18) Taylor, M. L., J. D. Astwood, M. Breeze and C. Stone (2001). Pesticide profile, mycotoxin and compositional analysis of corn event MON 863 and control lines LH82xA634 produced in Kihei, Hawaii in 2000. MSL-16953, an unpublished study conducted by Monsanto Company.

Study Title

Pesticide Profile, Mycotoxin, and Compositional Analyses of Corn Event MON 863 and Control Line LH82xA634 Produced in Kihei, Hawaii in 2000

Authors

Mary L. Taylor, James A. Astwood, Matthew Breeze*, and Charla Stone**

Study Completed On

June 20, 2001

Performing Laboratories

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Laboratory Project ID

Study Number: 00-01-39-35
Monsanto Report No: MSL-16953

Statement of No Data Confidentiality Claims

No claim of confidentially 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), and (C).

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

Company

Company Agent

Title

Signature

Statement of Compliance

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

The mycotoxin analyses completed at Romer Labs was conducted using a non-GLP assay. These analyses were a part of the pre-study requirement for subsequent animal feeding studies and were conducted under high scientific standards.

For the compositional analyses at Covance, the reference standards were not characterized according to GLP standards, and reserve samples from each batch of the reference standards were not retained. These exceptions had no effect on the integrity or quality of the study.

Submitter — Date

Ravinder S Sidhu — June 20, 2001
Sponsor Representative — Date

Mary L Taylor — June 20, 2001
Study Director — Date

Quality Assurance Statement

Study Title: Pesticide Profile, Mycotoxin, and Compositional Analyses of Corn Event MON 863 and Control Line LH82xA634 Produced in Kihei, Hawaii in 2000

Study Number: 00-01-39-35

Reviews conducted by the Quality Assurance Unit confirm that the final report reflects the raw data for the portion of the study conducted by Monsanto Company, Biotechnology Regulatory Sciences. This confirmation excludes the following data:

- Mycotoxin Analyses from Romer Laboratories

Reviews which have been conducted by Covance Laboratories, Inc. are enclosed within the Covance Subreport and are specified on their individual QA Statement.

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

Dates of Inspection/Audit	Phase	Date Reported to Study Director	Date Reported to Management
February 20, 2001	Draft Report Review	May 18, 2001	May 18, 2001

Paula A. Price — June 20, 2001

Quality Assurance Unit
Monsanto Regulatory

Date

Signatures of Approval

Study Number: 00-01-39-35

MSL Number: MSL-16953

Title: Pesticide Profile, Mycotoxin, and Compositional Analyses of Corn Event MON 863 and Control Line LH82xA634 Produced in Kihei, Hawaii in 2000

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Study Director: Mary L. Taylor

Principal Investigators: Matthew Breeze and Charla Stone

Experimental Start Date: 7/5/00

Experimental Completion Date: 6/20/01

Records Retention: The study specific raw data, protocols, final reports and facility records will be retained at Monsanto, St. Louis, Covance Labs, and Romer Labs.

Sample Storage: Any unused study samples that are not destroyed will be stored at Monsanto, St. Louis. Ground samples were destroyed at Covance and Romer Labs.

Signatures of Final Report Approval:

Mary L. Taylor — June 20, 2001
Study Director — Date

Rawnda S Sidhu — June 20, 2001
Sponsor Representative — Date

Product Safety Center Representative — June 20, 2001
Date

Abbreviations and Definitions

SOP	Standard Operating Procedure
g, mg	gram, milligram
AACC	American Association of Cereal Chemists
AOCS	American Oil Chemists Society
AOAC	Association of Official Analytical Chemists
NRC	National Research Council
fw	fresh weight
ppm	Parts per million
NDFE	Neutral Detergent Fiber, Enzyme Method
ADF	Acid Detergent Fiber
PAM	Pesticide Analytical Manual
FDA	Food and Drug Administration
Cry3Bb1	A natural isolate, and holotype, of the Cry3Bb class of *B.t.* Cry proteins
v/v	volume to volume
EPA	Environmental Protection Agency
FIFRA	Federal Insecticide Fungicide and Rodenticide Act

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Tables

Attachments

1.0 Introduction

Compositional data generated during this study was used to formulate animal diets in subsequent animal feeding studies. The test event MON863 produces a variant of the wild-type Cry3Bb1 protein (Hileman and Astwood, 2001), which protects against corn rootworm (CRW, *Diabrotica*). The study includes analyses of the non-transgenic parental control line, LH82xA634, that has background genetics representative of the MON863 test event but does not express the Cry3Bb1 insect control gene. Diets in subsequent feeding studies were formulated based on the individual nutrient analyses for the grain from the test event and control line.

2.0 Purpose

The purpose of this study was to conduct pesticide profile, mycotoxin, and compositional analyses of grain from corn event MON863 and its parental control line, LH82xA634. The pesticide and mycotoxin analyses were conducted to screen for potential contaminants in the grain, and the compositional data was used to formulate the diets in subsequent feeding studies.

3.0 Timelines

3.1 Study Start Date: July, 2000

3.2 Study Termination Date: June, 2001

4.0 Test and Control Substances

4.1 *Test Substance.* The test substance is the corn event MON863 produced in Kihei, Hawaii, USA under Production Plan #00-01-39-04 during the 2000 field season. It was assigned LIMS ID# 00ZMGRO01389 for the production of the test substance and Lot # TIO-0006-10408-I for tracking upon receipt at Monsanto.

4.2 *Control Substance.* The (negative) control substance, LH82xA634, is the non-transgenic control corn event for MON 863. It was also produced in Kihei, Hawaii, USA under Production Plan #00-01-39-04 during the 2000 field season. It was assigned LIMS ID# 00ZMGRO01390 for the production of the test substance and Lot # TPC-0006-10409-I for tracking upon receipt at Monsanto.

4.3 *Test and Control Substance Characterization.* The identity of the test and control substances were confirmed by molecular PCR analysis and by field and chain-of-custody records. These characterizations are filed under study #00-01-39-04.

4.4 *Test and Control Substance Seed Production and Shipment.* Test and control substance were produced during the 2000 growing season in the US. Bulk samples of test and control grain were shipped at ambient temperature to Colorado Quality Research (Wellington, CO) for subsequent broiler feeding studies. Chain of custody documentation accompanied all shipments.

5.0 Analytical Methods

5.1 *Pesticide Profile and Compositional Analyses at Covance.* All corn grain samples were analyzed for the presence of pesticides using the FDA PAM 304 pesticide screen (M304)-Appendix 1.

The following compositional analyses were performed on the test and control samples: proximates [moisture (M100), protein (PGEN), fat (FSOX), ash (ASHM)], crude fiber (CFIB), amino acid composition (TAAP), fatty acid composition (FAPM), acid detergent fiber (ADF), neutral detergent fiber (NDFE), sulfur (SULA), calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc (ICPS), cadmium (CDA), selenium (SEAS), and chloride (CLA). Carbohydrate (CHO) values were estimated by calculation.

a) **Acid detergent fiber (ADF).** The method used was based on a modified version of a USDA method (1970). The sample was placed in a fritted vessel and washed with an acidic boiling detergent solution that dissolved the protein, carbohydrate, and ash. An acetone wash removed the fats and pigments. The lignocellulose fraction was collected on the frit and determined gravimetrically. The limit of detection of the method for this study was 0.1% fresh weight (fw). There was no analytical reference substance for this analysis.

b) **Amino acid composition (TAAP).** The method used was based on a modified version of AOAC method 982.30 (2000) which estimates the levels of 18 amino acids in the sample: alanine, arginine, aspartic acid (including asparagine), cystine (including cysteine), glutamic acid (including glutamine), glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. The sample was assayed by three methods to obtain the full profile. Tryptophan required a base hydrolysis with sodium hydroxide. The sulfur containing amino acids required an oxidation with performic acid prior to hydrolysis with hydrochloric acid. Analysis of the samples for the remaining amino acids was accomplished through direct acid hydrolysis with hydrochloric acid. Once hydrolyzed, the individual amino acids were quantitated using an automated amino acid analyzer. The limit of detection of the method was 0.1 mg/g fw. The reference standards were: Beckman K18, 2.5

µmol/mL per constituent except cystine (1.25 µmol/mL), lot no. S911165; Aldrich L-tryptophan, 99%, lot no. 12729HS; Aldrich L-cysteic acid monohydrate, 98.0%, lot no. 04615MS; and Sigma L-methionine sulfone, 100%, lot no. 012H3349.

c) **Ash (ASHM).** The method used was based on a modified version of AOAC method 923.03 (2000). The sample was placed in an electric furnace at 550 °C and ignited to drive off all volatile organic matter. The nonvolatile matter remaining was quantitated gravimetrically and calculated to determine percent ash. The limit of detection for this study was 0.1% fw. There is no analytical reference for this analysis.

d) **Cadmium (CDA).** The method used was based on modified versions of AOAC method 974.27 (2000), U.S. EPA method Metals 1-19 and Method 213.1 (1979), and Perkin-Elmer method (1982). The sample was either dry-ashed, wet-ashed, or read directly. If dry-ashed, the sample was dried, pre-charred and ashed at 500 °C ± 50 °C in a muffle furnace for 5 to 16 hours. The sample was removed from the muffle furnace, cooled, treated with nitric acid, re-ashed, and dissolved in hydrochloric acid solution. If wet-ashed, the sample was digested on a hot plate with nitric acid, hydrochloric acid, and/or hydrogen peroxide. The amount of cadmium was determined by comparing the signal of the unknown sample, measured by the atomic absorption (AA) spectrophotometer, with the signal of the standard solutions. The limit of detection for this assay is 0.04 ppm. Reference Standard: Fisher Scientific, 1000 ppm cadmium, Lot Number 981734-24.

e) **Carbohydrates (CHO).** The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation:

% carbohydrates = 100% - (% protein + % fat + % moisture + % ash)

The limit of detection for this study was 1.0%. There was no analytical reference standard for the analysis.

f) **Chloride (CLA).** The method used was based on modified versions of AOAC methods 963.05, 969.10, and 971.27 (2000). The sample was put into solution with double deionized water and then made acidic with nitric acid. Chloride was determined potentiometrically by titrating with a standard silver nitrate solution to a predetermined endpoint. The limit of detection for this assay was 0.004%. The analytical reference standard used for this method was Mallinckrodt, 1,000 ppm sodium chloride, 99.9%, Lot Number 7581 KPAK.

g) **Crude Fiber (CFIB).** The method used was based on a modified version of

AOAC method 962.09 (2000). Crude fiber was quantitated as the loss on ignition of dried residue remaining after digestion of the sample with 1.25% sulfuric acid and 1.25% sodium hydroxide solutions under specific conditions. The limit of detection for this study was 0.1% fw. There is no analytical reference substance for this analysis.

h) **Fat by Soxhlet Extraction (FSOX).** The method used was based on a modified version of AOAC method 960.39 (2000). The sample was weighed into a cellulose thimble containing sand or sodium sulfate and dried to remove excess moisture. Pentane was dripped through the sample to remove the fat. The extract was evaporated, dried and weighed. The limit of detection of this method for this study was 0.1% fw. There was no analytical reference substance for this analysis.

i) **Fatty Acid Profile (FAPM).** The method used was based on a modified version of AOCS method Ce 1-62 (1997) which estimates the levels of 22 fatty acids in the sample: 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 16:0 palmitic acid, 16:1 palmitoleic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:0 stearic acid, 18:1 oleic acid, 18:2 linoleic acid, 18:3 linolenic, 18:3 gamma linolenic acid, 20:0 arachidic acid, 20:1 eicosenoic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, 20:4 arachidonic acid, and 22:0 behenic acid. The lipid in grain was extracted and saponified with 0.5 N sodium hydroxide in methanol. The saponification mixture was methylated with 14% (v/v) boron trifluoride:methanol. The resulting methyl esters were extracted with heptane containing an internal standard. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation. The limit of detection of this method for this study was 0.004%. The analytical reference standards (purity 100%) were: Nu Chek Prep Hazelton special prep no. 1 (lot no. A4-K), no. 2 (lot no. S10-G), no. 3 (lot no. F23-J) and no. 4 (lot no. JY26-J), and Nu Chek Prep methyl gamma linolenate (lot nos. U-63M-F25-J).

j) **Minerals/ICP emission spectrometry (ICPS).** The method used was based on modified versions of AOAC methods 984.27 and 985.01 (2000) and a modified version of a literature method (Dahlquist *et al.*, 1978). This method estimates the levels of nine minerals in the sample: calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc. The sample was dried, precharred, and ashed overnight at 500 °C ± 50 °C . The ashed sample was treated with hydrochloric acid, taken to dryness, and put into a solution of 5% (v/v) hydrochloric acid. The amount of each element was determined at appropriate wavelengths by comparing the emission of the unknown sample, measured by the inductively coupled plasma, with the emission of the standard solutions described below.

Table A
Spex Certiprep Reference Standards and Limits of Detection

Mineral	Lot Numbers	Concentration (ppm)	Limit of Detection (ppm)
Calcium	L6-59CA	10,000	20.0
Copper	6-242CU	1,000	0.500
Iron	7-97FE	1,000	2.00
Magnesium	L5-187MG	10,000	20.0
Manganese	6-201MN	1,000	0.300
Phosphorus	K6-54P	10,000	20.0
Potassium	M6-16K	10,000	100
Sodium	N6-151NA	10,000	100
Zinc	6-264ZN	1,000	0.400

k) **Moisture (M100).** The method used was based on a modified version of AOAC methods 926.08 and 925.09 (2000). The sample was dried in a vacuum oven at 100 °C to a constant weight. The moisture loss was determined and converted to percent moisture. The limit of detection of this method for this study was 0.1% fw. There was no analytical reference substance for this analysis.

l) **Neutral detergent fiber, enzyme method (NDFE).** The method used was based on modified versions of an AACC method 32.20 (1998) and a USDA method (1970). The sample was placed in a fritted vessel and washed with a neutral boiling detergent solution that dissolved the protein, carbohydrate, enzyme and ash. An acetone wash removed the fats and pigments. The hemicellulose, cellulose and lignin fractions were collected on the frit and determined gravimetrically. The limit of detection of this method for this study was 0.1% fw. There was no analytical reference substance for this analysis.

m) **Pesticide Profile (M304).** The method used was based on a modified version of a FDA method (1999). The sample was blended with ethyl acetate and cleaned up by gel permeation chromatography. The extract was analyzed for organophosphates, chlorinated, and nitrogen on a gas chromatography system. A high performance liquid chromatography system was used for the analysis of carbamates. The limits of detection (ppm) for this assay were: Organophosphates (0.050), Organonitrogens (0.500), Organochlorinated (0.200), and N-Methylcarbamates (0.100). Reference standards include:
Restek Corporation Custom Chlorinated Pesticide Mix, catalog # 54609, lot number A011108;
Restek Corporation Custom Organophosphorus Pesticides Mix, catalog # 54610, lot number A011117;
Restek Corporation Custom Nitrogen List catalog # 54611, lot number A011122;

Restek Corporation Carbamates I Mixture catalog # 54612, lot number A016802 and Restek Corporation Carbamates II Mixture catalog # 54613, lot number A016804.

n) **Protein (PGEN).** The method used was based on modifications of AOAC methods 955.04 and 979.09 (2000) and literature methods (Bradstreet, 1965; Kalthoff and Sandell, 1948). Protein and other nitrogenous compounds in the sample were reduced to ammonia by digesting the sample with sulfuric acid containing a mercury catalyst mixture. The acid digest was made alkaline, and the ammonia was distilled and titrated with a standard acid. The percent nitrogen was determined and converted to percent protein by multiplication with 6.25. The limit of detection for this study was 0.1%. There was no analytical reference substance for this analysis.

o) **Selenium (SEAS).** The method used was based on a modified version of AOAC methods 969.06 and 986.15 (2000) and modified literature methods (Watkinson, J.H., 1966; Haddad, P.R. and Smythe, L.E., 1974; and Bayfield, R.F. and Romalis, L.F., 1985). The sample was digested in a nitric-perchloric-hydrochloric acid mixture, in which any selenium present formed selenous acid. The selenous acid reacts with 2,3 diaminonaphtalene to form 2,3-4,5-benzopiazselenol. This compound was extracted into an organic solvent. The amount of selenium is then determined by comparing the absorbance of the unknown sample, measured by fluorescence spectroscopy, with the absorbance of standard solutions. The limit of detection for this assay was 0.05 ppm. Reference Standard: Fisher Scientific, 1000 ppm selenium, Lot Number 994379-18.

p) **Sulfur (SULA).** The method used was based on a modification of a literature method (Soil Society of America Proceedings, 1965). The sample was weighed into a volumetric flask and refluxed with nitric acid. Perchloric acid was added and refluxed again. Hydrochloric acid was added and the sample was heated to break down nitroso compounds. Sulfur seed and sulfur buffer solution were added. The analysis was completed by measuring the extent of turbidity in the sample after the addition of barium chloride. The percent transmittance of the samples is compared to that of standards for determining sulfur concentrations. The limit of detection for this study was 0.015%. Reference Standard: Spex CertiPrep, 1,000 mcg/ml sulfur, used as 100%, Lot Number 6-202S.

5.2 *Mycotoxin Analysis at Romer Labs.* Grain samples from the test event and control line were analyzed at Romer Labs, for potential mycotoxin contamination according to the methods employed for the 'Mycotoxin Screen': *Aflatoxin By HPLC*, Version: AFL-LC-01-00. 1 (formerly AFLAHPLC); *Ochratoxin by HPLC*, Version: O/C-DB-01-00.1, (formerly Version 97.4 OCHRAHPLC); *Analysis of*

Mixed Feed for Type A and B Trichohecenes By TLC, Version TRI-TL-01-00.1 (formerly Version: 95.4 FD Method); *HPLC Analyses for Zearalenonel*, Version ZON-LC-01-00.2 (formerly Version: 95.5 ZOLZONLOWER); *Fumonisin By HPLC*, Version FUM-LC-01-00.1 (formerly Version: 99.1 FUMHPLC) and *Citrinin By TLC*, Version O/C-DB-01-00.1 (formerly Version 99.1 (CITTLC). These non-GLP assays were a part of the pre-study requirement for subsequent animal feeding studies.

Test descriptions with limits of dectection are as follows: Aflatoxin B1, B2, G1, and G2, 1.0 ppb; Ochratoxin A, 5 ppb; Citrinin, 0.2 ppm; T-2 and HT-2 Toxin, 0.1 ppm; Diacetoxyscirpenol, 0.3 ppm; Neosolaniol, 0.5 ppm; Fusarenon X, 0.5 ppm; Deoxynivalenol, 0.1 ppm; 15 Acetyl-DON and 3-Acetyl-DON, 0.1 ppm, Nivalenol, 0.5 ppm, Zearalenone, 100 ppb; and Fumonisin B1, B2, and B3, 0.1 ppm.

6.0 Control of Bias and Quality Control Measures

Samples were treated in a similar manner for the test event and control line. Chain of custody documentation accompanied all shipments.

7.0 Results and Discussion

The identity of the test and control substances were confirmed by molecular PCR analyses and by field and chain of custody records. Both samples tested as expected, thereby confirming identity before use in subsequent analyses.

Initially, the corn grain was measured for potential pesticide and mycotoxin contamination. All values for the pesticide screen were below the limits of detection (see attached Covance subreport). All values for the mycotoxin screen were acceptable for the test event and control line (see attached Romer Labs data summary). The limits of detection (ppm) for the pesticide screen were: Organophosphates (0.050), Organonitrogens (0.500), Organochlorinated (0.200), and N-Methylcarbamates (0.100). Mycotoxin test descriptions with limits of dectection are as follows: Aflatoxin B1, B2, G1, and G2, 1.0 ppb; Ochratoxin A, 5 ppb; Citrinin, 0.2 ppm; T-2 and HT-2 Toxin, 0.1 ppm; Diacetoxyscirpenol, 0.3 ppm; Neosolaniol, 0.5 ppm; Fusarenon X, 0.5 ppm; Deoxynivalenol, 0.1 ppm; 15 Acetyl-DON and 3-Acetyl-DON, 0.1 ppm, Nivalenol, 0.5 ppm, Zearalenone, 100 ppb; and Fumonisin B1, B2, and B3, 0.1 ppm.

Compositional analyses were conducted on test and control grain to aid in formulating diets for subsequent feeding studies. The data for proximates (protein, moisture, fat, ash, and carbohydrates), crude fiber, neutral detergent fiber, and acid detergent fiber, sulfur, chloride, fatty acids, amino acids, selenium, cadmium, and minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc) is summarized in

Tables 1-5. All values are on a fresh weight basis. Compositional data on test and control grain were generally similar to literature ranges for corn (Ridley, et al., 2000, NRC, 1993, NRC 1994, NRC 1998, and Dale, 2000). All excess grain was disposed of at Romer and Covance Laboratories.

8.0 Conclusion

The test and control corn grain was analyzed for potential pesticide and mycotoxin contamination. All values for the pesticide screen were below the limit of detection. All values for the mycotoxin screen were acceptable for both samples. Compositional data on the test event and the control line were generally within normal ranges for corn and were suitable for formulating diets for animal nutrition or toxicology studies.

9.0 Acknowledgments

The authors would like to acknowledge Will Ridley and Ravinder Sidhu for critical review of this report and Donna Perrino-Stephens for assistance with the tabular presentation herein.

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Appendix 1.
Standard Compounds for M304 Pesticide Screen Provided by Covance Labs

Organochlorinateds	Organophosphates	Organonitrogens	n-Methyl Carbamates
Cypermethrin	Demeton-S	Ethalfluralin	3-Hydroxycarbofuran
Aldrin	Vapona	Fenpropathrin	Aldicarb
Endosulfan I	Dichlofenthion	Benfluralin	Aldicarb Sulfone
Endosulfan II	Methyl Chlorpyrifos	Ametryne	Aldicarb Sulfoxide
Oxadiazon	Prothiophos	Methoprotryne	Bendiocarb
DCNA	Dimethoate	Ethoxyquin	Butocarboxim
p,p'-DDE	Ethion	Aminocarb	Butoxycarboxim
Delta-BHC	Propetamphos	Myclobutanil	Carbaryl
DCPA	Fonofos	Metribuzin	Carbofuran
Captan	Acephate	Ethiolate	Dioxacarb
Chlorothalonil	Thimet	Nitralin	Ethiofencarb
Beta-BHC	Mevinphos	Pendimethalin	Fenobucarb
Endosulfan Sulfate	Parathion	Oxythioquinox	Isoprocarb
Folpet	Fenitrothion	Primacarb	Methiocarb
Technazene	Coumaphos	Diphenylamine	Methomyl
Endrin	Ronnel	Fluazifop-butyl	Metolcarb
Heptachlor Epoxide	Ethyl Parathion	Dinitramine	Oxamyl
Propyzamide	Phosalone	Procyazine	Promecarb
Alpha-BHC	Methamidiphos	Metalaxyl	Propoxur
p,p-DDT	Phosmet	Napropamide	Thiofanox
Mirex	Methidathion	Prometryne	
Permethrin	Azinphos-methyl	Propham	
Dicofol	Disulfoton	Simazine	
HCB	Malathion	Simetryn	
PCNB	EPN	Terbumeton	
Heptachlor	Ethyl Chlorpyrifos	Terbuthylazine	
Gamma-BHC (Lindane)	Methyl Pirimiphos	Terbutryn	
p,p-DDD	Trithion	Tetramethrin	
Captifol	Omethoate	Thiabendazole	
Methoxychlor	Chlorfenvinphos	THPI	
Dieldrin	Diazinon	Trifluralin	
Tetradifon			
Vinclozolin			

TABLE 1
Content of Proximates in Test and Control Corn Grain (% FW)*

Events	Moisture	Protein	Fat	Ash	Carbohydrates
(Test) MON863	10.6	11.3	2.71	1.50	73.9
(Control) LH82xA634	10.8	9.93	3.32	1.35	74.6

TABLE 2
Content of Fiber, Sulfur, Chloride, Cadmium, and Selenium in Test and Control Corn Grain

Events	Crude Fiber (% FW)*	NDFE (% FW)	ADF (% FW)	Sulfur (% FW)	Chloride (% FW)	Selenium (ppm)**	Cadmium (ppm)
(Test) MON863	2.15	9.16	2.66	0.088	0.046	0.08	<0.04
(Control) LH82xA634	1.72	11.6	2.29	0.093	0.049	0.09	<0.04

TABLE 3
Content of Minerals in Test and Control Corn Grain (ppm)**

Events	Calcium	Copper	Iron	Magnesium	Manganese	Phosphorus	Potassium	Sodium	Zinc
(Test) MON863	34.2	1.68	22.5	1290	7.49	3680	3850	<100	21.4
(Control) LH82xA634	33.3	1.90	22.8	1260	6.96	3650	3790	<100	21.0

*% = [g/g fresh weight] × 100
**ppm = parts per million

TABLE 4
Content of Fatty Acids in Test and Control Corn Grain (%FW)*

Events	8:0 caprylic	10:0 capric	12:0 lauric	14:0 myristic	14:1 myristoleic	15:0 pentadecanoic	15:1 pentadecenoic	16:0 palmitic
(Test) MON863	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	0.234
(Control) LH82xA634	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	0.298

Events	16:1 palmitoleic	17:0 heptadecanoic	17:1 heptadecenoic	18:0 stearic	18:1 oleic	18:2 linoleic	18:3 gamma linoleic
(Test) MON863	<0.004	<0.004	<0.004	0.0509	0.558	1.64	<0.004
(Control) LH82xA634	0.00462	<0.004	<0.004	0.0626	0.675	2.07	<0.004

Events	18:3 linoleic	20:0 arachidic	20:1 eicosenoic	20:2 eicosadienoic	20:3 eicosatrienoic	20:4 arachidonic	22:0 behenic
(Test) MON863	0.0265	0.0105	0.00731	<0.004	<0.004	<0.004	0.00498
(Control) LH82xA634	0.035	0.0132	0.00885	<0.004	<0.004	<0.004	0.00580

*% = [g/g fresh weight] × 100

TABLE 5
Content of Amino Acids in Test and Control Corn Grain (mg/g FW)

Events	Aspartic Acid	Threonine	Serine	Glutamic Acid	Proline	Glycine	Alanine	Cystine	Valine
(Test) MON863	7.70	3.74	5.27	22.2	9.93	4.20	9.01	2.30	5.88
(Control) LH82xA634	6.77	3.35	4.63	20.1	9.04	3.70	8.18	2.11	5.24

Events	Methionine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Histidine	Lysine	Arginine	Tryptophan
(Test) MON863	2.26	4.52	15.7	3.63	6.07	3.31	3.84	5.14	0.279
(Control) LH82xA634	2.14	4.09	14.2	3.63	5.34	2.86	3.19	4.48	0.242

Attachment 1.

Protocol
pp. 24-32

Study Number: 00-01-39-35
Covance Study Number: 6103-276

Study Title: Pesticide Profile, Mycotoxin, and Compositional Analyses of Corn Event MON 863 and Control Line LH82xA634 Produced in the U.S. in 2000

Sponsor:
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700 Chesterfield Parkway North
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Compositional Analysis Testing Facility:
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Wisconsin Facility
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Covance
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Mycotoxin Analysis
Testing Facility:

Romer Labs Inc.
1301 StyleMaster Drive
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Phone: (636) 583-8600
FAX: (636) 583-6553
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[redacted]

Approved By:

Patrick T. Weston
Testing Facility Management Representative
Monsanto Company
Biotechnology Regulatory Sciences

July 5, 2000
Date

Mary L. Taylor
Study Director
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Biotechnology Regulatory Sciences

July 5, 2000
Date

Ravinder S Sidhu

Ravinder S. Sidhu
Sponsor Representative
Monsanto Company
Regulatory Affairs

July 5, 2000
Date

James D. Astwood
Director, Product Safety Center
Monsanto Company
Biotechnology Regulatory Sciences

July 7, 2000
Date

Reviewed By:

Barbara Day

Quality Assurance Specialist
Monsanto Company
Monsanto Regulatory

7/5/00
Date

[redacted]

1.0 Regulatory Compliance

1.1 *GLP Compliance.* This is a product characterization study as defined by section §160.135(b) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards (40 CFR Part 160) intended to characterize the physical and/or chemical properties of a potential commercial product. This study will be conducted in compliance with all requirements of section §160.135 (b) with the following exception: Romer Labs is not a GLP facility and mycotoxin analyses performed there will not be conducted according to GLPs. However, the Monsanto Quality Assurance Unit (QAU) periodically visits Romer Labs to conduct facility and data audits.

2.0 Purpose

The purpose of this study is to conduct pesticide profile, mycotoxin, and compositional analyses of grain from of corn event MON 863 and control line LH82xA634. The test event MON863 expresses the insect control protein, Cry3Bb1.11098. The study includes analyses of non-transgenic parental control corn line LH82xA634 that has background genetics representative of their corresponding test event but does not express the Cry3Bb1.11098 insect control protein and was grown at the same location as the test event in the US in 2000.

Compositional data on test, control, and reference substances will be used to formulate animal diets in subsequent animal feeding studies.

3.0 Timelines

3.1 Proposed Experimental Start Date: July, 2000
3.2 Proposed Experimental Termination Date: December, 2000

[redacted]

4.0 Test, Parental Control, and Reference Control Substances

4.1 *Test Substance.* The test substance is the corn event MON863 produced in Kihei, Hawaii, USA under Production Plan #00-01-39-04 during the 2000 field season. The test substance will be analyzed at Romer Labs for mycotoxin screen and at Covance for pesticide screen (see section 5.1). If results demonstrate no unacceptable contamination of mycotoxins or pesticides, additional analyses in section 5.1 will be conducted on this sample.

4.2 *Parental Control Substance.* The parental (negative) control substance LH82xA634 is the non-transgenic parental control corn line produced in Kihei, Hawaii, USA under Production Plan #00-01-39-04 during the 2000 field season. The parental control substance will be analyzed at Romer Labs for mycotoxin screen and at Covance for pesticide screen (see section 5.1). If results demonstrate no unacceptable contamination of mycotoxins or pesticides, additional analyses in section 5.1 will be conducted on this sample.

4.3 *Reference Substances.* Appropriate standards will be used in each assay as reference standards for the analytical procedures or calibration of equipment.

4.4 *Characterization of Test, Control and Reference Substances.* The test substance identity was confirmed by molecular and immunochemical analyses and by field and chain-of-custody records. The parental (negative) control line was identified by immunochemical analysis, chain-of-custody records, and other documentation. All characterization data will be archived under Production Plan #00-01-39-04.

5.0 Experimental Design

Corn grain samples from the test and parental control lines will be analyzed for pesticide profile, mycotoxins and composition described in section 5.1. A sub-sample representative of the bulk whole grain samples was shipped at ambient temperature to Monsanto for identity confirmation. The bulk grain from each line will be shipped at ambient temperature to the Colorado Quality Research for use in subsequent animal feeding studies. Grain samples for pesticide, mycotoxin, and compositional analyses will be ground at Monsanto prior to shipment on dry ice to the appropriate testing facility (200 g to Covance and 100 g to Romer Labs) for analysis. Grain samples will be appropriately labeled (e.g. line #, Study #, tissue type, etc.) and identified in worksheets and/or sample transfer forms. Not all analyses will necessarily be performed on all grain samples from all lines. Grain samples will be returned or discarded at the end of the study at the direction of the study director.

[redacted]

5.1 *Sample Analyses.* The test and parental control corn grain samples will be analyzed according to the following methodology. Any additional compositional analyses or re-analyses will be documented and justified in the raw data file.

5.1.1 *Pesticide Profile and Compositional Analyses at Covance.* All corn grain samples will be analyzed for the presence of pesticides using the FDA PAM 304 pesticide screen (M304).

The following compositional analyses will be performed on all composite grain samples: proximates [moisture (M100), protein (PGEN), fat (FSOX), ash (ASHM)], crude fiber (CFIB), amino acid composition (TAAP), fatty acid composition (FAPM), acid detergent fiber (ADF), neutral detergent fiber (NDFE), sulfur (SULA), selenium (SEAS), cadmium (CDA), calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc (ICPS), and chloride (CLA). Carbohydrate (CHO) values will be estimated by calculation.

5.1.2 *Mycotoxin Analysis at Romer Labs.* Grain samples from all lines will be analyzed at Romer Labs, Union, MO for potential mycotoxin contamination according to the methods employed for the 'Mycotoxin Screen' test presented in Appendix 1. This non-GLP assay is referenced in this protocol, as it is an integral part of the pre-study requirement for subsequent animal feeding studies.

6.0 Records to be Maintained

Records will be maintained of all sample transfers, analyses, the protocol and all deviations and amendments thereto and copies of all letters memoranda and other correspondence related to this study. Upon completion of the study, all Monsanto study records and final report will be archived by the Sponsor. Original data will be archived at the following facilities: Monsanto facility (TCR characterization), Covance facility (pesticide profile and compositional analysis), and Romer Labs facility (mycotoxin analyses).

6.1 *Covance Subreport.* Original data or copies will be available at Covance to facilitate auditing the study during its progress and before acceptance of Covance's final subreport. The subreport will audited and accepted by the Covance quality assurance unit which will include: (1) a spreadsheet that summarizes the analytical report for each sample; (2) information on reference standards used (where applicable); and (3) analytical method summaries. One copy of the draft report and two copies of the final subreport will be submitted to the Study Director.

When the final subreport is completed, original study documentation, such as: paper data, computer printouts, chromatograms, worksheets, data sheets, notes by investigators, forms specified by SOP and magnetically encoded records, will be

retained in the archives of Covance in accordance with 40 CFR Part 160. Ten years after signing of the final report, all original or copies of data will be sent to the Sponsor. Supporting facility records will be retained at Covance but will not be archived with the study data, including refrigerator and freezer temperature records, instrument calibration and maintenance records.

6.2 *Romer Labs Data Summary.* Original data or copies will be available at Romer Labs to facilitate auditing the study during its progress, if warranted, before acceptance of Romer Lab's final data summary. Facility records will be stored indefinitely at Romer Labs. A certified copy of the raw data generated at Romer Labs will be archived at Monsanto.

7.0 Changes to the Protocol

Planned changes to the protocol will be documented in the form of written protocol amendments and signed by the Study Director. Amendments 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]

Appendix 1

Mycotoxin Screen

Aflatoxin B1, B2, G1, G2
Ochratoxin A
Citrinin
T-2 toxin
HT-2 toxin
Diacetoxyscirpenol
Neosolaniol
Fusarenon-x
Deoxynivalenol (DON)
15 Acetyl DON
3 Acetyl DON
Nivalenol
Zearalenone
Fumonisin B1, B2, B3

Attachment 2.

Covance Subreport pp. 33-59

Final Analytical Subreport

Pesticide Profile and Compositional Analyses of Corn Event MON863 and Control Line LH82xA634 Produced in the U.S. in 2000

PREPARED FOR:
Monsanto Company

COVANCE STUDY NUMBER:
6103-276

ISSUE DATE:
January 5, 2001

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Sponsor

Monsanto Company
St. Louis, MO

FINAL ANALYTICAL SUBREPORT

Subreport Title

Pesticide Profile and Compositional Analyses of Corn Event MON863 and Control Line LH82xA634 Produced in the U.S. in 2000

Author

Matthew L. Breeze

Subreport Completion Date

January 5, 2001

Performing Laboratory

Covance Laboratories Inc.
3301 Kinsman Blvd.
Madison, WI 53704

Laboratory Study Identification

Covance 6103-276

Monsanto Study Number

00-01-39-35

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories Inc., in accordance with the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 160. The following inspections were conducted and findings reported to the principal investigator (PI), study director (SD), and associated management.

Inspection Dates			Date Reported to PI and	Date Reported to SD and
From	To	Phase	PI Management	SD Management
07/18/00	07/18/00	Analytical Laboratory Inspection	07/21/00	11/02/00
08/02/00	08/04/00	Data/Table Review	08/04/00	09/18/00
08/02/00	08/04/00	Data/Table Review	08/04/00	09/18/00
12/07/00	12/07/00	Data/Table Review	12/13/00	12/13/00
12/06/00	12/13/00	Report Review	12/13/00	01/04/01
01/03/01	01/04/01	Report Review	01/04/01	01/04/01

Amy Forsyth
Representative, Quality Assurance Unit

01/05/01
Date

TABLE OF CONTENTS

STUDY IDENTIFICATION

Pesticide Profile and Compositional Analyses of Corn Event MON863 and Control Line LH82xA634 Produced in the U.S. in 2000

Test Substance:	Corn Event MON863 produced in Kihei, Hawaii, USA under Production Plan #00-01-39-04 during the 2000 field season
Sponsor Study No.:	00-01-39-35
Sponsor Study Title:	Pesticide Profile, Mycotoxin, and Compositional Analyses of Corn Event MON863 and Control Line LH82xA634 Produced in the U.S. in 2000
Sponsor:	Monsanto Company Biotechnology Regulatory Sciences 700 Chesterfield Parkway North St. Louis, MO 63198
Study Director:	Mary L. Taylor Monsanto Company - BB5F 700 Chesterfield Parkway North St. Louis, MO 63198 Phone: 636.737.6229 FAX: 636.737.6189 e-mail: mary.l.taylor@monsanto.com
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Monsanto Alternate Contact:	Gary F. Hartnell Monsanto Company - BB5G Regulatory Sciences 700 Chesterfield Parkway North St. Louis, MO 63198 Phone: 636.737.5915 FAX: 636.737.6189 e-mail: gary.f.hartnell@monsanto.com
Compositional Analysis Testing Facility:	Covance Laboratories Inc. 3301 Kinsman Blvd. Madison, WI 53704
Covance Principal Investigator:	Matthew L. Breeze Covance Laboratories Inc. 3301 Kinsman Blvd. Madison, WI 53704 Phone: 608.242.2712 ext. 2254 FAX: 608.242.7903 e-mail: matthew.breeze@covance.com
Study Timetable	
Study Initiation Date:	July 5, 2000
Analytical Start Date:	July 14, 2000
Analytical Completion Date:	July 27, 2000
Study Completion Date:	January 5, 2001

COVANCE KEY PERSONNEL

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Supervisor

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Proximate and Lipid Chemistry

Andrew J. Kohn
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Microbiological Vitamin Chemistry

Theodore W. Pritchard
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Robert G. Allen
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James R. Wehrmann
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Marc L. Pesselman
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Quality Assurance Unit

Nancy M. Centanni
Manager

Sample Management

Angela J. Underberg
Supervisor

INTRODUCTION

The purpose of this portion of the study was to conduct pesticide profiles and compositional analyses of grain from corn event MON863 and control line LH82xA634. The test event MON863 expressed the insect control protein, Cry3Bb1.11098. The study included analyses of non-transgenic parental control corn line LH82xA634 that had background genetics representative of their corresponding test event but did not express the Cry3Bb1.11098 insect control protein. The parental control line was grown at the same location as the test event in the U.S. in 2000.

Specifically, the study was designed to estimate the levels of pesticides, proximates (moisture, protein, fat, ash), crude fiber, amino acid composition, fatty acid composition, acid detergent fiber, neutral detergent fiber, sulfur, selenium, cadmium, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc, and chloride. The carbohydrate values were also estimated by calculation.

REGULATORY COMPLIANCE

This study was conducted in compliance with the Environmental Protection Agency (EPA) Good Laboratory Practice (GLP) Standards as set forth in Title 40 of the US Code of Federal Regulations Part 160 with the exceptions that the reference standards were not characterized according to GLP standards and that the final analytical subreport format is not in full accordance with EPA Pesticide Regulation Notice 86-5. These exceptions had no effect on the integrity or quality of the study.

TEST, CONTROL, AND REFERENCE SUBSTANCES

Identification

Test Substance

The test substance was the corn event MON863 produced in Kihei, Hawaii, U.S. under Production Plan #00-01-39-04 during the 2000 field season.

Parental Control Substance

The parental (negative) control substance LH82xA634 was the non-transgenic parental corn line produced in Kihei, Hawaii, U.S. under Production Plan #00-01-39-04 during the 2000 field season.

Reference Substance

There was no reference substance. Appropriate reference standards were used in each assay as reference standards for the analytical procedures and equipment calibrations. See Appendix A for reference standards identification (if applicable).

Characterization, Purity, and Stability

Information on characterization, purity, stability, synthesis methods, composition, or other characteristics that define the test, control, and reference substances was the responsibility of the sponsor.

Storage/Retention

Upon arrival in the analytical laboratory, all samples were stored in a secured freezer set to maintain -20° ±10°C. Excess samples will be returned or discarded at the end of the study at the direction of the study director. Remaining reference standards may be used for other testing.

Safety Precautions

Safety precautions were taken as required by Covance Policies and Procedures.

SAMPLE RECEIPT AND HANDLING

The samples were entered into the Covance Laboratory Information Management Systems (LIMS) with unique LIMS numbers. Each sample identification was matched with the LIMS information.

PROCEDURES

This study was conducted in accordance with Monsanto Study No. 00-01-39-35. All analyses were performed according to methods and standard operating procedures (SOPs) approved by Covance. See Appendix A for a summary of the analytical methods referenced by the method mnemonic. Listed in the following text table are the components analyzed and units reported by the assay. The following analyses were performed on the samples:

Analyte	Method Mnemonic	Units Reported by Assay
Proximates		
Moisture	M100	%[a]
Protein	PGEN	%[a]
Fat	FSOX	%[a]
Ash	ASHM	%[a]
Crude Fiber	CFIB	%[a]
Amino Acid Composition	TAAP	mg/g fresh weight
Fatty Acid Profile	FAPM	%[a]
Acid Detergent Fiber	ADF	%[a]
Neutral Detergent Fiber	NDFE	%[a]
Sulfur	SULA	%[a]
Selenium	SEAS	ppm[b]
Cadmium	CDA	ppm[b]
Minerals: Calcium, Copper, Iron, Magnesium, Manganese, Phosphorus, Potassium, Sodium, Zinc	ICPS	ppm[b]
Chloride	CLA	%[a]
Pesticide Profile	M304	ppm[b]

[a] % = [g/g fresh weight] x 100
[b] ppm = µg/g fresh weight

Carbohydrate (CHO) values were determined by calculation and reported as % = (g/g fresh weight) x 100.

A minimum frequency of 10% quality control samples (duplicates, recoveries, certified reference standards, blanks, or validated control samples) were prepared and analyzed at Covance. Additional analyses or re-analyses were documented and justified in the raw data.

STATISTICAL METHODS

No statistical analysis of the data was performed at Covance.

MAINTENANCE OF RAW DATA AND RECORDS

A final analytical subreport, including a compositional analyses summary spreadsheet accepted by the Covance Quality Assurance Unit, will be sent to the sponsor. All data relating to or generated by the project, including (if applicable) protocol, protocol amendments, a copy of the final analytical subreport, results, laboratory notebooks, applicable SOPs lists and any other information or records relating to the project will be retained in the archives of Covance in accordance with 40 CFR Part 160. Ten years after signing of the final report, all of the aforementioned materials will be returned to the sponsor with the exception of the magnetically encoded records.

The supporting records retained at Covance, but not archived with the study data, include the following items:

Magnetically encoded records
Storage area temperature records
Instrument calibration and maintenance records
Employee training records

RESULTS

The results for the pesticide and compositional analyses are presented in Table 1. All of the results are on a fresh-weight basis.

SIGNATURES

Matthew L. Breeze
Principal Investigator
Vitamin Chemistry
Covance Laboratories Inc.

1-5-01
Date

James R. Wehrmann
Associate Director
Food and Drug Analysis
Covance Laboratories Inc.

1-5-01
Date

Table 1
Pesticide and Compositional Analyses

Monsanto ID	MON863	LH82xA634
Covance ID	00702792	00702793
Pesticides (ppm)		
Organophosphates	<0.050	<0.050
Organonitrogens	<0.500	<0.500
Organochlorinated	<0.200	<0.200
N-Methylcarbamates	<0.100	<0.100
Proximate (%)		
Protein	11.3	9.93
Moisture	10.6	10.8
Total Fat	2.71	3.32
Ash	1.50	1.35
Carbohydrates	73.9	74.6
Neutral Detergent Fiber (%)	9.16	11.6
Acid Detergent Fiber (%)	2.66	2.29
Crude Fiber (%)	2.15	1.72
Cadmium (ppm)	<0.04	<0.04
Chloride (%)	0.046	0.049
Selenium (ppm)	0.08	0.09
Sulfur (%)	0.088	0.093

Table 1 (Continued)
Pesticide and Compositional Analyses

Monsanto ID	MON863	LH82xA634
Covance ID	00702792	00702793
Minerals (ppm)		
Calcium	34.2	33.3
Copper	1.68	1.90
Iron	22.5	22.8
Magnesium	1290	1260
Manganese	7.49	6.96
Phosphorus	3680	3650
Potassium	3850	3790
Sodium	<100	<100
Zinc	21.4	21.0

Table 1 (Continued)
Pesticide and Compositional Analyses

Monsanto ID	MON863	LH82xA634
Covance ID	00702792	00702793
Fatty Acids (%)		
8:0 caprylic	<0.00400	<0.00400
10:0 capric	<0.00400	<0.00400
12:0 lauric	<0.00400	<0.00400
14:0 myristic	<0.00400	<0.00400
14:1 myristoleic	<0.00400	<0.00400
15:0 pentadecanoic	<0.00400	<0.00400
15:1 pentadecenoic	<0.00400	<0.00400
16:0 palmitic	0.234	0.298
16:1 palmitoleic	<0.00400	0.00462
17:0 heptadecanoic	<0.00400	<0.00400
17:1 heptadecenoic	<0.00400	<0.00400
18:0 stearic	0.0509	0.0626
18:1 oleic	0.558	0.675
18:2 linoleic	1.64	2.07
18:3 gamma linolenic	<0.00400	<0.00400
18:3 linolenic	0.0265	0.0350
20:0 arachidic	0.0105	0.0132
20:1 eicosenoic	0.00731	0.00885
20:2 eicosadienoic	<0.00400	<0.00400
20:3 eicosatrienoic	<0.00400	<0.00400
20:4 arachidonic	<0.00400	<0.00400
22:0 behenic	0.00498	0.00580

Table 1 (Continued)
Pesticide and Compositional Analyses

Monsanto ID	MON863	LH82xA634
Covance ID	00702792	00702793
Amino Acids (mg/g)		
Aspartic Acid	7.70	6.77
Threonine	3.74	3.35
Serine	5.27	4.63
Glutamic Acid	22.2	20.1
Proline	9.93	9.04
Glycine	4.20	3.70
Alanine	9.01	8.18
Cystine	2.30	2.11
Valine	5.88	5.24
Methionine	2.26	2.14
Isoleucine	4.52	4.09
Leucine	15.7	14.2
Tyrosine	3.63	3.63
Phenylalanine	6.07	5.34
Histidine	3.31	2.86
Lysine	3.84	3.19
Arginine	5.14	4.48
Tryptophan	0.279	0.242

APPENDIX A

Analytical Method Summaries and Reference Standards

ANALYTICAL METHOD SUMMARIES AND REFERENCE STANDARDS

Protein (PGEN)
Nitrogenous compounds in the sample were reduced in the presence of boiling sulfuric acid and a mercury catalyst mixture to form ammonia. The acid digest was made alkaline. The ammonia was distilled and then titrated with a standard acid. The percent nitrogen was calculated and converted to protein using the factor 6.25. The limit of detection for this study was 0.1%. There is no analytical reference standard for this analysis.

References:
Official Methods of Analysis of AOAC INTERNATIONAL, 17th Ed., Methods 955.04 and 979.09, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2000), modified.

Bradstreet, R. B., *The Kjeldahl Method for Organic Nitrogen*, Academic Press: New York, New York, (1965), modified.

Kalthoff, I.M., and Sandell, E.B., *Quantitative Inorganic Analysis*, MacMillan: New York, (1948), modified.

Moisture (M100)
The sample was dried in a vacuum oven at 100°C to a constant weight. The moisture weight loss was determined and converted to percent moisture. The limit of detection for this study was 0.1%. There is no analytical reference standard for this analysis.

Reference:
Official Methods of Analysis of AOAC INTERNATIONAL, 17th Ed., Methods 926.08 and 925.09, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2000), modified.

Fat by Soxhlet Extraction (FSOX)
The sample was weighed into a cellulose thimble containing sand or sodium sulfate and dried to remove excess moisture. Pentane was dripped through the sample to remove the fat. The extract was then evaporated, dried, and weighed. The limit of detection for this study was 0.1%. There is no analytical reference standard for this analysis.

Reference:
Official Methods of Analysis of AOAC INTERNATIONAL, 17th Ed., Method 960.39, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2000), modified.

Ash (ASHM)
The sample was placed in an electric furnace at 550°C and ignited to drive off all volatile organic matter. The nonvolatile matter remaining was quantitated gravimetrically and calculated to determine percent ash. The limit of detection for this study was 0.1%. There is no analytical reference standard for this analysis.

Reference:
Official Methods of Analysis of AOAC INTERNATIONAL, 17th Ed., Method 923.03, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2000), modified.

Crude Fiber (CFIB)
Crude fiber was quantitated as the loss on ignition of dried residue remaining after digestion of the sample with 1.25% sulfuric acid and 1.25% sodium hydroxide solutions under specific conditions. The limit of detection for this study was 0.1%. There is no analytical reference substance for this analysis.

Reference:
Official Methods of Analysis of AOAC INTERNATIONAL, 17th Ed., Method 962.09, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2000), modified.

Carbohydrates (CHO)
The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation:

% carbohydrates = 100 % - (% protein + % fat + % moisture + % ash)

The limit of detection for this study was 1.0%. There is no analytical reference standard for this analysis.

Reference:
United States Department of Agriculture, "Energy Value of Foods", *Agriculture Handbook No. 74,* pp. 2-11, (1973).

Neutral Detergent Fiber, Enzyme Method (NDFE)
The sample was placed in a fritted vessel and washed with a neutral boiling detergent solution that dissolved the protein, carbohydrate, enzyme, and ash. An acetone wash removed the fats and pigments. Hemicellulose, cellulose, and lignin fractions were collected on the frit and determined gravimetrically. The limit of detection for this study was 0.1%. There is no analytical reference standard for this analysis.

References:
Approved Methods of the American Association of Cereal Chemists, 9th Ed., Method 32.20, (1998), modified.

Forage Fiber Analyses, Agriculture Handbook No.379, United States Department of Agriculture, (1970), modified.

Acid Detergent Fiber (ADF)
The sample was placed in a fritted vessel and washed with an acidic boiling detergent solution that dissolved the protein, carbohydrate, and ash. An acetone wash removed the fats and pigments. Lignocellulose fraction was collected on the frit and determined gravimetrically. The limit of detection for this study was 0.1%. There is no analytical reference standard for this analysis.

Reference:
Forage Fiber Analyses, Agriculture Handbook No.379, United States Department of Agriculture, (1970), modified.

Amino Acid Composition (TAAP)

Total aspartic acid (including asparagine)
Total threonine
Total serine
Total glutamic acid (including glutamine)
Total proline
Total glycine
Total alanine
Total valine
Total isoleucine
Total leucine
Total tyrosine
Total phenylalanine
Total histidine
Total lysine
Total arginine
Total tryptophan
Sulfur-containing amino acids: Total methionine
Total cystine (including cysteine)

The sample was assayed by three methods to obtain the full profile. Tryptophan required a base hydrolysis with sodium hydroxide. The sulfur containing amino acids required an oxidation with performic acid prior to hydrolysis with hydrochloric acid. Analysis of the samples for the remaining amino acids was accomplished through direct acid hydrolysis with hydrochloric acid. Once hydrolyzed, the individual amino acids were then quantitated using an automated amino acid analyzer. The limit of detection for this study was 0.1 mg/g.

Reference Standards:
Beckman K18, 2.5 µmol/mL per constituent except cystine (1.25 µmol/mL), Lot Number S911165
Aldrich L-Tryptophan, 99%, Lot Number 12729HS
Aldrich L-Cysteic Acid Monohydrate, 98%, Lot Number 04615MS
Sigma L-Methionine Sulfone, used as 100%, Lot Number 012H3349

Reference:
Official Methods of Analysis of AOAC INTERNATIONAL, 17th Ed., Method 982.30, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2000), modified.

Fatty Acids (FAPM)
The lipid was extracted and saponified with 0.5 N sodium hydroxide in methanol. The saponification mixture was methylated with 14% boron trifluoride:methanol. The resulting methyl esters were extracted with heptane containing an internal standard. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation. The limit of detection was 0.00400%.

Reference Standards:
Nu Chek Prep GLC Reference Standard Hazelton No. 1, used as 100%, Lot Number A4-K
Nu Chek Prep GLC Reference Standard Hazelton No. 2, used as 100%, Lot Number S10-G
Nu Chek Prep GLC Reference Standard Hazelton No. 3, used as 100%, Lot Number F23-J
Nu Chek Prep GLC Reference Standard Hazelton No. 4, used as 100%, Lot Number JY26-J
Nu Chek Prep Methyl Gamma Linolenate, used as 100%, Lot Number U-63M-F25-J

Reference:
Official Methods and Recommended Practices of the AOCS, 5th Ed., Method Ce 1-62, American Oil Chemists' Society: Champaign, Illinois, (1997), modified.

ICP Emission Spectrometry (ICPS)
Calcium
Copper
Iron
Magnesium
Manganese
Phosphorus
Potassium
Sodium
Zinc

The sample was dried, precharred, and ashed overnight at 500° ± 50°C. The ashed sample was treated with hydrochloric acid, taken to dryness, and put into a solution of 5% hydrochloric acid. The amount of each element was determined at appropriate wavelengths by comparing the emission of the unknown sample, measured by the inductively coupled plasma, with the emission of the standard solutions.

Spex CertiPrep Reference Standards and Limits of Detection:

Mineral	Lot Numbers	Concentration (ppm)	Limit of Detection (ppm)
Calcium	L6-59CA	10,000	20.0
Copper	6-242CU	1,000	0.500
Iron	7-97FE	1,000	2.00
Magnesium	L5-187MG	10,000	20.0
Manganese	6-201MN	1,000	0.300
Phosphorus	K6-54P	10,000	20.0
Potassium	M6-16K	10,000	100
Sodium	N6-151NA	10,000	100
Zinc	6-264ZN	1,000	0.400

References:

Dahlquist, R.L., and Knoll, J.W., "Inductively Coupled Plasma-Atomic Emission Spectrometry: Analysis of Biological Materials and Soils for Major, Trace, and Ultra Trace Elements," *Applied Spectroscopy*, *32*:1-29, (1978), modified.

Official Methods of Analysis of AOAC INTERNATIONAL, 17th Ed., Methods 984.27 and 985.01, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2000), modified.

Chloride (CLA)

The sample was put into solution with double deionized water and then made acidic with nitric acid. Chloride was determined potentiometrically by titrating with a standard silver nitrate solution to a predetermined endpoint. The limit of detection for this assay was 0.004%.

Reference Standard:

Mallinckrodt, 1000 ppm sodium chloride, 99.9% purity, Lot Number 7581 KPAK

Reference:
Official Methods of Analysis of AOAC INTERNATIONAL, 17th Ed., Methods 963.05, 969.10, and 971.27, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2000), modified.

Sulfur (SULA)
The sample was weighed into a volumetric flask and refluxed with nitric acid. Perchloric acid was added and refluxed again. Hydrochloric acid was added and the sample was heated to break down nitroso compounds. Sulfur seed and sulfur buffer solution were added. The analysis was completed by measuring the extent of turbidity in the sample after the addition of barium chloride. The percent transmittance of the samples is compared to that of standards for determining sulfur concentrations. The limit of detection for this study was 0.015%.

Reference Standard:
Spex CertiPrep, 1,000 mcg/mL sulfur, used as 100%, Lot Number 6-202S

Reference:
Soil Society of America Proceedings, 29:71-72, (1965), modified.

Selenium (SEAS)
The sample was digested in a nitric-perchloric-hydrochloric acid mixture, in which any selenium present formed selenous acid. The selenous acid is reacted with 2,3-diaminonaphthalene to form 2,3-4,5-benzopiazselenol. This compound was extracted into an organic solvent. The amount of selenium is then determined by comparing the absorbance of the unknown sample, measured by fluorescence spectroscopy, with the absorbance of standard solutions. The limit of detection for this assay was 0.05 ppm.

Reference Standard:
Fisher Scientific, 1000 ppm selenium, Lot Number 994379-18

References:
Official Methods of Analysis of AOAC INTERNATIONAL, 17th Ed., Methods 969.06 and 986.15, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2000), modified.

Watkinson, J. H., "Fluorometric Determination of Selenium in Biological Material with 2,3-Diaminonaphthalene," *Analytical Chemistry*, *38*(1):92-7, (1966), modified.

Haddad, P. R. and Smythe, L. E., "A Critical Evaluation of Fluorometric Methods for Determination of Selenium in Plant Materials with 2,3-Diaminonaphthalene," *Talanta*, *21*:859-865, (1974), modified.

Bayfield, R. F. and Romalis, L. F., "pH Control in the Fluorometric Assay for Selenium with 2,3-diaminonaphthalene," *Analytical Biochemistry*, *144*(2):569-576, (1985), modified.

Cadmium (CDA)

The sample was either dry-ashed, wet-ashed, or read directly. If dry-ashed, the sample was dried, pre-charred and ashed at 500°C ±50° in a muffle furnace for 5 to 16 hours. The sample was removed from the muffle furnace, cooled, treated with nitric acid, re-ashed, and dissolved in hydrochloric acid solution. If wet-ashed, the sample was digested on a hot plate with nitric acid, hydrochloric acid, and/or hydrogen peroxide. The amount of cadmium was determined by comparing the signal of the unknown sample, measured by the atomic absorption (AA) spectrophotometer, with the signal of the standard solutions. The limit of detection for this assay was 0.04 ppm.

Reference Standard:
Fisher Scientific, 1000 ppm cadmium, used as 100%, Lot Number 981734-24

References:

Official Methods of Analysis of AOAC INTERNATIONAL, 17th Ed., Method 974.27, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2000), modified.

Analytical Methods for Atomic Absorption Spectrophotometry, Perkin-Elmer: Norwalk, Connecticut, (January 1982), modified.

Methods for Chemical Analysis of Water and Wastes, Metals 1-19 and Method 213.1, U. S. EPA: Cincinnati, Ohio, (1979), modified.

Pesticide Profile (M304)

The sample was blended with ethyl acetate and cleaned up by gel permeation chromatography. The extract was injected for organophosphates, chlorinated, and nitrogen on a gas chromatography system. The carbamates were injected using a high performance liquid chromatography system. The limits of detection (ppm) for this assay were:

Organophosphates	0.050
Organonitrogens	0.500
Organochlorinated	0.200
N-Methylcarbamates	0.100

Reference Standards:

Restek Corporation Custom Chlorinated Pesticide Mix, Catalog # 54609, Lot Number A011108

Restek Corporation Custom Phosphorus Pesticides Mix, Catalog # 54610, Lot Number A011117

Restek Corporation Custom Nitrogen List Catalog # 54611, Lot Number A011122

Restek Corporation Carbamates I Mixture Catalog # 54612, Lot Number A016802

Restek Corporation Carbamates II Mixture Catalog # 54613, Lot Number A016804

Reference:

Pesticide Analytical Manual Volume 1: Multiresidue Methods, 3rd Ed., Chapter 3 Multiclass Multiresidue Methods: 304 Method for Fatty Foods, Food and Drug Administration, (1999), modified.

Attachment 3.

Romer Labs Summary
pp. 60-61

ROMER

Romer™ Labs, Inc.

1301 Stylemaster Drive ▲ Union, MO 63084-1156
Tel: (636) 583 8600 ▲ Fax: (636) 583 6553 ▲ www.romerlabs.com

Client: Monsanto Co.
700 Chesterfield Pkwy N.
St. Louis, MO 63198

Contact: Mary Taylor BB5K

Sample Number: 17578
Invoice Number: 15831
Receive Date: 6 July 00
Report Date: 13 July 00

Sample Description:
1=Corn Ground, MON863+, T10-0006-10408-I, 138g
2=Corn Ground, MON863-, TPC-0006-10409-I, 136g

Test Description:	Detection Limits	Sample Numbers 1	2
Aflatoxin B1	1.0 ppb	ND	ND
Aflatoxin B2	1.0 ppb	ND	ND
Aflatoxin G1	1.0 ppb	ND	ND
Aflatoxin G2	1.0 ppb	ND	ND
Ochratoxin A	5 ppb	ND	ND
Citrinin	0.2 ppm	ND	ND
T-2 Toxin	0.1 ppm	ND	ND
HT-2 Toxin	0.1 ppm	ND	ND
Diacetoxyscirpenol	0.3 ppm	ND	ND
Neosolaniol	0.5 ppm	ND	ND
Fusarenon X	0.5 ppm	ND	ND
Deoxynivalenol	0.1 ppm	ND	ND
15 Acetyl-DON	0.1 ppm	ND	ND
3 Acetyl-DON	0.1 ppm	ND	ND
Nivalenol	0.5 ppm	ND	ND
Zearalenone	100 ppb	ND	ND
Fumonisin B1	0.1 ppm	ND	ND
Fumonisin B2	0.1 ppm	ND	ND
Fumonisin B3	0.1 ppm	ND	ND

Approved By:

ND = NONE DETECTED
For Unusual Samples Detection Limits May Be Higher
We Sincerely appreciate your business. Please feel free to call (636) 583-8600,
if you have any questions regarding these results
All reports on the mycotoxin analysis of food, feed, and grain samples apply only to the samples submitted. Reports are not a guarantee of quality of the material of product from which the samples were taken for submission for analysis.