

STUDY TITLE

Microbial B.t. Cry1F(truncated) Delta-Endotoxin: Maize-Insect-Pest Susceptibility Study

DATA REQUIREMENTS

None

AUTHORS

[REDACTED]

STUDY COMPLETED ON

October 12, 1999

PERFORMING LABORATORY

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

LABORATORY STUDY ID

990029

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: Microbial Cry1F(truncated) Delta-endotoxin

Title: Microbial B.t. Cry1F(truncated) Delta-Endotoxin: Maize-Insect-Pest  
Susceptibility Study

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: 10/5/99

\*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: Microbial B.t. Cry1F(truncated) Delta-Endotoxin: Maize-Insect-Pest Susceptibility Study

Study Initiation Date: April 26, 1999      Study Completion Date: October 12, 1999  
Experimental Start Date: May 12, 1999      Experiment Termination Date: July 26, 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

All aspects of this study were conducted in accordance with the requirements for Good Laboratory Practice Standards, 40 CFR 160, with the following exceptions: Management-approved SOPs specific to the insect bioassays were not in place. Purity, solubility, stability, uniformity and carrier-interference analyses were not conducted on the treatment mixtures applied to the test substrate or for the treated test substrates. The test substrates were not analyzed for unknown contaminants. The purity of the imidacloprid standard was not re-assayed under GLP.

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Date

Dow AgroSciences Quality Assurance Unit  
Good Laboratory Practice Statement Page

Compound: Cry 1F

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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
04/22/99	04/26/99	Protocol review
05/12/99	05/13/99	Spiking, TS weighing, and insect infestation
05/19/99	05/28/99	Grading of sugarcane borer
06/04/99	06/07/99	Preparation of formulation for sucking insect assay (corn leaf aphid)
06/10/99	06/18/99	Insect grading and raw data entries
09/20/99	10/12/99	Report and raw data

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.

[Redacted Signature]

Date


October 12, 1999


Dow AgroSciences, Quality Assurance





SIGNATURE PAGE

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Microbial B.t. Cry1F(truncated) Delta-Endotoxin: Maize-Insect-Pest Susceptibility Study

ABSTRACT

Maize plants have been modified through the insertion of a gene that produces an insect-active Cry1F delta-endotoxin. In order to comply with anticipated regulatory guidelines, a study was conducted to measure the biological activity of the Cry1F(truncated) protein to a range of insects that feed on maize plants. Tests were conducted by exposing insects to artificial dietary substrates that had been treated with aqueous formulations of Cry1F protein produced from a microbial source. The equivalency of microbe-produced and plant-produced Cry1F protein is the subject of another study (1). As part of the aforementioned equivalency study, the sensitivities of four additional insect pests of maize (European corn borer, *Ostrinia nubilalis*, fall armyworm, *Spodoptera frugiperda*, corn earworm, *Helicoverpa zea*, and black cutworm, *Agrotis ipsilon*) were measured. The insect-sensitivity data reported here compliments the data previously reported for these insect pests.

Six species of insect were chosen for inclusion in this study based on their pest status on maize and their taxonomic diversity. Insects from three orders (Lepidoptera, Homoptera and Coleoptera) and four families (Pyralidae, Chrysomelidae, Aphididae and Cicadellidae) are represented. The insects evaluated in this study were lesser cornstalk borer (LCSB) (*Elasmopalpus lignosellus*), sugarcane borer (SCB) (*Diatraea saccharalis*), southwestern corn borer (SWCB) (*Diatraea grandiosella*), western corn rootworm (WCR) (*Diabrotica virgifera virgifera*), corn leaf aphid (CLA) (*Rhopalosiphum maidis*) and corn leafhopper (CLH) (*Dalbulus maidis*).

The test system for the chewing insects (LCSB, SCB, SWCB and WCR) consisted of treating the surface of solid agar-based diets with various concentrations of Cry1F, allowing the treated diets to dry, and infesting the treated diets with neonate larvae. The test system for the sucking insects (CLA and CLH) consisted of mixing the test substance with an aqueous liquid diet at a single concentration of Cry1F, covering aliquots of the diet with a thin membrane, and containing

nymphs (CLA) or adults (CPH) such that they had access to the diet by feeding through the membrane. Mortality data were collected after 7 days for the Lepidopteran insects (LCSB, SCB and SWCB), after 5 days for WCR, and after 3 days for the sucking insects (CLH and CLA).

There was no observed activity of the microbial Cry1F against western corn rootworm, corn leafhopper or corn leaf aphid. Lesser corn stalk borer was the most susceptible insect tested followed by southwestern corn borer and sugarcane borer.

## INTRODUCTION

Maize plants have been modified through the insertion of a gene that produces an insect-active Cry1F delta-endotoxin. In order to comply with anticipated regulatory guidelines, a study was conducted to measure the biological activity of the Cry1F(truncated) protein to a range of insects that feed on maize plants. Tests were conducted by exposing insects to artificial dietary substrates that had been treated with aqueous formulations of Cry1F protein produced from a microbial source. The equivalency of microbe-produced and plant-produced Cry1F protein is the subject of another study (1). As part of the aforementioned equivalency study, the sensitivities of four additional insect pests of maize (European corn borer, *Ostrinia nubilalis*, fall armyworm, *Spodoptera frugiperda*, corn earworm, *Helicoverpa zea*, and black cutworm, *Agrotis ipsilon*) were measured. The insect-sensitivity data reported here compliments the data previously reported for these insect pests.

## LABORATORY PHASE

### Test Substance and Standards

Microbially-produced Cry1F(truncated) powder containing 11.4% Cry1F delta-endotoxin was used in this study. Analyses of the test substance by enzyme-linked immuno sorbant assays (ELISA) before and after the bioassays indicated that the test substance was stable over the course of the study. The word "(truncated)" following the "Cry1F" designation above indicates that this is a truncated protein as opposed to a full-length protein. Spinosad or imidacloprid were used as positive standards in each bioassay.

Material	TSN Number	% AI (w/w)	Reference
Cry1F Powder	TSN 101788	11.4%	MYCO98-001 (1)
spinosad	TSN 101400	89.9%	FA&PC 973045 (2)
imidacloprid	TSN 101766	99%	Chem Service (3)

### Bioassays

Six species of insect were chosen for inclusion in this study based on their pest status on maize and their taxonomic diversity. Insects from three orders (Lepidoptera, Homoptera and Coleoptera) and four families (Pyralidae, Chrysomelidae, Aphididae and Cicadellidae) are represented. The insects evaluated in this study were lesser cornstalk borer (LCSB) (*Elasmopalpus lignosellus*), sugarcane borer (SCB) (*Diatraea saccharalis*), southwestern corn borer (SWCB) (*Diatraea grandiosella*), western corn rootworm (WCR) (*Diabrotica virgifera virgifera*), corn leaf aphid (CLA) (*Rhopalosiphum maidis*) and corn leafhopper (CLH) (*Dalbulus maidis*).

The test system for the chewing insects (LCSB, SCB, SWCB and WCR) consisted of treating the surface of solid agar-based diets with various concentrations of Cry1F, allowing the treated diets to dry, and infesting the treated diets with neonate larvae. The test system for the sucking insects (CLA and CLH) consisted of mixing the test substance with an aqueous liquid diet at a single concentration of Cry1F, covering aliquots of the diet with a thin membrane, and containing nymphs (CLA) or adults (CPH) such that they had access to the diet by feeding through the membrane. Mortality data were collected after 7 days for the Lepidopteran insects (LCSB, SCB and SWCB), after 5 days for WCR, and after 3 days for the sucking insects (CLH and CLA) (Tables 1-6).

The formulation for the test material consisted of 10 mM potassium phosphate buffer (pH 7.5) for the chewing-insect assays. Thirteen percent sucrose was added to this formulation for the sucking-insect assays. A small amount (~10 µg/mL) of yellow dye (tartrazine CAS# 1934-21-0) was also added to the sucking-insect formulation to encourage feeding. Formulations of the test substance were prepared fresh on each bioassay date. The concentrations tested in each test are listed in Tables 1-6.

Spinosad at a concentration of 0.267 µg ai/cm<sup>2</sup> was included as a positive control for the chewing-insect bioassays, and imidacloprid at a concentration of 10 µg ai/mL was included as a

positive control for the sucking-insect assays. Stock solutions of spinosad and imidacloprid were initially formulated at 1000 µg/mL in acetone. In order to obtain the 10-µg/mL solution, an aliquot of the acetone stock solution was transferred to a glass vial and the acetone was allowed to evaporate. The residue of imidicloprid was reconstituted in the aforementioned sucking-insect formulation and the spinosad residue was reconstituted in 2:1 acetone:water. Appropriate formulation blanks were included in each bioassay (Tables 1-6).

For the lepidopteran insects (LCSB, SCB and SWCB), 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<sup>2</sup>. Forty microliters of each treatment was applied to each of 16 wells on each bioassay date. The surface of the diet was allowed to dry before infesting each well with a single neonate larva. The wells were covered using vented lids provided with the bioassay trays. The same procedure was used for the WCR except that a proprietary diet was used. For the sucking insects, the formulations were pipetted into the concave underside of a vial or microcentrifuge tube lid and the liquid was sealed in with a piece of stretched parafilm. Five CLA or three CLH were placed in each test arena. Five test arenas were prepared for each treatment on each test date. Two bioassays were conducted for each insect species except for LCSB and SCB where three bioassays were conducted. The bioassay trays, and racks for vials and tubes, were labeled with the test date, insect, and protocol number. The tray sections, and individual vials and tubes were labeled with a treatment number. A grading sheet identifying the treatments accompanied each test setup. Additional test system specifications are listed in Table 7.

#### Statistical Analysis

All data collected from treatments that included the test substance were used in calculating potency estimates for the microbial Cry1F delta-endotoxin. The concentrations of the test substance estimated to kill 50% of the insects (LC<sub>50</sub>s) along with 95% fiducial limits were

calculated for sensitive species using Probit Analysis (4, 5). The mortality of insensitive species, at the highest concentration tested, was compared to the negative controls using Fisher's Exact Test (5).

## RESULTS AND DISCUSSION

### Bioassay Results

The positive controls, spinosad and imidacloprid, controlled greater than 93% of the insects in each bioassay with the exception of the western corn rootworm tests where spinosad controlled 62% to 81% of the insects (Tables 1-6). Thus, results with the positive standards indicate that the test insects were exposed to the treatments. Bioassay results from tests conducted with Cry1F are in Tables 1-6. Estimated LC<sub>50</sub>s generated from insect data collected in this study for the microbial Cry1F delta-endotoxin, are as follows:

#### Potency of Microbial Cry1F Protein Against Several Insect Pests of Maize

Insect	LC <sub>50</sub> (95% Fiducial Limits)
	( $\mu\text{g ai/cm}^2$ )
Lesser cornstalk borer (LCSB)	0.108 (0.019-0.294)
Southwestern corn borer (SWCB)	0.701 (0.489-1.000)
sugarcane borer (SCB)	1.457 (1.068-2.019)
Western corn rootworm (WCR)	>53.8 <sup>a</sup>
	( $\mu\text{g ai/mL}$ )
corn leaf aphid (CLA)	>70.0 <sup>b</sup>
corn leafhopper (CLH)	>70.0 <sup>c</sup>

<sup>a</sup> No mortality at 53.8  $\mu\text{g ai/cm}^2$ .

<sup>b</sup> Two percent additional mortality occurred at 70.0  $\mu\text{g ai/mL}$  compared to the negative control (18% vs. 16% kill).

<sup>c</sup> Less mortality than negative control at 70  $\mu\text{g ai/mL}$ .



There was no statistical difference between the mortality observed for WCR, CLA and CLH at the highest concentration tested, and the mortality observed for the negative controls based on Fisher's Exact Test (95% confidence level). Lesser corn stalk borer is statistically more susceptible than southwestern corn borer, which in turn is statistically more susceptible than sugarcane borer (based on non-overlap of the 95% fiducial limits for the  $LC_{50}$ s).

The relative susceptibilities of the insects to the Cry1F delta-endotoxin are as follows:

LCSB > SWCB > SCB >> WCR, CLA, CLH

#### Conduct of Study

Several factors related to the conduct of the study are worthy of discussion. Management-approved SOPs specifically related to the bioassays were not in place as this was the first study of this type conducted at this facility. A management-approved SOP covering general aspects of insect toxicity assays was available by the end of the study. Furthermore, since SOPs were not established at the initiation of the study, experimental detail normally placed in an SOP was included in the study protocol, amendments and deviations, and is further supported by documentation in the study file. Therefore, the lack of SOPs specifically related to bioassays did not negatively impact the study.

Purity, solubility, stability, uniformity and carrier-interference analyses were not conducted on the treatment mixtures applied to the test substrate or for the treated test substrates. The stability of the Cry1F powder used in this study was monitored. A fresh sample of the test substance was weighed out on each bioassay date and mixed with the simplest appropriate carriers to minimize any possible interferences or stability concerns. The conclusions of the study reflect comparisons made among the activities seen against the test insects and therefore any systematic interference or lack of stability should not affect the integrity of the conclusions drawn from this study. Any lack of uniformity in the formulations is captured as part of the error term in the experimental model and is reflected in the fiducial limits around the  $LC_{50}$ s. Appropriate positive

and negative controls were also included in every bioassay. Therefore, the aforementioned factors did not negatively impact the study.

Imidacloprid was included in the sucking-insect assays as a qualitative, positive control. The purity of the imidacloprid was determined to be 99% pure by Chem Services in Winchester, Pennsylvania. The GLP status of this analysis is not known. The purity was not re-assayed under GLP. Since the potency of this compound in the sucking-insect bioassays was used to help establish the suitability of each test for exposing the insects to the treatments, the observation of activity in each test confirmed sufficient purity to achieve this end.

#### CONCLUSIONS

The study demonstrated the relative sensitivity of six maize-feeding insects to the microbial Cry1F delta-endotoxin. There was no observed activity of the microbial Cry1F against western corn rootworm, corn leafhopper or corn leaf aphid. Lesser corn stalk borer was the most susceptible insect tested followed by southwestern corn borer and sugarcane borer.

#### ARCHIVING

Raw data and the original copy of the final report will be filed in the Dow AgroSciences' testing facility archives, Indianapolis, Indiana.

## 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.
2. Hamilton, T. D., 1997, FA&PC 973045, Dow AgroSciences internal report (not published).
3. Chem Service, 1999, Sigma Certificate of Analysis.
4. Finney, D. L., 1971, Probit Analysis, 3<sup>rd</sup> ed., Cambridge University Press, London.
5. SAS Institute Inc. 1990, SAS/STAT User's Guide, Version 6, Fourth Edition, SAS Institute Inc., Cary, NC.

Table 1. Bioassay Results: Sugarcane Borer

Test Date	Insect	Treatment	Concentration	Dead	Total
			ng ai/cm2		
05/12/1999	sugarcane borer	Cry1F	3,040.00	9	16
05/12/1999	sugarcane borer	Cry1F	1,013.33	5	16
05/12/1999	sugarcane borer	Cry1F	337.78	0	16
05/12/1999	sugarcane borer	Cry1F	112.59	0	16
05/12/1999	sugarcane borer	Cry1F	37.53	0	16
05/12/1999	sugarcane borer	Cry1F	12.51	0	15
05/12/1999	sugarcane borer	Cry1F	4.17	0	16
05/12/1999	sugarcane borer	Cry1F	1.39	0	16
05/12/1999	sugarcane borer	spinosad	266.67	16	16
05/12/1999	sugarcane borer	phosphate buffer	NA	0	16
05/12/1999	sugarcane borer	2:1 acetone:water	NA	0	16
05/19/1999	sugarcane borer	Cry1F	53,808.00	15	16
05/19/1999	sugarcane borer	Cry1F	17,936.00	15	16
05/19/1999	sugarcane borer	Cry1F	5,978.67	13	16
05/19/1999	sugarcane borer	Cry1F	1,992.89	13	16
05/19/1999	sugarcane borer	Cry1F	664.30	7	16
05/19/1999	sugarcane borer	Cry1F	221.43	2	16
05/19/1999	sugarcane borer	Cry1F	73.81	0	16
05/19/1999	sugarcane borer	Cry1F	24.60	0	16
05/19/1999	sugarcane borer	spinosad	266.67	16	16
05/19/1999	sugarcane borer	phosphate buffer	NA	0	16
05/19/1999	sugarcane borer	2:1 acetone:water	NA	1	15
05/27/1999	sugarcane borer	Cry1F	53,808.00	16	16
05/27/1999	sugarcane borer	Cry1F	17,936.00	14	16
05/27/1999	sugarcane borer	Cry1F	5,978.67	12	16
05/27/1999	sugarcane borer	Cry1F	1,992.89	9	16
05/27/1999	sugarcane borer	Cry1F	664.30	8	16
05/27/1999	sugarcane borer	Cry1F	221.43	4	16
05/27/1999	sugarcane borer	Cry1F	73.81	1	16
05/27/1999	sugarcane borer	Cry1F	24.60	1	16
05/27/1999	sugarcane borer	spinosad	266.67	15	16
05/27/1999	sugarcane borer	phosphate buffer	NA	0	16
05/27/1999	sugarcane borer	2:1 acetone:water	NA	0	16

Table 2. Bioassay Results: Southwestern Corn Borer

Test Date	Insect	Treatment	Concentration	Dead	Total
			ng ai/cm2		
05/17/1999	southwestern corn borer	Cry1F	53,808.00	16	16
05/17/1999	southwestern corn borer	Cry1F	17,936.00	16	16
05/17/1999	southwestern corn borer	Cry1F	5,978.67	16	16
05/17/1999	southwestern corn borer	Cry1F	1,992.89	10	16
05/17/1999	southwestern corn borer	Cry1F	664.30	3	16
05/17/1999	southwestern corn borer	Cry1F	221.43	1	16
05/17/1999	southwestern corn borer	Cry1F	73.81	1	16
05/17/1999	southwestern corn borer	Cry1F	24.60	0	16
05/17/1999	southwestern corn borer	spinosad	266.67	16	16
05/17/1999	southwestern corn borer	phosphate buffer	NA	0	16
05/17/1999	southwestern corn borer	2:1 acetone:water	NA	0	16
05/26/1999	southwestern corn borer	Cry1F	53,808.00	16	16
05/26/1999	southwestern corn borer	Cry1F	17,936.00	14	16
05/26/1999	southwestern corn borer	Cry1F	5,978.67	15	16
05/26/1999	southwestern corn borer	Cry1F	1,992.89	15	16
05/26/1999	southwestern corn borer	Cry1F	664.30	8	16
05/26/1999	southwestern corn borer	Cry1F	221.43	8	15
05/26/1999	southwestern corn borer	Cry1F	73.81	3	16
05/26/1999	southwestern corn borer	Cry1F	24.60	0	16
05/26/1999	southwestern corn borer	spinosad	266.67	16	16
05/26/1999	southwestern corn borer	phosphate buffer	NA	0	16
05/26/1999	southwestern corn borer	2:1 acetone:water	NA	0	15

Table 3. Bioassay Results: Western Corn Rootworm

Test Date	Insect	Treatment	Concentration	Dead	Total
			ng ai/cm2		
06/09/1999	western corn rootworm	Cry1F	53,808.00	0	16
06/09/1999	western corn rootworm	spinosad	266.67	13	16
06/09/1999	western corn rootworm	phosphate buffer	NA	0	16
06/09/1999	western corn rootworm	2:1 acetone:water	NA	2	16
06/16/1999	western corn rootworm	Cry1F	53,808.00	0	16
06/16/1999	western corn rootworm	spinosad	266.67	10	16
06/16/1999	western corn rootworm	phosphate buffer	NA	0	16
06/16/1999	western corn rootworm	2:1 acetone:water	NA	0	16

Table 4 Bioassay Results: Lesser Cornstalk Borer

Test Date	Insect	Treatment	Concentration	Dead	Total
			ng ai/cm2		
05/21/1999	lesser cornstalk borer	Cry1F	53,808.00	15	16
05/21/1999	lesser cornstalk borer	Cry1F	17,936.00	15	15
05/21/1999	lesser cornstalk borer	Cry1F	5,978.67	16	16
05/21/1999	lesser cornstalk borer	Cry1F	1,992.89	12	15
05/21/1999	lesser cornstalk borer	Cry1F	664.30	11	16
05/21/1999	lesser cornstalk borer	Cry1F	221.43	9	16
05/21/1999	lesser cornstalk borer	Cry1F	73.81	4	16
05/21/1999	lesser cornstalk borer	Cry1F	24.60	0	16
05/21/1999	lesser cornstalk borer	spinosad	266.67	16	16
05/21/1999	lesser cornstalk borer	phosphate buffer	NA	0	15
05/21/1999	lesser cornstalk borer	2:1 acetone:water	NA	1	16
06/16/1999	lesser cornstalk borer	Cry1F	53,808.00	16	16
06/16/1999	lesser cornstalk borer	Cry1F	17,936.00	16	16
06/16/1999	lesser cornstalk borer	Cry1F	5,978.67	15	16
06/16/1999	lesser cornstalk borer	Cry1F	1,992.89	14	16
06/16/1999	lesser cornstalk borer	Cry1F	664.30	15	16
06/16/1999	lesser cornstalk borer	Cry1F	221.43	15	16
06/16/1999	lesser cornstalk borer	Cry1F	73.81	11	16
06/16/1999	lesser cornstalk borer	Cry1F	24.60	3	16
06/16/1999	lesser cornstalk borer	spinosad	266.67	15	16
06/16/1999	lesser cornstalk borer	phosphate buffer	NA	2	16
06/16/1999	lesser cornstalk borer	2:1 acetone:water	NA	2	16
07/14/1999	lesser cornstalk borer	Cry1F	53,808.00	14	16
07/14/1999	lesser cornstalk borer	Cry1F	17,936.00	15	16
07/14/1999	lesser cornstalk borer	Cry1F	5,978.67	13	16
07/14/1999	lesser cornstalk borer	Cry1F	1,992.89	16	16
07/14/1999	lesser cornstalk borer	Cry1F	664.30	10	16
07/14/1999	lesser cornstalk borer	Cry1F	221.43	12	16
07/14/1999	lesser cornstalk borer	Cry1F	73.81	8	16
07/14/1999	lesser cornstalk borer	Cry1F	24.60	4	16
07/14/1999	lesser cornstalk borer	spinosad	266.67	15	16
07/14/1999	lesser cornstalk borer	phosphate buffer	NA	3	16
07/14/1999	lesser cornstalk borer	2:1 acetone:water	NA	1	16



Table 5. Bioassay Results: Corn Leaf Aphid

Test Date	Insect	Treatment	Concentration	Dead	Total
			ng ai/mL		
06/04/1999	corn leaf aphid	Cry1F	69,996.00	2	5
06/04/1999	corn leaf aphid	Cry1F	69,996.00	1	5
06/04/1999	corn leaf aphid	Cry1F	69,996.00	0	5
06/04/1999	corn leaf aphid	Cry1F	69,996.00	1	5
06/04/1999	corn leaf aphid	Cry1F	69,996.00	0	5
06/04/1999	corn leaf aphid	imidacloprid	10,000.00	4	5
06/04/1999	corn leaf aphid	imidacloprid	10,000.00	5	5
06/04/1999	corn leaf aphid	imidacloprid	10,000.00	5	5
06/04/1999	corn leaf aphid	imidacloprid	10,000.00	5	5
06/04/1999	corn leaf aphid	imidacloprid	10,000.00	5	5
06/04/1999	corn leaf aphid	diet blank	NA	0	5
06/04/1999	corn leaf aphid	diet blank	NA	0	5
06/04/1999	corn leaf aphid	diet blank	NA	3	5
06/04/1999	corn leaf aphid	diet blank	NA	0	5
06/04/1999	corn leaf aphid	diet blank	NA	1	5
06/07/1999	corn leaf aphid	Cry1F	69,996.00	2	5
06/07/1999	corn leaf aphid	Cry1F	69,996.00	0	5
06/07/1999	corn leaf aphid	Cry1F	69,996.00	2	5
06/07/1999	corn leaf aphid	Cry1F	69,996.00	1	5
06/07/1999	corn leaf aphid	Cry1F	69,996.00	0	5
06/07/1999	corn leaf aphid	imidacloprid	10,000.00	5	5
06/07/1999	corn leaf aphid	imidacloprid	10,000.00	5	5
06/07/1999	corn leaf aphid	imidacloprid	10,000.00	5	5
06/07/1999	corn leaf aphid	imidacloprid	10,000.00	5	5
06/07/1999	corn leaf aphid	imidacloprid	10,000.00	4	5
06/07/1999	corn leaf aphid	diet blank	NA	1	5
06/07/1999	corn leaf aphid	diet blank	NA	1	5
06/07/1999	corn leaf aphid	diet blank	NA	0	5
06/07/1999	corn leaf aphid	diet blank	NA	2	5
06/07/1999	corn leaf aphid	diet blank	NA	0	5



Table 6. Bioassay Results: Corn Leafhopper

Test Date	Insect	Treatment	Concentration	Dead	Total
			ng ai/mL		
07/19/1999	corn leafhopper	Cry1F	69,996.00	0	3
07/19/1999	corn leafhopper	Cry1F	69,996.00	0	3
07/19/1999	corn leafhopper	Cry1F	69,996.00	0	3
07/19/1999	corn leafhopper	Cry1F	69,996.00	0	3
07/19/1999	corn leafhopper	Cry1F	69,996.00	0	3
07/19/1999	corn leafhopper	imidacloprid	10,000.00	3	3
07/19/1999	corn leafhopper	imidacloprid	10,000.00	3	3
07/19/1999	corn leafhopper	imidacloprid	10,000.00	3	3
07/19/1999	corn leafhopper	imidacloprid	10,000.00	3	3
07/19/1999	corn leafhopper	imidacloprid	10,000.00	3	3
07/19/1999	corn leafhopper	diet blank	NA	0	3
07/19/1999	corn leafhopper	diet blank	NA	1	3
07/19/1999	corn leafhopper	diet blank	NA	0	3
07/19/1999	corn leafhopper	diet blank	NA	0	3
07/19/1999	corn leafhopper	diet blank	NA	1	3
07/23/1999	corn leafhopper	Cry1F	69,996.00	1	3
07/23/1999	corn leafhopper	Cry1F	69,996.00	0	3
07/23/1999	corn leafhopper	Cry1F	69,996.00	0	3
07/23/1999	corn leafhopper	Cry1F	69,996.00	0	2
07/23/1999	corn leafhopper	Cry1F	69,996.00	0	3
07/23/1999	corn leafhopper	imidacloprid	10,000.00	3	3
07/23/1999	corn leafhopper	imidacloprid	10,000.00	3	3
07/23/1999	corn leafhopper	imidacloprid	10,000.00	3	3
07/23/1999	corn leafhopper	imidacloprid	10,000.00	3	3
07/23/1999	corn leafhopper	imidacloprid	10,000.00	3	3
07/23/1999	corn leafhopper	diet blank	NA	0	3
07/23/1999	corn leafhopper	diet blank	NA	0	3
07/23/1999	corn leafhopper	diet blank	NA	1	3
07/23/1999	corn leafhopper	diet blank	NA	0	3
07/23/1999	corn leafhopper	diet blank	NA	2	2

Table 7. Test Systems

Insect common name: southwestern corn borer  
Insect species: *Diatraea grandiosella*  
Insect supplier: Frank Davis, Crop Science Research Laboratory, PO Box 5367 Mississippi State, MS 39762  
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 27 °C and 40% RH.

Insect common name: lesser cornstalk borer  
Insect species: *Elasmopalpus lignosellus*  
Insect supplier: Bob Lynch, USDA-ARS-IBPMRL, PO Box 748 Tipton, GA 31793 0748  
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 27 °C and 40% RH.

Insect common name: sugarcane borer  
Insect species: *Diatraea saccharalis*  
Insect supplier: Bill White, USDA-ARS Sugarcane Research Unit, 5883 USDA Rd. Houma, LA 70360  
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 27 °C and 40% RH.

Insect common name: western corn rootworm  
Insect species: *Diabrotica virgifera virgifera*  
Insect supplier: Lee French, French Agricultural Research Inc., RR 2 Box 294, Lamberton, MN 56152  
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 27 °C and 40% RH.

Insect common name: corn leafhopper  
Insect species: *Dalbulus maidis*  
Insect supplier: William Styer, 1680 Madison Ave., Wooster, OH 44691  
Insect test stage: adults  
Insect pre-test holding condition: Insects will be reared at ambient room-temperature on maize plants.  
Test holding conditions: Tests held at approximately 25 °C and 60% RH.

Insect common name: corn leaf aphid  
Insect species: *Rhopalosiphum maidis*  
Insect supplier: Kevin A. Shufron, USDA-ARS, 1301 N. Western Rd. Stillwater, OK 74075  
Insect test stage: mixed nymphs  
Insect pre-test holding condition: Insects reared at ambient room-temperature on barley plants.  
Test holding conditions: Tests held at approximately 25 °C and 60% RH.