AN ACUTE ORAL TOXICITY STUDY IN MICE WITH E. coli PRODUCED Cry3Bb1.11098(Q349R) PROTEIN

AMENDED FINAL REPORT

Data Guidelines

FDA, EPA-OPPTS, OECD, EEC

Authors

Kimberly L. Bonnette, M.S., LATG (Springborn Laboratories, Inc.)
Paul D. Pyla, B.S. (Monsanto Company)

Original Study Completion Date

July 9, 2001

Amended Study Completion Date

July 10, 2001

Performing Laboratory

Springborn Laboratories, Inc. (SLI)
Ohio Research Center
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3044.856

Monsanto Study No.

SB-2001-085

Submitted to

Monsanto Company
800 N. Lindbergh Blvd.
St. Louis, MO 63141

Springborn 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 submitted this material to the United States Environmental Protection Agency specifically under provisions contained in FIFRA as amended, and thereby consent to the use and disclosure of the material by EPA according to FIFRA. Some pages of the report may be stamped with the following: CONTAINS TRADE SECRET OR OTHERWISE CONFIDENTIAL INFORMATION OF MONSANTO COMPANY. This claim of confidentiality is not meant to convey supplemental claims of confidentiality regarding data subject to disclosure under sections 10 (d) and 10 (e) of FIFRA. In submitting this material to the EPA according to method and format requirements contained in PR Notice 86-5, we do not waive any protection rights involving this material that would have been claimed by the company if this material had not been submitted to the EPA."

COMPANY: _____

COMPANY AGENT: _____

_____ _____

TITLE DATE

1) Bonnette, K. L. and P. D. Pyla (2001). An acute oral toxicity study in mice with E. coli produced Cry3Bb1.11098(Q349R) Protein. MSL-17382, an unpublished study conducted for Monsanto Company.

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the FDA (21 CFR Part 58), the EPA (40 CFR Part 160) and the OECD [ENV/MC/CHEM(98)17].

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 7/9/01

Sponsor/Submitter
Monsanto Company

Date 7/11/01

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	04/16/01
Dose Preparation	04/17/01
Data Audit	05/17/01
Draft Report Review	05/18/01
Protocol Amendment Review	05/18/01
Final Report Review	07/09/01
Amended Final Report Review	07/10/01
Reports to Study Director and Management	05/18/01, 07/09/01, 07/10/01

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.

Rebecca A. Young Date 7/10/01
Rebecca A. Young
Quality Assurance Auditor

for Anita M. Bosau, RQAP-GLP Date 7/10/01
Director of Compliance Assurance

The following revised page has been incorporated into this report.

Page No.	Revision	Reason for Change
9	Added lot numbers for the vehicle control, protein control and the test article.	Formerly omitted from the final report.

Issue of the Report Amendment:

Kimberly L. Bonnette Date: 7/10/01
Kimberly L. Bonnette, M.S., LATG
Study Director

4. TABLE OF CONTENTS

5. LIST OF TABLES AND APPENDICES

Tables

Appendices

6. SUMMARY

The oral toxicity of *E. coli* Produced Cry3Bb1.11098(Q349R) Protein was evaluated in CD-1 mice. This study was performed in which groups of animals received the test article at varied dose levels as indicated below:

Treatment	Target Dose Level[a] (mg/kg)	Analytically Confirmed Dose level[b] (mg/kg)	No. of Animals	
			Male	Female
Vehicle Control	0	0	10	10
Protein Control (BSA)	2700[c]	2900	10	10
E. coli Produced Cry3Bb1.11098(Q349R) Protein	300	400	10	10
E. coli Produced Cry3Bb1.11098(Q349R) Protein	900	1100	10	10
E. coli Produced Cry3Bb1.11098(Q349R) Protein	2700	3200	10	10

[a]A dose volume of 33.3 mL/kg, which was based on the maximum solubility of the protein, was dosed twice (approximately 4 hours apart) on day 0.
[b]The analytical dose confirmation was provided by the Sponsor and is presented in Appendix B.
[c]The protein control was dosed at a level equivalent to the highest protein level.

Following dosing, the mice were observed daily and weighed weekly. A gross necropsy examination was performed on all animals at the time of scheduled euthanasia (day 14). Samples of the dosing solutions were collected on day 0 and subsequently analyzed for concentration, stability, homogeneity, and functional activity. These analyses confirmed that the dosing solutions were at the appropriate concentrations, stable over the dosing period, homogeneous and biologically active.

No mortality occurred during the study. No significant clinical observations were noted during the study. No statistical differences were observed in the body weight or the body weight gain data. The 300 mg/kg *E. coli* Produced Cry3Bb1.11098(Q349R) Protein males and the 900 mg/kg *E. coli* Produced Cry3Bb1.11098(Q349R) Protein males had a significant increase in food consumption as compared to the control group during the day 0 to 7 food consumption interval only. No significant gross internal findings were observed at necropsy on study day 14.

There were no adverse effects attributed to the oral administration of *E. coli* Produced Cry3Bb1.11098(Q349R) Protein in male and female mice at doses of 400, 1100 or 3200 mg/kg (target levels of 300, 900 or 2700 mg/kg, respectively) of body weight. Therefore, the No-Observed-Effect-Level (NOEL) of *E. coli* Produced Cry3Bb1.11098(Q349R) Protein administered as an acute dose by gavage to mice was determined to be at least 3200 (target level of 2700 mg/kg) of body weight, the highest tested dose.

7. INTRODUCTION

This study was performed to assess the short-term toxicity of *E. coli* Produced Cry3Bb1.11098(Q349R) Protein in CD-1 mice when administered by gavage as two separate oral doses administered approximately four hours apart. This study was intended to provide information on the potential health hazards of the test article with respect to oral exposure. Oral administration of the test protein was chosen since it is the potential route of exposure for humans and farm animals. The mouse is one of the preferred species for acute toxicity testing by various U.S. and international regulatory agencies. The CD-1 mouse has been used extensively for toxicological testing and a large database of historical information is available. This study was performed at Springborn Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on April 13, 2001 (GLP initiation date). The in-life phase of the study was initiated with test article administration on April 17, 2001 (day 0) and concluded with necropsy on May 2, 2001.

8. MATERIALS AND METHODS

8.1. Experimental Protocol

This study was performed in general conformance with the US Food and Drug Administration; US EPA Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity, August 1998; the OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects, Subsection 401, February 1987; and the EEC Part B: Methods for the Determination of Toxicity, B.1, No L 383 A/110, December 1992.

8.2. Test, Protein Control and Vehicle Control Articles

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

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date
Vehicle Control Lot No.: 6839197A	V01.002.3044	Clear colorless liquid	04/17/01
Protein Control (BSA) Lot No.: B38089	S01.025.3044	Pale yellow liquid	04/17/01
E. coli Produced CryY3Bb1.11098(Q349R) Protein 300 mg/kg Lot No.: 6962478	S01.022.3044	Cloudy white liquid	04/17/01
E. coli Produced Cry3Bb1.11098(Q349R) Protein 900 mg/kg Lot No.: 6962478	S01.023.3044	White liquid	04/17/01
E. coli Produced Cry3Bb1.11098(Q349R) Protein 2700 mg/kg Lot No.: 6962478	S01.024.3044	White liquid	04/17/01

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 FDA (21 CFR Part 58) and EPA (40 CFR Part 160). A Certificate of Analysis for the Protein Control (BSA) was provided by the Sponsor and is presented in Appendix A. Detailed information on the test article composition was provided by the Sponsor and is presented in Appendix B.

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 bulk test and control articles along with any remaining test and control article solutions were stored frozen (~-70° C) and will be returned to the Sponsor at report finalization.

After completion of dosing, samples of each bulk material were collected, frozen and returned to the Sponsor.

8.5. Method of Test/Control Article Preparation

The test and control articles were administered as received from the Sponsor and were stirred continuously for approximately 20 to 30 minutes to allow them to reach room temperature prior to dosing. The test and control articles were dispensed fresh on the days of dosing and were stirred continuously during the dosing procedures.

8.6. Concentration, Stability Analysis, and Homogeneity of the Test Article in Dosing Solutions

The Sponsor, in accordance with their study specific work procedure, performed verification of concentration and stability of the test article in dosing solutions over the course of the dosing period. Analyses were performed on Day 0 samples collected each day of dosing prior to and following dosing. Following visual inspection it was determined that the test article dosing preparations were not solutions and that homogeneity analysis would need to be performed. Samples for homogeneity were collected each day of dosing on all predose preparations of the test article, packed on dry ice and shipped to the Sponsor for analysis. The results of these analyses are presented in Appendix B.

8.7. Animals and Animal Husbandry

8.7.1. Description, Identification and Housing

Young adult, CD-1® Mice Crl: CD1®(ICR)BR (VAF/Plus ®) were received on April 10, 2001 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].

8.7.2. Environment

The animal room temperature and relative humidity ranges were 68-70°F (20-21°C) and 43-61%, 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.

8.7.3. 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.

8.7.4. 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).

8.7.5. 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.

8.7.6. Animal Selection

All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. Prior to randomization, at least 120 animals were weighed and examined in detail for adverse clinical signs. Animals determined to be suitable as test subjects were assigned randomly to groups based on body weights. The animal numbers and the respective body weight values were entered into the computer (MicroVax 3100). Homogeneity of groups by weight was the criteria of acceptance of the randomization. Disposition of animals not selected for study were documented in the study records. Females were nulliparous and nonpregnant. The male animals were approximately 7 weeks of age and weighed 27.0-32.7 g and the female animals were approximately 8 weeks of age and weighed 23.8-27.1 g prior to fasting.

9. EXPERIMENTAL PROCEDURES

9.1. Dosing

On day 0, the animals chosen for use on study were weighed and fasted 2-3 hours prior to dose administration. The test article was administered orally as two equal separate doses administered 4 hours apart (± 10 minutes) using a ball tipped stainless steel gavage needle attached to a syringe at the following levels:

Group No.	Treatment	Target Dose Level (mg/kg)	Dose Volume (mL/kg)[a, b]	No. of Animals	
				Male	Female
1	Vehicle Control	0	≤ 33.3	10	-
1a	Vehicle Control	0	≤ 33.3	-	10
2	Protein Control (BSA)	2700	≤ 33.3	10	-
2a	Protein Control (BSA)	2700	≤ 33.3	-	10
3	*E. coli* Produced Cry3Bb1.11098(Q349R) Protein	300	≤ 33.3	10	-
3a	*E. coli* Produced Cry3Bb1.11098(Q349R) Protein	300	≤ 33.3	-	10
4	*E. coli* Produced Cry3Bb1.11098(Q349R) Protein	900	≤ 33.3	10	-
4a	*E. coli* Produced Cry3Bb1.11098(Q349R) Protein	900	≤ 33.3	-	10
5	*E. coli* Produced Cry3Bb1.11098(Q349R) Protein	2700	≤ 33.3	10	-
5a	*E. coli* Produced Cry3Bb1.11098(Q349R) Protein	2700	≤ 33.3	-	10

[a]Dose volume was based on the maximum solubility of the proteins. The vehicle control groups received a dose volume equivalent to the highest test article dose volume. The protein control group received a dose equivalent to the highest dose of the test article.
[b]The total dose volume was 66.6 mL/kg per animal (2 doses x 33.3 mL/kg).

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

One animal (A1108/M) from group 4 (900 mg/kg *E. coli* Produced Cry3Bb1.11098(Q349R) Protein) was replaced after an unknown amount of test article was expelled from its mouth during administration of the first dose. The animal appeared normal but was replaced so that the entire target dose could be administered.

9.2. Clinical Observations

Study animals were observed for clinical abnormalities two times on study day 0 (post-dose) and daily thereafter (days 1-14). 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 day 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 entire carcass of each animal was retained in 10% neutral buffered formalin.

9.6. Protocol Deviations

No protocol deviations occurred during this study.

10. ANALYSIS OF DATA

Since less than 50% mortality occurred during the study, the LD50 was estimated to be greater than the administered dose.

Inferential statistical analyses were performed by the SLI MicroVax 3100 TASC 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 control group, when appropriate. All statistical comparisons were two-tailed with a minimum level of significance of 5% ($p < 0.05$).

11. MAINTENANCE OF RAW DATA, RECORDS AND SPECIMENS

The following records were transferred to the SLI archives for a period of 7 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.

12. RESULTS

12.1. Analytical Dose Analysis

The analytical results of the dosing solutions were provided by the Sponsor and are presented in Appendix B. The results are as follows:

Treatment	Target Dose Level[a] (mg/kg)	Analytically Confirmed Dose level[b] (mg/kg)
Vehicle Control	0	0
Protein Control (BSA)	2700[c]	2900
E. coli Produced Cry3Bb1.11098(Q349R) Protein	300	400
E. coli Produced Cry3Bb1.11098(Q349R) Protein	900	1100
E. coli Produced Cry3Bb1.11098(Q349R) Protein	2700	3200

[a]A dose volume of 33.3 mL/kg, which was based on the maximum solubility of the protein, was dosed twice (approximately 4 hours apart) on day 0.
[b]The analytical dose confirmation was provided by the Sponsor and is presented in Appendix B.
[c]The protein control was dosed at a level equivalent to the highest protein level.

12.2. Mortality

Summary Data: Table 1
Individual Data: Appendix C

No mortality occurred during the study.

12.3. Clinical Observations

Summary Data: Table 2
Individual Data: Appendix C

No significant clinical observations were noted during the study. During the study, one animal (Animal No. A1072/M) given 300 mg/kg *E. coli* Produced Cry3Bb1.11098(Q349R) Protein had a raised area on the abdomen which progressed to a scab beginning on day 4 and clearing on day 13. No other findings for any of the groups were noted during the 14 days of observation.

12.4. Body Weight Data

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

No statistical differences were observed in the body weight or the body weight gain data. Slight body weight loss was noted for one female (A1166/F) during the day 0 to 7 body weight interval given the Protein Control (BSA). During the day 7 to 14 body weight interval, a slight body weight loss was noted for one male (A1079/M) given *E. coli* Produced Cry3Bb1.11098(Q349R) Protein (2700 mg/kg), one female (A1125/F) given *E. coli* Produced Cry3Bb1.11098(Q349R) Protein (300 mg/kg) and one female (A1148/F) given *E. coli* Produced Cry3Bb1.11098(Q349R) Protein (900 mg/kg); however, these animals all exceeded their initial body weight by study termination (day 14). Body weight gain was noted for all other animals during the test period.

12.5. Food Consumption Data

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

The 300 mg/kg *E. coli* Produced Cry3Bb1.11098(Q349R) Protein males and the 900 mg/kg *E. coli* Produced Cry3Bb1.11098(Q349R) Protein males had a significant increase in food consumption compared to the vehicle control group during the day 0 to 7 food consumption interval.

12.6. Gross Necropsy

Summary Data: Table 7
Individual Data: Appendix H

No significant gross internal findings were observed at necropsy on study day 14. Two incidents of reddened lungs were noted in females (A1144/F and A1150/F) from the Protein Control (BSA) group. Periovarian cysts were also observed

across all five of the groups and were therefore not considered to be significant.

13. CONCLUSION

There were no adverse effects attributed to the oral administration of *E. coli* Produced Cry3Bb1.11098(Q349R) Protein in male and female mice at doses of 400, 1100 or 3200 mg/kg (target levels of 300, 900 or 2700 mg/kg, respectively) of body weight. Therefore, the No-Observed-Effect-Level (NOEL) of *E. coli* Produced Cry3Bb1.11098(Q349R) Protein administered as an acute dose by gavage to mice was determined to be at least 3200 (target level of 2700 mg/kg) of body weight, the highest tested dose.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 7/9/01

14. REPORT REVIEW

for Rusty E. Rush, M.S., LAT, DABT
Associate Director of Toxicology

Date 7/9/01

15. REFERENCE

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

SLI Study No. 3044.856
CLIENT: MONSANTO COMPANY

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

Sex	Treatment	Target 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	Vehicle Control	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
	Protein Control	2700	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
	E. coli Produced Cry3Bb1.11098(Q349R) Protein	300	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
	E. coli Produced Cry3Bb1.11098(Q349R) Protein	900	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
	E. coli Produced Cry3Bb1.11098(Q349R) Protein	2700	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
Female	Vehicle Control	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
	Protein Control	2700	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
	E. coli Produced Cry3Bb1.11098(Q349R) Protein	300	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
	E. coli Produced Cry3Bb1.11098(Q349R) Protein	900	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
	E. coli Produced Cry3Bb1.11098(Q349R) Protein	2700	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10

SLI STUDY NO.: 3044.856
CLIENT: MONSANTO COMPANY

TABLE 2
AN ACUTE ORAL TOXICITY STUDY IN MICE
SUMMARY OF SURVIVAL AND CLINICAL OBSERVATIONS (OCCURRENCE/ANIMALS AFFECTED)

----- M A L E -----

TABLE RANGE:	DAY 0 TO DAY 14				
GROUP:	1	2	3	4	5
TARGET LEVEL (MG/KG):	0	2700	300	900	2700
NORMAL					
-NO CLINICAL SIGNS	160/10	160/10	151/10	160/10	160/10
DEAD					
-SCHEDULED EUTHANASIA	10/10	10/10	10/10	10/10	10/10
BODY					
-RAISED AREA	0/ 0	0/ 0	8/ 1	0/ 0	0/ 0
-SMALL SCAB(S)	0/ 0	0/ 0	5/ 1	0/ 0	0/ 0

NOTE: DATA REFLECT THE TOTAL OCCURRENCE OF EACH CLINICAL FINDING OVER THE NUMBER OF ANIMALS EXHIBITING THE FINDING.

TABLE 2

SLI STUDY NO.: 3044.856 — AN ACUTE ORAL TOXICITY STUDY IN MICE —

CLIENT: MONSANTO COMPANY — SUMMARY OF SURVIVAL AND CLINICAL OBSERVATIONS (OCCURRENCE/ANIMALS AFFECTED)

----- F E M A L E -----

TABLE RANGE:	DAY 0 TO DAY 14				
GROUP:	1A	2A	3A	4A	5A
TARGET LEVEL (MG/KG):	0	2700	300	900	2700
NORMAL					
-NO CLINICAL SIGNS	160/10	160/10	160/10	160/10	160/10
DEAD					
-SCHEDULED EUTHANASIA	10/10	10/10	10/10	10/10	10/10

NOTE: DATA REFLECT THE TOTAL OCCURRENCE OF EACH CLINICAL FINDING OVER THE NUMBER OF ANIMALS EXHIBITING THE FINDING.

SLI STUDY NO.: 3044.856
CLIENT: MONSANTO COMPANY

TABLE 3
AN ACUTE ORAL TOXICITY STUDY IN MICE
SUMMARY OF BODY WEIGHT DATA (GRAMS)

----- M A L E -----

			GROUP:	1	2	3	4	5
			TARGET LEVEL (MG/KG):	0	2700	300	900	2700
DAY	0	(PREFASTED)	MEAN	29.1	29.2	29.1	29.5	29.3
			S.D.	1.39	1.60	1.42	1.89	1.66
			N	10	10	10	10	10
DAY	0		MEAN	27.8	28.0	27.7	28.3	27.5
			S.D.	1.40	1.40	1.48	1.94	1.28
			N	10	10	10	10	10
DAY	7		MEAN	30.5	31.2	30.8	31.4	30.4
			S.D.	1.82	1.76	1.67	1.84	1.58
			N	10	10	10	10	10
DAY	14		MEAN	32.1	32.6	32.5	32.8	31.9
			S.D.	1.91	1.96	1.86	2.20	1.75
			N	10	10	10	10	10

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL

SLI STUDY NO.: 3044.856
CLIENT: MONSANTO COMPANY

TABLE 3
AN ACUTE ORAL TOXICITY STUDY IN MICE
SUMMARY OF BODY WEIGHT DATA (GRAMS)

----- F E M A L E -----

		GROUP:	1A	2A	3A	4A	5A
		TARGET LEVEL (MG/KG):	0	2700	300	900	2700
DAY	0 (PREFASTED)	MEAN	25.2	25.3	25.4	25.4	25.4
		S.D.	1.12	0.96	1.02	0.95	0.95
		N	10	10	10	10	10
DAY	0	MEAN	24.2	23.9	24.2	24.2	24.2
		S.D.	1.01	0.94	1.23	0.93	0.93
		N	10	10	10	10	10
DAY	7	MEAN	25.9	25.2	25.7	25.4	25.4
		S.D.	1.04	1.10	0.90	1.09	0.96
		N	10	10	10	10	10
DAY	14	MEAN	27.0	26.3	26.9	27.0	26.9
		S.D.	0.95	1.37	1.15	1.68	1.24
		N	10	10	10	10	10

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL

SLI STUDY NO.: 3044.856
CLIENT: MONSANTO COMPANY

TABLE 4
AN ACUTE ORAL TOXICITY STUDY IN MICE
SUMMARY OF BODY WEIGHT GAIN DATA (GRAMS)

----- M A L E -----

		GROUP:	1	2	3	4	5
		TARGET LEVEL (MG/KG):	0	2700	300	900	2700
DAY	0 TO 7	MEAN	2.7	3.2	3.1	3.1	2.9
		S.D.	0.85	0.96	0.94	1.30	1.77
		N	10	10	10	10	10
DAY	7 TO 14	MEAN	1.6	1.5	1.7	1.4	1.5
		S.D.	0.86	0.52	0.54	0.65	0.85
		N	10	10	10	10	10

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL

TABLE 4
AN ACUTE ORAL TOXICITY STUDY IN MICE
SUMMARY OF BODY WEIGHT GAIN DATA (GRAMS)

----- F E M A L E -----

	GROUP:	1A	2A	3A	4A	5A
	TARGET LEVEL (MG/KG):	0	2700	300	900	2700
DAY 0 TO 7	MEAN	1.7	1.3	1.5	1.2	1.2
	S.D.	0.40	0.95	0.85	0.68	0.46
	N	10	10	10	10	10
DAY 7 TO 14	MEAN	1.1	1.1	1.2	1.6	1.5
	S.D.	0.59	0.46	0.85	1.31	0.64
	N	10	10	10	10	10

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL

SLI STUDY NO.: 3044.856
CLIENT: MONSANTO COMPANY

TABLE 5
AN ACUTE ORAL TOXICITY STUDY IN MICE
SUMMARY OF FOOD CONSUMPTION DATA (GRAMS/KG/DAY)

----- M A L E -----

		GROUP:	1	2	3	4	5
		TARGET LEVEL (MG/KG):	0	2700	300	900	2700
DAY	0 TO 7	MEAN	187.5	197.4	206.0*	204.9*	202.3
		S.D.	9.16	11.50	10.48	9.18	18.34
		N	10	10	10	10	10
DAY	7 TO 14	MEAN	184.1	179.0	189.8	182.6	187.4
		S.D.	18.41	12.96	11.95	13.44	10.99
		N	10	10	10	10	10

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05

SLI STUDY NO.: 3044.856
CLIENT: MONSANTO COMPANY

TABLE 5
AN ACUTE ORAL TOXICITY STUDY IN MICE
SUMMARY OF FOOD CONSUMPTION DATA (GRAMS/KG/DAY)

-----F E M A L E -----

	GROUP:	1A	2A	3A	4A	5A
	TARGET LEVEL (MG/KG):	0	2700	300	900	2700
DAY 0 TO 7	MEAN	210.3	202.8	207.3	208.8	208.4
	S.D.	16.79	19.84	19.41	16.19	12.03
	N	10	10	10	10	10
DAY 7 TO 14	MEAN	202.3	198.7	218.9	213.9	201.3
	S.D.	15.61	18.39	31.08	26.88	12.93
	N	10	10	10	10	10

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL

TABLE 6

SLI STUDY NO.: 3044.856 — AN ACUTE ORAL TOXICITY STUDY IN MICE — PAGE 1

CLIENT: MONSANTO COMPANY — SUMMARY OF FOOD CONSUMPTION DATA (GRAMS/ANIMAL/DAY)

----- M A L E -----

		GROUP:	1	2	3	4	5
		TARGET LEVEL (MG/KG):	0	2700	300	900	2700
DAY	0 TO 7	MEAN	5.2	5.5	5.7*	5.8*	5.5
		S.D.	0.37	0.35	0.41	0.34	0.38
		N	10	10	10	10	10
DAY	7 TO 14	MEAN	5.6	5.6	5.8	5.7	5.7
		S.D.	0.48	0.46	0.28	0.49	0.34
		N	10	10	10	10	10

SIGNIFICANTLY DIFFERENT FROM CONTROL: * = P<0.05

TABLE 6

SLI STUDY NO.: 3044.856
CLIENT: MONSANTO COMPANY

AN ACUTE ORAL TOXICITY STUDY IN MICE
SUMMARY OF FOOD CONSUMPTION DATA (GRAMS/ANIMAL/DAY)

----- F E M A L E -----

	GROUP:	1A	2A	3A	4A	5A
	TARGET LEVEL (MG/KG):	0	2700	300	900	2700
DAY 0 TO 7	MEAN	5.1	4.8	5.0	5.1	5.0
	S.D.	0.54	0.48	0.36	0.46	0.28
	N	10	10	10	10	10
DAY 7 TO 14	MEAN	5.2	5.0	5.6	5.4	5.1
	S.D.	0.43	0.52	0.77	0.63	0.35
	N	10	10	10	10	10

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL

SLI STUDY NO.: 3044.856
CLIENT: MONSANTO COMPANY

TABLE 7
AN ACUTE ORAL TOXICITY STUDY IN MICE
SUMMARY OF GROSS NECROPSY OBSERVATIONS
SCHEDULED EUTHANASIA

----- M A L E -----

GROUP:	1	2	3	4	5
TARGET LEVEL (MG/KG):	0	2700	300	900	2700
NUMBER OF ANIMALS EXAMINED	10	10	10	10	10
ALL TISSUES WITHIN NORMAL LIMITS	10	10	10	10	10

SLI STUDY NO.: 3044.856
CLIENT: MONSANTO COMPANY

TABLE 7
AN ACUTE ORAL TOXICITY STUDY IN MICE
SUMMARY OF GROSS NECROPSY OBSERVATIONS
SCHEDULED EUTHANASIA

----- F E M A L E -----

GROUP:	1A	2A	3A	4A	5A
TARGET LEVEL (MG/KG):	0	2700	300	900	2700
NUMBER OF ANIMALS EXAMINED	10	10	10	10	10
ALL TISSUES WITHIN NORMAL LIMITS	9	3	8	9	9
LUNG					
-DARK RED AREA(S)	0	1	0	0	0
-REDDENED	0	1	0	0	0
OVARY					
-PERIOVARIAN CYST(S)	1	5	2	1	1

APPENDIX A

Certificate of Analysis
(Provided by the Sponsor)

CALBIOCHEM NOVABIOCHEM

CERTIFICATE OF ANALYSIS

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

Product Number: 126609

Lot Number: B38089

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

TEST	RESULT
Appearance	Beige, flaky powder
Solubility (A_{550nm} of 1% solution)	0.1
SDS-PAGE	≥ 98%
Loss on drying	2.6%
Heavy Metals	≤ 10 ppm
pH	6.9
Sulfated ash	0.8%
Nuclease	None detected
Protease	None detected
Free Fatty Acid	0.016%
Storage and Handling	Refrigerator (+4°C)

Srirama Bhairi, Ph.D.
Quality Manager, Biologics

3/14/01
Date

FOR RESEARCH USE ONLY; NOT FOR DRUG OR HUMAN USE

APPENDIX B

Analytical Chemistry Report
(Provided by the Sponsor)

Analytical Study Title

Preparation, Formulation and Confirmation of Doses for an Acute Oral Toxicity Study with *E. coli*-Produced Cry3Bb1 Protein (lot 6962478) in Mice Performed at Springborn Laboratories, Inc.

Authors

Paul D. Pyla, B.S., Janet C. Obert, B.S., Richard S. Thoma, M.S., Christopher R. Brown, B.S., Ronald E. Hileman, Ph.D., Larry A. Turner, M.S., and James D. Astwood, Ph.D.

Study Completed On

July 6, 2001

Performing Laboratories

Monsanto Company
Product Safety Center
700 Chesterfield Parkway North
St. Louis, MO 63198

Monsanto Company
Ecology Technology Center
800 North Lindbergh Blvd.
St. Louis, MO 63141

Laboratory Project ID

SB-2001-085
SLI Study 3044.856

Statement of Compliance for Analytical Study Titled:

Preparation, Formulation and Confirmation of Doses for an Acute Oral Toxicity Study with *E. coli*-Produced Cry3Bb1 Protein (lot 6962478) in Mice Performed at Springborn Laboratories, Inc.

This study meets the GLP requirements for 40 CFR Part 160 (EPA) except for the following:

Documentation for the characterization of the Test Protein, *E. coli*-produced Cry3Bb1.11098(Q349R) protein (Study 00-01-39-30), was not completed prior to the initiation of this study. Preliminary pre-study non-GLP data indicated that the Test Protein was *E. coli*-produced Cry3Bb1.11098(Q349R) protein. Study 00-01-39-30, which characterized the Test Protein, was completed on July6, 2001. There was no impact to the study

Principal Investigator: _____ Date: 06 July 2001

Quality Assurance Statement for Analytical Study Titled:

Preparation, Formulation and Confirmation of Doses for an Acute Oral Toxicity Study with *E. coli*-Produced Cry3Bb1 Protein (lot 6962478) in Mice Performed at Springborn Laboratories, Inc.

Reviews conducted by the Quality Assurance Unit confirm that this 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 portion of the study reported herein.

Dates of Audit / Inspection	Phase	Dates reported to Study Director	Dates reported to Management
May 2, 2001	SDS-Page	June 6, 2001	June 6, 2001
May 7, 2001	Amino Acid Analysis	June 11, 2001	June 11, 2001
June 15, 2001	Data Review	June 18, 200	June 18, 200
June 26, 2001	Data Review	July 5, 2001	July 5, 2001
June 26, 2001	Data Review	July 5, 2001	July 5, 2001
June 28, 2001	Data Review	July 5, 2001	July 5, 2001
June 28, 2001	Data Review	July 5, 2001	July 5, 2001
July 2, 100	Draft Report Review	July 5, 2001	July 5, 2001

Paula A. Price

July 6, 2001

Paula A. Price
Quality Assurance Unit
Monsanto Regulatory, Monsanto Company

Date

Signatures of Final Analytical Report Approval

Study Number: SB-2001-085

Title: Preparation, Formulation and Confirmation of Doses for an Acute Oral Toxicity Study with *E. coli*-Produced Cry3Bb1 Protein (lot 6962478) in Mice Performed at Springborn Laboratories, Inc.

Facilities and Addresses:

Monsanto Company
Product Safety Center
700 Chesterfield Pkwy N.
St. Louis, MO 63198

Monsanto Company
Ecology Technology Center
800 N. Lindbergh Blvd.
St. Louis, MO 63141

Study Director: Kimberly L. Bonnette, Springborn Laboratories, Inc.

Principal Investigator: Paul D. Pyla

Contributors: Paul D. Pyla, Janet C. Obert, Richard S. Thoma, Christopher R. Brown, Ronald E. Hileman, Larry A. Turner, Christopher M. Dalton and James D. Astwood.

Study Start Date: April 17, 2001

Study Completion Date: July 6, 2001

Records Retention: All study specific raw data, protocols, final reports and facility records will be retained at Monsanto-St. Louis.

06 July 2001

Paul D. Pyla, B.S.
Principal Investigator, Author

July 6th, 2001

James D. Astwood, Ph.D.
Co-Director, Product Safety Center

Table of Contents

Tables

Figures

Appendix

Abbreviations

aa	amino acid
BSA	Bovine Serum Albumin
BW	Body weight
CFR	Code of Federal Regulations
Conc.	Concentration
DTT	Dithiothreitol
EPA	Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GLP	Good Laboratory Practice
kDa	Kilodalton
kg	Kilogram
mA	Milliampere
MALDI-TOF	Matrix Assisted Laser Desorption Ionization - Time of Flight
mL	Milliliter
mM	Millimolar
mg	Milligram
MSL	Monsanto St. Louis report
NBP	Monsanto notebook page
nmol	nanoMolar
OD	Optical Density, measure of light absorbance
PAGE	Polyacrylamide Gel Electrophoresis
Protein Control	BSA lot B38089
PVDF	Polyvinylidene difluoride
SDS	Sodium dodecyl sulfate
SLI	Springborn Laboratories, Inc.
SOP	Standard operating procedure
Test Protein	*E. coli* produced Cry3Bb1.11098(Q349R) lot 6962478
µL	Microliter
µg	Microgram
(v/v)	Volume to volume ratio
(w/v)	Weight to volume ratio
~ or ≈	Approximately

1.0 Summary

This study describes the formulation and preparation of test dosing solutions, confirmation of the administered dose levels, stability, and homogeneity of *E. coli* produced Cry3Bb1.11098(Q349R) protein (Test Protein), lot 6962478, used for an acute oral toxicity study conducted in mice. Data are provided that experimentally confirmed the Test Protein identity (N-terminal sequence analysis), protein concentration and homogeneity (amino acid composition analysis), and stability (densitometric analysis of colloidal Brilliant Blue G stained SDS-polyacrylamide gels and insect diet incorporation assay with the highest Test Protein dose to assess functional activity) of each dose administered to the mice.

Three targeted doses of 300, 900 and 2700 mg/kg mouse body weight (BW) of the Test Protein (referred to as the low, mid and high Test Protein doses) were prepared in phosphate buffer. These doses were based on the solubility of the Test Protein in buffer and what was capable of being delivered through a gavage needle as a free flowing suspension. The target dose level of the high test dose was unattainable in a single dose due to the limited solubility of the Test Protein. Consequently, two-one mL aliquots of the Test Protein were administered by gavage on the same day, four hours apart. Males were dosed on one day and females were dosed on the following day. Bovine serum albumin (BSA) served as the protein control and phosphate buffer was the vehicle control. Samples of the three formulated Test Protein doses and the protein control dose were collected prior to administration to mice. Samples were also collected after administration of the final dose. These samples were stored at approximately –80 °C until analyzed for homogeneity, stability and dose confirmation.

Results from this study indicated that a stable, homogenous formulation of *E.coli*-produced Cry3Bb1 protein (Test Protein) was achieved at all three dosing concentrations. The identity of the Test Protein and Protein Control was confirmed by N-terminal sequence analysis. The concentration of the test dosing solutions was calculated from amino acid composition analysis and percent purity data. The experimentally confirmed Test Protein dose concentrations were 400, 1100 and 3200 mg/kg (BW).

All test dose preparations were comparable to their respective targeted doses. Homogeneity, by amino acid composition analysis, was assessed for all Test Protein doses and confirmed. Purity, as assessed by densitometric analysis of test and control dose formulations separated on SDS-PAGE, and stained with Colloidal Brilliant Blue G, was comparable across all doses. The highest Test Protein dose, from which the other doses were formulated, was tested in an insect diet incorporation assay to assess functional activity. The insecticidal activity assay indicated that the Test Protein was functionally active against Colorado potato beetle.

The total amount of protein administered in the BSA protein control dose was confirmed by amino acid analysis to be comparable [2900 mg BSA/ kg (BW)] to the highest targeted Test Protein dose.

These data established the homogeneity, stability (as assessed by SDS-PAGE and functional activity by an insect bioassay), and the dose levels for the Test Protein used within the oral acute gavage study.

2.0 Introduction

Genetically modified corn, corn event MON 863, produces a variant of the *Bacillus thuringiensis* (*B.t.*) Cry3Bb1 protein. Corn plants producing this Cry3Bb1 protein variant are resistant to larval feeding damage from the coleopteran insect, corn rootworm (Coleoptera, Chrysomelidae, *Diabrotica* sp). The physical and functional equivalence of the modified Cry3Bb1 produced in corn event MON 863 to the Cry3Bb1.11098 protein produced by *B.t.* strain EG11098 was previously demonstrated (Holleschak *et al.*, 2001a; Holleschak *et al.*, 2001b). Recent DNA sequencing of the *cry3Bb1* coding region in corn event MON 863 has shown that it encodes a Q349R substitution in the expected Cry3Bb1.11098 protein (Cavato and Lirette, 2001). This was subsequently verified using MALDI-TOF mass spectrometry of the Cry3Bb1 protein produced in corn event MON 863 (Thoma *et al.*, 2001).

Food, feed and environmental safety evaluations that utilize purified protein require gram quantities. Because of the relatively low level of the Cry3Bb1 protein variant in tissues from corn event MON 863, it was not feasible to isolate protein directly from plants. Therefore, an *E. coli* heterologous protein production system was designed using the same *cry3Bb1.11098(Q349R)* DNA sequence present in corn event MON 863. The deduced amino acid sequences of the *E. coli*- and corn-produced proteins are thus identical.

The Test Protein was purified and characterized (Hileman *et al.*, 2001) as a test substance for the mouse acute oral toxicity study. To justify substitution of a plant-produced protein with a microbially-produced surrogate, the Test Protein was shown to be biochemically and functionally equivalent to the Cry3Bb1 protein produced in corn event MON 863. (Hileman et al., 2001).

3.0 Purpose

The purpose of this study was to formulate and confirm the dose levels, homogeneity and stability of the Test Protein doses. The identity of the Test and Control Proteins was also assessed by N-terminal sequence analysis. The test and control doses were prepared for use in an acute oral toxicity study conducted in mice.

4.0 Materials

4.1 *Test Substance.* The test substance was a variant of the wild type Cry3Bb1 protein. The test substance was *E. coli*-produced Cry3Bb1.11098(Q349R) protein (lot 6962478), isolated using chromatographic methods from a large-scale fermentation of *E. coli* containing the pET24d(+)/25097 expression plasmid (Hileman *et al.*, 2001).

4.2 *Control Substances.*

4.2.1 *Protein Control.* The protein control substance was bovine serum albumin (BSA) (Calbiochem/NovaBiochem, Prod. No. 126609, lot B38089), which was characterized by the manufacturer. A purity of 98% (supplied by the manufacturer) and a total protein content assessed at ~83.7% of the powdered material was used and corresponds to the pre-study value determined for BSA.

4.2.2 *Vehicle Control.* The vehicle control substance for this study was phosphate buffer, lot 6839197A.

4.3 *Reference substance.* Analytical references were used where appropriate for the various analytical tests employed and are described in the relevant sections of this report.

Fresh test substance, *E.coli* produced Cry3Bb1.11098(Q349R) protein (lot 6962478) was removed from each dose during the oral toxicity study, and stored at approximately –80 °C. Upon initiating experiments, a stock supply of the high test dose was placed in 2× Laemmli (Laemmli, 1970) buffer and used as a reference standard (described as the dose control in this report) for SDS-PAGE/imaging/densitometry stability gels. Protein concentration from initial amino acid composition analysis and a purity of 94% from preliminary pre-study data were used.

5.0 Test System

There was no test system for this study specific work procedure. Analytical procedures were employed to assess and confirm the test and control doses.

6.0 Justification of the Analytical Methods

The analytical methods chosen to confirm dose concentrations, homogeneity analysis and stability are accepted methods that are commonly used and extensively referenced in the scientific literature relevant to the purposes for which they were employed (Deutcher, 1990). The insect bioassay used to assess the functional activity of the Test Protein and was designed as an accurate and reproducible assay for the coleopteran pest, Colorado potato beetle (*Leptinotarsa decemlineata*). The Test Protein exhibits insecticidal activity against both the corn rootworm and the Colorado potato beetle (CPB). Because the CPB insect assay is quantitative, accurate and reproducible, this was an appropriate assay to assess the relative functional activity for the Test Protein for this study.

7.0 Methods

7.1 *Method for Calculation of Test Protein Doses.* Twenty mice, 10 male and 10 female (approximate body weight of 0.03 kg), were used for each dose level. Three targeted Test Protein doses of 300, 900 and 2700 mg/kg mouse body weight (BW) of the Test Protein (referred to as the low, mid and high Test Protein doses) were selected. These doses were selected based on the solubility of the Test Protein and what was capable of being delivered through a gavage needle as a free flowing suspension. The target dose level of the high test dose was unattainable in a single dose due to the limited solubility and the highly viscous nature of the Test Protein preparation. Consequently, two equal volume aliquots of the Test Protein doses were administered by gavage on the same day, four hours apart. Since the solubility and viscosity made it impossible to dose at a rate of 66.66 ml/kg (BW), the total dose was divided into two equal volumes which were then administered to mice in the following manner: mice were given a first dose of approximately one milliliter, then dosed again four hours later on the same day with approximately one milliliter (dose volume was adjusted for body weight and delivered at a rate of 33.33 ml/kg). Males were dosed on one day and the females the next.

Approximately 80 mL of each dose was prepared, of which approximately 40 mL was required for dosing, the excess was used for sampling and provided reserve material in the event of an average mouse body weight being greater than 0.03 kg. The doses were prepared one day in advance of the start of the mouse acute oral toxicity study.

The calculations for Test Protein doses were based on pre-study data using values of 43.5 mg/mL total protein and a purity of 94%. A BSA purity of 98% was supplied by the manufacturer. An example calculation for the high Test Protein dose is described below. For the high test dose, which was targeted for 2700 mg/kg Test Protein, 81 mg of the Test Protein was needed:

$$\frac{\text{High Test Protein dose Target}}{\text{Dose rate}} = \frac{2700 \text{ mg/kg (BW)}}{33.33 \text{ mL/kg}} = 81 \text{ mg/mL}$$

The high Test Protein dose could not be formulated in a single one mL dose at 81 mg/mL, due to limited solubility of the protein; the preparation would have had the consistency of paste and could not be administered as a free flowing suspension through the gavage needle. To achieve the targeted total dose, the high Test Protein dosing suspension was administered as two equal doses given four hours apart on the same day. Since the Test Protein was divided into two doses, 40.5 mg was needed per dose:

Test Protein Per Dose = 81 mg ÷ 2 doses = **40.5 mg**

Base upon pre-study data, the concentration of the Test Protein was at a total protein concentration of 43.5 mg/mL with purity at approximately 94%. Based on this information, the purity corrected dose was at 40.9 mg/mL (43.5 mg/mL× 0.94 = 40.9 mg/mL).

To account for variability and allow sampling, 80 mL (40 mL for dosing, and 40 mL for reserve) of dosing suspension was transferred to a separate container from the master batch of Test Protein. The remaining Test Protein doses were similarly calculated and diluted with phosphate buffer to achieve the targeted dose concentrations (see Table 2).

7.2 *Formulation of the Test and Control Protein Doses for the Mouse Acute Oral Toxicity Study.* The preparation of doses from the Test Protein was conducted as described below. The Test Protein was removed from the 4 °C refrigerator and allowed to warm to room temperature and stirred continuously. Doses of the Test Protein were independently formulated in 125 mL clear polypropylene sample containers with a triangular magnetic stir bar and a screw cap lid. Each dose was labeled with the study number, dose description and target dose level. The Test Protein doses were prepared by adding a known volume of the Test Protein to the appropriately labeled dose container. Then, if appropriate, a known volume of phosphate buffer was added into each container. The actual amounts of protein added are shown in Table 2.

For preparation of the Protein Control dose, 3.97 g of bovine serum albumin was dissolved in a final volume of 80 mL of phosphate buffer, giving an actual concentration of 40.8 mg/mL (based upon the purity supplied from the manufacturer, 98%, and using preliminary pre-study data for total protein content assessed at ~83.7% of the powdered material). As with the Test Protein doses, the control protein dose was formulated in an appropriately labeled 125 mL container with a magnetic stir bar. Finally, an appropriately labeled container containing phosphate buffer (vehicle control) with a magnetic stir bar was prepared. All doses were stored at approximately 4 °C, then on wet ice for transfer to the facility conducting the in-life phase of this study (Springborn Laboratories, Inc.)

7.3 *Sample Collection Before and After the Mouse Gavage for Dose Analysis.* At the Springborn Laboratories, Inc. (SLI) facility, prior to dosing, the dose solutions/suspensions were stirred at room temperature for at least 20 minutes and then throughout the dosing period. In the morning of each day of dosing, just prior to administration of the first dose, sample aliquots for stability/concentration measurement were collected from each dosing solution/suspension, and immediately frozen on dry ice. Each sample aliquot collected was approximately 0.5 mL. An identical second set of sample aliquots for each dose was taken just after administration of the second dose (approximately four hours later). Because Test Protein doses were visually assessed as suspensions, homogeneity samples were taken from the Test Protein doses. A set of three homogeneity sample aliquots from the Test Protein doses were taken immediately before dosing in the morning (aliquots were removed from the top left, middle center and bottom right of each dose container for each dose level) on each day of dosing, and immediately frozen on dry ice. Each sample aliquot was approximately 0.5 mL. Sample tubes containing aliquots were labeled with sampling date, study number, dose description, dose level, "Pre-Dose" or "Post Dose", and whether they were stability/concentration or homogeneity samples. Homogeneity samples labels further described the sample as being taken from top left (TL), middle center (MC), or bottom right (BR) of the Test Protein dosing container. The BSA Protein Control was visually assessed as to be a true solution. Thus, homogeneity sampling was not required for the Protein Control.

After completion of the first dosing in the morning, doses were stored at 4 °C. Prior to the afternoon dosing, the doses were stirred at room temperature for at least 20 minutes and then throughout the dosing period. A final stability sample from each dose was taken at the completion of dosing; the first day for males, the second day for females. A summary of sample collection appears below.

Dose Time	Sample	
	Stability/Concentration	Homogeneity
Males, Day 1, AM	before dosing	before dosing
Males, Day 1, PM	after final dosing	not sampled
Females, Day 2, AM	before dosing	before dosing
Females, Day 2, PM	after final dosing	not sampled

The unused dose solution/suspension portions (at the completion of the dosing day of the last group of mice) were stored frozen at approximately –80 °C and were retained at the Springborn Laboratories, Inc. (SLI) facility. Upon completion of the study, the unused portions, in their original containers, were packed on dry ice and transported back to Monsanto Company (Chesterfield Village site).

7.4 *Amino Acid Composition Analysis.* Frozen samples labeled "Pre-Dose" and "Post-Dose" stability/concentration and homogeneity samples were thawed. Aliquots from each sample were removed and diluted with water based upon the targeted dose protein concentration to approximately 1 mg/mL. All samples were prepared independently and analyzed in triplicate. The mean of the total protein concentration of each dose was reported. The 2700 mg Test Protein/kg (BW) dose samples were diluted approximately 40-fold prior to analysis; the 900 mg Test Protein/kg (BW) dose samples were diluted approximately 10-fold prior to analysis; the 300 mg Test Protein/kg (BW) dose samples were diluted approximately 4-fold prior to analysis; and the BSA control protein dose samples were diluted approximately 50-fold prior to analysis

Protein concentration was assessed by amino acid composition analysis, according to SOP No. SOP BR-EQ-0376-01. Data was obtained by utilizing a Hitachi L-8800 Amino Acid Analyzer with AAA System Manager. All samples were weighed on an analytical balance for increased accuracy. Protein samples and a norvaline internal control were added to hydrolysis tubes and evaporated to dryness in a Speed Vac Concentrator. A batch consisted of a maximum of 14 hydrolysis tubes placed in a reaction vial. Each hydrolyzed batch included a National Institute for Standards and Technology (NIST, Gaithersburg, MD) amino acid standard for the calibration curve, a NIST BSA hydrolysis control and twelve or fewer test samples. Five hundred microliters of 6N Hydrochloric acid (HCl) containing 1% Phenol, was added to the bottom of the reaction vial. The reaction vial was placed in Pico-Tag Workstation (Waters) and subjected to a vacuum (~30 s) step followed by a nitrogen purge (~5 s).

The vial was evacuated under vacuum and nitrogen purged three times to minimize oxygen levels. A final 30 s vacuum application was performed before sealing the vial. The vial was heated at 150 °C for ~90 min for protein hydrolysis. After 90 min, the vial was removed from the oven and allowed to cool before venting to the atmosphere. The hydrolysis tubes were evaporated to dryness in a speed-vac concentrator to remove any remaining HCl. Prior to amino acid analysis, the amino acid mixture was reconstituted in protein hydrolyzate-1 buffer (Hitachi). The Hitachi L-8800 amino acid analyzer post column analysis system was used for the analysis. The amino acids were separated over an ion-exchange HPLC pH analysis column and reacted with the derivatizing reagent, ninhydrin for detection. Sixteen amino acids in elution position order, aspartic acid (which represents both asparagine and aspartic acid), threonine, serine, glutamic acid (which represents both glutamine and glutamic acid), glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine were monitored at 570 nm. The imino acid proline was monitored at 440 nm. Measurement of peak areas was used for the determination of amino acid concentration. No peak area for tryptophan was determined because this amino acid degrades under acid hydrolysis conditions.

Prior to data analysis of a batch, a calibration curve for each amino acid was generated based on a 0.5, 1.5, 3.0 and 4.5 nmol injection of the NIST amino acid standard. From these calibration curves, peak areas were converted to nanomolar concentrations for each amino acid. A mole percent ratio was generated by dividing the nanomolar yield of an amino acid by the total yield. Picomoles of protein analyzed was then determined by averaging the seven best resolved amino acids (asparagine, glutamine, alanine, leucine, phenylalanine, lysine and arginine) picomolar yield per amino acid. This value was then used to generate an amino acid profile which could be compared to the theoretical amino acid composition based on the amino acid sequence of the protein. Percent error for each amino acid was determined by comparing the difference between the experimental and theoretical number of amino acids. Average percent error was determined by averaging the percent error for each amino acid (cystine not included). After using the internal control, norvaline, to account for injection variability, protein concentration was determined by accounting for sample volumes, dilutions and the norvaline correction.

7.5 *SDS-PAGE/Colloidal Blue Staining/Densitometry.* This technique was one of two ways (SDS-PAGE and insect bioassay) used to assess the stability of the test and control protein doses. Proteins contained in each sample were separated by SDS-PAGE according to SOP No. PB-EQP-005-01 and detected by colloidal Brilliant Blue G staining.

A detailed description of the imaging/densitometry method was archived with the raw data. Aliquots of each dose were diluted to provide three concentrations of Test Protein that were compatible with the SDS-PAGE technique. These samples were compared by densitometry to assess the stability of the Test Protein during the mouse gavage experiment.

These analyses were used to confirm the stability of the dosing solutions, and estimate the percent purity of Test Protein in the test dosing solutions. An aliquot of "Pre-Dose" and "Post Dose" concentration/stability samples was analyzed by SDS-PAGE and densitometry at three protein load levels (1, 2 and 3 µg/lane loadings). Male and female dose samples (which originated from the same container at each dose) were analyzed on separate SDS-polyacrylamide gels. Prior to analysis, samples of the test and control protein doses were diluted with an appropriate amount of 2× Laemmli (Laemmli, 1970) buffer and deionized water. Samples were then heated at approximately 100 °C for approximately 5 minutes. Samples were subjected to SDS-PAGE using precast 4→20% Tris-glycine mini-gels for approximately 2 hours at 120 V (constant voltage) according to SOP No. PB-EQP-005-01. The mini-gels were fixed in 40% v/v methanol, 7% v/v acetic acid for at least 30 minutes at room temperature on an orbital shaker. The fixing solution was replaced with Colloidal Brilliant Blue G (Neuhoff *et al.*, 1988) dye solution and shaken overnight. The gels were de-stained with 25% v/v methanol, for approximately 6 hours, or until the proteins appeared as blue bands against a clear background. A permanent record of the results were generated using a Bio-Rad densitometer (Model GS-710) interfaced with a computer workstation using QuantityOne software version 4.0.3 from Bio-Rad Corporation. The purity/stability of the Test Protein in the dosing solutions were estimated by densitometry.

7.6 *Insect Bioassay.* A Colorado potato beetle diet incorporation bioassay (SOP No. BR-ME-0044-02) was used to assess the functional activity of the formulated Test Protein. The final unused portion of the high test dose, female post dose, was allowed to thaw on ice and an insect bioassay sample was prepared by dilution in phosphate buffer. A test dilution of 1 mg/mL was prepared for bioassay. Further dilutions bracketing the expected LC_{50} value (Hileman *et al.*, 2001) for the Test Protein (0.2, 0.4, 0.9, 1.8, 3.5, and 7 µg/ml) were prepared. All dilutions that were prepared for the target dose concentrations were calculated based upon the final protein concentration and purity data from this work procedure. The Test Protein and two negative controls (Protein Control (BSA) and phosphate buffer), were prepared concomitantly. This analysis was included by amendment to the study specific work procedure (appendix 1). The addition of this assay positively affects the outcome of study specific work procedure results.

7.7 *Statistical Methods for Insect Bioassay.* The LC_{50} and the 95% confidence interval (CI) was estimated for each Test Protein dose response replicate using SAS Probit Procedure (release 6.12, SAS Institute 1989-1996). The probit model is as follows:

$$p = C + (1 - C)F(x'b)$$

where p is the observed probability of mortality given dose (x) considering parameter estimates (b), C is the natural response rate and F is the cumulative distribution function. The BSA Protein Control and the phosphate buffer control treatments were compared to the water-only control using survival percentage. No statistical comparison was performed on these treatments as a result of minimal statistical error (i.e. less than 5% mortality was observed in any of the control treatments).

7.8 *SDS-PAGE and Subsequent N-terminal sequence analysis.* Aliquots of the Test Protein and Protein Control were diluted with 2× Laemmli (Laemmli, 1970) sample buffer. Pre-stained molecular weight markers (~1 µg/band) were used to verify electrotransfer of protein to the membrane. These samples were heated at approximately 100 °C for 4 minutes and applied to a 4→20% pre-cast polyacrylamide gradient mini-gel. The Test Protein (~12 µg) and Protein Control samples (~12 µg) were loaded into 4 separate lanes. Electrophoresis was performed according to SOP PB-EQP-005-01 at constant voltage (190 V) for approximately one hour (until the dye front reached the bottom of the gel). Proteins were electrotransferred to a 0.2 µm PVDF membrane for 1.1 h at a constant 30 V. Protein bands were detected by briefly staining the membranes with Ponceau S reagent [0.1% (w/v) Ponceua S in 5% (v/v) acetic acid] followed by several one minute washes in water.

Bands that appeared at approximately 74 kDa were cut from the membranes. N-terminal sequence analysis was performed using automated cycles of Edman degradation (Hunkapillar *et al.*, 1983) using an Applied Biosystems Procise™ 494 Protein Sequencer with 140C Microgradient System, 785A Programmable Absorbance Detector and Procise Control Software (version 1.1a) according to SOP BR-EQ-0263-01. Prior to and after sequencing the Test Protein and Protein Control, the sequence and repetitive yield of a β-lactoglobulin sample was analyzed and used as an acceptance criteria.

8.0 Control of Bias and Quality Control Measures

Appropriate sets of dosing samples were analyzed concurrently on SDS-PAGE gels to eliminate any run-to-run variability. Colloidal Brilliant Blue G stained SDS-PAGE gels included freshly prepared samples of the Test Protein serving as a reference standard. The amino acid composition analysis and the functional assay (insect bioassay) included replicates for each treatment. Dosing samples were clearly labeled with the study number, dosing description, dosing level, and "Pre-Dose" and "Post-Dose" to assure the correct tubes were sampled at the time of analysis.

9.0 Results and Discussion

The analytical phase of the mouse oral acute toxicity study was performed to formulate and assess target dose concentrations, dose stability, and dose homogeneity of the Test Protein doses throughout the study. Three targeted doses of 300, 900 and 2700 mg/kg mouse body weight (BW) of the Test Protein (referred to as the low, mid and high Test Protein doses) were prepared in phosphate buffer. The Test Protein was not administered in a single dose due to its highly viscous nature, which prevented formulation of a single high-concentration dose of 81 mg/mL that could be delivered as a free flowing suspension through a gavage needle. Two-one mL doses at the highest concentration, 40.5 mg/mL, were used as an alternative to a single dose. A Protein Control dose (BSA) was used, targeted at the same total protein concentration as the high Test Protein dose. Analyses were obtained to experimentally confirm the Test Protein concentration (amino acid composition analysis), stability (densitometric analysis of colloidal Brilliant Blue G stained SDS-polyacrylamide gels and insect bioassay), homogeneity (amino acid composition analysis) and functional activity (insect bioassay) in each dose administered to the mice. Analysis to assess identity (N-terminal sequence analysis) of the Test and Control Proteins from the high Test Protein doses was also performed.

9.1 *Total Protein Concentration of Test and Control Protein Doses.* The results for the experimentally estimated protein concentrations in the test and control doses are shown in Table 3. The experimentally estimated protein concentrations for test and control protein doses were comparable to the targeted total protein concentrations, indicating that these doses were appropriately formulated and prepared. For all test and control protein doses, there was a less than a six percent difference between the "Pre-Dose" and "Post Dose" samples, indicating that the dose preparations/concentrations remained constant throughout the dosing procedure.

9.2 *Stability and Purity of Test and Control Doses.* Dosing samples were analyzed using densitometry of Colloidal Brilliant Blue G stained SDS-polyacrylamide gels (Figures 2-4) to assess the stability of the Test Protein in Test Protein doses for the duration of the acute oral toxicity study. Visual inspection of the gels indicated that the Test Protein was stable throughout the course of the acute oral toxicity study (Figures 2-4). Purity was calculated as the sum of the area percent for the major band present. The stability of the protein control dose (BSA) was also established for the duration of the study (Figure 5). The purity of the Test Protein in the dosing samples was estimated using densitometry. A summary of the purity data (percent area) for the Test Protein doses is summarized in Table 4. The average percent purity of Test Protein ranged from approximately 96.2% to 99.2% (the average purity was 97.5 ± 1.1%). These purity values are similar to the initial estimate of 94% obtained pre-study. The data also supports the stability of the Test Protein throughout the course of the acute oral toxicity study. There was little difference between the percent purity for the "Pre-Dose" and "Post Dose" samples at all dosing levels. The lack of changes in purity establish physical stability of the Test Protein throughout the course of the acute oral toxicity study.

9.3 *Homogeneity Assessment of Test and Control Protein Doses by Amino Acid Composition Analysis.* The results for the experimentally estimated protein concentration in the test and control protein doses are shown in Table 5. The experimentally estimated protein concentrations for all test and control protein doses were comparable to the targeted total protein concentrations, indicating that these doses were appropriately formulated, prepared and homogeneous. Based upon the limits of the analysis, it is concluded from this data that the protein concentration for all Test Protein doses were homogeneous at the time of dosing.

9.4 *Functional Activity of the Test Protein.* The functional activity of the Test Protein was assessed after dosing was completed. The Test Protein for the "Post-Dose" high test dose female sample (which was the last sample taken during dosing) was functionally active in the insect diet incorporation assay, indicating that the test protein was active through the duration of dosing. The LC_{50} value derived from the assay data is shown below and is comparable to data reported from the characterization report of the Test Protein (Hileman *et al.*, 2001).

Table 1. Insect bioassay results using the larvae of CPB. Doses were corrected for purity prior to analysis.

Protein Source	Assay Replicate[a]	LC_{50} (µg/mL)	95% CI
E. coli	1	0.95	0.60-1.44
	2	1.06	0.84-1.33
	Pooled[b]	1.09	0.89-1.31

[a] A total of 128 insects were tested for each replicate.
[b] Calculated using the all data from assay replicates 1 and 2.

9.5 *Identity of Test Protein and Protein Control by N-Terminal Sequence Analysis.* N-terminal sequence analysis, was used to assess the identity of the Test Protein and the Protein Control (BSA). N-terminal sequence analysis results for the Test Protein, shown below, indicated a clear primary sequence which was consistent with the expected sequence for the N-terminus of the Test Protein. These results demonstrated that the major band observed in the Test Protein was the Cry3Bb1 protein.

Figure 1. N-terminal amino acid sequence analysis results. The expected amino acid (residues 1-16) sequence of the Cry3Bb1.11098 protein and observed sequences obtained from the Test Protein are shown. The sequence information is shown using the single letter amino acid code[a].

```
Expected      1M A N P N N R S E H D T I K V T ..
E. coli         2A N P N N R S E H D T I K V T16
```

[a] The single letter IUPAC-IUB amino acid code is A, alanine; D, aspartic acid; E, glutamic acid; F, phenylalanine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, Asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; Y, tyrosine.

N-terminal sequence analysis results for the Protein Control indicated a clear primary sequence that was consistent with the expected sequence for the N-terminus of bovine serum albumin (BSA). These results indicate the correct proteins were used for the acute oral toxicity study.

9.6 *Experimentally Estimated Dosing Levels for the Test and Protein Control Doses.* A summary of the dosing information for the Test Protein used in the mouse gavage study is shown in Table 6. The calculation of doses was based on the total protein concentration and percent purity of the Test Protein in the dosing solutions. The Test Protein was shown to be stable during the course of the mouse gavage study. Therefore, the observed dose was calculated as the average of the observed dose concentrations for the "Pre-Dose" and "Post Dose" samples.

The concentration of the test dose formulations calculated from the amino acid composition analysis and percent purity data averaged 400, 1100 and 3200 mg Test Protein/kg (BW), compared to targeted concentrations of 300, 900 and 2700 mg Test Protein/kg (BW), respectively.

10.0 Conclusions

Results from this study indicated that a stable, homogenous formulation of *E.coli*-produced Cry3Bb1 (lot 6962478) protein (Test Protein) was achieved at all three dosing concentrations. The identity of the Test Protein and Protein Control were confirmed by N-terminal sequence analysis. The concentration of the test dosing solutions was calculated from amino acid composition analysis and percent purity data. The experimentally confirmed Test Protein dose concentrations were 400, 1100 and 3200 mg/kg (BW).

All test dose preparations were comparable to their respective targeted doses. Homogeneity by amino acid composition analysis was assessed for all Test Protein doses and confirmed. Purity, as assessed by densitometric analysis of test and control dose formulations separated on SDS-PAGE, and stained with Colloidal Brilliant Blue G, was comparable across all doses. The highest Test Protein dose, from which the other doses were formulated, was tested using an insect diet incorporation assay to assess functional activity. The insecticidal activity assay indicated that the Test Protein was functionally active against Colorado potato beetle. The total amount of protein administered in the BSA protein control dose was confirmed by amino acid composition analysis to be comparable [2900 mg BSA/ kg (BW)] to the highest targeted Test Protein dose.

These data established the homogeneity, stability (as assessed by SDS-PAGE and functional activity by an insect bioassay), and the dose levels for the Test Protein used within the oral acute gavage study.

11.0 References

Cavato, T. A., and Lirette, R. P. (2001). PCR analysis and DNA sequence of the insert in corn rootworm event MON 863. (Monsanto Company, St. Louis, MO) Study 01-01-39-02, Technical Report MSL-17108.

Deutcher, M. P. (1990). Guide to protein purification. *In* Methods Enzymol (New York: Academic Press, Inc.).

Hileman, R. E., Turner, L. A., Holleschak, G., Thoma, R. S., Brown, C. R., and Astwood, J. D. (2001). Characterization and Equivalence of the Cry3Bb1 Protein Produced by *E. coli* Fermentation and Corn Event MON 863. (Monsanto Company, St. Louis, MO) Study 01-01-39-30, Technical Report MSL-17274.

Holleschak, G., Hileman, R. E., and Astwood, J. D. (2001a). Amended report for MSL-16596: Assessment of the physicochemical equivalence of Cry3Bb1.11098 and NPTII proteins in corn event MON 863 to microbial sources. (Monsanto Company, St. Louis, MO) Study 99-01-39-31, Technical Report MSL-17220.

Holleschak, G., Lee, T. C., Pyla, P. D., Hileman, R. E., and Astwood, J. D. (2001b). Amended report for MSL-15835: Assessment of the equivalence of *B.t.* protein 11098, *B.t.* protein 11231 and NPTII protein expressed in corn events MON 853 and MON 860 to microbial sources. (Monsanto Company, St. Louis, MO) Study 98-01-39-18, Technical Report MSL-17222.

Hunkapillar, M. W., Hewick, R. M., Dreyer, W. J., and Hood, L. E. (1983). High-sensitivity sequencing with gas-phase sequenator. *Methods Enzymol* 91:399-413.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680-685.

Neuhoff, V., Norbert, A., Taube, D., and Wolfgang, E. (1988). Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 and R-250. *Electrophoresis* 9:255-262.

SAS Institute, Inc.. 1989-1996. SAS Statistical Software, release 6.12., SAS Institute, Cary, NC.

Thoma, R. S., Holleschak, G., and Astwood, J. D. (2001). Primary structural protein characterization of Cry3Bb1.11098 protein from insect protected corn event MON 863 using MALDI time of flight mass spectrometric and N-terminal sequencing techniques. (Monsanto Company, St. Louis, MO) Study Technical Report MSL-17154.

Table 2. Summary of Dose Formulations

Dose:	Target Dose (mg/kg (BW))	Substance Amount	Vehicle Amount (mL)
High Dose Test Protein	2700	80 mL	0
Mid-Dose Test Protein	900	26.4 mL	53.6
Low Dose Test Protein	300	8.8 mL	71.2
Protein Control (BSA)	2700	3.97g	80
Phosphate Buffer	0	-[a]	80

[a] not applicable

Table 3. Observed Protein Concentration of Test and Control Protein Doses Compared to Targeted Concentrations

Sample	Target Dose (mg/kg BW)	Target Protein Conc. (mg/mL)	Observed [a,b] Protein Conc. (mg/mL)
Pre-Dose Samples			
High Dose Test Protein	2700	40.5	46.2
Mid-Dose Test Protein	900	13.5	16.3
Low Dose Test Protein	300	4.5	6.0
BSA Protein Control	2700	40.5	44.2
Post Dose Samples			
High Dose Test Protein	2700	40.5	48.8
Mid-Dose Test Protein	900	13.5	16.6
Low Dose Test Protein	300	4.5	6.2
BSA Protein Control	2700	40.5	44.1

[a] For calculation of observed concentration of the Test Protein, the following equation was used :

Total Protein Concentration × Percent Purity (97.5%) = Observed Concentration

[b] For calculation of observed concentration of Protein Control (BSA) the following equation was used :
Total Protein Concentration × Percent Purity of Protein Control (BSA) (95.0%) = Observed Concentration

Table 4. Summary of the Purity Analysis Data for the Test Protein Doses

Sample	% Purity[a] 1.0 µg Load	% Purity[a] 2.0 µg Load	% Purity[a] 3.0 µg Load	Avg. % Purity
Pre-Dose Samples				
High Dose Test Protein Male	97.5	96.3	95.5	**96.4**
High Dose Test Protein Female	98.8	98.9	99.0	**98.9**
Mid-Dose Test Protein Male	97.7	96.4	96.1	**96.7**
Mid-Dose Test Protein Female	96.9	96.5	96.1	**96.5**
Low Dose Test Protein Male	99.0	98.0	98.1	**98.4**
Low Dose Test Protein Female	97.6	97.8	98.5	**98.0**
Post-Dose Samples				
High Dose Test Protein Male	96.9	96.1	95.5	**96.2**
High Dose Test Protein Female	99.5	99.0	99.2	**99.2**
Mid-Dose Test Protein Male	96.8	96.2	96.2	**96.4**
Mid-Dose Test Protein Female	97.0	96.8	96.8	**96.9**
Low Dose Test Protein Male	98.7	98.4	98.5	**98.5**
Low Dose Test Protein Female	97.5	98.8	98.4	**98.2**

[a]Percent purity was calculated as the sum of the major band (~74 kDa) present in the Test Protein dose samples.

Table 5. Summary of the Homogeneity Samples for the Test Protein Doses

Targeted Dose Conc. [mg/mL]	Observed [a] Test Protein Top Left Sample [mg/mL]	Observed [a] Test Protein Middle Center Sample [mg/mL]	Observed [a] Test Protein Bottom Right Sample [mg/mL]	Observed [a] Test Protein Average [mg/mL]
Males:				
40.5	49.3	48.7	51.1	49.7
13.5	16.3	16.8	16.7	16.6
4.5	6.2	6.2	6.5	6.30
Females:				
40.5	44.8	47.4	45.4	45.9
13.5	16.4	16.5	16.3	16.4
4.5	5.9	5.9	6.1	6.0

[a] For calculation of observed concentration of the Test Protein, the following equation was used :

Total Protein Concentration × Percent Purity (97.5%) = Observed Concentration

Table 6. Summary of the Test and Control Protein Doses

Targeted Dose Test Protein (mg/kg BW)	"Pre-Dose" Dosing Test Protein (mg /kg BW)	"Post-Dose" Dosing Test Protein (mg /kg BW)	Avg. Dosing Test Protein (mg /kg BW)
300	400	400	400
900	1100	1100	1100
2700	3100	3200	3200
Targeted Dose BSA Control (mg BSA/ kg (BW)	**"Pre-Dose" Dosing BSA Control (mg BSA/ kg (BW)**	**"Post-Dose" Dosing BSA Control (mg BSA/ kg (BW)**	**Average Dosing BSA Control (mg BSA/ kg (BW)**
2700	2900	2900	2900

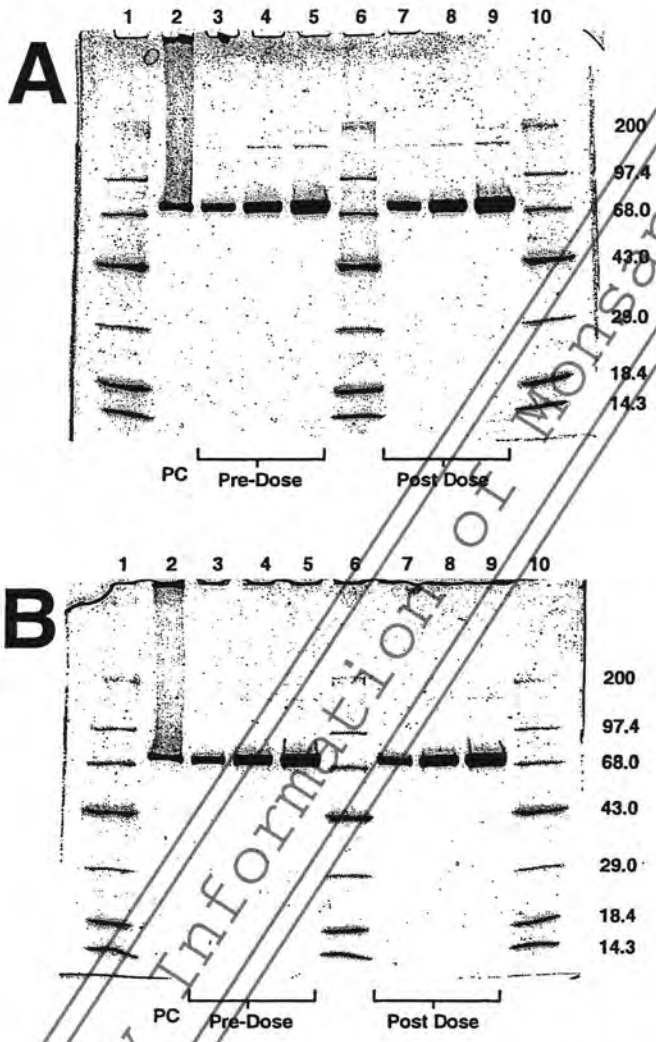

Figure 2. Gradient SDS-PAGE (4→20%) analysis of Colloidal Brilliant Blue G stained gels comparing high Test Protein dose before and after administration to mice. Panel **A** refers to pre- and post dose protein samples of High Test Dose for male mice. Panel **B** refers to pre- and post dose protein samples of High Test Dose for female mice. Lanes 1, 6 and 10 correspond to protein markers with molecular weights (kDa) indicated on the right. Lane 2 corresponds to sample freshly prepared from stock solution of Test Protein[~2 µg load Test Protein, labeled as **PC**]. Lanes 3, 4 and 5 correspond to pre-dose samples at 1, 2 and 3 µg Test Protein per lane. Lanes 7, 8 and 9 correspond to post dose samples at 1, 2 and 3 µg Test Protein per lane.

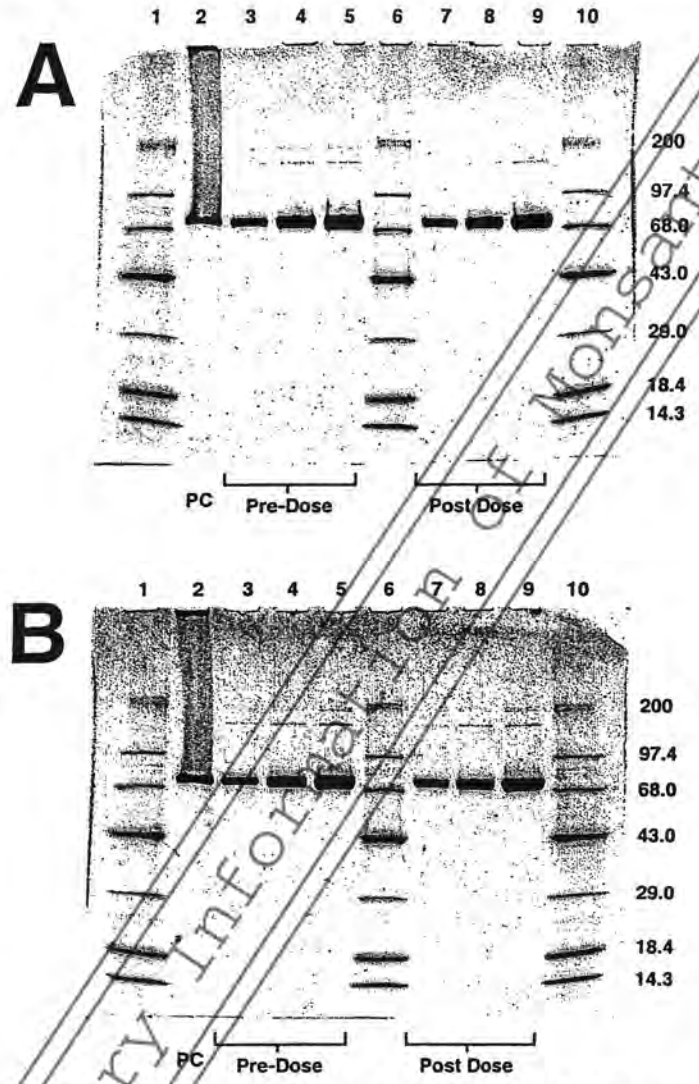

Figure 3. Gradient SDS-PAGE (4→20%) analysis of Colloidal Brilliant Blue G stained gels comparing mid-Test Protein dose before and after administration to mice. Panel **A** refers to pre- and post dose protein samples of Mid-Test Dose for male mice. Panel **B** refers to pre- and post dose protein samples of Mid-Test for female mice. Lanes 1, 6 and 10 correspond to protein markers with molecular weights (kDa) indicated on the right. Lane 2 corresponds to sample freshly prepared from stock solution of Test Protein [~2 µg load Test Protein, labeled as **PC**]. Lanes 3, 4 and 5 correspond to pre-dose samples at 1, 2 and 3 µg Test Protein per lane. Lanes 7, 8 and 9 correspond to post dose samples at 1, 2 and 3 µg Test Protein per lane.

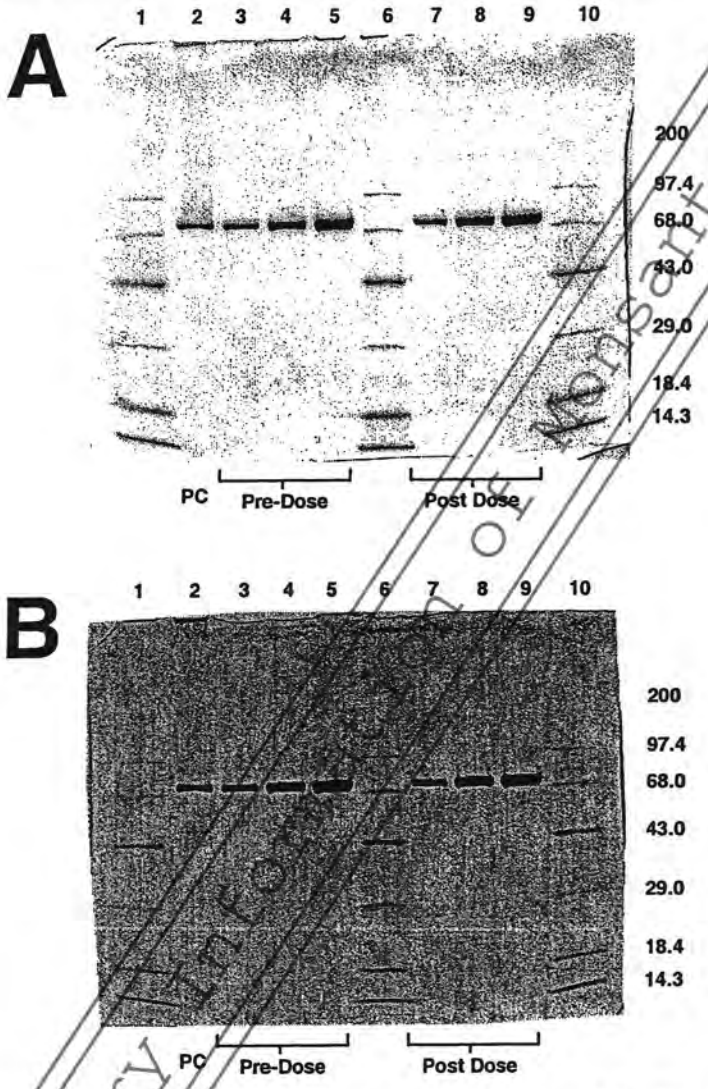

Figure 4. Gradient SDS-PAGE (4→20%) analysis of Colloidal Brilliant Blue G stained gels comparing low Test Protein dose before and after administration to mice. Panel **A** refers to pre- and post dose protein samples of Low Test for male mice. Panel **B** refers to pre- and post dose protein samples of Low Test Dose for female mice. Lanes 1, 6 and 10 correspond to protein markers with molecular weights (kDa) indicated on the right. Lane 2 corresponds to sample freshly prepared from stock solution of Test Protein [~2 µg load Test Protein, labeled as **PC**]. Lanes 3, 4 and 5 correspond to pre-dose samples at 1, 2 and 3 µg Test Protein per lane. Lanes 7, 8 and 9 correspond to post dose samples at 1, 2 and 3 µg Test Protein per lane.

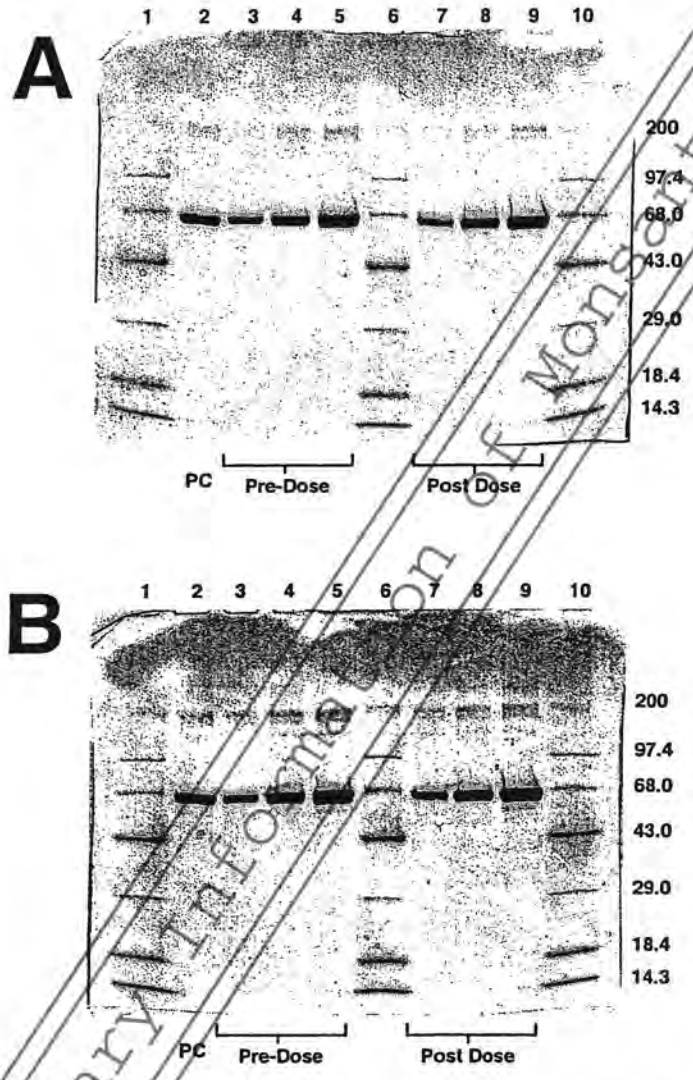

Figure 5. Gradient SDS-PAGE (4→20%) analysis of Colloidal Brilliant Blue G stained gels comparing the BSA Protein Control dose before and after administration to mice. Panel **A** refers to pre- and post dose 2700 mg/kg (BW) BSA protein control samples for male mice. Panel **B** refers to pre- and post dose 2700 mg/kg (BW) BSA protein control samples for female mice. Lanes 1, 6 and 10 correspond to protein markers with molecular weights (kDa) indicated on the right. Lane 2 corresponds to sample freshly prepared from stock solution BSA protein control sample [~2 µg load BSA protein control sample, labeled as **PC**]. Lanes 3, 4 and 5 correspond to pre-dose samples at 1, 2 and 3 µg BSA protein control sample per lane. Lanes 7, 8 and 9 correspond to post dose samples at 1, 2 and 3 µg BSA protein control per lane.

Appendix 1

List of Applicable Method SOPs

SOP Number	SOP Title
BR-EQ-0265-01	Applied Biosystems 494 Procise™ Protein Sequencing System
BR-EQ-0376-01	Hitachi L-8800 Amino Acid Analysis System
BR-ME-0044-02	Diet Incorporation Insect Bioassay for the Biological Activity Measurement of *Bacillus thuringiensis* & Other Insecticidal Proteins
PB-EQP-005-01	SDS Polyacrylamide Gel Electrophoresis

Appendix 2

Preparation and Confirmation of Doses for an Acute Oral Toxicity Study with *E. coli* Produced Cry3Bb1.11098(Q349R) Protein Performed at Springborn Laboratories, Inc.

Study Specific Procedure and Amendments

The following 13 pages are the Study Protocol and all amendments

Monsanto Study #: SB-2001-085
Springborn Labs, Inc. Study #: 3044.856

Title: Study Specific Work Procedure for the Preparation, Formulation and Confirmation of Doses for An Acute Oral Toxicity Study With *E. coli* Produced Cry3Bb1.11098(Q349R) Protein Performed at Springborn Labs

Sponsor:
Monsanto Company
700 Chesterfield Parkway North
St. Louis, MO 63198

Analytical Principal Investigator:
Paul D. Pyla
Monsanto Company - BB5G
Product Safety Center
700 Chesterfield Parkway North
St. Louis, MO 63198
Phone: (636) 737-5324
FAX: (636) 737-6189
e-mail: paul.d.pyla@monsanto.com

Acute Oral Toxicity Study Testing Facility:
Springborn Laboratories, Inc.
Ohio Research Center
553 N. Broadway
Spencerville, Ohio 45887
Phone: (419) 647-4196
Fax: (419) 647-6560

Acute Oral Toxicity Study Director:
Kimberly L. Bonnette, M.S., LATG
Springborn Laboratories, Inc.
Ohio Research Center
553 N. Broadway
Spencerville, Ohio 45887
Phone: (419) 647-4196
Fax: (419) 647-6560

Approved By:

Patrick T. Weston
Testing Facility Management Representative
Monsanto Company
April 16, 2001
Date

Paul D. Pyla
Analytical Principal Investigator
Monsanto Company
Product Safety Center
17 April 2001
Date

Terry A. Kaempfe
Sponsor Representative
Monsanto Company
April 16, 2000
Date

James D. Astwood
Co-Director, Product Safety Center
Monsanto Company
April 16, 2001
Date

Reviewed By:

changed QA 4-16-01 VRZ

~~Paula A. Price~~ Valerie A. Zink
Quality Assurance Specialist
Monsanto Company
April 16, 2001
Date

Kimberly L. Bonnette, M.S., LATG
Study Director
Springborn Laboratories, Inc.
April 17, 2001
Date

[redacted]

[redacted]

1.0 Purpose

The purpose of this document is to capture the procedure for the formulation, characterization and confirmation of the doses of the test and control substances used in a mouse acute oral toxicity study performed at Springborn Labs, Inc. (Spencerville, OH). Results from this study specific work procedure will be used to support future studies from Monsanto Company performed at Springborn Labs, Inc..

2.0 Test and Control (T/C) Substances

2.1 Test Substances

The test substance was the *E. coli* produced Cry3Bb1.11098(Q349R) Protein, Lot 6962478, isolated and purified at Monsanto Company. For future reference, *E. coli* produced Cry3Bb1.11098(Q349R) Protein, Lot 6962478 will be designated as the Test Protein. The test substance was formulated in phosphate buffer as the vehicle. Based on the on pre-study data, the concentration of the Test Protein stock solution was estimated at 43.5 mg/ml. The purity of the Test Protein was 94% (*e.g.*, the Test Protein represents 94% of the total protein value).

Test Substance:
E. coli produced Cry3Bb1.11098(Q349R) Protein, Lot 6962478

2.2 Control Substance(s)

The protein control substance was purchased from Calbiochem and is as follows:

Protein Control:	Description:
Bovine Serum Albumin (BSA) Lot B38089	Catalogue # 126609

The vehicle control substance was produced by Monsanto Company :

Vehicle Control: Phosphate Buffer (Lot:6839197A)

[redacted]

2.3 Characterization of Test and Control (T/C) Substances

Characterization of the test substance will be performed by the Sponsor in order to assess identity, purity and protein content within the protein solution. The analytical methods used will be described within the final characterization report.

3.0 Description of Experimental Design

The test substance was produced and purified at Monsanto Company and supplied as a solution in phosphate buffer. The test substance will then be further diluted in phosphate buffer (Lot:6839197A) to produce targeted dose levels described below. The control substance was purchased from the manufacturer as a protein powder which will then be suspended/dissolved in phosphate buffer (Lot:6839197A) to produce the targeted dose level described below:

Protein:	Substance Description	Doses(mg/kg)
E. coli produced Cry3Bb1.11098(Q349R) Protein, Lot 6962478	Test Protein	2700, 900, 300
Bovine Serum Albumin (BSA) Lot B38089	Protein Control	2700

The vehicle control will be phosphate buffer (Lot:6839197A).

3.1 Dose Preparation

The doses will be formulated Monday, April 16, 2001 (one day in advance of the start of the mouse acute oral toxicity study) as follows. The calculations for formulation are based on pre-study data and information supplied from the manufacturer. The target doses described in section 5.0 will be prepared with the following assumptions.

a) The average mouse body weight (BW) is 0.030 kg.
b) Doses will be administered at 33.33 mL/kg BW, twice daily.
c) 20 mice will be dosed at each target protein concentration.
d) The target dose will be administered in approximately two equal 1 mL doses.

The dose volume (mL) is based on the following information:

0.030 kg BW(mouse wt.) × 33.33 mL/kg (dose rate) ≡ 1 mL/dose

For 20 mice (dosed twice) the minimum total volume of sample required:

20 mice × 2 doses × 1 mL/dose = 40 mL

Based upon the purity information supplied from the manufacturer, as well as preliminary pre-study data for the Test Protein, purity for the Test Protein and the protein control will be factored into an equation for the amount of each protein needed to formulate each dose.

Protein:	Pre-Study SDS-PAGE Purity:
E. coli produced Cry3Bb1.11098 (Q349R) Protein	$X \geq 94\%$
Bovine Serum Albumin (BSA)	$X \geq 98\%$

For a 2700 mg/kg *E. coli* produced Cry3Bb1.11098(Q349R) Protein Test dose, 81 mg of test substance is needed:

2700 mg/kg BW × 0.030 kg BW = 81 mg

Since the Test Protein will be divided into two doses, 40.5 mg is needed per dose:

81 mg ÷ 2 doses = 40.5 mg/dose

Based upon Pre study data, the concentration of the Test Protein is 43.5 mg/ml.

The purity corrected test protein concentration is 40.9 mg/ml
(43.5 mg/ml × 0.94 ≈ 40.9 mg/ml)

To account for variability and allow sampling, 80 ml of dose will be used. Solution (80 ml) will be measured from the Test Protein and placed in a separate container.

For a 900 mg/kg *E. coli* produced Cry3Bb1.11098(Q349R) Protein Test dose, 27 mg of test substance is needed:

900 mg/kg BW × 0.030 kg BW = 27 mg

Since the test substance will be divided into two doses, 13.5 mg is needed per dose:

27 mg ÷ 2 doses = 13.5 mg/dose

Based upon Pre study data, the concentrated supply of the Test Protein is at a protein concentration of 43.5 mg/ml.

The purity corrected test protein concentration is 40.9 mg/ml
(43.5 mg/ml × 0.94 ≈ 40.9 mg/ml)

A 13.5 mg/dose concentration is achieved by diluting the Test Protein stock to 33 % of its original concentration.

13.5 mg/dose ÷ 40.9 mg/dose ≈ 0.33

To account for variability and allow sampling, 80 ml of dose will be used. A solution (80 ml) will be prepared from the stock of Test Protein which will be diluted to the appropriate concentration described above with phosphate buffer.

For 80 ml of 900 mg/kg dose (13.5 mg/dose), 26.4 ml of the Test Protein stock will need to be diluted with 53.6 ml of phosphate buffer.

80 ml total volume × 0.33 (dilution factor) = 26.4 ml of the Test Protein stock needed.

26.4 ml – 80 ml total dose volume = 53.6 ml phosphate buffer needed for dilution.

For a 300 mg/kg *E. coli* produced Cry3Bb1.11098(Q349R) Protein Test dose, 9 mg of test substance is needed:

300 mg/kg BW × 0.030 kg BW = 9 mg

Since the test substance will be divided into two doses, 4.5 mg is needed per dose:

9 mg ÷ 2 doses = 4.5 mg/dose

Based upon Pre study data, the concentrated supply of the Test Protein is at a protein concentration of 43.5 mg/ml.

The purity corrected test protein concentration is 40.9 mg/ml
(43.5 mg/ml × 0.94 ≈ 40.9 mg/ml)

A 4.5 mg/dose concentration is achieved by diluting the Test Protein stock to 11 % of its original concentration.

4.5 mg/dose ÷ 40.9 mg/dose ≈ 0.11

To account for variability and allow sampling, 80 ml of dose will be used. A solution (80 ml) will be prepared from the stock of Test Protein which will be diluted to the appropriate concentration described above with phosphate buffer.

For 80 ml of 300 mg/kg dose (4.5 mg/dose), 8.8 ml of the Test Protein stock will need to be diluted with 71.2 ml of phosphate buffer (Lot:6839197A).

80 ml total volume × 0.11 (dilution factor) = 8.8 ml of the Test Protein stock needed.

8.8ml – 80 ml total dose volume = 71.2 ml phosphate buffer needed for dilution.

For a 2700 mg/kg BSA Protein Control dose, 81 mg of test substance is needed:

2700 mg/kg BW × 0.030 kg BW = 81 mg

Since the control substance will be divided into two doses, 40.5 mg is needed per dose:

81 mg ÷ 2 doses = 40.5 g/dose

The total dry weight of powder needed (per dose) is then calculated based upon pre-study purity and protein assessment (Purity was assessed as X ≥ 98%, protein content within the protein powder from the manufacturer was 83.7%):

40.5 mg ÷ (0.98 × 0.837) ≅ 49 mg

To account for variability and allow sampling, 80 ml of dose will be prepared. The amount of test material required is then:

80 doses × 0.049 g/dose = 3.95 g

Assuming the density of the solution/suspension is equal to water, phosphate buffer will be added to a final mass of 80 g, in order to create 80 ml of control protein concentration of 40.5 mg/ml.

The formulations of the dose solutions/suspensions will be stored at 4 °C until transported from Monsanto Company, Chesterfield, MO, to Springborn Labs, Inc., Spencerville, OH. Doses will be transported on wet ice, or packaged "Blue ice" and will arrive at Springborn Labs, Inc. on Tuesday, April 17, 2001.

3.2 Dose Analytical Samples

After stirring 20 minutes, one sample will be taken for concentration/stability from each dose. Samples for homogeneity will be taken prior to each dosing day from any dose where a suspension is observed by removing an aliquot from the top left, middle center and bottom right. All sample aliquots (~500 µL) will be transferred to uniquely labeled vials and immediately frozen on dry ice.

After the morning dosing is complete, the dose solutions/suspensions will be stored in a refrigerator or placed on wet ice. Prior to the afternoon dosing, the dose solutions/suspensions will be stirred at room temperature for at least 20 minutes. A final stability sample from each dose will be taken when the dosing is completed for the day. At the conclusion of the acute oral gavage dosing, unused dose and analytical samples are to be transported frozen (approx. –80 °C) from Springborn Labs, Inc., Spencerville, OH to Monsanto Company, Chesterfield, MO on dry ice, and stored frozen (approx. –80 °C) until analyzed.

3.2.1 Summary of Sample Collection:

	Sample	
Time	Stability/Concentration	Homogeneity
Day 1, morning	before dosing	If a suspension, before dosing
Day 1, afternoon	after dosing	Not sampled
Day 2, morning	before dosing	If a suspension, before dosing
Day 2, afternoon	after dosing	Not sampled

3.3 Analytical Methods

Within four weeks of sample collection, the concentration and integrity of the test substance in these aliquots will be assessed using the panel of analytical methods described below.

3.3.1 N-Terminal Sequence Analysis

The identity of the dosed proteins will be determined using N-terminal sequence analysis in order to assess the integrity of the proteins which were dosed.

3.3.2 Amino Acid Analysis

The protein concentration of the dosing samples will be determined using amino acid analysis in order to assess both the final dose concentration and homogeneity. Aliquots of the samples from each dose will be removed and diluted to a concentration range which is compatible with this analytical technique.

3.3.3 SDS-PAGE/Coomassie Staining/Imaging/Densitometry

This technique will assess the integrity of the test and control substances. Proteins contained in each sample will be separated by SDS-PAGE according to SOP No. PB-EQP-005-01 and detected by colloidal Brilliant blue G staining. A detailed description of the imaging/densitometry method will be archived with the raw data.

Aliquots of each dose will be diluted to provide at least two concentrations of test substance that is compatible with the SDS-PAGE technique. These samples will be compared by densitometry to assess the integrity of the test substance during the duration of the mouse gavage.

4.0 Final Report

The final analytical sub-report will be appended to the study report

5.0 Records to be Maintained

5.1 Monsanto Company

Records will be maintained of all sample transfers, analyses, the protocol and all deviations and amendments. These documents may include: photocopies, computer generated hard copies, or hand-written notes that describe the procedures used to generate data for this study. Upon completion of the study, the final report will be retained by the Sponsor in the Monsanto Regulatory Archives.

[redacted]

6.0 Changes to the Protocol

Planned changes to the study specific work procedure will be documented in the form of written protocol amendments and signed by the Study Director. Amendments will become part of the protocol and will be archived with the protocol. All other changes will be in the form of written protocol deviations and will be filed with the raw data. All changes to the protocol will be addressed in the final report.

[redacted]

Monsanto Company
Springborn Study #: 3044.856
Monsanto Study #: SB-2001-085
Monsanto Regulatory

Study Specific Work Procedure Amendment Form **Amendment #: 1**
Monsanto Study #: SB-2001-085

Date changes implemented: See effective dates
Effective Date: April 17, 2001
Page number(s) and section(s):
Section 3.1, Page 8 of 10, Dose Preparation,

Study Specific Work Procedure originally stated:

> Assuming the density of the solution/suspension is equal to water, phosphate buffer will be added to a final mass of 80 g, in order to create 80 ml of control protein concentration of 40.5 mg/ml.

Amended as Follows:

> Phosphate buffer will be added to a final volume of 80 ml, in order to create a total of 80 ml of control protein with a final protein concentration of 40.5 mg/ml.

Reason for Amendment and what impact will result from this change:
The amended version of how the powdered material was to be resuspended was the intended way of to prepare the dose. This section was inadvertently left in from a previous study specific work procedure version. No impact will result from this change.

Effective Date: April 17, 2001
Page number(s) and section(s):
Section 3.3.1, Page 9 of 10, N-Terminal Sequence Analysis

Study Specific Work Procedure originally stated:

> **3.3.1 N-Terminal Sequence Analysis**
> The identity of the dosed proteins will be determined using N-terminal sequence analysis in order to assess the integrity of the proteins which were dosed.

Amended as Follows:

> **3.3.1 N-Terminal Sequence Analysis**
> The identity of the dosed proteins will be determined using N-terminal sequence analysis in order to assess the integrity of the proteins which were dosed. A sample of the Test Protein and the Protein Control from the highest dose will be used for this analysis.

Monsanto Company
Springborn Study #: 3044.856
Monsanto Study #: SB-2001-085
Monsanto Regulatory

Study Specific Work Procedure Amendment Form **Amendment #: 1**

Reason for Amendment and what impact will result from this change:
This was to clarify that only one sample from the Test Protein dosing regimen and one sample from the Protein Control were to be analyzed by N-terminal sequence analysis for this the study. No impact will result from this change.

Effective Date: May 9, 2001
Page number(s) and section(s):
New Section 3.3.4, Page 9 of 10,

Amended as Follows:

3.3.4 Insect Bioassay
This analysis will be used to estimate and compare the bioactivity (measured as a LC_{50} value) of the test substance incorporated into a diet fed to CPB larvae, a susceptible insect to that of the Protein Control. The LC_{50} is defined as the concentration of protein (µg/mL diet) required to kill 50% of the test larvae relative to control. Insect bioassays will be conducted at the Ecological Technology Center laboratories, Creve Coeur campus of Monsanto Company. The Test Protein Protein Control and Vehicle Control will be assayed according to SOP BR-ME-0044-02. Data will be analyzed using SAS Probit and/or logistic regression procedures.

Reason for Amendment and what impact will result from this change:
This assayed was needed in order to show that during the acute oral toxicity the Test Protein was biologically active. This will positively impact the study in that this data strengthens the overall study data, by showing that the Test Protein was fully active during the time of dosing

Effective Date: May 9, 2001
Page number(s) and section(s):
Section 3.3, Page 9 of 10, Analytical Methods

Study Specific Work Procedure originally stated:

3.3 Analytical Methods
Within four weeks of sample collection, the concentration and integrity of the test substance in these aliquots will be assessed using the panel of analytical methods described below.

[redacted]

Monsanto Company
Springborn Study #: 3044.856
Monsanto Study #: SB-2001-085
Monsanto Regulatory

Study Specific Work Procedure Amendment Form **Amendment #: 1**

Amended as Follows:

3.3 Analytical Methods
Within six weeks of sample collection, the concentration and integrity of the test substance in these aliquots will be assessed using the panel of analytical methods described below.

Reason for Amendment and what impact will result from this change:
Due to including the insect bioassay analytical method within the study specific work procedure, the time allowed for all analyses needs to be increased to accommodate this necessary analytical method. By allowing the time extension, the insect bioassay analysis can be performed and the results reported.

Approved By:

Patrick T. Weston
Testing Facility Management Representative
May 18, 2001
Date

Terry A. Kaempfe
Sponsor Representative
Monsanto Company
May 21, 2001
Date

Paul D. Pyla
Analytical Principal Investigator
Monsanto Company
Product Safety Center
21 MAY 2001
Date

Reviewed By:

Paula A. Price
Quality Assurance Specialist
Monsanto Company
May 18, 2001
Date

[redacted]

APPENDIX C

Individual Clinical Observations

SLI STUDY NO.: 3044.856
CLIENT: MONSANTO COMPANY

AN ACUTE ORAL TOXICITY STUDY IN MICE
INDIVIDUAL CLINICAL OBSERVATIONS

(POSITIVE FINDINGS)

ANIMAL NO.	CLINICAL OBSERVATIONS	DAY OF STUDY 0 1 2 3 4 5 6 7 8 9 1 1 1 1 1 0 1 2 3 4
A1054 M	GROUP 1 (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1063 M	GROUP 1 (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1065 M	GROUP 1 (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1070 M	GROUP 1 (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1078 M	GROUP 1 (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1086 M	GROUP 1 (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1092 M	GROUP 1 (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1101 M	GROUP 1 (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P

GRADE CODE: P-PRESENT

(POSITIVE FINDINGS)

ANIMAL NO.	CLINICAL OBSERVATIONS	DAY OF STUDY 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
A1106 M	GROUP 1 (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1110 M	GROUP 1 (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1056 M	GROUP 2 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1057 M	GROUP 2 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1062 M	GROUP 2 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1076 M	GROUP 2 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1077 M	GROUP 2 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1081 M	GROUP 2 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P

GRADE CODE: P-PRESENT

(POSITIVE FINDINGS)

ANIMAL NO.	CLINICAL OBSERVATIONS	DAY OF STUDY 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4
A1090 M	GROUP 2 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1102 M	GROUP 2 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1104 M	GROUP 2 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1107 M	GROUP 2 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1059 M	GROUP 3 (300 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1072 M	GROUP 3 (300 MG/KG)	
	BODY	
	RAISED AREA ABDOMINAL REGION	P P P P P P P P
	SMALL SCAB(S)	P P P P P
	DEAD	
	SCHEDULED EUTHANASIA	P
A1075 M	GROUP 3 (300 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P

GRADE CODE: P-PRESENT

(98)

(POSITIVE FINDINGS)

ANIMAL NO.	CLINICAL OBSERVATIONS	DAY OF STUDY 0 1 2 3 4 5 6 7 8 9	1 1 1 1 1 0 1 2 3 4
A1080 M	GROUP 3 (300 MG/KG)		
	DEAD		
	SCHEDULED EUTHANASIA		P
A1082 M	GROUP 3 (300 MG/KG)		
	DEAD		
	SCHEDULED EUTHANASIA		P
A1084 M	GROUP 3 (300 MG/KG)		
	DEAD		
	SCHEDULED EUTHANASIA		P
A1087 M	GROUP 3 (300 MG/KG)		
	DEAD		
	SCHEDULED EUTHANASIA		P
A1091 M	GROUP 3 (300 MG/KG)		
	DEAD		
	SCHEDULED EUTHANASIA		P
A1093 M	GROUP 3 (300 MG/KG)		
	DEAD		
	SCHEDULED EUTHANASIA		P
A1111 M	GROUP 3 (300 MG/KG)		
	DEAD		
	SCHEDULED EUTHANASIA		P
A1052 M	GROUP 4 (900 MG/KG)		
	DEAD		
	SCHEDULED EUTHANASIA		P

GRADE CODE: P-PRESENT

(POSITIVE FINDINGS)

ANIMAL NO.	CLINICAL OBSERVATIONS	DAY OF STUDY 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 (1 1 1 1 1)
A1053 M	GROUP 4 (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1058 M	GROUP 4 (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1066 M	GROUP 4 (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1067 M	GROUP 4 (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1085 M	GROUP 4 (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1089 M	GROUP 4 (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1097 M	GROUP 4 (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1105 M	GROUP 4 (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P

GRADE CODE: P-PRESENT

(POSITIVE FINDINGS)

ANIMAL NO.	CLINICAL OBSERVATIONS	DAY OF STUDY 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
A1055 M	GROUP 4 (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1051 M	GROUP 5 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1069 M	GROUP 5 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1073 M	GROUP 5 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1079 M	GROUP 5 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1083 M	GROUP 5 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1094 M	GROUP 5 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1095 M	GROUP 5 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P

GRADE CODE: P-PRESENT

SLI STUDY NO.: 3044.856
CLIENT: MONSANTO COMPANY

AN ACUTE ORAL TOXICITY STUDY IN MICE
INDIVIDUAL CLINICAL OBSERVATIONS

(POSITIVE FINDINGS)

ANIMAL NO.	CLINICAL OBSERVATIONS	DAY OF STUDY 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4
A1096 M	GROUP 5 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1098 M	GROUP 5 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1103 M	GROUP 5 (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P

GRADE CODE: P-PRESENT

(89)

(POSITIVE FINDINGS)

ANIMAL NO.	CLINICAL OBSERVATIONS	DAY OF STUDY 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 (11111)
A1113	F GROUP 1A (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1116	F GROUP 1A (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1119	F GROUP 1A (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1122	F GROUP 1A (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1130	F GROUP 1A (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1132	F GROUP 1A (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1145	F GROUP 1A (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1138	F GROUP 1A (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P

GRADE CODE: P-PRESENT

(06)

(POSITIVE FINDINGS)

ANIMAL NO.	CLINICAL OBSERVATIONS	DAY OF STUDY 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4
A1162 F	GROUP 1A (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1172 F	GROUP 1A (0 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1118 F	GROUP 2A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1120 F	GROUP 2A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1124 F	GROUP 2A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1135 F	GROUP 2A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1141 F	GROUP 2A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1144 F	GROUP 2A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P

GRADE CODE: P-PRESENT

(POSITIVE FINDINGS)

ANIMAL NO.	CLINICAL OBSERVATIONS	DAY OF STUDY 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4
A1150 F	GROUP 2A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1163 F	GROUP 2A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1166 F	GROUP 2A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1171 F	GROUP 2A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1125 F	GROUP 3A (300 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1126 F	GROUP 3A (300 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1127 F	GROUP 3A (300 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1133 F	GROUP 3A (300 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P

GRADE CODE: P-PRESENT

(POSITIVE FINDINGS)

ANIMAL NO.	CLINICAL OBSERVATIONS	DAY OF STUDY 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4
A1134 F	GROUP 3A (300 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1143 F	GROUP 3A (300 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1149 F	GROUP 3A (300 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1156 F	GROUP 3A (300 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1158 F	GROUP 3A (300 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1159 F	GROUP 3A (300 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1117 F	GROUP 4A (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1123 F	GROUP 4A (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P

GRADE CODE: P-PRESENT

(POSITIVE FINDINGS)

ANIMAL NO.	CLINICAL OBSERVATIONS	DAY OF STUDY 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4
A1136 F	GROUP 4A (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1140 F	GROUP 4A (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1142 F	GROUP 4A (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1148 F	GROUP 4A (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1152 F	GROUP 4A (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1155 F	GROUP 4A (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1160 F	GROUP 4A (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1169 F	GROUP 4A (900 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P

GRADE CODE: P-PRESENT

(POSITIVE FINDINGS)

ANIMAL NO.	CLINICAL OBSERVATIONS	DAY OF STUDY 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
A1112 F	GROUP 5A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1114 F	GROUP 5A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1115 F	GROUP 5A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1137 F	GROUP 5A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1151 F	GROUP 5A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1157 F	GROUP 5A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1161 F	GROUP 5A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1164 F	GROUP 5A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P

GRADE CODE: P-PRESENT

SLI STUDY NO.: 3044.856
CLIENT: MONSANTO COMPANY

AN ACUTE ORAL TOXICITY STUDY IN MICE
INDIVIDUAL CLINICAL OBSERVATIONS

(POSITIVE FINDINGS)

ANIMAL NO.	CLINICAL OBSERVATIONS	DAY OF STUDY 0 1 2 3 4 5 6 7 8 9 1 1 1 1 1 0 1 2 3 4
A1165 F	GROUP 5A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P
A1170 F	GROUP 5A (2700 MG/KG)	
	DEAD	
	SCHEDULED EUTHANASIA	P

GRADE CODE: P-PRESENT

(96)

APPENDIX D

Individual Body Weight Data

SLI STUDY NO.: 3044.856
CLIENT: MONSANTO COMPANY

AN ACUTE ORAL TOXICITY STUDY IN MICE
INDIVIDUAL BODY WEIGHT DATA (GRAMS)

	(PREFASTED)			
DAY	0	0	7	14
GROUP 1 (0 MG/KG)				
A1054 M	27.8	26.6	28.8	32.3
A1063 M	27.7	26.7	28.5	29.6
A1065 M	29.5	28.1	30.0	30.6
A1070 M	27.4	26.0	29.6	31.5
A1078 M	30.1	28.8	31.0	32.0
A1086 M	31.5	30.3	34.3	36.3
A1092 M	29.0	27.2	29.6	32.1
A1101 M	28.3	27.2	29.2	30.5
A1106 M	30.9	29.7	32.4	34.0
A1110 M	29.2	27.7	31.6	32.6
MEAN	29.1	27.8	30.5	32.2
S.D.	1.39	1.40	1.82	1.91
N	10	10	10	10

	(PREFASTED)			
DAY	0	0	7	14
GROUP 2 (2700 MG/KG)				
A1056 M	29.6	28.1	31.5	32.3
A1057 M	29.2	27.9	31.9	33.6
A1062 M	30.1	28.9	33.3	34.8
A1076 M	27.6	26.4	27.7	28.6
A1077 M	29.2	28.0	32.2	33.6
A1081 M	28.2	27.2	30.9	33.1
A1090 M	32.2	30.7	33.1	34.4
A1102 M	31.0	29.4	31.8	34.2
A1104 M	27.2	26.2	29.2	30.8
A1107 M	27.7	26.9	30.0	31.1
MEAN	29.2	28.0	31.2	32.7
S.D.	1.60	1.40	1.76	1.96
N	10	10	10	10

DAY	(PREFASTED) 0	0	7	14
GROUP 3 (300 MG/KG)				
A1059 M	29.3	27.4	30.7	32.8
A1072 M	27.0	25.8	28.5	30.9
A1075 M	27.7	26.1	28.3	30.1
A1080 M	28.4	26.5	31.3	32.7
A1082 M	30.2	29.2	31.6	33.5
A1084 M	29.5	27.7	31.5	33.4
A1087 M	27.7	26.8	30.6	31.9
A1091 M	31.1	29.6	33.2	35.3
A1093 M	29.1	27.7	29.3	29.8
A1111 M	31.1	30.0	32.8	34.8
MEAN	29.1	27.7	30.8	32.5
S.D.	1.42	1.48	1.67	1.86
N	10	10	10	10

	(PREFASTED)			
DAY	0	0	7	14
GROUP 4 (900 MG/KG)				
A1052 M	29.5	28.1	31.1	32.4
A1053 M	29.2	29.1	29.9	30.7
A1058 M	30.8	28.9	33.8	36.0
A1066 M	27.9	26.8	30.0	32.0
A1067 M	29.9	28.4	30.2	31.7
A1085 M	32.0	30.4	33.4	35.1
A1089 M	27.0	26.5	29.4	29.6
A1097 M	27.4	25.6	30.8	32.9
A1105 M	28.7	27.2	30.7	31.5
A1055 M	32.7	32.1	34.6	35.9
MEAN	29.5	28.3	31.4	32.8
S.D.	1.89	1.94	1.84	2.20
N	10	10	10	10

	(PREFASTED)			
DAY	0	0	7	14
GROUP 5 (2700 MG/KG)				
A1051 M	27.7	26.7	29.2	31.6
A1069 M	28.0	26.7	30.5	33.1
A1073 M	31.1	29.4	32.4	33.0
A1079 M	29.4	27.6	31.1	31.0
A1083 M	27.0	25.6	29.2	30.6
A1094 M	29.8	28.2	28.4	29.5
A1095 M	28.0	26.8	28.6	30.3
A1096 M	32.2	26.6	33.1	35.5
A1098 M	30.5	29.6	30.4	31.9
A1103 M	28.9	27.5	31.1	32.9
MEAN	29.3	27.5	30.4	31.9
S.D.	1.66	1.28	1.58	1.75
N	10	10	10	10

		(PREFASTED)			
DAY		0	0	7	14
GROUP 1A (0 MG/KG)					
A1113	F	24.1	23.7	25.1	26.9
A1116	F	26.7	25.7	27.0	27.5
A1119	F	26.0	24.9	26.1	27.0
A1122	F	23.9	22.9	24.5	25.6
A1130	F	25.8	24.6	26.3	28.4
A1132	F	26.3	25.0	27.6	28.5
A1145	F	24.6	24.0	25.7	26.8
A1138	F	23.8	22.5	24.5	25.7
A1162	F	26.4	25.0	26.8	26.9
A1172	F	24.8	23.8	25.6	27.2
MEAN		25.2	24.2	25.9	27.0
S.D.		1.12	1.01	1.04	0.95
N		10	10	10	10

		(PREFASTED)			
DAY		0	0	7	14
GROUP 2A (2700 MG/KG)					
A1118	F	24.2	22.8	24.5	26.0
A1120	F	23.9	22.7	23.6	24.4
A1124	F	26.0	24.3	25.2	26.9
A1135	F	26.6	25.4	26.3	27.6
A1141	F	24.8	23.9	25.3	25.9
A1144	F	26.3	25.0	26.7	27.7
A1150	F	25.4	23.9	26.8	28.2
A1163	F	24.9	23.4	24.2	24.7
A1166	F	26.2	24.8	24.3	24.8
A1171	F	24.4	23.1	25.5	27.0
MEAN		25.3	23.9	25.2	26.3
S.D.		0.97	0.94	1.10	1.37
N		10	10	10	10

		(PREFASTED)			
DAY		0	0	7	14
GROUP 3A (300 MG/KG)					
A1125	F	24.0	22.4	25.0	24.9
A1126	F	25.6	24.1	27.0	27.5
A1127	F	24.5	22.9	24.7	27.0
A1133	F	24.7	23.5	25.4	26.5
A1134	F	24.1	23.0	24.5	25.7
A1143	F	25.9	25.3	26.3	27.4
A1149	F	25.2	24.3	25.1	27.1
A1156	F	26.1	25.1	26.1	28.5
A1158	F	26.3	25.3	25.5	25.7
A1159	F	27.1	26.1	27.0	28.2
MEAN		25.4	24.2	25.7	26.9
S.D.		1.02	1.23	0.90	1.15
N		10	10	10	10

		(PREFASTED)			
DAY		0	0	7	14
GROUP 4A (900 MG/KG)					
A1117	F	24.6	23.4	24.6	26.2
A1123	F	24.4	23.8	26.0	27.1
A1136	F	26.1	24.6	25.0	27.5
A1140	F	26.6	25.5	26.3	28.4
A1142	F	23.9	22.7	24.3	27.3
A1148	F	25.8	23.9	25.3	23.5
A1152	F	25.9	25.0	25.4	27.5
A1155	F	25.2	24.3	25.4	27.3
A1160	F	26.5	25.5	27.8	29.9
A1169	F	24.6	23.5	24.1	25.8
MEAN		25.4	24.2	25.4	27.0
S.D.		0.95	0.93	1.09	1.68
N		10	10	10	10

		(PREFASTED)			
DAY		0	0	7	14
GROUP 5A (2700 MG/KG)					
A1112	F	26.4	25.6	26.3	27.7
A1114	F	26.1	24.8	26.1	27.7
A1115	F	24.7	23.7	25.1	26.9
A1137	F	25.2	24.0	25.9	26.9
A1151	F	26.2	24.8	26.7	29.3
A1157	F	24.6	23.3	24.6	26.8
A1161	F	25.6	24.5	25.4	25.8
A1164	F	24.4	23.1	24.0	25.7
A1165	F	23.8	23.1	24.0	24.9
A1170	F	26.5	25.4	26.0	27.4
MEAN		25.4	24.2	25.4	26.9
S.D.		0.95	0.93	0.96	1.24
N		10	10	10	10

APPENDIX E

Individual Body Weight Gain Data

DAY	0-7	7-14
GROUP 1 (0 MG/KG)		
A1054 M	2.2	3.5
A1063 M	1.8	1.1
A1065 M	1.9	0.6
A1070 M	3.6	1.9
A1078 M	2.2	1.0
A1086 M	4.0	2.0
A1092 M	2.4	2.5
A1101 M	2.0	1.3
A1106 M	2.7	1.6
A1110 M	3.9	1.0
MEAN	2.7	1.6
S.D.	0.85	0.86
N	10	10

DAY	0-7	7-14
GROUP 2 (2700 MG/KG)		
A1056 M	3.4	0.8
A1057 M	4.0	1.7
A1062 M	4.4	1.5
A1076 M	1.3	0.9
A1077 M	4.2	1.4
A1081 M	3.7	2.2
A1090 M	2.4	1.3
A1102 M	2.4	2.4
A1104 M	3.0	1.6
A1107 M	3.1	1.1
MEAN	3.2	1.5
S.D.	0.96	0.52
N	10	10

DAY	0-7	7-14
GROUP 3 (300 MG/KG)		
A1059 M	3.3	2.1
A1072 M	2.7	2.4
A1075 M	2.2	1.8
A1080 M	4.8	1.4
A1082 M	2.4	1.9
A1084 M	3.8	1.9
A1087 M	3.8	1.3
A1091 M	3.6	2.1
A1093 M	1.6	0.5
A1111 M	2.8	2.0
MEAN	3.1	1.7
S.D.	0.94	0.54
N	10	10

DAY	0-7	7-14
GROUP 4 (900 MG/KG)		
A1052 M	3.0	1.3
A1053 M	0.8	0.8
A1058 M	4.9	2.2
A1066 M	3.2	2.0
A1067 M	1.8	1.5
A1085 M	3.0	1.7
A1089 M	2.9	0.2
A1097 M	5.2	2.1
A1105 M	3.5	0.8
A1055 M	2.5	1.3
MEAN	3.1	1.4
S.D.	1.30	0.65
N	10	10

DAY	0-7	7-14
GROUP 5 (2700 MG/KG)		
A1051 M	2.5	2.4
A1069 M	3.8	2.6
A1073 M	3.0	0.6
A1079 M	3.5	-0.1
A1083 M	3.6	1.4
A1094 M	0.2	1.1
A1095 M	1.8	1.7
A1096 M	6.5	2.4
A1098 M	0.8	1.5
A1103 M	3.6	1.8
MEAN	2.9	1.5
S.D.	1.77	0.85
N	10	10

DAY	0-7	7-14
GROUP 1A (0 MG/KG)		
A1113 F	1.4	1.8
A1116 F	1.3	0.5
A1119 F	1.2	0.9
A1122 F	1.6	1.1
A1130 F	1.7	2.1
A1132 F	2.6	0.9
A1145 F	1.7	1.1
A1138 F	2.0	1.2
A1162 F	1.8	0.1
A1172 F	1.8	1.6
MEAN	1.7	1.1
S.D.	0.40	0.59
N	10	10

DAY	0-7	7-14
GROUP 2A (2700 MG/KG)		
A1118 F	1.7	1.5
A1120 F	0.9	0.8
A1124 F	0.9	1.7
A1135 F	0.9	1.3
A1141 F	1.4	0.6
A1144 F	1.7	1.0
A1150 F	2.9	1.4
A1163 F	0.8	0.5
A1166 F	-0.5	0.5
A1171 F	2.4	1.5
MEAN	1.3	1.1
S.D.	0.95	0.46
N	10	10

DAY	0-7	7-14
GROUP 3A (300 MG/KG)		
A1125 F	2.6	-0.1
A1126 F	2.9	0.5
A1127 F	1.8	2.3
A1133 F	1.9	1.1
A1134 F	1.5	1.2
A1143 F	1.0	1.1
A1149 F	0.8	2.0
A1156 F	1.0	2.4
A1158 F	0.2	0.2
A1159 F	0.9	1.2
MEAN	1.5	1.2
S.D.	0.85	0.85
N	10	10

DAY	0-7	7-14
GROUP 4A (900 MG/KG)		
A1117 F	1.2	1.6
A1123 F	2.2	1.1
A1136 F	0.4	2.5
A1140 F	0.8	2.1
A1142 F	1.6	3.0
A1148 F	1.4	-1.8
A1152 F	0.4	2.1
A1155 F	1.1	1.9
A1160 F	2.3	2.1
A1169 F	0.6	1.7
MEAN	1.2	1.6
S.D.	0.68	1.31
N	10	10

DAY	0-7	7-14
GROUP 5A (2700 MG/KG)		
A1112 F	0.7	1.4
A1114 F	1.3	1.6
A1115 F	1.4	1.8
A1137 F	1.9	1.0
A1151 F	1.9	2.6
A1157 F	1.3	2.2
A1161 F	0.9	0.4
A1164 F	0.9	1.7
A1165 F	0.9	0.9
A1170 F	0.6	1.4
MEAN	1.2	1.5
S.D.	0.46	0.64
N	10	10

APPENDIX F

Individual Food Consumption Data
(g/kg/day)

SLI STUDY NO.: 3044.856
CLIENT: MONSANTO COMPANY

AN ACUTE ORAL TOXICITY STUDY IN MICE
INDIVIDUAL FOOD CONSUMPTION DATA (GRAMS/KG/DAY)

DAY	0-7	7-14
GROUP 1 (0 MG/KG)		
A1054 M	188.5	198.4
A1063 M	173.9	172.4
A1065 M	184.5	177.1
A1070 M	183.5	222.0
A1078 M	179.6	183.9
A1086 M	193.3	169.5
A1092 M	199.6	201.3
A1101 M	187.0	179.1
A1106 M	181.3	158.3
A1110 M	203.7	179.0
MEAN	187.5	184.1
S.D.	9.16	18.41
N	10	10

DAY		0-7	7-14
GROUP 2 (2700 MG/KG)			
A1056	M	183.0	166.4
A1057	M	183.3	167.9
A1062	M	212.6	199.9
A1076	M	195.3	188.8
A1077	M	202.0	171.7
A1081	M	214.3	194.2
A1090	M	188.0	164.9
A1102	M	189.0	178.8
A1104	M	200.7	188.8
A1107	M	206.1	168.1
MEAN		197.4	179.0
S.D.		11.50	12.96
N		10	10

DAY	0-7	7-14
GROUP 3 (300 MG/KG)		
A1059 M	207.0	183.8
A1072 M	207.6	212.5
A1075 M	198.7	202.9
A1080 M	211.9	186.2
A1082 M	206.9	198.0
A1084 M	193.4	174.6
A1087 M	214.3	187.7
A1091 M	223.9	188.9
A1093 M	208.9	189.2
A1111 M	187.6	174.2
MEAN	206.0	189.8
S.D.	10.48	11.95
N	10	10

DAY	0-7	7-14
GROUP 4 (900 MG/KG)		
A1052 M	197.3	183.7
A1053 M	208.1	200.2
A1058 M	217.5	194.4
A1066 M	197.8	197.1
A1067 M	202.2	193.9
A1085 M	205.4	180.5
A1089 M	209.2	171.0
A1097 M	218.2	161.4
A1105 M	204.8	175.0
A1055 M	188.3	168.9
MEAN	204.9	182.6
S.D.	9.18	13.44
N	10	10

DAY	0-7	7-14
GROUP 5 (2700 MG/KG)		
A1051 M	194.2	204.0
A1069 M	223.6	202.8
A1073 M	197.3	171.5
A1079 M	193.1	174.6
A1083 M	212.6	186.9
A1094 M	180.3	184.1
A1095 M	203.6	196.3
A1096 M	236.8	189.5
A1098 M	177.6	180.0
A1103 M	204.2	184.7
MEAN	202.3	187.4
S.D.	18.34	11.00
N	10	10

DAY		0-7	7-14
GROUP 1A (0 MG/KG)			
A1113	F	188.7	195.2
A1116	F	224.0	216.4
A1119	F	232.9	222.8
A1122	F	217.1	220.4
A1130	F	220.1	214.6
A1132	F	226.3	193.6
A1145	F	203.0	184.5
A1138	F	207.0	201.7
A1162	F	202.3	177.5
A1172	F	181.3	195.9
MEAN		210.3	202.3
S.D.		16.79	15.61
N		10	10

DAY		0-7	7-14
GROUP 2A (2700 MG/KG)			
A1118	F	210.5	195.3
A1120	F	198.2	193.1
A1124	F	208.1	195.0
A1135	F	200.8	191.7
A1141	F	217.0	245.6
A1144	F	217.1	189.4
A1150	F	211.6	191.9
A1163	F	178.3	195.4
A1166	F	160.1	178.1
A1171	F	225.7	211.8
MEAN		202.8	198.7
S.D.		19.84	18.39
N		10	10

DAY	0-7	7-14
GROUP 3A (300 MG/KG)		
A1125 F	235.3	278.9
A1126 F	238.3	224.9
A1127 F	217.7	256.8
A1133 F	197.0	187.9
A1134 F	210.6	197.1
A1143 F	207.8	202.6
A1149 F	205.8	229.9
A1156 F	194.7	210.2
A1158 F	176.7	177.6
A1159 F	189.4	223.3
MEAN	207.3	218.9
S.D.	19.41	31.08
N	10	10

DAY		0-7	7-14
GROUP 4A (900 MG/KG)			
A1117	F	202.7	221.8
A1123	F	220.3	197.8
A1136	F	191.1	213.1
A1140	F	221.3	229.2
A1142	F	227.8	281.0
A1148	F	212.2	205.5
A1152	F	193.7	204.2
A1155	F	195.2	182.2
A1160	F	233.1	203.0
A1169	F	190.3	201.5
MEAN		208.8	213.9
S.D.		16.19	26.88
N		10	10

DAY		0-7	7-14
GROUP 5A (2700 MG/KG)			
A1112	F	212.1	191.7
A1114	F	182.6	181.2
A1115	F	210.4	222.5
A1137	F	226.2	185.9
A1151	F	210.8	210.8
A1157	F	207.8	205.6
A1161	F	204.7	211.5
A1164	F	209.0	192.9
A1165	F	222.0	206.0
A1170	F	198.5	204.9
MEAN		208.4	201.3
S.D.		12.03	12.93
N		10	10

APPENDIX G

Individual Food Consumption Data
(g/animal/day)

SLI STUDY NO.: 3044.856 AN ACUTE ORAL TOXICITY STUDY IN MICE PAGE 1
CLIENT: MONSANTO COMPANY INDIVIDUAL FOOD CONSUMPTION DATA (GRAMS/ANIMAL/DAY)

DAY	0-7	7-14
GROUP 1 (0 MG/KG)		
A1054 M	5.0	5.7
A1063 M	4.6	4.9
A1065 M	5.2	5.3
A1070 M	4.8	6.6
A1078 M	5.2	5.7
A1086 M	5.9	5.8
A1092 M	5.4	6.0
A1101 M	5.1	5.2
A1106 M	5.4	5.1
A1110 M	5.6	5.7
MEAN	5.2	5.6
S.D.	0.37	0.48
N	10	10

DAY	0-7	7-14
GROUP 2 (2700 MG/KG)		
A1056 M	5.1	5.2
A1057 M	5.1	5.4
A1062 M	6.1	6.7
A1076 M	5.2	5.2
A1077 M	5.7	5.5
A1081 M	5.8	6.0
A1090 M	5.8	5.5
A1102 M	5.6	5.7
A1104 M	5.3	5.5
A1107 M	5.5	5.0
MEAN	5.5	5.6
S.D.	0.35	0.46
N	10	10

DAY	0-7	7-14
GROUP 3 (300 MG/KG)		
A1059 M	5.7	5.6
A1072 M	5.4	6.1
A1075 M	5.2	5.7
A1080 M	5.6	5.8
A1082 M	6.0	6.3
A1084 M	5.4	5.5
A1087 M	5.7	5.7
A1091 M	6.6	6.3
A1093 M	5.8	5.5
A1111 M	5.6	5.7
MEAN	5.7	5.8
S.D.	0.41	0.28
N	10	10

DAY	0-7	7-14
GROUP 4 (900 MG/KG)		
A1052 M	5.5	5.7
A1053 M	6.1	6.0
A1058 M	6.3	6.6
A1066 M	5.3	5.9
A1067 M	5.7	5.9
A1085 M	6.2	6.0
A1089 M	5.5	5.0
A1097 M	5.6	5.0
A1105 M	5.6	5.4
A1055 M	6.0	5.8
MEAN	5.8	5.7
S.D.	0.34	0.49
N	10	10

DAY	0-7	7-14
GROUP 5 (2700 MG/KG)		
A1051 M	5.2	6.0
A1069 M	6.0	6.2
A1073 M	5.8	5.6
A1079 M	5.3	5.4
A1083 M	5.4	5.5
A1094 M	5.1	5.2
A1095 M	5.5	5.6
A1096 M	6.3	6.3
A1098 M	5.3	5.5
A1103 M	5.6	5.7
MEAN	5.5	5.7
S.D.	0.38	0.34
N	10	10

DAY	0-7	7-14
GROUP 1A (0 MG/KG)		
A1113 F	4.5	4.9
A1116 F	5.8	5.8
A1119 F	5.8	5.8
A1122 F	5.0	5.4
A1130 F	5.4	5.6
A1132 F	5.7	5.3
A1145 F	4.9	4.7
A1138 F	4.7	4.9
A1162 F	5.1	4.8
A1172 F	4.3	5.0
MEAN	5.1	5.2
S.D.	0.54	0.43
N	10	10

DAY	0-7	7-14
GROUP 2A (2700 MG/KG)		
A1118 F	4.8	4.8
A1120 F	4.5	4.6
A1124 F	5.1	4.9
A1135 F	5.1	5.0
A1141 F	5.2	6.2
A1144 F	5.4	5.1
A1150 F	5.1	5.1
A1163 F	4.2	4.7
A1166 F	4.0	4.3
A1171 F	5.2	5.4
MEAN	4.8	5.0
S.D.	0.48	0.52
N	10	10

DAY	0-7	7-14
GROUP 3A (300 MG/KG)		
A1125 F	5.3	7.0
A1126 F	5.7	6.1
A1127 F	5.0	6.3
A1133 F	4.6	4.8
A1134 F	4.8	4.8
A1143 F	5.3	5.3
A1149 F	5.0	5.8
A1156 F	4.9	5.5
A1158 F	4.5	4.5
A1159 F	4.9	6.0
MEAN	5.0	5.6
S.D.	0.36	0.77
N	10	10

DAY	0-7	7-14
GROUP 4A (900 MG/KG)		
A1117 F	4.7	5.5
A1123 F	5.2	5.1
A1136 F	4.7	5.3
A1140 F	5.6	6.0
A1142 F	5.2	6.8
A1148 F	5.1	5.2
A1152 F	4.8	5.2
A1155 F	4.7	4.6
A1160 F	5.9	5.6
A1169 F	4.5	4.9
MEAN	5.1	5.4
S.D.	0.46	0.63
N	10	10

DAY	0-7	7-14
GROUP 5A (2700 MG/KG)		
A1112 F	5.4	5.0
A1114 F	4.5	4.7
A1115 F	5.0	5.6
A1137 F	5.4	4.8
A1151 F	5.2	5.6
A1157 F	4.8	5.1
A1161 F	5.0	5.4
A1164 F	4.8	4.6
A1165 F	5.1	4.9
A1170 F	5.0	5.3
MEAN	5.0	5.1
S.D.	0.28	0.35
N	10	10

APPENDIX H

Individual Gross Necropsy Observations

ANIMAL NO.	A1054 GROUP 1 (0 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1063 GROUP 1 (0 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1065 GROUP 1 (0 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1070 GROUP 1 (0 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1078 GROUP 1 (0 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1086 GROUP 1 (0 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1092 GROUP 1 (0 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1101 GROUP 1 (0 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1106 GROUP 1 (0 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1110 GROUP 1 (0 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1056 GROUP 2 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS

ANIMAL NO.	A1057 GROUP 2 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1062 GROUP 2 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1076 GROUP 2 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1077 GROUP 2 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1081 GROUP 2 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1090 GROUP 2 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1102 GROUP 2 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1104 GROUP 2 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1107 GROUP 2 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1059 GROUP 3 (300 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1072 GROUP 3 (300 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS

ANIMAL NO.	A1075 GROUP 3 (300 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1080 GROUP 3 (300 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1082 GROUP 3 (300 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1084 GROUP 3 (300 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1087 GROUP 3 (300 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1091 GROUP 3 (300 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1093 GROUP 3 (300 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1111 GROUP 3 (300 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1052 GROUP 4 (900 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1053 GROUP 4 (900 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1058 GROUP 4 (900 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS

ANIMAL NO.	A1066 GROUP 4 (900 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1067 GROUP 4 (900 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1085 GROUP 4 (900 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1089 GROUP 4 (900 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1097 GROUP 4 (900 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1105 GROUP 4 (900 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1055 GROUP 4 (900 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1051 GROUP 5 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1069 GROUP 5 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1073 GROUP 5 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1079 GROUP 5 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS

ANIMAL NO.	A1083 GROUP 5 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1094 GROUP 5 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1095 GROUP 5 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1096 GROUP 5 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1098 GROUP 5 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1103 GROUP 5 (2700 MG/KG)	MALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS

ANIMAL NO.	A1113 GROUP 1A (0 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1116 GROUP 1A (0 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1119 GROUP 1A (0 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1122 GROUP 1A (0 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1130 GROUP 1A (0 MG/KG) OVARY	FEMALE DAY 14 PERIOVARIAN CYST(S); PRESENT BILATERAL, EACH APPROXIMATELY 0.5 CM DIAMETER, CLEAR FLUID FILLED ALL OTHER TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1132 GROUP 1A (0 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1145 GROUP 1A (0 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1138 GROUP 1A (0 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1162 GROUP 1A (0 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1172 GROUP 1A (0 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS

ANIMAL NO.	A1118 GROUP 2A (2700 MG/KG) OVARY	FEMALE DAY 14 PERIOVARIAN CYST(S); PRESENT LEFT, 0.3 CM IN DIAMETER, CLEAR FLUID FILLED; RIGHT, 0.3 CM IN DIAMETER, RED FLUID FILLED ALL OTHER TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1120 GROUP 2A (2700 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1124 GROUP 2A (2700 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1135 GROUP 2A (2700 MG/KG) OVARY	FEMALE DAY 14 PERIOVARIAN CYST(S); PRESENT RIGHT, ONE, 0.4 CM DIAMTER, CLEAR FLUID FILLED ALL OTHER TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1141 GROUP 2A (2700 MG/KG) OVARY	FEMALE DAY 14 PERIOVARIAN CYST(S); PRESENT LEFT, ONE, 0.4 CM DIAMETER, CLEAR FLUID FILLED ALL OTHER TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1144 GROUP 2A (2700 MG/KG) LUNG	FEMALE DAY 14 DARK RED AREA(S); PRESENT LEFT LOBE, ONE, 0.4 CM X 0.3 CM ALL OTHER TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1150 GROUP 2A (2700 MG/KG) LUNG	FEMALE DAY 14 REDDENED; PRESENT ALL LOBES ALL OTHER TISSUES WERE WITHIN NORMAL LIMITS

ANIMAL NO.	A1163 GROUP 2A (2700 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1166 GROUP 2A (2700 MG/KG) OVARY	FEMALE DAY 14 PERIOVARIAN CYST(S); PRESENT RIGHT, ONE, 0.4 CM DIAMETER, CLEAR FLUID FILLED ALL OTHER TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1171 GROUP 2A (2700 MG/KG) OVARY	FEMALE DAY 14 PERIOVARIAN CYST(S); PRESENT LEFT, ONE, 0.1 CM IN DIAMETER, CLEAR FLUID FILLED ALL OTHER TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1125 GROUP 3A (300 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1126 GROUP 3A (300 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1127 GROUP 3A (300 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1133 GROUP 3A (300 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1134 GROUP 3A (300 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1143 GROUP 3A (300 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1149 GROUP 3A (300 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS

ANIMAL NO.	A1156 GROUP 3A (300 MG/KG) OVARY	FEMALE DAY 14 PERIOVARIAN CYST(S); PRESENT BILATERAL, EACH APPROXIMATELY 0.4 CM IN DIAMETER, CLEAR FLUID FILLED ALL OTHER TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1158 GROUP 3A (300 MG/KG) OVARY	FEMALE DAY 14 PERIOVARIAN CYST(S); PRESENT RIGHT, ONE, 0.5 CM DIAMETER, CLEAR RED FLUID FILLED ALL OTHER TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1159 GROUP 3A (300 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1117 GROUP 4A (900 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1123 GROUP 4A (900 MG/KG) OVARY	FEMALE DAY 14 PERIOVARIAN CYST(S); PRESENT RIGHT, ONE, 0.4 CM DIAMETER, CLEAR FLUID FILLED ALL OTHER TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1136 GROUP 4A (900 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1140 GROUP 4A (900 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1142 GROUP 4A (900 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1148 GROUP 4A (900 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS

ANIMAL NO.	A1152 GROUP 4A (900 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1155 GROUP 4A (900 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1160 GROUP 4A (900 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1169 GROUP 4A (900 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1112 GROUP 5A (2700 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1114 GROUP 5A (2700 MG/KG) OVARY	FEMALE DAY 14 PERIOVARIAN CYST(S); PRESENT BILATERAL, EACH APPROXIMATELY 0.4 CM DIAMETER, CLEAR FLUID FILLED ALL OTHER TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1115 GROUP 5A (2700 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1137 GROUP 5A (2700 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1151 GROUP 5A (2700 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1157 GROUP 5A (2700 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS

SLI STUDY NO.: 3044.856
CLIENT: MONSANTO COMPANY

AN ACUTE ORAL TOXICITY STUDY IN MICE
INDIVIDUAL GROSS NECROPSY OBSERVATIONS
SCHEDULED EUTHANASIA

ANIMAL NO.	A1161 GROUP 5A (2700 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1164 GROUP 5A (2700 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1165 GROUP 5A (2700 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS
ANIMAL NO.	A1170 GROUP 5A (2700 MG/KG)	FEMALE DAY 14 ALL TISSUES WERE WITHIN NORMAL LIMITS

APPENDIX I

SLI Personnel Responsibilities

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Manager of Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Assistant Toxicologist
Robert C. Springborn, Ph.D.	Chairman, President and CEO
Malcolm Blair, Ph.D.	Senior Vice President and Managing Director
Joseph C. Siglin, Ph.D., DABT	Vice President, Director of Research
Rusty E. Rush, M.S., LAT, DABT	Associate Director of Toxicology
Christopher W. Wilson, B.S.	Assistant Toxicologist
Pamela S. Smith, ALAT	Primary Technician/Supervisor of Acute Toxicology
Delores P. Knippen	Supervisor of Pharmacy
Steven H. Magness, B.S., LATG	Supervisor of Gross and Fetal Pathology
Anita M. Bosau, RQAP-GLP	Director of Compliance Assurance
Deanna M. Talerico, RQAP-GLP	Supervisor of Quality Assurance
J. Dale Thurman, D.V.M., M.S., DACVP	Director of Pathology

Statistical Evaluation of Composition Data
Terminology for Tolerance Interval

Recent conversations with our statistician, Margaret Nemeth, have resulted in a revision in the terminology to be used for the designation of tolerance intervals for the commercial reference varieties in the evaluation of composition data.

A tolerance interval is an interval with a specified degree of confidence, $100(1-\alpha)\%$, which contains at least a specified proportion, p, of an entire population for the parameter measured. For each compositional analysis component, tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the values expressed in the population of commercial lines.

In the previous statistical reports from Certus International, Inc. this interval has been called the 95% Tolerance Interval specified to contain 99% of the commercial population. It has been decided that in subsequent reports the interval will be referred to as the 99% Tolerance Interval. In this revised nomenclature the numeric value identifies the range of values contained within the interval rather than the level of confidence. The 95% level of confidence for the 99% Tolerance Interval will be explained in the text of the final reports and the Certus statistical subreports as well as footnotes to data tables in both reports.

This change in terminology will not have any effect on the way in which the analysis is conducted or the interpretation of the results.

Will

William P. Ridley, Ph.D.
Product Safety Center
Composition Team

February 2, 2001