MSL 14326

Report Title

Compositional Analyses of Insect Protected and Insect Protected Roundup Ready™ Corn Lines from the 1994 US Field Trials

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Report Date

March 14, 1996

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Laboratory Project ID

CHW 6103-180
94-01-50-18

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).

Company Monsanto Company

Company Agent Kent Croon Date 3/14/96

Title Mgr., Regulatory Affairs Signature

Monsanto Company
CEREGEN
Regulatory Sciences

Study #: 94-01-50-18
CHW #: 6103-180

STATEMENT OF COMPLIANCE

This study meets the requirements for 40 CFR Part 160.

Submitter — Date 3/14/96

Sponsor — Date 3/14/96

Patricia R Sanders — Study Director — Date 3/14/96

Monsanto Company
CEREGEN
Regulatory Sciences

Study #: 94-01-50[redacted]
CHW #: 6103-180

Quality Assurance Unit Statement

This signed statement indicates that the Quality Assurance Unit has monitored a portion of this study and reviewed the final report, that the report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study.

The following is a list of reviews conducted at Monsanto on the study reported herein. Additional reviews conducted by contract facility Quality Assurance Units are reported in the contract facility reports.

Date of Inspection	Type of Inspection	Date Reported to Study Director	Date Reported to Management
Jul 5, 1995	Protocol amendment	Jul 5, 1995	Jul 5, 1995
Sep 7, 1995	Protocol amendment	Sep 7, 1995	Sep 7, 1995
Feb 8-12, 1996	Final report draft	Feb 12, 1996	Feb 12, 1996
Mar 13, 1996	Final review	Mar 13, 1996	Mar 13, 1996

Clyde L. Livingston — 13 Mar 1996

Clyde L. Livingston
Quality Assurance Representative
Monsanto Company

Date

SIGNATURES OF APPROVAL

Study Number: CHW 6103-180
94-01-50-18

Title: Compositional Analyses of Insect Protected and Insect Protected Roundup Ready™ Corn Lines from the 1994 Field Trials

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

Study Director: Patricia R. Sanders

Principal Investigator: Diane M. Henning

Records Retention: All study specific raw data, protocols and final reports will be retained at Monsanto-St. Louis except raw data and facility records for Corning Hazleton, Inc., Wisconsin Facility.

Sample Retention: Any study samples that are to be retained will be stored at Monsanto, St. Louis.

Study Initiation Date: November 30, 1994

Signatures of Approval

Patricia R Sanders Date: 3/14/96
Study Director

Sponsor Date: 3/14/96

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ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
B.t.k.	*Bacillus thuringiensis* subsp. *kurstaki*
B.t.k. HD-1	*Bacillus thuringiensis* subsp. *kurstaki* HD-1 protein
CP4 EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase isolated from *Agrobacterium species* strain designated CP4
CryIA(b)	*Bacillus thuringiensis* subsp. *kurstaki* CryIA(b) protein, also referred to as *B.t.k.* HD-1 protein
cryIA(b)	*Bacillus thuringiensis* subsp. *kurstaki* HD-1 gene
ECB	European Corn Borer
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
fwt	fresh weight
g	gram
GLP	Good Laboratory Practices
GOX	Glyphosate oxidoreductase protein
gox	Glyphosate oxidoreductase gene
HPLC	high performance liquid chromatography
IPC	insect protected corn
MRID	Master Record Identification Number
MSL	Monsanto St. Louis Report
NPTII	neomycin phosphotransferase II protein
nptII	neomycin phosphotransferase II gene
ppm	parts per million
SOP	standard operating procedure
subsp.	subspecies

I. SUMMARY

Corn lines have been modified to express a protein from *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1 [Cry IA(b)] (Höfte and Whiteley, 1989). The CryIA(b) protein has insecticidal activity against the European Corn Borer (ECB) insect pest. In addition to the *cryIA(b)* gene, genes encoding CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) (Padgette *et al.*, 1993) and glyphosate oxidoreductase (*gox*) (Padgette *et al.*, 1994) may also be present in some lines. The CP4 EPSPS and *gox* genes are present to enable selection of cells in tissue culture that contain the *cryIA(b)* gene and to confer glyphosate tolerance to the corn plant for some lines. This study includes insect protected (IPC) and insect protected Roundup Ready™ (IPC/RR) corn lines. The corn transformation vectors used to produce the corn lines include a gene cassette containing a bacterial specific promoter and the coding region for neomycin phosphotransferase, NPTII. Neomycin phosphotransferase protein allows selection of bacteria containing the vector in media containing kanamycin. The *nptII* gene is under the control of a bacterial-specific promoter and, therefore, does not produce the NPTII protein in plant cells. The control line has background genetics representative of the test lines, but has not been genetically modified and therefore, does not express the CryIA(b), CP4 EPSPS or GOX proteins.

The purpose of this study was to assess compositional parameters in grain from the insect protected (IPC) and insect protected Roundup Ready™ (IPC/RR) corn lines compared to grain from the parental corn lines. Grain was harvested from the 1994 GLP field trials at 6 geographically distinct locations and analyzed for corn grain components: proximates (moisture, protein, ash, fat), calories, crude fiber, carbohydrate, starch, fatty acid profile, sugar profile, amino acid composition, tocopherols, phytic acid, and minerals (calcium and phosphorus).

Compositional data on all the test lines was comparable to the control line, MON 818, and within the published literature ranges, with few exceptions. These exceptions do not represent meaningful differences and are attributable to the variability in the noncommercial corn genotype used as the experimental line in the study. Based on these data, it was concluded that there are no meaningful compositional differences between the insect protected corn lines (MON 801, MON 809 and MON 810), the insect protected Roundup Ready corn lines (MON 802 and MON 805) and the control line, MON 818.

II. INTRODUCTION

A. Background

Corn lines have been modified to express a protein from *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1 [Cry IA(b)] (Höfte and Whiteley, 1989). The CryIA(b) protein has insecticidal activity against the European Corn Borer (ECB) insect pest. In addition to the *cryIA(b)* gene, genes encoding CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) (Padgette *et al.*, 1993) and glyphosate oxidoreductase (*gox*) (Padgette *et al.*, 1994) may also be present in some lines. The CP4 EPSPS and *gox* genes are present to enable selection of cells in tissue culture that contain the *cryIA(b)* gene and to confer glyphosate tolerance to the corn plant for some lines. This study includes insect protected (IPC) and insect protected Roundup Ready™ (IPC/RR) corn lines. The corn transformation vectors used to produce the corn lines include a gene cassette containing a bacterial specific promoter and the coding region for neomycin phosphotransferase, NPTII. Neomycin phosphotransferase protein allows selection of bacteria containing the vector in media containing kanamycin. The *nptII* gene is under the control of a bacterial-specific promoter and, therefore, does not produce the NPTII protein in plant cells. The control line has background genetics representative of the test lines, but has not been genetically modified and therefore, does not express the CryIA(b), CP4 EPSPS or GOX proteins.

B. Purpose

The purpose of this study was to assess compositional parameters in grain from the insect protected (IPC) and insect protected Roundup Ready™ (IPC/RR) corn lines compared to grain from the parental corn lines. Grain was harvested from the 1994 GLP field trials at 6 geographically distinct locations and analyzed for corn grain components: proximates (moisture, protein, ash, fat), calories, crude fiber, carbohydrate, starch, fatty acid profile, sugar profile, amino acid composition, tocopherols, phytic acid, and minerals (calcium and phosphorus).

C. Protocol Amendments

The protocol for this study is contained in Appendix 1. There were two Amendments to this study. Amendment #1 changed the study title and study number, changed the Study Director designation from Hazleton Wisconsin, Inc. to Monsanto Company, expanded the test line samples which could be analyzed, added the calcium and phosphorus analyses of all grain samples and changed the data requirements for the Analytical subreport. Amendment #2 deleted the MON 812 samples from the study, since this corn line was dropped as a lead line due to poor efficacy. These Amendments had no adverse effect on the study. There was one protocol deviation during the study, the MON 801 grain samples were analyzed for proximates and amino acids only rather than all the composition parameters mentioned above.

III. MATERIALS

A. Test Substance

The test substances for this study were grain of the IPC corn lines MON 801, 809, and 810; and the IPC/RR corn lines MON 802 and 805. The grain was harvested from the 1994 field trials conducted under GLP (EPA MRID #43665502). The batch of grain from each field site was identified with a 5-digit MON number as listed in Table 1.

B. Control Substance

The control substance for this study was grain of the corn line MON 818 harvested from the 1994 field trials conducted under GLP (EPA MRID #43665502). The 5-digit grain sample identifiers as well as site locations are listed in Table 1.

C. Test and Control Substance Characterization

This study was conducted using grain harvested from Study 94-01-39-01. The test and control corn grain was harvested at 6 locations: Jerseyville, IL (JV), Monmouth, IL (MN), Johnston, IA (JH), Sheldahl, IA (SH), Windfall, IN (WN) and York, NE (YK). Line purity was maintained in two ways during the field trial: 1) ear shoots were bagged to prevent open pollination; and 2) each plant was self-pollinated by hand.

Grain collected from the 1994 field trial was tested for the presence of the CryIA(b) protein before being used as the test substance in this study. Control grain collected from the field was tested for the absence of the CryIA(b) protein before being used as the control substance in this study. The results are included in the study data files of Study 94-01-39-01.

The identity of the test substance harvested was confirmed by Southern blot analysis of leaf material collected from greenhouse grown plants from the same grain batch harvested from the Jerseyville, IL (JV) site. Southern blot analysis gave a unique DNA pattern for each corn line remaining in the trial. The unique DNA pattern for each line was identical for seed planted and grain harvested, indicating that line identity had been maintained. The control line, MON 818 did not contain a *cryIA(b)* fragment, confirming its identity as a control. The results are included in the study data files of Study 94-01-39-01.

D. Collection, Storage, and Transfer of Test and Control Substances

Grain from Study 94-01-39-01 was harvested in bulk for each line and shipped to Monsanto Company in November 1994 (Halsey *et al*, 1995). Harvested grain was stored at approximately 6°C until initiation of this study. These storage conditions were appropriate to maintain seed quality (Wych, 1988). The grain was ground to a fine powder, shipped on dry ice to Corning Hazleton Inc. and stored at approximately -20°C during this study. Grain of corn lines

MON 802, 810, and 818 was shipped November 30, 1994. MON 805 grain was shipped June 29, 1995; MON 809 on July 11, 1995; and MON 801 on July 13, 1995.

A reference substance was not used in this study. Appropriate analytical standards were used in each assay as reference standards for the analytical procedure. The reference standards are described with the method for each analysis where appropriate.

E. Stability of the Test, Control and Reference Substances

Stability of test and control grain was ensured by storing the samples under conditions appropriate to maintain seed quality (Wych, 1988). The analytical standards were known to be stable for the experimental period; assay parameters were monitored for each procedure to ensure integrity of analyses. Samples of the test and control substances were archived under Monsanto Study 94-01-39-01 until final notification of disposition by the Sponsor.

IV. METHODS

A. Test System

The panel of analytical biochemical methods employed in this study was considered the test system. These are standard published analytical methods which are currently used to evaluate nutritional quality and composition parameters in corn for commercial purposes. All analyses were performed at Corning Hazleton, Inc. on ground corn grain. The method references are listed in Appendix 2.

B. Proximate Analysis of Corn Grain

Moisture (M100). The sample was dried in a vacuum oven at 100°C to a constant weight (approximately 5 hours) (AOAC methods 926.08 and 925.09, 1990). The moisture loss was determined gravimetrically. There was no analytical reference substance for these analyses.

Protein (PGEN). Protein and other organic nitrogen in the sample was converted 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 protein using the factor 6.25 (AOAC methods 955.04C and 979.09, 1990; Bradstreet, R.B. 1965; Kalthoff and Sandell, 1948). There was no analytical reference substance for these analyses.

Fat (FSOX). The sample was weighed into a cellulose thimble containing sand or sodium sulfate. The thimble was dried to remove excess moisture. Pentane was dripped through the sample to remove the fat. The extract was evaporated, dried and weighed (AOAC methods 960.39). This method was used

for the grain samples. There was no analytical reference substance for these analyses.

Ash (ASHM). Volatile organic matter was driven off when the sample was ignited at 550°C in an electric furnace. The residue was quantitated gravimetrically and calculated to determine percent ash (AOAC method 923.03, 1990). There was no analytical reference substance for this analysis.

Carbohydrates (CHO). Carbohydrates were calculated by difference using the fresh weight-derived data and the following equation (USDA Agricultural Handbook No. 8, 1975):

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

There was no analytical reference substance for these analyses.

Calories (CALC). Calories were calculated using the Atwater factors with the fresh weight-derived data and the following equation (USDA Agricultural Handbook No. 8, 1975):

calories (kcal/100g) = (4 * % protein) + (9 * % fat) + (4 * % carbohydrates)

There was no analytical reference substance for these analyses.

Crude Fiber (CFIB). Crude fiber is the loss on ignition of dried residue remaining after digestion of the samples with 1.25% sulfuric acid and 1.25% sodium hydroxide solutions under specfic conditions (AOAC method 962.09, 1990). There was no reference substance for this method.

C. Amino Acid Composition (TAAP) of Corn Grain

Grain samples were hydrolyzed with hydrochloric acid, and adjusted to pH 2.2. The individual amino acids were quantitated using an automated amino acid analyzer. This assay was based on previously published references (AOAC method 982.30, 1990). The reference substances used for these analyses were: K18 (Beckman, lot #A304008), L-Tryptophan (Sigma Chemical, lot #52H0717), Cysteic Acid Monohydrate (Sigma Chemical, lot #50H2616 and 83H2607), Methionine Sulfone (Sigma Chemical, lot #49F0113 and 12H3349).

D. Fatty Acid Profile (FAC) of Corn Grain

The lipid in the grain samples was extracted, saponified with 0.5N sodium hydroxide in methanol, and 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 (AOCS method Ce 1-62, 1981). The reference substances are listed in the study data files.

E. Sugar Profile (SUGN) of Corn Grain

The sugars were extracted from the grain samples in deionized water, treated with a hydroxylamine hydrochloride solution in pyridine containing phenyl-β-D-

glucoside as the internal standard. The resulting oximes were converted to silyl derivatives with hexamethyldisilazane (HMDS) and trifluoroacetic acid (TFA) treatment and analyzed by gas chromatography using a flame ionization detector (Mason and Slover, 1971; Brosbt, K., 1972). The reference substances were obtained from Sigma Chemical: fructose (lot #109F0752), glucose (lot #41H0313), sucrose (lot #90H0679), maltose (lot #51H0476), lactose (lot #29F0105), and galactose (lot #43H03631).

F. Starch Analysis (STCH) of Corn Grain

Grain samples were extracted with alcohol to remove the carbohydrates other than starches. The starches were gelatinized, hydrolyzed with enzymes and titrated as reducing sugars (AOAC methods 979.10, 906.03 and 929.09, 1990). There was no analytical reference substance for these analyses.

G. Tocopherol Analysis (EFD2) of Corn Grain

The grain samples were saponified to release the tocopherols, which were then extracted with organic solvent, followed by quantitation on a high performance liquid chromatography (HPLC) silica column using fluorescence detection (Cort *et al.*, 1983; Speek *et al.*, 1985; McMurray *et al.*, 1980). The reference substances for this method were alpha tocopherol (USP, lot L), gamma-tocopherol (Sigma Chemical lot #43H0475 and 114H0720).

H. Phytic Acid Analysis (A1XX) of Corn Grain

The grain samples were weighed, extracted with 0.5N hydrochloric acid, filtered and treated with ferric chloride. The amount of phosphorus was determined colorimetrically at a wavelength of 660 nm by comparison of the sample, reacted with the color reagent to form a blue complex, to standards prepared in an identical manner. Phytic acid values are determined by calculation:

$$\frac{\text{ppm phosphorus X } 0.0001}{0.2816} = \%\text{ phytic acid}$$

(McChance and Widdewson, 1935; Fiske and Subbarrow, 1925). The reference substance for this method was a 10,000 ppm phosphorus solution (SPEX lot #D-261P and E-87P).

I. Inorganic Components of Corn Grain

Calcium (CAA). The grain samples were dried, pre-charred, and ashed overnight at 500° to 550°C. The samples were treated with nitric acid, dried, reashed and solubilized in 4% hydrochloric acid. The amount of calcium was determined at a wavelength of 422.7 nm by comparison of test sample to the signal of the standard solution. All solutions contain 1% lanthanum and 5% hydrochloric acid (AOAC method 965.09, 968.08 and 985.35). The reference substance for this method was 1000 ppm calcium solution (Fisher, lot #940982-24).

Phosphorus (PTA). The grain samples were dried, pre-charred, and ashed overnight at 500° to 550°C. The samples were treated with nitric acid, dried, reashed and solubilized in 4% hydrochloric acid. The amount of phosphorus was determined colorimetrically at a wavelength of 420 nm by comparison of test sample to the signal of the standard solution, each reacted with molybdovanadate solution (AOAC method 965.17 and 962.11). The reference substance for this method was 10,000 ppm phosphorus solution (SPEX, lot #E-87P).

J. Data Reduction and Statistical Analysis

All data reduction and statistical analyses were performed using the SAS statistical program (SAS Institute, 1990).

V. RESULTS AND DISCUSSION

The values reported for the compositional analyses at Corning Hazleton Inc. were expressed as percent dry weight of the sample using the measured moisture content. The analytical data was summarized in an Analytical Subreport which has been archived. A paired t-test was used to examine for statistically significant differences between the values for each test line and the control line. For each analyte, the differences between the values for test and control line at each of six locations were calculated. These differences were then used in the SAS MEANS procedure (t-test option) to test whether the mean differences were equal to zero. The mean differences from control were statistically significant at the 5% level if the p-value was less than 0.05. The statistical analysis report is included in Appendix 3.

For many analytes, values were reported as "<X", where X is the limit of quantitation for the assay. All analytes showing one or more locations as "<X" were excluded from statistical analysis. In addition, because of rounding of values near the reporting limit, a few analytes show almost no variability. Analysis of such data would not be expected to be meaningful. Therefore, four fatty acids, for which the majority of the numbers were the same (palmitoleic, arachidic, eicosenoic and behenic) were also excluded from statistical analysis.

A. Proximate Analysis of Corn Grain

The levels of the major components of corn grain (protein, fat, ash, carbohydrates, calories and moisture) were determined for grain of five test corn lines and one control line harvested from six field trials conducted under GLP in 1994 (EPA MRID #43665502). Table 2a summarizes the results of these analyses. The values for both the test and control lines were also compared to the published and reported literature ranges (Table 2b).

1. Corn Line MON 801. There were no statistically significant differences between the values for the test, MON 801, and the control line, MON 81S, for

fat, ash, and moisture. The mean protein, carbohydrate and calorie levels for the test line, MON 801 were statistically significantly different from the control line, MON 818. The mean value for protein in MON 801 was 13.6% compared to 12.8% for MON 818. However, the protein values for both lines were within the ranges reported in the literature (Jugenheimer, 1976) (Table 2b). Commercial hybrids typically have lower protein levels, 6.0-12.0% (Table 2b). Considerable genetic variability of protein content in hybrids has been reported (Dudley and Lambert, 1992).

Ciba Seeds reported similar protein values for their inbred and hybrid lines, with ranges for the control lines from 10.0-12.8% protein, and 10.8-13.6% protein for the transgenic *Bt* lines (Lotstein, 1995). DEKALB Genetics Corp. reported protein values of 12.1% for the B16 hybrid and 9.2% for the unconverted hybrid. The range of protein derived from analysis of commercial DEKALB hybrids grown throughout the midwest in 1993 and 1994 was 6.8 to 13.4% (Flick, 1995).

The mean value for carbohydrate in MON 801 was 82.0% compared to 82.7% for MON 818. The carbohydrate values were derived by calculation : 100 - protein - ash - moisture - fat = carbohydrate. The higher protein levels for MON 801 in the 1994 field tests resulted in a statistically significant lower carbohydrate value. The carbohydrate levels for both MON 801 and MON 818 lines are within the range observed for two lines of similar genetic backgrounds from a previous study (Sanders and Patzer, 1995) (Table 2b).

2. **Corn Line MON 802.** There were no statistically significant differences between the protein, fat, ash, carbohydrate, calories and moisture values for the test, MON 802, and the control line, MON 818 (Table 2a).

3. **Corn Line MON 805.** There were statistically significant differences between the protein, fat, carbohydrate, calories and moisture values for the test, MON 805, and the control line, MON 818. However, the mean values for both lines were within the published and reported literature ranges for the components measured (Table 2b).

4. **Corn Line MON 809.** There were no significant differences between the values for the test, MON 809, and the control line, MON 818, for protein, ash, carbohydrate and calories. The mean fat and moisture levels for the test line, MON 809 were statistically significantly different from the control line, MON 818 at the 95% confidence level using a paired t-test. The mean fat value for the test line, MON 809, was slightly lower (2.6%) than the published ranges, 2.9 to 6.1% (Table 2b). However, the values for the test line ranged from 2.1 to 3.2%, indicating significant variability across the field sites. The moisture values for both the test and control lines were within the published literature ranges (Table 2b).

5. Corn Line MON 810. There were no significant differences between the values for the test, MON 810, and the control line, MON 818, for protein, fat, ash, carbohydrates, calories and moisture. The values for both the test and control lines were also within the published and reported literature ranges (Table 2b).

B. Amino Acid Composition of Corn Grain

Amino acid composition was completed on corn grain samples and the results are presented in Table 3. The reported values for each amino acid (mg/g) were converted to percent of total protein and the statistical analyses performed.

1. Corn Line MON 801. The test line, MON 801 contained three amino acids, lysine, threonine and glycine, whose values were statistically significantly different from the values for the control line, MON 818. The mean values for these amino acids are within the published literature range (Watson, 1982).

2. Corn Line MON 802. The test line, MON 802 contained six amino acids, tryptophan, threonine, valine, glycine, alanine and tyrosine, whose values were statistically significantly different from the values for the control line, MON 818. The mean values for these amino acids, with the exception of threonine, are within the published literature range (Watson, 1982) or reported range for two lines (MON 800/801) of similar genetic backgrounds from a previous study (Sanders and Patzer, 1995) (Table 3).

3. Corn Line MON 805. The test line, MON 805 contained six amino acids, methionine, cystine, lysine, threonine, histidine and glycine, whose values were statistically significantly different from the values for the control line, MON 818 (Table 3). The mean values for these amino acids are within the published literature range (Watson, 1982) or reported range for two lines (MON 800/801) of similar genetic backgrounds from a previous study (Sanders and Patzer, 1995) (Table 3).

4. Corn Line MON 809. The test line, MON 809 contained two amino acids, cystine and threonine, which were statistically significantly different from the values for the control line, MON 818 (Table 3). The mean value for cystine for both the test and control line was higher than the literature range. However, the level of cystine for both the MON 809 (2.0%) and MON 818 (1.9%) lines are within the range (1.9-2.3%) observed for two lines (MON 800/801) of similar genetic backgrounds from a previous study (Sanders and Patzer, 1995) (Table 3). The mean values for threonine are within the literature range.

5. Corn Line MON 810. The test line, MON 810 contained eight amino acids (cystine, tryptophan, histidine, phenylalanine, alanine, proline, serine and tyrosine) which were statistically significantly different from the values for the control line, MON 818 (Table 3). The mean values for six of these amino acids

(tryptophan, phenylalanine, alanine, proline, serine and tyrosine) are within the ranges reported in the literature (Watson, 1982). The mean values for cystine and histidine for both the test and control line were higher than the literature range. However, the level of cystine for both the MON 810 (2.0%) and MON 818 (1.9%) lines are within the range (1.9-2.3%) observed for two lines (MON 800/801) of similar genetic backgrounds from a previous study (Sanders and Patzer, 1995). In addition, the level of histidine for MON 810 (3.1%) line is within the range (2.8-3.3%) observed for two lines of similar genetic backgrounds from a previous study (Sanders and Patzer, 1995).

C. Fatty Acid Profile of Corn Grain

The fatty acid composition was determined for the grain of the four test lines and the results are summarized in Table 4. Statistical analysis was completed on the five fatty acids which gave meaningful values in the assay: palmitic, stearic, oleic, linoleic and linolenic (Table 4). Four fatty acids, for which the majority of the values were the same (palmitoleic, arachidic, eicosenoic and behenic) were excluded from statistical analysis as previously discussed. A fatty acid profile was not generated for corn line MON 801 since this had been done previously (Sanders and Patzer, 1995).

1. Corn Line MON 802. One of the five fatty acids measured, linolenic, showed statistically significant differences between the test (MON 802) and control (MON 818) lines in a paired t-test analysis at the 95% confidence level. The mean value for linolenic, 0.93%, is within the literature range (Watson, 1982) and within the range (0.9-1.1%) previously reported for two corn lines (MON 800/801) of similar genetic backgrounds (Sanders and Patzer, 1995) (Table 4).

2. Corn Line MON 805. There were no significant differences between the values for the test line, MON 805, and the control line, MON 818, for the measured fatty acids (Table 4).

3. Corn Line MON 809. Only one of the five fatty acids measured, linolenic, showed statistically significant difference between the test and control lines in a paired t-test analysis at the 95% confidence level. The mean value for linolenic, 1.0%, is within the literature range and within the range (0.9-1.1%) previously reported for two corn lines (MON 800/801) of similar genetic backgrounds (Sanders and Patzer, 1995) (Table 4).

4. Corn Line MON 810. All of the five fatty acids measured showed no statistically significant differences between the test line, MON 810, and the control line, MON 818, in a paired t-test analysis at the 95% confidence level.

D. Carbohydrates in Corn Grain

Carbohydrate components, namely starch, crude fiber, sugars and phytic acid were evaluated and the results are presented in Table 5. In addition to fructose, glucose and sucrose, the sugars galactose, lactose and maltose were also assayed but the values were below the limit of detection of the assay.

1. Corn Line MON 802. No statistically significant differences between crude fiber and sugars of the test line, MON 802, and the control line, MON 818, were observed in a paired t-test analysis at the 95% confidence level (Table 5). The mean starch and phytic acid levels for the test line were statistically significantly different from the control line values but were within the ranges reported in the literature (Watson, 1982).

2. Corn Line MON 805. No statistically significant differences between starch, crude fiber and fructose of the test line, MON 805, and the control line, MON 818, were observed in a paired t-test analysis at the 95% confidence level (Table 5). The mean glucose, sucrose and phytic acid levels for the test line were statistically significantly different from the control line values. The glucose level (0.56 g/100g) was similar to the range (0.47-1.03 g/100g) previously reported for two corn lines (MON 800/801) of similar genetic backgrounds (Sanders and Patzer, 1995). The sucrose and phytic acid levels for test line MON 805 were slightly higher than those previously reported (Sanders and Patzer, 1995).

3. Corn Line MON 809. No statistically significant differences between the carbohydrate components of the test line, MON 809, and the control line, MON 818, were observed in a paired t-test analysis at the 95% confidence level (Table 5).

4. Corn Line MON 810. There were no statistically significant differences between the values for the test, MON 810, and the control line, MON 818, for starch, fructose, glucose, sucrose and phytic acid (Table 5). Crude fiber values in MON 810 grain (2.6%) were statistically significantly different than MON 818 values (2.4%) but the values are within the range reported in the literature (2.0-5.5%) (Watson, 1982).

E. Tocopherol Analysis of Corn Grain

Tocopherols are naturally present in corn oil and have vitamin E potency (Watson, 1982). The alpha, beta and gamma tocopherols were measured and the results summarized in Table 5. The gamma tocopherol is one-tenth as active as the alpha tocopherol (Watson, 1982) and therefore, is not an important component of corn grain. Literature values for beta and gamma tocopherol levels in corn grain were not reported (Watson, 1982).

1. **Corn Line MON 802.** The mean values for the alpha and beta tocopherols were significantly different between the test and control samples. However, the alpha tocopherol values for the test (9.7%) and control (10.9%) line are well within the range reported in the literature (3.0-12.1%).

2. **Corn Line MON 805.** There were no statistically significant differences between the values for the test, MON 805, and the control line, MON 818, for alpha tocopherol. The mean values for the beta and gamma tocopherols were significantly different between the test and control samples but these are minor components in corn grain.

3. **Corn Line MON 809.** The mean values for the tocopherols were significantly different between the test line, MON 809, and the control line, MON 818. However, the alpha tocopherol values for the test (9.5%) and control (10.9%) line are well within the range reported in the literature (3.0-12.1%).

4. **Corn Line MON 810.** There were no statistically significant differences between the values for the test, MON 810, and the control line, MON 818, for alpha and gamma tocopherols. The mean value for the beta tocopherol was statistically significantly different between the test and control samples.

F. Inorganic Components in Corn Grain

The calcium and phosphorus levels were determined and the results summarized in Table 5.

1. **Corn Line MON 802.** Calcium was statistically significantly lower in the test line, MON 802, compared to the control line, MON 818, although the differences are minor (0.0029% compared to 0.0033%). These values are very near the range reported for two corn lines, MON 800/801, (0.003-0.004%) of similar genetic backgrounds (Sanders and Patzer, 1995). No statistically significant difference was observed in the phosphorus levels of the test and control line.

2. **Corn Line MON 805.** There were no statistically significant differences between the values for the test, MON 805, and the control line, MON 818, for calcium and phosphorus values (Table 5).

3. **Corn Line MON 809.** Calcium was statistically significantly lower in the test compared to the control line, although the differences are minor (0.0030% compared to 0.0033%). These values are within the range reported for two corn lines, MON 800/801 (0.003-0.004%) of similar genetic background (Sanders and Patzer, 1995). No statistically significant difference was observed in the phosphorus levels of the test and control line.

4. Corn Line MON 810. Calcium was statistically significantly higher in the test compared to the control line, although the differences are minor (0.0036% compared to 0.0033%). These values are within the range reported for two corn lines, MON 800/801 (0.003-0.004%) of similar genetic backgrounds (Sanders and Patzer, 1995). No statistically significant difference was observed in the phosphorus levels of the test and control line.

VI. CONCLUSIONS

Compositional data on the test lines was comparable to the control line, MON 818, and within the published or reported literature ranges, with few exceptions. These few exceptions do not represent meaningful differences and are attributable to the variability in the noncommercial corn genotype used as the experimental genotype in the study. Based on these data, it was concluded that there are no meaningful compositional differences between the insect protected corn lines, MON 801, MON 809 and MON 810, the insect protected Roundup Ready corn lines, MON 802 and MON 805 and the control line, MON 818.

VII. ACKNOWLEDGEMENTS

The author would like to thank Larry Holden and Anthony Carella for the statistical analysis, Hope Dodson for tables preparation, Clyde Livingston for the Quality Assurance review, and Roy Fuchs for his critical review of this report.

VIII. REFERENCES

The references for the analytical methods are listed in Appendix 2.

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Table 1. Grain Sample Identifiers

Corn Line	Site Code					
MON #	JV	MN	JH	SH	WN	YK
801	80171	80172	80173	80174	80175	80176
802	80281	80282	80283	80284	80285	80286
805	80581	80582	80583	80584	80585	80586
809	80981	80982	80983	80984	80985	80986
810	81081	81082	81083	81084	81085	81086
818	81881	81882	81883	81884	81885	81886

Table 2a: Summary of Proximate Analysis of Corn Grain

Characteristic	MON 801	MON 802	MON 805	MON 809	MON 810	MON 818
	Mean[a] (Range)[b]	Mean (Range)	Mean (Range)	Mean (Range)	Mean (Range)	Mean (Range)
Protein[c]	13.6[d] (12.6-14.1)	12.9 (11.8-13.7)	13.4[d] (12.8-14.0)	13.1 (12.5-13.6)	13.1 (12.7-13.6)	12.8 (11.7-13.6)
Fat	2.7 (2.6-2.7)	3.1 (2.8-3.2)	3.3[d] (2.9-3.7)	2.6[d] (2.1-3.2)	3.0 (2.6-3.3)	2.9 (2.6-3.2)
Ash[c]	1.7 (1.6-1.7)	1.6 (1.5-1.8)	1.6 (1.5-1.7)	1.5 (1.3-1.7)	1.6 (1.5-1.7)	1.5 (1.5-1.6)
Carbohydrate[c]	82.0[d] (81.4-83.1)	82.4 (81.5-83.2)	81.7[d] (81.0-82.9)	82.8 (82.3-83.6)	82.4 (81.8-82.9)	82.7 (81.7-83.8)
Calories/100g	406.5[d] (405.8-407.0)	409.2 (408.6-409.8)	410.4[d] (408.7-411.5)	407.0 (404.4-409.7)	408.4 (407.0-410.1)	408.5 (406.0-410.1)
Moisture %	11.4 (10.0-12.5)	12.6 (11.2-14.8)	13.2[d] (12.4-14.8)	13.2[d] (10.6-14.6)	12.4 (11.0-14.4)	12.0 (10.6-14.2)

[a] Value reported is mean of six samples, one from each field site (Sanders et. al., 1995).

[b] Range denotes the lowest and highest individual values across sites for each line.

[c] Percent dry weight of sample.

[d] Values are significantly different from control line, MON 818, at the 95% confidence level (paired t-test).

Table 2b: Literature References

Component	Literature Mean	Literature Range	Literature Reference	MON 800/801[a] Range
Protein %	9.5	6.0-12.0	Watson, 1987	11.2-13.6
	12.3	9.7-16.1	Jugenheimer, 1976	
Fat (oil) %	4.3	3.1-5.7	Watson, 1987	3.8-4.2
	4.6	2.9-6.1	Jugenheimer, 1976	
Ash %	1.4	1.1-3.9	Watson, 1987	1.5-1.8
Carbohydrate		not reported		80.8-83.0
Calories/100g		not reported		412.6-415.7
Moisture %	16.0	7-23	Watson, 1987	13.0-15.8

[a] Sanders and Patzer (1995), range for two lines with similar genetic backgrounds, one sample of each line from each of five field sites, Study 93-01-39-02 (Ream and Sanders, 1995).

Table 3: Amino Acid Composition of Corn Grain[a]

Amino Acids	MON 801	MON 802	MON 805	MON 809	MON 810	MON 818	Literature[b]	MON 800/801[c]
	Mean[d] (Range)[e]	Mean (Range)	Mean (Range)	Mean (Range)	Mean (Range)	Mean (Range)	Range	Range
Nutritionally Essential								
Methionine	1.6 (1.5-1.7)	1.7 (1.4-1.9)	1.8[f] (1.6-1.9)	1.7 (1.6-1.8)	1.7 (1.6-1.9)	1.7 (1.6-1.7)	1.0-2.1	2.0-2.6
Cystine	2.0 (1.9-2.1)	2.0 (1.8-2.1)	2.0[f] (1.9-2.1)	2.0[f] (2.0-2.1)	2.0[f] (1.9-2.1)	1.9 (1.8-2.0)	1.2-1.6	1.9-2.3
Lysine	2.6[f] (2.5-2.8)	2.8 (2.7-3.0)	2.6[f] (2.4-2.9)	2.7 (2.4-3.0)	2.8 (2.5-2.9)	2.8 (2.7-2.9)	2.0-3.8	2.6-3.4
Tryptophan	0.6 (0.6-0.6)	0.5[f] (0.4-0.5)	0.6 (0.5-0.6)	0.6 (0.5-0.6)	0.6[f] (0.5-0.7)	0.6 (0.4-0.6)	0.5-1.2	0.5-0.6
Threonine	3.4[f] (3.3-3.6)	4.4[f] (4.2-4.6)	3.4[f] (3.2-3.5)	3.5[f] (3.4-3.6)	3.9 (3.7-4.4)	3.8 (3.7-3.9)	2.9-3.9	3.9-4.2
Isoleucine	3.8 (3.7-3.9)	3.8 (3.6-4.1)	3.7 (3.1-4.0)	3.8 (3.5-4.0)	3.7 (3.3-4.1)	3.8 (3.6-4.0)	2.6-4.0	3.5-3.8
Histidine	2.9 (2.8-2.9)	3.0 (2.9-3.1)	2.8[f] (2.6-3.0)	2.9 (2.7-3.0)	3.1[f] (2.9-3.3)	2.9 (2.8-3.0)	2.0-2.8	2.8-3.3

[a] Values are expressed as percent of total protein.

[b] Watson, 1982. Values are percent of total protein [10.1% total protein (Nx6.25)].

[c] Sanders and Patzer (1995), range for two lines with similar genetic background. Range from values of five samples of each line, one from each field site, Study 93-01-39-02 (Ream and Sanders, 1995)

[d] Value reported is mean of six samples, one from each field site, Study 94-01-39-01 (EPA MRID #43665502).

[e] Range denotes the lowest and highest individual values across sites for each line.

[f] Significantly different from the control line, MON 818 at the 5% level (paired t-test).

Table 3: Amino Acid Composition of Corn Grain[a] continued

Amino Acids	MON 801	MON 802	MON 805	MON 809	MON 810	MON 818	Literature[b]	MON 800/801[c]
	Mean[d] (Range)[e]	Mean (Range)	Mean (Range)	Mean (Range)	Mean (Range)	Mean (Range)	Range	Mean Range
Nutritionally Essential								
Valine	4.6	4.1[f]	4.4	4.6	4.5	4.6		
	(4.3-4.7)	(3.8-4.5)	(3.9-4.8)	(4.4-4.9)	(4.1-4.9)	(4.3-4.8)	2.1-5.2	4.2-4.8
Leucine	14.3	14.5	14.3	14.4	15.0	14.5		
	(13.9-14.7)	(14.0-15.4)	(14.1-14.8)	(13.4-15.2)	(14.1-16.7)	(13.8-15.0)	7.8-15.2	13.6-14.5
Arginine	4.3	4.5	4.4	4.5	4.5	4.5		
	(4.2-4.6)	(4.4-4.7)	(4.2-4.9)	(4.1-5.0)	(4.1-4.7)	(4.2-4.7)	2.9-5.9	4.1-5.0
Phenylalanine	5.5	5.4	5.4	5.5	5.6[f]	5.4		
	(5.3-5.7)	(5.3-5.7)	(5.3-5.6)	(5.3-5.7)	(5.4-6.1)	(5.2-5.6)	2.9-5.7	5.2-5.6
Glycine	3.4[f]	4.1[f]	3.4[f]	3.5	3.7	3.7		
	(3.3-3.5)	(4.0-4.3)	(3.2-3.7)	(3.3-3.9)	(3.4-4.0)	(3.5-3.8)	2.6-4.7	3.4-4.2

[a] Values are expressed as percent of total protein.

[b] Watson, 1982. Values are percent of total protein [10.1% total protein (Nx6.25)].

[c] Sanders and Patzer (1995), range for two lines with similar genetic background. Range from values of five samples of each line, one from each field site, Study 93-01-39-02 (Ream and Sanders, 1995).

[d] Value reported is mean of six samples, one from each field site, Study 94-01-39-01 (EPA MRID #43665502).

[e] Range denotes the lowest and highest individual values across sites for each line.

[f] Significantly different from the control line, MON 818 at the 5% level (paired t-test).

Table 3: Amino Acid Composition of Corn Grain[a] continued

Amino Acids	MON 801	MON 802	MON 805	MON 809	MON 810	MON 818	Literature[b]	MON 800/801[c]
	Mean[d] (Range)[e]	Mean (Range)	Mean (Range)	Mean (Range)	Mean (Range)	Mean (Range)	Range	(Range)
Nonessential								
Alanine	7.9 (7.7-8.2)	7.5[f] (7.2-7.9)	7.9 (7.7-8.0)	8.0 (7.7-8.3)	8.2[f] (7.8-8.9)	7.8 (7.5-8.0)	6.4-9.9	7.8-8.2
Aspartic Acid	6.3 (6.1-6.8)	6.9 (6.4-7.9)	6.4 (6.0-6.7)	6.5 (6.3-6.8)	7.1 (6.4-8.2)	6.6 (6.3-6.8)	5.8-7.2	6.7-7.3
Glutamic Acid	21.0 (20.5-21.7)	21.7 (20.7-23.0)	21.0 (20.5-21.3)	21.2 (20.4-22.1)	21.9 (20.4-24.4)	21.1 (20.1-21.6)	12.4-19.6	19.9-21.4
Proline	9.4 (9.2-9.7)	9.4 (8.6-10.0)	9.5 (9.3-9.8)	9.8 (9.4-10.3)	9.9[f] (9.7-10.5)	9.6 (9.4-9.8)	6.6-10.3	9.0-9.4
Serine	5.1 (5.0-5.4)	5.4 (5.1-5.6)	5.2 (5.0-5.4)	5.2 (4.8-5.4)	5.5[f] (5.3-5.9)	5.2 (5.1-5.4)	4.2-5.5	5.5-6.1
Tyrosine	3.9 (3.8-4.1)	4.3[f] (4.1-4.5)	4.1 (4.0-4.2)	4.0 (3.9-4.1)	4.4[f] (4.1-4.8)	4.0 (3.9-4.1)	2.9-4.7	3.8-4.3

[a] Values are expressed as percent of total protein.

[b] Watson, 1982. Values are percent of total protein [10.1% total protein (Nx6.25)].

[c] Sanders and Patzer (1995), range for two lines with similar genetic backgrounds. Range from values of five samples of each line, one from each field site, Study 93-01-39-02 (Ream and Sanders, 1995).

[d] Value reported is mean of six samples, one from each field site, Study 94-01-39-01 (EPA MRID #43665502).

[e] Range denotes the lowest and highest individual values across sites for each line.

[f] Significantly different from the control line, MON 818 at the 5% level (paired t-test).

Table 4: Fatty Acid Composition of Corn Grain[a]

Fatty Acids	MON 802	MON 805	MON 809	MON 810	MON 818	Literature[b] Range	MON 800/801[c]
	Mean[d] (Range)[e]	Mean (Range)	Mean (Range)	Mean (Range)	Mean (Range)		(Range)
Palmitic (16:0)	10.3 (10.1-10.4)	10.5 (10.2-10.8)	10.6 (10.5-11.0)	10.5 (10.2-11.1)	10.5 (10.2-10.7)	7-19	(10.2-10.9)
Stearic (18:0)	1.8 (1.7-1.8)	1.9 (1.8-2.0)	1.9 (1.8-2.0)	1.9 (1.7-2.1)	1.8 (1.8-1.9)	1-3	(1.6-3.1)
Oleic (18:1)	23.4 (22.2-24.4)	23.2 (22.0-24.3)	22.9 (22.4-23.5)	23.2 (21.5-25.4)	22.8 (21.6-23.9)	20-46	(21.2-25.9)
Linoleic (18:2)	62.6 (61.6-63.8)	62.4 (60.8-64.1)	62.6 (61.9-63.3)	62.6 (59.5-64.7)	63.0 (61.8-64.6)	35-70	(58.0-65.0)
Linolenic (18:3)	0.9[f] (0.9-1.0)	0.9 (0.7-0.9)	1.0[f] (0.9-1.0)	0.8 (0.7-0.9)	0.9 (0.8-0.9)	0.8-2	(0.9-1.1)

[a] Value of fatty acid is % of total lipid. Other fatty acids were below the limit of detection of the assay.

[b] Watson, 1982.

[c] Sanders and Patzer (1995), range for two lines with similar genetic backgrounds.

[d] Value reported is mean of six samples, one from each field site, Study 94-01-39-01 (EPA MRID #43665502).

[e] Range denotes the lowest and highest individual values across sites for each line.

[f] Significantly different from the control line, MON 818 at the 5% level (paired t-test).

Table 5: Analysis of Carbohydrates, Tocopherols and Inorganic Components of Corn Grain[a]

Component	MON 802	MON 805	MON 809	MON 810	MON 818	Literature[b] Range	MON 800/801[c]
	Mean[d] (Range)[e]	Mean (Range)	Mean (Range)	Mean (Range)	Mean (Range)		Range
A. Carbohydrates							
Starch %	70.5[f] (68.6-72.4)	66.1 (63.6-68.6)	66.8 (65.2-68.9)	67.6 (65.3-69.7)	66.9 (64.6-69.0)	64-78.0	63.7-71.5
Crude Fiber %	2.4 (2.1-2.6)	2.3 (2.2-2.5)	2.3 (1.9-2.7)	2.6[f] (2.5-2.8)	2.4 (2.3-2.5)	2.0-5.5	1.98-2.61
Sugars[g] g/100g							
Fructose	0.31 (0.23-0.35)	0.25 (0.23-0.34)	0.25 (0.22-0.34)	0.32 (0.23-0.35)	0.27 (0.22-0.40)		0.47-0.96
Glucose	0.44 (0.34-0.57)	0.56[f] (0.46-0.69)	0.36 (0.34-0.45)	0.44 (0.34-0.47)	0.41 (0.34-0.46)		0.47-1.03
Sucrose	0.97 (0.82-1.03)	1.09[f] (0.91-1.27)	0.86 (0.70-1.02)	0.93 (0.79-1.12)	0.93 (0.68-1.11)		0.40-0.94
Phytic Acid %	0.97[f] (0.88-1.06)	0.92[f] (0.82-1.01)	0.85 (0.76-0.91)	0.86 (0.81-0.91)	0.84 (0.79-0.91)	0.7-1.0	0.45-0.57

[a] Values on a dry weight basis.
[b] Watson, 1982. Literature values provided if available.
[c] Value reported is mean of six samples, one from each field site.
[d] Range denotes the lowest and highest individual values across sites for each line.
[e] Sanders and Patzer (1995), range for two lines with similar genetic backgrounds.
[f] Galactose, lactose and maltose were also measured but values were below the limit of detection of the assay.
[g] Values are significantly different from control line, MON 818, at the 95% confidence level (paired t-test).

Table 5: Analysis of Carbohydrates, Tocopherols and Inorganic Components of Corn Grain[a] continued

Component	MON 802	MON 805	MON 809	MON 810	MON 818	Literature[b]	MON 800/801[c]
	Mean[c] (Range)[d]	Mean (Range)	Mean (Range)	Mean (Range)	Mean (Range)	Range	Range
B. Tocopherols							
Tocopherols mg/kg							
alpha	9.7[g] (9.1-10.6)	10.5 (9.3-11.9)	9.5[g] (8.8-10.1)	10.4 (9.7-11.3)	10.9 (9.9-12.1)	3.0-12.1	7.3-12.3
beta	8.1[g] (7.4-8.6)	6.3[g] (5.6-6.9)	6.4[g] (6.0-6.9)	8.5[g] (8.1-9.2)	7.5 (7.0-7.9)		7.9-10.7
gamma	23.8 (21.7-31.3)	17.8[g] (15.7-20.4)	14.8[g] (13.0-16.9)	20.2 (15.3-24.8)	21.6 (18.8-27.8)		21.7-42.5
C. Inorganic Components							
Calcium %	0.0029[g] (0.0025-0.0033)	0.0035 (0.0031-0.0037)	0.0030[g] (0.0027-0.0033)	0.0036[g] (0.0033-0.0039)	0.0033 (0.0029-0.0037)	0.01-0.1	0.003-0.004
Phosphorus %	0.336 (0.321-0.356)	0.348 (0.332-0.362)	0.334 (0.287-0.357)	0.358 (0.334-0.377)	0.348 (0.327-0.363)	0.26-0.75	0.311-0.363

[a] Values on a dry weight basis.

[b] Watson, 1982. Literature values provided if available.

[c] Value reported is mean of six samples, one from each field site.

[d] Range denotes the lowest and highest individual values across sites for each line.

[e] Sanders and Patzer (1995), range for two lines with similar genetic backgrounds.

[f] Galactose, lactose and maltose were also measured but values were below the limit of detection of the assay.

[g] Values are significantly different from control line, MON 818, at the 95% confidence level (paired t-test).

Appendix 1

Protocol

Sponsor:

The Agricultural Group of Monsanto Company
St. Louis, Missouri

Study Title:

Composition and Quality Analysis of Cornseed from European Corn Borer Resistant Corn Line Grown in 1994 GLP Trials

Date:

November 30, 1994

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6103-180

STUDY IDENTIFICATION

Composition and Quality Analysis of Cornseed from European Corn Borer Resistant Corn Line Grown in 1994 GLP Trials

HWI Study No.	6103-180
Test Material	Cornseed
Sponsor Study Title	Composition and Quality Analysis of Cornseed from European Corn Borer Resistant Corn Line Grown in 1994 GLP Trials
Sponsor	The Agricultural Group of Monsanto Company 700 Chesterfield Parkway North St. Louis, MO 63198
Study Monitor	Patricia Sanders The Agricultural Group of Monsanto Company 700 Chesterfield Parkway North GG4K St. Louis, MO 63198 (314) 537-6412 Fax No. (314) 537-6567
Study Director	Shari S. Patzer Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707 (608) 241-4471 Fax No. (608) 241-7227
Study Location	Hazleton Wisconsin, Inc. 3301 Kinsman Boulevard Madison, WI 53704
Proposed Study Schedule	
Samples to be Received	December 1, 1994
Initial Sample Analysis	December 5, 1994
Completion of Sample Analysis	December 30, 1994

1. Study	Precision Determination of Cornseed for Composition/Quality Parameters
2. Purpose	To analyze cornseed samples for proximate anlaysis (moisture, protein, fat, ash, calories and carbohydrates), crude fiber, amino acids, starch, sugar, fatty acid profile, vitamin E and phytic acid according to Hazleton Wisconsin (HWI) approved methods.
3. Regulatory Compliance	This study will be conducted in accordance with the Food and Drug Administration Good Laboratory Practice Regulations for Nonclinical Laboratory Studies, 21 CFR 58.
4. Quality Assurance	The protocol, study conduct, data and final report will be audited by the Quality Assurance Unit in accordance with Hazleton Wisconsin Standard Operating Procedures (SOPs).
5. Test Material	
A. Test Material	
(1) Identification	Cornseed
(2) Purity	Responsibility of the Sponsor
(3) Stability	Responsibility of the Sponsor
(4) Storage	In a freezer set to maintain -20°C ±10°
(5) Characteristics	Information on synthesis methods, composition, or other characteristics that define the test material is the responsibility of the Sponsor.
(6) Reserve Samples	Responsibility of the Sponsor.

6. Experimental Design

A. Samples

(1) Source — Samples will be shipped to HWI by Monsanto Company.

(2) Number of Samples — Twenty-four test samples and six control samples.

(3) Identification — The test samples are as follows: MON80281, MON80282, MON80283, MON80284, MON80285, MON80286, MON80581, MON80582, MON80583, MON80584, MON80585, MON80586, MON81081, MON81082, MON81083, MON81084, MON81085, MON81086, MON81281, MON81282, MON81283, MON81284, MON81285, MON81286. The control samples are as follows: MON81881, MON81882, MON81883, MON81884, MON81885 and MON81886.

(4) Storage — In a freezer set to maintain -20°c ±10°. Any remaining test material, including original sample receipt containers will be returned to the Sponsor after completion of the study.

B. Analytical Methods — Each of the samples will be assayed by the following HWI methods: PGEN, M100, FSOX, ASHM, CFIB, CALC, CHO, TAAP, STCH, SUGN, FAPM, EFD2, AND A1XX.

7. Statistical Evaluation — Statistical evaluation of the data will be performed by the Sponsor.

8. Reports — A final report including, but not limited to, those items listed below will be submitted. One copy of the draft report and two copies of the final report will be provided.

A copy of the protocol and any protocol amendments

A copy of the Laboratory Information Management Systems (LIMS) reports

Statistical analysis and test material stability, purity and characterization will be provided by the Sponsor as an attachment(s) to the final report.

9. Maintenance of Raw Data and Records

Original data or copies will be available at HWI to facilitate auditing the study during its progress and before acceptance of the final report. When the final report is completed, original paper data and magnetically encoded records, including those items listed below will be retained in the archives of HWI in accordance with 21 CFR 58.

Protocol, and any protocol amendments, computer printouts, chromatographs, worksheets, data sheets, original notes by investigators, forms specified by SOP, transfer forms and final report, original signed copy.

The following supporting records will be retained at HWI but will not be archived with the study data: refrigerator and freezer temperature records, instrument calibration and maintenance records.

PROTOCOL APPROVAL

Patricia Sanders — 12/1/94

Patricia Sanders
Study Monitor
The Agricultural Group of Monsanto Company

Date

Shari S. Patzer — 11/30/94

Shari S. Patzer
Study Director
Hazleton Wisconsin, Inc.

Date

Kim Weber — 11/30/94

Representative
Quality Assurance Unit
Hazleton, Wisconsin, Inc.

Date

Study Number: 94-01-50-18 Amendment #: 1

Date Change Implemented: 6-30-95

Project: Corn

Page No/s. &/or Section/s: see below

Protocol originally stated:
Page 1. Study Title: Composition and Quality Analysis of Cornseed from European Corn Borer Resistant Corn Line Grown in 1994 GLP Trials

Laboratory Project Indentification: HWI 6103-180

Page 2. Sponsor Study Title: Composition and Quality Analysis of Cornseed from European Corn Borer Resistant Corn Line Grown in 1994 GLP Trials

Study Monitor: Patricia Sanders
The Agricultural Group of Monsanto Company
700 Chesterfield Parkway North GG4K
St. Louis, MO 63198
(314) 637-6412
Fax No. (314) 537-6567

Study Director: Shari S. Patzer
Hazleton Wisconsin, Inc.
P.O. Box 7545
Madison, WI 53707
(608) 241-4471
Fax No. (608) 241-7227

Proposed Study Schedule: Completion of Sample Analysis December 30, 1994

Page 3. 1. Study: Precision Determination of Cornseed for Composition/Quality Parameters

2. Purpose: To analyze cornseed samples for proximate analysis (moisture, protein, fat, ash, calories and carbohydrates), crude fiber, amino acids, starch, sugar, fatty acid profile, vitamin E and phytic acid according to Hazleton Wisconsin (HWI) approved methods.

4. Quality Assurance: The protocol, study conduct, data and final report will be audited by the Quality Assurance Unit in accordance with Hazleton Wisconsin Standard Operating Procedures (SOPs).

Page 4:

B. Analytical Methods: (additional methods for Ca and P as noted below)

8. Reports: A final report including, but not limited to, those items listed below will be submitted. One copy of the draft report and two copies of the final report will be provided.

A copy of the Laboratory Information Management Systems (LIMS) reports

Statistical analysis and test material stability, purity and characterization will be provided by the Sponsor as an attachment(s) to the final report.

9. Maintenance of Raw Data and Records: When the final report is completed, original paper data and magnetically encoded records, including those items listed below will be retained in the archives of HWI in accordance with 21 CFR 58.

Protocol, and any protocol amendments, computer printouts, chromatographs, worksheets, data sheets, original notes by investigators, forms specified by SOP, transfer forms and final report, original signed copy.

Amended as Follows:
Page 1. Study Title: Compositional Analyses of Corn Seed from the 1994 Corn Field Trials of Insect Protected and Insect Protected Roundup Ready™ Corn Lines

Laboratory Project Identification:
HWI 6103-180/Monsanto Study #94-01-50-18

Page 2. Sponsor Study Title: Compositional Analyses of Corn Seed from the 1994 Corn Field Trials of Insect Protected and Insect Protected Roundup Ready™ Corn Lines

Study Director: Patricia Sanders
Monsanto Company
CEREGEN
700 Chesterfield Parkway North GG4K
St. Louis, MO 63198
(314) 637-6412
Fax No. (314) 537-6567

Principal Investigator: Diane Henning
Corning Hazleton, Inc.
Wisconsin Facility
P.O. Box 7545
Madison, WI 53707
(608) 242-2712 Ext 2386
Fax No. (608) 241-7227

Add-

Primary Testing Facility
Monsanto Company
CEREGEN
700 Chesterfield Parkway North
St. Louis, MO 63198

Proposed Study Schedule: Completion of Sample Analysis September 29, 1995

Page 3. 1. Study: Compositional Analyses of Corn Seed from the 1994 Corn Field Trials of Insect Protected and Insect Protected Roundup Ready™ Corn Lines

2. Purpose: To analyze cornseed samples for proximate analysis (moisture, protein, fat, ash, calories and carbohydrates), crude fiber, amino acids, starch, sugar, fatty acid profile, vitamin E, phytic acid, calcium and phosphorus according to Corning Hazleton, Inc. (CHW) approved methods.

4. Quality Assurance: The protocol, study conduct, data and analytical subreport will be audited by the CHW Quality Assurance Unit in accordance with Corning Hazleton, Inc. Wisconsin Facility, Standard Operating Procedures (SOPs).

Page 4:
A. Samples (addition)
(2) Additional samples may be added to the study if necessary.

B. Analytical Methods: Additional analyses: CAA and PTA.

8. Reports: The final report will be generated by the Monsanto Study Director which includes or references statistical analysis and test material stability, purity and characterization. A quality control checked and Quality Assurance accepted analytical subreport generated by the CHW Principal Investigator will be submitted to the Monsanto Study Director to be used in preparation of the final report. The analytical subreport will include method summaries,

reference standards where applicable and a summary spreadsheet (which condenses the LIMS report data on a fresh weight basis and results calculated on dry weight basis). The Monsanto Quality Assurance unit will assume responsibility for audit of the final report.

9. Maintenance of Raw Data and Records: When the analytical subreport is completed, the original signed subreport, original paper data, computer printouts, chromatographs, worksheets, data sheets, original notes by investigators, forms specified by SOP and magnetically encoded records will be retained in the archives of CHW in accordance with 21 CFR 58.

The protocol, all protocol amendments, test and control substance transfer forms and the original signed final report will be retained in the Monsanto Archives.

Reason for Amendment and how this change will impact the Study: Reevaluation of responsibilities of the Study Monitor and Study Director by Monsanto Testing Facility Management. Corning Hazleton personnel will generate the data, assure that the data has been verified and checked by their Quality Assurance Department and supply copies of LIMS reports and protocols to Monsanto as part of analytical subreport. Monsanto Quality Assurance will do the final report review. The original report will be archived at Monsanto.

Monsanto added calcium and phosphorus analysis to the analytical methods requested.

These changes will have no adverse impact on the study.

Signatures of Approval

Study Director:

Patricia Sanders Date: 6/29/95

Patricia Sanders

Sponsor/Testing Facilities Management Representative:

Date: 6/29/95

Roy Fuchs

[redacted]

Signatures of Acknowledgement

Diane Henning
Principal Investigator: Diane Henning — Date: 7/3/95

Hazleton Facility Management — Date: 7-3-95

Signature of Review by QA

QA Rep.: Clyde L. Livingston
Monsanto — Date: 5 July 1995

QA Rep.: Jonathan C Kellum
Corning Hazleton, Inc. — Date: 7/3/95

Monsanto
CEREGEN
Regulatory Sciences

Protocol Amendment Form
SOP Reference: GEN-POL-005 Page 1 of 2

[redacted]

Study Number: 94-01-50-18 Amendment #: 2

Date Change Implemented: 4/11/95

Project: B.t.k. Corn

Page No/s. &/or Section/s: Pg 4, Section 6 (3)

Protocol originally stated: The test samples are as follows ..... MON81281, MON81282, MON81283, MON81284, MON81285 and MON81286.

Amended as Follows: (These test samples have been deleted from the study).

Reason for Amendment and how this change will impact the Study: On 4/11/95, the corn line MON 812 was dropped as a lead line commercialization. The experimental work was in progress (and completed) on these samples and the data archived as part of the Corning Hazelton Analytical Subreport. No statistical analysis will be performed on the data collected on these test samples. This will have no impact on the study, only data on lead lines will be included in the final report.

Signatures of Approval

Study Director:

Patricia Sanders Date: 9/7/95
Patricia Sanders

Sponsor/Testing Facilities Management Representative:

Roy L. Fuchs Date: 9/7/95
Roy L. Fuchs

Signatures of Acknowledgement

Diane Henning Date: 9/19/95
Diane Henning

Signature of Review by QA

QA Rep.: Clyde L. Livingston
Ceregen
Date: 7 Sep 1995

QA Rep.: Scott C Rumsey
Corning Hazelton, Inc.
Date: 9-22-95

cc:

Appendix 2

Method References

AOAC Method 982.30. 1990. *In* Official Methods of Analysis, 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia, modified.

AOAC Method 979.10, 906.03, 929.09. 1990. *In* Official Methods of Analysis, 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia, modified.

AOAC Method 965.17, 962.11. 1990. *In* Official Methods of Analysis, 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia, modified.

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AOAC Method 955.04C, 979.09. 1990. *In* Official Methods of Analysis, 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia, modified.

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AOAC Method 923.03. 1990. *In* Official Methods of Analysis, 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia, modified.

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Fiske, C.H. and Subbarrow, Y. 1925. *Journal of Biochemical Chemistry* 66:375, modified.

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Mason, B.S., and Slover, H.T. 1971. *Journal of Agricultural Food Chemistry* 19(3):551-554, modified.

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Official and Tentative Methods of the American Oil Chemists Society. 1981. Method Ce 1-62, American Oil Chemists Society, Champaign, Illinois, modified.

Speek, A.J., Schrijver, J., and Schreurs, W.H.P. 1985. *Journal of Food Science* 50:121-124 modified.

USDA Agriculture Handbook No. 8. 1975. Composition of Foods. *In* Agricultural Hadnbook No. 8. United States Department of Agriculture, Washington, D.C. pp. 159-165.

Appendix 3

Statistical Analysis Report for Compositional Data

Study 94-01-50-18

Statistical Analysis of Composition Results from Five IPC Corn Lines Compared to Control Line MON 818.

Data Processing

Composition data from five transgenic lines of corn (801, 802, 805, 809 and 810) and a control line (818) were provided in a diskette. All data were contained in a number of Microsoft Excel spreadsheets. Each spreadsheet contained data from a single line and a single analysis group (see below). Two categories of information were available in each spreadsheet: actual values (rounded by the laboratory) and formulas. The spreadsheet formulas were created by the analytical laboratory to compute moisture or protein adjusted values. Since these adjustments are performed completely within the SAS analysis program (see below) and some errors were present in the spreadsheet formulas (and some labels) provided to us, only the actual numbers contained in the spreadsheets were used as data. In this way, statistical analysis started with reliable raw data which was not confounded by other information supplied on the original spreadsheets. These data were then organized into a single file and transferred to a VAX model 6000-610 for processing by the SAS statistical program (Release 6.08, SAS Institute Inc., 1990).

Each value in the data used for statistical analysis corresponded to an analytical response from material grown at one of 6 locations (coded 1-6 for convenience) and assayed by the laboratory in one of 3 analysis groups. Analyte groupings present in the supplied data were retained. An additional group, miscellaneous, was added for consistency and convenience in data processing. Thus, there are a maximum of 7 analyte categories: amino acids, fatty acids, proximates, starch (1 analyte), sugars, tocopherols, and miscellaneous.

For some analytes, values in the original spreadsheet were given as "<X," where X is a laboratory data reporting threshold, presumably the limit of quantitation (LOQ). In all of these cases the majority of results for that analyte were unavailable; therefore, no analysis was possible. For this reason, all analytes containing such "<X" values were excluded from statistical analysis. In addition, because of rounding of values near the reporting limit, a few analytes showed almost no variability. Analysis of such data would not be expected to be meaningful. Thus, 4 fatty acids, for which an overwhelming majority of the numbers were the same (i.e., 16:1 palmitoleic, 20:0 arachidic, 20:1 eicosenoic, and 22:0 behenic), were also excluded from statistical analysis. Table I lists all the excluded analytes.

As noted above, it was requested that some analytes be re-expressed on either a dry weight or per-protein basis. All re-expressions were performed within SAS and detailed in Table II. All calculations were designed to

duplicate those (correct) formulas supplied in the original spreadsheets. Although the statistical model used for the final analysis of the data was straightforward (see the following section), some complicated data processing was necessary to prepare the data for analysis. This need arose because samples were assayed in three groups, which we coded for convenience as 0, 1, and 2. Some lines were assayed in more than one group and not all analytes were available from each line within a group. Table III illustrates the grouping of analyses on a line-by-line basis. Some analytes of the control line (818) were present in all three groups and only two categories in line 801 (amino acids and proximates) were assayed, each category in a different analysis group.

This complicated analysis structure affected the corrections for moisture and protein. For consistency, it was decided to follow the same procedures indicated on the original spreadsheets given to us. That is:

(a) Adjust samples at each location for moisture and protein using the results in the same analysis group, if available.

(b) In the case of line 801 amino acids in group 1, use the protein results obtained in group 2.

Following the protein and/or moisture adjustments, the results from all groups were collapsed into a single value per analyte per line per location. That is, any multiple values for any line and location combination were averaged. This seems reasonable since the multiple values merely represent additional analyses of the same sample. This process resulted in a data set that is completely balanced. That is, for each analyte, there are the same number of locations (6) and lines (either 5 or 6).

Description of Statistical Analysis

For each analyte, the following randomized complete block model was used:

(1) $$Y_{ij} = \mu_j + L_i + E_{ij}$$

where

Y_{ij} = collapsed value obtained for line j in location i
μ_j = true mean of line j over all locations (i.e., overall mean)
L_i = random effect of location i
E_{ij} = random effect for line j within location i.

Model (1) was fit to the re-expressed data using the GLM procedure in SAS.

The overall F-test of equal means in GLM was ignored, since the purpose of the study was only the comparison of each transgenic corn line to the control. The SAS LSMEANS statement in the GLM procedure, however, was used to obtain treatment means, standard errors and covariances. These results were then used to construct a t-statistic, T_j, for comparing each line, j, with the control line (818):

$$(2) \qquad T_j = \frac{D_j}{SED_j}$$

In (2), D_j is the difference between the means of line j and line 818 and SED_j is the standard error of this difference. Each SED_j is computed within SAS from the standard errors of each mean (SE_j, SE_{818}) and from the covariance ($COV_{j,818}$) as follows:

$$(3) \qquad SED_j = [\,(SE_j)^2 + (SE_{818})^2 - 2\,COV_{j,818}\,]^{0.5}$$

(Note: since there were no missing location values in these data, $COV_{j,818}$ was actually zero in all cases.) For a test of zero average difference from control, each line's T_j value can be compared with the distribution of a Student's t statistic having (I-1)(J-1) degrees of freedom. Here, I and J are the number of locations and lines, respectively, available for each analyte. The SAS function PROBT was used to obtain the Student's t p-value corresponding to each T_j. The p-value is the observed significance level for a two-sided t-test of zero difference. For example, a p-value less than 0.05 indicates statistical significance of the difference at the 5% level.

For each analyte, means, differences, and p-values are given in Tables IV through VIII. Other summary statistics for the means, such as ranges, standard errors, and confidence intervals, were computed in SAS for each line separately. Since these statistics are not relevant to the comparison of the transgenic and control lines, however, they are not duplicated in this report. (These results can, however, be found in the SAS computer listing, which is part of the statistical analysis package.)

Summary of Overall Statistical Results

Tables IV through VIII show the mean differences from control for each of the five transgenic lines. Since 184 individual tests are being performed in this analysis, some false significant differences are expected. At the conventional 5% level of significance ($p<0.05$), if no transgenic corn line differed from control, we would expect on average about $0.05 \times 184 \approx 9$ significant results by chance alone. In fact, due to correlations among

analyte results, false significances may occur in clusters and exceed the average of 9. As a conservative estimate, however, p-values smaller than 0.05/184=0.00027 would indicate strong evidence of a true difference from control. There are only 7 instances of p-values smaller than 0.00027: glycine (802), threonine (802, 805), tyrosine (810), beta tocopherol (805, 809), and gamma tocopherol (809). Each of these shows the strongest evidence of statistical significance.

For 54 line-analyte combinations, the test results were statistically significant at the conventional 5% level (i.e., the observed p-value was less than 0.05). The more consistent differences, i.e., those occurring on multiple lines and with the same algebraic sign, are also less likely to be false positives. The following analytes appeared to show such consistent differences:

Analyte	Differences from control ($p<0.05$)	Lines
cystine	increase	805, 809, 810
lysine	decrease	801, 805
tyrosine	increase	802, 810
18:3 linolenic	increase	802, 809
phytic acid	increase	802, 805
carbohydrates	decrease	801, 805
moisture	increase	805, 809
protein	increase	801, 805
alpha tocopherol	decrease	802, 809
gamma tocopherol	decrease	805, 809

Summary of Results By Line:

The following analytes were found to have statistically significant differences from control at the conventional $p<0.05$. Some of these are most certainly false positives:

Line 801 (Table IV). The levels of amino acids glycine, lysine, and threonine were lower than in the control with percent differences between -7% to -10% . For proximates both calories and carbohydrates were about 1% lower than control, and protein levels were 6% higher.

Line 802 (Table V). The following amino acid percent differences were significant: alanine (-4%), glycine (13%), threonine (17%), tryptophan (-10%), tyrosine (9%), and valine (-10%). The fatty acid 18:3 linolenic gave a 10% increase over control. This line also had lower calcium (-13%) and higher levels of phytic acid (16%) than control. There was an increase in enzymatic starch of 5%. Two tocopherols were significant at $p<0.05$: alpha

(-10%) and beta (9%). Gamma tocopherol gave a 10% increase over control and with a p-value of exactly 0.05 is very close to significant (at the 5% level).

Line 805 (Table VI). The levels of 4 of the 6 significant amino acids were significantly lower than control: glycine (-7%), histidine (-5%), lysine (-7%), and threonine (-11%). Cystine and methionine, however, showed significant increases of 5% and 7%, respectively. Increases over control were also seen for phytic acid (10%), calories (1%), moisture (10%), protein (4%), total fat (15%), and the two sugars glucose (37%) and sucrose (18%). Decreases from control were found in carbohydrates (-1%), beta tocopherol (-16%), and gamma tocopherol (-18%).

Line 809 (Table VII). Two amino acid differences were significant: cystine (7%) and threonine (-9%). The fatty acid 18:3 linolenic showed a 16% increase over control. Except for an increase in moisture of 10%, the other significant differences found for line 809 were negative. These are calcium (-10%), total fat (-11%), alpha tocopherol (-12%), beta tocopherol (-15%), and gamma tocopherol (-32%).

Line 810 (Table VIII). Only significant increases from the control were seen for line 810. Eight of these were increases for the amino acids alanine (5%), cystine (6%), histidine (6%), phenylalanine (4%), proline (4%), serine (7%), tryptophan (9%), and tyrosine (10%). Other analytes giving significant increases over the control were: calcium (8%), crude fiber (10%) and beta tocopherol (14%).

References

SAS Institute Inc. 1990. SAS/STAT® User's Guide, Version 6, Fourth Edition, Volumes 1 and 2; SAS Procedures Guide®, Version 6, Third Edition; Cary NC.

Table I.

Analytes Excluded from Statistical Analysis.

Analyte Category	Analyte Excluded	Reason for Exclusion
Fatty Acids	10:0 Capric	<LOQ Values Present
	12:0 Lauric	<LOQ Values Present
	14:0 Myristic	<LOQ Values Present
	14:1 Myristoleic	<LOQ Values Present
	15:0 Pentadecanoic	<LOQ Values Present
	15:1 Pentadecenoic	<LOQ Values Present
	16:1 Palmitoleic	Insufficient Variation
	17:0 Heptadecanoic	<LOQ Values Present
	17:1 Heptadecenoic	<LOQ Values Present
	18:3 Gamma Linolenic	<LOQ Values Present
	20:0 Arachidic	Insufficient Variation
	20:1 Eicosenoic	Insufficient Variation
	20:2 Eicosadienoic	<LOQ Values Present
	20:3 Eicosatrienoic	<LOQ Values Present
	20:4 Arachidonic	<LOQ Values Present
	22:0 Behenic	Insufficient Variation
	8:0 Caprylic	<LOQ Values Present
Sugars	Galactose	<LOQ Values Present
	Lactose	<LOQ Values Present
	Maltose	<LOQ Values Present
Tocopherols	Delta	<LOQ Values Present

Table II.

Re-expressions of Analyte Values Requested prior to Statistical Analysis.

Analyte Category	Original Units	Analyzed Units	Re-expression Formula
Proximates (except moisture and Calories)	% seed fresh wt.	% seed dry wt.	$Y = X/(1-M/100)$
Proximates (moisture only)	% seed fresh wt.	same	none
Proximates (Calories only)	Cal./100g of seed fresh wt.	Cal./100g of seed dry wt.	$Y = X/(1-M/100)$
Sugars and Starch	% seed fresh wt.	% seed dry wt.	$Y = X/(1-M/100)$
Miscellaneous	% seed fresh wt.	% seed dry wt.	$Y = X/(1-M/100)$
Fatty Acids	% total fatty acid weight	same	none
Amino Acids	mg/g of seed fresh wt.	% total protein	$Y = 10X/P$
Tocopherols	mg/g of seed fresh wt.	mg/kg of seed dry wt.	$Y = 1000X/(1-M/100)$

KEY:

X = value in the original units
Y = value in the units desired for statistical analysis
M = Moisture as a percent of seed fresh weight
P = Percent protein expressed on a fresh weight basis.

Table III.

Organization of Lines and Analytes by Analysis Group.

	Analysis Group		
Line	0	1	2
801	–	Amino Acids only	Proximates only (except crude fiber)
802	all analytes	–	–
805	–	all analytes	–
809	–	all analytes	–
810	all analytes	–	–
818	all analytes (except Calcium and Phosphorus)	all analytes	Proximates only (except crude fiber)

Table IV.

Statistical Comparison of Line 801 to Control Line 818.†

Analyte	818 Mean	801 Mean	Mean Diff.	% Diff.	SE Diff.	p-Value*
Amino Acids						
alanine	7.80	7.91	0.11	1.5	0.14	0.41038
arginine	4.49	4.31	-0.18	-3.9	0.12	0.15022
aspartic acid	6.60	6.34	-0.27	-4.0	0.26	0.31697
cystine	1.91	1.99	0.08	4.4	0.04	0.05136
glutamic acid	21.10	21.00	-0.10	-0.5	0.45	0.81749
glycine	3.65	3.37	-0.28	-7.6	0.10	0.00930
histidine	2.90	2.87	-0.03	-1.0	0.06	0.61068
isoleucine	3.82	3.81	-0.00	-0.1	0.13	0.98370
leucine	14.54	14.32	-0.22	-1.5	0.31	0.48014
lysine	2.76	2.58	-0.18	-6.5	0.09	0.04613
methionine	1.65	1.59	-0.06	-3.5	0.04	0.21406
phenylalanine	5.42	5.47	0.05	0.9	0.10	0.63758
proline	9.55	9.41	-0.14	-1.4	0.18	0.45853
serine	5.17	5.14	-0.04	-0.7	0.10	0.71867
threonine	3.79	3.41	-0.38	-10.1	0.09	0.00039
tryptophan	0.56	0.58	0.02	4.4	0.02	0.29676
tyrosine	3.95	3.94	-0.02	-0.4	0.09	0.85785
valine	4.58	4.59	0.00	0.0	0.14	0.99180
Proximates						
ash	1.54	1.66	0.12	7.5	0.06	0.05743
calories	408.5	406.5	-2.0	-0.5	0.7	0.00998
carbohydrates	82.71	82.04	-0.67	-0.8	0.27	0.01914
moisture	12.00	11.43	-0.57	-4.7	0.41	0.17426
protein	12.83	13.63	0.80	6.2	0.22	0.00129
total fat	2.92	2.67	-0.24	-8.4	0.14	0.10096

† For clarity of presentation, the number of decimal places reported for means, differences and standard errors was one more than the number of decimal places in the original data provided. The same number of decimal places was used for each analyte within a category whenever it was practical to do so. For all analytes, percent differences were reported to a single decimal place.

* The observed significance level from a test of mean difference equal to zero. Values of $p<0.05$ indicate statistical significance at the 5% level.

Table V.

Statistical Comparison of Line 802 to Control Line 818.†

Analyte	818 Mean	802 Mean	Mean Diff.	% Diff.	SE Diff.	p-Value*
Amino Acids						
alanine	7.80	7.47	-0.33	-4.2	0.14	0.02479
arginine	4.49	4.53	0.05	1.0	0.12	0.69553
aspartic acid	6.60	6.89	0.29	4.3	0.26	0.28291
cystine	1.91	1.97	0.06	3.1	0.04	0.15929
glutamic acid	21.10	21.69	0.58	2.8	0.45	0.20380
glycine	3.65	4.12	0.47	12.9	0.10	0.00007
histidine	2.90	3.01	0.11	3.8	0.06	0.05596
isoleucine	3.82	3.84	0.02	0.6	0.13	0.86567
leucine	14.54	14.54	0.00	0.0	0.31	0.99719
lysine	2.76	2.83	0.07	2.6	0.09	0.40998
methionine	1.65	1.67	0.03	1.5	0.04	0.57599
phenylalanine	5.42	5.40	-0.02	-0.3	0.10	0.85694
proline	9.55	9.44	-0.10	-1.1	0.18	0.57668
serine	5.17	5.35	0.17	3.4	0.10	0.08343
threonine	3.79	4.43	0.64	16.8	0.09	0.00000
tryptophan	0.56	0.50	-0.05	-9.7	0.02	0.02549
tyrosine	3.95	4.33	0.37	9.4	0.09	0.00054
valine	4.58	4.14	-0.44	-9.6	0.14	0.00558
Fatty Acids						
16:0 palmitic	10.48	10.28	-0.20	-1.9	0.10	0.06759
18:0 stearic	1.84	1.78	-0.06	-3.2	0.04	0.16915
18:1 oleic	22.79	23.42	0.63	2.7	0.32	0.06553
18:2 linoleic	62.96	62.58	-0.38	-0.6	0.42	0.38081
18:3 linolenic	0.85	0.93	0.08	9.8	0.03	0.01901
Miscellaneous						
Calcium	0.003340	0.002912	-0.000428	-12.8	0.000113	0.00110
Phosphorus	0.3479	0.3360	-0.0120	-3.4	0.0096	0.22631
Phytic Acid	0.8351	0.9689	0.1338	16.0	0.0350	0.00108

Table V (Continued).

Analyte	818 Mean	802 Mean	Mean Diff.	% Diff.	SE Diff.	p-Value*
Proximates						
ash	1.54	1.60	0.06	3.9	0.06	0.30826
calories	408.5	409.2	0.7	0.2	0.7	0.33685
carbohydrates	82.71	82.44	-0.28	-0.3	0.27	0.31046
crude fiber	2.39	2.40	0.01	0.5	0.10	0.90745
moisture	12.00	12.58	0.58	4.9	0.41	0.16239
protein	12.83	12.91	0.08	0.6	0.22	0.70940
total fat	2.92	3.05	0.13	4.6	0.14	0.36084
Sugars and Starch						
fructose	0.27	0.31	0.03	11.0	0.03	0.32928
glucose	0.41	0.44	0.03	7.6	0.04	0.49560
sucrose	0.93	0.97	0.04	4.5	0.06	0.49661
starch (enzymatic)	66.86	70.49	3.63	5.4	0.87	0.00049
Tocopherols						
alpha	10.87	9.74	-1.13	-10.4	0.39	0.00836
beta	7.47	8.10	0.64	8.5	0.25	0.01823
gamma	21.64	23.77	2.13	9.9	1.02	0.05000

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* The observed significance level from a test of mean difference equal to zero. Values of $p<0.05$ indicate statistical significance at the 5% level.

Table VI.

Statistical Comparison of Line 805 to Control Line 818.†

Analyte	818 Mean	805 Mean	Mean Diff.	% Diff.	SE Diff.	p-Value*
Amino Acids						
alanine	7.80	7.90	0.10	1.3	0.14	0.46388
arginine	4.49	4.39	-0.09	-2.1	0.12	0.43471
aspartic acid	6.60	6.36	-0.25	-3.7	0.26	0.35447
cystine	1.91	2.00	0.09	4.9	0.04	0.03100
glutamic acid	21.10	20.98	-0.13	-0.6	0.45	0.77586
glycine	3.65	3.38	-0.27	-7.3	0.10	0.01241
histidine	2.90	2.76	-0.14	-4.7	0.06	0.01951
isoleucine	3.82	3.67	-0.14	-3.8	0.13	0.28260
leucine	14.54	14.34	-0.20	-1.4	0.31	0.52628
lysine	2.76	2.58	-0.19	-6.7	0.09	0.03976
methionine	1.65	1.76	0.11	6.6	0.04	0.02268
phenylalanine	5.42	5.44	0.02	0.3	0.10	0.86950
proline	9.55	9.50	-0.05	-0.5	0.18	0.80538
serine	5.17	5.22	0.05	1.0	0.10	0.60526
threonine	3.79	3.37	-0.43	-11.3	0.09	0.00011
tryptophan	0.56	0.59	0.03	6.2	0.02	0.14436
tyrosine	3.95	4.09	0.13	3.3	0.09	0.17234
valine	4.58	4.39	-0.19	-4.2	0.14	0.19425
Fatty Acids						
16:0 palmitic	10.48	10.47	-0.02	-0.2	0.10	0.87368
18:0 stearic	1.84	1.90	0.06	3.2	0.04	0.16915
18:1 oleic	22.79	23.15	0.36	1.6	0.32	0.27719
18:2 linoleic	62.96	62.42	-0.54	-0.9	0.42	0.21024
18:3 linolenic	0.85	0.85	0.00	0.0	0.03	1.00000
Miscellaneous						
Calcium	0.003340	0.003527	0.000186	5.6	0.000113	0.11353
Phosphorus	0.3479	0.3476	-0.0003	-0.1	0.0096	0.97337
Phytic Acid	0.8351	0.9167	0.0816	9.8	0.0350	0.03058

Table VI (Continued).

Analyte	818 Mean	805 Mean	Mean Diff.	% Diff.	SE Diff.	p-Value*
Proximates						
ash	1.54	1.61	0.07	4.7	0.06	0.21959
calories	408.5	410.4	2.0	0.5	0.7	0.01109
carbohydrates	82.71	81.71	-1.00	-1.2	0.27	0.00096
crude fiber	2.39	2.30	-0.09	-3.6	0.10	0.42342
moisture	12.00	13.22	1.22	10.1	0.41	0.00600
protein	12.83	13.35	0.52	4.1	0.22	0.02626
total fat	2.92	3.34	0.43	14.6	0.14	0.00656
Sugars and Starch						
fructose	0.27	0.25	-0.03	-9.3	0.03	0.40990
glucose	0.41	0.56	0.15	36.6	0.04	0.00327
sucrose	0.93	1.09	0.16	17.7	0.06	0.01317
starch (enzymatic)	66.86	66.06	-0.80	-1.2	0.87	0.37323
Tocopherols						
alpha	10.87	10.52	-0.35	-3.2	0.39	0.38157
beta	7.47	6.26	-1.21	-16.1	0.25	0.00009
gamma	21.64	17.76	-3.88	-17.9	1.02	0.00115

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* The observed significance level from a test of mean difference equal to zero. Values of $p<0.05$ indicate statistical significance at the 5% level.

Table VII.

Statistical Comparison of Line 809 to Control Line 818.†

Analyte	818 Mean	809 Mean	Mean Diff.	% Diff.	SE Diff.	p-Value*
Amino Acids						
alanine	7.80	8.03	0.23	3.0	0.14	0.09818
arginine	4.49	4.45	-0.03	-0.7	0.12	0.78360
aspartic acid	6.60	6.52	-0.08	-1.3	0.26	0.75374
cystine	1.91	2.04	0.13	7.0	0.04	0.00303
glutamic acid	21.10	21.22	0.11	0.5	0.45	0.80303
glycine	3.65	3.53	-0.12	-3.4	0.10	0.22636
histidine	2.90	2.89	-0.01	-0.3	0.06	0.88184
isoleucine	3.82	3.82	0.00	0.1	0.13	0.97632
leucine	14.54	14.39	-0.15	-1.0	0.31	0.62349
lysine	2.76	2.66	-0.10	-3.7	0.09	0.24489
methionine	1.65	1.72	0.07	4.3	0.04	0.12362
phenylalanine	5.42	5.51	0.08	1.6	0.10	0.40072
proline	9.55	9.80	0.25	2.6	0.18	0.17976
serine	5.17	5.24	0.07	1.3	0.10	0.47759
threonine	3.79	3.45	-0.34	-9.0	0.09	0.00119
tryptophan	0.56	0.56	0.00	0.3	0.02	0.94263
tyrosine	3.95	3.98	0.02	0.6	0.09	0.79627
valine	4.58	4.64	0.06	1.3	0.14	0.69226
Fatty Acids						
16:0 palmitic	10.48	10.60	0.12	1.1	0.10	0.27297
18:0 stearic	1.84	1.88	0.04	2.3	0.04	0.32041
18:1 oleic	22.79	22.92	0.13	0.5	0.32	0.70089
18:2 linoleic	62.96	62.57	-0.39	-0.6	0.42	0.36043
18:3 linolenic	0.85	0.98	0.13	15.7	0.03	0.00058
Miscellaneous						
Calcium	0.003340	0.002993	-0.000347	-10.4	0.000113	0.00581
Phosphorus	0.3479	0.3335	-0.0144	-4.1	0.0096	0.14840
Phytic Acid	0.8351	0.8460	0.0108	1.3	0.0350	0.76062

Table VII (Continued).

Analyte	818 Mean	809 Mean	Mean Diff.	% Diff.	SE Diff.	p-Value*
Proximates						
ash	1.54	1.50	-0.04	-2.8	0.06	0.45733
calories	408.5	407.0	-1.4	-0.4	0.7	0.05230
carbohydrates	82.71	82.82	0.11	0.1	0.27	0.67871
crude fiber	2.39	2.27	-0.13	-5.2	0.10	0.24436
moisture	12.00	13.15	1.15	9.6	0.41	0.00888
protein	12.83	13.07	0.24	1.9	0.22	0.28506
total fat	2.92	2.61	-0.31	-10.6	0.14	0.04100
Sugars and Starch						
fructose	0.27	0.25	-0.03	-9.3	0.03	0.40590
glucose	0.41	0.36	-0.04	-10.6	0.04	0.34463
sucrose	0.93	0.86	-0.07	-7.3	0.06	0.27907
starch (enzymatic)	66.86	66.81	-0.05	-0.1	0.87	0.95858
Tocopherols						
alpha	10.87	9.53	-1.33	-12.3	0.39	0.00247
beta	7.47	6.35	-1.12	-15.0	0.25	0.00020
gamma	21.64	14.80	-6.84	-31.6	1.02	0.00000

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* The observed significance level from a test of mean difference equal to zero. Values of $p<0.05$ indicate statistical significance at the 5% level.

Table VIII.

Statistical Comparison of Line 810 to Control Line 818.†

Analyte	818 Mean	810 Mean	Mean Diff.	% Diff.	SE Diff.	p-Value*
Amino Acids						
alanine	7.80	8.18	0.38	4.9	0.14	0.01015
arginine	4.49	4.50	0.02	0.3	0.12	0.89988
aspartic acid	6.60	7.10	0.50	7.5	0.26	0.06921
cystine	1.91	2.03	0.12	6.3	0.04	0.00697
glutamic acid	21.10	21.94	0.83	4.0	0.45	0.07262
glycine	3.65	3.70	0.06	1.5	0.10	0.58268
histidine	2.90	3.08	0.18	6.2	0.06	0.00312
isoleucine	3.82	3.69	-0.13	-3.3	0.13	0.35058
leucine	14.54	15.03	0.50	3.4	0.31	0.11865
lysine	2.76	2.81	0.04	1.6	0.09	0.60760
methionine	1.65	1.72	0.08	4.7	0.04	0.09930
phenylalanine	5.42	5.64	0.22	4.1	0.10	0.03319
proline	9.55	9.94	0.40	4.2	0.18	0.03701
serine	5.17	5.54	0.37	7.2	0.10	0.00073
threonine	3.79	3.94	0.14	3.7	0.09	0.14098
tryptophan	0.56	0.61	0.05	9.4	0.02	0.03106
tyrosine	3.95	4.36	0.40	10.1	0.09	0.00025
valine	4.58	4.46	-0.13	-2.8	0.14	0.38849
Fatty Acids						
16:0 palmitic	10.48	10.53	0.05	0.5	0.10	0.63426
18:0 stearic	1.84	1.87	0.02	1.4	0.04	0.54785
18:1 oleic	22.79	23.18	0.39	1.7	0.32	0.23628
18:2 linoleic	62.96	62.55	-0.41	-0.6	0.42	0.34079
18:3 linolenic	0.85	0.78	-0.07	-7.8	0.03	0.05464
Miscellaneous						
Calcium	0.003340	0.003622	0.000282	8.4	0.000113	0.02101
Phosphorus	0.3479	0.3578	0.0098	2.8	0.0096	0.31718
Phytic Acid	0.8351	0.8599	0.0247	3.0	0.0350	0.48849

Table VIII (Continued).

Analyte	818 Mean	810 Mean	Mean Diff.	% Diff.	SE Diff.	p-Value*
Proximates						
ash	1.54	1.56	0.02	1.2	0.06	0.75275
calories	408.5	408.4	-0.0	-0.0	0.7	0.97901
carbohydrates	82.71	82.35	-0.36	-0.4	0.27	0.18898
crude fiber	2.39	2.62	0.23	9.7	0.10	0.03783
moisture	12.00	12.35	0.35	2.9	0.41	0.39593
protein	12.83	13.14	0.31	2.4	0.22	0.16838
total fat	2.92	2.95	0.03	1.0	0.14	0.83651
Sugars and Starch						
fructose	0.27	0.32	0.05	17.6	0.03	0.12603
glucose	0.41	0.44	0.03	7.3	0.04	0.51475
sucrose	0.93	0.93	0.00	0.2	0.06	0.97781
starch (enzymatic)	66.86	67.62	0.76	1.1	0.87	0.39261
Tocopherols						
alpha	10.87	10.35	-0.52	-4.8	0.39	0.19448
beta	7.47	8.54	1.07	14.4	0.25	0.00031
gamma	21.64	20.23	-1.41	-6.5	1.02	0.18384

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* The observed significance level from a test of mean difference equal to zero. Values of $p<0.05$ indicate statistical significance at the 5% level.

Study 94-01-50-18

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