FINAL REPORT

REPORT NO.: MSL-18711

JOB/PROJECT NO.: SB-2003-002

DATE: September 26, 2003

TITLE: An Acute Oral Toxicity Study in Mice with *E. coli*-Produced Cry3Bb1.pvzmir39 Protein

AUTHORS: [redacted]

STUDY DIRECTOR: [redacted]

ABSTRACT: This study was conducted to assess the acute oral toxicity of *E. coli*-produced Cry3Bb1.pvzmir39 protein (lot 30-100002) in CD1 mice. Two groups (test protein and control protein) of ten animals per sex were used in this study. The test and control (bovine serum albumin) proteins were formulated in a sodium carbonate-bicarbonate buffer. One group of ten male and ten female mice were given an acute oral target dose of 2100 mg of test protein per kg body weight. The target dose of the test protein was based on the maximum attainable protein concentration of the dosing solution (estimated at 37 mg/mL) and a total dose volume of 66.6 mL/kg body weight. The solubility of the test protein precluded administration as a single dose and, therefore, two doses of 33.3 mL/kg body weight (66.6 mL/kg total) were given to achieve the target dose. On the day of dosing (Day 0) the two individual doses of 33.3 mg/mL body weight were separated by approximately 4 hours. A separate group of ten male and ten female protein control animals received bovine serum albumin (BSA) at a target dose of 2500 mg/kg.

A 14-day observation period followed dosing. During this period the mice were observed daily, weighed weekly, and had food consumption measured weekly. A gross necropsy examination was performed on all animals at termination (Day 14).

The analysis of the dosing suspensions/solutions indicated that the formulated doses were stable, of the appropriate concentration, homogeneous, and biologically active. The experimentally confirmed test protein dose was 1930 mg/kg body weight compared to the target dose of 2100 mg/kg weight. The experimentally confirmed control protein dose was 1900 mg/kg body weight compared to the target dose of 2500 mg/kg body weight.

ABSTRACT (CONTINUED):

No mortality occurred and there were no significant clinical abnormalities observed during the routine examinations. Body weight was increased ($p<0.05$) on Day 7 for the test protein males compared to the vehicle control and on Day 14 for the test protein females compared to the vehicle control. Additionally, body weight change for the Day 0 to Day 7 interval was significantly increased ($p<0.01$) for both test protein males and females compared to their respective protein control groups. No significant differences were observed in food consumption during the study. No gross pathological findings considered to be test article related were observed at necropsy at study termination.

Under the conditions of this test, no toxicity was observed in any of the groups. Therefore, the acute oral LD50 of *E. coli*-produced Cry3Bb1.pvzmir39 protein in the mouse is greater than 1930 mg/kg body weight.

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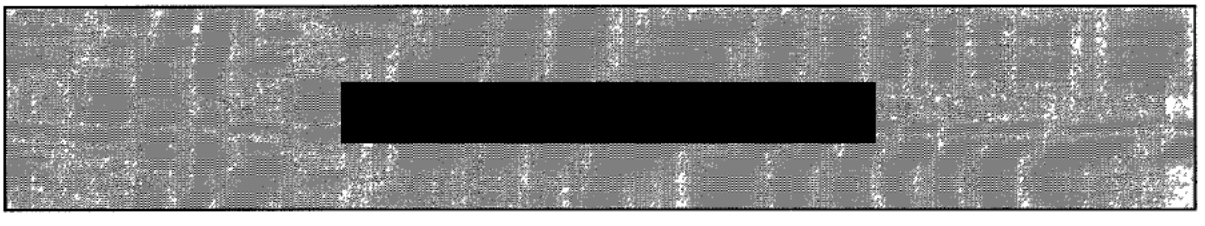

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Report Amendment No. 1

AN ACUTE ORAL TOXICITY STUDY IN MICE WITH *E. coli*-PRODUCED CRY3Bb1.pvzmir39 PROTEIN

AMENDED FINAL REPORT

Guidelines

EPA (OPPTS 870.1100), OECD (401), EEC (B.1)

Author

[redacted]

Original Study Completion Date

September 10, 2003

Amended Study Completion Date

September 26, 2003

Performing Laboratory

Springborn Laboratories (SLI),
a division of Charles River Laboratories, Inc.
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3044.931

Monsanto Study No.

SB-2003-002

Submitted to

Monsanto Company
800 N. Lindbergh Blvd.
St. Louis, MO 63167

CHARLES RIVER
LABORATORIES

1. 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 §10(d)(1)(A), (B), or (C).

"We submit this material to the United States Environmental Protection Agency specifically under requirements set forth in FIFRA as amended, and consent to the use and disclosure of the material by EPA strictly in accordance with FIFRA. By submitting this material to EPA in accordance with the method and format requirements contained in PR Notice 86-5, we reserve and do not waive any rights involving this material that are or can be claimed by the company notwithstanding this submission to EPA.

COMPANY: Monsanto Company

COMPANY AGENT: _____

_____ _____
SIGNATURE DATE

_____ _____
TITLE DATE

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Part 160) with the following exception:

The BSA protein control article was not characterized according to the EPA GLP regulations, however, a certificate of analysis from the manufacturer was available and is archived with the analytical sub-report data.

[redacted] Date 9/26/03

Study Director/Author
Springborn Laboratories

[redacted] Date 9/26/03

Monsanto Company

_____ Date _____

Submitter
Monsanto Company

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	01/28/03
Animal Receipt	02/11/03
Dosing	02/19/03
Protocol Amendment Review	03/20/03, 09/03/03
Data Audit	03/20/03
Draft Report Review	03/20/03
Revised Draft Report Review	05/19/03
Second Revised Draft Report Review	07/07/03
Third Revised Draft Report Review	07/23/03
Final Report Review	09/10/03
Amended Final Report Review	09/26/03
Reports to Study Director and Management	02/11/03, 03/20/03, 05/19/03, 07/07/03, 07/23/03, 09/10/03, 09/26/03

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

[redacted] Date 9/26/03

Quality Assurance Auditor

[redacted] Date 9/26/03

Senior Director, Compliance Assurance

The following revised pages have been incorporated into this report.

Page No.	Revision	Reason for Change
4	Protocol Amendment Review date of 09/03/03 added, date corrected for Third Revised Draft Review (07/23/03).	To provide correct dates
8, 9	Replace the test article identification on this page with *E. coli*-produced Cry3Bb1.pvzmir39 protein.	To correct a typographical error
37, 41, 44, 53-56, 57	Replace with correct pages from the final version of the Analytical Sub-Report.	To include appropriate pages from the final Analytical Sub-Report

[redacted] Date 9/26/03

Study Director

4. TABLE OF CONTENTS

5. LIST OF TABLES AND APPENDICES

Tables

Appendices

6. SUMMARY

The oral toxicity of *E. coli*-produced Cry3Bb1.pvzmir39 protein was evaluated in CD-1 mice. Two groups of animals received either the test article or the protein control article as indicated below:

Group No.	Treatment	Target Dose Level [a] (mg/kg)	Analytically-Confirmed Dose Level[c] (mg/kg)	No. of Animals	
				Male	Female
1	Protein Control (BSA)	2500[b]	1900	10	10
2	*E. coli*-produced Cry3Bb1.pvzmir39 protein	2100	1930	10	10

[a] The target dose level of the test protein was adjusted from 3000 to 2100 mg/kg based on the maximum attainable protein concentration of the dosing solutions (estimated at 37 mg/mL) and a total dose volume of 66.6 mL/kg. The total dose volume was administered as two individual doses of 33.3. mL/kg separated by approximately 4 hours on day 0.
[b] The target dose level of the protein control was adjusted from 3000 to 2500 mg/kg to meet or exceed the adjusted target dose level of the test protein.
[c] The analytical dose confirmation was provided by the Sponsor and is presented in Appendix B.

Following dosing, the mice were observed daily, weighed weekly and had food consumption measured weekly. A gross necropsy examination was performed on all animals at the time of scheduled euthanasia (day 14).

No mortality occurred during the study and there were no significant clinical abnormalities during routine observations. The only notable clinical findings were very minor/transient incidences of rough coat (1/10 test article males on day 11 only), swelling of the urogenital region (1/10 protein control females on days 6-14) and urine staining (1/10 test article females on day 11).

Individually, for the protein control group, a slight body weight loss was noted for three females during the day 0 (fasted) to 7 interval and for one male and one female during the day 7 to 14 interval. A slight body weight loss was also noted in two males and three females from the test article group during the day 7 to 14 interval. Body weight gain/maintenance was noted for all other animals during the test period. Overall, body weight was increased ($p<0.05$) on day 7 for the test article group males compared to the protein control males and on day 14 for the test article group females compared to the protein control females. In addition, body weight change was statistically increased ($p<0.01$) for both the test article group males and females for the day 0 to 7 interval compared to their respective control groups.

No statistical differences were observed in food consumption during the study. No gross pathological findings were observed at necropsy on day 14 that could be considered test article related.

Under the conditions of this test, no toxicity was observed in any of the groups. Therefore, the acute oral LD50 of the *E. coli*-produced Cry3Bb1.pvzmir39 protein is greater than 1930 mg/kg in the mouse.

7. INTRODUCTION

This study was performed to assess the short-term toxicity of *E. coli*-produced Cry3Bb1.pvzmir39 protein in CD-1 mice when administered by gavage as two separate oral doses approximately four hours apart. This study was performed at Springborn Laboratories, 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on February 17, 2003 (GLP initiation date). The in-life phase of the study was initiated with test article administration on February 19, 2003 (day 0) and concluded with necropsy on March 5, 2003.

8. MATERIALS AND METHODS

8.1. Experimental Protocol

This study was performed at Springborn Laboratories in general conformance with the US EPA Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity, December 2002; the OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects, Subsection 401, February 24, 1987; and the EEC Part B: Methods for the Determination of Toxicity, B.1, No L 383 A/110, December 29, 1992.

8.2. Test and Protein Control Articles

The test and protein control articles were received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Protein Control (BSA) BSA Lot No.: B49935 BSA Purity: 95%	S03.001.3044	Pale yellow liquid	02/18/03	None provided
E. coli-produced Cry3Bb1.pvzmir39 protein Lot No. 30-100002	S03.002.3044	Pale yellow liquid	02/18/03	None provided

The test and vehicle control articles were stored refrigerated. The Sponsor was responsible for any necessary evaluations related to chemical composition, purity, strength, stability and other data required by EPA (40 CFR Part 160). The Principal Investigator for the characterization of the test article was Heather K.S. Bonner, Ph.D. The characterization of the test article was performed by the

Monsanto Product Safety Center (PSC), 800 North Lindbergh Blvd. St. Louis, MO 63167. The Certificates of Analysis for the test protein and protein control (BSA) were provided by the Sponsor and are presented in Appendix A.

8.3. Retention Sample

The Sponsor was responsible for maintaining a retention sample of the test article.

8.4. Test/Control Article Disposition

The remaining test and control articles were returned to the Sponsor following completion of the study.

8.5. Method of Test/Control Article Preparation

The test and control articles were administered as received from the Sponsor and dispensed fresh on the day of dosing. The test and control articles were stirred continuously during the dosing procedures including 20 to 30 minutes prior to dosing to allow them to reach room temperature. Subsamples of the dosing solutions were retained for concentration verification, homogeneity and stability analysis. In addition, 1.0 mL end of study samples were collected for each group preparation.

8.6. Stability Analysis

The Sponsor, in accordance with their study specific work procedure, performed the stability analysis of the test articles in the solution, over the course of the dosing period. The samples for stability were collected on day 0, prior to and after the dosing procedure. All predose samples were collected after the required stirring time had been completed.

8.7. Concentration and Homogeneity of the Test Article in Dosing Solutions

The Sponsor, in accordance with their study specific work procedure, performed verification of concentration of the test articles in the dosing solutions. Analyses were performed on day 0 samples collected prior to and following dosing. Samples for homogeneity were collected on all predose preparations that did not visually appear to be solutions. The results of these analyses are presented in Appendix B.

8.8. Animals and Animal Husbandry

8.8.1. Description, Identification and Housing

Young adult, Crl: CD-1®(ICR)BR (VAF/Plus®) Mice were received on February 11, 2003 at SLI from Charles River Laboratories, Inc., Portage, Michigan. Upon receipt, metal ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.8.2. Environment

The animal room temperature and relative humidity ranges were 67-70°F (19-21°C) and 36-55%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

8.8.3. Food

PMI Certified Rodent Meal #5002 (Purina Mills, Inc.) was provided *ad libitum* to the animals throughout the study (except during fasting). The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed and certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.8.4. Water

Municipal tap water treated by reverse osmosis was available *ad libitum* throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.8.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with metal ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.8.6. Animal Selection

Only healthy animals were chosen for study use. Prior to randomization, at least 60 animals were weighed and examined in detail for adverse clinical signs. Animals determined to be suitable as test subjects were assigned randomly to two groups of ten animals per sex, based on body weights. The animal numbers and the respective body weight values were entered into the computer. Homogeneity of groups by weight was the criteria of acceptance of the randomization. The homogeneity of groups criteria was met for this study. Disposition of animals not selected for study was documented in the study records as returned to stock. Females were nulliparous and nonpregnant. The male animals were approximately 7 weeks of age and weighed 27.1-29.6 g and the female animals were approximately 11 weeks of age and weighed 27.5-30.3 g prior to fasting.

9. EXPERIMENTAL PROCEDURES

9.1. Dosing

On day 0, the animals chosen for use on study were weighed and fasted approximately 2 hours and 35 minutes to 2 hours and 55 minutes prior to the first dose administration. The test article was administered orally as two separate doses administered approximately 4 hours apart using a ball tipped stainless steel gavage needle attached to a syringe at the following levels:

Group No.	Treatment	Target Dose Level [a] (mg/kg)	Analytically-Confirmed Dose Level[c] (mg/kg)	No. of Animals	
				Male	Female
1	Protein Control (BSA)	2500[b]	1900	10	10
2	*E. coli*-produced Cry3Bb1.pvzmir39 protein	2100	1930	10	10

[a] The target dose level of the test protein was adjusted from 3000 to 2100 mg/kg based on the maximum attainable protein concentration of the dosing solutions (estimated at 37 mg/mL) and a total dose volume of 66.6 mL/kg. The total dose volume was administered as two individual doses of 33.3. mL/kg separated by approximately 4 hours on day 0.
[b] The target dose level of the protein control was adjusted from 3000 to 2500 mg/kg to meet or exceed the adjusted target dose level of the test protein.
[c] The analytical dose confirmation was provided by the Sponsor and is presented in Appendix B.

Individual doses were calculated based on the animal's nonfasted (day 0) body weight. Animals were returned to *ad libitum* feeding after dosing.

9.2. Clinical Observations

Study animals were observed for clinical abnormalities two times on study day 0 (post-dose, one following the first dosing interval and one following the second dosing interval) and daily thereafter (days 1-14) according to Appendix C. Clinical observations included, but were not limited to, changes in the skin and fur, eyes and mucous membranes, respiratory system, circulatory system, autonomic and central nervous systems, including tremors and convulsions, changes in level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength and stereotypies or bizarre behavior. A general health/mortality check was performed twice daily (in the morning and in the afternoon).

9.3. Body Weights

Individual body weights were obtained for the study animals prior to fasting (day 0), prior to dosing on day 0 and on days 7 and 14.

9.4. Food Consumption

Individual food consumption was measured on study days 0, 7 and 14.

9.5. Scheduled Euthanasia

All study animals were euthanized by carbon dioxide inhalation at study termination (day 14) and necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) were opened and examined. The whole animal was retained in 10% buffered neutral formalin for possible future examination. The lungs and GI tract were flushed with 10% neutral buffered formalin prior to immersion.

9.6. Protocol Deviations

No protocol deviations occurred during this study.

10. DATA ACQUISITION AND ELECTRONIC RECORDS

Electronic data were recorded on a Compaq Alpha Server DS10 utilizing the Toxicology Analysis System Customized, Acute Toxicology Module, Version 1.0.0 or higher. The SLI study number assigned to this study is 3044.931. The computer study number used to collect data for the study phases was 3044931. The tables within the report display the applicable computer number.

11. ANALYSIS OF DATA

Less than 50% mortality occurred during the study, therefore, the LD50 was estimated to be greater than the administered dose:

Inferential statistical analyses were performed by the SLI Alpha DS-10 computer system. Body weights, body weight changes and food consumption were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey Kramer test for group-wise comparisons to the protein control group, when appropriate. Summary data tables display calculated group means, standard deviations (S.D.) and group sizes (N), as appropriate. All statistical comparisons were two-tailed with a minimum level of significance of 5% ($p < 0.05$).

12. MAINTENANCE OF RAW DATA, RECORDS AND SPECIMENS

The following records were transferred to the SLI archives for a period of seven years:

-Protocol, protocol amendments and protocol deviations (if any)
-Study-related correspondence
-Test article receipt, utilization and preparation data
-Animal husbandry data
-In-life and pathology data
-Specimens
-Final report

The Sponsor will be contacted prior to final disposition of these items. The dosing solution characterization data and corresponding subreport will be archived at Monsanto, St. Louis, MO.

13. RESULTS

13.1. Mortality

Summary Data: Table 1
Individual Data: Appendix D

No mortality occurred during the study.

13.2. Clinical Observations

Summary Data: Table 2
Individual Data: Appendix D

There were no significant clinical abnormalities. The only notable clinical findings were very minor/transient incidences of rough coat (1/10 test article males on day 11 only), swelling of the urogenital region (1/10 protein control females on days 6-14) and urine staining (1/10 test article females on day 11).

13.3. Body Weight Data

Summary Data: Tables 3 and 4
Individual Data: Appendices E and F

Individually, for the protein control group, a slight body weight loss was noted for three females during the day 0 (fasted) to day 7 body weight interval and for one male and one female during the day 7 to 14 body weight interval. A slight body weight loss was also noted in two males and three females from the test article group during the day 7 to 14 body weight interval. Body weight gain/maintenance was noted for all other animals during the test period. Overall, body weight was increased ($p<0.05$) on day 7 for the test article group males compared to the protein control males and on day 14 for the test article group females compared to the protein control females. In addition, body weight change was statistically increased ($p<0.01$)) for both the test article group males and females for the day 0 to 7 body weight interval compared to their respective control groups.

13.4. Food Consumption Data

Summary Data: Tables 5 and 6
Individual Data: Appendices G and H

No statistical differences were observed in the food consumption data during the study. Food consumption for all groups was slightly less overall during the second week.

13.5. Gross Necropsy

Summary Data: Table 7
Individual Data: Appendix I

No gross pathological findings were observed at necropsy on day 14 that are considered to be test article related. Two protein control females exhibited periovarian cysts at the time of scheduled necropsy on study day 14. This finding is commonly observed in this strain of mouse. In addition, one protein control female exhibited a subcutaneous abscess that was consistent with the urogenital swelling observed during the in-life phase. One test article female was noted as having an enlarged mandibular lymph node. However, since there was only a single occurrence of this finding, it is not considered to be test article related.

14. CONCLUSION

Under the conditions of this test, no toxicity was observed in any of the groups. Therefore, the acute oral LD50 of the *E. coli*-produced Cry3Bb1.pvzmir39 protein is greater than 1930 mg/kg in the mouse.

[redacted]

Date 9/26/03

Study Director

15. REPORT REVIEW

[redacted]

Date 9-26-03

Director of Toxicology

16. REFERENCE

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

Study No. 3044.931
MONSANTO COMPANY: SB-2003-002

TABLE 1
AN ACUTE ORAL TOXICITY STUDY IN MICE
SUMMARY OF MORTALITY

Sex	Treatment	Dose Level (mg/kg)	No. of Animals	Study Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mortality
Male	Protein Control	1900	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
	E. coli-produced Cry3Bb1.pvzmir39 protein (Test)	1930	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
Female	Protein Control	1900	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
	E. coli-produced Cry3Bb1.pvzmir39 protein (Test)	1930	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10

TABLE 2

AN ACUTE ORAL TOXICITY STUDY IN MICE

MALES SUMMARY OF CLINICAL OBSERVATIONS (FREQUENCY/ANIMALS)

GROUP:	1	2
LEVEL(MG/KG):	BSA POSITIVE CONTROL (1900)	TEST (1930)
DAY 0 to 14		
NORMAL		
WITHIN NORMAL LIMITS	160/10	159/10
SCHEDULED EUTHANASIA	10/10	10/10
BODY		
ROUGH COAT	0/ 0	1/ 1

TABLE 2

AN ACUTE ORAL TOXICITY STUDY IN MICE

FEMALES — SUMMARY OF CLINICAL OBSERVATIONS (FREQUENCY/ANIMALS)

GROUP:	1	2
LEVEL(MG/KG):	BSA POSITIVE CONTROL (1900)	TEST (1930)
DAY 0 to 14		
NORMAL		
WITHIN NORMAL LIMITS	149/10	159/10
SCHEDULED EUTHANASIA	10/10	10/10
BODY		
SWELLING UROGENITAL REGION	8/ 1	0/ 0
HAIRLOSS	3/ 1	0/ 0
URINE STAIN	0/ 0	1/ 1

TABLE 3

AN ACUTE ORAL TOXICITY STUDY IN MICE

MALES

SUMMARY OF BODY WEIGHT DATA (GRAMS)

GROUP:			1	2
LEVEL(MG/KG):			BSA PROTEIN CONTROL (1900)	TEST (1930)
DAY	0 (PREFASTED)	MEAN	28.5 t	28.4
		S.D.	0.74	0.61
		N	10	10
DAY	0	MEAN	27.1 t	27.2
		S.D.	0.79	0.53
		N	10	10
DAY	7	MEAN	28.7 t	29.9*
		S.D.	1.07	1.21
		N	10	10
DAY	14	MEAN	29.6 t	30.6
		S.D.	1.84	1.72
		N	10	10

STATISTICAL KEY: t=ANOVA/TUKEY-KRAMER * = P<0.05

TABLE 3

AN ACUTE ORAL TOXICITY STUDY IN MICE

FEMALES

SUMMARY OF BODY WEIGHT DATA (GRAMS)

GROUP:			1	2
LEVEL(MG/KG):			BSA PROTEIN CONTROL (1900)	TEST (1930)
DAY	0 (PREFASTED)	MEAN	28.8 t	28.8
		S.D.	0.85	0.88
		N	10	10
DAY	0	MEAN	27.6 t	27.5
		S.D.	0.99	0.99
		N	10	10
DAY	7	MEAN	28.3 t	29.1
		S.D.	1.15	1.21
		N	10	10
DAY	14	MEAN	28.9 t	30.0*
		S.D.	0.73	1.47
		N	10	10

STATISTICAL KEY: t=ANOVA/TUKEY-KRAMER * = P<0.05

TABLE 4

AN ACUTE ORAL TOXICITY STUDY IN MICE

MALES

SUMMARY OF BODY WEIGHT CHANGES (GRAMS)

	GROUP:	1	2
	LEVEL(MG/KG):	BSA PROTEIN CONTROL (1900)	TEST (1930)
DAY 0 TO 7	MEAN	1.6 t	2.7**
	S.D.	0.55	1.04
	N	10	10
DAY 7 TO 14	MEAN	1.0 t	0.7
	S.D.	0.86	0.78
	N	10	10

STATISTICAL KEY: t=ANOVA/TUKEY-KRAMER ** = P<0.01

TABLE 4

AN ACUTE ORAL TOXICITY STUDY IN MICE

FEMALES

SUMMARY OF BODY WEIGHT CHANGES (GRAMS)

		GROUP:	1	2
		LEVEL(MG/KG):	BSA PROTEIN CONTROL (1900)	TEST (1930)
DAY	0 TO 7	MEAN	0.7 t	1.6**
		S.D.	0.75	0.71
		N	10	10
DAY	7 TO 14	MEAN	0.6 t	0.9
		S.D.	0.95	0.90
		N	10	10

STATISTICAL KEY: t=ANOVA/TUKEY-KRAMER ** = P<0.01

TABLE 5

AN ACUTE ORAL TOXICITY STUDY IN MICE

MALES

SUMMARY OF FOOD CONSUMPTION DATA (GRAMS/KG/DAY)

	GROUP:	1	2
	LEVEL(MG/KG):	BSA PROTEIN CONTROL (1900)	TEST (1930)
DAY 0 to 7	MEAN	236.5 t	235.3
	S.D.	37.90	36.19
	N	10	10
DAY 7 to 14	MEAN	201.1 t	197.2
	S.D.	21.31	14.80
	N	10	10

Statistical key: t=ANOVA/TUKEY-KRAMER

TABLE 5

AN ACUTE ORAL TOXICITY STUDY IN MICE

FEMALES

SUMMARY OF FOOD CONSUMPTION DATA (GRAMS/KG/DAY)

	GROUP:	1	2
	LEVEL(MG/KG):	BSA PROTEIN CONTROL (1900)	TEST (1930)
DAY 0 to 7	MEAN	252.5 t	259.1
	S.D.	88.42	83.16
	N	10	10
DAY 7 to 14	MEAN	218.2 t	225.7
	S.D.	23.24	37.00
	N	10	10

STATISTICAL KEY: t=ANOVA/TUKEY-KRAMER

TABLE 6

AN ACUTE ORAL TOXICITY STUDY IN MICE

MALES SUMMARY OF FOOD CONSUMPTION DATA (GRAMS/ANIMAL/DAY)

	GROUP:	1	2
	LEVEL(MG/KG):	BSA PROTEIN CONTROL (1900)	TEST (1930)
DAY 0 to 7	MEAN	6.6 t	6.5
	S.D.	1.04	1.04
	N	10	10
DAY 7 to 14	MEAN	5.9 t	6.0
	S.D.	0.69	0.52
	N	10	10

Statistical key: t=ANOVA/TUKEY-KRAMER

TABLE 6

AN ACUTE ORAL TOXICITY STUDY IN MICE

FEMALES SUMMARY OF FOOD CONSUMPTION DATA (GRAMS/ANIMAL/DAY)

	GROUP:	1	2
	LEVEL(MG/KG):	BSA PROTEIN CONTROL (1900)	TEST (1930)
DAY 0 to 7	MEAN	7.1 t	7.3
	S.D.	2.48	2.33
	N	10	10
DAY 7 to 14	MEAN	6.2 t	6.7
	S.D.	0.53	1.02
	N	10	10

STATISTICAL KEY: t=ANOVA/TUKEY-KRAMER

TABLE 7

AN ACUTE ORAL TOXICITY STUDY IN MICE

SUMMARY OF GROSS NECROPSY OBSERVATIONS

GROUP:	1	2
LEVEL(MG/KG):	BSA PROTEIN CONTROL (1900)	TEST (1930)
MALES: TOTAL NUMBER EXAMINED	10	10
WITHIN NORMAL LIMITS	10	10

TABLE 7

AN ACUTE ORAL TOXICITY STUDY IN MICE

SUMMARY OF GROSS NECROPSY OBSERVATIONS

GROUP:		1	2
LEVEL(MG/KG):		BSA PROTEIN CONTROL (1900)	TEST (1930)
FEMALES: TOTAL NUMBER EXAMINED		10	10
WITHIN NORMAL LIMITS		6	9
HAIRCOAT	N	1	0
HAIRLOSS	N	1	0
LYMPH NODE, MANDIBULAR	N	0	1
ENLARGED	N	0	1
OVARY	N	2	0
PERIOVARIAN CYST(S)	N	2	0
SKIN - SUBCUTANEOUS	N	1	0
ABSCESS	N	1	0

APPENDIX A

Certificates of Analysis
(Provided by the Sponsor)

CALBIOCHEM®

CERTIFICATE OF ANALYSIS

Product: Albumin, Bovine Serum, Fraction V, Fatty Acid-Poor, Nuclease- and Protease-Free

Product Number: 126609

Lot Number: B49935

Molecular Weight: 66,000 **CAS Number:** 9048-46-8

TEST	RESULT
Appearance	White powder
SDS-PAGE	98%
Loss on drying	1.5%
Heavy Metals	≤ 10 ppm
pH	6.9
Nuclease	None detected
Sulfated ash	1.0%
Protease	None detected
DNase activity	None detected
Free Fatty Acid (w/w)	0.011%

Storage and Handling	Refrigerator (+4°C)

[redacted]

March 24, 2003
Date

FOR RESEARCH USE ONLY; NOT FOR DRUG OR HUMAN USE

This document has been generated electronically and is valid without signature.
Date: 10/23/02

Page: 1 of 1

Analytical Protein Standard
Certificate of Analysis

MONSANTO ANALYTICAL PROTEIN STANDARDS

Re-certification No. 1

Sample Information:

Name of APS	APS Lot Number	Re-certification Date
E. coli-produced Cry3Bb1.pvzmir39	30-100002	(6 months from APSO signature date)
Common or Alias Name(s)	**Historical APS Lot Number(s)**	**Storage Requirements (until use)**
CCR Cry3Bb1	7160635	–80 °C
Source: Fermentation of *Escherichia coli* containing the pMON70855 expression plasmid		Comment(s): None
Additional Background Information: None		

Characteristic	Method	SOP(s)	Analysis Date	Result
Concentration	Bio-Rad	BR-ME-0525-01	8/19/2002	0.9 mg/mL (total protein) Based on BSA Std. Curve
Concentration	Amino Acid Composition	BR-EQ-0376-01	02/05/2003 10/25/2002	1.2 mg/mL (total protein)
Purity[a]	SDS-PAGE Densitometry	BR-ME-0388-01 BR-ME-0527-01 BR-EQ-0599-01	03/25/2003 02/13/2003 10/24/2002	98%
Molecular weight Major Band (MB) Minor Band 1 (M1) Minor Band 2 (M2)	SDS-PAGE Densitometry	BR-ME-0388-01 BR-ME-0527-01 BR-EQ-0599-01	(MB)9/12/2002 (M1) 9/12/2002 (M2) 9/12/2002	(MB) 74.9 KDa (M1) 69.5 kDa (M2) 66.0 kDa
Identity	Immunoblot	BR-ME-0388-01 GEN-PRO-002-03	8/30/2002	Confirmed[b]
Identity	N-terminal sequence	BR-ME-0388-01 BR-EQ-0265-01	8/26/2002 9/4/2002	Confirmed: ANPXXR Internal sequence fragments identified
Identity Major Band (MB) Minor Band 1 (M1) Minor Band 2 (M2)	MALDI-TOF MS (Trypsinized)	BR-ME-0388-01 BR-EQ-0783-01	(MB) 9/4/2002 (M1) 9/20/2002 (M2) 9/19/2002	% Confirmed sequence (MB) 65.1% (M1) 29.1% (M2) 7.8%
Identity	MALDI-TOF MS (Native)	BR-EQ-0783-01	10/4/2002	74342.98 Daltons
Activity	Insect Bioassay	BR-ME-0044-02	03/31/2003 10/23/2002	CPB[c] LC_{50}: 0.91 µg of Cry3Bb1.pvzmir39/mL diet

[a] Denotes purity is the summation of values from the Major (MB), and Minor bands [(M1), (M2)]
[b] Denotes Immunoreactive bands were observed.
[c] Denotes Colorado Potato Beetle

Buffer composition: 50 mM Sodium Carbonate/Bicarbonate, 1 mM EDTA, pH ~10.1
Purity corrected concentration is 1.2 mg/mL (1.2 mg/mL x 0.98 ≈ 1.2 mg/mL)

[redacted]

April 2, 2003
Date

April 2, 2003
Date

April 2, 2003
Date

APPENDIX B

Analytical Chemistry Report
(Provided by the Sponsor)

Title

Formulation and Confirmation of Doses for An Acute Oral Toxicity Study Performed at Springborn Laboratories Using *E. coli*-Produced Cry3Bb1.pvzmir39 Protein

Authors

[redacted]

Analytical Sub-Report Completed On

September 8, 2003

Monsanto Company Analytical Testing Facilities

Product Characterization Center
800 North Lindbergh Boulevard
Saint Louis, Missouri 63167

Ecological Technology Center
800 North Lindbergh Boulevard
Saint Louis, Missouri 63167

Statistical Technology Center
700 Chesterfield Parkway North
Saint Louis, Missouri 63017

Laboratory Project ID

MSL-18711
Springborn Study #: 3044.931
Monsanto Study #: SB-2003-002

Statement of No Data Confidentiality Claim

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

We submit this material to the United States Environmental Protection Agency specifically under the requirements set forth in FIFRA as amended, and consent to the use and disclosure of this material by EPA strictly in accordance with FIFRA. By submitting this material to EPA in accordance with the method and format requirements contained in PR Notice 86-5, we reserve and do not waive any rights involving this material that are or can be claimed by the company notwithstanding this submission to EPA.

Company: Monsanto Company

Company Agent: _____

Title: _____

Signature: _____ Date: _____

Statement of Compliance

This portion of the study meets the GLP requirements for 40 CFR Part 160 with the following exceptions:

The BSA protein control article was not characterized according to the EPA GLP regulations, however, a certificate of analysis from the manufacturer was available and is archived with the analytical sub-report data.

Submitter: _____ Date: _____

Sponsor Representative: [redacted] Date: 9/8/2003

Analytical Principal Investigator: [redacted] Date: 9/8/2003

Quality Assurance Statement

Study Title: Formulation and Confirmation of Doses for An Acute Oral Toxicity Study Performed at Springborn Laboratories Using *E. coli*-Produced Cry3Bb1.pvzmir39 Protein

Springborn Study No: 3044.931

Monsanto Study No.: SB-2003-002

Reviews conducted by the Quality Assurance Unit confirm that this final sub-report accurately describes the methods and standard operating procedures followed and accurately reflects the raw data for this portion of the study.

Following is a list of reviews conducted by the Monsanto Regulatory Quality Assurance Unit on the study reported herein.

Dates of Inspection / Audit	Phase	Date Reported To: Study Director	Management
April 1, 2003	Amino Acid Analysis	April 14, 2003	April 14, 2003
July 11, 2003	Raw Data Audit	July 25, 2003	July 25, 2003
July 31, 2003	Draft Report Audit	August 14, 2003	August 14, 2003

[redacted]

September 8, 2003
Date

Monsanto Regulatory, Monsanto Company

Sub-Report Information Page

MSL Number: 18711

Title: Formulation and Confirmation of Doses for An Acute Oral Toxicity Study Performed at Springborn Laboratories Using *E. coli*-Produced Cry3Bb1.pvzmir39 Protein

Analytical Testing Facilities:

Product Characterization Center
800 North Lindbergh Boulevard
Saint Louis, Missouri 63167, USA

Ecological Technology Center
800 North Lindbergh Boulevard
Saint Louis, Missouri 63167, USA

Statistical Technology Center
700 Chesterfield Parkway North
Saint Louis, MO 63017, USA

Analytical Principal Investigator: [redacted]

Contributors: [redacted]

Study Specific Work Procedure Initiation Date: February 17, 2003

Analytical Sub-Report Completion Date: September 8, 2003

Records and Sample Retention: All raw data generated at Monsanto, study specific work procedure and amendments, final sub-report and facility records are retained at Monsanto, Saint Louis. Any unused study samples that were not disposed of were stored at Monsanto, Saint Louis.

Sub-Report Certification Page

The results reported in this Analytical Sub-Report accurately reflect the data generated under study specific work procedure number SB-2003-002.

[redacted]

Analytical Principal Investigator

9/8/03
Date

[redacted]

Sponsor Representative

9/8/2003
Date

[redacted]

Technical Center Lead

9-8-2003
Date

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Tables

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Appendices

Abbreviations and Definitions[1]

~	approximately
BSA	bovine serum albumin
BW	body weight
CAPS	3-(cyclohexylamino)-1-propane sulfonic acid
CI	confidence interval
COA	certificate of analysis
CPB	Colorado potato beetle (*Leptinotarsa decemlineata*)
CV	coefficient of variation
E. coli	*Escherichia coli*
ECL	enhanced chemiluminescence
EDTA	ethylenediaminetetraacetic acid
EPA	Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
HRP	horseradish peroxidase
kDa	kilodaltons
LC_{50}	concentration of protein (µg/mL diet) required to kill 50% of the test larvae relative to the negative control
PBST	phosphate buffered saline with Tween 20
PTH	phenylthiohydantoin
PVDF	polyvinylidene difluoride
SD	standard deviation
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SOP	Standard Operating Procedure
Tris	tris(hydroxymethyl)aminomethane
Tween 20	polyoxyethylenesorbitan monolaurate

[1] Standard abbreviations, e.g. units of measure, are used according to the format described in "Instructions to Authors" in the *Journal of Biological Chemistry*.

1.0 Summary

The purpose of this analytical sub-report is to describe the formulation and analysis of the Cry3Bb1.pvzmir39 protein test and bovine serum albumin (BSA) control doses used in a mouse acute oral toxicity study performed at Springborn Laboratories, Inc. (SLI Study No. 3044.931, Spencerville, OH).

Cry3Bb1.pvzmir39 and BSA doses were both formulated in vehicle buffer (50 mM sodium carbonate-bicarbonate, pH 10, 1 mM EDTA). Materials were transported on dry ice to the testing facility and samples taken prior to and after dosing were returned to Monsanto on dry ice and stored in a −80 °C freezer until analysis. Samples of Cry3Bb1.pvzmir39 protein were taken from the test article dose immediately before administration in order to assess homogeneity. Samples of both proteins were also taken immediately before and after administration of the doses to the mice in order to assess concentration, purity, and stability of both proteins and functional activity of and verification of identity for the Cry3Bb1.pvzmir39 protein.

The total protein concentration of the samples collected for homogeneity analysis from the Cry3Bb1.pvzmir39 protein dose was determined using amino acid analysis. Three locations (top left, middle center, and bottom right) of the dosing solution were sampled. The Cry3Bb1.pvzmir39 protein dose was confirmed to be uniform throughout the solution.

Total protein concentration for the analytical samples was determined using amino acid analysis and was 36.1 mg/mL for the Cry3Bb1.pvzmir39 protein dose and 35.6 mg/mL for the BSA dose.

The purity of the analytical samples was assessed using image analysis of Colloidal Brilliant Blue G stained gels. Purity of the Cry3Bb1.pvzmir39 protein in the test article dosing solution was estimated to be an average of 80% for all analytical samples taken during this study. Purity of the BSA protein in the control article dosing solution was estimated to be an average of 80% for all analytical samples taken during this study. Stability of the proteins in the dose solutions was inferred from the purity analyses. Comparison of the purities of samples taken before and after dosing indicated that both the Cry3Bb1.pvzmir39 protein and the BSA were stable throughout dosing.

The functional activity of the analytical samples from the Cry3Bb1.pvzmir39 protein dose was determined using an insect bioassay. Statistical analyses confirmed that the Cry3Bb1.pvzmir39 protein was stable throughout the experiment because no significant

differences in LC_{50} values were observed between the Cry3Bb1.pvzmir39 protein samples taken before and after dosing at the 0.05 level with a p-value of 0.37.

The identity of the Cry3Bb1.pvzmir39 protein dose was verified by N-terminal sequencing and Western blot analysis.

The experimentally determined dose levels were calculated based on the total protein concentration and purity estimates for the Cry3Bb1.pvzmir39 protein and the BSA protein. The confirmed dosing level for the Cry3Bb1.pvzmir39 protein test dose was 1930 mg/kg BW. The confirmed dosing level for the BSA protein control dose was 1900 mg/kg BW.

2.0 Introduction

Monsanto has developed MON 88017 corn that is protected from feeding damage by the coleopteran pest corn rootworm (CRW; *Diabrotica* spp.), and is tolerant to Roundup® agricultural herbicides. MON 88017 expresses a variant of the Cry3Bb1 protein isolated from *B. thuringiensis* that provides protection from feeding damage by corn rootworm, as well as a 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium sp.* strain CP4 (CP4 EPSPS), which confers tolerance to glyphosate, the active ingredient in the Roundup® family of agricultural herbicides.

An acute oral toxicity study was performed on mice using the Cry3Bb1 protein expressed in MON 88017. The *E. coli*-produced Cry3Bb1 protein, designed to be equivalent to the plant-produced Cry3Bb1 protein, was utilized due to the large protein quantities required for *in vivo* mouse toxicity studies. This sub-report includes descriptions of the formulation and analysis of the test and control article dose solutions for that study. Analysis of the test article dose solution included identity, protein concentration, purity, stability, functional activity, and homogeneity both before and after dosing of mice. Analysis of the control article dose solution included protein concentration, purity, and stability both before and after dosing of mice. These procedures were performed to confirm dose concentrations and to assess if any changes in the test or control articles occurred during the performance of the acute oral toxicity study.

3.0 Purpose

The purpose of this sub-report was to describe the formulation and analysis of the Cry3Bb1.pvzmir39 protein test article and BSA control article doses used in a mouse

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acute oral toxicity study performed at Springborn Laboratories, Inc. (SLI Study No. 3044.931, Spencerville, OH).

4.0 Materials

4.1 Test Article

E. coli-produced Cry3Bb1.pvzmir39 protein (lot 30-100002) was isolated at Monsanto Company and had a total protein concentration of 1.2 mg/mL and an estimated purity of 98%. Cry3Bb1.pvzmir39 protein was stored and formulated in a buffer solution containing 50 mM sodium carbonate-bicarbonate, pH 10-10.1, 1 mM EDTA.

4.2 Control Article

Bovine serum albumin (BSA) protein (lot B49935) was purchased from Calbiochem (catalog # 126609). BSA was formulated in a buffer solution containing 50 mM sodium carbonate-bicarbonate, pH 10, 1 mM EDTA.

4.3 Characterization of Test and Control Articles

The identity, concentration, purity, stability, and functional activity of the Cry3Bb1.pvzmir39 protein (lot 30-100002) was previously described by Monsanto Company and a copy of the certificate of analysis (COA) was archived at Monsanto with the sub-report data. The BSA protein was characterized by the manufacturer and determined to have a purity of 98%; a copy of the COA was archived at Monsanto with the sub-report data.

4.4 Assay Controls

Controls and/or standards were included with each analysis. Protein standards were used to calibrate SDS-PAGE gels and verify protein transfer to PVDF membranes. An analytical reference protein (10 pmole β-lactoglobulin) was analyzed before and after the test proteins to verify that the N-terminal sequencer met acceptable performance criteria for repetitive yield and sequence identity. Phenylthiohydantoin amino acid standards were used during N-terminal sequencing analysis. The following standards and controls were used during amino acid analysis; NIST BSA, NIST AA Standards, and norvaline standard.

5.0 Methods

5.1 Formulation of the Test and Control Article Doses

The test and control doses were formulated in the vehicle buffer (50 mM sodium carbonate-bicarbonate, pH 10, 1 mM EDTA). Doses were formulated in a volume sufficient to allow for sampling. To achieve the desired test article dose level, the *E. coli*-produced Cry3Bb1.pvzmir39 protein (lot 30-100002) was concentrated following a concentration plan using both a hollow fiber filtration system and a centrifugal filter device. After concentration using the hollow fiber filtration system, the protein concentration was estimated to be 39 mg/mL by amino acid analysis. To reach the target concentration of ≥40 mg/mL, an attempt to further concentrate the protein was carried out using a Centricon Plus-80 centrifugal filter device. During use of the Centricon Plus-80 device, precipitation of the protein was observed and therefore concentration was ceased. Approximately 96 mL of the test article was prepared (Feb. 14, 2003) at a calculated concentration of approximately 37 mg/mL total protein, representing an estimated target dose of 2100 mg/kg BW.

$$\frac{((37mg/mL)\times(0.86))\times(1mL/dose)\times(2doses)}{0.03kgBW} \cong 2100\frac{mg}{kgBW}$$

The control dose was formulated (Feb. 17, 2003) by adding 60 mL of vehicle buffer to 2.8 g of BSA (Calbiochem lot B49935). After the BSA was in solution, the final volume was brought to 80 mL with additional vehicle buffer. The control article was prepared at a target concentration of approximately 34 mg/mL, representing an estimated target dose of 2500 mg/kg BW.

Doses were independently formulated in small, labeled jars. A triangular magnetic stir bar (1/2" × 1 ¾") was placed in each container. Each dose (jar) was labeled with its identity, lot number of protein from which the dose was prepared, preparation date, and storage conditions. Doses were shipped (Feb. 17, 2003) on dry ice to the testing facility (Springborn Laboratories, Inc.).

5.2 Collection of Dose Samples for Analysis

Two types of dose samples were collected for analysis: analytical (test and control articles) and homogeneity (test article). Prior to administration to mice, doses were thawed and stirred for 20-34 minutes at room temperature to allow for temperature equilibration. A 500 µL analytical sample of each dose was taken

both before administration of the morning dose and after administration of the afternoon dose. Homogeneity samples (500 µL) were taken from the test article dose solution prior to morning dosing by removing an aliquot from the top left, middle center, and bottom right locations of the container while stirring. The study specific work procedure was amended to include an end of study sample (1000 µL) to be taken after completion of all dosing and removal of all analytical samples from the dose solutions. All samples were stored in a freezer until shipped to the sponsor (Monsanto). The remainder of each dose was stored frozen at the testing facility (Springborn Laboratories, Inc., Spencerville, OH) until completion of the study and the analytical and homogeneity samples were returned to Monsanto Company, Creve Coeur, MO on dry ice. Once received at Monsanto, the analytical and homogeneity samples were stored in a –80 °C freezer until analyzed.

Prior to analysis all samples were thawed in a 4 °C refrigerator and four separate 100 µL aliquots of each sample were prepared on ice. Each aliquot was labeled consistent with the initial sample tube labels, with the addition of an aliquot number. The aliquots and original sample tubes (containing remaining sample) were returned to the –80 °C freezer until analyzed.

5.3 Determination of Protein Concentration Using Amino Acid Analysis

The total protein concentration of all the analytical and homogeneity samples was determined using amino acid analysis. During dose formulation, one batch of amino acid analysis data was rejected due to the system not performing to the guidelines specified. Each sample was thawed and diluted to a concentration range compatible with amino acid analysis. Samples were subjected to vapor phase acid hydrolysis followed by amino acid analysis on a Hitachi L-8800 amino acid analysis system. Amino acids were detected using post-column ninhydrin derivatization. Each protein sample was analyzed in triplicate. Total protein concentrations were calculated as an average of the triplicate analysis. The final dose levels for the test and control articles were calculated based on the total protein concentration, corrected for purity.

5.4 Determination of Purity Using SDS-PAGE Analysis

The purity of the test and control article in the doses was estimated by densitometric analysis of Colloidal Brilliant Blue G stained gels. Aliquots of the analytical samples taken before and after dosing were diluted in 1.5× sample buffer, resulting in a final concentration of 1× sample buffer, and heated for 5

minutes at 101-106 °C. Proteins were separated under reducing conditions by electrophoresis on 4-20% polyacrylamide gels. For each sample, three amounts (1, 2, and 3 µg) of total protein were loaded onto the gel. Electrophoresis was carried out for 90-105 minutes at a constant voltage of 125 V. Gels were fixed for 60 minutes in a solution of 40% (v/v) methanol, 7% (v/v) acetic acid, and then stained for 2 hours in Colloidal Brilliant Blue G stain. Gels were de-stained for 45-60 seconds in a solution of 10% (v/v) acetic acid, 25% (v/v) methanol, followed by 2 hours in a solution of 25% (v/v) methanol. Imaging and densitometry were performed using a Bio-Rad GS-710 Calibrating Imaging Densitometer with the supplied Quantity One software version 4.3.0. Purity of the Cry3Bb1.pvzmir protein was calculated as the sum of three bands ranging from ~66 kDa to ~74 kDa.

5.5 Determination of Functional Activity Using Insect Bioassays

Aliquots of analytical samples of the test dose were transferred to the Entomology Laboratory of the Ecological Technology Center, Monsanto Company. These aliquots were used to estimate the bioactivity (measured as an LC_{50} value) of the test article incorporated into a diet fed to Colorado potato beetle (CPB) larvae, a susceptible insect. See Appendix 2 of this sub-report for the method used.

5.6 Verification of Identity Using N-Terminal Sequence Analysis

The identity of the Cry3Bb1.pvzmir39 protein in the dose solution was evaluated using N-terminal amino acid sequence analysis. The initial blot was rejected because individual bands could not be distinguished. Aliquots of the test article analytical samples taken before and after dosing were diluted in 1.5× sample buffer, resulting in a final concentration of 1× sample buffer, and heated for 5 minutes at 100-101 °C. Proteins were separated under reducing conditions by electrophoresis on a 10% polyacrylamide gel. For each sample, five lanes were loaded with 4 µg total protein in each lane. Electrophoresis was carried out for 95-110 minutes at a constant voltage of 125 V. Proteins were transferred from the gel to a PVDF membrane for either 30 minutes at 500 mA or 90 minutes at 300 mA in CAPS buffer (10 mM CAPS, pH 11 and 10% (v/v) methanol). Pre-stained molecular weight markers were included during electrophoresis to verify transfer of proteins to the PVDF membrane. Proteins were visualized on the PVDF membrane by first staining with Coomassie Brilliant Blue stain and subsequently washing with destaining solution (40% (v/v) methanol, 10% (v/v) acetic acid).

Three protein bands, migrating at approximately 74, 71, and 66 kDa, were excised from the PVDF membrane and sequenced using an Applied Biosystems 494

Procise Sequencing System with 140C Microgradient, 785A Programmable Absorbance Detector, Procise Control Software (version 1.1a). Chromatographic data were collected using Atlas99 software (version 3.59a). Chromatography calibration was performed for each analysis using PTH-amino acid standards (Applied Biosystems, Foster City, CA). This mixture also served to verify system criteria such as percent peak resolution and relative amino acid chromatographic retention times. A control protein (10 pmole β-lactoglobulin) was analyzed before and after the test proteins to verify that the sequencer met acceptable performance criteria for repetitive yield and sequence identity.

5.7 Verification of Identity Using Western Blot Analysis

Because the goat anti-Cry3Bb1 serum was shown previously to be specific for the Cry3Bb1 protein (data archived under antibody lot 6844582), Western blot analysis was done to verify the identity of the Cry3Bb1.pvzmir39 protein in the test dose. This procedure was added by amendment to the original study specific work procedure for Monsanto study number SB-2003-002. The initial Western blot was rejected because the gel was torn through the protein band of interest, making the data uninterpretable. Aliquots of the test article analytical samples taken before and after dosing were diluted in 1.5× sample buffer, resulting in a final concentration of 1× sample buffer, and heated for 4-5 minutes at 104-105 °C. Proteins were separated by electrophoresis on a 10% polyacrylamide gel. For each sample, three amounts (1, 5, and 10 ng) of total protein were loaded into three separate wells. Electrophoresis was carried out for 105 minutes at a constant voltage of 125 V. Proteins were transferred from the gel to the PVDF membrane for 60-90 minutes at 300 mA in Tris-glycine transfer buffer (12 mM Tris, pH 8.5, 96 mM glycine, and 10% (v/v) methanol). Prestained molecular weight markers were included in the gel to verify transfer of proteins to the PVDF membrane. After transfer, the membrane was blocked at room temperature with 5% (w/v) non-fat dry milk in PBST for 90-100 minutes or overnight. The primary antibody (goat anti-Cry3Bb1 serum lot 6844582) was added to the blot at a 1/5000 dilution in 1% (w/v) non-fat dry milk in PBST and incubated for 60-70 minutes at room temperature. The blot was washed three times, 10-15 minutes each, with 30 mL PBST. The secondary antibody (anti-Goat IgG, HRP conjugated, Sigma) was added to the blot at a 1/10,000 dilution in 1% (w/v) non-fat dry milk in PBST and incubated for 60-80 minutes. The blot was again washed three times, 10-15 minutes each, with 30 mL PBST. The blot was developed with ECL Reagent (AP Biotech) and exposed to Hyperfilm (AP Biotech) for 10 seconds, 30 seconds, and 1 minute. Films were developed using a Konica SRX-101A automated film processor.

5.8 Calculation of the Test and Control Article Dose Concentrations

The dose concentrations were calculated using the following assumptions.

a) The average mouse body weight (BW) is 0.030 kg.
b) Doses was administered at 33.33 mL/kg BW (~1 mL), twice daily.
c) The concentration and purity of the Cry3Bb1.pvzmir39 protein are 36.1 mg/mL and 80%, respectively.
d) The concentration and purity of the BSA protein are 35.6 mg/mL and 80%, respectively.

The final test and control article dose concentration values were calculated based upon the respective total protein concentration, corrected for purity, and rounded to three significant figures, as follows.

Test article dose:

$$\frac{((36.1mg/mL)\times(0.80))\times(1mL/dose)\times(2doses)}{0.03kgBW} \cong 1930\frac{mg}{kgBW}$$

Control article dose:

$$\frac{((35.6mg/mL)\times(0.80))\times(1mL/dose)\times(2doses)}{0.03kgBW} \cong 1900\frac{mg}{kgBW}$$

6.0 Control of Bias and Quality Measures

Purity and Western blot analysis gels were analyzed with multiple dilutions of protein on each gel. Three separate aliquots of each sample were hydrolyzed then analyzed by amino acid analysis for protein concentration determination.

7.0 Statistical Methods

Probit analysis was used by the Monsanto Statistics Technology Center to estimate LC_{50} values and the associated 95% confidence intervals (CV) for the insect bioassay data (Appendix 2).

8.0 Results and Discussion

8.1 Total Protein Concentration of the Test and Control Article Doses

The total protein concentration in the analytical samples of the Cry3Bb1.pvzmir39 test dose was 36.5 mg/mL before dosing and 35.7 mg/mL after dosing. The average total protein concentration in the analytical samples of the Cry3Bb1.pvzmir39 test dose was 36.1 mg/mL.

The total protein concentration in the analytical samples of the BSA control dose was 35.0 mg/mL before dosing and 36.2 mg/mL after dosing. The average total protein concentration of the BSA control article in the analytical sample was 35.6 mg/mL.

8.2 Verification of Test Article Identity

The identity of the test article was verified using N-terminal sequence analysis and Western blot analysis. N-terminal sequence analysis of the three major protein bands observed in the analytical samples taken before and after dosing resulted in an amino acid sequence (Ala-Asn-Pro) consistent with the N-terminal sequence of Cry3Bb1.pvzmir39 for one of the bands (~74 kDa) and was inconclusive for the ~71 and ~66 kDa bands. Western blot analysis was also performed with aliquots of the analytical analysis samples taken before and after dosing. Because the antibody used had been characterized previously and shown to be specific for Cry3Bb1 protein, the Western blot (Figure 1) using goat anti-Cry3Bb1 polyclonal serum (lot 6844582) confirmed the identity of the three major protein bands (~74, ~71, and ~66 kDa) in the analytical samples as representing the Cry3Bb1.pvzmir39 protein. The ~74 kDa band corresponded to the expected size for the Cry3Bb1.pvzmir39 protein, the ~71 and ~66 kDa bands corresponded to previously identified degradation products for the Cry3Bb1.pvzmir39 protein.

8.3 Purity and Stability of the Test and Control Article Doses

The purity of the Cry3Bb1.pvzmir39 and the BSA proteins contained in the test and control article doses was assessed using image analysis of the Colloidal Brilliant Blue G stained gels shown in Figures 2 and 3.

Cry3Bb1.pvzmir39 protein in samples taken before (Figure 2, lanes 2-4) and after (Figure 2, lanes 6-8) dosing were both estimated to be 80% pure, calculated as the sum of all three bands (~74, ~71, and ~66 kDa) identified as Cry3Bb1.pvzmir39 protein. While the experimentally determined purity for the Cry3Bb1.pvzmir39

protein was lower than that on the COA, the relative abundance of each of the three individual bands was similar in the samples taken both before and after dosing. The final purity of 80% was the average of the purities determined for each protein amount loaded. Because the staining pattern and purity of the Cry3Bb1.pvzmir39 protein samples taken before and after dosing were similar, no degradation of the test article occurred during dosing. The experimentally determined average BSA protein purity of 80% was used to calculate the final dose concentration.

BSA protein in samples taken before (Figure 3, lanes 2-4) and after (Figure 3, lanes 6-8) dosing was estimated to be 79% and 80% pure, respectively. While the experimentally determined purity for the BSA protein was lower than that provided by the manufacturer (98%), the purity estimated both before and after dosing were similar. Therefore, no degradation of the control article occurred during dosing. The experimentally determined average BSA protein purity of 80% was used to calculate the final dose concentration.

8.4 Functional Stability of the Cry3Bb1.pvzmir39 Protein

The LC_{50} estimates for the samples taken before and after dosing and the corresponding 95% CI for the analytical test dose samples are summarized in Table 1 of Appendix 2. No statistically significant difference in the LC_{50} values for CPB (Colorado potato beetle) was observed between the Cry3Bb1.pvzmir39 analytical samples taken before (0.485 µg Cry3Bb1/mL diet) and after (0.455 µg Cry3Bb1/mL diet) dosing, indicating that no significant loss of activity had occurred.

8.5 Homogeneity of the Cry3Bb1.pvzmir39 Protein Dose Solution

Homogeneity samples collected from the test article dose solution prior to dosing were evaluated for concentration. Three locations of the dosing solution (top left, middle center, and bottom right) were sampled from the container. The total protein concentrations of the test article homogeneity samples were determined by amino acid analysis to be 35.6, 36.6, and 36.3 mg/mL (Table 1). This corresponds to a 1.4% CV, and indicates that the test article dose was homogeneous.

8.6 Experimentally Confirmed Values for the Test and Control Articles in the Prepared Doses

The experimentally determined dose levels were calculated based on the total protein concentration and purity estimates for the Cry3Bb1.pvzmir39 protein in the test article and the BSA protein in the control article as well as an assumed body weight of 0.03 kg/ mouse. The confirmed dosing level for the test article (Cry3Bb1.pvzmir39 protein) was 1930 mg/kg BW. The confirmed dosing level for the control article protein (BSA) was 1900 mg/kg BW.

9.0 Conclusions

The BSA control article dose used in the mouse acute oral toxicity study performed at Springborn Laboratories, Inc. (SLI Study No. 3044.931, Spencerville, OH) was formulated and analyzed. The total protein concentration of the control article dose was determined to be 35.6 mg/mL. The purity of the BSA protein in the control article dose was estimated to be 80%. Stability of the protein control article during dosing was inferred from the purity analysis. The dosing level for the control article protein (BSA) was 1900 mg/kg BW.

The Cry3Bb1.pvzmir39 protein test article dose used in the mouse acute oral toxicity study performed at Springborn Laboratories, Inc. (SLI Study No. 3044.931, Spencerville, OH) was formulated and analyzed. The homogeneity of the test article dosing solution was confirmed. N-terminal sequence and Western blot analysis were used to confirm the identity of the test article as Cry3Bb1.pvzmir39 protein. The total protein concentration of the test article dose was determined to be 36.1 mg/mL. The purity of the Cry3Bb1.pvzmir39 protein in the test article dose solution was estimated to be 80%. Stability of the protein test article during dosing was inferred from the purity analysis. The functional stability of the test article during dosing was confirmed using insect bioassays. The dosing level for the test article protein (Cry3Bb1.pvzmir39) was 1930 mg/kg BW.

Table 1. Homogeneity Analysis of Test Article Dose Solution. Three locations of the dosing solution (top left, middle center, and bottom right) were sampled from the container prior to dosing. The total protein concentration of the test article homogeneity samples was determined by amino acid analysis of each sample in triplicate.

Homogeneity Sample	Replicate	Protein Concentration (mg/mL)	Average Protein Concentration (mg/mL)
Top Left	1	35.1	35.6
	2	35.1	
	3	36.5	
Middle Center	1	36.7	36.6
	2	—[a]	
	3	36.4	
Bottom Right	1	36.0	36.3
	2	36.0	
	3	37.1	

Calculated % CV for Average Protein Concentration = 1.4%

[a] The second replicate analysis sample from the middle center homogeneity sample had no data obtained.

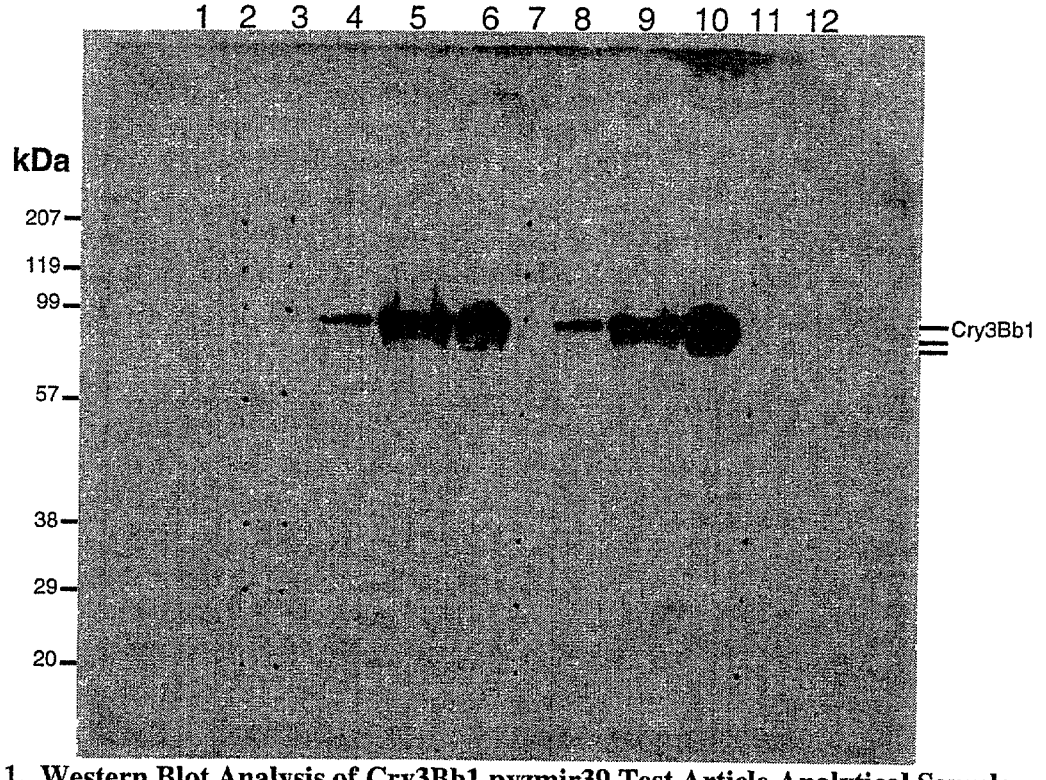

Figure 1. Western Blot Analysis of Cry3Bb1.pvzmir39 Test Article Analytical Samples Taken Before and After Dosing. (Blot shown not actual size.) A Western blot was performed in order to verify the identity of the test article in the dosing solution. Samples (1, 5, 10 ng) taken before and after dosing were electrophoresed on a 10% polyacrylamide gel under reducing and denaturing conditions. The dots (Lanes 2, 3, 7, and 11) represent the location of the molecular weight markers on the membrane. Bands corresponding to ~74, ~71, and ~66 kDa are indicated on the right. A 30 second exposure is shown.

Lane	Sample
1	Sample buffer
2	Prestained SDS-PAGE Molecular Weight Standards (Bio-Rad)
3	Prestained SDS-PAGE Molecular Weight Standards (Bio-Rad)
4	1 ng Test Article Before Dosing
5	5 ng Test Article Before Dosing
6	10 ng Test Article Before Dosing
7	Prestained SDS-PAGE Molecular Weight Standards (Bio-Rad)
8	1 ng Test Article After Dosing
9	5 ng Test Article After Dosing
10	10 ng Test Article After Dosing
11	Prestained SDS-PAGE Molecular Weight Standards (Bio-Rad)
12	Sample buffer

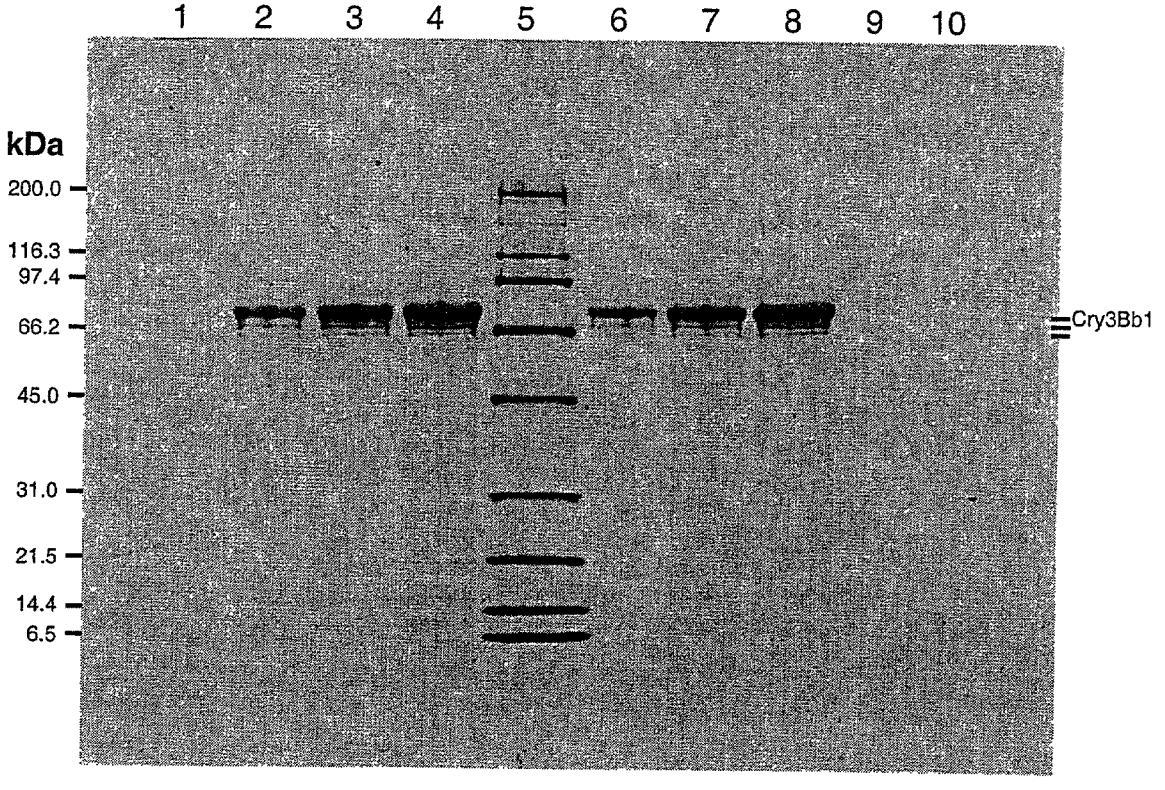

Figure 2. SDS-PAGE Showing Purity of the Cry3Bb1.pvzmir39 Protein in the Analytical Samples Taken Before and After Dosing. (Gel shown not actual size.) Three amounts (1, 2, and 3 µg total protein) of each sample were electrophoresed on a 4-20% polyacrylamide gel under reducing and denaturing conditions. Bands corresponding to ~74, ~71, and ~66 kDa are indicated on the right. Image analysis was used to determine the purity of the Cry3Bb1.pvzmir39 protein in the dosing solutions. Molecular weights (kDa) shown correspond to the markers in lane 5.

Lane	Sample
1	Sample buffer
2	1 µg Before Dosing Sample
3	2 µg Before Dosing Sample
4	3 µg Before Dosing Sample
5	SDS-PAGE Molecular Weight Standards, Broad Range (Bio-Rad)
6	1 µg After Dosing Sample
7	2 µg After Dosing Sample
8	3 µg After Dosing Sample
9	Sample buffer
10	Sample buffer

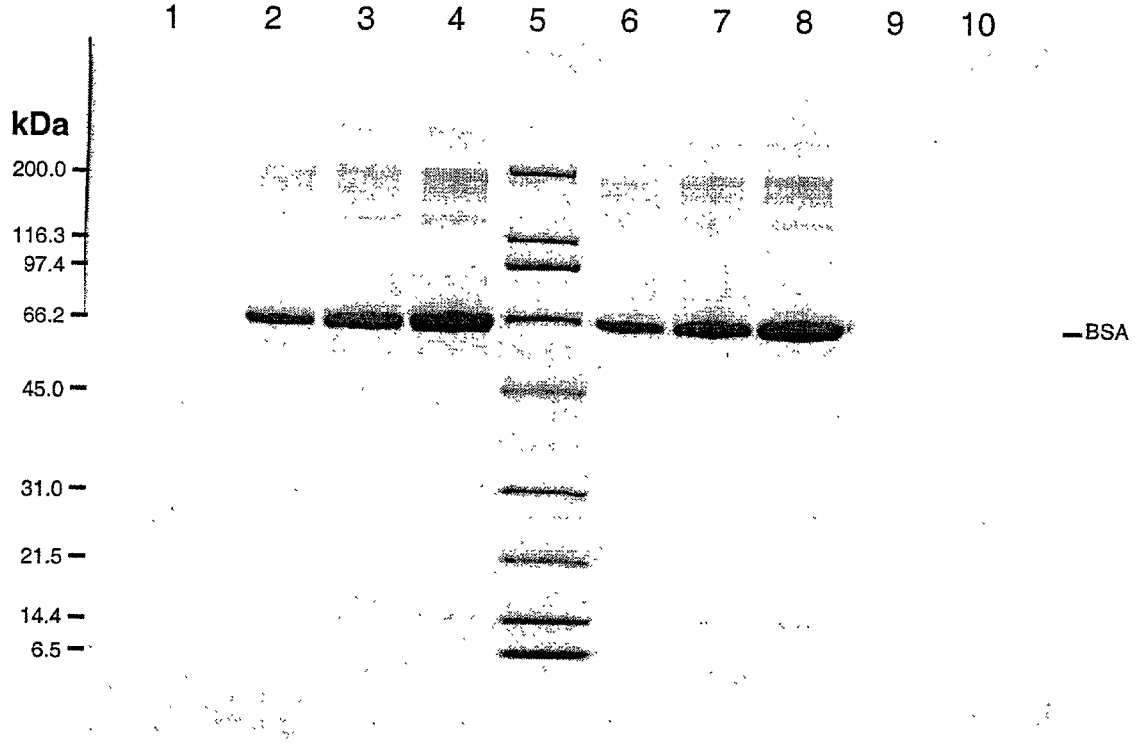

Figure 3. SDS-PAGE Showing Purity of the BSA Protein in the Analytical Samples Taken Before and After Dosing. (Gel shown not actual size.) Three amounts (1, 2, and 3 µg total protein) of each sample were electrophoresed on a 4-20% polyacrylamide gel under reducing and denaturing conditions. Image analysis was used to determine the purity of the BSA protein in the dosing solution. Molecular weights (kDa) shown correspond to the markers in lane 5.

Lane	Sample
1	Sample buffer
2	1 µg Before Dosing Sample
3	2 µg Before Dosing Sample
4	3 µg Before Dosing Sample
5	SDS-PAGE Molecular Weight Standards, Broad Range (Bio-Rad)
6	1 µg After Dosing Sample
7	2 µg After Dosing Sample
8	3 µg After Dosing Sample
9	Sample buffer
10	Sample buffer

Appendix 1

List of Applicable SOPs

AG-EQ-0379-01	Atlas™ Chromatography Data System
BR-EQ-0265-01	Applied Biosystems 494 ProciseTM Protein Sequencing System
BR-EQ-0376-01	Hitachi L-8800 Amino Acid Analysis System
BR-EQ-0599-01	Bio-Rad GS-710 Densitometer
BR-EQ-0935-01	Konica SRX X-Ray Film Processor
BR-ME-0044-02	Diet Incorporation Insect Bioassay for the Biological Activity Measurement of *Bacillus thuringiensis* and Other Insecticidal Proteins
BR-ME-0388-01	SDS Polyacrylamide Gel Electrophoresis (PAGE) using Pre-Cast Gels in Mini Gel Electrophoresis Apparatus
BR-ME-0527-01	Brilliant Blue G-Colloidal Staining of Polyacrylamide Gels
GEN-PRO-002-03	Western Blot Analysis (Immunoblotting)

Appendix 2

Insect Bioassay Summary

Study Specific Work Procedure for the Preparation, Formulation and Confirmation of Doses for an Acute Oral Toxicity Study Performed at Springborn Labs using *E. coli* Produced Cry3Bb1.pvzmir39 Protein

Summary: Insect bioassay portion of work procedure:

Objective:

The objective of this analysis was to determine the biological activity of Cry3Bb1.pvzmir39 in samples collected before and after dosing for study SB-2003-002. Aliquots of samples were incorporated in a diet fed to the Colorado potato beetle (CPB), *Leptinotarsa decemlineata*, larvae, a known susceptible insect. Biological activity was measured as an LC_{50} which is defined as the estimated lethal concentration of the protein (µg/ml of diet) required to kill 50% of the test CPB larvae.

Samples received and analyzed:

The samples analyzed were received from the Product Characterization Center on 19 March 2003. The test samples of Cry3Bb1.pvzmir39 (Lot # 30-100002) were labeled as before and after dosing. The Cry3Bb1 was suspended in 50mM sodium carbonate buffer with a pH of 10. A buffer sample (lot # 7148946A) was also received for use in preparing diluted sub-samples for each bioassay. Upon receipt, the test samples were transferred to approximately -80° C. The buffer was stored at approximately 5° C.

Methods:

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata*, was used to measure activity of the Cry3Bb1 protein samples in accordance with the Monsanto SOP # BR-ME-0044-02 entitled "Diet Incorporation Insect Bioassay for the Biological Activity Measurement of *Bacillus thuringiensis* and Other Insecticidal Proteins". The bioassay was replicated three times on different days each using separate batches of insects. Each bioassay replicate consisted of a series of dilutions from each Cry3Bb1.pvzmir39 sample yielding a dose series ranging from 0-7 µg Cry3Bb1 protein/ml diet plus a water control. Water was inadvertently used as a negative control in place of the vehicle buffer, however this had no impact on the results. The Cry3Bb1 protein doses were prepared by diluting the protein with purified water and incorporating the dilution into an agar based insect diet. This dose series in diet was expected to elicit a response range from CPB larvae that would allow for an estimate of the LC_{50} of the Cry3Bb1 protein. Colorado potato beetle larvae were placed on these diets, approximately 24 insects per treatment, and allowed to feed for a period of seven days. The number and weight of survivors were recorded seven days after initiation of the bioassays. Insect weight was recorded for quality control purposes only and was not used in the analysis.

Statistical analysis:

The bioassay data analyzed consisted of the number of surviving CPB larvae for each treatment for each replicate. The data were entered into an Excel spreadsheet and transferred to the Statistics Technology Center for analysis (See also, "Statistical Report" for Study SB-2003-002). Statistical analysis was performed with Release 8.2 of the SAS statistical program running under Windows 2000 Professional (SAS Institute Inc., 1999-2001).
Probit analysis was used to estimate LC_{50} values and the associated 95% confidence intervals (CI) for each Cry3Bb1 protein dose response.

Probit analysis was performed with the following model:

$$p_j = C + (1-C)F(Dose_j + e_j)$$

p_j: Observed proportion of mortality under dose level j;
C: Natural mortality;
F: Normal cumulative distribution function;
$Dose_j$: Dose level j in logarithm scale of base 10;
e_j: Random residual effect.

A joint analysis was also performed to test the differences between two treatments (before and after samples) over replicates with the following model:

$$p_{ijk} = C + (1-C)F(Rep_i + Treat_j + Dose_k + e_{ijk})$$

p_{ijk}: Observed proportion of mortality under dose level k and treatment j in the i^{th} replicate;
C: Natural mortality;
F: Normal cumulative distribution function;
Rep_i: Effect of the replicate;
$Treat_j$: Effect of the j^{th} treatment;
$Dose_k$: Dose effect for k^{th} dose level;
e_{ijk}: Random residual effect.

Results:

Table 1 lists the LC_{50} estimates and the corresponding 95% CI for Cry3Bb1.pvzmir39 samples collected before and after dosing sample. The mean LC_{50} and standard deviation for the three replicates of both treatments are also given. No significant difference in LC_{50}s was observed between the samples of Cry3Bb1.pvzmir39 collected before and after dosing at the 0.05 level with a p-value of 0.375.

Table 1

Date Bioassay Initiated	Cry3Bb1.pvzmir39 Treatment	Sample ID	Rep	LC_{50} (µg/mL diet)	95% CI (µg/mL diet)
3/21/03	After Dosing	TA-AA-AD	1	0.436	0.349 – 0.542
	Before Dosing	TA-AA-BD	1	0.431	0.333 – 0.552
3/27/03	After Dosing	TA-AA-AD	2	0.534	0.427 – 0.666
	Before Dosing	TA-AA-BD	2	0.488	0.384 – 0.616
3/28/03	After Dosing	TA-AA-AD	3	0.395	0.333 – 0.454
	Before Dosing	TA-AA-BD	3	0.535	0.434 – 0.659
After Dosing Mean LC_{50} 0.455 µg Cry3Bb1/mL diet **SD** 0.0715					
Before Dosing Mean LC_{50} 0.485 µg Cry3Bb1/mL diet **SD** 0.0519					

Conclusions:

No significant difference in LC_{50} values for CPB was observed between the Cry3Bb1.pvzmir39 samples collected before and after dosing indicating that no significant degradation occurred.

Sources:

SAS®, Version 8.2 on-line documentation, 1999-2001 SAS Institute, Inc., Cary, NC, USA.

Prepared by Changjian Jiang and Christopher R. Brown:

[redacted] 05/12/03
Date

03/12/03
Date

APPENDIX C

Detailed Clinical Observation Parameters

Detailed Clinical Observation Parameters

Cage-side Observations	Recorded As
Abnormal movements or behavior	See Categorical
Resistance to removal from cage	Score

Hand-held Observations	Recorded As
Palpebral closure	Score
Lacrimation (non-colored periocular wetness)	Score
Pupil Size	Score
Salivation (non-colored perioral wetness)	Score
Muscle tone	Score
Extensor-thrust response	Score
Reactivity to handling	Score

Open-field observations	Recorded As
Responsiveness to touch	Score
Gait evaluation	Score

Categorical observations (anytime during the DCO)	Recorded As
Abnormal behavior	Description
Abnormalities of the eye	Description
Abnormal urine or feces	Description
Abnormalities of the gastrointestinal (GI) tract	Description
Injury	Description
Missing extremity	Description
Abnormal muscle movements	Description
Palpable mass/swellings	Description
Abnormal posture	Description
Abnormalities of the reproductive system	Description
Abnormal respiration	Description
Abnormal skin or hair-coat/mucous membranes	Description
Excessive soiling	Description
General abnormalities	Description

Explicitly Defined Scales for DCOs

DCO Examination Conduct

The clinical examination is conducted in a careful and systematic format. The examination begins at the head of the animal and gradually works towards the tail as outlined below.

Cage-side observations are made first.

Categorical observations include: Unusual body movements (e.g., tremors, convulsions), abnormal behaviors (e.g., circling, stereotypy) and changes in posture (e.g., arched back, splayed stance).

Resistance to Removal: The degree to which the animal attempts to escape capture is scored. The observer will slowly present a gloved hand into the cage and will grasp the animal over the shoulder area or by the tail.

1 = Decrease – clearly less resistance to capture than typical
2 = Typical – minimally to actively avoids capture and may be mildly aggressive
3 = Increase – clearly more resistance to capture than typical and is very aggressive (attempts to bite).

Eye observations: Eyes are bilaterally examined for these effects; however, if a unilateral observation is made, a concurrent observation is not made for the other eye if it is within normal limits.

Palpebral closure:

1 = Closed (50% to completely closed)
2 = Open
3 = Protruding eyes

Pupil size (aided by penlight): Under typical examination conditions (white light), the typical appearance of the pupils in albino animals is complete constriction. Therefore a decrease in pupil size cannot be observed.

0 = Unable to evaluate
1 = Decrease – clearly decreased pupil size compared to typical
2 = Typical – completely constricted pupils
3 = Increase – clearly increased pupil size compared to typical

Lacrimation (clear wetness): Under typical examination conditions, corneal dryness is not observed in rodents, nor are the eyelids excessively wet.

1 = Decrease – extremely dry appearance of cornea
2 = Typical – glistening cornea (moderate dryness or wetness)
3 = Increase – extensive wetness around the eyes

Degree of salivation: Under typical examination conditions, dryness of the oral cavity is not observed in rodents.

1 = Decrease – oral dryness
2 = Typical – limited to moderate perioral wetness, but lips and chin are dry
3 = Increase – extensive wetness around the mouth and lips

Muscle tone: An assessment of muscle tone at the time of the hand-held observations.

1 = Decrease – clearly less muscle tone than typical
2 = Typical – animal is neither very relaxed nor very tense
3 = Increase – clearly more muscle tone than typical

Extensor-thrust response: Extent of reflex response to brisk pushes (by finger) on the plantar surface of the hindfeet.

1 = Decrease – clearly less response than typical
2 = Typical – clearly detectable extensor-thrust response
3 = Increase – clearly more response than typical

Reactivity to handling: The degree to which an animal struggles to get free from hand-held restraint is ranked.

1 = Decrease – very slight or no struggling
2 = Typical – mild to moderate struggling, animal may vocalize
3 = Increase – aggressive escape behavior, may try to bite observer and usually vocalizes

Observations made in the open-field.

Responsiveness to touch: The ventral aspect of the tail is lightly stroked using a finger. Typically, the animal will lift its tail and wrap it around the finger when lightly touched.

1 = Decrease – does not lift tail, but may briefly hold tail in the air when manually lifted; no response to touch
2 = Typical – lifts tail when touched
3 = Increase – lifts tail and acts startled, may turn towards finger in an attack response

Gait evaluation: Open-field observations are used for gait evaluation. If the animal remains motionless in the open-field, it may be forced to walk on its forelegs while the hindlegs are held off the floor of the observation box ("the wheel-barrow test").

1 = Unable to walk
2 = Clear knuckling, stumbling and poor coordination, may include falling and/or dragging of one or more limbs
3 = Typical – smooth and coordinated gait

Categorical Observations: These observations can be made at anytime during the DCOs. For the categories listed below, the observer directly records the positive observation.

1.	Abnormal behavior	Description
2.	Abnormalities of the eye	Description
3.	Abnormal urine or feces	Description
4.	Abnormalities of the gastrointestinal tract	Description
5.	Injury	Description
6.	Missing extremity	Description
7.	Abnormal muscle movements	Description
8.	Palpable mass/swellings	Description
9.	Abnormal posture	Description
10.	Abnormalities of the reproductive system	Description
11.	Abnormal respiration	Description
12.	Abnormal skin or hair coat/mucous membranes	Description
13.	Excessive soiling	Description
14.	General abnormalities	Description

APPENDIX D

Individual Clinical Observations

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL CLINICAL OBSERVATIONS

MALES BSA (1900 MG/KG)

ANIMAL#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A2965	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A2966	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A2970	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A2971	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A2982	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A2983	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A2984	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A2986	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A2991	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL CLINICAL OBSERVATIONS

MALES BSA (1900 MG/KG)

ANIMAL#	OBSERVATIONS	DAY OF STUDY	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A2994	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA																P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL CLINICAL OBSERVATIONS

MALES TEST (1930 MG/KG)

ANIMAL#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A2969	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A2972	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A2977	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P		P	P	P
	SCHEDULED EUTHANASIA															P
	BODY: ROUGH COAT											P				
A2979	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A2985	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A2987	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A2988	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A2992	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL CLINICAL OBSERVATIONS

MALES TEST (1930 MG/KG)

ANIMAL#	OBSERVATIONS	DAY OF STUDY	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A2993	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA																P
A2995	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA																P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES BSA (1900 MG/KG)

ANIMAL#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A0004	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A0009	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P				P	P
	SCHEDULED EUTHANASIA															P
	BODY: HAIRLOSS											1	1	1		
A0015	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A0016	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A0018	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A0020	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A0021	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P								
	SCHEDULED EUTHANASIA															P
	BODY: SWELLING UROGENITAL REGION								P	P	P	P	P	P	P	P
A0023	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES BSA (1900 MG/KG)

ANIMAL#	OBSERVATIONS	DAY OF STUDY	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A0025	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA																P
A2998	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA																P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES TEST (1930 MG/KG)

ANIMAL#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A0001	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A0002	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A0006	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A0011	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A0012	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A0013	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P
A0014	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P		P	P	P	P
	SCHEDULED EUTHANASIA															P
	BODY: URINE STAIN											1				
A0022	WITHIN NORMAL LIMITS	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA															P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES TEST (1930 MG/KG)

ANIMAL#	OBSERVATIONS	DAY OF STUDY	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A0024	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA																P
A3000	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	SCHEDULED EUTHANASIA																P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

APPENDIX E

Individual Body Weight Data

STUDY NO.: 3044931
MONSANTO COMPANY: SB-2003-002

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL BODY WEIGHT DATA (GRAMS)

MALES BSA (1900 MG/KG)

ANIMAL#	(PREFASTED) 0	DAY OF STUDY 0	7	14
A2965	28.6	26.8	29.0	29.6
A2966	28.3	26.9	28.8	29.7
A2970	29.6	28.4	30.7	33.1
A2971	27.5	25.8	26.8	26.0
A2982	29.0	27.8	28.8	30.5
A2983	28.9	27.8	28.6	29.0
A2984	28.5	27.2	28.6	29.5
A2986	28.1	26.8	28.7	29.7
A2991	27.1	26.2	27.3	28.3
A2994	28.9	27.6	29.5	31.1
MEAN	28.5	27.1	28.7	29.6
S.D.	0.74	0.79	1.07	1.84
N	10	10	10	10

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL BODY WEIGHT DATA (GRAMS)

MALES TEST (1930 MG/KG)

ANIMAL#	(PREFASTED) 0	DAY OF STUDY 0	7	14
A2969	28.7	26.9	30.5	30.1
A2972	27.8	27.1	29.9	30.0
A2977	27.2	26.3	28.2	28.8
A2979	28.2	27.1	29.1	28.7
A2985	28.0	26.8	28.9	30.0
A2987	28.4	27.1	30.2	31.2
A2988	28.8	27.3	30.5	31.5
A2992	29.0	28.1	30.1	30.8
A2993	28.9	27.8	29.2	30.3
A2995	29.1	27.7	32.6	34.8
MEAN	28.4	27.2	29.9	30.6
S.D.	0.61	0.53	1.21	1.72
N	10	10	10	10

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL BODY WEIGHT DATA (GRAMS)

FEMALES BSA (1900 MG/KG)

ANIMAL#	(PREFASTED) 0	DAY OF STUDY 0	7	14
A0004	27.8	26.2	27.5	28.3
A0009	30.1	28.7	29.7	30.3
A0015	28.9	27.3	26.7	27.7
A0016	28.8	28.1	28.7	28.9
A0018	28.4	27.2	28.8	28.9
A0020	28.6	27.6	28.9	28.4
A0021	27.7	26.4	26.3	29.3
A0023	29.8	29.0	28.8	29.0
A0025	28.0	26.9	28.1	28.6
A2998	29.7	28.7	29.6	29.6
MEAN	28.8	27.6	28.3	28.9
S.D.	0.85	0.99	1.15	0.73
N	10	10	10	10

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL BODY WEIGHT DATA (GRAMS)

FEMALES TEST (1930 MG/KG)

ANIMAL#	(PREFASTED) 0	0	DAY 7	OF STUDY 14
A0001	27.9	27.2	28.4	28.3
A0002	28.5	27.1	28.4	30.2
A0006	27.5	26.0	27.5	28.5
A0011	30.3	29.0	30.4	32.0
A0012	29.8	28.5	31.3	32.9
A0013	28.1	26.0	28.6	29.4
A0014	28.8	27.6	29.0	28.8
A0022	29.4	27.8	28.3	30.2
A0024	29.3	28.3	30.6	30.0
A3000	28.6	27.6	29.0	30.0
MEAN	28.8	27.5	29.1	30.0
S.D.	0.88	0.99	1.21	1.47
N	10	10	10	10

APPENDIX F

Individual Body Weight Changes

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL BODY WEIGHT CHANGES (GRAMS)

MALES BSA (1900 MG/KG)

ANIMAL#	DAY OF STUDY 0-7	7-14
A2965	2.2	0.6
A2966	1.9	0.9
A2970	2.3	2.4
A2971	1.0	-0.8
A2982	1.0	1.7
A2983	0.8	0.4
A2984	1.4	0.9
A2986	1.9	1.0
A2991	1.1	1.0
A2994	1.9	1.6
MEAN	1.6	1.0
S.D.	0.55	0.86
N	10	10

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL BODY WEIGHT CHANGES (GRAMS)

MALES TEST (1930 MG/KG)

ANIMAL#	DAY OF STUDY 0-7	7-14
A2969	3.6	-0.4
A2972	2.8	0.1
A2977	1.9	0.6
A2979	2.0	-0.4
A2985	2.1	1.1
A2987	3.1	1.0
A2988	3.2	1.0
A2992	2.0	0.7
A2993	1.4	1.1
A2995	4.9	2.2
MEAN	2.7	0.7
S.D.	1.04	0.78
N	10	10

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL BODY WEIGHT CHANGES (GRAMS)

FEMALES BSA (1900 MG/KG)

ANIMAL#	DAY OF STUDY 0-7	7-14
A0004	1.3	0.8
A0009	1.0	0.6
A0015	-0.6	1.0
A0016	0.6	0.2
A0018	1.6	0.1
A0020	1.3	-0.5
A0021	-0.1	3.0
A0023	-0.2	0.2
A0025	1.2	0.5
A2998	0.9	0.0
MEAN	0.7	0.6
S.D.	0.75	0.95
N	10	10

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL BODY WEIGHT CHANGES (GRAMS)

FEMALES TEST (1930 MG/KG)

ANIMAL#	DAY OF STUDY 0-7	7-14
A0001	1.2	-0.1
A0002	1.3	1.8
A0006	1.5	1.0
A0011	1.4	1.6
A0012	2.8	1.6
A0013	2.6	0.8
A0014	1.4	-0.2
A0022	0.5	1.9
A0024	2.3	-0.6
A3000	1.4	1.0
MEAN	1.6	0.9
S.D.	0.71	0.90
N	10	10

APPENDIX G

Individual Food Consumption Data
(g/kg/day)

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL FOOD CONSUMPTION DATA (GRAMS/KG/DAY)

MALES BSA (1900 MG/KG)

ANIMAL#	DAY OF STUDY 0-7	7-14
A2965	268.2	175.5
A2966	203.9	176.8
A2970	216.3	201.1
A2971	234.8	191.0
A2982	227.4	201.4
A2983	208.1	190.0
A2984	319.1	232.6
A2986	270.1	239.7
A2991	213.9	209.7
A2994	203.3	193.3
MEAN	236.5	201.1
S.D.	37.90	21.31
N	10	10

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL FOOD CONSUMPTION DATA (GRAMS/KG/DAY)

MALES TEST (1930 MG/KG)

ANIMAL#	DAY OF STUDY 0-7	7-14
A2969	237.9	214.5
A2972	235.8	164.6
A2977	210.4	205.0
A2979	196.3	188.8
A2985	222.1	214.4
A2987	310.9	204.7
A2988	229.7	195.4
A2992	207.7	186.7
A2993	286.2	199.8
A2995	216.3	198.4
MEAN	235.3	197.2
S.D.	36.19	14.80
N	10	10

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL FOOD CONSUMPTION DATA (GRAMS/KG/DAY)

FEMALES BSA (1900 MG/KG)

ANIMAL#	DAY OF STUDY 0-7	7-14
A0004	258.7	241.2
A0009	260.0	205.2
A0015	493.6	268.9
A0016	193.8	189.5
A0018	243.1	204.0
A0020	249.6	231.9
A0021	201.7	219.4
A0023	204.1	210.6
A0025	214.4	207.1
A2998	205.5	204.2
MEAN	252.5	218.2
S.D.	88.42	23.24
N	10	10

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL FOOD CONSUMPTION DATA (GRAMS/KG/DAY)

FEMALES TEST (1930 MG/KG)

ANIMAL#	DAY OF STUDY 0-7	7-14
A0001	201.2	221.2
A0002	214.8	233.5
A0006	349.8	191.3
A0011	214.4	209.7
A0012	252.4	196.7
A0013	268.3	231.5
A0014	230.5	309.9
A0022	456.0	223.7
A0024	187.0	182.5
A3000	217.1	256.7
MEAN	259.1	225.7
S.D.	83.16	37.00
N	10	10

APPENDIX H

Individual Food Consumption Data
(g/animal/day)

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL FOOD CONSUMPTION DATA (GRAMS/ANIMAL/DAY)

MALES BSA (1900 MG/KG)

ANIMAL#	DAY OF STUDY 0-7	7-14
A2965	7.4	5.1
A2966	5.6	5.2
A2970	6.3	6.4
A2971	6.3	5.0
A2982	6.5	6.0
A2983	5.9	5.5
A2984	8.9	6.8
A2986	7.4	7.0
A2991	5.7	5.8
A2994	5.7	5.9
MEAN	6.6	5.9
S.D.	1.04	0.69
N	10	10

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL FOOD CONSUMPTION DATA (GRAMS/ANIMAL/DAY)

MALES TEST (1930 MG/KG)

ANIMAL#	DAY OF STUDY 0-7	7-14
A2969	6.6	6.5
A2972	6.5	4.9
A2977	5.6	5.8
A2979	5.4	5.5
A2985	6.1	6.3
A2987	8.6	6.3
A2988	6.4	6.1
A2992	5.9	5.7
A2993	8.1	5.9
A2995	6.1	6.7
MEAN	6.5	6.0
S.D.	1.04	0.52
N	10	10

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL FOOD CONSUMPTION DATA (GRAMS/ANIMAL/DAY)

FEMALES BSA (1900 MG/KG)

ANIMAL#	DAY OF STUDY 0-7	7-14
A0004	7.0	6.7
A0009	7.6	6.2
A0015	13.9	7.3
A0016	5.5	5.5
A0018	6.8	5.9
A0020	7.0	6.6
A0021	5.5	6.1
A0023	6.0	6.1
A0025	5.9	5.9
A2998	6.0	6.0
MEAN	7.1	6.2
S.D.	2.48	0.53
N	10	10

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL FOOD CONSUMPTION DATA (GRAMS/ANIMAL/DAY)

FEMALES TEST (1930 MG/KG)

ANIMAL#	DAY OF STUDY 0-7	7-14
A0001	5.5	6.3
A0002	6.0	6.8
A0006	9.4	5.4
A0011	6.4	6.5
A0012	7.4	6.3
A0013	7.3	6.7
A0014	6.5	9.0
A0022	13.0	6.5
A0024	5.4	5.5
A3000	6.1	7.6
MEAN	7.3	6.7
S.D.	2.33	1.02
N	10	10

APPENDIX I

Individual Gross Necropsy Observations

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

MALES BSA (1900 MG/KG)

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A2965	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2966	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2970	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2971	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2982	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2983	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2984	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2986	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2991	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2994	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

MALES TEST (1930 MG/KG)

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A2969	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2972	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2977	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2979	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2985	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2987	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2988	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2992	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2993	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2995	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

FEMALES BSA (1900 MG/KG)

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A0004	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A0009	5-MAR-03	14	HAIRCOAT: HAIRLOSS; PRESENT FORELIMBS	SCHEDULED EUTHANASIA
A0015	5-MAR-03	14	OVARY: PERIOVARIAN CYST(S); PRESENT LEFT, ONE, 0.3 CM DIAMETER, CLEAR FLUID FILLED	SCHEDULED EUTHANASIA
A0016	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A0018	5-MAR-03	14	OVARY: PERIOVARIAN CYST(S); PRESENT LEFT, ONE, 0.5 X 0.3 X 0.2 CM, CLEAR FLUID FILLED	SCHEDULED EUTHANASIA
A0020	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A0021	5-MAR-03	14	SKIN - SUBCUTANEOUS: ABSCESS; PRESENT RIGHT UROGENITAL AREA, ONE, APPROXIMATELY 0.5 CM DIAMETER, GREEN CASEOUS CONTENTS, PROBABLE CLITORAL GLAND	SCHEDULED EUTHANASIA
A0023	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A0025	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A2998	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

AN ACUTE ORAL TOXICITY STUDY IN MICE

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

FEMALES TEST (1930 MG/KG)

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A0001	5-MAR-03	14	LYMPH NODE, MANDIBULAR: ENLARGED; PRESENT FEW, UP TO 0.5 X 0.5 X 0.2 CM	SCHEDULED EUTHANASIA
A0002	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A0006	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A0011	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A0012	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A0013	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A0014	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A0022	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A0024	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A3000	5-MAR-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

APPENDIX J

SLI Personnel Responsibilities

SLI PERSONNEL RESPONSIBILITIES

[redacted]	Study Director/Manager of Acute Toxicology
	Alternate Contact/Toxicologist
	Managing Director Emeritus
	General Manager
	Director of Toxicology
	Assistant Toxicologist
	Primary Technician/Study Supervisor, Acute Toxicology
	Supervisor, Pharmacy
	Senior Supervisor, Pathology
	Senior Director, Compliance Assurance
	Senior Supervisor, Report Writing
	Senior Supervisor, Quality Assurance
	Senior Director, Pathology
	Archivist