data generated during the conduct of this study.

D. Keyes — Date: 11/18/99

D. Keyes
Dow AgroSciences, Quality Assurance

SIGNATURE PAGE

D. L. Young — Date: 11/18/99
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Characterization of Expressed Cry1F Protein in Maize Tissues (Pollen, Grain, Grain-Containing Feed, and Purified Maize-Expressed Cry1F Protein) and Microbial Expressed Cry1F Delta Endotoxin by Biological and Biochemical Procedures

ABSTRACT

This report contains characterization information used in support of regulatory submissions for maize lines that have been modified to express the Cry1F protein. The activity of maize tissues expressing Cry1F protein (pollen, grain, grain-containing feed and purified maize-expressed Cry1F protein) and microbial derived Cry1F protein were evaluated and characterized by biological and biochemical analysis.

Biological analysis of the purified maize-expressed Cry1F protein, the bacterially derived Cry1F protein, and maize pollen test substances demonstrates that the Cry1F protein present in all test substances was active against European corn borer (ECB) at all time points tested. Activity of each test substance analyzed is summarized in the following table:

ECB Potency

Test Substance	Activity
1507 – Maize pollen	100% mortality at high dose of 0.2 mg Cry1F/µL buffer diet overlay
5XH751 – Control pollen	No activity
1568-022A – Purified Maize-expressed Protein Control	0-36% Mortality
1568-022B – Purified Maize-expressed Cry1F Protein	LC_{50} = <0.03 µg Cry1F/mL diet
101788 – Microbial Cry1F Powder	LC_{50} = <0.02 µg - 0.06 µg Cry1F/mL diet

The potency of the test substance against tobacco budworm (TBW) was measured by determining the GI_{50} (concentration that inhibits growth by 50%). LC_{50}s (concentration that kills 50% of the insects) were not useful for indexing the potency of the test substances due to insufficient mortality at the highest concentrations tested. Biological analysis of the maize grain and feeds containing maize grain with TBW are summarized in the following tables:

TBW Potency Estimates with Cry1F Maize Grain, Quail Feed, and Fish Feed

Test Substance	GI_{50} (95% confidence limits) in % Cry1F Maize Grain[a]
maize grain expressing Cry1F	0.15 (0.07-0.32)
0-day quail feed containing Cry1F expressing maize	0.15 (0.06-0.41)
5-day quail feed containing Cry1F expressing maize	0.20 (0.05-0.77)
fish feed containing Cry1F expressing maize	>7.7

[a]Expressed as a percent of maize grain expressing Cry1F applied in the treatment suspensions.

TBW Weights with Fish Feed at 7.7% Maize

Test Substance	Insect Weight (mg)
Cry1F fish feed	875.7[a]
control fish feed	1032.3[a]
agar control	1214.9[a]
2:1 acetone:water	1253.7[a]

[a]The means were not significantly different ($\alpha = 0.05$) based on analysis of variance (1).

TBW results demonstrate comparable activity between the maize grain and the maize grain component of the quail feed. No statistically significant difference in activity was observed between fish feed containing Cry1F and the three controls.

Biochemical analysis by ELISA of the purified maize-expressed Cry1F protein, microbial derived Cry1F protein, maize grain, feeds containing maize grain and maize pollen test substances demonstrate that the Cry1F protein was present in all Cry1F expressed test substances. The range of quantitation of extractable Cry1F protein is summarized in the following table:

Test and Control Substances (sample number and identification)	Cry1F Concentration (ng Cry1F/mg)[a]
1507 – Maize pollen	30.7 – 32.8
5XH751 – Control pollen	ND[b]
1568-022A – Purified Maize-expressed Protein Control	ND
1568-022B – Purified Maize-expressed Cry1F Protein	1511.33 ± 268.9
101788 – Microbial Cry1F Powder	114,000
TSN101791 – maize grain containing Cry1F	2.2 - 3.5
TSN101792 – Control maize	ND
TSN101834 – fish feed containing control maize	ND
TSN101835 – fish feed containing Cry1F expressing maize	ND
TSN101862 – quail feed, Day 0 containing Cry1F expressing maize	0.2 - 1.1
TSN101863 – quail feed, Day 0 containing control maize	ND
TSN101864 – quail feed, Day 5 containing Cry1F expressing maize	0.2 - 0.6

[a] ng Cry1F/mg of tissue or powder weighed.
[b] ND = not detectable, below the limit of detection of the ELISA (0.04 ng/mg), 5 mg sample extracted.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western immunoblotting results indicated an expected immunoreactive molecular weight band of ~64kDa as previously reported (2) in both the microbial expressed Cry1F protein and the maize grain expressed Cry1F protein.

APPENDIX II

Diet Preparation

Nominal weights or volumes of constituents used to prepare test diets:

Nominal Concentration (ppm a.i.)	Test Substance Stock		Moth Egg Diet		Final Weight of Diet (g)
	Test Substance Weight (g)	Final Volume in Water (mL)	Volume of Stock (mL)	Weight of Moth Eggs (g)	
Control	---	---	1	2.5000	3.5
480	0.0735	5	1	2.5000	3.5

Test diets were prepared according to the following calculation:

Desired Concentration (ppm or $\mu g/g$) = Test Substance (g) ÷ Final Volume of Stock Suspension (mL) x Volume of Stock Suspension Mixed with Moth Eggs (mL) ÷ Final Weight of Diet (g) x 10^6 $\mu g/g$

To prepare the stock, the test substance was weighed into a tared 5-mL volumetric flask. The flask was brought to volume with deionized water, was covered and inverted to mix. To prepare the diet, one mL of the stock was measured using a 1 cc syringe and was added to 2.5 g moth eggs, stirring with a metal spatula. The control diet was prepared in the same manner, without the addition of test substance to the deionized water. Fresh diets were prepared weekly in the same manner.

APPENDIX III

Changes to Protocol

This study was conducted in accordance with the approved Protocol with the following changes:

1. Redundant information on page 3 of the protocol was removed by amendment.
2. The diet preparation section of the protocol was corrected by amendment to base the calculations on the concentration of the test substance in the diet.
3. The incorrect insect identification on page 7 of the protocol was corrected by amendment.

APPENDIX IV

Personnel Involved in the Study

The following key Wildlife International Ltd. personnel were involved in the conduct or management of this study:

(1) Henry O. Krueger, Ph.D., Director, Aquatic Toxicology and Non-Target Plants
(2) John Porch, Senior Biologist
(3) Kimberly A. Hoxter, Senior Biologist
(4) Mark Jaber, Wildlife Toxicologist

STUDY TITLE

Supplement to MRID 45020109: Cry 1F *Bacillus Thuringiensis* Var. *Aizawai* Delta Endotoxin: A Dietary Toxicity Study with Green Lacewing Larvae

DATA REQUIREMENTS

None

AUTHOR

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STUDY COMPLETED ON

January 16, 2001

SUBMITTED BY

Mycogen Seeds c/o
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LABORATORY STUDY ID

GH-C 5168

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: Cry 1F

Title: Supplement to MRID 45020109: Cry 1F *Bacillus Thuringiensis* Var. *Aizawai* Delta Endotoxin: A Dietary Toxicity Study with Green Lacewing Larvae

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: P. L. Hunst

Title: Regulatory Manager

Signature: Dennis K Hunst

Date: 1/16/01

*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: Supplement to MRID 45020109: Cry 1F *Bacillus Thuringiensis* Var. *Aizawai* Delta Endotoxin: A Dietary Toxicity Study with Green Lacewing Larvae

Study Initiation Date:	1/12/01	Study Completion Date:	1/16/01
Experimental Start Date:	1/12/01	Experiment Termination Date:	1/12/01

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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

No aspect of this study is subject to Good Laboratory Practice Standards.

Penny K Hunst — 1/16/01
P. L. Hunst — Date
Sponsor
Dow AgroSciences LLC

Penny K Hunst — 1/16/01
P. L. Hunst — Date
Submitter
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Study Monitor/Author
Dow AgroSciences LLC

QUALITY ASSURANCE STATEMENT

Compound: Cry 1F

Title: Supplement to MRID 45020109: Cry 1F *Bacillus Thuringiensis* Var. *Aizawai* Delta Endotoxin: A Dietary Toxicity Study with Green Lacewing Larvae

Study Initiation Date: 1/12/01 Study Completion Date: 1/16/01

NON-GLP STUDY

SIGNATURE PAGE

Marie A Mayes 1/16/01

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Date

for DLE 1/16/01

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Date

A reviewer noted that based on current pollen expression data that the Margin of Exposure (MOE) as reported in this study is in error. The original protocol and subsequent report based the MOE on preliminary information that indicated that the expression of Cry1F delta-endotoxin in pollen was 16 μg/g. A definitive study appended to the original report indicated that the expression of Cry1F delta-endotoxin in pollen is 32 μg/g. Therefore, the MOE should be 15 rather than 30 as indicated in the original report. This change should be noted on pages 8 and 11 of the original report.