Food and Feed Safety and Nutritional Assessment of Roundup Ready® Flex Cotton MON 88913, (OECD Unique Identifier MON-88913-8)

Conclusion Based on Data and Information Evaluated According to FDA's Policy on Foods from New Plant Varieties

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Certification

Monsanto Company is submitting this food and feed safety and nutritional assessment in compliance with the FDA's 1992 policy statement regarding foods derived from new plant varieties (57 FR 22984). At the Agency's request, and where appropriate, this submission also complies with the recommendations contained in the proposed rule for Premarket Biotechnology Notice (PBN) Concerning Bioengineered Foods (66 FR 4706).

Specifically, as recommended in the proposed 21 C.F.R. §192.25(a), the undersigned attests to the following:

1. It is the view of Monsanto Company (hereafter referred to as Monsanto) that: (i) Roundup Ready® Flex cotton MON 88913 is as safe as commercially available conventional varieties of cotton; and (ii) the intended uses of the food and feed derived from Roundup Ready Flex cotton MON 88913 are in compliance with all applicable requirements of the Federal Food, Drug and Cosmetic Act.
2. Monsanto will make available to the FDA, upon request, relevant data or other information not included in this submission, either during the course of FDA's evaluation of the submission, or for cause.
3. Monsanto will make relevant data or other information not included in this submission available to the FDA either: (i) by allowing FDA to review and copy these data or information at Monsanto's offices in St. Louis, MO, during customary business hours; or (ii) by sending a copy of these data or information to FDA.
4. Monsanto makes no claim of confidentiality regarding either the existence of this submission, or any of the data or other information contained herein. However, Monsanto reserves the right to make a claim of confidentiality regarding any relevant data or other information not included in this submission, but requested by FDA, either in the course of its review of this submission, or for cause. Any such claim of confidentiality will be made at the time such data or information is provided, along with an explanation for the basis of the claim.
5. To the best of Monsanto's knowledge, this submission is representative and balanced, including information, unfavorable as well as favorable, pertinent to the evaluation of the safety, nutritional, or other regulatory issues that may be associated with Roundup Ready Flex cotton MON 88913.

Signature:

Date:

[redacted]
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Release of Information

Monsanto is submitting the information in this assessment for review by the FDA as part of the regulatory process. By submitting this information, Monsanto does not authorize its release to any third party except to the extent it is requested under the Freedom of Information Act (FOIA), 5 U.S.C., § 552; FDA complies with the provisions of FOIA and FDA's implementation regulations (21 CFR Part 20); and this information is responsive to the specific request. Except in accordance with the Freedom of Information Act, Monsanto does not authorize the release, publication or other distribution of this information (including website posting) without Monsanto's prior notice and consent.

Abbreviations and Definitions[1]

~	Approximately
AA	Amino acid
aad	Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase, from the transposon Tn7
ADF	Acid detergent fiber
ALLERGEN 3	Allergen and gliadin protein sequence database, compiled by Monsanto Company
ALLPEPTIDES	Protein sequence database comprised of GenPept, PIR and Swiss Prot, as curated by Monsanto Company
ANOVA	Analysis of variance
APS	Analytical protein standard
Avg	Average
B	Border region
BSA	Bovine serum albumin
C	Celsius
CAPS	3-[cyclohexylamino]-1-propanesulfonic acid
CFR	Code of Federal Regulations
CI	Confidence interval
CP4	*Agrobacterium sp.* strain CP4
CP4-EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium sp.* strain CP4
cp4 epsps	Coding sequence for the CP4 EPSPS protein from *Agrobacterium sp.* strain CP4 present in plasmid PV-GHGT35
CR	Coding region
CTAB	Cetyltrimethylammonium bromide
CTP	Chloroplast transit peptide
*ctp*2	Chloroplast transit peptide, isolated from *Arabidopsis thaliana* L. EPSPS
CV	Coefficient of variation
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
DDE	Daily dietary exposure
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol

DWCF	Dry weight conversion factor
dwt	Dry weight
ECL	Enhanced chemiluminescence
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase
FA	Fatty acid
FASTA	Algorithm used to find local high scoring alignments between a pair of protein or nucleotide sequences
FDA	United States Food and Drug Administration
FFDCA	Federal Food, Drug and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FMV	Figwort mosaic virus
fwt	Fresh weight
GenBank	A public genetic database maintained by the National Center for Biotechnology Information at the National Institutes of Health, Bethesda, MD
GLP	Good Laboratory Practice
HCl	Hydrochloric acid
HEPES	N-[2-(Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)]
HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase
I	Intron
I-ACT8	Intron and flanking exon sequence from the *act8* gene of *Arabidopsis thaliana*
IgG	Immunoglobulin G
I-TSF1	Intron from the *Arabidopsis thaliana tsf1* gene encoding elongation factor EF-1alpha
IUPAC-IUB	International Union of Pure and Applied Chemistry - International Union of Biochemistry
Kb	Kilobase pair
KCl	Potassium chloride
kDa	Kilodalton
KH_2PO_4	Potassium phosphate monobasic
L	Leader
L-ACT8	Leader sequence from the *act8* gene of *Arabidopsis thaliana*

LB	Left border region
LOQ	Limit of quantitation
LOD	Limit of detection
L-TSF1	Leader (exon 1) from the *Arabidopsis thaliana tsf1* gene encoding elongation factor EF-1alpha
mA	Milliampere
MALDI-TOF MS	Matrix assisted laser desorption ionization time of flight mass spectrometry
MES	2-[N-Morpholino]ethanesulfonic acid
$MgCl_2$	Magnesium chloride
MH+	Protonated mass ion
mM	Millimolar
MOE	Margin of exposure
MS	Mass spectrometry
MW	Molecular weight
na	Not available
$Na_2B_4O_7$	Sodium tetraborate
NaCl	Sodium chloride
Na_2CO_3	Sodium carbonate
$NaHCO_3$	Sodium bicarbonate
Na_2HPO_4	Sodium phosphate
NaOAc	Sodium acetate
NDF	Neutral detergent fiber
NFDM	Non-fat dried milk
NIST	National Institute of Standards and Technology
NOEL	No observable effect level
OD	Optical density
OECD	Organization for Economic Co-operation and Development
OR	Origin of replication
OR-ORI-PBR322	Origin of replication from pBR322 for maintenance of plasmid in *E. coli*
OR-ORI V	Origin of replication for *Agrobacterium* derived from the broad host range plasmid RK2
OSL	Overseason leaf - Leaf material collected from different time points during the growing season
P	Promoter
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline

PBST	Phosphate buffered saline containing 0.05% (v/v) Tween-20
PCR	Polymerase chain reaction
PEP	Phosphoenolpyruvate
P-FMV/TSF1	Chimeric promoter containing the *Arabidopsis thaliana tsf1* gene promoter, encoding elongation factor EF-1alpha, and enhancer sequences from the Figwort Mosaic virus 35S promoter
PMSF	Phenylmethylsulfonyl fluoride
ppm	Parts per million (µg of analyte/g of sample)
P-35S/ACT8	Chimeric promoter containing the promoter of the *act8* gene of *Arabidopsis thaliana* combined with the enhancer sequences of the Cauliflower mosaic virus (CaMV) 35S promoter
PTH	Phenylthiohydantoin
PVDF	Polyvinylidene difluoride
PVPP	Polyvinylpolypyrrolidone
RB	Right border
rbc	Ribulose-1, 5-bisphosphate carboxylase
RNA	Ribonucleic acid
Rop	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli*
RQTY	Relative quantity
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SE	Standard error
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
sp	Species
S3P	Shikimate-3-phosphate
SwissProt	A public protein database maintained by the Swiss Institute of Bioinformatics, Geneva, Switzerland, and the European Molecular Biology Laboratory at the European Bioinformatics Institute, Hinxton, England
TBA	Tris-borate buffer with L-ascorbic acid
TDF	Total dietary fiber
T-DNA	Transfer(ed) DNA
TE	Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)

T-E9	DNA sequences derived from *Pisum sativum L.*, containing the 3' nontranslated region of the pea ribulose-1,5-bisphosphate carboxylase, small subunit E9 gene
TFA	Trifluoroacetic acid
TI	Tolerance interval
Tris	Tris(hydroxymethyl)aminomethane
TS	Targeting sequence
TSSP	Tissue-specific site pool
U	Units
USDA-APHIS	United States Department of Agriculture – Animal and Plant Health Inspection Service
v/v	Volume per volume
w/v	Weight per volume

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

Narrative Summary

Assessment of Food and Feed Safety for Roundup Ready® Flex Cotton MON 88913

The first cotton varieties with biotechnology traits were commercialized in the United States in the mid-1990s. The most successful in terms of farmer adoption has been Roundup Ready cotton 1445 (hereinafter referred to as Roundup Ready cotton), which is tolerant to glyphosate, the active ingredient in Roundup® agricultural herbicides. Roundup Ready cotton has been rapidly adopted by U.S. cotton farmers (95% grower satisfaction, Monsanto unpublished survey results) and has been a significant part of U.S. annual cotton production since its market introduction. Cotton with the Roundup Ready trait is currently cultivated on approximately 59% of the U.S. cotton acres (USDA-NASS, 2003). However, a constraint within the current Roundup Ready cotton system is the limitation of in-crop, over-the-top herbicide application to Roundup Ready cotton plants with no more than four true leaves. Applications at the fifth true leaf stage and beyond require specialized spray equipment to apply the herbicide between the rows and away from the cotton plant.

Monsanto has developed a second-generation glyphosate-tolerant cotton product, Roundup Ready Flex cotton MON 88913, (hereinafter referred to as MON 88913) which provides increased tolerance to glyphosate during the critical reproductive phases of growth compared to Roundup Ready cotton. Use of MON 88913 will enable the application of a Roundup agricultural herbicide over the top of the cotton crop at later stages of development than is possible with the current Roundup Ready cotton product. This will provide more effective weed control options during crop production, because Roundup agricultural herbicides are highly effective against the majority of annual and perennial weeds that can be problematic during the later stages of crop development, with minimal risk of crop injury. The increased level of glyphosate tolerance in MON 88913 is achieved through the use of improved promoter sequences that regulate the expression of the *cp4 epsps* (5-enolpyruvylshikimate-3-phosphate synthase) coding sequence (Fincher et al., 2003). The CP4 EPSPS protein produced in MON 88913 is identical to that produced in the initial Roundup Ready cotton product.

Control of weeds in a cotton crop is essential because weeds compete with the crop for the same limited resources in the field including sunlight, water and nutrients (Ross and Lembi, 1985; Wilcut et al., 2003). Because failure to control weeds within the crop can result in decreased yields and reduced crop quality, an intensive program for weed control is essential to ensure profitability (Wilcut et al., 2003; Hayes et al., 2001). Losses from weeds in cotton result in a $300 million crop loss per year (Abernathy and McWhorter, 1992). In addition, weeds present at cotton harvest reduce the efficiency of the mechanical harvest of the crop and can reduce both the quality and value of the lint because of staining by vegetation.

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The data and information presented in this summary demonstrate that MON 88913, and the feeds and foods derived from it, are as safe and nutritious as commercial conventional varieties of cotton and the comparable feeds and foods derived from them. This is based on three categories of analysis. The first is the detailed molecular characterization of the inserted DNA and a detailed biochemical characterization of the CP4 EPSPS protein produced in MON 88913. These data support a history of safe use and experience with the EPSPS family of proteins, including the CP4 EPSPS protein in food and feed consumption. The second is a direct assessment of the toxicity and allergenicity potential of the CP4 EPSPS protein produced in MON 88913 based on information and studies that were performed on the CP4 EPSPS protein. The third is a safety and nutritional assessment that demonstrates that MON 88913 is compositionally equivalent to commercial conventional cotton.

MON 88913 was developed using the same *cp4 epsps* coding sequence and chloroplast targeting sequence and produces the same CP4 EPSPS protein as Roundup Ready cotton. The methodology used to produce MON 88913, *Agrobacterium tumefaciens*-mediated plant transformation, is comparable to the method used in the development of Roundup Ready cotton. MON 88913 utilizes the same *cp4 epsps* coding sequence in the same crop and confers the same glyphosate-tolerant phenotype as the current commercial Roundup Ready cotton product.

As determined by Southern analysis, MON 88913 contains a single, intact DNA insert from the binary plasmid PV-GHGT35 at a single integration locus within the cotton genome. The DNA insert in MON 88913 contains two intact *cp4 epsps* gene expression cassettes containing identical *cp4 epsps* coding sequences. Polymerase chain reaction was performed to confirm the 5' and 3' insert-to-genomic DNA junctions and the organization of the elements within the insert in MON 88913. No backbone sequences from the plasmid are present. The DNA insert and the Roundup Ready trait are stable across multiple generations. Phenotypic segregation data confirmed that the single insert locus and Roundup Ready trait behave as a single dominant locus, resulting in the expected Mendelian segregation pattern across multiple generations.

The protein characterization studies show that seed derived from MON 88913 contains the CP4 EPSPS protein of the expected molecular weight, amino acid sequence, immunological activity and functional activity. The CP4 EPSPS protein level in MON 88913 seed was 340 µg/g dwt, which represents less than 0.12% of the total protein in cottonseed. The CP4 EPSPS protein in MON 88913 has the same functional and enzymatic activity as naturally occurring EPSPSs and as the CP4 EPSPS in other Roundup Ready crops, including the current Roundup Ready cotton product. The CP4 EPSPS protein in MON 88913 is structurally homologous to EPSPSs naturally present in food crops (e.g., soybean and corn) and in microbial food sources such as Baker's yeast. The amino acid sequence of the CP4 EPSPS protein in MON 88913 is identical to, or greater than 99% identical to, the CP4 EPSPS protein in Roundup Ready crops, such as soybean, corn (NK603), canola, and cotton, which have already completed the FDA consultation process. All of these data and information taken together demonstrate a history of safe use with respect to the family of EPSPS proteins which naturally occur in

crops and microbially-based foods that have a long history of safe consumption by humans and animals. These data also include a history of safe experience with respect to Roundup Ready crops that have been consumed in significant amounts, either directly or as processed products, by humans and animals since their initial commercialization in 1996.

Information and data from studies support the safety of the CP4 EPSPS protein and demonstrate that it is unlikely to be an allergen or toxin. This is based on:

1. the source of the *cp4 epsps* coding sequence, a soil bacterium, which is not a known human or animal pathogen and for which there are no reports of allergies;
2. rapid digestion of the CP4 EPSPS protein;
3. low levels of the CP4 EPSPS protein present in MON 88913 seed, representing less than 0.12% of the total protein on a dry weight basis;
4. lack of significant structural similarities of the CP4 EPSPS protein to known allergens, or pharmacologically active proteins known to cause adverse health effects, based on bioinformatic searches of amino acid sequence databases;
5. no acute toxicity, based on a mouse gavage study; and
6. lack of any documented reports of allergy or adverse effects from the consumption of food products derived from other Roundup Ready crops that have been in the food supply since 1996.

Furthermore, there is no significant human consumption of cotton, since the only significant sources of food from cotton is oil and linters, neither of which contains significant protein.

The composition and nutritional assessment compared MON 88913 to MON 88913(-), a negative segregant of MON 88913 that contains similar background genetics but does not contain the DNA insert, as well as to 16 commercial conventional cotton varieties, using cottonseed collected from replicated field trials. The results of these compositional analyses show that, for the 53 components statistically evaluated, there were no statistically significant differences ($p \leq 0.05$) in 236 of the 265 comparisons made between MON 88913 and MON 88913(-). Of the 29 statistically significant differences, all MON 88913 values fell within the population of commercial conventional cottonseed as described by the 99% tolerance interval and/or within published ranges for conventional cottonseed. Additionally, a cottonseed oil and meal composition assessment is provided. Cottonseed oil derived from MON 88913 was compared to oil from MON 88913(-) and six commercial conventional cotton varieties. There were no statistically significant differences for 11 of 13 comparisons for cottonseed oil. Of the two statistically significant differences, the range of values for those components were found to all fall within the 99% tolerance interval for the commercial conventional varieties grown alongside MON 88913 and MON 88913(-) or produced separately and then processed alongside MON 88913 and MON 88913(-) cotton. There were no statistically significant differences for 40 of 41 comparisons for cottonseed meal, and the range of values for this one statistically significant component fell within the 99% tolerance interval for commercial conventional cotton varieties. These data support the conclusion that cottonseed, cottonseed oil, and meal derived from MON 88913 are compositionally

equivalent to cottonseed, cottonseed oil and meal derived from cotton grown commercially today.

In conclusion, we have determined, based on the information provided in this summary, that MON 88913, and the foods and feeds derived from MON 88913, are as safe and nutritious as conventional cotton and the comparable foods and feeds derived from them. Sales and consumption of MON 88913 cottonseed and the food and feed derived from it would be fully consistent with FDA's Food Policy and be in compliance with all applicable requirements of the Federal Food, Drug and Cosmetic Act.

PART II: SYNOPSIS OF CONSULTATION SUMMARY

Section 1. Name and Address of the Submitter

The submitter of this safety and nutritional assessment summary for Roundup Ready Flex cotton MON 88913 is:

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

Communications with regard to this submission should be directed to [redacted], Regulatory Affairs Manager, at the Monsanto address. He can also be contacted by [redacted].

Section 2. The Subject of this Summary and the Plant Species from which it was Derived

The subject of this summary is Roundup Ready Flex cotton MON 88913 derived from the cotton variety Coker 312.

Section 3. Distinctive Designations Given to the Subject of this Summary

The Roundup Ready Flex cotton that is subject of this summary is given a designation MON 88913. In subsequent discussions in this submission Roundup Ready Flex cotton will be referred to as "MON 88913". In accordance with OECD's "Guidance for the Designation of a Unique Identifier for Transgenic Plants", MON 88913 has been assigned the unique identifier MON-88913-8.

Section 4. Identity and Sources of the Genetic Material Introduced into Cotton to Produce MON 88913

Agrobacterium-mediated transformation, utilizing plasmid vector PV-GHGT35 (Figures IV-1a and IV-1b) was used to generate MON 88913. The genetic elements present in PV-GHGT35 are listed in Table IV-1. Molecular analysis, described in Part IV, demonstrated that MON 88913 contains a single copy of the DNA insert that is approximately 8.1 kb in length, and is inserted at a single locus. This insert contains two intact *cp4 epsps* gene expression cassettes containing identical *cp4 epsps* coding sequences. There are no detectable plasmid backbone sequences, and no additional elements, linked or unlinked to intact cassettes, from the transformation vector PV-GHGT35.

The T-DNA is comprised of two *cp4 epsps* gene expression cassettes. The first *ctp2/cp4 epsps* coding sequence is directed by the P-FMV/TSF1 chimeric promoter, the leader (exon 1) and intron sequences from the *Arabidopsis thaliana tsf1* gene, and the transcriptional termination and polyadenylation sequence derived from the 3' nontranslated region of the pea (*P. sativum*) ribulose-1, 5-bisphosphate carboxylase small subunit (*rbc*) E9 gene. The second *ctp2/cp4 epsps* coding sequence, identical to the first, is directed by the P-35S/ACT8 promoter, the leader, intron and flanking sequences from the *act8* gene of *Arabidopsis thaliana* and the transcriptional termination and polyadenylation sequence derived from the 3' nontranslated region of the pea (*P. sativum*) ribulose-1,5-bisphosphate carboxylase small subunit *(rbc)* E9 gene.

Section 5. The Intended Technical Effect of MON 88913

MON 88913 produces a CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium sp.* strain CP4) that provides tolerance to the action of Roundup agricultural herbicides.

The CP4 EPSPS protein is structurally and functionally similar to native plant EPSPS enzymes, but has a much reduced affinity for glyphosate (Padgette et al., 1996). Typically, glyphosate binds to the plant EPSPS enzyme and blocks the biosynthesis of aromatic amino acids, thereby depriving plants of these essential components (Steinrücken and Amrheim, 1980; Haslam, 1993). In Roundup Ready plants producing the CP4 EPSPS protein, requirements for growth and development are met by the continued action of the CP4 EPSPS enzyme in the presence of glyphosate. MON 88913 produces the CP4 EPSPS protein, and is therefore tolerant to Roundup agricultural herbicides applied over the top of cotton during the growing season.

Section 6. The Applications and Uses of MON 88913

Monsanto has developed a second-generation glyphosate-tolerant cotton product, MON 88913, that provides increased tolerance to glyphosate compared to the current product, Roundup Ready cotton, through the use of recombinant DNA techniques. Use of MON 88913 will enable the application of a Roundup agricultural herbicide over the top of the cotton crop at later stages of development than is possible with Roundup Ready cotton. Therefore, MON 88913 will offer growers an expanded window for application of Roundup agricultural herbicides and enhanced flexibility in weed control options relative to the current Roundup Ready cotton product.

The introduction of MON 88913 is expected to continue to provide the grower with economic and environmental benefits and superior weed control benefits compared to those currently provided by Roundup Ready cotton. These benefits include:

1. *Effective weed control:* The Roundup Ready cotton system provides growers with effective weed control and equivalent yields while reducing the number of herbicide applications required (Culpepper and York, 1998, 2000; Gianessi et al.,

2002). Growers experience improved flexibility in weed control compared to herbicide programs used in conventional cotton, as specific preemergent herbicides that are used for prevention are replaced by a broad-spectrum post-emergent herbicide that can be used on an as-needed basis (Welch et al., 1997; Culpepper and York, 1998).

2. *Convenience and simplicity:* The Roundup Ready cotton system increases farming convenience and production simplicity (Culpepper and York, 1998; McCloskey, 1998), which was a major driver for the adoption of Roundup Ready cotton (Kalaitzandonakes and Suntornpithug, 2001). Additionally, the Roundup Ready cotton system offers crop rotation options over other weed control systems, is an easier system to manage, and more acreage can be covered by the same equipment (Kalaitzandonakes and Suntornpithug, 2001; Culpepper and York, 1998; McCloskey, 1998). Less labor is often required because of the elimination of hand weeding and the high cost of early, postdirected sprays that require specialized equipment (McCloskey, 1998).

3. *Increased grower income:* Use of the Roundup Ready cotton system has resulted in reduced production costs, net economic advantage, and reduced production risks (Gianessi et al., 2002; Kalaitzandonakes and Suntornpithug, 2001). In 2001, herbicide-tolerant cotton increased the total net value of U.S. cotton production by $133 million dollars (Gianessi et al., 2002).

4. *Increased adoption of reduced tillage practices:* Use of the Roundup Ready cotton system encourages adoption of reduced tillage practices by growers (Gianessi et al., 2002; Kalaitzandonakes and Suntornpithug, 2001). It is estimated that reduced tillage is practiced on one out of every two new acres of Roundup Ready cotton (Kalaitzandonakes and Suntornpithug, 2001). The use of conservation tillage practices reduces water runoff by 30% compared to conventional tillage practices, thereby improving the quality of surface water (Baker and Johnson, 1979). Additionally, conservation tillage improves water quality and creates habitat for wildlife (CTIC, 1999; Fawcett and Towry, 2002). Use of the Roundup Ready cotton system significantly improves overall weed control in conservation tillage cotton (Keeling et al., 1998).

5. *Compatibility with Integrated Pest Management (IPM) and soil conservation techniques:* Roundup Ready cotton is highly compatible with integrated pest management and soil conservation techniques (Keeling et al., 1998; Patterson et al., 1998; Smart and Bradford, 1999), resulting in a number of important environmental benefits including reduced soil erosion and improved water quality (Baker and Laflen, 1979; Hebblethewaite, 1995; CTIC, 1998), improved soil structure with higher organic matter (Kay, 1995), improved wildlife habitat (Phatak et al., 1999), improved carbon sequestration (Reicosky, 1995; Reicosky and Lindstrom, 1995), and reduced CO_2 emissions (Kern and Johnson, 1993).

6. *History of safe use:* The U.S. EPA (1993) concluded that the use of Roundup agricultural herbicides does not pose unreasonable risks to humans, birds, mammals, aquatic organisms, bees and invertebrates. Glyphosate, the active ingredient in Roundup agricultural herbicides, has favorable environmental characteristics compared to some other herbicides (Nelson and Bullock, 2003).

Section 7. Applications for which MON 88913 is not Suitable

Monsanto Company is aware of no food or feed uses of conventional cotton that are not applicable to MON 88913.

PART III: STATUS OF SUBMISSIONS TO OTHER REGULATORY AGENCIES

Section 1. Status of Submission to USDA-APHIS

Monsanto requested a Determination of Nonregulated Status for MON 88913, including all progenies derived from crosses between MON 88913 and other cotton varieties, from USDA-APHIS on March 25, 2004. Under regulations administered by USDA-APHIS (7 CFR 340), MON 88913 is currently considered a "regulated article". Monsanto will continue to conduct all field tests for MON 88913 in strict compliance with USDA field regulations until a Determination of Nonregulated Status is obtained for MON 88913.

Section 2. Status of Submission to EPA

The United States Environmental Protection Agency has authority over the use of pesticidal substances under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), as amended (7 U.S.C. § 136 *et seq.*). A submission of glyphosate residue data and proposed labeling for the expanded use of Roundup UltraMAX® herbicide (EPA Reg. No. 524-537) over Roundup Ready Flex cotton, MON 88913, was made to the EPA on March 27, 2003.

Pursuant to section 408(d) of the Federal Food Drug and Cosmetic Act (FFDCA), 21 U.S.C. 346 a(d), the EPA has previously reviewed and established an exemption from the requirement for a tolerance for the CP4 EPSPS protein and the genetic material necessary for the production of this protein in or on all raw agricultural commodities (40 CFR § 180.1174).

Section 3. Status of Submissions to Foreign Governments

Regulatory submissions will be made to countries that import food and feed products derived from U.S. cotton and have regulatory approval processes in place. These will include submissions to a number of foreign governmental regulatory agencies including Japan's Ministry of Health, Labor and Welfare (MHLW), Ministry of Agriculture, Forestry and Fisheries (MAFF), as well as Canadian Food Inspection Agency (CFIA) and Health Canada. As appropriate, notifications will be made to countries that do not have a formal approval process.

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PART IV: DEVELOPMENT OF ROUNDUP READY FLEX COTTON MON 88913

Section 1. History and Biology of Cotton

Cotton is the leading plant fiber crop produced in the world and the most important in the U.S. Cotton has been extensively characterized and has a long history of agricultural production (Supak et al., 1992; USDA, 2001; USDA-ERS, 2003; USDA, 2003a). A short review of the biology and use of cotton in the United States is available from USDA-APHIS at http://www.aphis.usda.gov/brs/. The USDA estimated that cotton was planted on 13.4 million acres in the United States in 2003, and that 18.2 million bales were produced (National Cotton Council, 2004).

In the U.S., cotton has a long history of agricultural production (USDA, 2001; USDA-ERS, 2003; USDA, 2003a). Cotton production in the U.S. is located primarily in a region including 17 southern states across the cottonbelt, which extends across the southern and western U.S. from Virginia south and west to California. Cultivated cotton is noted for its general adaptability and high productivity. Cotton fiber is used for cordage and other nonwoven products, as well as for textiles. In addition, cotton linters, which are the short fibers removed from seeds prior to crushing, are a major source of industrial cellulose.

Food and feed uses of cotton are limited due to the natural toxicants present in cottonseed: gossypol and cyclopropenoid fatty acids. Thus, only highly refined products are used for human consumption (refined oil and linters). Whole cottonseed, cottonseed meal, and processing by-products (hulls and gin trash) are fed primarily to ruminants, which can tolerate gossypol. Cottonseed meal is also fed to non-ruminant farm animals in limited quantities.

Refined cottonseed oil is a premium-quality oil used for a variety of food uses, including frying oil, salad and cooking oil, mayonnaise, salad dressing, shortening, margarine, and packing oil. The short fibers on the cottonseed, or linters, consist primarily of cellulose. The linters, after extensive processing, are used in a wide variety of food and industrial products (NCPA, 2002a). Linter fiber is used to improve the viscosity of food dressings. Viscose, a linters product, is utilized in bologna and sausage casings. Thus, both refined cottonseed oil, and to a lesser extent, processed cotton linters, are routinely used for human food products and have a history of safe use that is well documented (NCPA, 1999).

Whole cottonseed, cottonseed meal, crude cottonseed oil, hulls and gin trash are used in animal feeds for cattle, sheep, goats, horses, poultry, swine, fish and shrimp (NCPA, 1999). Meal is typically sold as a 38 - 44% protein product, or a 35% protein cottonseed cake. Cottonseed meal can be used alone, or mixed with other feed materials. Cottonseed hulls are separated from the meal cake in preparation for the oil extraction process, are use as fiber to aid in cattle digestion, and are nutritionally comparable to good quality grass hay. Nearly all of the cottonseed produced in the U.S. is used for feed

in various forms. Approximately 50-60% of the cottonseed is processed into oil, meal and hulls. The remaining cottonseed is fed to cattle as whole cottonseed (USDA-NASS, 2002; National Cotton Council, 2004).

1.1. Scientific Name and Taxonomic Classification of Cotton

Cotton belongs to the genus *Gossypium* of the tribe Gossypieae of the family Malvaceae of the order Malvales (Fryxell, 1979; Munro, 1987). Some authors have grouped species differently, and *Gossypium* has been included in the tribe Hibisceae (Smith, 1977). The genus *Gossypium* is currently comprised of 49 species that are widely distributed and occur predominately in tropical and subtropical regions around the world (Percival et al., 1999). The taxonomic status of a number of noncultivated species, especially in Africa and the Middle East, is still under development. Several primary centers of diversity have developed, and the greatest species diversity occurs in northwestern Australia, North Eastern Africa, and the Arabian Peninsula, and the western and northern part of Mexico (Percival et al., 1999).

Worldwide, four *Gossypium* species are collectively known as cotton and are grown commercially. These include two diploid species (2n=2x=26) *G. arboreum* L. and *G. herbaceum* L., which evolved in Africa and the Middle East, and two allotetraploid species (2n=4X=52) *G. barbadense* L. and *G. hirsutum* L., which evolved in the Americas (reviewed in Brubaker et al., 1999; Percival et al., 1999; Supak et al., 1992).

There are four species of *Gossypium* in the U.S. Two of them, *G. hirsutum* (upland cotton) and *G. barbadense* (pima), are introduced species and are grown commercially. The two noncommercial species native to the U.S. are *G. thurberi* Todaro and *G. tomentosum* Nuttall ex Seeman (Brown and Ware, 1958; Fryxell, 1979; Munro, 1987).

Gossypium thurberi Todaro (*Thurberia thespesiodes* Gray) is found in the mountainous regions of southern Arizona in the counties of Graham, Gila, Pinal, Maricopa, Cochise, Santa Cruz and Pima, and also in the Bradshaw Mountains of Yavapai County (Fryxell, 1979). *G. thurberi* is generally found at elevations of 2,500 to 5,000 feet and is isolated from areas of cotton production.

G. tomentosum is a tetraploid and is found on Hawaii (Degener, 1946). The local range is on the larger islands as well as on Nihau and Kahoolawe. It grows on arid, rocky, or clay plains not far from the sea. Thus, on the larger islands, it is found chiefly on the dry, leeward side. On Oahu it is common near Koko Crater, and grows scattered between Honolulu and Markus Balley. On Molokai it is common on the southwestern end; elsewhere it is rare except near Kamalo. On Maui the species may be found in one of the valleys south of Wailuku.

1.2. History of Cotton Development

As discussed above, cotton belongs to the genus *Gossypium* of the tribe Gossypieae of the family Malvaceae of the order Malvales and the genus *Gossypium* is currently comprised of 49 species that are widely distributed and occur predominately in tropical

and subtropical regions around the world. Several primary centers of diversity have developed, and the greatest species diversity occurs in northwestern Australia, North Eastern Africa, and the Arabian Peninsula, and the western and northern part of Mexico (Percival et al., 1999).

Worldwide, cotton taxonomy still remains to be fully elucidated; however, the phylogeny of the two commercial species in the U.S. is well established. Because of the purposeful selection and transport of *Gossypium* species by humans over thousands of years in order to develop a high-quality and high-yielding marketable plant, "its morphology, genetic composition, and indigenous ranges have been altered significantly by human activity," basically transforming perennial shrubs or trees into a compact annual row crop producing a high-quality white fiber (Brubaker et al., 1999).

Improved modern varieties of *G. hirsutum* and *G. barbadense* are currently cultivated in the southern U.S., with *G. barbadense* grown primarily in the western states of Arizona, California, New Mexico, and Texas, and *G. hirsutum* produced throughout the 17 states comprising the U.S. cottonbelt. *G. hirsutum* comprises the vast majority of U.S. cotton production, 13.7 million acres, compared to *G. barbadense* varieties, which were cultivated on less than 250,000 acres in 2002.

Phylogenetic classifications of the *Gossypium* genus have expanded in the last decade. There are three major lineages of the diploid *Gossypium* species: Australian (C, G, K genomes), the American continents (D genome), and Africa/Middle East (A, B, E, F genomes) (Percival et al., 1999). The tetraploid species (2n=4x=52) including *G. hirsutum*, *G. barbadense* and *G. tomentosum* (in Hawaii) are comprised of the A and D nuclear genomes (AADD) and contain only the A chloroplast genome, indicating the seed parent of the original hybridization was of African or Middle Eastern descent (Percival et al., 1999). Diploid species, AA, BB, *etc.* (2n=2x=26), are distributed among tropical and subtropical regions worldwide. As mentioned above, two of the diploid species, *G. herbaceum* and *G. arboreum*, are of regional agronomic importance outside of the U.S.

Among cultivated cotton (*G. arboreum, G. herbaceum, G. hirsutum* and *G. barbadense*), introgression within ploidy/genome type is historically common because of expansion of the natural range through human intervention and cultivation. Interspecific exchange of genes is responsible for some of the genetic diversity found within each cultivated species (Brubaker et al., 1999).

1.3. Characteristics of the Recipient Plant

The cotton variety used as the recipient for the DNA insert in MON 88913 was Coker 312. Coker 312 is an older commercial variety of upland cotton (*G. hirsutum*) and is the same recipient variety used for development of the current commercial Roundup Ready cotton product.

In developing the data in support of this Food and Feed Safety and Nutritional Assessment, appropriate test and control materials were developed and, where feasible, use of reference cotton materials were used to establish a range of expected responses for commercial conventional cotton in the U.S. Cotton, unlike hybrid crops, is a varietal crop in the U.S., and exhibits a significant amount of seed-to-seed genetic variability within a given variety. This variability is a natural genetic resource effectively utilized by cotton breeders. Thus, the production of positive inbreds (test) and negative inbreds or true isolines (control) commonly utilized for hybrid crops, are not necessarily feasible for cotton. In this regard, taking advantage of conventional genetics, negative segregants derived from the genotype-positive MON 88913 were developed as appropriate controls [MON 88913(-)] for product characterization studies. MON 88913(-) plants used for the characterization data in this assessment were selected at the R2 stage where they were segregating for the DNA insert. MON 88913 was first identified at the R0 stage in the growth chamber and greenhouse by antibody strip tests that identify the presence of the CP4 EPSPS protein. These results were confirmed by PCR analysis specifically designed to detect the DNA insert in MON 88913. MON 88913(-) plants were identified at the R2 stage by negative results in the antibody strip test and by PCR analysis specifically designed to detect the DNA insert in MON 88913. The genetic background of MON 88913(-) is expected to be very close, but not 100% identical, to that of MON 88913. Therefore, MON 88913(-) was considered a more appropriate negative control material than the generic conventional cottonseed of the recipient variety (Coker 312).

Section 2. Characterization of the Vector Used in Transformation

MON 88913 was developed through *Agrobacterium*-mediated transformation of cotton hypocotyl tissue using the double-border, binary vector PV-GHGT35 shown in Figures IV-1a, IV-1b. This vector contains two joined *cp4 epsps* gene expression cassettes delineated by left and right border regions. This T-DNA of approximately 8.1 kb contains two tandem *cp4 epsps* gene expression cassettes that were transferred into the cotton genome by *Agrobacterium tumefaciens* during the *in vitro* transformation process. From the right border region, the first *cp4 epsps* coding sequence is under the regulation of a chimeric transcriptional promoter P-FMV/TSF1, L-TSF1 leader and intron sequences, a chloroplast transit peptide (TS-*ctp2*) sequence and a T-E9 polyadenylation sequence. The second *cp4 epsps* coding sequence is regulated by a P-35S/ACT8 chimeric transcriptional promoter, L-Act8 leader and intron sequences, and the same chloroplast targeting and polyadenylation sequences as utilized in the first *cp4 epsps* gene expression cassette. The *cp4 epsps* coding sequence used to produce MON 88913 is the same as that in the current Roundup Ready cotton product. A description of the genetic elements in vector PV-GHGT35 is provided in Table IV-1.

2.1. The *cp4 epsps* Coding Sequence and the CP4 EPSPS Protein

The *cp4 epsps* gene from *Agrobacterium sp.* strain CP4, a common soil-borne bacterium, has been sequenced and shown to encode a 47.6 kDa EPSPS protein consisting of a

single polypeptide of 455 amino acids (Padgette et al., 1996). In plants, the EPSPS enzyme is located within the chloroplast. The CP4 EPSPS protein produced in Roundup Ready plants is functionally identical to endogenous plant EPSPS enzymes with the exception that CP4 EPSPS naturally displays reduced affinity for glyphosate, the active ingredient in Roundup agricultural herbicides, relative to endogenous plant EPSPSs (Padgette et al., 1996). The deduced amino acid sequence of the mature CP4 EPSPS protein is shown in Figure IV-2.

In conventional plants, glyphosate binds to the endogenous plant EPSPS enzyme and blocks the biosynthesis of the 5-hydroxyl of shikimate-3-phosphate, thereby depriving plants of essential amino acids (Steinrücken and Amrhein, 1980; Haslam, 1993). In Roundup Ready plants, which have been improved through biotechnology to be tolerant to Roundup agricultural herbicides, aromatic amino acids that are necessary for growth and development are produced by the continued action of the glyphosate-tolerant CP4 EPSPS enzyme (Padgette et al., 1996).

2.1.a. The *Arabidopsis thaliana* EPSPS Transit Peptide

Within the expression cassettes, the *cp4 epsps* coding sequence is joined to a chloroplast transit peptide sequence, designated *ctp2*, derived from the *Arabidopsis thaliana epsps* gene (Klee and Rogers, 1987). This transit peptide directs the transport of the CP4 EPSPS protein to the chloroplast, which is the location of EPSPS in plants, and the site of aromatic amino acid biosynthesis (Klee and Rogers, 1987; Kishore et al., 1988). Transit peptides are typically cleaved from the mature protein following delivery to the plastid (Della-Cioppa et al., 1986). The *ctp2* present in PV-GHGT35 is the same *ctp2* transit peptide sequence used in the development of the existing cotton product, Roundup Ready cotton.

2.1.b. Regulatory Sequences

Starting from the right border region of plasmid PV-GHGT35, the *ctp2/cp4 epsps* coding sequence in the first gene expression cassette is under the regulation of the P-FMV/TSF1 transcriptional promoter. P-FMV/TSF1 is a chimeric promoter containing the *Arabidopsis thaliana* TSF1 gene promoter (encoding elongation factor EF-1 alpha, Axelos et al., 1989) and enhancer sequences from the figwort mosaic virus 35S promoter (Richins et al., 1987). Located between the P-FMV/TSF1 promoter and the *ctp2/cp4 epsps* coding sequence are the nontranslated L-TSF1 leader sequence (exon 1) and the I-TSF1 nontranslated intron (Axelos et al., 1989). The *ctp2/cp4 epsps* coding sequence is linked at the 3' end to the T-E9 DNA sequence derived from *P. sativum*, containing the 3' nontranslated region of the pea ribulose-1,5-bisphosphate carboxylase, small subunit (*rbc*) E9 gene (Coruzzi et al., 1984) for transcriptional termination and polyadenylation of the *cp4 epsps* mRNA.

Following tandem to the first gene expression cassette described above, the second *ctp2/cp4 epsps* gene expression cassette is under the regulation of the P-35S/ACT8 transcriptional promoter. P-35S/ACT8 is a chimeric promoter containing the promoter of

the ACT8 gene of *A. thaliana* (An et al., 1996) combined with the enhancer sequences of the cauliflower mosaic virus (CaMV) 35S promoter (Kay et al., 1987). Located between the P-35S/ACT8 promoter and the *ctp2/cp4 epsps* coding sequence is the nontranslated leader sequence L-ACT8 from the ACT8 gene of *A. thaliana*, the I-ACT8 intron, and flanking exon sequence from the ACT8 gene of *A. thaliana* (An et al., 1996). The *ctp2/cp4 epsps* coding sequence is linked at the 3' end to the T-E9 DNA sequence (Coruzzi et al., 1984), identical to the first *cp4 epsps* gene expression cassette, for transcriptional termination and polyadenylation of the *cp4 epsps* mRNA.

2.2. T-DNA Borders

Plasmid vector PV-GHGT35 contains border regions that delineate the T-DNA to be transferred into cotton and are necessary for the efficient transfer of the T-DNA into the plant cell. These are termed the right border and left border regions (Figures IV-1a, IV-1b and Table IV-1). The right and left border regions are derived from *Agrobacterium* (Depicker et al., 1982; Barker et al., 1983).

2.3. Genetic Elements Outside of the T-DNA Borders

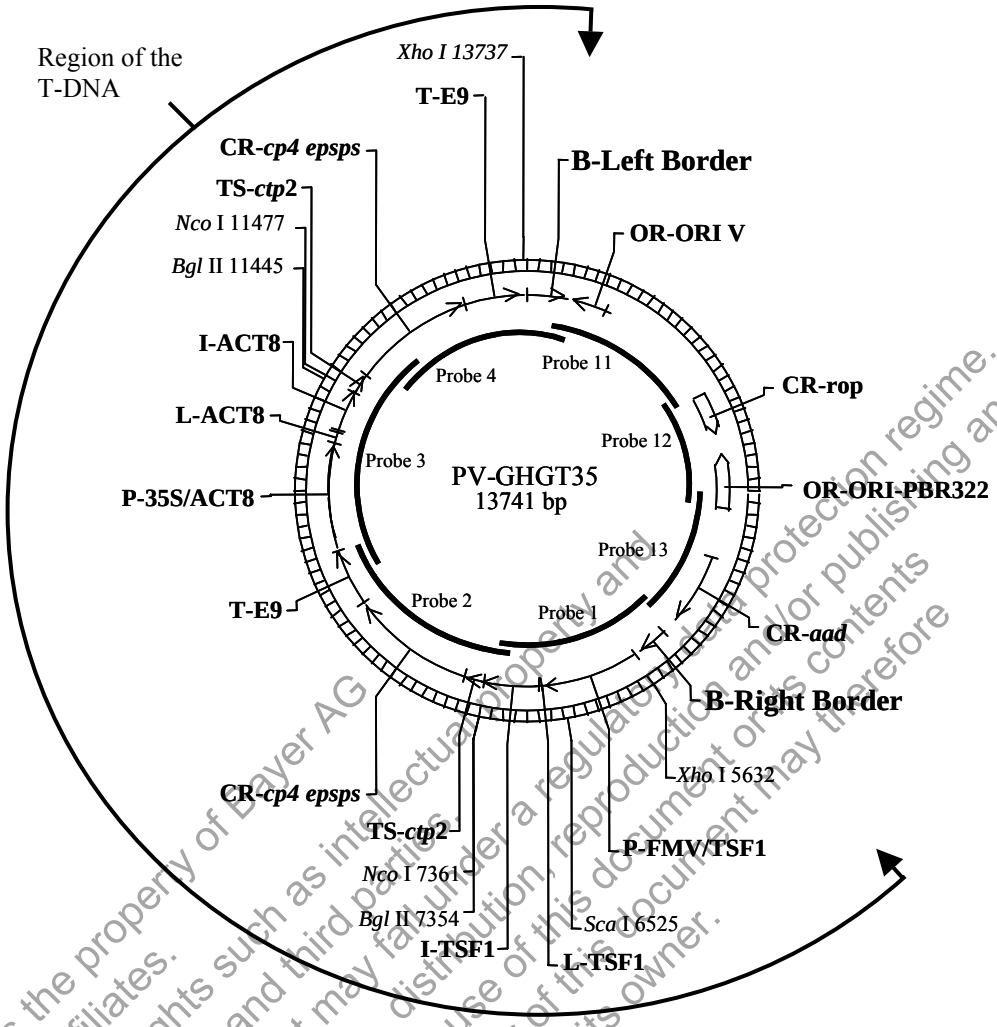
The elements described below are present on plasmid vector PV-GHGT35 (Figures IV-1a and IV-1b), but exist outside the T-DNA borders. Hence, they were not expected to be transferred into the cotton genome, and their absence in MON 88913 has been confirmed by data presented in later in this section.

- <u>*OR-ORIV:*</u> Origin of replication for maintenance of plasmid in *Agrobacterium* derived from the broad host range plasmid RK2 (Stalker et al., 1981).

- <u>*CR-rop:*</u> Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989).

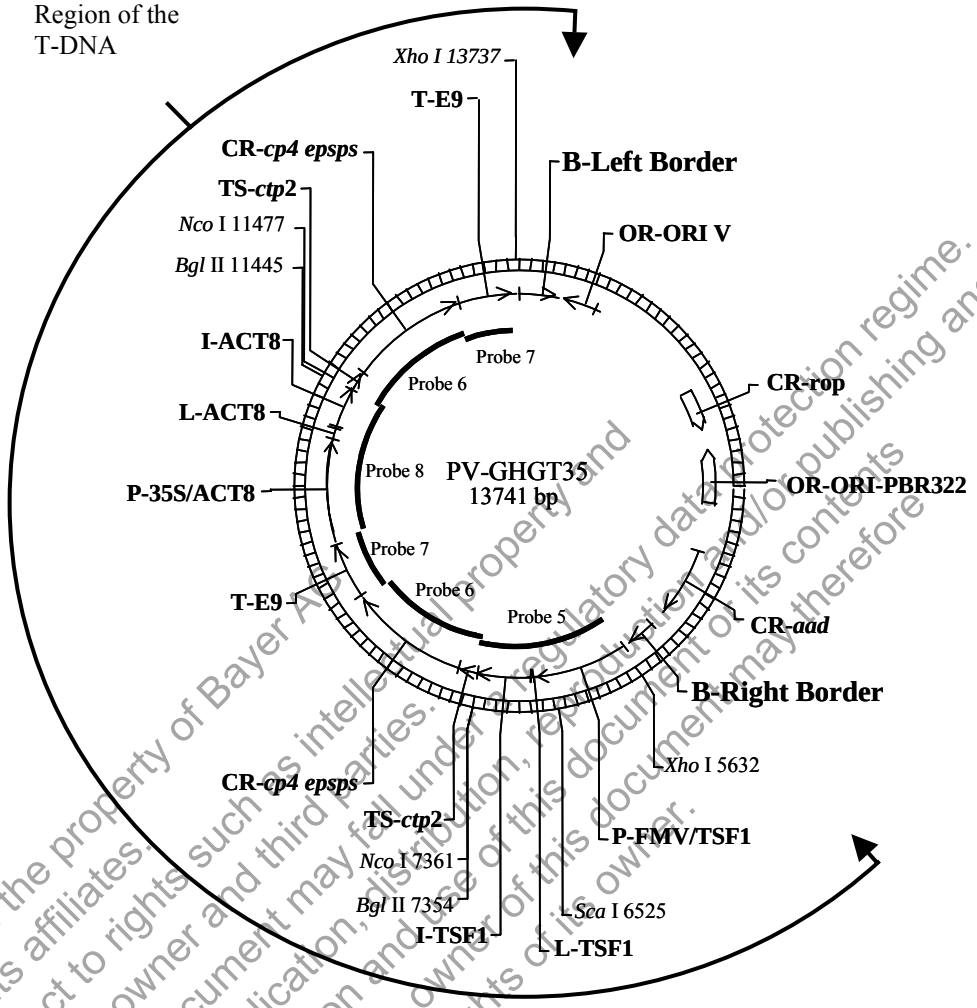
- <u>*OR-ORI-PBR322:*</u> Origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1978).

- <u>*CR-aad:*</u> Coding sequence for Tn7 adenyltransferase conferring spectinomycin and streptomycin resistance (Fling et al., 1985).



Probe	DNA Probe	Start Position	End Position	Total Length (~kb)
1	T-DNA Probe 1	5521	8049	2.5
2	T-DNA Probe 2	7324	9829	2.5
3	T-DNA Probe 3	9518	12024	2.5
4	T-DNA Probe 4	294	11673	2.4
11	Backbone Probe 1	276	2069	1.8
12	Backbone Probe 2	1976	4109	2.1
13	Backbone Probe 3	4019	5525	1.5

Figure IV-1a. Plasmid Vector PV-GHGT35 and Plasmid Backbone Probes
Circular map of the plasmid vector PV-GHGT35 containing the T-DNA used via *Agrobacterium*-mediated transformation to create MON 88913. Four overlapping probes corresponding to the T-DNA and three overlapping probes corresponding to the backbone are drawn on the interior of the map. Genetic elements and restriction sites for enzymes used in the Southern analysis (with positions relative to the size of the plasmid vector) are shown on the exterior of the map. Probes used in the Southern analysis are detailed in the accompanying list.



Probe	DNA Probe	Start Position	End Position	Total Length (~kb)
5	P-FMV/TSF1 + L-TSF1/I-TSF1 Probe	7350	5633	1.7
6	TS-*ctp2*/CR-*cp4 epsps* Probe	7361	8958	1.6
7	T-E9 Probe	9001	9643	0.6
8	P-35S/ACT8 + L-ACT8/I-ACT8 Probe	9672	11469	1.8
6	TS-*ctp2*/CR-*cp4 epsps* Probe	11477	13074	1.6
7	T-E9 Probe	13081	13723	0.6

Figure IV-1b. Plasmid Vector PV-GHGT35 and Individual Element Probes
Circular map of the plasmid vector PV-GHGT35 containing the T-DNA used via *Agrobacterium*-mediated transformation to create MON 88913. Probes corresponding to each of the elements are drawn on the interior of the map. Genetic elements and restriction sites for enzymes used in the Southern analysis (with positions relative to the size of the plasmid vector) are shown on the exterior of the map. Probes used in the Southern analysis are detailed in the accompanying list. Probes six and seven each hybridize to two different sections of the T-DNA.

Table IV-1. Summary of Genetic Elements in PV-GHGT35

Genetic Element	Location in Plasmid	Function (Reference)
Intervening Sequence	1-8	Intervening linker sequences
B[1]- Left Border Region	9-450	DNA sequence derived from *Agrobacterium* containing the left border (LB) sequence for the efficient transfer of the T-DNA (Barker et al., 1983).
Intervening Sequence	451-536	Intervening linker sequences
OR[1]-ORI V	537-1174	Origin of replication for *Agrobacterium* derived from the broad host range plasmid RK2 (Stalker et al., 1981).
Intervening Sequence	1175-2329	Intervening linker sequences
CR[2]-rop	2330-2802	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989).
Intervening Sequence	2803-3050	Intervening linker sequences
OR–ORI-PBR322	3051-3679	Origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1978).
Intervening Sequence	3680-4221	Intervening linker sequences
CR - *aad*	4222-5010	Coding sequence for Tn7 adenylyltransferase conferring spectinomycin and streptomycin resistance (Fling et al., 1985).
Intervening Sequence	5011-5204	Intervening linker sequences
B-Right Border Region	5205-5535	DNA sequences derived from *Agrobacterium* containing the right border (RB) sequence for the efficient transfer of the T-DNA (Depicker et al., 1982).
Intervening sequence	5536-5645	Intervening linker sequences
P[3]-FMV/TSF1	5646-6685	Chimeric promoter containing the *Arabidopsis thaliana tsf1* gene promoter (encoding elongation factor EF-1alpha [Axelos, et al., 1989]) and enhancer sequences from the Figwort Mosaic virus 35S promoter (Richins et al., 1987).
L[4]-TSF1	6686-6731	Leader (exon 1) from the *Arabidopsis thaliana tsf1* gene encoding elongation factor EF-1alpha (Axelos et al., 1989).
I[5]-TSF1	6732-7353	Intron from the *Arabidopsis thaliana tsf1* gene encoding elongation factor EF-1alpha (Axelos et al., 1989).
Intervening Sequence	7354-7362	Intervening linker sequences

[1] B - Border
[1] OR - Origin of replication
[2] CR – Coding region
[3] P - Promoter
[4] L - Leader
[5] I - Intron

Table IV-1 (Continued). Summary of Genetic Elements in PV-GHGT35

Genetic Element	Location in Plasmid	Function (References)
TS[6]- *ctp2*	7363-7590	DNA sequences derived from *Arabidopsis thaliana*. chloroplast transit peptide, derived from the *Arabidopsis thaliana epsps* gene, present to direct the CP4 EPSPS protein to the chloroplast, the site of aromatic amino acid synthesis (Klee and Rogers, 1987).
cp4 epsps	7591-8958	DNA sequence containing synthetic coding sequence for the CP4 EPSPS protein from *Agrobacterium* sp. strain CP4 (Padgette et al., 1996; Barry et al., 1997).
Intervening Sequence	8959-9000	Intervening linker sequences
T[7]-E9	9001-9643	DNA sequences derived from *Pisum sativum,* containing the 3' nontranslated region of the pea ribulose-1, 5-bisphosphate carboxylase, small subunit (*rbc*) E9 gene (Coruzzi et al., 1984).
Intervening sequence	9644-9681	Intervening linker sequences
P-35S/ACT8	9682-10856	Chimeric promoter containing the promoter of the *act8* gene of *Arabidopsis thaliana* (An et al., 1996) combined with the enhancer sequences of the Cauliflower mosaic virus (CaMV) 35S promoter (Kay et al., 1987).
L-ACT8	10857-10997	Leader sequence from the *act8* gene of *Arabidopsis thaliana* (An et al., 1996).
I-ACT8	10998-11470	Intron and flanking exon sequence from the *act8* gene of *Arabidopsis thaliana* (An et al., 1996).
Intervening Sequence	11471-11478	Intervening linker sequences
TS-*ctp2*	11479-11706	DNA sequences derived from *Arabidopsis thaliana*. Chloroplast transit peptide, derived from the *Arabidopsis thaliana epsps* gene, present to direct the CP4 EPSPS protein to the chloroplast, the site of aromatic amino acid synthesis (Klee and Rogers, 1987).
CR - *cp4 epsps*	11707-13074	DNA sequence containing synthetic coding sequence for the CP4 EPSPS protein from *Agrobacterium sp.* strain CP4 (Padgette et al., 1996; Barry et al., 1997).
Intervening Sequence	13075-13080	Intervening linker sequences
T-E9	13081-13723	DNA sequences derived from *Pisum sativum,* containing the 3' nontranslated region of the pea ribulose-1, 5-bisphosphate carboxylase, small subunit (*rbc*) E9 gene (Coruzzi et al., 1984).
Intervening Sequence	13724-13741	Intervening linker sequences

[6] TS - Targeting sequence

[7] T - 3' untranslated transcriptional termination sequence and polyadenylation signal sequences

```
  1     MLHGASSRPA TARKSSGLSG TVRIPGDKSI SHRSFMFGGL ASGETRITGL
 51     LEGEDVINTG KAMQAMGARI RKEGDTWIID GVGNGGLLAP EAPLDFGNAA
101     TGCRLTMGLV GVYDFDSTFI GDASLTKRPM GRVLNPLREM GVQVKSEDGD
151     RLPVTLRGPK TPTPITYRVP MASAQVKSAV LLAGLNTPGI TTVIEPIMTR
201     DHTEKMLQGF GANLTVETDA DGVRTIRLEG RGKLTGQVID VPGDPSSTAF
251     PLVAALLVPG SDVTILNVLM NPTRTGLILT LQEMGADIEV INPRLAGGED
301     VADLRVRSST LKGVTVPEDR APSMIDEYPI LAVAAAFAEG ATVMNGLEEL
351     RVKESDRLSA VANGLKLNGV DCDEGETSLV VRGRPDGKGL GNASGAAVAT
401     HLDHRIAMSF LVMGLVSENP VTVDDATMIA TSFPEFMDLM AGLGAKIELS
451     DTKAA
```

Figure IV-2. Deduced Amino Acid Sequence of the CP4 EPSPS Protein Present in MON 88913. The amino acid sequence of the plant-produced CP4 EPSPS protein in MON 88913 was deduced from the full-length *cp4 epsps* coding sequence present in PV-GHGT35 and in MON 88913.

Section 3. Characterization of the Introduced Genetic Material

3.1. Molecular Analysis

This section details the molecular analyses that were performed to characterize the integrated DNA insert in MON 88913, verify the DNA insert junction with the cotton genome, and verify the insert stability across generations.

Genomic DNA from MON 88913 was digested with restriction enzymes and subjected to Southern blot analyses to characterize the DNA that was integrated into the cotton genome. A map of plasmid vector PV-GHGT35 annotated with the probes used in the Southern analyses is presented in Figures IV-1a and IV-1b. A linear map depicting restriction sites within the DNA insert, as well as within the cotton genomic DNA flanking the insert is shown in Figure IV-3. The materials and methods used in the analyses are presented in Appendix A.

3.2. Results of Molecular Analysis

Insert and Copy Number

The insert number (the number of integration sites of the T-DNA in the cotton genome) was evaluated by digesting DNA of MON 88913 and MON 88913(-) with the restriction enzyme *Spe* I that does not cleave within the T-DNA insert. This enzyme should release a restriction fragment containing the entire DNA insert and adjacent plant genomic DNA (Figure IV-3). The number of restriction fragments detected indicates the number of inserts present in MON 88913.

Plasmid PV-GHGT35 DNA previously digested with *Nco* I mixed with MON 88913(-) DNA digested with *Spe* I (lanes 7 and 8) produced the expected size bands of ~9.6 kb and 4.1 kb (Figure IV-3). MON 88913 DNA digested with *Spe* I (lanes 3 and 9) produced a single band of ~13.0 kb. This indicates that MON 88913 contains one DNA insert located on an ~13.0 kb *Spe* I restriction fragment. MON 88913 DNA digested with a combination of *Spe* I and *Sca* I (lanes 4 and 10) produced two unique bands at ~12.0 kb and ~1.2 kb in lane 10, representing the expected two border fragments that indicate only a single copy of DNA insert is present. The ~1.2 kb band expected in lane 4 (long run) ran off the gel and is not visible in the figure. The concept of using both long and short gel electrophoresis run times (runs) for the Southern blots was to assist in elucidating closely migrating DNA restriction fragments and to ensure that small molecular weight fragments were retained at the bottom of the agarose gel. Long runs provide enhanced resolution for higher molecular weight restriction fragments, and short runs provide retention and resolution of smaller molecular weight restriction fragments. MON 88913(-) DNA digested with *Spe* I alone (lanes 1 and 5) or a combination of *Spe* I and *Sca* I (lanes 2 and 6) produced no hybridization signal (Figure V-3). The faint mark observed at ~40 kb in lane 4 is a nonspecific hybridization artifact. Because this appears only in lane 4 of the long run and not in lane 10 of the short run and does not obscure any expected hybridization signals, it does not affect the interpretation of this Southern blot.

The number of copies of the T-DNA integrated at a single locus was determined by digesting MON 88913 DNA with the combination of restriction enzymes *Spe* I and *Sca* I. *Spe* I alone should release a restriction fragment containing the DNA insert and adjacent plant genomic DNA, while the *Sca* I cleaves once within the DNA insert (Figure IV-3). If MON 88913 contains one copy of the T-DNA, probing with the T-DNA will result in two bands, each band representing a portion of the DNA insert along with adjacent plant genomic DNA. The blot was examined with four overlapping radiolabeled probes (probes 1 – 4, Figure IV-1a) that spanned the entire T-DNA. The results of this analysis are presented in Figure IV-4. For estimating the sizes of bands present in the long-run lanes of Southern blots, the molecular weight markers on the left side of the figure were used. For estimating the sizes of bands present in the short-run lanes, the molecular weight markers on the right side of the figure were used.

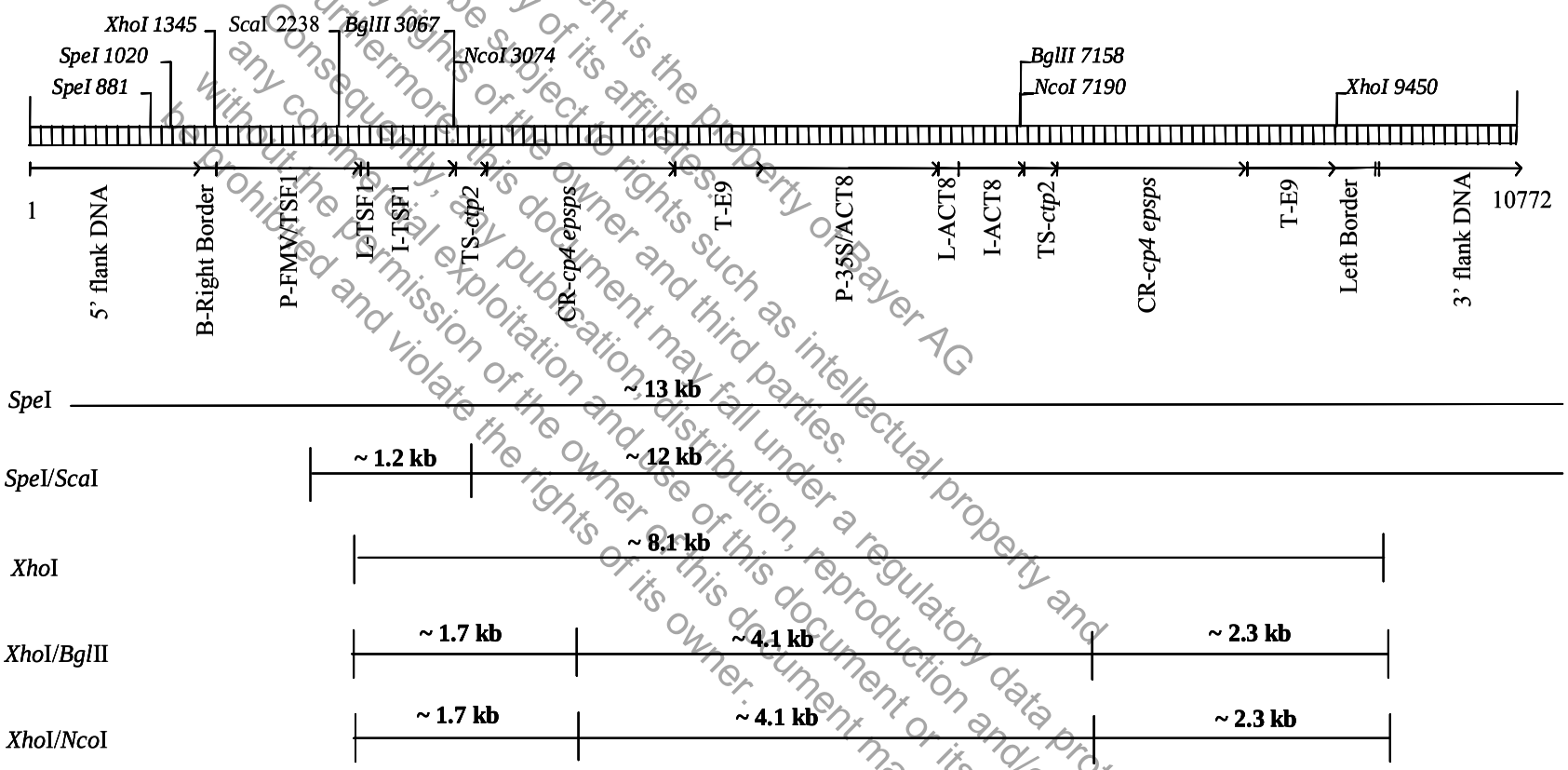


Figure IV-3. Map of the DNA Insert in MON 88913

A linear map of the DNA insert and adjacent DNA flanking the insert in MON 88913 is shown. Identified on the map are genetic elements within the DNA insert, as well as restriction sites with positions relative to the size of the linear map for enzymes used in the Southern analysis. Arrows indicate the direction of transcription. MON 88913 contains one copy of the DNA insert at a single integration locus, so this schematic is also a representation of the T-DNA of PV-GHGT35. However, intact left and right borders are not implied. The expected size of the full DNA insert upon digestion with *Xho* I is ~8.1 kb. Base pairs 1232 – 9743 represent the inserted DNA corresponding to base pairs 5518 – 288 from PV-GHGT35.

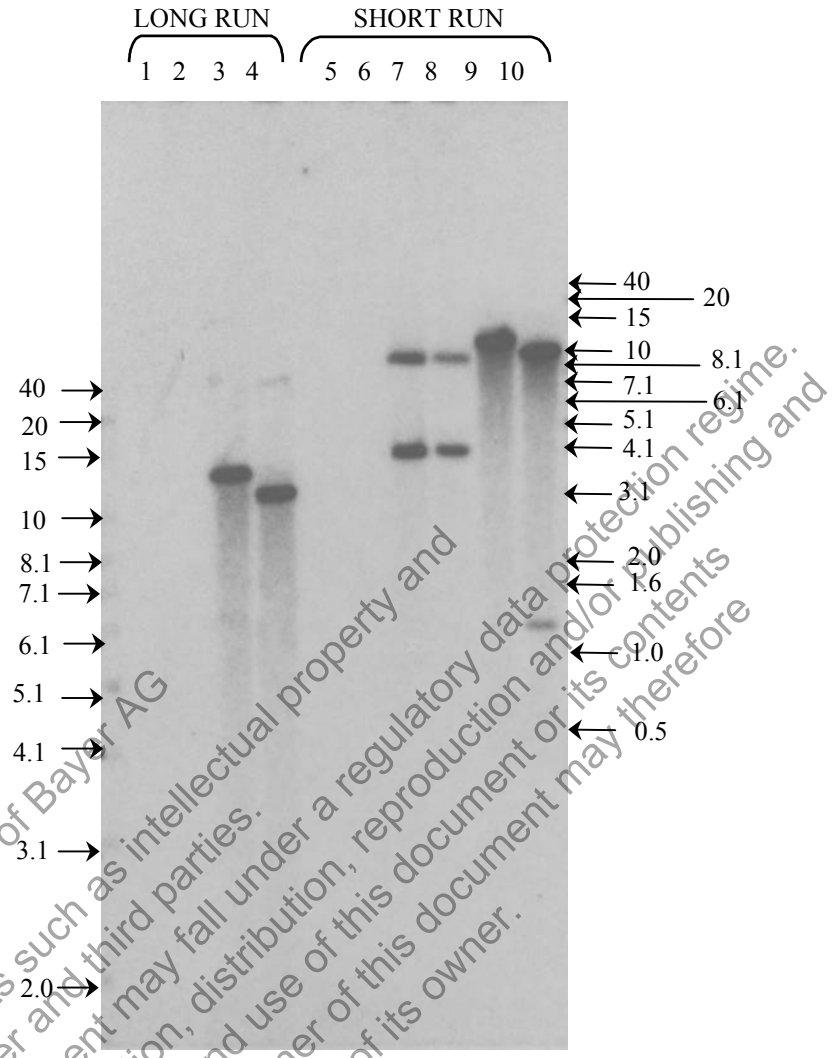


Figure IV-4. Southern Blot Analysis of MON 88913: Insert and Copy Number Analyses. The blot was probed simultaneously with four ^{32}P-labeled probes that spanned the entire length of the T-DNA (probes 1, 2, 3, and 4, Figure IV-1a). Each lane contains ~10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

Lane 1: MON 88913(-) (*Spe* I)
2: MON 88913(-) (*Spe* I and *Sca* I)
3: MON 88913 (*Spe* I)
4: MON 88913 (*Spe* I and *Sca* I)
5: MON 88913(-) (*Spe* I)
6: MON 88913(-) (*Spe* I and *Sca* I)
7: MON 88913(-) (*Spe* I) spiked with PV-GHGT35 (*Nco* I) [1.0 copy]
8: MON 88913(-) (*Spe* I) spiked with PV-GHGT35 (*Nco* I) [0.5 copy]
9: MON 88913 (*Spe* I)
10: MON 88913 (*Spe* I and *Sca* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

3.3. *cp4 epsps* Expression Cassette Integrity

The integrity of the two inserted *cp4 epsps* gene expression cassettes and their associated genetic elements was assessed by digestion of MON 88913 DNA with the restriction enzyme *Xho* I, the combination of restriction enzymes *Xho* I and *Bgl* II, or the combination of restriction enzymes *Xho* I and *Nco* I. Digestion with *Xho* I generates a single ~8.1 kb restriction fragment containing both expression cassettes of the entire T-DNA (Figure IV-3). Digestion of MON 88913 DNA with the combination of *Xho* I and *Bgl* II when examined with the P-FMV/TSF1 + L-TSF1/I-TSF1 probe was expected to generate a single restriction fragment of ~1.7 kb containing the P-FMV/TSF1 promoter, the L-TSF1 leader, and the I-TSF1 intron. Digestion of the MON 88913 DNA with the combination of *Xho* I and *Nco* I was expected to generate two restriction fragments of ~4.1 kb and ~2.3 kb when examined with the TS-*ctp2/cp4 epsps* probe (Figure IV-3). Digestion of the MON 88913 DNA with the combination of *Xho* I and *Nco* I was expected to generate a single restriction fragment of ~4.1 kb when examined with the P-35S/ACT8 + L-ACT8/I-ACT8 probe (Figure IV-3). The individual Southern blots were probed with P-FMV/TSF1 + L-TSF1/I-TSF1, TS-*ctp2*/*cp4 epsps* coding region, T-E9, or P-35S/ACT8 + L-ACT8/I-ACT8 (probes 5, 6, 7, and 8 respectively; Figure IV-1b). Because the TS–*ctp2*/*cp4 epsps* coding region and T-E9 are identical in both cassettes, the same banding pattern is expected to be produced with each of these probes for the two *cp4 epsps* gene expression cassettes.

<u>*P-FMV/TSF1 + L-TSF1/I-TSF1*</u>

When examined with the P-FMV/TSF1 + L-TSF1/I-TSF1 probe (probe 5, Figure IV-1b), plasmid PV-GHGT35 DNA previously digested with *Nco* I and mixed with MON 88913(-) DNA digested with *Xho* I (lanes 7 and 8) produced the expected size band at ~9.6 kb. The results are shown in Figure IV-5. The probe was expected to cross-hybridize with the molecular weight marker bands because of common genetic elements. Therefore, these lanes were removed from the blot prior to hybridization. Aligning these lanes to the corresponding blot after hybridization allowed for appropriate annotation of the molecular weight markers on the film. MON 88913 DNA digested with *Xho* I (lanes 3 and 9) produced the expected band of ~8.1 kb. MON 88913 DNA digested with the combination of *Xho* I and *Bgl* II (lanes 4 and 10) produced a single predicted size band of ~1.7 kb. MON 88913(-) DNA digested with *Xho* I (lanes 1 and 5), or the combination of *Xho* I and *Bgl* II (lanes 2 and 6) showed no detectable hybridizing bands, as expected. Thus, based on the results presented in Figure IV-5, no unexpected bands were detected in MON 88913, indicating that MON 88913 contains no additional, detectable P-FMV/TSF1 + L-TSF1/I-TSF1 elements other than those associated with the intact *cp4 epsps* gene expression cassettes.

<u>*TS-ctp2/cp4 epsps*</u>

Southern blot analysis was performed using the TS-*ctp2*/*cp4 epsps* probe (probe 6, Figure IV-1b), and the results are shown in Figure IV-6. Plasmid PV-GHGT35 DNA previously digested with *Nco* I mixed with MON 88913(-) DNA digested with *Xho* I (lanes 7 and 8) produced the expected size bands at ~9.6 kb and 4.1 kb. MON 88913 DNA digested with *Xho* I (lanes 3 and 9) produced the expected size band of ~8.1 kb. MON 88913 DNA

digested with a combination of *Xho* I and *Bgl* II (lanes 4 and 10) produced the expected size bands of ~4.1 kb and 2.3 kb. MON 88913(-) DNA digested with *Xho* I (lanes 1 and 5) or a combination of *Xho* I and *Bgl* II (lanes 2 and 6) showed no detectable hybridizing bands, as expected. The migration of the ~8.1 kb *Xho* I fragment containing the entire DNA insert is slightly lower than indicated by the molecular weight marker band sizes. The migration of the ~4.1 kb plasmid fragment is slightly higher than indicated by the molecular weight marker band sizes. These slightly altered migrations may be due to the difference in salt concentrations between the MON 88913 DNA sample and the molecular weight marker (Sambrook and Russell, 2001). No unexpected bands were detected, indicating that MON 88913 contains no additional, detectable TS-*ctp2/cp4 epsps* elements other than those associated with the intact *cp4 epsps* gene expression cassettes. The aberrant signal observed at ~5.1 kb spanning lanes 5 and 6 is a background hybridization artifact and does not obscure any expected hybridization signals. Therefore, it does not affect the interpretation of this Southern blot.

T-E9

Southern blot analysis was performed using the T-E9 probe (probe 7, Figure IV-1b) and the results are shown in Figure IV-7. Plasmid PV-GHGT35 DNA previously digested with *Nco* I mixed with MON 88913(-) DNA digested with *Xho* I (lanes 7 and 8) produced the expected size bands at ~9.6 kb and ~4.1 kb. MON 88913 DNA digested with *Xho* I (lanes 3 and 9) produced the expected size band of ~8.1 kb. MON 88913 DNA digested with a combination of *Xho* I and *Bgl* II (lanes 4 and 10) produced the expected size bands of ~4.1 kb and ~2.3 kb. MON 88913(-) DNA digested with *Xho* I (lanes 1 and 5) or a combination of *Xho* I and *Bgl* II (lanes 2 and 6) showed no detectable hybridization bands, as expected. No unexpected bands were detected, indicating that MON 88913 contains no additional, detectable T-E9 elements other than those associated with the intact *cp4 epsps* gene expression cassettes.

P-35S/ACT8 + L-ACT8/I-ACT8

When examined with the P-35S/ACT8 + L-ACT8/I-ACT8 probe (probe 8, Figure IV-1b), plasmid PV-GHGT35 DNA previously digested with *Nco* I and mixed with MON 88913(-) DNA digested with *Xho* I (lanes 7 and 8) produced one expected size band at ~4.1 kb. The results are shown in Figure IV-8. The probe was expected to cross-hybridize with the molecular weight marker bands because of common genetic elements. Therefore, these lanes were removed from the blot prior to hybridization. Aligning these lanes to the corresponding blot after hybridization allowed for appropriate annotation of the molecular weight markers on the film. MON 88913 DNA digested with *Xho* I (lanes 3 and 9) produced the expected size band of ~8.1 kb. The migration of the ~8.1 kb *Xho* I fragment containing the entire DNA insert is slightly higher than indicated by the molecular weight marker band sizes. This slightly altered migration may be due to the difference in salt concentrations between the MON 88913 DNA sample and the molecular weight marker (Sambrook and Russell, 2001). MON 88913 DNA digested with a combination of *Xho* I and *Nco* I (lanes 4 and 10) produced the expected band of ~4.1 kb. MON 88913(-) DNA digested with *Xho* I (lanes 1 and 5), or a combination of *Xho* I and *Nco* I (lanes 2 and 6) showed no detectable hybridization bands, as expected. No unexpected bands were detected, indicating that MON 88913 contains no additional,

detectable P-35S/ACT8 + L-ACT8/I-ACT8 elements other than those associated with the intact *cp4 epsps* gene expression cassettes.

3.4. Confirmation of the Absence of Plasmid PV-GHGT35 Backbone

MON 88913 and MON 88913(-) DNA were digested with either *Spe* I or a combination of *Spe* I and *Sca* I. Plasmid PV-GHGT35 DNA previously digested with *Nco* I was mixed with MON 88913(-) genomic DNA digested with *Spe* I and then loaded on the gel to serve as a positive hybridization control. The blot was examined simultaneously with three overlapping probes (probes 11, 12, and 13, Figure IV-1a) that span the backbone (sequences outside of the T-DNA) present in PV-GHGT35. The backbone probes were expected to cross-hybridize with the molecular weight markers because of common genetic elements. Therefore, these lanes were removed from the blot prior to hybridization. Aligning these lanes to the blot after hybridization allowed for appropriate annotation of the molecular weight markers on the film. MON 88913(-) DNA digested with *Spe* I (lanes 1 and 5) or a combination of *Spe* I and *Sca* I (lanes 2 and 6) showed no detectable hybridization bands, as expected for MON 88913(-) (Figure IV-9). Plasmid PV-GHGT35 *Nco* I restriction fragments mixed with MON 889139(-) DNA digested with *Spe* I (lanes 7 and 8) produced one expected size band at ~9.6 kb. MON 88913 DNA digested with either *Spe* I (lanes 3 and 9) or a combination of *Spe* I and *Sca* I (lanes 4 and 10) showed no detectable hybridization signal, indicating that MON 88913 does not contain any detectable backbone sequence from the transformation vector PV-GHGT35.

3.5. Stability of the DNA Insert

In order to demonstrate the stability of the DNA insert in MON 88913, Southern blot analysis was performed using DNA from multiple generations from the MON 88913 breeding tree. For reference, the breeding history of MON 88913 is presented in Figure IV-10. The specific generations tested are indicated in the legends of Figures IV-10 and IV-11. For these analyses, MON 88913 and MON 88913(-) DNA samples were digested with the combination of restriction enzymes *Spe* I and *Sca* I. Digestion of MON 88913 with the combination of *Spe* I and *Sca* I produced two restriction fragments of ~12.0 kb and ~1.2 kb (lanes 4 – 8, Figure IV-11). This is the same restriction pattern observed for the R3 generation shown in Figure IV-4. Plasmid PV-GHGT35 DNA previously digested with *Nco* I and mixed with MON 88913(-) DNA digested with *Spe* I and *Sca* I produced the expected size bands of ~9.6 kb and ~4.1 kb for the positive hybridization control (lanes 2 and 3). The results of this analysis establish the stability of the inserted DNA over the selected generations of MON 88913 representing multiple generations of the breeding tree.

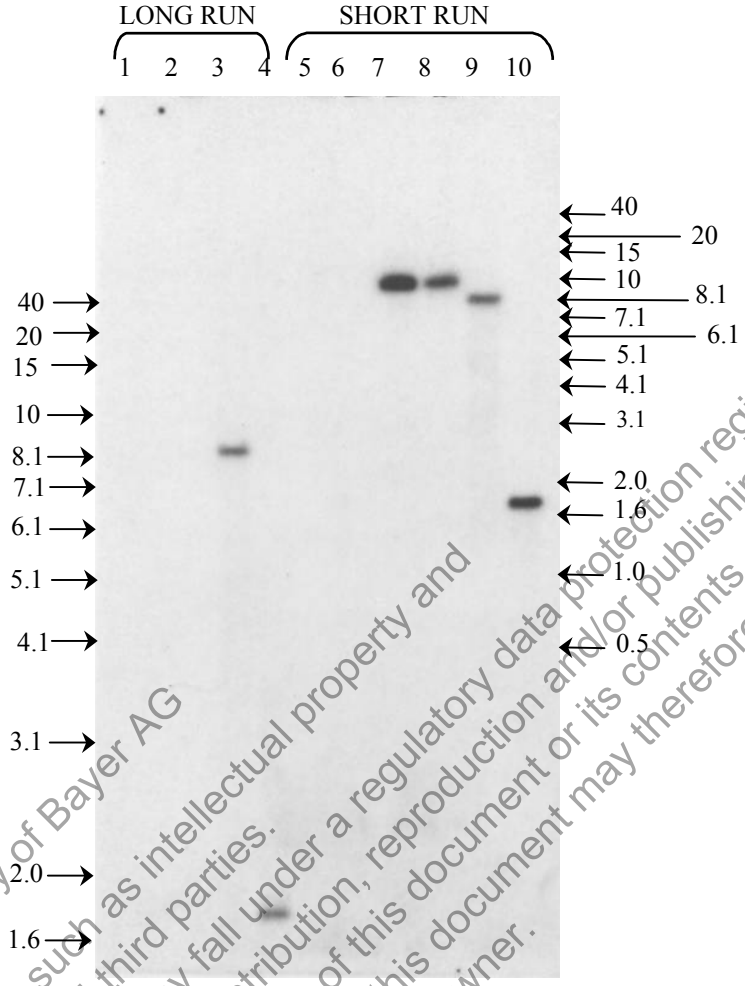


Figure IV-5. Southern Blot Analysis of MON 88913: Gene Cassette Intactness: P-FMV/TSF1 + L-TSF1/I-TSF1 Probe. The blot was probed with a ^{32}P-labeled probe that spanned the P-FMV/TSF1 promoter, L-TSF1 leader and I-TSF1 intron (probe 5, Figure IV-1b). Each lane contains ~10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

Lane 1: MON 88913(-) (*Xho* I)
2: MON 88913(-) (*Xho* I and *Bgl* II)
3: MON 88913 (*Xho* I)
4: MON 88913 (*Xho* I and *Bgl* II)
5: MON 88913(-) (*Xho* I)
6: MON 88913(-) (*Xho* I and *Bgl* II)
7: MON 88913(-) (*Xho* I) spiked with PV-GHGT35 (*Nco* I) [1.0 copy]
8: MON 88913(-) (*Xho* I) spiked with PV-GHGT35 (*Nco* I) [0.5 copy]
9: MON 88913 (*Xho* I)
10: MON 88913 (*Xho* I and *Bgl* II)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

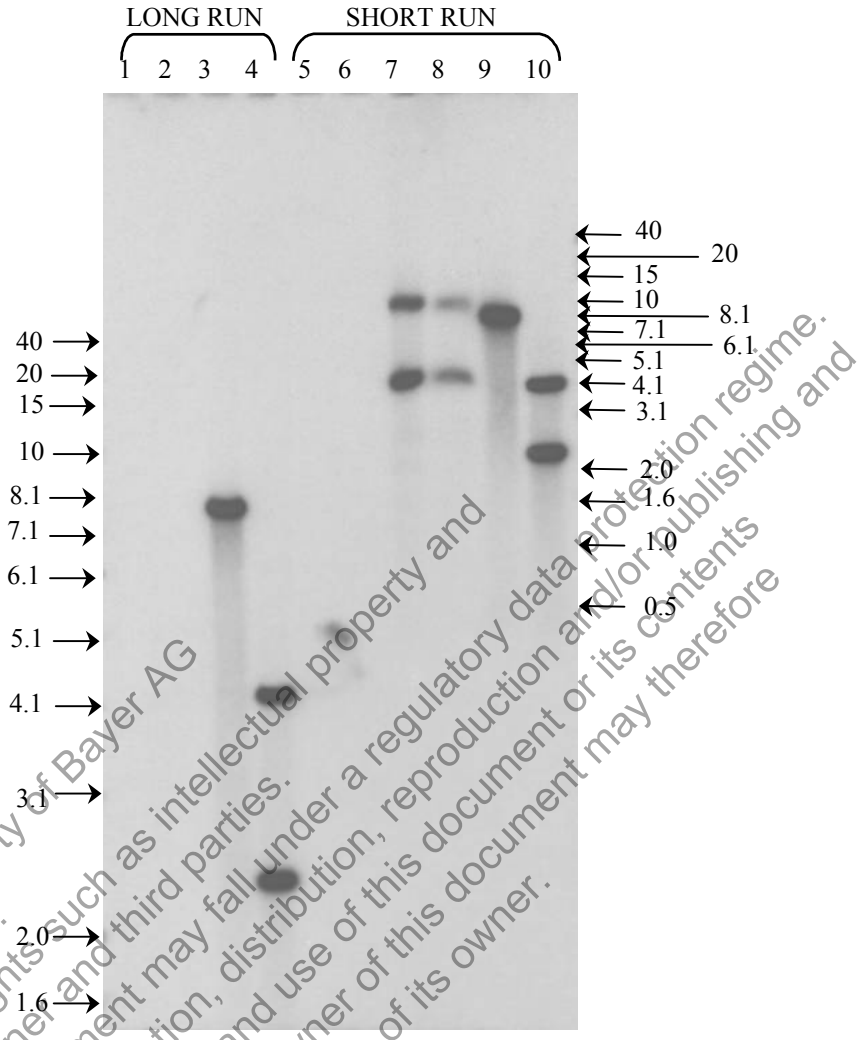


Figure IV-6. Southern Blot Analysis of MON 88913: Gene Cassette Intactness: TS-*ctp2*/*cp4 epsps* Probe. The blot was probed with a ^{32}P-labeled probe that spanned the TS-*ctp2* (chloroplast transit peptide) and *cp4 epsps* (probe 6, Figure IV-1b). Each lane contains ~10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

Lane 1: MON 88913(-) (*Xho*I)
2: MON 88913(-) (*Xho* I and *Bgl* II)
3: MON 88913 (*Xho* I)
4: MON 88913 (*Xho* I and *Bgl* II)
5: MON 88913(-) (*Xho* I)
6: MON 88913(-) (*Xho* I and *Bgl* II)
7: MON 88913(-) (*Xho* I) spiked with PV-GHGT35 (*Nco* I) [1.0 copy]
8: MON 88913(-) (*Xho* I) spiked with PV-GHGT35 (*Nco* I) [0.5 copy]
9: MON 88913 (X*ho* I)
10: MON 88913 (*Xho* I and *Bgl* II)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

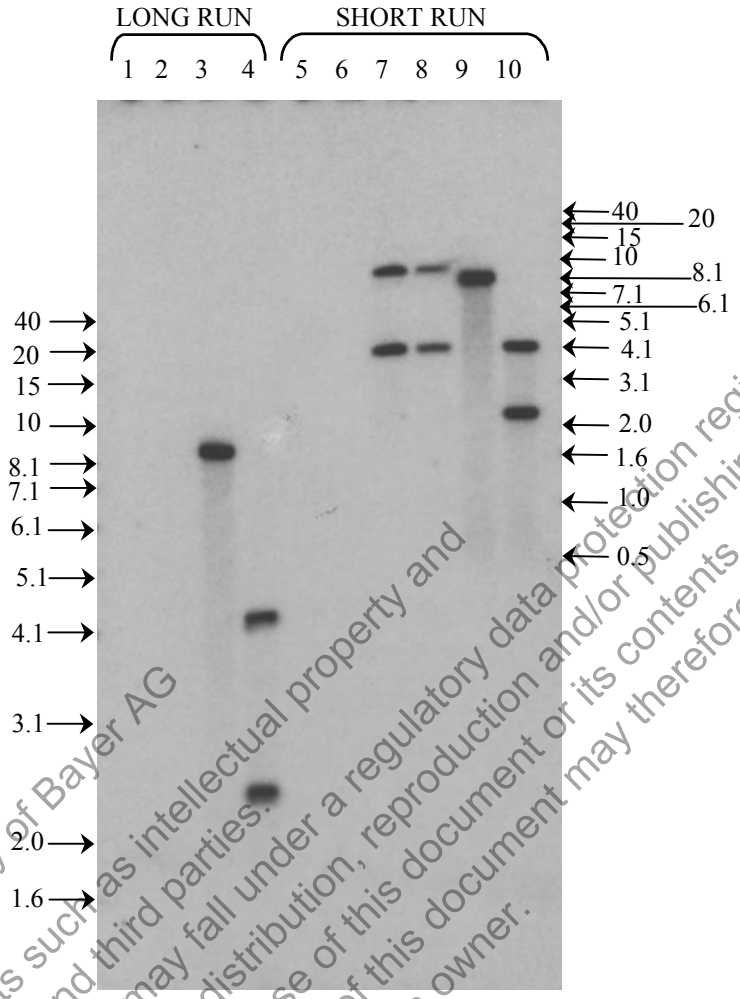


Figure IV-7. Southern Blot Analysis of MON 88913: Gene Cassette Intactness: T-E9 Probe. The blot was probed with a ^{32}P-labeled probe that spanned the T-E9 (probe 7, Figure IV-1b). Each lane contains ~10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

Lane 1: MON 88913(-) (*Xho*I)
2: MON 88913(-) (*Xho* I and *Bgl* II)
3: MON 88913 (*Xho* I)
4: MON 88913 (*Xho* I and *Bgl* II)
5: MON 88913(-) (*Xho* I)
6: MON 88913(-) (*Xho* I and *Bgl* II)
7: MON 88913(-) (*Xho* I) spiked with PV-GHGT35 (*Nco* I) [1.0 copy]
8: MON 88913(-) (*Xho* I) spiked with PV-GHGT35 (*Nco* I) [0.5 copy]
9: MON 88913 (X*ho* I)
10: MON 88913 (*Xho* I and *Bgl* II)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

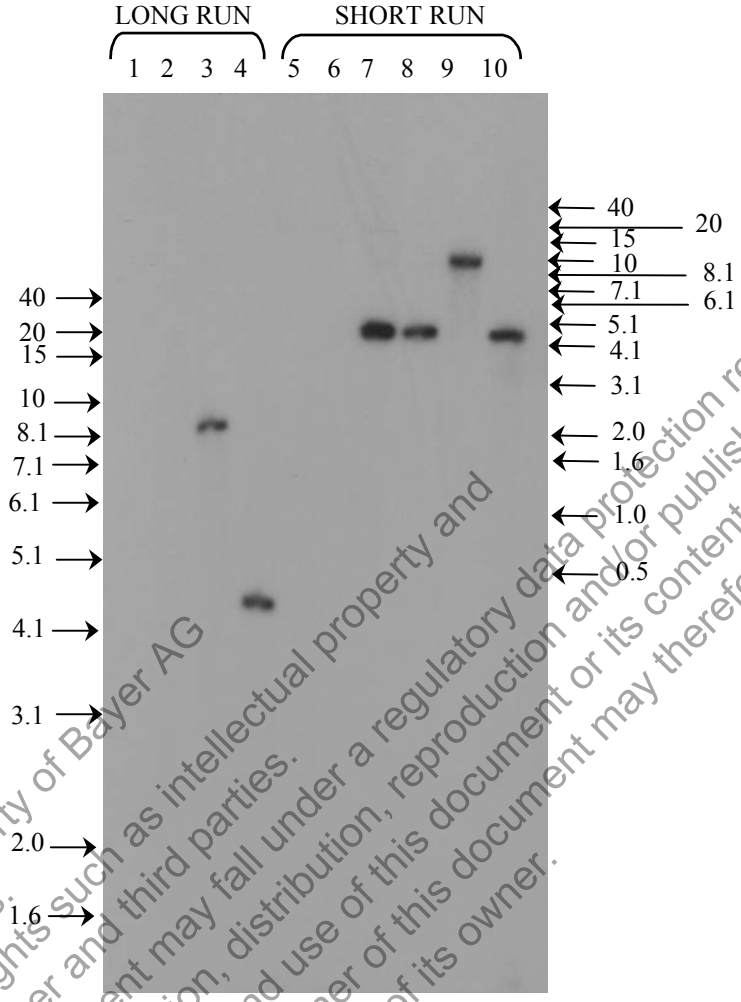


Figure IV-8. Southern Blot Analysis of MON 88913: Gene Cassette Intactness: P-35S/ACT8 + L-ACT8/I-ACT8 Probe. The blot was probed with a ^{32}P-labeled probe that spanned the P-35S/ACT8 promoter, L-ACT8 leader and I-ACT8 intron (probe 8, Figure IV-1b). Each lane contains ~10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

Lane 1: MON 88913(-) (*Xho* I)
2: MON 88913(-) (*Xho* I and *Nco* I)
3: MON 88913 (*Xho* I)
4: MON 88913 (*Xho* I and *Nco* I)
5: MON 88913(-) (*Xho* I)
6: MON 88913(-) (*Xho* I and *Nco* I)
7: MON 88913(-) (*Xho* I) spiked with PV-GHGT35 (*Nco* I) [1.0 copy]
8: MON 88913(-) (*Xho* I) spiked with PV-GHGT35 (*Nco* I) [0.5 copy]
9: MON 88913 (*Xho* I)
10: MON 88913 (*Xho* I and *Nco* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

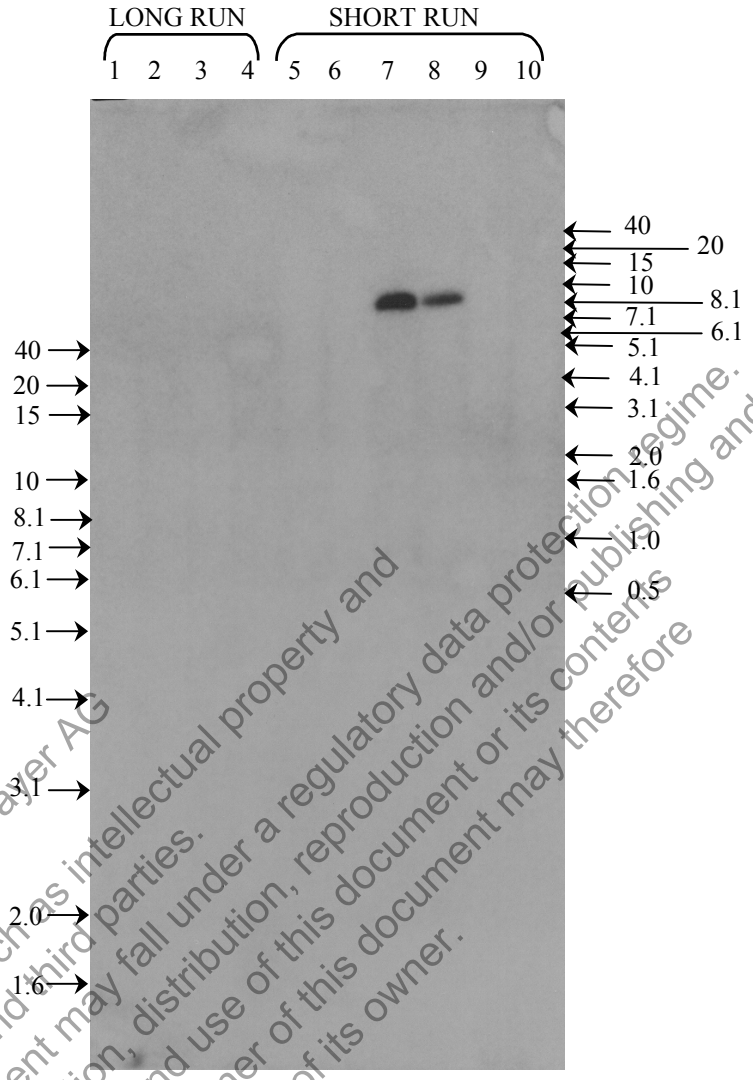


Figure IV-9. Southern Blot Analysis of MON 88913: PV-GHGT35 Backbone Analysis. Each blot was probed simultaneously with three ^{32}P-labeled probes that span the entire backbone sequence (probes 11, 12, and 13, Figure IV-1a) of plasmid PV-GHGT35. Each lane contains ~10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

Lane 1: MON 88913(-) (*Spe* I)
2: MON 88913(-) (*Spe* I and *Sca* I)
3: MON 88913 (*Spe* I)
4: MON 88913 (*Spe* I and *Sca* I)
5: MON 88913(-) (*Spe* I)
6: MON 88913(-) (*Spe* I and *Sca* I)
7: MON 88913(-) (*Spe* I) spiked with PV-GHGT35 (*Nco* I) [1.0 copy]
8: MON 88913(-) (*Spe* I) spiked with PV-GHGT35 (*Nco* I) [0.5 copy]
9: MON 88913 (*Spe* I)
10: MON 88913 (*Spe* I and *Sca* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

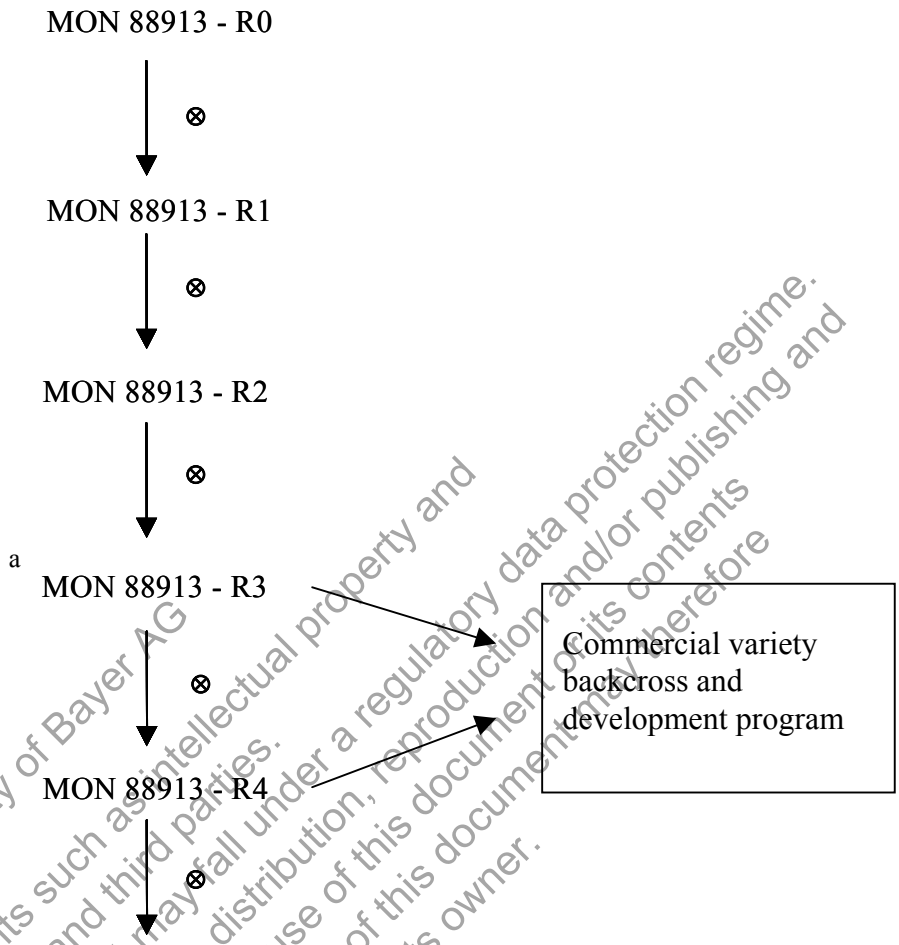


Figure IV-10. Breeding Tree for MON 88913
Generations R1 through R5 were selected for generational stability by Southern blot analyses. Generations R3 and R4 were used as donors for commercial variety development.

R0 = Initial MON 88913 plant
⊗ = Crossed to self
a = Generation used for seed composition, molecular characterization, protein characterization, seed germination, and protein level determinations
b = Generation used for replicated agronomic field tests

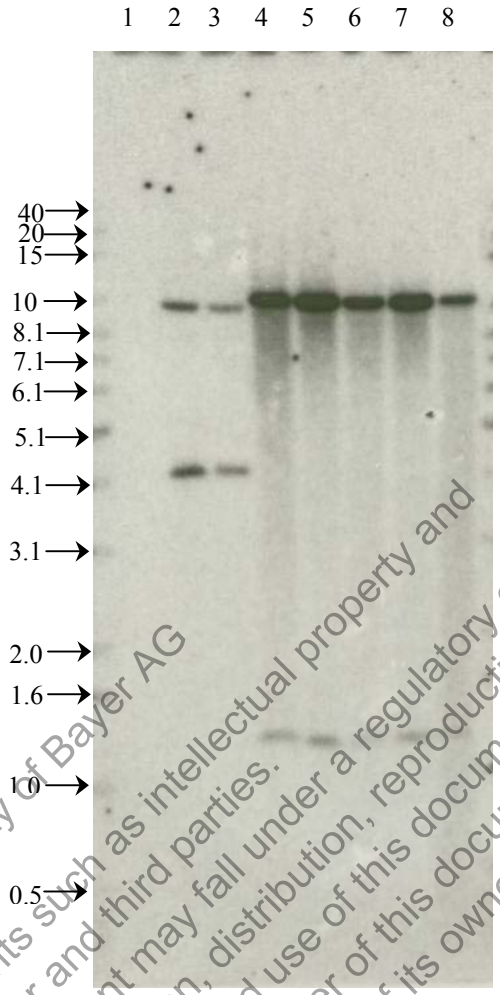


Figure IV-11. Generational Stability of MON 88913: Insert and Copy Number Analysis. The blot was probed simultaneously with four ^{32}P-labeled probes that span the T-DNA of PV-GHGT35 (probes 1, 2, 3 and 4, Figure IV-1a). Each lane contains ~10 µg of digested genomic DNA isolated from seed or leaf material. The breeding history of MON 88913 is illustrated in Figure IV-10. Lane designations are as follows:

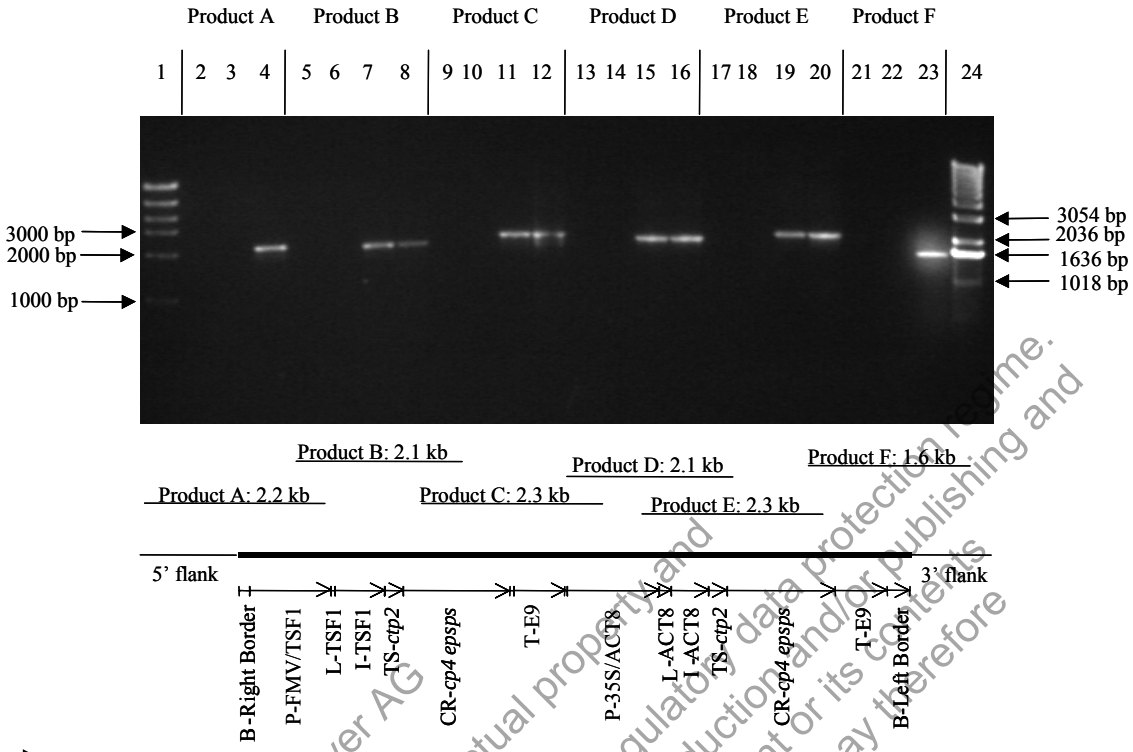
Lane 1: MON 88913(-) (*Spe* I and *Sca* I)
2: MON 88913(-) (*Spe* I and *Sca* I) spiked with PV-GHGT35 (*Nco* I) [1.0 copy]
3: MON 88913(-) (*Spe* I and *Sca* I) spiked with PV-GHGT35 (*Nco* I) [0.5 copy]
4: MON 88913 - R1 (*Spe* I and *Sca* I)
5: MON 88913 - R2 (*Spe* I and *Sca* I)
6: MON 88913 - R3 (*Spe* I and *Sca* I)
7: MON 88913 - R4 (*Spe* I and *Sca* I)
8: MON 88913 - R5 (*Spe* I and *Sca* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

3.6. Confirmation of the Organization of the DNA Insert

The organization of the elements within the DNA insert in MON 88913 was confirmed using PCR analysis by amplifying six overlapping regions of DNA that span the entire length of the insert and the immediate flanking cotton genomic DNA at the 5' and 3' junctions. The locations of the PCR products generated in relation to the insert, as well as the results of the PCR analyses, are shown in Figure IV-12. The DNA sequence at the 5' and 3' ends of the insert was verified by PCR using MON 88913 genomic DNA as a template. The PCR for the 5' insert-to-plant junction was performed using one primer designed to the 5' genomic flanking DNA sequence, paired with a second primer in the 5' end of the DNA insert. The PCR for the 3' insert-to-plant junction was conducted using a primer designed to the 3' genomic flanking DNA sequence, paired with a second primer located in the 3' end of the DNA insert.

The control reactions containing no template DNA (lanes 2, 5, 9, 13, 17, and 21) did not generate PCR products with any of the primer sets, as expected. The MON 88913(-) reactions (lanes 3, 6, 10, 14, 18, and 22) also did not generate any PCR products, as expected. The plasmid PV-GHGT35 was used as a positive control in the four PCR analyses (Products B-E) that amplified products containing only the inserted DNA rather than the genomic DNA flanking the insert. In these four analyses, cotton genomic DNA from MON 88913, as well as the plasmid PV-GHGT35, generated the expected size PCR products of ~2.1 kb for Product B (lanes 7 and 8); ~2.3 kb for Product C (lanes 11 and 12); ~2.1 kb for Product D (lanes 15 and 16); and ~2.3 kb for Product E (lanes 19 and 20). MON 88913 DNA also generated the expected size PCR products of ~2.2 kb for Product A (lane 4) and ~1.6 kb for Product F (lane 23). The generation of the predicted size PCR products from MON 88913 establishes that the arrangement and linkage of elements in the insert are the same as those in plasmid PV-GHGT35 and that the elements within each *cp4 epsps* gene expression cassette are arranged as depicted in the schematic of the insert in Figure IV-3.



→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide stained gel.

Figure IV-12. Overlapping PCR Analysis Across the DNA Insert in MON 88913
PCR analyses demonstrating the linkage of the individual genetic elements within the DNA insert in MON 88913 were performed on MON 88913 genomic DNA extracted from seed. Lanes are marked to show which and how much product was loaded and is visualized on the agarose gel. The expected product size for each amplicon is highlighted in the illustration of the insert in MON 88913 that appears at the bottom of the figure.

Lane		Lane	
1:	Invitrogen High Mass DNA ladder	13:	No template (5 µl)
2:	No template (5 µl)	14:	MON 88913(-) negative segregant (5 µl)
3:	MON 88913(-) negative segregant (5 µl)	15:	MON 88913 genomic DNA (20 µl)
4:	MON 88913 genomic DNA (10 µl)	16:	plasmid PV-GHGT35 (3 µl)
5:	No template (5 µl)	17:	No template (5 µl)
6:	MON 88913(-) negative segregant (5 µl)	18:	MON 88913(-) negative segregant (5 µl)
7:	MON 88913 genomic DNA (5.5 µl)	19:	MON 88913 genomic DNA (9 µl)
8:	plasmid PV-GHGT35 (3 µl)	20:	plasmid PV-GHGT35 (3 µl)
9:	No template (5 µl)	21:	No template (5 µl)
10:	MON 88913(-) negative segregant (5 µl)	22:	MON 88913(-) negative segregant (5 µl)
11:	MON 88913 genomic DNA (3.5 µl)	23:	MON 88913 genomic DNA (12 µl)
12:	plasmid PV-GHGT35 (1 µl)	24:	Invitrogen 1Kb DNA ladder

3.7. Inheritance of the Glyphosate Tolerance Trait in MON 88913

During the development of the MON 88913, analysis of phenotypic segregation data was conducted across several generations. A summary of results of these analyses is presented in Tables IV-2, IV-3, and IV-4. The glyphosate tolerance of individual plants was determined by antibody strip-test for the CP4 EPSPS protein and/or tolerance to a Roundup agricultural herbicide spray. After self pollinating the MON 88913 plant regenerated from tissue culture, the R1 seed were planted, and the resulting plants were expected to segregate in a 3:1 ratio in favor of the glyphosate-tolerant phenotype as a single, dominant trait loci. In the R1 plants, the calculated Chi-Square value for phenotype was less than the critical value of 3.84 at the 5% level of error, and therefore MON 88913 demonstrated the expected 3:1 segregation in the R1 generation (Table IV-2). The R2 generation represents a point in the breeding process where homozygous seed can be identified. Individual glyphosate-tolerant R1 plants were identified, self-pollinated to produce R2 seed, and then subjected to progeny screens to identify homozygous seed lots. Individual R2 families are expected to segregate 1:2 for homozygosity after glyphosate-sensitive individuals are removed from the population. Seventy-six R2 families were generated and tested for homozygosity. Chi-square analysis for homozygote recovery is presented in Table IV-3. The calculated Chi-square statistic is less than the critical value of 3.84 at the 5% level of error. Therefore, the expected number of homozygous families were recovered during the breeding process. Selection of homozygous plant seed lots was successful in the R3 generation and was confirmed in generations R4 and R5. Homozygous MON 88913 seed lots are expected to segregate 1:0 for glyphosate tolerance. Glyphosate tolerance data from the R4 and R5 generations are summarized in Table IV-4. These data confirm homozygosity and generational stability of MON 88913 and, thus, the stability of the DNA insert.

Table IV-2. Segregation Ratio for the MON 88913 Phenotype in the R1 Generation

Generation	Phenotype	Expected Ratio	Expected No. of Plants(E)	Observed No. of Plants (O)	$(O-E)^2/E$*
R1	Glyphosate tolerant	0.75	111.8	111	0.005
	Non-glyphosate tolerant	0.25	37.3	38	0.0151
		Total	149	149	0.0201

* Critical value at 0.05 = 3.84; 1 degree of freedom.

Table IV-3. Homozygous Recovery Ratio for the MON 88913 Phenotype in R2 Families

Generation	Phenotype	Expected Ratio	Expected No. of Families (E)	Observed No. of Families (O)	$(O-E)^2/E$*
R2	Homozygous	0.3333	25.3308	24	0.1310
	Segregating	0.6666	50.6616	52	0.0675
		Total	76	76	0.1985

*Critical value at 0.05 = 3.84; 1 degree of freedom.

Table IV-4. Confirmation of Homozygous Status in the R4 and R5 Generations

Generation	Number Glyphosate Tolerant	Number Non-Glyphosate Tolerant	Test Method
R4	322	0	Roundup spray
R5	310	0	Roundup spray

3.8. Conclusions for Molecular Characterization

Molecular analyses were performed to characterize the integrated DNA insert in MON 88913. Southern blot genomic analyses were used to determine the DNA insert number (number of integration sites within the cotton genome), copy number (the number of copies within one insert), the intactness of the *cp4 epsps* gene expression cassettes, and to establish the absence of plasmid backbone sequences in the plant. The stability of the DNA insert across multiple generations was also demonstrated by Southern blot fingerprint analysis. Polymerase chain reaction analysis was performed to identify the 5' and 3' insert-to-genomic DNA junctions, and to confirm the organization of the elements within the DNA insert.

The data show that MON 88913 contains a single integration locus on an ~13.0 kb *Spe* I restriction fragment containing one copy of the DNA insert, and that the DNA insert contains two intact *cp4 epsps* gene expression cassettes. No additional elements from the transformation vector PV-GHGT35, linked or unlinked to the intact DNA insert, were detected in the genome of MON 88913. Additionally, backbone sequence from PV-GHGT35 was not detected. Generational stability analysis demonstrated that the expected Southern blot fingerprint of MON 88913 has been maintained across five generations of breeding, thereby confirming the stability of the DNA insert over multiple generations. These generations were also shown not to contain any detectable backbone sequence from plasmid PV-GHGT35. The PCR analysis confirmed the organization of the elements within the DNA insert of MON 88913. The generation of the predicted size PCR products from MON 88913 established that the arrangement and linkage of elements in the insert are the same as those in plasmid PV-GHGT35 and that the elements within each *cp4 epsps* gene expression cassette are arranged as depicted in the

schematic of the insert in Figure IV-3. Finally, Mendelian segregation of the expected MON 88913 phenotype across multiple generations and families corroborates the molecular insert stability analysis and establishes the genetic behavior of the DNA insert as a single locus.

Section 4. Other Data or Information Regarding the Development of MON 88913

All relevant information regarding development of MON 88913 is described in Parts II - VII of this summary.

PART V: PRESENCE OF GENES THAT ENCODE RESISTANCE TO ANTIBIOTICS

No genes that encode resistance to an antibiotic were inserted into the cotton genome during the development of MON 88913. Molecular characterization data presented in Part IV, Sections 3.2, 3.3 and 3.4 demonstrate the absence of the *aad* antibiotic resistance marker in MON 88913.

PART VI: CHARACTERIZATION OF THE CP4 EPSPS PROTEIN PRODUCED IN ROUNDUP READY FLEX COTTON MON 88913

Section 1. The CP4 EPSPS Protein Present in MON 88913

MON 88913 contains the 5-enolpyruvylshikimate-3-phosphate synthase gene derived from *Agrobacterium sp*. strain CP4 (*cp4 epsps*). The *cp4 epsps* coding sequence encodes a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids (Padgette et al., 1996). The CP4 EPSPS protein is structurally similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate, the active ingredient in Roundup agricultural herbicides, relative to endogenous plant EPSPS (Padgette et al., 1996). In conventional plants, glyphosate binds to the endogenous plant EPSPS enzyme and blocks the biosynthesis of shikimate-3-phosphate, thereby depriving plants of essential amino acids (Steinrücken and Amrhein, 1980; Haslam, 1993). In Roundup Ready plants, which are tolerant to Roundup agricultural herbicides, requirements for production of aromatic amino acids and other metabolites that are necessary for growth and development are met by the continued action of the CP4 EPSPS enzyme in the presence of glyphosate (Padgette et al., 1996).

In plants, the chloroplast is the site of the EPSPS enzyme and its activity. Therefore, the CP4 EPSPS protein produced in MON 88913 is targeted to the chloroplasts via an N-terminal fusion with the CTP2 transit peptide to form a CTP2-CP4 EPSPS precursor protein. The precursor protein, produced in the cytoplasm, is then processed to remove the transit peptide upon translocation into the plant chloroplast, resulting in the mature protein (Chua and Schmidt, 1978; Highfield and Ellis, 1978; Oblong and Lamppa, 1992). A safety assessment of the CP4 EPSPS protein has been previously described in the literature (Harrison et al., 1996) and a general review of the genes used to confer tolerance to glyphosate, and their respective enzymes, is contained in an OECD consensus document (OECD, 1999).

1.1. Identity and Characterization Summary of the CP4 EPSPS Protein Present in MON 88913

A panel of analytical tests, some utilizing the *E. coli*-produced CP4 EPSPS protein as a reference standard, was used to establish the identity and characterize the plant-produced CP4 EPSPS protein. These analytical tests were: (1) immunoblot analysis and densitometry, (2) matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry, (3) N-terminal sequence analysis, (4) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and densitometry, (5) CP4 EPSPS enzymatic activity assay, and (6) glycosylation analysis. The results from each of these analyses are summarized below. Details on materials and methods can be found in Appendix B.

1.1.a. Immunoblot Analysis and Densitometry

An immunoblot analysis followed by densitometry was conducted using the plant-produced CP4 EPSPS protein and the *E. coli*-produced CP4 EPSPS reference standard to provide evidence supporting the identity of the plant-produced protein.

As expected, the immunoreactive signal increased with increasing levels of the CP4 EPSPS protein and the mobilities of the plant- and *E. coli*-produced CP4 EPSPS proteins were very similar (Figure VI-1). Furthermore, densitometric analysis of the western blot showed that the CP4 EPSPS protein isolated from MON 88913 bound equivalent amounts of goat anti-CP4 EPSPS serum (≤ 10% difference) as the *E. coli*-produced CP4 EPSPS reference standard.

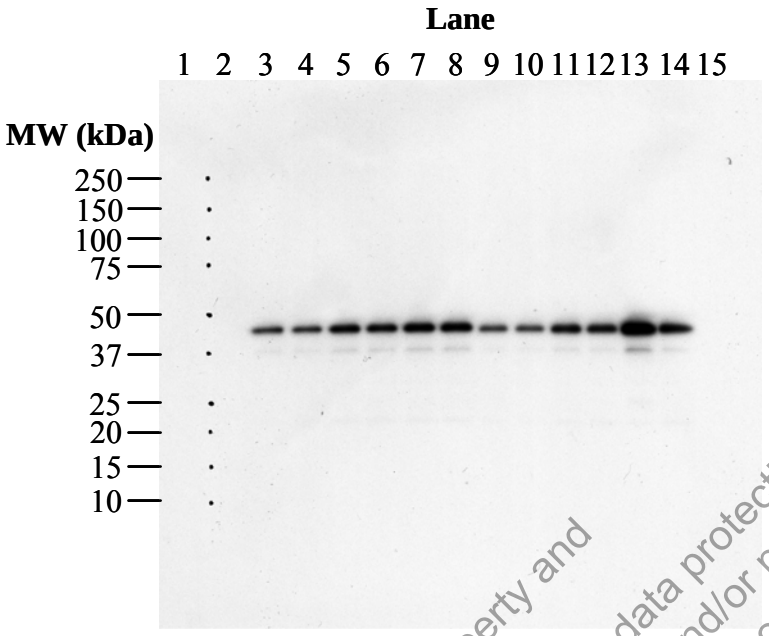
There was one difference in the immunoreactive signals among duplicates for the plant-produced CP4 EPSPS protein loaded at 3 ng. This observed difference was likely due to an error in loading a duplicate sample in lane # 13. Thus, the densitometric value for this lane was excluded from the average calculation of immunoreactivity of plant-produced CP4 EPSPS protein. Also visible are lower molecular weight immunoreactive bands in lanes 3-8 and 9-14 that migrate at approximately 23 kDa and 37 kDa. The lower molecular weight immunoreactive bands, visible with increasing levels of the loaded proteins, may have been formed by proteolytic degradation of the CP4 EPSPS protein during the protein extraction process.

The observed similarity in protein mobility and immunoreactivity for the plant- and *E. coli*-produced CP4 EPSPS proteins provides evidence that the plant-produced CP4 EPSPS protein is equivalent to the *E. coli*-produced CP4 EPSPS reference standard.

1.1.b. MALDI-TOF Mass Spectrometry

The plant-produced CP4 EPSPS was also analyzed by MALDI-TOF mass spectrometry. The ability to identify a protein using this method is dependent upon matching a sufficient number of observed tryptic peptide fragment masses with predicted tryptic peptide fragment masses. In practice, protein identity can be determined when tryptic peptide fragment masses, derived from > 40% of the amino acid sequence of the protein of interest, are matched with the predicted tryptic peptide fragment masses (Jiménez et al. 1998). Peptides are considered to match when differences in molecular weight of less than one Dalton are found between the observed and predicted fragment masses.

The amino acid sequence of the plant-produced CP4 EPSPS protein was deduced from the coding region of the full-length *cp4 epsps* gene present in MON 88913. A coverage map was generated using the identified masses from the MALDI-TOF tryptic mass analysis. Approximately 52.7% (240 of 455 amino acids) of the expected protein sequence was identified (Figure VI-2) and the plant-produced CP4 EPSPS protein was shown to have a molecular weight of 47346.5 Da. This value compares well with 47613.7 Da, the calculated molecular weight of amino acids 1 to 455 of the CP4 EPSPS protein.



Lane	Sample	Amount (ng)
1	Blank lane containing 10 µl Laemmli sample buffer	—
2	MW Markers	—
3	*E. coli*-produced CP4 EPSPS reference standard	1
4	*E. coli*-produced CP4 EPSPS reference standard	1
5	*E. coli*-produced CP4 EPSPS reference standard	2
6	*E. coli*-produced CP4 EPSPS reference standard	2
7	*E. coli*-produced CP4 EPSPS reference standard	3
8	*E. coli*-produced CP4 EPSPS reference standard	3
9	Plant-produced CP4 EPSPS protein from MON 88913	1
10	Plant-produced CP4 EPSPS protein from MON 88913	1
11	Plant-produced CP4 EPSPS protein from MON 88913	2
12	Plant-produced CP4 EPSPS protein from MON 88913	2
13	Plant-produced CP4 EPSPS protein from MON 88913	3
14	Plant-produced CP4 EPSPS protein from MON 88913	3
15	Blank lane containing 10 µl Laemmli sample buffer	—

Figure VI-1. Immunoblot Analysis of the CP4 EPSPS Protein Isolated from MON 88913. Samples of plant-produced CP4 EPSPS protein and *E. coli*-produced CP4 EPSPS reference standard were separated by 4→20% SDS-PAGE, electrotransferred to a PVDF membrane and detected using CP4 EPSPS polyclonal antiserum followed by development using the ECL system (15 sec exposure shown). Amount refers to CP4 EPSPS protein (corrected for purity) loaded per lane. Approximate molecular weights (kDa) correspond to the markers loaded in Lane 2.

```
  1   MLHGASSRPA TARKSSGLSG TVRIPGDKSI SHRSFMFGGL ASGETRITGL
 51   LEGEDVINTG KAMQAMGARI RKEGDTWIID GVGNGGLLAP EAPLDFGNAA
101   TGCRLTMGLV GVYDFDSTFI GDASLTKRPM GRVLNPLREM GVQVKSEDGD
151   RLPVTLRGPK TPTPITYRVP MASAQVKSAV LLAGLNTPGI TTVIEPIMTR
201   DHTEKMLQGF GANLTVETDA DGVRTIRLEG RGKLTGQVID VPGDPSSTAF
251   PLVAALLVPG SDVTILNVLM NPTRTGLILT LQEMGADIEV INPRLAGGED
301   VADLRVRSST LKGVTVPEDR APSMIDEYPI LAVAAAFAEG ATVMNGLEEL
351   RVKESDRLSA VANGLKLNGV DCDEGETSLV VRGRPDGKGL GNASGAAVAT
401   HLDHRIAMSF LVMGLVSENP VTVDDATMIA TSFPEFMDLM AGLGAKIELS
451   DTKAA
```

Figure VI-2. MALDI-TOF Coverage Map of the CP4 EPSPS Protein Isolated from MON 88913. Shaded regions correspond to peptide masses identified by MALDI-TOF. Approximately 52.7% (240 of 455 amino acids) of the expected protein sequence was identified.

1.1.c. N-terminal Sequence Analysis

The results of the N-terminal sequence analysis of the plant-produced CP4 EPSPS protein are summarized in Table VI-1. The experimentally determined N-terminal sequence for the plant-produced CP4 EPSPS protein isolated from MON 88913 confirmed the expected amino acid sequence. Three sequences, all of which are consistent with the N-terminus of the CP4 EPSPS protein, were observed in the CP4 EPSPS protein isolated from MON 88913 seed. The first sequence originates at residue four, glycine, and the other two sequences start at residues two and six (leucine and serine, respectively). The observation of a staggered N-terminal sequence for the plant-produced CP4 EPSPS protein has previously been reported for soybean, canola and cotton (Harrison et al., 1996). Such a finding is not uncommon since the initiator methionine is normally removed from proteins in eukaryotic organisms by an endogenous methionine aminopeptidase (Arfin and Bradshaw, 1988) and the loss of several N-terminal amino acid residues may be due to protease action when plant cells are homogenized. Despite the staggered N-terminus, the sequence data confirm that the ~43 kDa protein isolated from MON 88913 is the CP4 EPSPS protein and that this sequence is consistent with the N-terminal sequence of the *E. coli*-produced CP4 EPSPS reference standard.

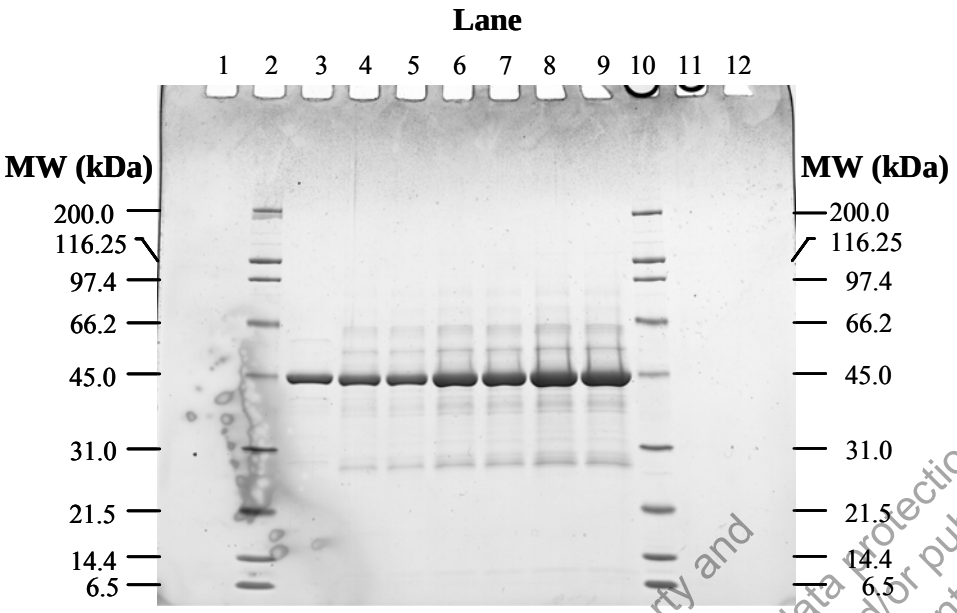
Table VI-1. N-terminal Amino Acid Sequence Analysis of the CP4 EPSPS Protein Purified from MON 88913

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Predicted	M	L	H	G	A	S	S	R	P	A	T	A	R	K	S	S	G	L	S	G
Observed-1				G	A	S	X	R	P	A	T	A	R	K	S	X	G	(L)		
Observed-2						S	X	R	P	A	T	A	X	K	S	S	G	L	S	(G)
Observed-3		L	H	G	A	X	X	R	X	A	X	X	X	X	S	X				

The predicted amino acid sequence (residues 1-20 of 455) of the plant-produced CP4 EPSPS protein was deduced from the coding region of the full-length *cp4 epsps* gene present in MON 88913. Three sequences were observed (1, 2, and 3) from N-terminal sequencing of the ~43 kDa band, all of which are consistent with plant-produced CP4 EPSPS protein. For all sequences undesignated amino acid assignments are shown as an "X", tentative assignments are shown in brackets ( ) and amino acids are assigned using the single letter amino acid code. The single letter IUPAC-IUB amino acid code is **A**, alanine; **G**, glycine; **H**, histidine; **K**, lysine; **L**, leucine; **M**, methionine; **P**, proline; **R**, arginine; **S**, serine; and **T**, threonine.

1.1.d. Electrophoresis and Densitometry

The plant-produced CP4 EPSPS protein was separated using SDS-PAGE and stained with Brilliant Blue G-Colloidal stain (Figure VI-3). The molecular weight and purity of the plant-produced CP4 EPSPS protein were estimated using SDS-PAGE and densitometric analysis, respectively, and the results are summarized below and in Table VI-2. The predominant band in the plant-purified sample had an average molecular weight of 43.1 kDa, estimated by comparison to molecular weight markers on the SDS-polyacrylamide gel. Since this protein migrated with a near identical molecular weight as that of the *E. coli*-produced CP4 EPSPS reference standard analyzed concurrently, the plant-produced CP4 EPSPS protein was concluded to have the same molecular weight as the *E. coli*-produced CP4 EPSPS protein. The average purity of the plant-produced CP4 EPSPS protein was estimated to be 81%.



Lane	Sample	Amount (µg)
1	Blank lane containing 10 µl Laemmli sample buffer	—
2	MW Markers (Bio-Rad, Cat #: 161-0317)	0.5 µg/band
3	*E. coli*-produced CP4 EPSPS reference standard	1
4	Plant-produced CP4 EPSPS protein from MON 88913	1
5	Plant-produced CP4 EPSPS protein from MON 88913	1
6	Plant-produced CP4 EPSPS protein from MON 88913	2
7	Plant-produced CP4 EPSPS protein from MON 88913	2
8	Plant-produced CP4 EPSPS protein from MON 88913	3
9	Plant-produced CP4 EPSPS protein from MON 88913	3
10	MW Markers (Bio-Rad, Cat #: 161-0317)	0.5 µg/band
11	Blank lane containing 10 µl Laemmli sample buffer	—
12	Empty lane (nothing loaded into the well)	—

Figure VI-3. SDS-PAGE Purity and Molecular Weight Analysis of the CP4 EPSPS Protein Isolated from MON 88913. Samples of the plant-produced CP4 EPSPS protein and *E. coli*-produced CP4 EPSPS reference standard were loaded as indicated on a 4→20% polyacrylamide gel. Amount refers to total protein loaded per lane. Approximate molecular weights (kDa) correspond to the markers loaded in Lanes 2 and 10. Following electrophoresis, the Brilliant Blue G-Colloidal stained gel was analyzed densitometrically (see Table VI-2).

Table VI-2. Protein Molecular Weight and Purity Estimation of the CP4 EPSPS Protein Isolated from MON 88913

1 µg Load (Figure VI-3, Lanes 4 and 5)				2 µg Load (Figure VI-3, Lanes 6 and 7)				3 µg Load (Figure VI-3, Lanes 8 and 9)				Average Value	
Replicate 1		**Replicate 2**		**Replicate 1**		**Replicate 2**		**Replicate 1**		**Replicate 2**			
MW	**RQTY**	**MW**	**RQTY**	**MW**	**RQTY**	**MW**	**RQTY**	**MW**	**RQTY**	**MW**	**RQTY**	**MW**	**RQTY**
—	—	—	—	—	—	—	—	86.95	0.6	86.81	0.5	—	—
—	—	—	—	—	—	—	—	75.75	0.4	75.34	0.3	—	—
63.65	1.6	63.48	1.4	63.91	1.5	63.65	2.0	64.48	1.5	64.46	1.2	—	—
61.25	1.2	62.27	1.3	62.55	1.4	62.60	1.5	63.27	2.1	62.95	1.8	—	—
53.41	3.2	53.42	2.9	53.94	3.7	53.99	3.7	54.46	3.5	54.33	3.5	—	—
43.32	**83.5**	**43.33**	**83.8**	**42.92**	**79.8**	**43.04**	**80.1**	**42.94**	**78.6**	**42.96**	**78.9**	**43.1**	**81**
39.95	0.8	40.03	0.6	40.23	0.8	40.34	0.6	40.51	0.6	40.33	1.0	—	—
38.13	2.4	38.20	2.2	38.58	3.2	38.68	2.9	38.90	3.4	38.85	3.2	—	—
36.79	0.7	36.86	0.9	37.11	1.0	37.27	1.0	37.48	1.1	37.49	1.0	—	—
—	—	—	—	32.24	0.3	32.38	0.4	32.50	0.4	32.45	0.3	—	—
29.24	1.3	29.19	1.4	29.69	1.5	29.79	1.3	29.92	1.7	29.92	1.6	—	—
27.79	5.2	27.95	5.4	28.22	6.2	28	5.7	28.54	5.6	28.44	6.2	—	—
—	—	—	—	9.43	0.5	9.70	0.8	10.28	0.5	10.09	0.6	—	—

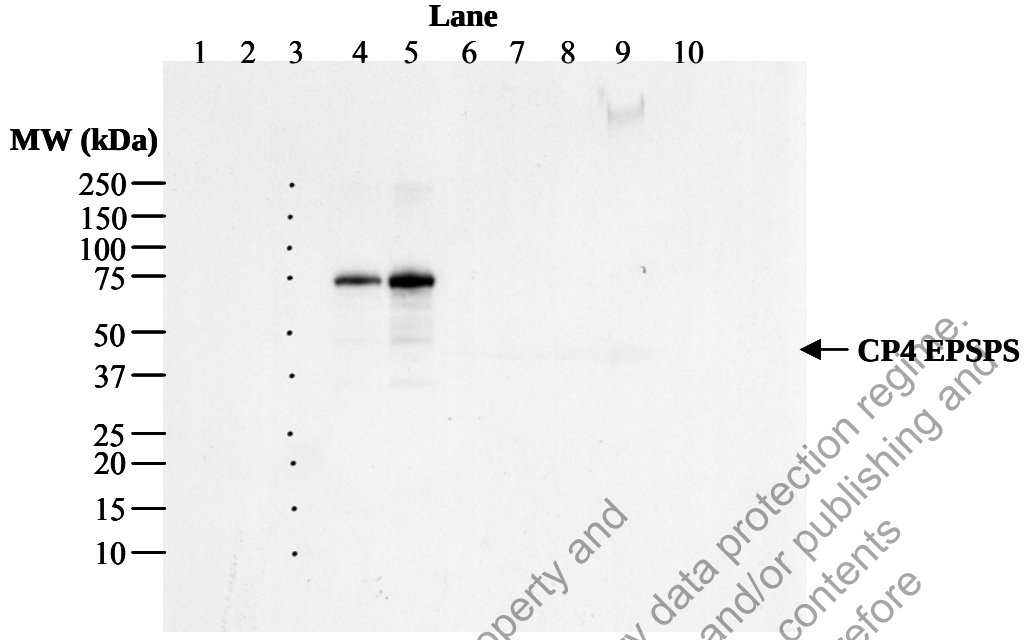
Relative percent quantities (RQTY) of visible bands in the protein isolated from MON 88913 were derived from densitometric analysis of the SDS polyacrylamide gel shown in Figure VI-3, Lanes 4 to 9. The protein molecular weights (MW) were calculated from the molecular weight markers (Figure VI-3, Lanes 2 and 10) using the manufacturer's supplied molecular weight values.

1.1.e. CP4 EPSPS Enzymatic Activity

The specific activity of the plant-produced CP4 EPSPS protein was estimated using a phosphate release assay. The estimated specific activity of the plant-produced CP4 EPSPS protein was 6.7 U/mg of CP4 EPSPS protein. The specific activity of the *E. coli*-produced CP4 EPSPS reference standard protein, which was analyzed concurrently, was 5.0 U/mg of CP4 EPSPS protein. The enzyme assay demonstrated that the plant-produced CP4 EPSPS protein was as active as the *E. coli*-produced CP4 EPSPS protein and, thus, the plant-produced protein is functionally equivalent to the *E. coli*-produced protein with respect to CP4 EPSPS enzyme-mediated release of the phosphate group from PEP.

1.1.f. Glycosylation Analysis

Many eukaryotic proteins are post-translationally modified with carbohydrate moieties (Rademacher et al., 1988). These carbohydrate moieties may be complex branched polysaccharide structures or simple monosaccharides. In contrast, prokaryotic organisms such as *E. coli* lack the necessary biochemical mechanisms required for protein glycosylation. To test whether potential post-translational glycosylation of the plant-produced CP4 EPSPS protein occurred, the isolated plant-produced CP4 EPSPS protein was analyzed for the presence of covalently bound carbohydrate. The *E. coli*-produced CP4 EPSPS reference standard, a negative control in this experiment, and transferrin protein, a positive control, were analyzed concurrently. The positive control (transferrin) was clearly detected in a concentration-dependent manner at loadings of 0.50 and 1.0 µg/lane (Lanes 4 and 5 of Figure VI-4). At the 30 sec exposure time, a barely discernable band, close to the expected position for the CP4 EPSPS, was observed for both the plant-produced CP4 EPSPS protein and the *E. coli*-produced CP4 EPSPS reference standard, the negative control. The very faint bands observed for both the plant-produced CP4 EPSPS protein and *E. coli*-produced CP4 EPSPS reference standard protein are likely due to a non-specific interaction between the detection reagent (Streptavidin-HRP conjugate) and protein mass bound to the blot and do not represent glycosylation of the CP4 EPSPS protein.



Lane	Sample	Amount (µg)
1	Empty lane (nothing loaded into the well)	—
2	Blank lane containing 10 µl Laemmli sample buffer	—
3	Pre-Stained MW Markers (Bio-Rad, Cat #: 161-0374)	—
4	Transferrin (Positive control)	0.5
5	Transferrin (Positive control)	1
6	*E. coli*-produced CP4 EPSPS reference standard	0.5
7	*E. coli*-produced CP4 EPSPS reference standard	1
8	Plant-produced CP4 EPSPS protein from MON 88913	0.5
9	Plant-produced CP4 EPSPS protein from MON 88913	1
10	Blank lane containing 10 µL Laemmli sample buffer	—

Figure VI-4. Glycosylation Analysis of the CP4 EPSPS Protein Isolated from MON 88913. Samples of the plant-produced CP4 EPSPS protein, *E. coli*-produced CP4 EPSPS reference standard (negative control), and transferrin (positive control) were separated by SDS-PAGE and electrotransferred to a PVDF membrane. If present, the protein-bound carbohydrate moiety is labeled with biotin, and detected with streptavidin-horseradish peroxidase and enhanced chemiluminescence (30 sec exposure shown). Amount refers to total protein loaded per lane, except for plant-produced and *E. coli*-produced CP4 EPSPS protein samples which were corrected for purity. Approximate molecular weights (kDa) correspond to the markers loaded in Lane 3.

1.1.g. Conclusions

The plant-produced CP4 EPSPS protein isolated from MON 88913 was identified and characterized using a battery of analytical tests. These analytical tests, some of which involved side-by-side comparisons with the *E. coli*-produced CP4 EPSPS reference standard, included: (1) immunoblot analysis and densitometry, (2) MALDI-TOF mass spectrometry, (3) N-terminal sequence analysis, (4) SDS-PAGE and densitometry, (5) CP4 EPSPS enzymatic activity analysis, and (6) glycosylation analysis.

On the basis of western blot analysis, the electrophoretic mobility and immunoreactive properties of the plant-produced CP4 EPSPS protein were demonstrated to be comparable to those of the *E. coli*-produced CP4 EPSPS reference standard. MALDI-TOF mass spectral analysis of the tryptic digest of the CP4 EPSPS protein isolated from MON 88913 yielded peptide masses consistent with peptide masses derived from the CP4 EPSPS protein. The N-terminus of the major protein band contained in the plant-produced CP4 EPSPS protein preparation was consistent with the predicted sequence of amino acids translated from the *cp4 epsps* coding sequence within MON 88913. Molecular weight and purity, estimated by SDS-PAGE and densitometric analysis, were observed to be 43.1 kDa and 81%, respectively. The molecular weight of the plant-produced CP4 EPSPS protein using MALDI-TOF mass spectrometry was also consistent with the molecular weight of the CP4 EPSPS protein calculated from the amino acid sequence. The functional activities of the plant-produced CP4 EPSPS protein and the *E. coli*-produced CP4 EPSPS reference standard were determined and found to be functionally equivalent.

These data provide a detailed characterization of the CP4 EPSPS protein isolated from MON 88913 and establishes the identity of the plant-produced CP4 EPSPS protein and its equivalence to the *E. coli*-produced CP4 EPSPS protein standard.

Section 2. Levels of the CP4 EPSPS Protein in MON 88913

CP4 EPSPS protein levels in MON 88913 were determined by a validated enzyme-linked immunosorbent assay (ELISA). The levels of the CP4 EPSPS protein in young leaf, overseason leaf (OSL), root, seed, and pollen tissues were determined in tissues collected from MON 88913 produced in replicated field trials across four U.S. field locations during 2002. CP4 EPSPS protein levels for all tissue types were calculated on a microgram (µg) per gram (g) fresh weight (fwt) basis. Moisture content was measured for young leaf, overseason leaf OSL-1, OSL-2, OSL-3; root; and seed tissues. Protein levels in these tissues were converted to a dry weight (dwt) basis by calculation. The mean CP4 EPSPS protein levels across four sites for young leaf, OSL1, OSL2, OSL3, root, and seed tissues of MON 88913 were 970, 1400, 690, 630, 99, and 340 µg/g dwt, respectively, (Table VI-3). The mean CP4 EPSPS protein level across four sites for pollen was 4.0 µg/g fwt. The levels of the CP4 EPSPS protein in all tissue types from MON 88913(-) were less than the assay limits of quantitation (LOQ) presented in Table VI-3.

Table VI-3. CP4 EPSPS Protein Levels in MON 88913 Tissues†

Tissue Type[1]	Mean CP4 EPSPS Protein Level in µg/g fwt (SD)[2]	Range[3] (µg/g fwt)	Mean CP4 EPSPS Protein Level in µg/g dwt (SD)[4]	Range (µg/g dwt)	LOQ / LOD (µg/g fwt)
Young Leaf	170 (64)	64 – 260	970 (460)	270 – 1700	0.23 / 0.069
OSL1[5]	270 (99)	77 – 410	1400 (540)	480 – 2600	0.23 / 0.069
OSL2	170 (44)	63 – 260	690 (210)	290 – 1000	0.23 / 0.069
OSL3	160 (61)	66 – 260	630 (230)	290 – 1100	0.23 / 0.069
Root	31 (11)	19 – 64	99 (40)	57 – 200	0.23 / 0.073
Seed	310 (110)	67 – 550	340 (120)	72 – 580	2.7 / 1.7
Pollen	4.0 (0.22)	3.8 – 4.3	n/a[6]	n/a[6]	0.23 / 0.11

†Field-produced tissues in 2002 were from Baldwin County, Alabama; Tulare County, California; Clarke County, Georgia; and Hockley County, Texas.

[1]Description of the tissue types is provided in Appendix C.

[2]Protein levels are expressed as micrograms (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis. The arithmetic mean and standard deviation (SD) were calculated for each tissue type across sites.

[3]Minimum and maximum values were determined for each tissue type across all sites.

[4]Protein levels are expressed as µg/g of tissue on a dry weight (dwt) basis. The dwt values were calculated by dividing the fwt values by the dry weight conversion factors (Appendix C) obtained from moisture analysis data.

[5]Tissues OSL1 – OSL3 represent overseason leaves collected at different time points throughout the growing season (Appendix C).

[6]Because of limited quantities of cotton pollen, moisture levels could not be determined in this tissue and values are presented on a fwt basis only.

Section 3. Estimate of Dietary Exposure

The safety assessment of the CP4 EPSPS protein involved the exposure of humans and farm animals to the protein in the diet. Specifically whether the protein will be consumed at similar levels to other foods, or as a macroconstituent of the diet. Farm animals may be exposed to the CP4 EPSPS protein through dietary intake of feed derived from MON 88913. Farm animals may consume whole cottonseed, cottonseed meal, hulls, and other processing byproducts, which are discussed in greater detail in Part VII.

Cottonseed is not consumed by humans in the U.S. due to the level of natural toxicants, primarily gossypol, in the majority of commercial cotton varieties. However, food products containing highly processed and refined fractions of cottonseed are consumed, primarily refined cottonseed oil (discussed in greater detail in Part VII). Linters are an industrial byproduct of ginning, but some fractions are consumed as a highly processed viscose product composed of nearly pure cellulose (NCPA, 2002a). There is virtually no human exposure to CP4 EPSPS protein through dietary intake of cottonseed oil derived from MON 88913. The quantity of food and feed derived from cotton and consumed on a daily basis by humans and livestock, as well as the levels of CP4 EPSPS protein in cotton food and feed products, are necessary to derive an estimate of daily dietary exposure (DDE). An estimate of the dietary exposure from the primary edible food product of cottonseed is produced below.

The mean adult consumption of cottonseed oil in the U.S. is 0.0786 g/kg body weight/day. This is based on the total consumption of cottonseed oil in food in the U.S., 9.3×10^8 lb/year (NCPA, 1993) and a U.S. adult population of 209 million. Cottonseed and hulls are consumed in the highest amounts by ruminant animals, typically dairy cows. The daily consumption of cottonseed by a dairy cow is calculated to be 5.3 g/kg/day (Hoard's Dairyman, 1984). The DDE is computed as follows:

DDE = Cotton product consumption (g/kg) × CP4 EPSPS protein concentration (µg/g)

For calculations regarding cottonseed oil, cottonseed protein was assumed to be present in cottonseed oil at the limit of detection (1.3 µg protein/ml of oil), although previous studies have found no detectable protein in cottonseed oil (Fuchs et al., 1993). The concentration of CP4 EPSPS protein in cottonseed was derived from the amount of CP4 EPSPS produced in cottonseed on a dry weight basis divided by the total percent of protein in MON 88913 cottonseed on a dry weight basis (see section 4.2, Part VI).

Using upper bound estimates of consumption of cottonseed, or food and feed products derived from cottonseed, it is possible to calculate the margin of exposure (MOE) for these proteins. The margin of exposure is defined as the ratio of the no observed effect level (NOEL) derived from toxicology tests to the estimate of human and animal DDE. The MOE is computed as follows:

MOE = NOEL (mg/kg)/DDE (µg/kg)

The exposure calculation makes the conservative assumption that there is no loss of the introduced protein during the processing of cottonseed into products derived from cottonseed. It also assumes that 100% of the cottonseed, or products derived from cottonseed, is derived from MON 88913. This scenario would be highly unlikely, given the number of commercial cotton varieties that exist in the marketplace.

3.1. Estimated Dietary Exposure to the CP4 EPSPS Protein in MON 88913

The maximum amount of CP4 EPSPS in cottonseed oil was calculated to be 1.6×10^{-3} µg/g cottonseed protein in oil, based on the limit of detection of cottonseed protein in oil [1.3 µg protein/mL oil x 0.12% CP4 EPSPS (Table VI-3, Part VI)]. Data presented in Section 2 of this Part VI show that the mean CP4 EPSPS protein levels in MON 88913 cottonseed collected during 2002 was 340 µg/g dwt for MON 88913 (Table VI-3). The average percent dry weight of total protein in MON 88913 is 28.23% dwt (Table VII-1). Using this average, the CP4 EPSPS protein would represent approximately 0.12 percent of the total protein in MON 88913.

3.2. Margins of Exposure

The highest dose of the CP4 EPSPS protein that was administered by gavage to mice, 572 mg/kg (572,000 µg/kg), produced no adverse effects; therefore the no observed effect level (NOEL) in the CP4 EPSPS mouse gavage study is 572,000 µg/kg. The calculated margins of exposure for consumption of CP4 EPSPS protein in MON 88913 are presented in Table VI-4 for humans that consume cottonseed oil or farm animals that consume whole cottonseed or the meal and hulls from MON 88913 cottonseed.

Large margins of exposure have been calculated for CP4 EPSPS protein in humans (4.6×10^{9}) and dairy cows (3.17×10^{5}). These calculated margins of exposure indicate that there is no risk to human and animal health that will be associated with dietary exposure to food and feed products derived from MON 88913.

Table VI-4. Margins of Exposure for Dietary Consumption of CP4 EPSPS Proteins in MON 88913 When Used as Food or Animal Feed

Parameter	Adult Human - Oil	Dairy Cow - Feed
Daily consumption (g/kg body wt)	0.0786	5.3
DDE (mg/ kg body wt/day)	1.26×10^{-4}	1.80×10^{-3}
MOE	4.6×10^{9}	3.17×10^{5}

Section 4. Assessment of the Potential for Allergenicity of the CP4 EPSPS Protein Produced in MON 88913

This assessment of the allergenic potential of the CP4 EPSPS protein addresses the following questions, which identify characteristics of known allergens:

1. Is the protein from a known allergenic source?
2. Does the protein represent a relatively large portion of the total protein in MON 88913?
3. Is the protein structurally similar, based on amino acid sequence, to known allergens?
4. Is the protein resistant to digestion in simulated mammalian gastric fluid?

The following sections address each of these questions and demonstrate that CP4 EPSPS is not allergenic. General information on the methods used in assessing the last two questions - structural similarity to known allergens and stability in simulated digestive fluids- is provided in Appendix D.

4.1. Source of the CP4 EPSPS Protein

As described in Part VI, Section 1, the *cp4 epsps* coding sequence was obtained from a naturally occurring bacterium and has been identified by the American Type Culture Collection as an *Agrobacterium* species. Because there are no reports of allergies to *Agrobacterium* species (see section 5.3 in Part VI), it can be concluded that the CP4 EPSPS protein is not from a known allergenic source. Furthermore, according to FAO/WHO (2001), there is no known population of individuals sensitized to bacterial proteins.

4.2. Proportion of Total Protein – CP4 EPSPS

The CP4 EPSPS protein was detected at relatively low levels in various plant tissues at a number of time points during the growing season. Among these tissues, cottonseed is the most relevant to an assessment of food allergenicity. Data presented in Section 2 of Part VI show that the mean average CP4 EPSPS protein level in MON 88913 seed was 340 µg/g dwt (Table VI-3), whereas the average percent dry weight of total protein in MON 88913 was 28.23% dwt (Table VII-1). Using these averages, the CP4 EPSPS protein would represent only approximately 0.12 percent of the total protein in MON 88913 (340 µg/g ÷282,300 µg x 100 = 0.12%). Therefore, the CP4 EPSPS protein represents only a small portion of the total protein in MON 88913.

4.3. Bioinformatic Analyses of Sequence Similarity of the CP4 EPSPS protein produced in MON 88913 to Allergens

A bioinformatic assessment of the CP4 EPSPS protein, using allergen and public domain protein sequences databases, has been performed and demonstrates the absence of sequence similarity to proteins known to pose human health risks. No immunologically relevant sequences (eight contiguous amino acid identities) were detected when the

amino acid sequence of the CP4 EPSPS protein was compared to the ALLERGEN3 sequence database. Together, these data demonstrate that the CP4 EPSPS protein present in MON 88913 does not share structurally relevant or immunologically relevant amino acid sequence similarities with allergens or gliadins. Therefore, it is highly unlikely that this protein may contain immunologically cross-reactive allergenic epitopes.

4.4. Stability of the CP4 EPSPS Protein in Simulated Digestive Fluids

Harrison et al. (1996) demonstrated that the CP4 EPSPS protein is rapidly degraded in simulated digestive fluids. The half-life for CP4 EPSPS was less than 15 seconds in the gastric system and less than 10 minutes in the intestinal system, based on western blot analysis. Therefore, if any of the CP4 EPSPS protein were to survive in the gastric system, it would be rapidly degraded in the intestine. As a comparison, 50% of solid food has been estimated to empty from the human stomach in two hours, while 50% liquid empties in approximately 25 minutes (Sleisenger and Fordtran, 1989). Based on this information, CP4 EPSPS protein is expected to degrade rapidly in the mammalian digestive tract.

Subsequent experiments were performed to assess the *in vitro* digestibility of the CP4 EPSPS protein in simulated gastric fluid (SGF). As with the previous study (Harrison et al., 1996), the CP4 EPSPS protein used was produced in and purified from *E. coli*. Digestibility was assessed by three methods, including SDS-PAGE gel staining, western blot analysis, and EPSPS enzymatic activity assay.

The results of these experiments demonstrate that the *E. coli*-produced mature CP4 EPSPS protein was rapidly digested after incubation in SGF. The SDS-PAGE colloidal blue gel staining method demonstrated that at least 98% of the *E. coli*-produced mature CP4 EPSPS protein was digested in SGF within 15 seconds (Figure VI-5). No degenerative bands due to digestion were observed. Western blot analysis (Figure VI-6) confirmed that greater than 95% of the *E. coli*-produced CP4 EPSPS protein was digested in SGF within 15 seconds. Likewise, it was demonstrated that the EPSPS activity was reduced to <10% within 15 seconds of incubation of the CP4 EPSPS protein in SGF (Table VI-5). In summary, and in complement to the earlier study by Harrison et al., the three methods (SDS PAGE, western blot, and functional assay) all demonstrate that the *E. coli*-produced CP4 EPSPS protein is rapidly degraded in simulated gastric fluid.

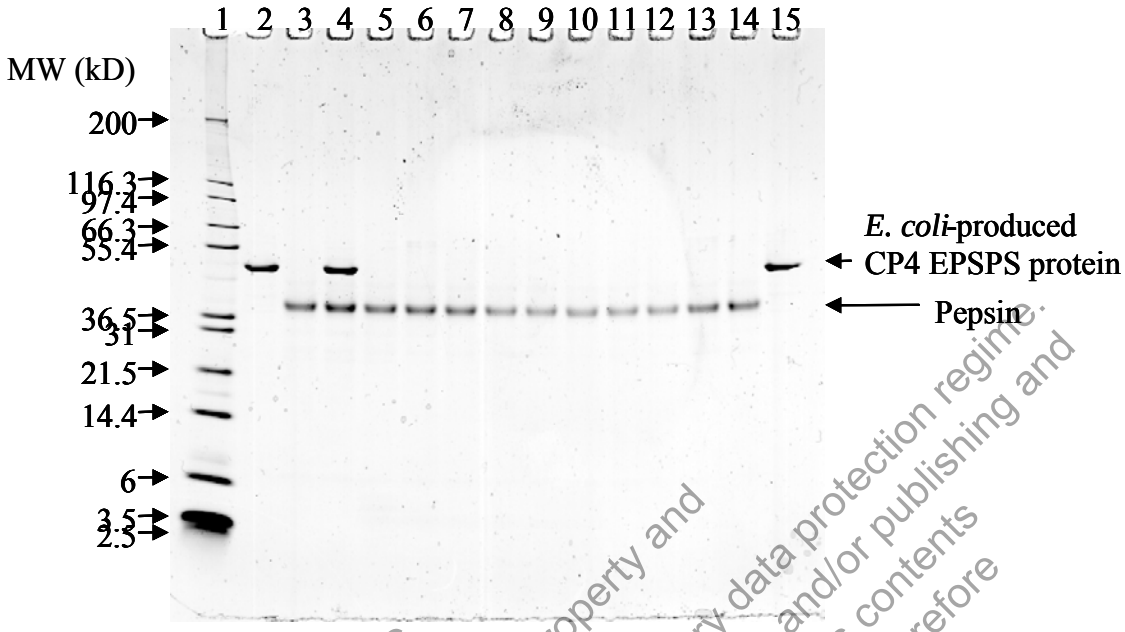


Figure VI-5. Colloidal Blue Stained SDS-PAGE Gel Showing the Digestion of Purified *E. coli*-produced CP4 EPSPS Protein in Simulated Gastric Fluid.
Proteins were separated by SDS-PAGE using a 10→20% polyacrylamide gradient in a tricine buffered gel. Proteins were detected by staining with Brilliant Blue G stain. *E. coli*-produced CP4 EPSPS protein was loaded at 500 ng per lane based on pre-digestion concentrations.

	Lane Description	Incubation Time
1	Molecular weight markers	
2	Experimental control without pepsin (P0)	0 s
3	Experimental control without CP4 EPSPS (N0)	0 s
4	CP4 EPSPS protein in SGF, T = 0	0 s
5	CP4 EPSPS protein in SGF, T = 1	15 s
6	CP4 EPSPS protein in SGF, T = 2	30 s
7	CP4 EPSPS protein in SGF, T = 3	1 min
8	CP4 EPSPS protein in SGF, T = 4	2 min
9	CP4 EPSPS protein in SGF, T = 5	4 min
10	CP4 EPSPS protein in SGF, T = 6	8 min
11	CP4 EPSPS protein in SGF, T = 7	15 min
12	CP4 EPSPS protein in SGF, T = 8	30 min
13	CP4 EPSPS protein in SGF, T = 9	60 min
14	Experimental control without CP4 EPSPS (N9)	60 min
15	Experimental control without pepsin (P9)	60 min

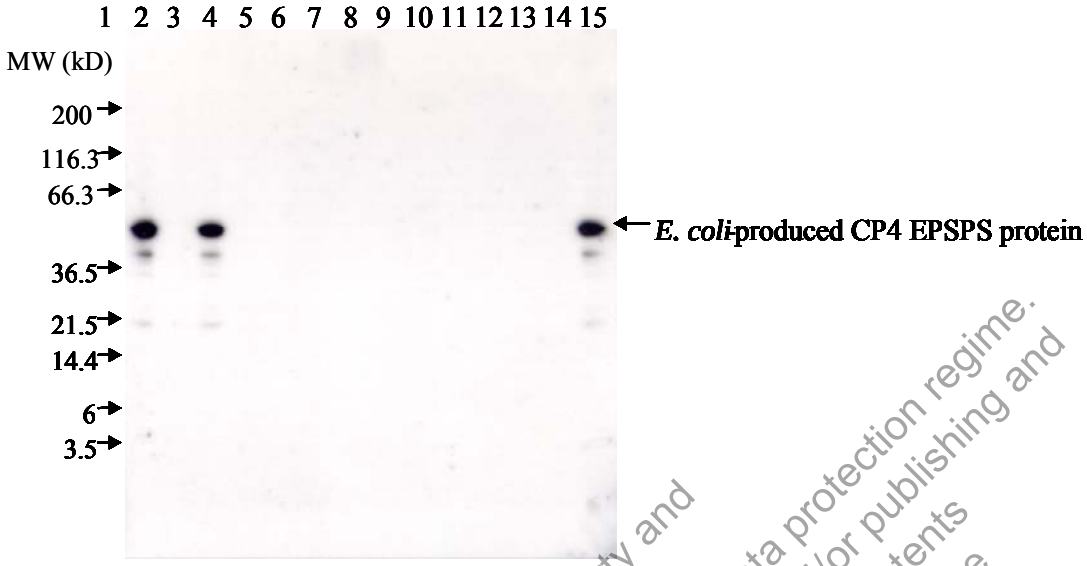


Figure VI-6. Western Blot Showing the Digestion of Purified *E. coli*-produced CP4 EPSPS Protein in Simulated Gastric Fluid. Proteins were separated by SDS-PAGE using a 10→20% polyacrylamide gradient in a tricine buffered gel. *E. coli*-produced CP4 EPSPS protein was loaded at 1 ng per lane based on 90% purity and pre-digestion concentrations.

Lane	Description	Incubation Time
1	Molecular weight markers	
2	Experimental control without pepsin (P0)	0 sec
3	Experimental control without CP4 EPSPS (N0)	0 sec
4	CP4 EPSPS protein in SGF, T = 0	0 sec
5	CP4 EPSPS protein in SGF, T = 1	15 sec
6	CP4 EPSPS protein in SGF, T = 2	30 sec
7	CP4 EPSPS protein in SGF, T = 3	1 min
8	CP4 EPSPS protein in SGF, T = 4	2 min
9	CP4 EPSPS protein in SGF, T = 5	4 min
10	CP4 EPSPS protein in SGF, T = 6	8 min
11	CP4 EPSPS protein in SGF, T = 7	15 min
12	CP4 EPSPS protein in SGF, T = 8	30 min
13	CP4 EPSPS protein in SGF, T = 9	60 min
14	Experimental control without CP4 EPSPS (N9)	60 min
15	Experimental control without pepsin (P9)	60 min

Table VI-5. Specific Activity of *E. coli*-produced CP4 EPSPS Protein after Digestion in Simulated Gastric Fluid

Sample	Specific Activity (Units/mg protein)
Experimental control without pepsin incubated for 0 seconds	4.92
Experimental control without pepsin incubated for 60 minutes	2.10
E. coli-produced CP4 EPSPS protein in SGF incubated for 0 seconds	5.63
E. coli-produced CP4 EPSPS protein in SGF incubated for 15 seconds	0.27
E. coli-produced CP4 EPSPS protein in SGF incubated for 30 seconds	0.15
E. coli-produced CP4 EPSPS protein in SGF incubated for 60 seconds	0.15
Experimental control without CP4 EPSPS incubated for 0 seconds	0.02
Experimental control without CP4 EPSPS incubated for 60 minutes	0.05
Buffer Blank	0.01

4.5. Conclusions

The data and information provided in this section address the questions important to an assessment of allergenic potential. There are no reports of allergies to the donor organism, an *Agrobacterium* species; thus, the CP4 EPSPS protein is not from a known allergenic source. The CP4 EPSPS protein represents no more than 0.12% of the total protein in MON 88913 cottonseed. Therefore, the CP4 EPSPS protein would represent a very small portion of the total protein present in food and feed derived from MON 88913. A bioinformatic analysis demonstrated that the CP4 EPSPS protein does not share structurally relevant or immunologically relevant amino acid sequence similarities with known allergens or gliadins. Therefore, it is highly unlikely that this protein may contain immunologically cross-reactive allergenic epitopes. Experiments with *E. coli*-produced CP4 EPSPS protein demonstrate that it was rapidly digested in simulated digestive fluids, a characteristic shared among proteins with a history of safe consumption. Thus, it is concluded that the CP4 EPSPS protein in Roundup Ready Flex cotton MON 88913 does not pose a significant allergenic risk.

Section 5. Safety Assessment of the CP4 EPSPS Protein in MON 88913

The previous section demonstrated the lack of structural similarity of the CP4 EPSPS protein to known allergens (including gliadins) and the rapid digestibility of the CP4 EPSPS protein in simulated gastric fluids. This section includes information on the structural similarity of the CP4 EPSPS protein to other known proteins and the acute oral toxicity of the CP4 EPSPS protein by mouse gavage. It also provides additional components of the safety assessment, including an evaluation of the donor organism, *Agrobacterium* sp. strain CP4, and an evaluation of the similarity of the CP4 EPSPS protein to EPSPSs naturally present in foods with a long history of safe use and to the CP4 EPSPS protein in Roundup Ready crops, for which there is experience in safe consumption.

5.1. Structural Similarity of the CP4 EPSPS Protein to All Known Proteins

Potential structural similarities shared between the CP4 EPSPS protein and proteins in the ALLPEPTIDES database were evaluated using the FASTA sequence alignment tool. Although the FASTA program directly compares amino acid sequences (i.e., primary protein structure), the alignment data may be used to infer higher order structural similarities (i.e., secondary and tertiary protein structures). Proteins that share a high degree of similarity throughout the entire length are often homologous. Homologous proteins share secondary structure and common three-dimensional folds. Identified proteins were ranked according to their degree of similarity. The most significant alignment was to the CP4 EPSPS protein found in biotechnology-derived *Glycine max* [Roundup Ready soybean] (Accession No. AY125353), demonstrating 100.0% identity over a 455 aa overlap window with an *E*-score of 1.4 e-165. This result was expected, as the CP4 EPSPS proteins in Roundup Ready soybean and MON 88913 are identical. All the remaining alignments with significant *E*-scores (i.e., < 1 e-5) were to other members of the EPSPS protein family and, therefore, do not present a risk of adverse biological activity toward humans and animals.

Potential structural similarities shared between the CP4 EPSPS protein and proteins in the toxin database were also evaluated using the FASTA sequence alignment tool. Identified proteins were ranked according to their degree of similarity. The most significant alignment was to the *Bacillus cereus* sphingomyelinase c precursor protein (Accession No. P11889), demonstrating only 28.2% identity over a 131 aa overlap window with an *E*-score of 0.26. Since the length and quality of the alignments are low, these data demonstrate that the CP4 EPSPS protein is highly unlikely to share any structural homology to any known toxin proteins.

Results of the FASTA sequence alignments demonstrated a lack of structurally relevant similarity between the CP4 EPSPS protein and any known toxic or pharmacologically active proteins relevant to human or animal health.

5.2. Evaluation of the Acute Oral Toxicity of the CP4 EPSPS Protein by Mouse Gavage

An oral acute toxicity study was conducted with *E. coli*-produced CP4 EPSPS protein (Harrison et al., 1996). The CP4 EPSPS protein produced in MON 88913 has been demonstrated to be equivalent to the *E. coli*-produced CP4 EPSPS protein standard as a part of the characterization of MON 88913 (refer to Section 1, Part VI). Acute administration was considered appropriate to assess the safety of the CP4 EPSPS protein since proteins that are toxic act via acute mechanisms (Sjoblad et al., 1992; Pariza and Foster, 1983; Jones and Maryanski, 1991). The no effect level (NOEL) for oral toxicity in mice was 572 mg/kg, the highest dose tested (Harrison et al., 1996). There were no statistically significant differences in body weight, cumulative body weight, or food consumption between the vehicle or bovine serum albumin protein control groups and CP4 EPSPS protein-treated groups.

5.3. Safety of the Donor Organism - *Agrobacterium* sp. Strain CP4

Agrobacterium sp. strain CP4 was chosen as the donor organism because this bacterium exhibited tolerance to glyphosate by producing a naturally glyphosate-tolerant EPSPS (Padgette et al., 1996). The bacterial isolate, CP4, was identified by the American Type Culture Collection as an *Agrobacterium* species. *Agrobacterium* species are not known for human or animal pathogenicity, and are not commonly allergenic (FAO/WHO, 1991). Furthermore, according to FAO/WHO, (2001), there is no known population of individuals sensitized to bacterial proteins.

The EPSPS from *Agrobacterium* sp. strain CP4 is highly tolerant to inhibition by glyphosate and has high catalytic efficiency, compared to most glyphosate-tolerant EPSPSs (Barry et al., 1992; Padgette et al., 1996). EPSPS is an enzyme of the shikimate pathway integral to aromatic amino acid biosynthesis in plants and microorganisms (Levin and Sprinson, 1964; Steinrücken and Amrhein, 1980). Therefore, this enzyme and its activity are not novel in food derived from plant sources. Genes for numerous EPSPSs have been cloned (Padgette et al., 1996) and active site domains are conserved among the known EPSPSs. Bacterial EPSPSs have been well characterized with respect to their three dimensional X-ray crystal structures (Stallings et al., 1991) and detailed kinetic and chemical mechanisms (Anderson and Johnson, 1990). The CP4 EPSPS protein thus represents one of many different EPSPSs found in nature; the CP4 and native EPSPS enzymes are functionally equivalent except for their affinity to glyphosate.

Agrobacterium sp. strain CP4 has been previously reviewed as a part of the safety assessment of the donor organism during Monsanto consultations with the FDA regarding Roundup Ready soybean (1994), Roundup Ready canola (1995), Roundup Ready cotton (1995), Roundup Ready corn NK603 (1996), and Roundup Ready sugar beet (1998). Further, the Environmental Protection Agency (EPA) has established an exemption from the requirement of a tolerance for residues of CP4 EPSPS and the

genetic material necessary for its production in all plants (40 CFR 180.1174; 61 FR 40340).

5.4. Similarity of CP4 EPSPS to EPSPSs Derived from Food Sources With a Long History of Safe Consumption

The CP4 EPSPS protein present in MON 88913 is similar to EPSPSs consumed in a variety of food and feed sources. The *cp4 epsps* coding region has been completely sequenced and encodes a 47.6 kDa protein consisting of a single polypeptide of 455 amino acids. As shown in Table VI-6, the CP4 EPSPS protein is homologous to EPSPSs naturally present in plants, including food crops (*e.g*., soybean and corn), and fungal and microbial food sources such as Baker's yeast (*Saccharomyces cerevisiae*), which have a history of safe human consumption (Padgette et al., 1996; Harrison et al., 1996). The similarity of the CP4 EPSPS protein to EPSPSs in a variety of foods supports extensive human consumption of the family of EPSPS proteins and the lack of health concerns. Further, the ubiquitous presence of homologous EPSPS enzymes in food crops and common microbes establishes that EPSPS proteins, and their enzyme activity, pose no hazards for human consumption.

Table VI-6. Comparison of the Deduced Amino Acid Sequence of Native CP4 EPSPS to That of Other EPSPSs

	soybean	**corn**	**petunia**	***E. coli***	***B. subtilis***	***S. cerevisiae***
CP4 EPSPS						
% sequence identity	26	24	23	26	41	30
% sequence similarity	51	49	50	52	59	54

5.5. Presence of the CP4 EPSPS Protein in Commercial Food and Feed Crops

Herbicide tolerant crops, primarily those with the Roundup Ready trait, were planted on 49.7 million hectares globally in 2003 (James, 2003). Roundup Ready soybean is a significant example of the growth in acceptance of these crops since its introduction in 1996. Commercially, Roundup Ready soybean has been rapidly adopted by growers, increasing from 1% of U.S. soybean acres (400,000 hectares) planted in 1996 to 79% of U.S. soybean acres in 2002. Globally in 2003, Roundup Ready soybean containing the CP4 EPSPS protein was produced on approximately 41.4 million hectares, including almost 13 million hectares in Argentina and over three million hectares in Canada. An additional approximately seven million hectares of other Roundup Ready crops, including corn, cotton and canola were planted globally (James 2003).

The amino acid sequence of the CP4 EPSPS protein produced in MON 88913 is identical to, or shares greater than 99% sequence identity with, the amino acid sequence of the CP4 EPSPS protein produced in these other Roundup Ready crops that have completed the FDA consultation process and are commercialized. As described above, humans and animals have consumed these crops, or their processed products, since 1996 (James,

2002). This demonstrates significant experience with the safe use of these Roundup Ready crops and the CP4 EPSPS protein they contain and anticipates the same safe use experience for MON 88913 and the CP4 EPSPS protein it contains.

5.6. Conclusions

Studies and evaluations were performed to obtain data and information to assess the safety of the CP4 EPSPS protein in MON 88913. Digestibility studies demonstrated that the CP4 EPSPS protein is rapidly degraded in simulated digestive fluids. In a bioinformatics analysis no biologically relevant structural similarities were observed between the CP4 EPSPS protein and pharmacologically active proteins that are known to cause adverse health effects in humans or animals. Results from the acute oral toxicity study demonstrated that the mature CP4 EPSPS protein is not toxic. The donor organism, *Agrobacterium* sp. strain CP4 is not known for human or animal pathogenicity, and is not commonly allergenic. The CP4 EPSPS protein produced is functionally equivalent to native EPSPSs except for its affinity for glyphosate. Additionally, *Agrobacterium* sp. strain CP4 and the CP4 EPSPS protein it produces, have been previously reviewed as a part of the safety assessment of the donor organism for other crops. Finally, a history of, and experience in, the safe use of CP4 EPSPS protein is demonstrated, based on the similarity of the CP4 EPSPS protein in MON 88913 to EPSPSs naturally present in food crops (e.g., soybean and corn) and in fungal and microbial food sources such as Baker's yeast (*Saccharomyces cerevisiae*) (Padgette et al., 1996; Harrison et al., 1996), and to the CP4 EPSPS protein produced in a number of other Roundup Ready food crops that have already completed the FDA consultation process, and are commercialized, including Roundup Ready soybean, Roundup Ready corn NK603, Roundup Ready canola, and Roundup Ready cotton.

Using guidance provided by the FDA, a conclusion of "no concern" is reached for the donor organism (Figure VI-7). Figure VI-8 is another of the decision trees reproduced from the FDA Food Policy (FDA, 1992) and identifies the considerations to be used in evaluating the safety of the proteins introduced from the donor. As with the donor, the information provided in this section and summarized above leads to a finding of "no concerns" for the CP4 EPSPS protein in MON 88913. It is concluded that, the data and information provided in Section 5, and supported by other data and information in this Part VI, demonstrate that the CP4 EPSPS protein in MON 88913 is safe for human and animal consumption.

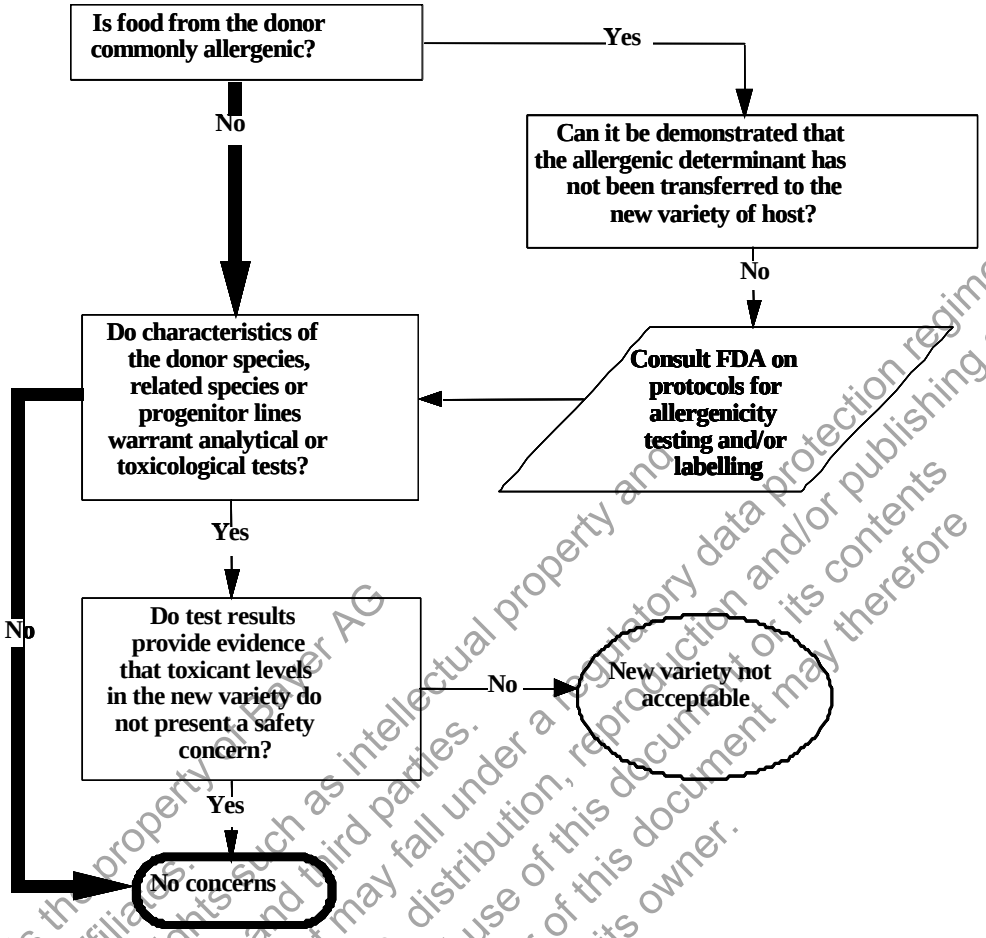


Figure VI-7. Safety Assessment of New Varieties: The Donor

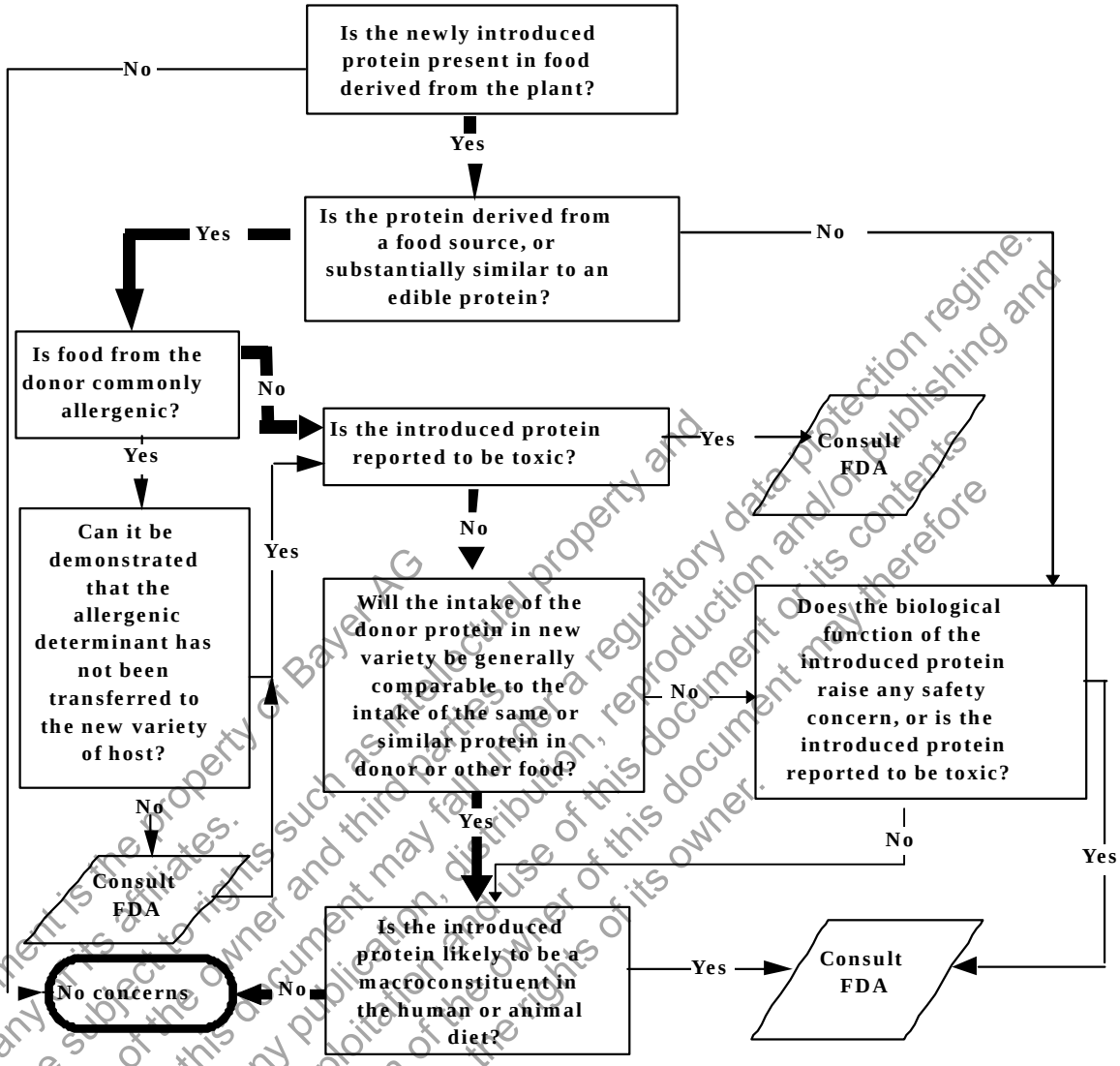


Figure VI-8. Safety Assessment of New Varieties: Proteins Introduced from the Donor

PART VII: FOOD/FEED SAFETY AND NUTRITIONAL ASSESSMENT OF MON 88913

Section 1. Cotton Varieties as the Comparable Food and Feed

In order to assess if the Roundup Ready trait in MON 88913 caused any unintended effects on the composition of the cottonseed, a compositional analysis was conducted of delinted cottonseed collected from MON 88913 grown under replicated field conditions at four U.S. locations sites. MON 88913 was compared to MON 88913(-), which has background genetics representative of the MON 88913 but does not contain the DNA insert or produce the CP4 EPSPS protein. Additionally, 16 commercial conventional cotton varieties produced in the same field trial alongside MON 88913 and MON 88913(-) were used in the compositional analyses. Values derived from these conventional varieties were used as references to produce a 99% tolerance interval for conventional cotton. In addition, cottonseed produced under replicated field conditions at two locations was used to produce refined, bleached and deodorized cottonseed oil and cottonseed meal. A compositional analysis was conducted on the oil and meal to compare MON 88913 to MON 88913(-) and to a 99% tolerance interval for oil and meal produced from conventional cotton.

Section 2. Historical Uses of Cotton

Cotton is the leading plant fiber crop produced in the world, and is grown worldwide, typically in arid regions of the tropical or subtropical areas (Niles and Feaster, 1984). Current hypotheses suggest that linted cottons developed in both the Old and New Worlds and that the presence of lint provided the chief impetus for domestication (Lee, 1984). Today, cotton is considered the most prominent source of textile fiber worldwide, and makes up over 40% of the total fiber used (USDA-ERS, 2002).

2.1. History and Utilization of Cotton

The history and development of cotton has been previously discussed in Part IV, Section 1.2. The USDA estimated that cotton was planted on 13.4 million acres in the United States in 2003, and that 18.2 million bales were produced (National Cotton Council, 2004). It is grown primarily for the value of the fiber, with cottonseed produced as a by-product. Cottonseed oil, which is extracted from cottonseed, has been a part of the U.S. diet for well over a century. Until the 1940s, it was the major vegetable oil produced in the United States. Today, annual production of cottonseed oil in the U.S. averages more than one billion pounds (NCPA, 2002c).

2.2. Cotton as a Food Source

After ginning to remove the lint, cottonseed is processed into four major products: oil, meal, hulls, and linters. Processing of cottonseed typically yields (by weight): 16% oil,

45% meal, 26% hulls, and 9% linters, with 4% lost during processing (Cherry and Leffler, 1984). Only cottonseed oil and linters are utilized as food sources.

Cottonseed is highly processed during the production of oil and meal. After hulling, the cottonseed is flaked by a rolling process to facilitate oil removal. Prior to oil extraction, the flakes are heated to break down the cell walls, reduce the viscosity of the oil, inactivate proteins, and detoxify gossypol. After heating, oil is typically removed from the meal by direct solvent extraction with hexane. Crude cottonseed oil is further processed, depending on the end use of the product

Further processing (refining) for all the uses of cottonseed oil includes deodorization and bleaching. Deodorization greatly reduces the cyclopropenoid fatty acid content of the oil due to extreme pH and temperature conditions (NCPA, 1993). A winterization step is added to produce cooking oil, whereas for solid shortening a hydrogenation step is added to transform the liquid oil into a solid fat. Previous studies have shown that the resulting oil contains no detectable protein (Fuchs et al., 1993). Cottonseed oil is a premium quality oil that is used for a variety of food uses, including frying oil, salad and cooking oil, mayonnaise, salad dressing, shortening, margarine, and packing oil.

The material left after the extraction of the crude cottonseed oil is the cottonseed meal. The gossypol levels in the meal after extraction are reduced by approximately half. Cottonseed meal is discussed further in Section 2.3.

The short fibers on the cottonseed after ginning, or linters, consist primarily of cellulose (>95%) (Wakelyn et al., 1998). After extensive processing at alkaline pH and high temperatures, the linters can be used as a high fiber dietary product. Food uses include casings for bologna, sausages, frankfurters, and to improve viscosity in products such as toothpaste, ice cream, and salad dressings (NCPA, 1999). Based on the composition of linters and the extensive processing undertaken prior to food use, cellulose used for food derived from cotton linters is not expected to contain any detectable protein (Sims et al., 1996).

2.3. Cotton as a Feed Source

Cottonseed meal is the second most valuable product of cottonseed, as reported by the National Cottonseed Products Association (2002a), and usually accounts for a third of total product value. Cottonseed meal is not used for human consumption in the U.S. (Morgan, 1990), but is principally sold as feed for livestock, with its primary value as a protein concentrate (NCPA, 2002a, 2002b; Cotton Incorporated, 2004). The presence of gossypol and cyclopropenoid fatty acids in cottonseed limits its use as a protein supplement in animal feed except for ruminants, which tolerate these components at higher levels than other animals. Inactivation or removal of these anti-nutritional components during processing enables the use of some cottonseed meal for catfish, poultry and swine.

The hull is the tough protective covering of the cottonseed removed prior to processing the seed for oil and meal. Cottonseed hulls contain 3-8% highly digestible cotton linters, and are an exceptional roughage source, with a high level of effective fiber. They are also very palatable and are commonly used in feedlot and dairy rations because they require no grinding and mix well with other feed ingredients (NCPA, 2002b). Gin trash, the dried plant material cleaned from the fiber during ginning, is also used as a source of roughage for livestock feeds.

Section 3. Comparison of the Composition and Nutritional Components of MON 88913

3.1. Levels of Significant Nutrients, Antinutrients and Other Components in Cottonseed

The composition of MON 88913 cottonseed was compared to MON 88913(-) cottonseed, which has background genetics representative of the MON 88913 but does not contain the DNA insert or produce the CP4 EPSPS protein. Sixteen commercial conventional cotton varieties produced in the same field trials alongside MON 88913 and MON 88913(-) were also analyzed as references to produce a 99% tolerance interval for commercial conventional cotton. A summary of the combined site statistical evaluation of the compositional data is presented in Table VII-1. The field experimental design and compositional methods, as well as individual site composition data are presented in Appendix E.

Analyses were conducted on the cottonseed to measure proximates (protein, total fat, ash, and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude fiber, total dietary fiber (TDF), amino acids, fatty acids (C8-C22), cyclopropenoid fatty acids (malvalic acid, sterculic acid, and dihydrosterculic acid), vitamin E, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), gossypol (free and total), and aflatoxins (B1, B2, G1, and G2). In addition, carbohydrates and calories were determined by calculation.

In all, 69 different components were evaluated as part of the nutritional assessment of MON 88913 cottonseed. Of the 69 components evaluated, 50% of the observations for 16 of the components were below the assay detection limit and were therefore excluded from the statistical analysis. As a result, 53 components were statistically analyzed. A total of 265 comparisons were made: 53 comparisons for each of the five statistical analyses (four sites individually plus all sites combined). MON 88913 was compared to MON 88913(-) to determine statistically significant differences at a significance level of $p \leq 0.05$. In addition, for those comparisons in which MON 88913 was statistically different from MON 88913(-), the range of values for MON 88913 was compared to the 99% tolerance interval (with 95% confidence) of the reference varieties to determine if the values fell within the population of commercial conventional cotton.

There were no statistically significant differences between MON 88913 and MON 88913(-) for 236 of the 265 comparisons, including fifteen of eighteen amino acids, six of the ten fatty acids statistically analyzed, dihydrosterculic acid, iron, magnesium, phosphorus, ash, protein, calories, carbohydrates, vitamin E, acid detergent fiber, neutral detergent fiber, total dietary fiber, free gossypol, and total gossypol (Appendix E). Of the 29 comparisons found to be statistically different, 5%, or approximately 13 (0.05 x 265), were expected based on chance alone. Statistically significant differences ($p \leq 0.05$) between MON 88913 and MON 88913(-) were observed in one of the five comparisons for tryptophan, glycine, 16:0 palmitic acid, 18:0 stearic acid, malvalic acid, sterculic acid, crude fiber, moisture, copper, and zinc; in two of the five comparisons for phenylalanine, calcium, manganese, and fat; in three of the five comparisons for sodium and 18:2 linoleic acid; and in all five comparisons for 18:1 oleic acid (Table VII-2).

These last two fatty acid components, 18:2 linoleic acid and 18:1 oleic acid, showed a difference between MON 88913 and MON 88913(-) in cottonseed produced at greater than half of the sites, and were statistically different in the combined site analysis. However, the differences between MON 88913 and MON 88913(-) for the components were small, 3.8 to 5.0% and 8.5 to 13.7%, respectively. Importantly, the oleic and linoleic acid content of cottonseed of MON 88913 are not outside the range of expected values for these components in conventional cotton (Table VII-3). These and the other observed differences are unlikely to be biologically meaningful because the range of values for all components associated with the statistically significant differences were found to fall within the 99% tolerance interval for the commercial conventional varieties planted in the same field trials as MON 88913 and MON 88913(-), with the exception of moisture in the combined site comparison. The range of values for moisture in MON 88913 cottonseed did, however, fall within published ranges for conventional cottonseed (Table VII-1).

In addition, the background genetics of MON 88913 and MON 88913(-) cottonseed are expected to be genetically similar but not 100% identical, further providing a practical explanation for minor differences noted between MON 88913 and MON 88913(-). In this context, minor differences within the range of expected values for conventional cotton were unlikely to be biologically meaningful. These results demonstrate that the levels of key nutrients and other components of cottonseed of MON 88913 are within the expected range for conventional cotton.

Table VII-1. Statistical Summary[1] of Combined Site Cottonseed Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% Confidence Interval (Lower, Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Interval[2]]**
Amino Acid (% Total AA)						
Alanine	4.28 ± 0.056	4.30 ± 0.056	-0.013 ± 0.030	-0.11,0.081	0.691	(4.08 - 4.46)
	(4.09 - 4.51)	(4.15 - 4.46)	(-0.27 - 0.24)			[4.01,4.58]
Arginine	11.78 ± 0.17	11.77 ± 0.17	0.0033 ± 0.12	-0.39,0.40	0.980	(11.08 - 12.77)
	(11.19 - 12.25)	(11.11 - 12.27)	(-0.81 - 0.99)			[10.57,12.96]
Aspartic Acid	9.82 ± 0.064	9.80 ± 0.064	0.020 ± 0.031	-0.080,0.12	0.567	(9.70 - 10.38)
	(9.59 - 10.08)	(9.59 - 9.99)	(-0.13 - 0.29)			[9.48,10.35]
Cystine	1.89 ± 0.042	1.92 ± 0.042	-0.035 ± 0.029	-0.097,0.027	0.243	(1.62 - 2.35)
	(1.69 - 2.10)	(1.76 - 2.10)	(-0.25 - 0.16)			[1.60,2.14]
Glutamic Acid	21.66 ± 0.13	21.55 ± 0.13	0.11 ± 0.096	-0.085,0.31	0.253	(20.92 - 22.18)
	(21.08 - 22.14)	(21.10 - 21.96)	(-0.63 - 1.03)			[20.88,22.49]
Glycine	4.42 ± 0.029	4.45 ± 0.029	-0.025 ± 0.018	-0.062,0.012	0.171	(4.29 - 4.66)
	(4.33 - 4.56)	(4.33 - 4.64)	(-0.24 - 0.13)			[4.21,4.64]

[1] Means in the table are least square means from SAS®. Cottonseed produced under field conditions in 2002 from Baldwin County, Alabama; Tulare County, California; Clarke County, Georgia; Hockley County, Texas.

[2] Tolerance Interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional cotton. Negative limits were set to zero.

Table VII-1 (Continued). Statistical Summary[1] of Combined Site Cottonseed Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% Confidence Interval (Lower, Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Interval[2]]**
Amino Acid (% Total AA)						
Histidine	3.15 ± 0.0079	3.14 ± 0.0079	0.0059 ± 0.011	-0.022,0.033	0.619	(3.01 - 3.22)
	(3.09 - 3.21)	(3.11 - 3.20)	(-0.070 - 0.10)			[3.04,3.23]
Isoleucine	3.43 ± 0.020	3.43 ± 0.020	-0.0040 ± 0.026	-0.086,0.078	0.887	(3.19 - 3.59)
	(3.31 - 3.54)	(3.34 - 3.56)	(-0.25 - 0.12)			[3.13,3.65]
Leucine	6.31 ± 0.048	6.27 ± 0.048	0.046 ± 0.026	-0.036,0.13	0.169	(6.03 - 6.48)
	(6.14 - 6.52)	(6.10 - 6.48)	(-0.20 - 0.20)			[5.84,6.66]
Lysine	4.99 ± 0.052	5.09 ± 0.052	-0.11 ± 0.053	-0.22,0.0020	0.053	(4.72 - 5.38)
	(4.77 - 5.23)	(4.89 - 5.48)	(-0.48 - 0.30)			[4.53,5.43]
Methionine	1.65 ± 0.040	1.69 ± 0.040	-0.042 ± 0.043	-0.18,0.094	0.397	(1.27 - 1.94)
	(1.47 - 1.90)	(1.49 - 1.95)	(-0.34 - 0.22)			[1.30,1.93]
Phenylalanine	5.64 ± 0.014	5.60 ± 0.014	0.044 ± 0.019	0.0042,0.083	0.031	(5.44 - 5.82)
	(5.53 - 5.75)	(5.45 - 5.72)	(-0.19 - 0.21)			[5.43,5.82]
Proline	4.17 ± 0.045	4.16 ± 0.045	0.010 ± 0.028	-0.079,0.099	0.739	(3.97 - 4.49)
	(3.92 - 4.39)	(3.93 - 4.25)	(-0.18 - 0.20)			[3.91,4.43]

Table VII-1 (Continued). Statistical Summary[1] of Combined Site Cottonseed Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% Confidence Interval (Lower, Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Interval[2]]**
Amino Acid (% Total AA)						
Serine	4.88 ± 0.096	4.90 ± 0.096	-0.017 ± 0.054	-0.19,0.15	0.773	(4.53 - 5.31)
	(4.35 - 5.32)	(4.65 - 5.32)	(-0.48 - 0.50)			[4.55,5.42]
Threonine	3.19 ± 0.094	3.20 ± 0.094	-0.0082 ± 0.067	-0.22,0.21	0.910	(2.67 - 3.50)
	(2.61 - 3.49)	(2.70 - 3.45)	(-0.49 - 0.48)			[2.73,3.74]
Tryptophan	1.10 ± 0.012	1.14 ± 0.012	-0.039 ± 0.016	-0.074,-0.0044	0.029	(0.97 - 1.31)
	(1.03 - 1.23)	(1.09 - 1.25)	(-0.14 - 0.089)			[0.94,1.26]
Tyrosine	2.79 ± 0.033	2.78 ± 0.033	0.017 ± 0.028	-0.071,0.11	0.576	(2.63 - 2.93)
	(2.70 - 2.90)	(2.62 - 2.89)	(-0.085 - 0.18)			[2.61,3.00]
Valine	4.84 ± 0.028	4.81 ± 0.028	0.032 ± 0.024	-0.019,0.084	0.202	(4.57 - 5.02)
	(4.68 - 5.00)	(4.68 - 4.96)	(-0.12 - 0.22)			[4.48,5.02]
Fatty Acid (% Total FA)						
14:0 Myristic	0.76 ± 0.040	0.75 ± 0.040	0.016 ± 0.019	-0.044,0.077	0.458	(0.64 - 1.03)
	(0.66 - 0.90)	(0.65 - 0.90)	(-0.092 - 0.20)			[0.44,1.14]
16:0 Palmitic	23.55 ± 0.40	23.09 ± 0.40	0.46 ± 0.19	-0.13,1.05	0.089	(21.47 - 25.36)
	(22.09 - 24.69)	(21.26 - 24.17)	(-0.41 - 1.67)			[20.76,26.19]

Table VII-1 (Continued). Statistical Summary[1] of Combined Site Cottonseed Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% Confidence Interval (Lower, Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Interval[2]]**
Fatty Acid (% Total FA)						
16:0 Palmitic	23.55 ± 0.40	23.09 ± 0.40	0.46 ± 0.19	-0.13,1.05	0.089	(21.47 - 25.36)
	(22.09 - 24.69)	(21.26 - 24.17)	(-0.41 - 1.67)			[20.76,26.19]
16:1 Palmitoleic	0.54 ± 0.0066	0.53 ± 0.0066	0.0098 ± 0.0086	-0.0079,0.027	0.265	(0.46 - 0.77)
	(0.51 - 0.59)	(0.50 - 0.59)	(-0.048 - 0.090)			[0.37,0.80]
18:0 Stearic	2.64 ± 0.073	2.65 ± 0.073	-0.0096 ± 0.041	-0.14,0.12	0.830	(2.38 - 3.03)
	(2.32 - 2.85)	(2.33 - 2.94)	(-0.28 - 0.17)			[2.18,3.17]
18:1 Oleic	18.61 ± 0.75	20.94 ± 0.75	-2.33 ± 0.27	-3.18,-1.48	0.003	(13.29 - 18.60)
	(16.35 - 20.72)	(18.34 - 23.29)	(-4.07 - -0.56)			[10.59,21.29]
18:2 Linoleic	52.36 ± 0.76	50.42 ± 0.76	1.94 ± 0.28	1.34,2.53	<0.001	(51.51 - 59.40)
	(49.66 - 54.32)	(47.89 - 53.27)	(-0.36 - 3.40)			[48.89,61.11]
18:3 Gamma Linolenic	0.12 ± 0.023	0.13 ± 0.023	-0.0059 ± 0.031	-0.10,0.092	0.860	(0.043 - 0.23)
	(0.045 - 0.28)	(0.049 - 0.20)	(-0.13 - 0.23)			[0,0.24]
18:3 Linolenic	0.18 ± 0.025	0.17 ± 0.025	0.0044 ± 0.0076	-0.020,0.029	0.602	(0.11 - 0.27)
	(0.11 - 0.26)	(0.12 - 0.24)	(-0.031 - 0.042)			[0.031,0.31]

Table VII-1 (Continued). Statistical Summary[1] of Combined Site Cottonseed Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% Confidence Interval (Lower, Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Interval[2]]**
Fatty Acid (% Total FA)						
20:0 Arachidic	0.27 ± 0.0057	0.28 ± 0.0057	-0.00064 ± 0.0054	-0.018,0.016	0.913	(0.22 - 0.33)
	(0.25 - 0.31)	(0.24 - 0.30)	(-0.031 - 0.043)			[0.21,0.34]
22:0 Behenic	0.15 ± 0.0048	0.15 ± 0.0048	0.00095 ± 0.0038	-0.0071,0.0090	0.804	(0.12 - 0.18)
	(0.13 - 0.17)	(0.12 - 0.17)	(-0.024 - 0.023)			[0.099,0.19]
Dihydrosterculic	0.15 ± 0.0081	0.17 ± 0.0081	-0.021 ± 0.0075	-0.045,0.0028	0.067	(0.075 - 0.24)
	(0.12 - 0.18)	(0.10 - 0.21)	(-0.062 - 0.031)			[0.056,0.25]
Malvalic	0.36 ± 0.040	0.39 ± 0.040	-0.035 ± 0.029	-0.13,0.056	0.310	(0.23 - 0.56)
	(0.24 - 0.56)	(0.23 - 0.55)	(-0.22 - 0.13)			[0.16,0.58]
Sterculic	0.31 ± 0.025	0.33 ± 0.025	-0.024 ± 0.016	-0.057,0.0097	0.157	(0.19 - 0.41)
	(0.24 - 0.41)	(0.21 - 0.44)	(-0.17 - 0.094)			[0.18,0.40]
Fiber (% dwt)						
Acid Detergent Fiber	31.31 ± 0.50	30.78 ± 0.50	0.53 ± 0.64	-0.77,1.83	0.409	(26.32 - 38.97)
	(27.72 - 34.98)	(28.08 - 34.42)	(-6.70 - 5.74)			[25.48,38.48]
Crude Fiber	17.76 ± 0.68	17.97 ± 0.68	-0.21 ± 0.38	-1.42,1.00	0.616	(15.96 - 23.10)
	(14.96 - 20.41)	(16.04 - 20.39)	(-2.25 - 1.94)			[13.34,24.17]

Table VII-1 (Continued). Statistical Summary[1] of Combined Site Cottonseed Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% Confidence Interval (Lower, Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Interval[2]]**
Fiber (% dwt)						
Neutral Detergent Fiber	42.26 ± 1.07	42.56 ± 1.07	-0.29 ± 0.94	-2.22,1.63	0.757	(38.49 - 51.84)
	(33.91 - 47.36)	(38.00 - 46.92)	(-6.11 - 9.36)			[34.51,53.25]
Total Dietary Fiber	40.23 ± 0.53	39.60 ± 0.53	0.63 ± 0.48	-0.36,1.61	0.202	(36.47 - 47.54)
	(37.85 - 43.17)	(36.55 - 43.27)	(-4.90 - 3.03)			[36.13,48.96]
Mineral						
Calcium (% dwt)	0.16 ± 0.012	0.16 ± 0.012	-0.0024 ± 0.0063	-0.022,0.017	0.722	(0.10 - 0.19)
	(0.13 - 0.19)	(0.11 - 0.19)	(-0.022 - 0.020)			[0.074,0.22]
Copper (mg/kg dwt)	6.72 ± 0.61	6.54 ± 0.61	0.18 ± 0.20	-0.45,0.80	0.436	(4.92 - 12.47)
	(5.15 - 8.51)	(4.53 - 9.47)	(-1.83 - 1.06)			[2.01,12.94]
Iron (mg/kg dwt)	52.65 ± 1.68	52.20 ± 1.68	0.45 ± 1.42	-2.45,3.36	0.751	(36.71 - 67.75)
	(41.27 - 58.87)	(46.77 - 62.47)	(-21.20 - 8.45)			[33.44,68.99]
Magnesium (% dwt)	0.41 ± 0.011	0.42 ± 0.011	-0.0040 ± 0.0089	-0.032,0.024	0.682	(0.35 - 0.47)
	(0.38 - 0.45)	(0.37 - 0.46)	(-0.062 - 0.050)			[0.31,0.51]
Manganese (mg/kg dwt)	15.34 ± 1.29	14.64 ± 1.29	0.70 ± 0.29	0.096,1.30	0.024	(10.68 - 21.96)
	(12.37 - 19.98)	(11.91 - 18.23)	(-2.25 - 2.64)			[4.69,26.45]

Table VII-1 (Continued). Statistical Summary[1] of Combined Site Cottonseed Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% Confidence Interval (Lower, Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Interval[2]]**
Mineral						
Phosphorus (% dwt)	0.68 ± 0.052	0.70 ± 0.052	-0.013 ± 0.028	-0.10,0.075	0.671	(0.48 - 0.99)
	(0.54 - 0.82)	(0.53 - 0.93)	(-0.17 - 0.11)			[0.31,1.08]
Potassium (% dwt)	1.21 ± 0.030	1.23 ± 0.030	-0.014 ± 0.018	-0.073,0.044	0.488	(1.07 - 1.39)
	(1.12 - 1.34)	(1.12 - 1.43)	(-0.24 - 0.083)			[0.96,1.46]
Sodium (% dwt)	0.062 ± 0.015	0.068 ± 0.015	-0.0062 ± 0.019	-0.068,0.055	0.767	(0.032 - 0.14)
	(0.027 - 0.12)	(0.033 - 0.11)	(-0.075 - 0.034)			[0,0.17]
Zinc (mg/kg dwt)	40.87 ± 3.72	39.42 ± 3.72	1.45 ± 0.99	-0.58,3.47	0.155	(30.11 - 59.51)
	(29.30 - 52.16)	(27.60 - 52.16)	(-11.11 - 7.50)			[17.12,58.50]
Proximate						
Ash (% dwt)	4.33 ± 0.17	4.37 ± 0.17	-0.036 ± 0.084	-0.30,0.23	0.697	(3.76 - 5.34)
	(3.94 - 4.81)	(3.76 - 5.19)	(-0.82 - 0.39)			[2.96,5.62]
Calories (Kcal/100g dwt)	460.31 ± 5.33	455.51 ± 5.33	4.80 ± 3.80	-2.99,12.60	0.216	(407.45 - 471.46)
	(424.36 - 481.93)	(415.74 - 475.23)	(-34.58 - 36.83)			[409.12,496.45]
Carbohydrates (% dwt)	44.74 ± 0.49	45.57 ± 0.49	-0.83 ± 0.60	-2.73,1.08	0.260	(40.06 - 52.01)
	(42.61 - 47.67)	(42.07 - 49.32)	(-5.22 - 3.76)			[38.23,56.70]

Table VII-1 (Continued). Statistical Summary[1] of Combined Site Cottonseed Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% Confidence Interval (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Interval[2]]**
Proximate						
Moisture (% fwt)	6.39 ± 0.26	6.22 ± 0.26	0.17 ± 0.063	0.038,0.30	0.013	(5.06 - 6.49)
	(5.65 - 7.34)	(5.32 - 7.12)	(-0.40 - 0.70)			[4.51,7.21]
Protein (% dwt)	28.23 ± 0.60	27.41 ± 0.60	0.82 ± 0.59	-0.39,2.03	0.175	(21.48 - 32.03)
	(24.08 - 31.13)	(21.64 - 29.53)	(-5.45 - 6.35)			[20.19,32.70]
Total Fat (% dwt)	22.70 ± 0.52	22.66 ± 0.52	0.046 ± 0.45	-1.38,1.48	0.925	(17.60 - 27.29)
	(21.00 - 25.25)	(19.99 - 24.82)	(-1.50 - 1.91)			[15.16,28.44]
Vitamin						
Vitamin E (mg/kg dwt)	150.85 ± 14.02	148.79 ± 14.02	2.06 ± 1.93	-4.07,8.19	0.363	(70.79 - 197.22)
	(103.60 - 179.33)	(107.81 - 182.23)	(-6.89 - 14.75)			[9.30,263.66]
Gossypol						
Free Gossypol (% dwt)	0.65 ± 0.032	0.68 ± 0.032	-0.029 ± 0.036	-0.14,0.086	0.480	(0.53 - 1.05)
	(0.51 - 0.77)	(0.51 - 0.86)	(-0.19 - 0.16)			[0.43,1.06]
Total Gossypol (% dwt)	0.81 ± 0.034	0.82 ± 0.034	-0.0050 ± 0.018	-0.062,0.052	0.799	(0.78 - 1.19)
	(0.70 - 0.91)	(0.69 - 0.96)	(-0.16 - 0.099)			[0.61,1.25]

Table VII-2. Summary of Statistical Differences *($p \leq 0.05$)* in Cottonseed for the Comparison of MON 88913 to MON 88913(-), Plus Commercial Conventional Varieties

Site / Component [a]	Mean [b] MON 88913	Mean [b] MON 88913(-)	Mean Difference [% of MON 88913(-)] [b]	Mean Difference Significance (*p-value*) [b]	MON 88913 Range [b]	Commercial 99% T. I. [c] [Lower, Upper] [b]	Literature Range [d]
Alabama							
18:1 Oleic (% Total FA)	16.60	18.89	-12.1	0.002	16.35 – 17.01	[10.59,21.29]	15.17 [1]–19.94 [1]
18:2 Linoleic (% Total FA)	53.88	51.69	4.2	<0.001	53.54 – 54.32	[48.89,61.11]	49.07 [1]–59.1 [2]
Manganese (mg/kg dwt)	19.31	17.77	8.7	0.031	18.45 – 19.98	[4.69,26.45]	10 [3]–20.1 [4]
Sodium (% dwt)	0.10	0.080	30.5	0.017	0.087 – 0.12	[0,0.17]	0.03 [4]–0.31 [3]
Zinc (mg/kg dwt)	43.39	40.96	5.9	0.032	41.22 – 44.70	[17.12,58.50]	28.9 [4]–37 [5]
Total Fat (% dwt)	21.33	22.41	-4.8	0.004	21.04 – 21.91	[15.16,28.44]	16.9 [4]–26.8 [1]
California							
16:0 Palmitic (% Total FA)	24.33	23.70	2.6	0.031	23.56 – 24.69	[20.76,26.19]	18.4 [2]–26.18 [1]
18:1 Oleic (% Total FA)	19.77	21.61	-8.5	0.026	19.05 – 20.35	[10.59,21.29]	15.17 [1]–19.94 [1]
Malvalic (% Total FA)	0.26	0.34	-25.3	0.003	0.24 – 0.27	[0.16,0.58]	0.7 [6]–1.5 [6]

[a] dwt=dry weight; AA=amino acids; FA=fatty acids; fwt=fresh weight

[b] As found in Appendix G. Mean is the least squares mean. Range is the range of the average duplicate analyses of single samples.

[c] Tolerance Interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional cotton. Negative limits were set to zero.

[d] Range of values found in published literature for cotton varieties. [1]Cherry et al., 1978 (as % oil); [2]Cherry, 1983 (as % lipid); [3] NRC, 1982 (fuzzy seed); [4] Belyea et al., 1989; [5] NRC, 2001 (fuzzy seed); [6] Shenstone and Vickery, 1961 (as % oil); [7] Lawhon et al., 1977 (as g/16gN defatted flour)

Table VII-2 (Continued). Summary of Statistical Differences *(p≤0.05)* in Cottonseed for the Comparison of MON 88913 to MON 88913(-), Plus Commercial Conventional Varieties

Site / Component[a]	Mean [b] MON 88913	Mean [b] MON 88913(-)	Mean Difference [% of MON 88913(-)] [b]	Mean Difference Significance (*p-value*) [b]	MON 88913 Range [b]	Commercial 99% T. I. [c] [Lower, Upper] [b]	Literature Range [d]
California							
Sterculic (% Total FA)	0.26	0.28	-7.9	0.020	0.25 – 0.28	[0.18,0.40]	0.3 [6]–0.5 [6]
Crude Fiber (% dwt)	16.06	16.87	-4.8	0.020	14.96 – 16.79	[13.34,24.17]	20.8 [3]
Calcium (% dwt)	0.16	0.18	-9.2	0.008	0.16 – 0.17	[0.074,0.22]	0.1 [4]–0.17 [5]
Sodium (% dwt)	0.060	0.037	61.5	0.002	0.053 – 0.068	[0,0.17]	0.03 [4]–0.31 [3]
Georgia							
Glycine (% Total AA)	4.34	4.37	-0.6	0.040	4.33 – 4.38	[4.21,4.64]	3.7 [7]–4.6 [7]
18:0 Stearic (% Total FA)	2.73	2.64	3.5	0.045	2.68 – 2.77	[2.18,3.17]	2.2 [2]–2.88 [1]
18:1 Oleic (% Total FA)	18.57	20.67	-10.2	0.015	17.93 – 19.14	[10.59,21.29]	15.17 [1]–19.94 [1]
Calcium (% dwt)	0.14	0.12	11.2	0.014	0.13 – 0.15	[0.074,0.22]	0.1 [4]–0.17 [5]
Sodium (% dwt)	0.045	0.10	-56.2	0.001	0.037 – 0.051	[0,0.17]	0.03 [4]–0.31 [3]
Texas							
Phenylalanine (% Total AA)	5.65	5.59	1.0	0.023	5.61 – 5.67	[5.43,5.82]	5.0 [7]–6.2 [7]
18:1 Oleic (% Total FA)	19.51	22.59	-13.7	0.012	18.71 – 20.72	[10.59,21.29]	15.17 [1]–19.94 [1]

Table VII-2 (Continued). Summary of Statistical Differences *(p≤0.05)* in Cottonseed for the Comparison of MON 88913 to MON 88913(-), Plus Commercial Conventional Varieties

Site / Component [a]	Mean [b] MON 88913	Mean [b] MON 88913(-)	Mean Difference [% of MON 88913(-)] [b]	Mean Difference Significance (*p-value*) [b]	MON 88913 Range [b]	Commercial 99% T. I. [c] [Lower, Upper] [b]	Literature Range [d]
Texas							
18:2 Linoleic (% Total FA)	51.63	49.17	5.0	0.005	50.80 – 52.61	[48.89,61.11]	49.07 [1]–59.1 [2]
Copper (mg/kg dwt)	6.78	6.28	7.9	0.036	6.59 – 6.96	[2.01,12.94]	9.9 [4]–54 [3]
Total Fat (% dwt)	22.96	21.84	5.1	0.024	22.52 – 23.54	[15.16,28.44]	16.9 [4]–26.8 [1]
Combined Site							
Phenylalanine (% Total AA)	5.64	5.60	0.8	0.031	5.53 – 5.75	[5.43,5.82]	5.0 [7]–6.2 [7]
Tryptophan (% Total AA)	1.10	1.14	-3.4	0.029	1.03 – 1.23	[0.94,1.26]	1.0 [7]–1.4 [7]
18:1 Oleic (% Total FA)	18.61	20.94	-11.1	0.003	16.35 – 20.72	[10.59,21.29]	15.17 [1]–19.94 [1]
18:2 Linoleic (% Total FA)	52.36	50.42	3.8	<0.001	49.66 – 54.32	[48.89,61.11]	49.07 [1]–59.1 [2]
Manganese (mg/kg dwt)	15.34	14.64	4.8	0.024	12.37 – 19.98	[4.69,26.45]	10 [3]–20.1 [4]
Moisture (% fwt)	6.39	6.22	2.7	0.013	5.65 – 7.34	[4.51,7.21]	5.4 [1]–10.1 [1]

Table VII-3. Literature Values for Cottonseed Compositional Analytes

Component	Literature Ranges [a]
Proximates, Fibers (% dwt)	
Protein	21.2 [1] – 29.5 [2]
Fat	16.9 [3] – 26.8 [2]
Ash	3.8 [3] – 4.5 [4]
Moisture	5.4 [2] – 10.1 [2]
Carbohydrates	Not Available
Calories (kcal/100g)	Not Available
Acid Detergent Fiber	29.0 [5] – 40.1 [6]
Crude Fiber	20.8 [5]
Neutral Detergent Fiber	48.7 [3] – 50.3 [6]
Total Dietary Fiber	Not Available
Amino Acids (% Total AA)	
Alanine	3.6 [1] – 4.2 [1]
Arginine	10.9 [1] – 13.2 [1]
Aspartic Acid	8.8 [1] – 9.5 [1]
Cystine	1.76 [6] – 3.4 [1]
Glutamic Acid	19.9 [1] – 22.4 [1]
Glycine	3.7 [1] – 4.6 [1]
Histidine	2.6 [1] – 3.11 [6]
Isoleucine	2.8 [1] – 3.4 [1]
Leucine	5.3 [1] – 6.1 [1]
Lysine	4.2 [1] – 4.6 [1]
Methionine	1.2 [1] – 1.8 [1]
Phenylalanine	5.0 [1] – 6.2 [1]
Proline	3.1 [1] – 4.0 [1]
Serine	3.9 [1] – 4.4 [1]
Threonine	2.8 [1] – 3.46 [6]
Tryptophan	1.0 [1] – 1.4 [1]
Tyrosine	1.6 [1] – 3.3 [1]
Valine	4.1 [1] – 4.8 [1]

[a]Ranges include literature values for conventional cotton and for both glanded and glandless cotton. [1] Lawhon et al., 1977 (amino acids as g/16gN defatted flour); [2] Cherry et al., 1978 (fatty acids as % oil); [3] Belyea et al., 1989; [4] Cherry and Leffler, 1984; [5] NRC, 1982 (fuzzy seed; [6] NRC, 2001 (fuzzy seed, amino acids as % protein); [7] Cherry, 1983 (fatty acids as % lipid, 20:0 arachidic acid as % phospholipids in oil); [8] Shenstone and Vickery, 1961 (fatty acids as % oil); [9] Basset et al., 1970; [10] Cherry et al., 1986; [11] Smith and Creelman, 2001 (vitamin E as ppm fwt).

Table VII-3 (Continued). Literature Values for Cottonseed Compositional Analytes

Component	Literature Ranges [b]
Fatty Acids (% Total FA)	
14:0 Myristic	0.56 [7] – 1.16 [2]
16:0 Palmitic	18.4 [7] – 26.18 [2]
16:1 Palmitoleic	0.56 [2] – 1.00 [7]
18:0 Stearic	2.2 [7] – 2.88 [2]
18:1 Oleic	15.17 [2] – 19.94 [2]
18:2 Linoleic	49.07 [2] – 59.1 [7]
18:2 Gamma Linoleic	Not Available
18:3 Linolenic	0.23 [7]
20:0 Arachidic	0.41 [7]
22:0 Behenic	Not Available
Dihydrosterculic	Not Available
Malvalic	0.7 [8] – 1.5 [8]
Sterculic	0.3 [8] – 0.5 [8]
Minerals	
Calcium (% dwt)	0.1 [3] – 0.17 [6]
Copper (ppm dwt)	9.9 [3] – 54 [5]
Iron (ppm dwt)	67.0 [3] – 151 [5]
Magnesium (% dwt)	0.34 [3] – 0.37 [6]
Manganese (ppm dwt)	10 [5] – 20.1 [3]
Phosphorus (% dwt)	0.56 [9] – 0.75 [5]
Potassium (% dwt)	0.96 [3] – 1.21 [5]
Sodium (% dwt)	0.03 [3] – 0.31 [5]
Zinc (ppm dwt)	28.9 [3] – 37 [6]
Miscellaneous	
Gossypol, Free (% dwt)	0.59 [10] – 2.35 [10]
Gossypol, Total (% dwt)	0.80 [7] – 1.09 [7]
Vitamin (ppm)	
Vitamin E	99 [11] – 224 [11]

3.2. Levels of Significant Nutrients, Antinutrients and Other Components in Refined Cottonseed Oil and Cottonseed Meal

Compositional analyses were conducted on refined, bleached, deodorized cottonseed oil and raw (untoasted) cottonseed meal. The oil and meal fractions were derived from MON 88913 and the control, MON 88913(-), grown at two sites in 2002. In addition, six commercial conventional cotton reference varieties were grown alongside MON 88913 and MON 88913(-) at these two sites, as well as at a third site, in 2002. The whole, unprocessed, delinted cottonseed was also compositionally analyzed as a confirmation that the cottonseed used for the production of oil and meal was compositionally equivalent to the control. The cottonseed was processed into refined, bleached and deodorized cottonseed oil, and raw, untoasted cottonseed meal at the Food and Protein Research and Development Center at Texas A&M University. The experimental design and analytical methods are presented in Appendix F.

Analyses were conducted on the cottonseed to measure proximates (ash, moisture, protein, and total fat), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude fiber, total dietary fiber (TDF), amino acids, fatty acids (C8-C22), cyclopropenoid fatty acids (malvalic acid, sterculic acid, and dihydrosterculic acid), vitamin E, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and gossypol (free and total). The cottonseed oil was analyzed for fatty acids, cyclopropenoid fatty acids, vitamin E, and gossypol. The raw cottonseed meal was analyzed for the following: proximates, ADF, NDF, crude fiber, amino acids, cyclopropenoid fatty acids, minerals, and gossypol. In addition, the carbohydrate and caloric content of cottonseed and raw cottonseed meal were determined by calculation.

For MON 88913 and MON 88913(-), the data from the two sites were combined and statistical analyses conducted using a mixed model analysis of variance. Analytes with 50% of the observations below the assay detection limit were excluded from the statistical analysis. For each matrix, MON 88913 was compared to MON 88913(-), to determine statistically significant differences ($p<0.05$). Fifty-two comparisons were made in cottonseed, forty-one in cottonseed meal and thirteen in cottonseed oil. When statistically significant differences were observed, the range of values for MON 88913 was compared to the 99% tolerance interval (with 95% confidence) for the reference varieties to determine if the values observed for MON 88913 fell within the population of commercial conventional cotton. The statistical evaluations of the cottonseed oil, cottonseed meal, and whole cottonseed fraction compositional data are presented in Table VII-4, Table VII-6, and Table VII-8, respectively. The literature values for cottonseed oil and cottonseed meal composition analysis are presented in Table VII-5 and Table VII-7, respectively. The analytes that are statistically different between MON 88913 and MON 88913(-) in the processed fractions are summarized in Table VII-9.

There were no statistically significant differences for 51 of 52 comparisons made for cottonseed, 40 of 41 comparisons for raw cottonseed meal, and 11 of 13 comparisons for cottonseed oil. The differences observed were for phenylalanine in cottonseed, total gossypol in raw cottonseed meal, and 14:0 myristic acid and 22:0 behenic acid in cottonseed oil (Table VII-9). The range of values for those components associated with

the statistically significant differences, were found to all fall within the 99% tolerance interval for commercial conventional cotton. These results demonstrated, with 95% confidence, that the levels of key nutrients and other components of cottonseed, raw cottonseed meal, and cottonseed oil derived from MON 88913 are within the same population as expected for commercial conventional cotton. Therefore, the cottonseed, raw cottonseed meal, and cottonseed oil derived from MON 88913, are considered to be compositionally equivalent to those derived from conventional cotton.

Table VII-4. Statistical Summary of Combined Site Cottonseed Oil Fatty Acid and Vitamin E Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% Confidence Interval (Lower, Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Fatty Acid (% Total FA)						
14:0 Myristic	0.61 ± 0.026	0.62 ± 0.026	-0.013 ± 0.0033	-0.023, 0.0025	0029	(0.52 - 0.74)
	(0.57 - 0.64)	(0.58 - 0.66)	(-0.017 - -0.0032)			[0.20,1.13]
16:0 Palmitic	23.21 ± 0.83	23.23 ± 0.83	-0.025 ± 0.052	-0.19,0.14	0.667	(22.51 - 25.61)
	(22.33 - 24.10)	(22.32 - 24.27)	(-0.17 - 0.066)			[16.41,30.45]
16:1 Palmitoleic	0.53 ± 0.020	0.52 ± 0.020	0.0093 ± 0.0019	-0.015,0.033	0.128	(0.49 - 0.78)
	(0.51 - 0.55)	(0.50 - 0.54)	(0.0070 - 0.011)			[0,1.24]
18:0 Stearic	2.53 ± 0.19	2.53 ± 0.19	-0.0025 ± 0.059	-0.76,0.75	0.973	(2.26 - 2.59)
	(2.29 - 2.78)	(2.34 - 2.73)	(-0.11 - 0.12)			[1.69,3.07]
18:1 Oleic	20.18 ± 0.63	20.15 ± 0.63	0.025 ± 0.055	-0.15,0.20	0.681	(13.10 - 15.83)
	(19.41 - 20.82)	(19.46 - 20.83)	(-0.066 - 0.17)			[8.44,20.60]
18:2 Linoleic	51.67 ± 1.66	51.72 ± 1.66	-0.045 ± 0.098	-1.29,1.20	0.723	(54.70 - 59.69)
	(50.00 - 53.53)	(49.84 - 53.55)	(-0.27 - 0.16)			[46.72,67.80]
18:3 Linolenic	0.15 ± 0.0067	0.16 ± 0.0067	-0.0072 ± 0.0033	-0.018,0.0032	0.114	(0.13 - 0.17)
	(0.13 - 0.16)	(0.14 - 0.17)	(-0.013 - 0.0023)			[0.048,0.24]
20:0 Arachidic	0.24 ± 0.0046	0.25 ± 0.0046	-0.0033 ± 0.0031	-0.013,0.0066	0.369	(0.23 - 0.25)
	(0.24 - 0.26)	(0.24 - 0.25)	(-0.0086 - 0.0038)			[0.19,0.30]

[1]Data were averaged across duplicates for each sample prior to statistical analysis and summary. Means in the table are least square means from SAS. Cottonseed produced under field conditions in 2002 in Arkansas, Arizona and Georgia.

[2]Tolerance Interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional cotton. Negative limits were set to zero.

Table VII-4 (Continued). Statistical Summary[1] of Combined Site Cottonseed Oil Fatty Acid and Vitamin E Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% Confidence Interval (Lower, Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Fatty Acid (% Total FA)						
22:0 Behenic	0.11 ± 0.0016	0.12 ± 0.0016	-0.0052 ± 0.0016	-0.010,-0.00018	0.045	(0.11 - 0.13)
	(0.11 - 0.12)	(0.12 - 0.12)	(-0.0096 - -0.0020)			[0.080,0.16]
Dihydrosterculic	0.19 ± 0.016	0.16 ± 0.016	0.027 ± 0.022	-0.070,0.12	0.347	(0.12 - 0.16)
	(0.17 - 0.21)	(0.14 - 0.18)	(-0.0066 - 0.064)			[0.058,0.23]
Malvalic	0.31 ± 0.031	0.28 ± 0.031	0.031 ± 0.030	-0.35,0.42	0.489	(0.27 - 0.30)
	(0.26 - 0.37)	(0.26 - 0.30)	(-0.014 - 0.084)			[0.21,0.38]
Sterculic	0.26 ± 0.020	0.25 ± 0.020	0.0091 ± 0.026	-0.11,0.13	0.758	(0.17 - 0.23)
	(0.22 - 0.29)	(0.22 - 0.29)	(-0.028 - 0.066)			[0.069,0.34]
Vitamin						
Vitamin E (mg/kg FW)	464.38 ± 25.92	498.50 ± 25.92	-34.13 ± 12.24	-73.09,4.84	0.068	(444.00 - 652.00)
	(406.00 - 507.50)	(454.50 - 532.00)	(-60.50 - -8.00)			[0,1089.43]

Table VII-5. Literature Values for Cottonseed Oil Compositional Analytes

Component	Literature Range [a]
Fatty Acids (%)	
14:0 Myristic	0.5 [1] – 2.5 [1]
16:0 Palmitic	17 [1] – 29 [1]
16:1 Palmitoleic	0.3 [2] – 1.5 [1]
18:0 Stearic	1.0 [1] – 4.0 [1]
18:1 Oleic	13 [1] – 44 [1]
18:2 Linoleic	33 [1] – 58 [1]
18:3 Linolenic	0.1 [1] – 2.1 [1]
20:0 Arachidic	0.2 [2] – 0.4 [2]
22:0 Behenic	0.2 [2]
Dihydrosterculic	Not Available
Malvalic	0.015 [1] – 0.98 [3]
Sterculic	0.005 [1] – 0.126 [1]
Vitamin (ppm)	
Vitamin E	320 [1] – 353 [4]

[a] Range of values found in published literature for cottonseed meal.
[1] Hui, 1996; [2] Rossell, 1991; [3] Cherry et. al., 1986; [4] USDA, 2003b.

Table VII-6. Statistical Summary[1] of Combined Site Cottonseed Meal Amino Acid, Fatty Acid, Fiber, Mineral, Proximate and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% Confidence Interval (Lower, Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Alanine	4.44 ± 0.13	4.42 ± 0.13	0.023 ± 0.060	-0.74,0.79	0.771	(4.17 - 4.40)
	(4.27 - 4.54)	(4.25 - 4.62)	(-0.083 - 0.15)			[3.84,4.77]
Arginine	11.77 ± 0.17	11.94 ± 0.17	-0.18 ± 0.21	-2.80,2.45	0.551	(11.91 - 12.70)
	(11.57 - 11.90)	(11.67 - 12.23)	(-0.54 - 0.23)			[10.31,14.04]
Aspartic Acid	9.69 ± 0.032	9.65 ± 0.032	0.047 ± 0.037	-0.14,0.24	0.354	(9.64 - 9.85)
	(9.64 - 9.77)	(9.59 - 9.67)	(-0.032 - 0.10)			[9.28,10.22]
Cystine						
	(1.78 - 1.85)	(1.75 - 1.85)	(-0.036 - 0.10)			[1.53,2.12]
Glutamic Acid	20.65 ± 0.18	20.74 ± 0.18	-0.090 ± 0.23	-3.04,2.86	0.765	(20.49 - 20.97)
	(20.11 - 21.13)	(20.55 - 20.98)	(-0.76 - 0.16)			[19.75,21.63]
Glycine	4.64 ± 0.041	4.61 ± 0.041	0.035 ± 0.030	-0.042,0.11	0.294	(4.54 - 4.61)
Histidine	3.20 ± 0.036	3.23 ± 0.036	-0.036 ± 0.052	-0.26,0.19	0.560	(3.15 - 3.25)
	(3.13 - 3.26)	(3.17 - 3.29)	(-0.16 - 0.093)			[2.93,3.46]
Isoleucine	3.37 ± 0.076	3.45 ± 0.076	-0.078 ± 0.091	-1.24,1.08	0.548	(3.10 - 3.53)
	(3.10 - 3.52)	(3.42 - 3.49)	(-0.34 - 0.086)			[2.26,4.50]

[1]Data were averaged across duplicates for each sample prior to statistical analysis and summary. Means in the table are least square means from SAS.
[2]Tolerance Interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional cotton. Negative limits were set to zero.

VII-6 (Continued). Statistical Summary[1] of Combined Site Cottonseed Meal Amino Acid, Fatty Acid, Fiber, Mineral, Proximate and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% Confidence Interval (Lower, Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Leucine	6.65 ± 0.11	6.48 ± 0.11	0.17 ± 0.15	-0.21,0.54	0.317	(6.37 - 7.07)
	(6.44 - 7.09)	(6.42 - 6.55)	(-0.039 - 0.66)			[4.93,8.34]
Lysine	5.19 ± 0.080	5.18 ± 0.080	0.0098 ± 0.035	-0.10,0.12	0.798	(4.98 - 5.23)
	(5.10 - 5.28)	(5.02 - 5.32)	(-0.064 - 0.081)			[4.50,5.62]
Methionine	1.68 ± 0.019	1.65 ± 0.019	0.028 ± 0.027	-0.037,0.093	0.335	(1.53 - 1.69)
	(1.64 - 1.72)	(1.61 - 1.70)	(-0.040 - 0.11)			[1.30,1.96]
Phenylalanine	5.57 ± 0.059	5.63 ± 0.059	-0.062 ± 0.084	-0.42,0.30	0.537	(5.62 - 5.75)
	(5.52 - 5.64)	(5.54 - 5.73)	(-0.17 - 0.094)			[5.36,5.99]
Proline	4.10 ± 0.039	4.07 ± 0.039	0.032 ± 0.044	-0.11,0.17	0.523	(3.94 - 4.18)
	(4.01 - 4.20)	(4.00 - 4.09)	(-0.086 - 0.11)			[3.53,4.55]
Serine	4.96 ± 0.043	4.91 ± 0.043	0.046 ± 0.061	-0.22,0.31	0.527	(4.72 - 4.83)
	(4.89 - 5.01)	(4.80 - 5.00)	(-0.11 - 0.15)			[4.51,5.01]
Threonine	3.54 ± 0.069	3.53 ± 0.069	0.015 ± 0.066	-0.82,0.85	0.852	(3.47 - 3.56)
	(3.41 - 3.64)	(3.49 - 3.58)	(-0.092 - 0.11)			[3.34,3.69]
Tryptophan	1.15 ± 0.018	1.18 ± 0.018	-0.032 ± 0.023	-0.33,0.26	0.398	(1.08 - 1.28)
	(1.12 - 1.18)	(1.17 - 1.19)	(-0.063 - -0.0070)			[0.75,1.57]

VII-6 (Continued). Statistical Summary[1] of Combined Site Cottonseed Meal Amino Acid, Fatty Acid, Fiber, Mineral, Proximate and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% Confidence Interval (Lower, Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Tyrosine	2.79 ± 0.062	2.83 ± 0.062	-0.034 ± 0.087	-0.41,0.34	0.733	(2.70 - 2.88)
	(2.69 - 2.89)	(2.72 - 2.91)	(-0.22 - 0.17)			[2.47,3.16]
Valine	4.82 ± 0.064	4.73 ± 0.064	0.089 ± 0.091	-0.20,0.38	0.396	(4.77 - 4.86)
	(4.68 - 5.06)	(4.69 - 4.80)	(-0.090 - 0.32)			[4.62,5.04]
Fatty Acid (µg/g dwt)						
Dihydrosterculic	59.05 ± 14.69	50.60 ± 14.69	8.45 ± 19.33	-85.30,102.21	0.709	(29.62 - 51.13)
	(35.71 - 87.02)	(36.25 - 61.90)	(-17.37 - 32.71)			[0,92.65]
Malvalic	112.95 ± 35.40	97.20 ± 35.40	15.76 ± 28.22	-342.85,374.37	0.675	(76.12 - 131.19)
	(61.16 - 175.47)	(76.93 - 136.04)	(-19.61 - 48.54)			[0,220.73]
Sterculic	100.79 ± 21.19	88.33 ± 21.19	12.46 ± 26.58	-139.09,164.00	0.696	(55.12 - 88.00)
	(61.99 - 143.93)	(70.63 - 104.24)	(-20.69 - 43.53)			[3.67,147.63]
Fiber (% dwt)						
Acid Detergent Fiber	18.87 ± 2.10	16.17 ± 2.10	2.70 ± 2.97	-10.20,15.59	0.460	(14.42 - 21.22)
	(17.95 - 19.71)	(11.70 - 20.59)	(-1.31 - 8.00)			[2.86,31.68]
Crude Fiber	13.46 ± 1.52	11.21 ± 1.52	2.24 ± 2.15	-6.99,11.47	0.405	(10.51 - 15.54)
	(12.87 - 13.97)	(8.36 - 13.79)	(-0.20 - 5.61)			[1.66,23.85]
Neutral Detergent Fiber	25.28 ± 2.75	21.15 ± 2.75	4.13 ± 3.89	-12.61,20.87	0.399	(19.08 - 29.04)
	(24.86 - 25.76)	(15.07 - 26.08)	(-1.22 - 10.68)			[2.46,44.39]

VII-6 (Continued). Statistical Summary[1] of Combined Site Cottonseed Meal Amino Acid, Fatty Acid, Fiber, Mineral, Proximate and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% Confidence Interval (Lower, Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Fiber (% dwt)						
Total Dietary Fiber	35.44 ± 3.25	32.12 ± 3.25	3.33 ± 4.59	-16.43,23.08	0.544	(31.70 - 37.78)
	(34.58 - 36.65)	(27.00 - 37.44)	(-2.85 - 9.66)			[22.75,45.80]
Mineral						
Calcium (% dwt)	0.24 ± 0.0048	0.26 ± 0.0048	-0.015 ± 0.0063	-0.035,0.0053	0.101	(0.19 - 0.22)
	(0.24 - 0.25)	(0.24 - 0.27)	(-0.025 - -0.0018)			[0.14,0.27]
Copper (mg/kg dwt)	13.59 ± 1.56	14.88 ± 1.56	-1.29 ± 0.66	-9.63,7.06	0.300	(11.12 - 15.02)
	(11.72 - 14.86)	(12.50 - 16.84)	(-2.13 - -0.48)			[5.89,21.52]
Iron (mg/kg dwt)	88.91 ± 7.70	101.80 ± 7.70	-12.89 ± 7.60	-109.32,83.54	0.339	(78.14 - 96.00)
	(76.11 - 100.01)	(84.24 - 113.57)	(-37.46 - -2.05)			[51.88,123.28]
Magnesium (% dwt)	0.75 ± 0.022	0.78 ± 0.022	-0.031 ± 0.027	-0.37,0.31	0.450	(0.70 - 0.81)
	(0.71 - 0.79)	(0.76 - 0.79)	(-0.066 - 0.0055)			[0.52,1.01]
Manganese (mg/kg dwt)	20.56 ± 1.21	20.52 ± 1.21	0.039 ± 0.65	-8.28,8.35	0.962	(18.63 - 21.05)
	(18.93 - 22.52)	(18.74 - 22.08)	(-1.71 - 0.94)			[14.52,24.91]
Phosphorus (% dwt)	1.49 ± 0.040	1.56 ± 0.040	-0.070 ± 0.056	-0.31,0.17	0.338	(1.40 - 1.59)
	(1.45 - 1.51)	(1.45 - 1.65)	(-0.20 - 0.065)			[1.11,1.89]
Potassium (% dwt)	1.87 ± 0.058	1.92 ± 0.058	-0.042 ± 0.038	-0.52,0.44	0.465	(1.83 - 1.97)
	(1.82 - 1.93)	(1.79 - 1.99)	(-0.095 - 0.032)			[1.57,2.17]

VII-6 (Continued). Statistical Summary[1] of Combined Site Cottonseed Meal Amino Acid, Fatty Acid, Fiber, Mineral, Proximate and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% Confidence Interval (Lower, Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Mineral						
Zinc (mg/kg dwt)	65.97 ± 12.70	71.17 ± 12.70	-5.20 ± 3.91	-54.84,44.44	0.410	(58.64 - 80.02)
	(55.15 - 76.85)	(56.03 - 87.00)	(-10.73 - -0.44)			[15.87,118.52]
Proximate						
Ash (% dwt)	7.39 ± 0.17	7.79 ± 0.17	-0.40 ± 0.24	-1.00,0.19	0.149	(6.97 - 7.53)
	(7.25 - 7.57)	(7.11 - 8.11)	(-0.86 - 0.29)			[6.19,8.45]
Calories (Kcal/100g dwt)	381.57 ± 1.33	382.04 ± 1.33	-0.48 ± 1.74	-8.69,7.74	0.812	(379.12 - 383.85)
	(378.52 - 385.01)	(380.11 - 383.13)	(-4.61 - 2.59)			[371.50,391.85]
Carbohydrates (% dwt)	45.49 ± 2.92	41.61 ± 2.92	3.87 ± 3.64	-42.37,50.12	0.480	(41.31 - 48.64)
	(43.97 - 46.09)	(37.04 - 45.95)	(-0.024 - 9.05)			[28.34,59.69]
Moisture (% fwt)	2.25 ± 1.93	4.82 ± 1.93	-2.57 ± 1.97	-27.57,22.44	0.416	(1.53 - 5.84)
	(1.46 - 4.10)	(0.76 - 9.43)	(-5.33 - 0.93)			[0,13.79]
Protein (% dwt)	44.94 ± 2.62	47.95 ± 2.62	-3.02 ± 2.70	-37.33,31.30	0.464	(42.09 - 49.43)
	(43.84 - 46.47)	(43.92 - 51.78)	(-6.58 - 0.32)			[31.32,61.57]
Total Fat (% dwt)	2.20 ± 0.32	2.65 ± 0.32	-0.45 ± 0.45	-2.41,1.50	0.422	(1.82 - 2.48)
	(1.48 - 2.85)	(2.20 - 3.09)	(-1.61 - 0.66)			[0.65,3.76]

VII-6 (Continued). Statistical Summary[1] of Combined Site Cottonseed Meal Amino Acid, Fatty Acid, Fiber, Mineral, Proximate and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% Confidence Interval (Lower, Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Gossypol						
Free Gossypol (% dwt)	0.18 ± 0.042	0.25 ± 0.042	-0.071 ± 0.024	-0.38,0.24	0.208	(0.17 - 0.39)
	(0.13 - 0.23)	(0.19 - 0.31)	(-0.13 - -0.034)			[0,0.76]
Total Gossypol (% dwt)	1.32 ± 0.027	1.48 ± 0.027	-0.16 ± 0.038	-0.25,-0.063	0.006	(1.10 - 2.02)
	(1.26 - 1.40)	(1.44 - 1.55)	(-0.22 - -0.041)			[0,3.35]

Table VII-7. Literature Values for Cottonseed Meal Compositional Analytes

Component	Literature Range[a]
Proximates, Fibers (% dwt)	
Protein	41.06 [1] – 49.1 [2]
Fat	0.33 [3] – 4.77 [2]
Ash	6.15 [1] – 7.1 [4]
Moisture (% fwt)	6.60 [1] – 9.5 [5]
Carbohydrates	38.43 [2]
Calories (kcal/100g)	367 [2]
Acid Detergent Fiber	19.9 [5]
Crude Fiber	9.64 [3] – 17.26 [1]
Neutral Detergent Fiber	30.8 [5]
Total Dietary Fiber	Not Available
Amino Acids (% Total AA)	
Alanine	4.21 [6] – 4.57 [6]
Arginine	9.97 [3] – 12.59 [6]
Aspartic Acid	9.84 [6] – 10.65 [6]
Cystine	1.34 [6] – 2.07 [6]
Glutamic Acid	20.05 [6] – 22.79 [6]
Glycine	3.78 [3] – 4.78 [6]
Histidine	2.55 [3] – 3.72 [6]
Isoleucine	2.91 [3] – 4.29 [6]
Leucine	5.33 [3] – 6.71 [6]
Lysine	3.58 [6] – 4.58 [6]
Methionine	1.06 [6] – 1.81 [6]
Phenylalanine	4.93 [3] – 6.32 [6]
Proline	2.22 [6] – 3.78 [6]
Serine	2.98 [3] – 8.42 [6]
Threonine	2.82 [3] – 3.82 [6]
Tryptophan	0.92 [3] – 1.48 [6]
Tyrosine	2.55 [3] – 3.61 [6]
Valine	4.08 [3] – 5.41 [6]

[a] Range of values found in published literature for cottonseed meal. [1] Papadopoulos and Ziras, 1987; [2] USDA, 2003b (fresh weight); [3]Waldroup and Kersey, 2002 (fiber, proximates, gossypol as % fwt, amino acids as % protein); [4]NRC, 1982; [5] NRC, 2001; [6] Fevrier et al., 2001; [7] Turner, 1967 (on 8% moisture basis).

Table VII-7 (Continued). Literature Values for Cottonseed Meal Compositional Analytes

Component	Literature Range[a]
Fatty Acids (ppm dwt)	
Dihydrosterculic	Not Available
Malvalic	Not Available
Sterculic	Not Available
Minerals	
Calcium (% dwt)	0.177 [1] – 0.5 [2]
Copper (ppm dwt)	0.01 [2] – 22 [4]
Iron (ppm dwt)	133.5 [2] – 630 [1]
Magnesium (% dwt)	0.486 [1] – 0.76 [2]
Manganese (ppm dwt)	4.30 [1] – 24 [5]
Phosphorus (% dwt)	0.815 [1] – 1.68 [2]
Potassium (% dwt)	1.09 [7] – 1.87 [2]
Sodium (% dwt)	0.027 [7] – 0.178 [7]
Zinc (ppm dwt)	46.70 [1] – 123 [2]
Miscellaneous	
Gossypol, Free (% dwt)	0.034 [1] – 0.14 [3]
Gossypol, Total (% dwt)	1.15 [3] – 1.45 [3]
Vitamin (ppm dwt)	
Vitamin E	17 [4]

Table VII-8. Statistical Summary[1] of Combined Site Cottonseed Fraction Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin E and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower, Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Alanine	4.43 ± 0.14	4.44 ± 0.14	-0.0077 ± 0.026	-0.075,0.060	0.783	(4.26 - 4.42)
	(4.29 - 4.56)	(4.28 - 4.64)	(-0.079 - 0.044)			[3.93,4.76]
Arginine	11.45 ± 0.18	11.49 ± 0.18	-0.042 ± 0.068	-0.22,0.13	0.567	(11.26 - 11.78)
	(11.24 - 11.76)	(11.28 - 11.70)	(-0.21 - 0.12)			[10.34,12.73]
Aspartic Acid	9.80 ± 0.050	9.74 ± 0.050	0.065 ± 0.037	-0.052,0.18	0.175	(9.79 - 10.01)
	(9.70 - 9.93)	(9.69 - 9.79)	(-0.0014 - 0.16)			[9.43,10.38]
Cystine	1.96 ± 0.050	1.95 ± 0.050	0.0045 ± 0.060	-0.19,0.20	0.945	(1.82 - 2.06)
	(1.92 - 1.99)	(1.80 - 2.13)	(-0.16 - 0.14)			[1.47,2.42]
Glutamic Acid	21.29 ± 0.36	21.18 ± 0.36	0.12 ± 0.17	-2.00,2.23	0.614	(21.18 - 22.07)
	(20.78 - 21.91)	(20.87 - 21.58)	(-0.16 - 0.33)			[19.66,23.32]
Glycine	4.55 ± 0.037	4.56 ± 0.037	-0.015 ± 0.044	-0.57,0.54	0.788	(4.41 - 4.55)
	(4.47 - 4.61)	(4.52 - 4.59)	(-0.067 - 0.035)			[4.19,4.76]
Histidine	3.15 ± 0.0077	3.17 ± 0.0077	-0.019 ± 0.0096	-0.049,0.012	0.144	(3.15 - 3.18)
	(3.14 - 3.17)	(3.16 - 3.20)	(-0.033 - 0.0095)			[3.08,3.24]

[1]Data were averaged across duplicates for each sample prior to statistical analysis and summary. Means in the table are least square means from SAS.
[2]Tolerance Interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional cotton. Negative limits were set to zero.

Table VII-8 (Continued). Statistical Summary of Combined Site Cottonseed Fraction Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin E and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Isoleucine	3.40 ± 0.042	3.43 ± 0.042	-0.025 ± 0.038	-0.51,0.46	0.624	(3.24 - 3.48)
	(3.31 - 3.49)	(3.36 - 3.48)	(-0.076 - 0.021)			[2.89,3.90]
Leucine	6.34 ± 0.052	6.39 ± 0.052	-0.052 ± 0.062	-0.84,0.74	0.554	(6.09 - 6.42)
	(6.21 - 6.42)	(6.33 - 6.43)	(-0.12 - 0.032)			[5.60,6.99]
Lysine	5.11 ± 0.17	5.14 ± 0.17	-0.024 ± 0.091	-1.19,1.14	0.833	(4.95 - 5.12)
	(4.85 - 5.40)	(4.96 - 5.27)	(-0.12 - 0.16)			[4.64,5.48]
Methionine	1.71 ± 0.053	1.71 ± 0.053	0.0022 ± 0.054	-0.17,0.17	0.969	(1.58 - 1.78)
	(1.64 - 1.78)	(1.57 - 1.86)	(-0.086 - 0.15)			[1.28,2.07]
Phenylalanine	5.53 ± 0.053	5.49 ± 0.053	0.034 ± 0.0069	0.012,0.056	0.015	(5.48 - 5.70)
	(5.44 - 5.58)	(5.40 - 5.57)	(0.015 - 0.045)			[5.10,6.02]
Proline	4.17 ± 0.045	4.23 ± 0.045	-0.060 ± 0.047	-0.66,0.54	0.422	(4.16 - 4.36)
	(4.10 - 4.28)	(4.16 - 4.27)	(-0.17 - 0.014)			[3.83,4.69]
Serine	5.03 ± 0.035	5.05 ± 0.035	-0.016 ± 0.0093	-0.046,0.014	0.181	(4.98 - 5.09)
	(4.97 - 5.12)	(4.99 - 5.15)	(-0.035 - 0.0063)			[4.76,5.26]
Threonine	3.35 ± 0.047	3.34 ± 0.047	0.011 ± 0.052	-0.39,0.41	0.865	(3.20 - 3.38)
	(3.25 - 3.44)	(3.26 - 3.40)	(-0.073 - 0.068)			[2.90,3.69]

Table VII-8 (Continued). Statistical Summary of Combined Site Cottonseed Fraction Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin E and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Tryptophan	1.13 ± 0.036	1.17 ± 0.036	-0.035 ± 0.042	-0.17,0.098	0.461	(1.07 - 1.12)
	(1.08 - 1.16)	(1.10 - 1.30)	(-0.16 - 0.033)			[0.99,1.19]
Tyrosine	2.89 ± 0.030	2.82 ± 0.030	0.066 ± 0.028	-0.11,0.24	0.186	(2.74 - 2.92)
	(2.84 - 2.96)	(2.78 - 2.89)	(0.014 - 0.12)			[2.42,3.20]
Valine	4.69 ± 0.024	4.69 ± 0.024	-0.00035 ± 0.013	-0.041,0.040	0.979	(4.61 - 4.72)
	(4.64 - 4.72)	(4.62 - 4.75)	(-0.034 - 0.021)			[4.44,4.93]
Fatty Acid (% Total FA)						
14:0 Myristic	0.61 ± 0.030	0.62 ± 0.030	-0.018 ± 0.012	-0.17,0.14	0.374	(0.51 - 0.74)
	(0.57 - 0.63)	(0.57 - 0.67)	(-0.040 - -0.0030)			[0.19,1.12]
16:0 Palmitic	23.21 ± 0.80	23.43 ± 0.80	-0.22 ± 0.12	-1.70,1.27	0.316	(22.61 - 25.86)
	(22.38 - 24.07)	(22.53 - 24.49)	(-0.42 - -0.050)			[16.40,30.85]
16:1 Palmitoleic	0.52 ± 0.022	0.53 ± 0.022	-0.0060 ± 0.0056	-0.024,0.012	0.359	(0.48 - 0.77)
	(0.50 - 0.54)	(0.50 - 0.56)	(-0.019 - 0.0079)			[0,1.24]
18:0 Stearic	2.73 ± 0.18	2.71 ± 0.18	0.023 ± 0.0089	-0.090,0.14	0.233	(2.41 - 2.68)
	(2.55 - 2.92)	(2.53 - 2.90)	(0.0033 - 0.046)			[1.91,3.20]
18:1 Oleic	19.92 ± 0.59	20.10 ± 0.59	-0.17 ± 0.066	-1.01,0.67	0.231	(13.25 - 15.38)
	(19.21 - 20.67)	(19.42 - 20.85)	(-0.30 - -0.0040)			[9.94,18.80]
18:2 Linoleic	51.35 ± 1.56	51.06 ± 1.56	0.29 ± 0.24	-2.75,3.34	0.437	(54.27 - 58.43)
	(49.71 - 53.02)	(49.04 - 52.82)	(-0.099 - 0.67)			[48.13,65.04]

Table VII-8 (Continued). Statistical Summary of Combined Site Cottonseed Fraction Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin E and Gossypol Content for MON 88913 Versus MON 88913(-)

Analytical Component	MON 88913 Mean ± S.E. (Range)	MON 88913(-) Mean ± S.E. (Range)	Difference [MON 88913 minus MON 88913(-)] Mean ± S.E. (Range)	95% CI (Lower,Upper)	*p-Value*	Commercial (Range) [99% Tolerance Int.[2]]
Fatty Acid (% Total FA)						
18:3 Gamma Linolenic	0.14 ± 0.019	0.095 ± 0.019	0.040 ± 0.023	-0.089,0.17	0.256	(0.090 - 0.16)
	(0.12 - 0.15)	(0.048 - 0.13)	(0.0094 - 0.076)			[0,0.26]
18:3 Linolenic	0.17 ± 0.015	0.17 ± 0.015	0.0012 ± 0.0091	-0.028,0.030	0.901	(0.15 - 0.20)
	(0.14 - 0.19)	(0.14 - 0.19)	(-0.022 - 0.021)			[0.067,0.27]
20:0 Arachidic	0.25 ± 0.0048	0.25 ± 0.0048	-0.0042 ± 0.0046	-0.062,0.054	0.525	(0.22 - 0.26)
	(0.24 - 0.25)	(0.24 - 0.26)	(-0.015 - 0.0032)			[0.17,0.32]
22:0 Behenic	0.099 ± 0.0070	0.086 ± 0.0070	0.013 ± 0.0079	-0.012,0.038	0.205	(0.089 - 0.13)
	(0.080 - 0.12)	(0.079 - 0.093)	(-0.0016 - 0.032)			[0.0086,0.19]
Dihydrosterculic	0.19 ± 0.0056	0.18 ± 0.0056	0.0094 ± 0.0079	-0.025,0.044	0.358	(0.14 - 0.19)
	(0.17 - 0.19)	(0.16 - 0.19)	(-0.0058 - 0.030)			[0.065,0.27]
Malvalic	0.46 ± 0.038	0.44 ± 0.038	0.021 ± 0.040	-0.49,0.53	0.691	(0.40 - 0.54)
	(0.39 - 0.51)	(0.42 - 0.45)	(-0.035 - 0.065)			[0.17,0.78]
Sterculic	0.36 ± 0.020	0.34 ± 0.020	0.016 ± 0.028	-0.11,0.14	0.621	(0.28 - 0.38)
	(0.32 - 0.41)	(0.32 - 0.36)	(-0.043 - 0.085)			[0.087,0.59]
Fiber						
Acid Detergent Fiber (% dwt)	31.08 ± 2.30	33.37 ± 2.30	-2.28 ± 1.49	-21.23,16.67	0.368	(31.20 - 33.71)
	(26.65 - 34.07)	(30.78 - 35.08)	(-6.44 - -0.57)			[27.02,38.03]

Table VII-8 (Continued). Statistical Summary of Combined Site Cottonseed Fraction Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin E and Gossypol Content for MON 88913 Versus MON 88913(-)

Analytical Component	MON 88913 Mean ± S.E. (Range)	MON 88913(-) Mean ± S.E. (Range)	Difference [MON 88913 minus MON 88913(-)] Mean ± S.E. (Range)	95% CI (Lower,Upper)	*p-Value*	Commercial (Range) [99% Tolerance Int.[2]]
Fiber						
Crude Fiber (% dwt)	16.90 ± 1.98	18.19 ± 1.98	-1.29 ± 1.17	-16.15,13.58	0.469	(19.04 - 21.01)
	(14.13 - 19.99)	(16.88 - 19.87)	(-2.76 - 0.86)			[15.19,24.26]
Neutral Detergent Fiber (% dwt)	41.54 ± 3.60	41.35 ± 3.60	0.19 ± 1.83	-23.07,23.45	0.932	(41.62 - 48.02)
	(34.66 - 47.16)	(37.07 - 45.33)	(-5.83 - 2.55)			[29.88,57.62]
Total Dietary Fiber (% dwt)	38.32 ± 4.00	42.91 ± 4.00	-4.60 ± 1.97	-9.65,0.46	0.066	(40.86 - 44.89)
	(34.55 - 42.70)	(38.86 - 51.29)	(-9.93 - 0.073)			[33.47,52.34]
Mineral						
Calcium (% dwt)	0.17 ± 0.0022	0.17 ± 0.0022	-0.0034 ± 0.0019	-0.0094,0.0027	0.173	(0.13 - 0.15)
	(0.16 - 0.17)	(0.17 - 0.18)	(-0.0082 - 0.00009)			[0.093,0.18]
Copper (mg/kg dwt)	8.56 ± 1.05	8.59 ± 1.05	-0.040 ± 0.10	-1.37,1.29	0.769	(6.28 - 8.38)
	(7.27 - 9.79)	(7.24 - 9.75)	(-0.32 - 0.094)			[3.27,11.71]
Iron (mg/kg dwt)	53.82 ± 6.52	62.30 ± 6.52	-8.48 ± 9.21	-49.80,32.84	0.458	(43.43 - 54.44)
	(44.21 - 63.45)	(49.40 - 77.36)	(-32.23 - 1.95)			[23.72,71.13]
Magnesium (% dwt)	0.48 ± 0.010	0.46 ± 0.010	0.012 ± 0.014	-0.049,0.073	0.498	(0.42 - 0.46)
	(0.47 - 0.48)	(0.45 - 0.48)	(-0.011 - 0.033)			[0.35,0.52]
Manganese (mg/kg dwt)	15.34 ± 0.73	16.10 ± 0.73	-0.77 ± 0.47	-2.26,0.73	0.201	(14.23 - 16.31)
	(14.43 - 16.62)	(14.53 - 16.94)	(-2.05 - 0.13)			[10.72,19.39]

Table VII-8 (Continued). Statistical Summary of Combined Site Cottonseed Fraction Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin E and Gossypol Content for MON 88913 Versus MON 88913(-)

Analytical Component	MON 88913 Mean ± S.E. (Range)	MON 88913(-) Mean ± S.E. (Range)	Difference [MON 88913 minus MON 88913(-)] Mean ± S.E. (Range)	95% CI (Lower,Upper)	*p-Value*	Commercial (Range) [99% Tolerance Int.[2]]
Mineral						
Phosphorus (% dwt)	0.87 ± 0.034	0.84 ± 0.034	0.025 ± 0.035	-0.42,0.47	0.603	(0.76 - 0.78)
	(0.81 - 0.92)	(0.82 - 0.86)	(-0.013 - 0.068)			[0.71,0.82]
Potassium (% dwt)	1.31 ± 0.061	1.31 ± 0.061	0.00044 ± 0.012	-0.038,0.038	0.972	(1.19 - 1.37)
	(1.23 - 1.38)	(1.24 - 1.40)	(-0.028 - 0.029)			[0.92,1.65]
Zinc (mg/kg dwt)	40.40 ± 7.95	39.86 ± 7.95	0.54 ± 1.52	-18.82,19.91	0.781	(29.73 - 44.31)
	(31.52 - 49.16)	(32.55 - 47.18)	(-1.34 - 2.31)			[3.47,69.46]
Proximate						
Ash (% dwt)	4.88 ± 0.16	4.67 ± 0.16	0.21 ± 0.20	-0.99,1.41	0.436	(4.61 - 4.86)
	(4.54 - 5.13)	(4.45 - 4.88)	(-0.099 - 0.54)			[4.10,5.29]
Calories (Kcal/100g dwt)	503.50 ± 2.29	500.28 ± 2.29	3.22 ± 2.79	-32.22,38.66	0.454	(483.77 - 499.89)
	(498.26 - 507.25)	(499.42 - 501.00)	(-1.16 - 6.25)			[457.45,527.86]
Carbohydrates (% dwt)	44.04 ± 2.93	45.95 ± 2.93	-1.92 ± 2.64	-35.43,31.59	0.599	(46.65 - 49.61)
	(39.76 - 48.49)	(44.35 - 47.67)	(-5.20 - 1.66)			[42.47,54.05]
Moisture (% fwt)	10.50 ± 2.97	10.35 ± 2.97	0.15 ± 0.48	-5.95,6.24	0.813	(7.50 - 12.05)
	(6.95 - 13.70)	(7.38 - 13.20)	(-0.43 - 0.75)			[0,20.51]
Protein (% dwt)	26.53 ± 2.25	25.62 ± 2.25	0.91 ± 1.66	-20.19,22.01	0.680	(23.30 - 26.55)
	(23.17 - 29.77)	(24.24 - 26.98)	(-1.31 - 2.99)			[17.15,32.46]
Total Fat (% dwt)	24.57 ± 0.57	23.76 ± 0.57	0.81 ± 0.75	-8.74,10.36	0.477	(20.48 - 23.69)
	(23.52 - 25.39)	(23.61 - 23.94)	(-0.27 - 1.67)			[15.28,29.21]

Table VII-8 (Continued). Statistical Summary of Combined Site Cottonseed Fraction Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin E and Gossypol Content for MON 88913 Versus MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Vitamin						
Vitamin E (mg/kg dwt)	144.38 ± 9.02	137.25 ± 9.02	7.13 ± 3.46	-36.86,51.12	0.287	(104.56 - 159.03)
	(135.88 - 152.95)	(125.93 - 152.07)	(-2.02 - 11.24)			[23.79,238.24]
Gossypol						
Free Gossypol (% dwt)	0.87 ± 0.024	0.84 ± 0.024	0.032 ± 0.027	-0.31,0.37	0.447	(0.58 - 1.10)
	(0.84 - 0.94)	(0.80 - 0.87)	(-0.027 - 0.10)			[0,1.97]
Total Gossypol (% dwt)	0.89 ± 0.030	0.86 ± 0.030	0.024 ± 0.030	-0.070,0.12	0.470	(0.61 - 1.13)
	(0.86 - 0.92)	(0.77 - 0.91)	(-0.051 - 0.089)			[0,1.99]

Table VII-9. Summary of Statistical Differences ($p<0.05$) in Combined Site Cottonseed, Cottonseed Oil and Cottonseed Meal For The Comparison of MON 88913 to MON 88913(-), and Commercial Conventional Reference Varieties

Matrix / Component[a]	Mean[b] MON 88913	Mean[b] MON 88913(-)	Mean Difference [% of MON 88913(-)][b]	Significance (*p-value*)[b]	MON 88913 Range[b]	Commercial 99% T. I.[c] [Lower , Upper][b]	Literature Values[d]
Cottonseed							
Phenylalanine (% Total AA)	5.53	5.49	0.6	0.015	(5.44-5.58)	[5.10,6.02]	5.0 [1] – 6.2 [1]
Cottonseed Oil							
14:0 Myristic Acid (% Total FA)	0.61	0.62	-2.1	0.029	(0.57-0.64)	[0.20,1.13]	0.5 [3] – 2.5 [3]
22:0 Behenic Acid (% Total FA)	0.11	0.12	-4.4	0.045	(0.11-0.12)	[0.080,0.16]	0.2[4]
Raw Cottonseed Meal							
Total Gossypol (% dwt)	1.32	1.48	-10.6	0.006	(1.26-1.40)	[0,3.35]	1.15 [2] – 1.45 [2]

[a] dwt=dry weight; AA=amino acids; FA=fatty acids

[b] As found in this section. Mean is the least squares mean. Range is the range of the average duplicate analyses of single samples.

[c] Tolerance Interval: with 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional cotton. Negative limits were set to zero.

[d] Range of values found in published literature for cotton varieties. [1]Lawhon et al., 1977; [2] Waldroup and Kersey, 2002 (% fwt); [3] Hui, 1996; [4] Rossell, 1991.

3.3. Levels of Naturally Occurring Toxicants and Anti-nutrients

Aflatoxins are toxins that are the by-product of several varieties of fungi known to contaminate cottonseed. Aflatoxin levels are closely monitored by the seed industry in cottonseed destined for feed use. Although aflatoxins are not produced directly by the cotton plant, MON 88913 cottonseed was evaluated for the presence of aflatoxins B1, B2, G1, and G2 as part of the overall compositional analysis. Greater than 50% of the samples from all of the cottonseed samples of MON 88913, MON 88913(-), and reference varieties were found to be below the LOQ of the assay and were thus excluded from the statistical analysis.

The cyclopropenoid fatty acids –malvalic acid, dihydrosterculic acid, and sterculic acid – are anti-nutrients found in cottonseed and cottonseed oil. Levels of the cyclopropenoid fatty acids were measured in MON 88913 cottonseed, cottonseed meal, and cottonseed oil and compared to MON 88913(-) cottonseed, meal, and oil (Tables VII-1, VII-2, VII-4, VII-6, VII-8, and VII-9). No statistical differences were observed in cyclopropenoid fatty acid content between MON 88913 and MON 88913(-) cottonseed, meal, or oil in the combined site analyses. A statistically significant difference ($p \leq 0.05$) between MON 88913 and MON 88913(-) was observed in the comparisons for malvalic acid and sterculic acid in cottonseed from a single location. However, no statistical differences were observed in cottonseed from the other three locations or in the combined site analyses. Values for these analytes from the location where differences were observed were within a tolerance interval that contains 99% of the values expressed in the population of conventional cotton at the 95% confidence level.

Gossypol is a naturally occurring toxicant found in cottonseed, cottonseed meal, and cottonseed oil. No statistical difference in the level of gossypol (free and total) was detected between MON 88913 and MON 88913(-) cottonseed. The level of gossypol in processed, refined cottonseed oil was below the limit of detection for all samples. One statistical difference was detected in the level of total gossypol in raw cottonseed meal between MON 88913 and MON 88913(-); however, the values fell within the range of values for the reference cotton, and within the 99% tolerance interval, and the level of total gossypol was less in cottonseed meal of MON 88913 than MON 88913(-) (Table VII-6). These results demonstrate, with 95% confidence, that the levels of gossypol in the raw cottonseed meal from MON 88913 are within the same population as expected for conventional cotton. Therefore, any minor differences noted were unlikely to be biologically meaningful.

Therefore, it is concluded that the levels of toxicants and anti-nutrients in MON 88913 are comparable to those found in conventional cotton.

3.4. Any Intended Changes to the Composition of Food and Feed

There have been no intended changes to the composition (including nutrients and anti-nutrients) of food or feed derived from MON 88913 compared to other conventional cotton varieties, other than the introduced *cp4 epsps* coding sequence and the production of CP4

EPSPS protein that confers tolerance to glyphosate. The analysis of 69 components of cottonseed, 13 components of cottonseed oil and 41 components of cottonseed meal have shown no biologically meaningful differences between MON 88913 and the negative segregant MON 88913(-), or conventional cotton varieties. Given this extensive compositional characterization, it is concluded that no pleiotropic changes have occurred in MON 88913.

Section 4. Other Information Relevant to the Safety and Nutritional Assessment of MON 88913

Having demonstrated the compositional equivalence of cottonseed derived from MON 88913, and considering the history of safe use of the host organism, cotton, no additional information was considered necessary to support the safety and nutritional assessment of MON 88913.

Section 5. Food and Feed Safety Assessment for MON 88913

5.1. Substantial Equivalence of MON 88913 to MON 88913(-) and Conventional Cotton Varieties

A summary of this assessment demonstrating that MON 88913 is as safe as comparable feed and food, and in compliance with all applicable requirements of the Federal Food, Drug, and Cosmetic Act, is presented in Part I. The data and information provided in Part VII, Section 3, establish that MON 88913, and the foods and feeds derived from it, are compositionally equivalent to conventional varieties of cotton and the comparable foods/feeds that are derived from them.

A detailed nutritional assessment of cottonseed, cottonseed oil and cottonseed meal by composition analyses statistically compared the levels of key nutrients and other components in MON 88913 to the negative segregant control, MON 88913(-). Additionally, a tolerance interval that contains 99% of the values expressed in the cotton population of conventional cotton, at 95% confidence was established for reference. The results establish that the levels of key nutrients and other components of MON 88913 are compositionally equivalent to those of conventional cotton. In the multi-site analysis of cottonseed, the majority of statistical differences identified did not occur across all sites, and for the few statistically different components that occurred across sites, the values for MON 88913 fell within the 99% tolerance interval for commercial cotton.

In the across site analysis for cottonseed processed fractions, only four statistical differences were observed between MON 88913 and MON 88913(-) — in cottonseed, phenylalanine content was higher in MON 88913 than the control; in raw cottonseed meal, levels of total gossypol were lower in MON 88913 than the control; and in cottonseed oil, levels of two minor fatty acids, myristic and behenic acid, were lower in MON 88913

compared to the control. All of these values fell within the 99% tolerance interval for conventional cotton, and within literature ranges for the components where available.

These results establish, with a confidence level of 95%, that the levels of key nutrients and other components of the cottonseed produced from MON 88913 are within the same population as expected for conventional cotton. Therefore, the few statistically significant differences are unlikely to be biologically meaningful, and the cottonseed from MON 88913 is considered compositionally equivalent to MON 88913(-) and commercial cotton. In summary, we have concluded, based on the data and information provided, that the intended uses of MON 88913, and the foods and feeds that would be derived from it, are compositionally equivalent to commercial conventional varieties of cotton, and the comparable foods and feeds that are derived from them.

5.2. Conclusions

Collectively, these data and a history of safe use of the host organism, cotton, as a common source of processed human food and animal feed, support a conclusion of "no concerns" for every criterion specified in the flowcharts outlined in the FDA's Food Policy document (Figure VII-1). Roundup Ready Flex cotton MON 88913 is not materially different in composition, safety or nutrition from conventional cotton, other than its tolerance to Roundup agricultural herbicides. Sales or consumption of cottonseed or processed products derived from MON 88913 cottonseed would be fully consistent with the FDA's Food Policy, the Federal Food, Drug and Cosmetic Act, and current practice for the development and introduction of new cotton varieties and biotechnology traits.

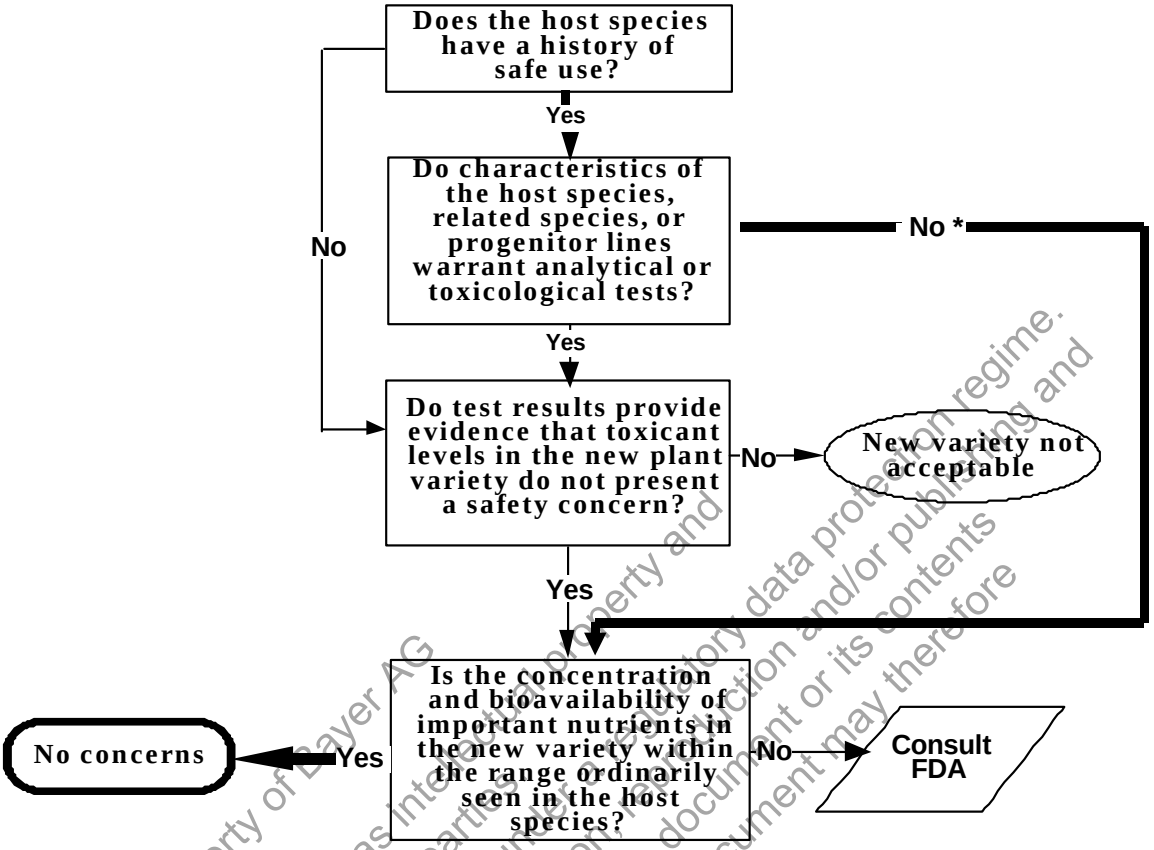


Figure VII-1. Safety Assessment of New Varieties: The Host Plant

REFERENCES

AACC. 1998. *In* Approved Methods of the American Association of Cereal Chemists, 9th Edition.

Abernathy, J.R. and C.G. McWhorter. 1992. Evolution of weed control. Pp 1-8. *In* Weeds of Cotton: Characterization and Control. C.G. McWhorter, J.R. Abernathy (eds.). The Cotton Foundation, Pub. Memphis, Tennessee.

Aebersold, R. 1993. Mass spectrometry of proteins and peptides in biotechnology. Curr. Opin. Biotechnol. 4:412-419.

Akeson, W.R. and M.A. Stahmann. 1964. A pepsin pancreatin digest index of protein quality evaluation. J. Nutrition 83:257-261.

Aalberse, R.C. 2000. Structural biology of allergens. J. Allergy Clin. Immunol. 106:228-38.

Aalberse, R.C, J. Akkerdaas, and R. Van Ree. 2001. Cross-reactivity of IgE antibodies to allergens. Allergy 56:478-90.

An, Y-Q, J.M. McDowell, S. Huang, E.C. McKinney, S. Chambliss, and R.B. Meagher. 1996. Strong, constitutive expression of the *Arabidopsis ACT2/ACT8* actin subclass in vegetative tissues. Plant Journal 10(1):107-121.

Anderson, K.S. and K.A. Johnson. 1990. Kinetic and structural analysis of enzyme intermediates: lessons from EPSP synthase. Chem. Rev. 90:1131-1149.

AOAC International. 2000. *In* Official Methods of Analysis of AOAC International, 17th Edition. Association of Official Analytical Chemists International, Gaithersburg, Maryland.

AOCS. 1997. *In* Official Methods and Recommended Practices of the American Oil Chemists Society, 5th Edition. American Oil Chemists' Society: Champaign, Illinois.

AOCS. 1998. *In* Official Methods and Recommended Practices of the American Oil Chemists' Society, 5th Edition. American Oil Chemists' Society: Champaign, Illinois.

Arfin, S.M., and R.A.Bradshaw. 1988. Cotranslational processing and protein turnover in eukaryotic cells. Biochem. 27:7984-7990.

Astwood, J.D., J.N. Leach, and R.L. Fuchs. 1996. Stability of food allergens to digestion *in vitro*. Nature Biotechnology 14:1269-1273.

Axelos, M., C. Bardet, T. Liboz, A. Le Van Thai, C. Curie, and B. Lescure. 1989. The gene family encoding the *Arabidopsis thaliana* translation elongation factor EF-1 alpha: molecular cloning, characterization and expression. Mol. Gen. Genet. 219(1-2): 106-112.

Bagshaw, R.D., J.W. Callahan, and D.J. Mahuran. 2000. Desalting of in-gel-digested protein sample with mini-C18 columns for matrix-assisted laser desorption ionization time of flight peptide mass fingerprinting. Anal. Biochem. 284:432-435.

Baker, J.L. and H.P. Johnson. 1979. The effect of tillage system on pesticides in runoff from small watersheds. Transaction of the American Society of Agricultural Engineers 25:554-559.

Baker, J.L. and J.M. Laflen. 1979. Runoff losses of surface-applied herbicides as affected by wheel tracks and incorporation. J. Environ. Qual. 8:602-607.

Barker, R.F., K.B. Idler, D.V. Thompson, and J.D. Kemp. 1983. Nucleotide sequence of the T-DNA Region from the *Agrobacterium tumefaciens* Octopine Ti Plasmid pTi15955. Plant Mol. Biol. 2:335-350.

Barry, G., G. Kishore, S. Padgette, M. Taylor, K. Kolacz, M. Weldon, D. Re, D. Eichholtz, K. Fincher, and L. Hallas. 1992. Inhibitors of amino acid biosynthesis: strategies for imparting glyphosate tolerance to crop plants. Pp. 139-145. *In* Biosynthesis and Molecular Regulation of Amino Acids in Plants, B.K. Singh, H.E. Flores, and J.C. Shannon (eds.). American Society of Plant Physiologists.

Barry, G.F., G.M. Kishore, S.R. Padgette, and W.C. Stallings. 1997. Glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthases. United States Patent 5,633,435.

Bassett, D.M., W.D. Anderson, and C.H.E. Werkoven. 1970. Dry matter production and nutrient uptake in irrigated cotton (*Gossypium hirsutum*). Agronomy J. 62:299-303.

Belyea, R. L., B.J. Steevens, R.J. Restrepo, and A.P. Clubb. 1989. Variation in composition of by-product feeds. J. Dairy Sci. 72:2339-2345.

Billeci, T.M. and J.T. Stults. 1993. Tryptic mapping of recombinant proteins by matrix-assisted laser desorption/ionization mass spectrometry. Anal. Chem. 65:1709-1716.

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

Brown, H.B., and J.O. Ware. 1958. Cotton. Pp 20-41. Third Edition. McGraw-Hill Book Company, Inc., New York.

Brubaker, C.L., F.M. Bourland, and J.F. Wendel. 1999. Chapter 1.1 The Origin and Domestication of Cotton. Pp 3-31. *In* Cotton: Origin, History, Technology, and Production. W.C. Smith (ed.). John Wiley & Sons, Inc., New York.

Cherry, J.P., J.G. Simmons, and R.J. Kohel. 1978. Potential for improving cottonseed quality by genetic and agronomic practices. Pp 343-364. *In* Nutritional Improvement of Food and Feed Proteins. M. Friedman (ed.). Plenum Press, New York.

Cherry, J.P. 1983. Cottonseed oil. J. Am. Oil Chem. Soc. 60:360-367.

Cherry, J.P. and H.R. Leffler. 1984. Seed. Pp. 512-558. *In* Cotton, R.J. Kohel and C.F. Lewis (eds.). Amer. Soc. Agron. Madison, WI.

Cherry, J. P., R.J. Kohel, L.A. Jones and W.H. Powell. 1986. Food and Feed Quality of Cottonseed. Pp 557-595. *In* Cotton Physiology. Mauney, J. R and Stewart, J. McD., (eds.). The Cotton Foundation: Memphis, TN.

Chua, N.H., and G.W. Schmidt. 1978. Post-translational transport into intact chloroplasts of a precursor to the small subunit of ribulose-1, 5-bisphosphate carboxylase. Proc. Natl. Acad. Sci. USA. 75:6110-6114.

Conservation Technology Information Center (CTIC). 1998. Crop Residue Management Survey. West Lafayette, Indiana. http://www.ctic.purdue.edu/Core4/CT/Archive.html

Conservation Technology Information Center (CTIC). 1999. What's Conservation Tillage. West Lafayette, Indiana. http://www.ctic.purdue.edu/Core4/WhatsC4.html

Cort, W.M., T.S. Vincente, E.H. Waysek, and B.D. Williams. 1983. Vitamin E Content of Feedstuffs Determined by High-Performance Liquid Chromatographic Fluorescence. Journal of Agricultural Food Chemistry 31:1330-1333.

Coruzzi, G., R. Broglie, C. Edwards, and N-H Chua. 1984. Tissue-specific and light-regulated expression of a pea nuclear gene encoding the small subunit of ribulose-1,5-bisphosphate carboxylase. EMBO J. 3:1671-1679.

Culpepper, A. and A. York. 2000. Weed management in ultra narrow row cotton (*Gossypium hirsutum*). Weed Technol. 14(1):19-29.

Culpepper, A. and A. York. 1998. Weed management: weed management in glyphosate-tolerant cotton. J. Cotton Sci. 2:174-185.

Cotton Incorporated. 2004. http://www.cottoninc.com/CottonSeed/homepage.cfm?PAGE=9#introduction.

Dahlquist, R.L. and J.W. Knoll. 1978. Inductively coupled plasma-atomic emission spectrometry: analysis of biological materials and soils for major, trace, and ultra trace elements. Applied Spectroscopy. 32:1-29.

Degener, O. 1946. Flora Hawaiiness, or, New Illustrated Flora of the Hawaiian Islands [Family 221, Genus *Gossypium*, species *tomentosum*]. O. Degener, Honolulu, Hawaii.

Della-Cioppa, G., S.C. Bauer, B.K. Klein, D.M. Shah, R.T. Fraley, and G.M. Kishore. 1986. Translocation of the precursor of 5-enolpyruvylshikimate-3-phosphate synthase into chloroplasts of higher plants *in vitro*. Proc. Natl. Acad. Sci. 83:6873-6877.

Depicker, A., S. Stachel., P. Dhaese, P. Zambryski, and H.M. Goodman. 1982. Nopaline Synthase: Transcript Mapping and DNA Sequence. J. Molec. Appl. Genet. 1:561-573.

Doherty, A.M., J.S. Kaltenbronn, J.P. Hudspeth, J.T. Repine, W.H. Roark, I. Sircar, F.J. Tinney, C.J. Connolly, J.C. Hodges, M.D. Taylor, B.L. Batley, M.J. Ryan, A.D. Essenburg, S.T. Rapundalo, R.E. Weishaar, C. Humblet, and E.A. Lunney. 1991. New inhibitiors of human renin that contain novel replacements at the P2 site. J. Med. Chem. 34:1258-1271.

FAO/WHO. 1991. Strategies for assessing the safety of foods produced by biotechnology. Report of joint FAO/WHO consultation. WHO, Geneva, Switzerland.

FAO/WHO. 2001. Evaluation of the allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology 22- 25 January 2001. WHO, Geneva, Switzerland.

Fawcett, R. and D. Towry. 2002. Conservation tillage and plant biotechnology: how new technologies can improve the environment by reducing the need to plow. Pp 1-24. Conservatory Technology Information Center, West Lafayette, Indiana.

FDA. 1992. Statement of Policy: Foods Derived from New Plant Varieties. U.S. Food and Drug Administration. Federal Register 57:22984-23005.

Fevrier, C., Y. Lechevestrier, Y. Lebreton, and Y. Jaguelin-Peyraud. 2001. Prediction of the standardized ideal true digestibility of amino acids from the chemical composition of oilseed meals in the growing pig. Animal Feed Sci. Technol. 90:103-115.

Fincher, K.L., S. Flasinski, and J.Q. Wilkinson. 2003. Plant expression constructs. United States Patent 6,660,911.

Fling, M., J. Kopf, and C. Richards. 1985. Nucleotide sequence of the transposon Tn7 gene encoding an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase. Nucleic Acids Res. 13:7095-7106.

Fryxell, P.A. 1979. The Natural History of the Cotton Tribe (*Malvaceae*, tribe *Gossypieae*). Texas A&M University Press, College Station, Texas.

Fuchs, R.L., S.A. Berberich, and F.S. Serdy. 1993. Safety Evaluation of Genetically Engineered Plants and Plant Products: Insect-Resistant Cotton. Pp 199-212. *In* Biotechnology and Safety Assessment, J.A. Thomas and L.A. Myers, (eds.). Raven Press, Ltd., New York.

Gianessi, L.P., C.S. Cressida, S. Sankula, and J.E. Carpenter. 2002. Herbicide tolerant cotton. *In* Plant Biotechnology: Current and Potential Impact for Improving Pest Management in U.S. Agriculture, An Analysis of 40 Case Studies. National Center for Food and Agricultural Policy. Washington, D.C.

Giza, P.E. and R.C. Huang. 1989. A self-inducing runaway-replication plasmid expression system utilizing the Rop protein. Gene 78:73-84.

Görisch, H. 1988. Drop dialysis: time course of salt and protein exchange. Anal. Biochem. 173:393-398.

Harrison, L., M. Bailey, M. Naylor, J. Ream, B. Hammond, D. Nida, B. Burnette, T. Nickson, T. Mitsky, M. Taylor, R. Fuchs, and S. Padgette. 1996. The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested *in vitro* and is not toxic to acutely gavaged mice. J. Nutr. 126:728-740.

Haslam, E. 1993. Pp 3-50. Shikimic Acid: Metabolism and Metabolites. John Wiley and Sons: Chichester, England.

Hayes, R.M., J.W. Wilcut, R.L. Nichols, B.J. Brecke, D.C. Bridges, J.M. Chandler, J.A. Kendig, D.K. Miller, K.L. Smith, and C.E. Snipes. 2001. Assessment of weed management in nontransgenic and transgenic *Gossypium hirsutum*. Pp 249-254. The British Crop Protection Conference: Weeds 2001.

Hebblethewaite, J.F. 1995. The contribution of no-till to sustainable and environmentally beneficial crop production: a global perspective. Conservation Technology Information Center, West Lafayette, Indiana.

Highfield, P.E., and R.J. Ellis. 1978. Synthesis and transport of the small subunit of chloroplast ribulose bisphosphate carboxylase. Nature 271:420-424.

Hoard's Dairyman. April 10, 1984. P 493.

Hui, E. 1996. Cottonseed Oil. Pp 159-240. *In* Bailey's Industrial Oil and Fat Products, Fifth Edition, Vol. 2; Edible Oil and Fat Products: Oils and Oilseeds. John Wiley and Sons, Inc., New York.

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

James, C. 2003. Preview: Global status of commercialized transgenic crops: 2003. ISAAA Briefs No. 30. Ithaca, New York.

James, C. 2002. Global Status of Commercialized Transgenic Crops: 2002. ISAAA Briefs No. 27. ISAAA: Ithaca, New York.

Jiménez, C.R., L. Huang, Y. Qui, and A.L. Burlingame. 1998. Pp 16.4.1-16.4.5. In-gel digestion of proteins for MALDI-MS fingerprint mapping. Current Protocols in Protein Science, Supplement 14.

Jones D.D. and J.H. Maryanski. 1991. Safety considerations in the evaluation of transgenic plants for human food. *In* Safety Considerations in the Evaluation of Transgenic Plants.

Kalaitzandonakes, N.G. and P. Suntornpithug. 2001. Why do farmers adopt biotech cotton? Pp 179-183. Proceedings of the Beltwide Cotton Conference. National Cotton Council, Memphis, Tennessee.

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

Kay, B.D. 1995. Soil Quality: Impact of tillage on the structure of tilth of soil. Pp 7-9. *In* Farming for a Better Environment. Soil and Water Conservation Society, Ankeny, Iowa.

Kay, R., A. Chan , M. Daly, and J. McPherson. 1987. Duplication of CaMV 35S promoter sequences created a strong enhancer for plant genes. Science 236:1299-1302.

Keeling, J.W., P.A. Dotray, T.S. Osborn, and B.S. Asher. 1998. Postemergence weed management with Roundup Ultra, Buctril and Staple in Texas high plains cotton. Pp 861-862. Proceedings of the Beltwide Cotton Conference. National Cotton Council, Memphis, Tennessee.

Kern, J.S. and M.G. Johnson. 1993. Conservation tillage impacts on national soil and atmospheric carbon levels. Soil Sc. Am. J. 57:200-210.

Kimber, I., N.I. Kerkvliet, S.L. Taylor, J.D. Astwood, K. Sarlo, and R.J. Dearman. 1999. Toxicology of Protein Allergenicity: Prediction and Characterization. Toxicological Sciences 48:157-162.

Kishore, G., D. Shah, S. Padgette, G. Della-Cioppa, C. Gasser, D. Re, C. Hironaka, M. Taylor, J. Wibbenmeyer, D. Eichholtz, M. Hayford, N. Hoffmann, X. Delannay, R. Horsch, H. Klee, S. Rogers, D. Rochester, L. Brandage, P. Sanders, and R.T. Fraley. 1988. 5-Enolpyruvylshikimate 3-phosphate synthase. Pp 37-48. From Biochemistry to Genetic Engineering of Glyphosate Tolerance: Biotechnology for Crop Protection. American Chemical Society.

Klee, H.J. and S.G. Rogers. 1987. Cloning of an *Arabidopsis* gene encoding 5-enolpyruvylshikimate-3-phosphate synthase: sequence analysis and manipulation to obtain glyphosate-tolerant plants. Mol. Gen. Genet. 210:437-442.

Lawhon, J. T., C.M. Cater, and K.F. Mattil. 1977. Evaluation of the food use potential of sixteen varieties of cottonseed. J. Am. Oil. Chem. Soc. 54:75-80.

Lee, J.A. 1984. Cotton as a World Crop. Pp 6-26. *In* Cotton. R.J. Kohel and C.F. Lewis (eds.). Agronomy No. 24. Soil Society of America, Inc., Madison, Wisconsin.

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

Levin, J.G. and D.B. Sprinson. 1964. The enzymatic formation and isolation of 3-enolpyruvylshikimate-5-phosphate. J. Biol. Chem. 239:1142-1150.

Marquez, U.M.L. and F.M. Lajolo. 1981. Composition and digestibility of albumins, globulins, and glutelins from *Phaseolus vulgaris*. J. Agric. Food Chem. 29:1068-1074.

McCloskey, W.B. 1998. Weed Management: Transgenics and New Technologies - A Weed Scientists Perspective. Pp 25-26. Proceedings of the Beltwide Cotton Conference. National Cotton Council, Memphis, Tennessee.

McMurray, C.H., W.J. Blanchflower, and D.A. Rice. 1980. Influence of extraction techniques on determination of α-tocopherol in animal feedstuffs. Journal of the Association of Official Analytical Chemists. 63(6):1258-1261.

Metcalfe, D. D., J.D. Astwood, R. Townsend, H.A. Sampson, S.L. Taylor, and R.L. Fuchs. 1996. Assessment of the allergenic potential of foods derived from genetically engineered crop plants. Crit. Rev. Food. Sci. Nutr. 36:S165-186.

Morgan, S.E. 1990. "Gossypol Residues in Organ Meats vs. Thresholds of Toxicity. Vet. Hum. Toxicol. 32S:76-80.

Munro, J.M. 1987. Cotton. 2nd Edition. John Wiley & Sons, New York.

National Cotton Council. 2004. http://risk.cotton.org/03intentions.htm.

National Cottonseed Products Association (NCPA). 2002a. Cottonseed and Its Products. http://www.cottonseed.com/publications/default.asp.

National Cottonseed Products Association (NCPA). 2002b. Cottonseed Feed Product Guide. http://www.cottonseed.com/publications/default.asp.

National Cottonseed Products Association (NCPA). 2002c. Twenty Facts About Cottonseed Oil. http://www.cottonseed.com/publications/default.asp.

National Cottonseed Products Association (NCPA). 1999. Cottonseed: The Inside Story. http://www.txfb.org/planetagriculture/educationalmaterials/cottonseed.asp.

National Cottonseed Products Association (NCPA). 1993. Cottonseed Oil. L.A. Jones and C.C. Clay (eds.) National Cottonseed Products Association and The Cotton Foundation, Memphis, Tennessee.

National Resource Council (NRC). 1982. United States-Canadian Tables of Feed Composition: Pp 3-124. Nutritional Data for United States and Canadian Feeds. National Academy Press, Washington, D. C.

National Resource Council (NRC). 2001. Nutrient Requirements of Dairy Cattle: Pp 1-7. National Academy Press, Washington, D. C.

Nelson, G.C. and D.S. Bullock. 2003. Environmental effects of glyphosate resistant soybeans in the United States. Pp 89-101. *In* The Economic and Environmental Impacts of Agbiotech: a global perspective. Kalaizandonakes, N. (ed.). Kluwer Academic/Plenum Publishers, New York.

Nielson, S.S. 1988. Degradation of bean proteins by endogenous and exogenous proteases - a review. Cereal Chemistry 65:435-442.

Niles, G.A. and C.V. Feaster. 1984. Breeding. Pp 201-231. *In* Cotton. R.J. Kohel and C.F. Lewis (eds.). Agronomy 24. Soil Society of America, Inc., Madison, Wisconsin.

Oblong, J.E., and G.K. Lamppa. 1992. Identification of two structurally related proteins involved in proteolytic processing of precursors targeted to the chloroplast. EMBOL J. 11:4401-4409.

OECD. 1999. Consensus document on general information concerning the genes and their enzymes that confer tolerance to glyphosate herbicide. OECD Environmental Health and Safety Publications. Paris ENV/JM/MONO (99)9.

Padgette, S.R., D.B. Re, G.F. Barry, D.E. Eichhlotz, X. Delannay, R.L. Fuchs, G.M. Kishore and R.T. Fraley. 1996. New weed control opportunities: Development of soybeans with a Roundup Ready™ gene. Pp 53-84. *In* Herbicide-Resistant Crops: Agricultural, Environmental, Economic, Regulatory, and Technical Aspects. Ed. S. O. Duke. CRC Press, New York.

Papadopoulos, G. and E. Ziras. 1987. Nutrient composition of Greek cottonseed meal. Animal Feed Sci. and Tech. 18:295-301.

Pariza, M.W. and E.M. Foster. 1983. Determining the safety of enzymes used in food processing. Journal of Food Protection 46:453-468.

Patterson, M.G., W.R. Goodman, C.D. Monks, and D.P. Delaney. 1998. Economic assessment of Roundup Ready cotton tillage systems. Pp 1:864. Proceedings of the Beltwide Cotton Conference. National Cotton Council, Memphis, Tennessee.

Pearson, W. R. 2000. Flexible sequence similarity searching with the FASTA3 program package. Methods Mol. Biol. 132:185-219.

Percival, A.E., J.F. Wendel, and J.M. Stewart. 1999. Taxonomy and Germplasm Resources. Pp 33-63. *In* Cotton: Origin, History, Technology, and Production. W.C. Smith (ed.). John Wiley & Sons, Inc., New York.

Pharmacopeia. 1990. The United States Pharmacopeia, Vol. XXII, NF XVII. United States Pharmacopeial Convention, Inc. Rockville, MD. P. 1788.

Phatak, S.C., R. Reed, W. Fussell, W.J. Lewis, and G.H. Harris. 1999. Crimson clover-cotton relay cropping with conservation tillage system. Pp 184-188. *In* Proceedings of the 22nd Annual Southern Conservation Tillage Conference for Sustainable Agriculture, Tifton, GA, 6-8 July 1999. Hook, J.E. (ed.). Georgia Agriculture Experiment Station Special Publication 95. Athens, Georgia.

Rademacher, T.W., R.B. Parekh, and R.A. Dwek. 1988. Glycobiology. Annu. Rev. Biochem. 57:785-838.

Reicosky, D.C. 1995. Impact of tillage on soil as a carbon sink. Pp 50-53. *In* Farming for a Better Environment. Soil and Water Conservation Society, Ankeny, Iowa.

Reicosky, D.C. and M.J. Lindstrom. 1995. Impact of fall tillage on short-term carbon dioxide flux. Pp 177-187. *In* Soils and global Change. R. Lal, J. Kimbal, E. Levine, and B.A. Steard (eds.). Lewis Publishers, Chelsea, Michigan.

Richins, R., H. Scholthof, and R. Shepard. 1987. Sequence of figwort mosaic virus DNA (Caulimovirus Group). Nucleic Acids Res. 15:8451-8466.

Rogers, S.O. and A.J. Bendich. 1985. Extraction of DNA from milligram amounts of fresh herbarium and mummified plant tissue. Plant Mol. Biol. Sci. 5:69-76.

Ross, M.A. and C.A. Lembi 1985. Applied Weed Science. Pp 1-19. Burgess Publishing Company, Minneapolis, Minnesota.

Rossell, J. B. 1991. Vegetable Oils and Fats. Pp 261-327 *In* Analysis of Oilseeds, Fats and Fatty Foods. Rossell, J. B. and Pritchard, J. L. R. (eds.) Elsevier Applied Science: New York, NY.

Sambrook, J., and D. Russell (eds.). 2001. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, New York.

SAS® software. SAS Institute Inc., Cary, NC.

Shenstone, F. S. and J.R. Vickery. 1961. Occurrence of cyclo-propene acids in some plants of the order Malvales. Nature 190:168-169.

Sims, S.R., S.A. Berberich, D.L. Nida, L.L. Segalini, J.N. Leach, C.C. Ebert, and R.L. Fuchs. 1996. Analysis of expressed proteins in fibre fractions from insect-protected and glyphosate-tolerant cotton varieties. Crop Sci. 36:1212-1216.

Sjoblad R.D., J.T. McClintock, and R. Engler. 1992. Toxicological considerations for protein components of biological pesticide products. Regulatory Toxicology and Pharmacology 15:3-9.

Sleisenger, M.H. and J.S. Fordtran. 1989. Gastrointestinal Disease, Vol 1. Pp 685-689. *In* Pathophysiology Diagnosis Management. W.B. Saunders Company, Philadelphia.

Smart, J.R. and J.M. Bradford. 1999. Conservation tillage with Roundup can decrease cotton production costs. Pp 1:735-738. Proceedings of the Beltwide Cotton Conference. National Cotton Council, Memphis, Tennessee.

Smith Jr., J.P. 1977. Vascular Plant Families. Pp 120-121. Mad River Press, Eureka, California.

Smith, C.W. and R.A. Creelman. 2001. Vitamin E concentration in upland cotton seeds. Crop Sci. 41:577-579.

Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503-517.

Speek, A.J., J. Schijver, and W.H.P. Schreurs. 1985. Vitamin E composition of some seed oils as determined by high-performance liquid chromatography with fluorometric quantitation. J. Food Sci. 50(1):121-124.

Stalker, D.M., C.M. Thomas, and D.R. Helinski. 1981. Nucleotide sequence of the region of the origin of replication of the broad host range plasmid RK2. Mol. Gen. Genetics. 181:8-12.

Stallings, W.C., S.S. Abdel-Meguid, L.W. Lim, H.S. Shieh, H.E. Dayringer, N.K. Leimgruber, R.A. Stegeman, K.S. Anderson, J.A. Sikorski, S.R. Padgette, and G.M. Kishore. 1991. Structure and topological symmetry of the glyphosate target 5-enolpyruvylshikimate-3-phosphate synthase: A distinctive protein fold. Proc. Natl. Acad. Sci. USA 88:5046-5050.

Steinrücken, H. and N. Amrhein. 1980. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase. Biochem. Biophys. Res. Commun. 94:1207-1212.

Supak, J.R., C.G, Anderson, W.D. Mayfield. 1992. Trends in Cotton Production: History, Culture, Mechanization and Economics. Pp 9-45. *In* Weeds of Cotton: Characterization and Control. C.G. McWhorter, J.R. Abernathy (eds.). The Cotton Foundation, Pub. Memphis, Tennessee.

Sutcliffe, J.G. 1978. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Symposia on Quantitative Biology. 43:77-103.

Tilch, C. and P.S. Elias. 1984. Investigation of the mutagenicity of thylphenylglycidate. Mutation Research 138:1-8.

Turner, W.W., Jr. 1967. Cottonseed meal trace element survey by atomic absorption spectrophotometry. JAOCS 44:129-130.

U.S. EPA. 1993. Reregistration eligibility decision (RED): Glyphosate. Office of Prevention, Pesticides and Toxic Substances, Washington, D.C.

USDA 2003a. http://www.aphis.usda.gov/brs/

USDA. 2003b. www.nal.usda.gov/fnic/cgi-bin/nut_search.pl, Searched on cotton, subtopic seed, cottonseed meal, partially defatted (glandless) and cotton, subtopic oil, vegetable, cottonseed, salad or cooking.

USDA. 2001. The Classification of Cotton. USDA Agricultural Handbook 566.

USDA-ERS. February 2003. Agricultural Resources and Environmental Indicators. Chap. 4.2, P 14. Ag Handbook No. (AH722).
http://www.ers.usda.gov/publications/arei/arei2001/

USDA-ERS. 2002. Cotton Background. USDA/ERS Washington D.C.
http:// www.ers.usda.gov/briefing/cotton/background.htm

USDA-NASS. 2003. Statistics of Cotton, Tobacco, Sugar Crops, and Honey. P II-1. Agricultural Statistics 2003.

USDA-NASS. 2002. http://www.nass.usda.gov/la/Ff051702.txt

USDA Agriculture Handbook No. 74. 1973. Energy Value of Foods. *In* Agriculture Handbook No. 7. United States Department of Agriculture: Washington, D.C.

USDA Agriculture Handbook No. 379. 1970. Forage Fiber Analyses. *In* Agriculture Handbook No. 379. United States Department of Agriculture: Washington, D.C.

Wakelyn, P.J., N.R. Bertoniere, A.D. French, S.H. Zeronian, T.P. Nevell, D.P. Thibodeaux, E.J. Blanchard, T.A. Calamari, B.A. Triplett, C.K. Bragg, C.M. Welch, J.D. Timpa, W.R. Goynes, Jr., W.E. Franklin, R.M. Reinhardt, and T.L. Vigo. 1998. Cotton Fibers. Pp. 577-705. *In* Handbook of Fiber Chemistry, Second Edition. M. Lewin & E.M. Pierce (eds.) Marcel Dekker Inc.

Waldroup, P.W. and J.H. Kersey. 2002. Nutrient composition of cottonseed meal surveyed. Feedstuffs 74:46.

Welch, A.K., P.R. Rahn, R.D. Voth, J.A. Mills, and C.R. Shumway. 1997. Evaluation of preplant and preemergence herbicides in Roundup Ready cotton. Pp 1:784-786. Proceedings of the Beltwide Cotton Conference. National Cotton Council, Memphis, Tennessee.

Wilcut, J.W., R.M. Hayes, R.L. Nichols, S.B. Clewis, J. Summerlin, D.K. Miller, A. Kendig, J.M. Chandler, D.C. Bridges, B. Brecke, C.E. Snipes, and S.M. Brown. 2003. Weed Management in Transgenic Cotton. N.C. State University. Technical Bulletin 319. January 2003.

Williams, K., M. LoPresti, and K. Stone. 1997. Internal protein sequencing of SDS-PAGE-separated proteins: optimization of an in-gel digest protocol. Tech. in Protein Chem. 8:79-98.

Wood, R. 1986. High performance liquid chromatography analysis of cyclopropene fatty acids. Biochemical Archives 2:63-71.

Zikakis, J.P., S.J. Rzucidlo, and N.O. Biasotto. 1977. Persistence of bovine milk xanthine oxidase activity after gastric digestion in vivo and in vitro. J. Dairy Science 60:533-541.

APPENDIX A

Materials and Methods Used for Molecular Analysis of MON 88913

Appendix A

Materials and Methods Used for Molecular Analysis of MON 88913

Materials

DNA for the analysis was isolated from MON 88913 cottonseed produced under field conditions in 2002 grown from seed lot GLP-0203-12170-S. Additional DNA extracted from cottonseed from various MON 88913 generations was used in the generational stability analyses. For these analyses, DNA was isolated from leaves or seed. The control was MON 88913(-). MON 88913(-) is a negative segregant derived from MON 88913 that does not contain the DNA insert. The references included plasmid PV-GHGT35 (Figures IV-1a and IV-1b) that was used to produce MON 88913. For Southern blot analyses of cotton genomic DNA, digested DNA of plasmid PV-GHGT35 (~0.5 and 1 genome copy equivalents) was mixed with digested DNA from MON 88913(-) and separated by electrophoresis on agarose gels. As additional reference standards, the 1 kb DNA Extension Ladder from Invitrogen was used for size estimations on Southern blots. The High Mass Ladder and 1 kb Ladder from Invitrogen were used for size estimations for the PCR analyses.

Characterization of the Materials

The identity of the field-produced cottonseed was confirmed by PCR analysis prior to use to confirm the presence or absence of MON 88913, as appropriate. The stability was determined in each Southern analysis by observation of the digested DNA sample on an ethidium bromide stained agarose gel. The identity of the materials used in generational stability analyses was confirmed by chain-of-custody documents and by Southern blot fingerprint.

DNA Isolation for Southern Blot and PCR Analyses

Genomic DNA from MON 88913 and MON 88913(-) was extracted from cottonseed by first grinding the seed to a meal and then following standard DNA extraction procedures based on the CTAB DNA extraction method of Rogers and Bendich (1985). Genomic DNA samples were incubated in a 65 °C water bath prior to quantification (typically for several hours). Leaf tissue used in the stability analyses was lyophilized for ~23 hours and then ground into a fine powder. The genomic DNA was extracted following standard procedures. Genomic DNA was stored in a 4 °C refrigerator. The DNA from plasmid PV-GHGT35 was purified from *E. coli* following standard procedures. Purified plasmid DNA was stored in a -20 °C freezer.

Quantification of Genomic DNA

Quantification of DNA samples was performed using a Hoefer DyNA Quant 200 Fluorometer with Roche Molecular Size Marker IX or Roche pBR322 DNA as a calibration standard.

Restriction Enzyme Digestion of Genomic DNA

Approximately 20 µg of genomic DNA from either MON 88913 or MON 88913(-) were used for restriction enzyme digestions. Overnight digests were performed at 37 °C

according to standard procedures based on Sambrook and Russell (2001) in a total volume of 500 µl using 100 units of the appropriate restriction enzyme(s). After digestion, the samples were precipitated by adding 1/10 volume (50 µl) of 3 M NaOAc (pH 5.2) and two volumes (1 ml relative to the original digest volume) of 100% ethanol, followed by incubation in a -20 °C freezer. The digested DNA was precipitated at maximum speed in a microcentrifuge, washed with 70% (v/v) ethanol, vacuum-dried, and re-dissolved in Tris-EDTA (TE) buffer.

DNA Probe Preparation for Southern Blot Analyses

Probe template DNA containing sequences of plasmid PV-GHGT35 (Figures IV-1a and IV-1b) was prepared by PCR amplification following a standard procedure based on Sambrook and Russell (2001). Approximately 25 ng of each probe template were labeled with ^{32}P-dCTP (~6000 Ci/mmol) at 65 °C or with ^{32}P-dATP (~6000 Ci/mmol) at 60 °C by the random priming method (RadPrime DNA Labeling System, Life Technologies).

Southern Blot Analyses of Genomic DNA

Samples of DNA digested with restriction enzymes were separated based on size using 0.8% (w/v) agarose gel electrophoresis according to standard procedure based on Sambrook and Russell (2001). A 'long run' and 'short run' were performed during the gel electrophoresis. The ~20 µg samples of digested MON 88913 DNA were divided in half for loading ~10 µg on the long run and ~10 µg on the short run. The long run enabled greater separation of higher molecular weight DNAs, while the short run allowed smaller molecular weight DNAs to be retained on the gel. The long-run samples were loaded onto the gel and typically subjected to electrophoresis for 14-16 hours at 35 volts. The short run samples were then loaded in adjacent lanes on the same gel, and typically the gel was subjected to electrophoresis for 4–5 additional hours at 85 volts. In the case of generational stability analyses, ~10 µg of digested genomic DNA were separated based on size using a 0.8% (w/v) agarose gel as a single run at 35 volts for ~18.5 hours. All Southern blot analyses were performed according to standard procedure based on the method of Southern (1975). Multiple exposures of each blot were then generated using Kodak Biomax MS-1 or MS-2 film in conjunction with Kodak Biomax MS intensifying screen in a -80 °C freezer.

PCR Analyses of the Insert

The organization of the elements within the DNA insert and verification of adjacent genomic cotton DNA in MON 88913 were confirmed using PCR analysis by amplifying six overlapping regions of DNA that span the entire length of the insert. The PCR analyses were conducted using 50 ng of genomic DNA template in a 50 µl reaction volume containing a final concentration of 1.5 mM $MgCl_2$, 0.2-1.22 µM of each primer, 0.2 mM each dNTP, and 2.5 µl of HotStarTaq DNA polymerase (Qiagen). The amplification of Products A-F (Part IV, Figure IV-12) was performed under the following cycling conditions: 95 °C for 15 minutes, 38 cycles at 94 °C for 1 minute, 60 °C for 1 minute, 72 °C for 2 minutes, and 1 cycle at 72 °C for 2 minutes. Aliquots of each product were separated on 1.0 % (w/v) agarose gels and visualized by ethidium bromide staining to verify the products were of the expected size.

APPENDIX B

Materials and Methods Used for Characterization of the CP4 EPSPS Protein Produced in MON 88913

Appendix B

Materials and Methods Used for Characterization of the CP4 EPSPS Protein Produced in MON 88913

Materials

The plant-produced CP4 EPSPS protein was isolated from ground seed of Roundup Ready Flex cotton MON 88913, produced under field conditions in 2002 and grown from seed lot GLP-0203-12170-S. The identity of the seed was confirmed by PCR analysis. The CP4 EPSPS protein was stored in a -80 °C freezer in a buffer solution containing 50 mM Tris-Cl, pH 7.5, 50 mM KCl, 2 mM DTT, 1 mM benzamidine, and 25% (v/v) glycerol at a total protein concentration of 0.5 mg/mL and archived under Monsanto Analytical Protein Standard lot 60-100027.

The *E. coli*-produced CP4 EPSPS protein (APS lot 20-100015) was used as a reference standard to establish equivalence in select analyses. These analyses included molecular weight determination by SDS-PAGE, immunoblot analysis, glycosylation analysis, and the functional enzymatic assay. The CP4 EPSPS protein was stored in a -80 °C freezer in a buffer solution [50 mM Tris-HCl, pH 7.5, 50 mM KCl, 2mM DTT, 1mM benzamidine-HCl, and 25% (v/v) glycerol] at a total protein concentration of 3.8 mg/mL.

Description of Assay Controls. Protein molecular weight standards were used to calibrate SDS-PAGE gels and verify protein transfer to PVDF membranes. β-lactoglobulin protein and PTH-amino acid standards were used to verify the performance of the amino acid sequencer. A peptide mixture and an analytical BSA standard were used to calibrate the MALDI-TOF mass spectrometer for tryptic mass analysis and molecular weight determination, respectively. Transferrin was used as the positive control in glycosylation analysis. The following standards and controls were used during amino acid analysis: NIST BSA, NIST AA standards, and norvaline standard.

Methods

The CP4 EPSPS protein was purified from extracts of ground MON 88913 seed using a combination of ammonium sulfate fractionation, hydrophobic interaction chromatography, anion exchange chromatography, and affinity chromatography.

Approximately 100 g of ground MON 88913 seed were defatted by acetone extraction before the sample was suspended and homogenized in 1 L of extraction buffer A [50 mM HEPES, pH 7.5, 1 mM urea, 10% (v/v) glycerol, 2 mM DTT, 1 mM EDTA, 0.01 mM ammonium molybdate, 1 mM benzamidine, 0.5 mM PMSF, and 1% (w/v) PVPP]. Following filtration and centrifugation, the supernatant was treated with 50% (w/v) polyethyleneimine to precipitate the nucleic acids. The precipitated nucleic acids were removed by centrifugation and the CP4 EPSPS protein in the supernatant was precipitated by 70% ammonium sulfate saturation. The pellet from the ammonium sulfate precipitation was dissolved in 275 mL of buffer B [20 mM HEPES, pH 7.2, 15% (v/v) glycerol, 0.5 mM PMSF, 0.1 mM EDTA, 1 mM DTT, 1 mM benzamidine and 0.01 mM sodium tungstate] containing 1.25 M ammonium sulfate.

The solubilized ammonium sulfate precipitate of 225 mL was loaded onto a 10 cm x 1.6 cm column (bed volume ~20 mL) of Phenyl Sepharose 6 Fast Flow (high sub) and equilibrated with buffer B containing 1.25 M ammonium sulfate. The column was washed with 40 mL of buffer B containing 1.25 M ammonium sulfate and then eluted with 120 mL using a linear gradient from 1.25 M to 0.00 M ammonium sulfate in buffer B, followed by a 40 mL wash with buffer B. The fractions containing the CP4 EPSPS proteins were identified based on the analyses of western blot and SDS-PAGE, were pooled (~15 mL) and dialyzed against 800 mL of buffer C [25 mM Bis-Tris propane, pH 6.5, 1 mM DTT, 1 mM benzamidine, 0.5 mM PMSF, and 10% (v/v) glycerol] with two buffer changes.

The dialyzed preparation was then loaded onto a 1.8 cm x 1 cm anion exchange column containing ~1.5 mL of Source 15Q resin equilibrated with buffer C and then washed with ~2 mL of buffer C. Bound proteins were then eluted with 7.5 mL using a linear gradient from 0 to 150 mM NaCl in buffer C; 6 mL using a linear gradient from 150 mM to 500 mM NaCl in buffer C; and finally 2 mL of 500 mM NaCl in buffer C. Based on the SDS-PAGE gel analysis, fractions containing CP4 EPSPS protein (major band co-migrating with the CP4 EPSPS reference standard) were pooled and dialyzed against buffer D [50 mM MES, pH 5.85, 1 mM DTT, 1 mM benzamidine, and 15% (v/v) glycerol] with two buffer changes.

The buffer-exchanged sample (final volume of 4 mL) recovered from the anion exchange column was loaded onto a ~2 mL column of pre-cycled cellulose phosphate equilibrated with buffer D. After loading, the column was washed with buffer D, and the CP4 EPSPS protein was eluted by washing with buffer D, containing 0.5 mM phosphoenolpyruvate (PEP) and 0.5 mM shikimate-3-phosphate (S3P). CP4 EPSPS-containing fractions were pooled based upon SDS-PAGE analysis. The pooled fractions were concentrated and then buffer exchanged into a storage buffer (66.7 mM Tris-HCl, pH 7.5, 66.7 mM KCl, 2.7 mM DTT, and 1.3 mM benzamidine) using a Millipore 10 kDa molecular weight cut off Amicon Ultra-15 centrifugal concentration device. The final volume of the concentrated sample was brought to ~1 mL with storage buffer. An aliquot of 150 µL of this sample was submitted for definitive amino acid compositional analysis. An aliquot of plant-produced CP4 EPSPS was hydrolyzed and analyzed (three replicates/sample) according to standard operating procedures. Amino acid standards were used to calibrate the amino acid analyzer and BSA was used to verify the vapor phase protein hydrolysis.

A second aliquot (0.85 mL) of the sample was diluted with glycerol to a concentration of 25% (v/v) resulting in a final sample (APS lot 60-100027) in 50 mM Tris-Cl, pH 7.5, 50 mM KCl, 2.0 mM DTT, and 1 mM benzamidine. This diluted sample was aliquotted into 100 µL portions and stored in a -80 °C freezer.

Molecular Weight and Purity Estimation – SDS-PAGE. Aliquots of stock solutions of the plant-produced CP4 EPSPS protein and reference standard CP4 EPSPS protein were diluted to a final concentration of 0.2 µg/µL. Molecular weight markers (Bio-Rad broad-range, cat # 161-0317, Hercules, CA) used to estimate the molecular weight of the plant-produced CP4 EPSPS protein were diluted to a final concentration per protein band of

0.1 µg/µL. The plant-produced protein was analyzed in duplicate at 1, 2, and 3 µg total protein per lane. The *E. coli*-produced protein was analyzed at 1 µg as a reference standard. All samples were heated at ~104 °C for 4 min and applied to a pre-cast Tris-Glycine 4→20% polyacrylamide gradient 12-well mini-gel (Invitrogen, Carlsbad, CA). Electrophoresis was performed at a constant voltage of 125 V for 15 min followed by a constant voltage of 170 V for 65 min until the dye front approached the bottom of the gel. Proteins were fixed in the gel by gentle shaking in a solution of 40% (v/v) methanol and 7% (v/v) glacial acetic acid for 30 min, stained (2 h) with Brilliant Blue G-Colloidal stain (Sigma Chemical Co., St. Louis, MO), destained ~ 30 sec with a solution containing 10% (v/v) acetic acid and 25% (v/v) methanol, and finally destained with 25% (v/v) methanol for 2 h.

Analysis of the gel was performed using a Bio-Rad Laboratories GS-710 densitometer with the supplied Quantity One software (version 4.3.0, Hercules, CA). Molecular weight values supplied by the manufacturer were used to estimate the molecular weight of each observed band. All visible bands within each lane were quantified. For the plant-produced CP4 EPSPS protein, purity was estimated as the percent optical density of the ~43 kDa band relative to all bands detected in the lane. Molecular weight and purity were reported as an average of all three loadings containing the plant-produced CP4 EPSPS protein.

<u>*MALDI-TOF Analysis.*</u> MALDI-TOF mass spectrometry was used to confirm the identity of the plant-produced CP4 EPSPS protein.

Molecular Weight Determination. Prior to analysis, the plant-produced CP4 EPSPS protein and BSA reference protein (NIST, Gaithersburg, MD) were desalted using drop dialysis (Görisch, 1988). Briefly, a Millipore 25 mm microdialysis disk (type VSWP, 0.025 µm pore size, Bedford, MA) was floated on HPLC-grade water, spotted with 4 µL of the protein, and dialyzed for 60-120 minutes. Portions of each protein sample (0.3 and 0.5 µL) were spotted on an analysis plate, mixed with 0.75 µL sinapinic acid solution, and air-dried. Mass spectral analysis of the plant-produced CP4 EPSPS protein was performed using an Applied Biosystems Voyager DE-Pro Biospectrometry Workstation MALDI-TOF instrument with the supplied Data Explorer software (version 4.0, Foster City, CA). Mass calibration of the instrument was performed using the desalted BSA reference protein. The mass of the plant-produced CP4 EPSPS protein was reported as an average of three separate mass spectral acquisitions. For comparison, the mass of the CP4 EPSPS protein was also calculated from the expected amino acid sequence of the protein using the EditSeq module of DNAstar software (version 5.02).

SDS-PAGE Separation of Proteins. Prior to the generation of tryptic fragments for MALDI-TOF mass spectroscopy, aliquots of plant-produced CP4 EPSPS protein in loading buffer were electrophoresed on a pre-cast Tris-Glycine 4→20% polyacrylamide gradient mini-gel (Invitrogen, Carlsbad, CA). Prior to loading, the plant-produced CP4 EPSPS protein and Bio-Rad (Hercules, CA) Broad Range

molecular weight markers were heated at ~100 °C for five minutes. The gel was loaded, electrophoresed at 140 V for 15 min followed by 200 V for 50 min, then fixed in 7% (v/v) acetic acid, 40% (v/v) methanol for 45 minutes. Following fixation, the gel was stained for 1.5 h with Brilliant Blue G-Colloidal stain (Sigma, St. Louis) and destained for 60 sec with 10% (v/v) acetic acid, 25% (v/v) methanol and for 2 h with 25% (v/v) methanol.

In-gel Protein Digestion. A band in each of four lanes, containing the plant-produced CP4 EPSPS that migrated at ~43 kDa was excised, destained, reduced, alkylated, and subjected to an in-gel trypsin (Promega, Madison, WI) digest (Williams et al., 1997). Briefly, each gel band was individually destained by incubation in 100 µL of 40% (v/v) methanol and 10% (v/v) glacial acetic acid in its own microfuge tube. Following destaining, the gel bands were incubated in 100 µL of 100 mM ammonium bicarbonate buffer for 30 min at room temperature. Proteins were reduced in 100 µL of 10 mM dithiothreitol solution for two hours at 37 °C. Proteins were then alkylated by the addition of 100 µL of buffer containing 100 mM iodoacetic acid. The alkylation reaction was allowed to proceed at room temperature for 20 min in the dark. The gel bands were incubated in 100 µL of 100 mM ammonium bicarbonate buffer for 30 min at room temperature at which time 100 µL of acetonitrile was added and the incubation was continued for an additional 30 minutes. The ammonium bicarbonate/acetonitrile incubations were repeated two additional times to remove the reducing and alkylating agents from the gel. The gel bands were dried in a SpeedVac concentrator (Savant, Holbrook, NY), rehydrated with 50 µL 25 mM ammonium bicarbonate containing 33 µg/mL trypsin, and the protein contained in the gel band was digested overnight at 37 °C. Digested peptides were extracted for one hour at room temperature with 50 µL 70% (v/v) acetonitrile containing 0.1% (v/v) TFA per gel band. The extraction supernatants were combined into a single tube and dried in a SpeedVac concentrator. This process of extracting the peptides was repeated two more times. The final dried material was reconstituted in 5 µL of 0.1% (v/v) TFA.

Sample Preparation. A portion (~4.7 µl) of the digested sample was desalted (Bagshaw et al., 2000) using Millipore (Bedford, MA) ZipTip$_{C18}$ pipette tips. Prior to desalting, the tips were wetted with methanol and equilibrated with 0.1% (v/v) TFA. The sample was applied to a ZipTip and eluted with 5 µL of Wash 1 [0.1% (v/v) TFA], 5 µL of Wash 2 [20% (v/v) acetonitrile containing 0.1% (v/v) TFA], 5 µL of Wash 3 [50% (v/v) acetonitrile containing 0.1% (v/v) TFA], and finally with 5 µL of Wash 4 [90% (v/v) acetonitrile containing 0.1% (v/v) TFA].

MALDI-TOF Instrumentation and Mass Analysis. Mass spectral analyses were performed as follows: mass calibration of the instrument was performed using an external peptide mixture from a Sequazyme Peptide Mass Standards kit (Applied Biosystems). Samples (0.3 µL) from each of the desalting steps, as well as a sample of solution taken prior to desalting, were co-crystallized with 0.75 µL α-cyano-4-hydroxy cinnamic acid (Ciphergen Biosystems, Palo Alto, CA) on the

analysis plate. All samples were analyzed in the 500 to 5000 Dalton range in reflector mode using 150 shots at a laser intensity setting of 3100 (a unit-less MALDI-TOF instrument specific value). Protonated (MH+) peptide masses were observed monoisotopically in reflector mode (Aebersold, 1993; Billeci and Stults, 1993). GPMAW32 software (Applied Biosystems, version 4.23) was used to generate a theoretical trypsin digest of the expected protein sequence. Masses were calculated for each theoretical peptide and compared to the raw mass data. Experimental masses (MH+) were assigned to peaks when three (or more) isotopically resolved ion peaks were observed in the raw mass data. Peaks were not assessed if there were less than three isotopically resolved peaks in the spectra, when peak heights were less than approximately twice the baseline noise or when a mass could not be assigned due to overlap with a stronger signal ±2 Daltons from the mass analyzed. Known autocatalytic fragments from trypsin digestion were identified in the raw data.

N-terminal Sequence Analysis. Prior to N-terminal sequence analysis, five 5 µg aliquots of the plant-produced CP4 EPSPS protein in Laemmli sample buffer (Laemmli, 1970) were electrophoresed and then electrotransferred to a 0.2 µm PVDF membrane (Bio-Rad). Prior to electrophoresis, the samples were first heated to ~100 °C for 5 min and cooled. These samples, along with pre-stained molecular weight markers (Bio-Rad Dual Color, cat # 161-0374, Hercules, CA), were loaded onto a pre-cast Tris-Glycine 4→20% polyacrylamide gradient 10-well mini-gel (Invitrogen, Carlsbad, CA). Electrophoresis was performed at a constant voltage of 140 V for 18 min followed by a constant voltage of 200 V for 52 min until the dye front approached the bottom of the gel. The gel was then electroblotted for 60 min at a constant current of 300 mA in a solution containing 10 mM CAPS diluted with 10% (v/v) methanol, pH 11. Protein bands were stained by soaking the membrane for 90 sec in Ponceau S stain (Sigma, St. Louis, MO) and destained by washing twice with Milli Q water each for 2 minutes. Two lanes of the CP4 EPSPS protein band at ~ 43 kDa were excised from the membrane and sequenced.

N-terminal sequence analysis was performed for 15 cycles using automated Edman degradation chemistry (Hunkapillar et al., 1983). An Applied Biosystems 494 Procise Sequencing System with 140C Microgradient system and 785A Programmable Absorbance Detector and Procise Control Software (version 1.1a) were used. Chromatographic data were collected using Atlas software (version 3.59a, LabSystems, Altrincham, Cheshire, England). A PTH-amino acid standard mixture (Applied Biosystems, Foster City, CA) was used to chromatographically calibrate the instrument for each analysis. This mixture served to verify system suitability criteria such as percent peak resolution and relative amino acid chromatographic retention times. A control protein (10 picomole β-lactoglobulin, Applied Biosystems) was analyzed before and after the ~43 kDa protein band to verify that the sequencer met acceptable performance criteria for repetitive yield and sequence identity.

Immunoblot Analysis – Immunoreactivity. Aliquots of the stock solutions of the plant-produced CP4 EPSPS and reference standard were diluted in Laemmli sample buffer (Laemmli, 1970) to final concentrations of 0.3, 0.2, and 0.1 ng/µL. Samples were then heated to 97 °C for five min and applied to a pre-cast Tris-Glycine 4→20% polyacrylamide gradient 15-well mini-gel (Invitrogen, Carlsbad, CA). Both plant- and *E. coli*-produced CP4 EPSPS proteins were loaded in duplicate at 1, 2, and 3 ng CP4 EPSPS protein per lane. Electrophoresis was performed at constant voltage of 125 V for 60 min followed by a constant voltage of 150 V for 30 min until the dye front reached the bottom of the gel. Pre-stained molecular weight markers included during electrophoresis (Bio-Rad Dual Color, cat # 161-0374, Hercules, CA) were used to verify electrotransfer of protein to the PVDF membrane and to estimate the molecular weight of the immunoreactive bands. Samples were electrotransferred to a 0.45 µm PVDF membrane (Invitrogen, Carlsbad, CA) for 70 min at a constant current of 300 mA.

The membrane was then blocked by incubation in 5% (w/v) NFDM in 1× PBST for 30 minutes. The membrane was first probed with 25 mL of a 1:4000 dilution of goat anti-CP4 EPSPS serum [lot 6844572, prepared using *E. coli*-produced CP4 EPSPS protein reference standard APS lot 20-100017 as the antigen] in 1% (w/v) NFDM in PBST for one hour. Excess serum was removed using three 5-min washes with PBST. The membrane was finally probed with HRP-conjugated rabbit anti-goat IgG (Sigma, St. Louis, MO) at a dilution of 1:10000 in 1% (w/v) NFDM in PBST for 45 min and again excess HRP-conjugate was removed using three 5-min washes with PBST. All incubations were performed at room temperature. Immunoreactive bands were visualized using the ECL detection system (Amersham Biosciences) and exposed (15, 20, 30 sec., and 1 min) to Hyperfilm ECL high performance chemiluminescence film (Amersham Biosciences). Films were developed using a Konica SRX-101A automated film processor (Tokyo, Japan).

Image Analysis of Blot Films. Image analysis of immunoreactive bands on blot films was conducted using a Bio-Rad model GS-710 calibrated imaging densitometer (Hercules, CA) equipped with Quantity One software Version 4.3.0. The level of signal for the principal band corresponding to the CP4 EPSPS protein detected in each lane was measured as band contour quantity (avg. band OD × band area in mm^2). The percent difference between the plant- and *E. coli*-produced CP4 EPSPS proteins was calculated as shown below:

$$\left|\frac{(E.coli\text{ - CP4 EPSPS}) - (\text{Plant - CP4 EPSPS})}{(E.coli\text{ - CP4 EPSPS})}\right| \times 100$$

The average overall percent difference was calculated and the immunoreactivities of the plant-produced and reference proteins were judged to be equivalent if the overall average percent difference was ≤ 20%.

Functional Activity Assay. Prior to analysis, the plant-produced CP4 EPSPS protein and the *E. coli*-produced CP4 EPSPS reference standard were diluted to fall within the range of the assay standard curve in a buffer solution containing 50 mM HEPES, pH 7.0.

Assays for both the plant-produced CP4 EPSPS protein and the *E. coli*-produced CP4 EPSPS reference standard protein were conducted in triplicate and each replicate was subsequently analyzed spectrophotometrically in duplicate. This end-point type colorimetric assay measures the release of inorganic phosphate from one of the substrates, PEP, which is released by the action of the EPSPS enzyme. Briefly, reaction mixtures contained the EPSPS enzyme with 2 mM S3P and were initiated with 5 mM PEP. The final reagent concentrations in the assay were 50 mM HEPES (pH 7.0), 0.1 mM ammonium molybdate, and 5 mM potassium fluoride. Reactions were incubated for two min at 25 °C to allow for product formation. The reactions were quenched with malachite green (phosphate assay) reagent and fixed after two min with 33% (w/v) sodium citrate. The EPSPS-catalyzed release of inorganic phosphate from PEP was determined at a wavelength of 660 nm using a PowerWave X_i (Bio-Tek) microplate reader, relative to a standard curve of inorganic phosphate treated with the malachite green (phosphate assay) reagent and 33% (w/v) sodium citrate. For EPSPS, one unit (U) of enzyme activity was defined as the amount of enzyme that produced 1 µmole of inorganic phosphate from PEP per min at 25 °C. Calculations of the specific activities were performed using Microsoft Excel 2000 version 9.0.4402 SR-1. Specific activity values were calculated based on the purity corrected concentration of the CP4 EPSPS protein. The plant-produced CP4 EPSPS protein was considered to be equivalent to the *E. coli*-produced CP4 EPSPS if the specific activity was within two fold of the *E. coli*-produced protein specific activity.

Glycosylation Analysis. This analysis was used to determine whether the plant-produced CP4 EPSPS protein was post-translationally modified with covalently bound carbohydrate moieties. Aliquots of the plant-produced CP4 EPSPS, the *E. coli*-produced CP4 EPSPS reference standard (in this instance, a negative control), and the positive control, transferrin, were all diluted with 1-2× Laemmli buffer to concentrations of 0.05 µg/µL and 0.1 µg/µL. The samples were heated to 97 °C for five min, cooled, and loaded, along with 10 µL of molecular weight marker (Bio-Rad Dual Color, cat # 161-0374, Hercules, CA), on a Tris-Glycine 4→20% polyacrylamide gradient 10-well mini-gel (Invitrogen, Carlsbad, CA). Electrophoresis was performed at a constant voltage of 150 V for 15 min followed by a constant voltage of 200 V for 55 min until the dye front reached the bottom of the gel. After electrophoresis, proteins were electrotransferred to a 0.45 µm PVDF membrane for one hour at a constant current of 300 mA.

Carbohydrate detection was performed directly on the PVDF membrane using the ECL detection system (Amersham Pharmacia). The PVDF membrane was incubated for 10 min in PBS and transferred to a solution of 100 mM sodium acetate buffer, pH 5.5, containing the oxidation reagent, 10 mM sodium metaperiodate. The membrane was incubated in the dark for 20 minutes. The oxidation solution was removed from the membrane by two brief rinses followed by three sequential 10 min washes in PBS. The membrane was transferred to a solution of 100 mM sodium acetate buffer, pH 5.5, containing 25 nM biotin hydrazide and incubated for 60 minutes. Biotin hydrazide solution was removed by washing in PBS, as previously described for the removal of the 10 mM sodium metaperiodate solution. The membrane was blocked for 60 min in 5% NFDM blocking agent in PBS. The blocking solution was removed by washing in PBS

as previously described. The membrane was incubated with streptavidin-HRP conjugate (diluted 1:6000) in PBS for 30 min to detect carbohydrate moieties bound to biotin. Excess streptavidin-HRP was removed by washing in PBS as previously described. Bands were visualized using the ECL detection system (Amersham Biosciences) and exposed (30 sec and one min) to Hyperfilm ECL high performance chemiluminescence film (Amersham Biosciences). Films were developed using a Konica SRX-101A automated film processor (Tokyo, Japan).

APPENDIX C

Materials and Methods Used for the Analysis of the Levels of CP4 EPSPS Protein in MON 88913

Appendix C

Materials and Methods Used for the Analysis of the Levels of CP4 EPSPS Protein in MON 88913

Materials

Tissue samples analyzed in this study were produced under field conditions in 2002 alongside the materials for molecular and protein characterization and were grown from seed lot GLP-0203-12170-S (MON 88913) and GLP-0203-12171-S [MON 88913(-)]. An *E. coli*-produced CP4 EPSPS protein standard (Monsanto APS lot # 20-100015) was used as a reference for analysis of CP4 EPSPS protein levels.

Characterization of the Materials

The identities of the field-produced tissues and cottonseed were confirmed by verifying the chain-of-custody documentation and the tissues were assayed prior to use by PCR analysis to confirm the presence or absence of MON 88913, as appropriate.

Summary of Field Design and Tissue Collection

MON 88913 and MON 88913(-) were grown at four field locations in the U.S in 2002: Baldwin County, Alabama; Tulare County, California; Clarke County, Georgia; and Hockley County, Texas. These field sites provided a range of environmental and agronomic conditions representative of locations where MON 88913 is expected to be produced commercially. At each site, four replicated plots of MON 88913 and MON 88913(-) were planted using a randomized complete block field design. Young leaf, overseason leaf (OSL1, OSL2, OSL3), root, seed, and pollen tissues were collected from each replicated plot at all field sites. Throughout the field production process, sample identity was maintained using unique sample identifiers and chain-of-custody documentation. Upon collection, all tissue samples were placed in uniquely labeled bags or containers. All tissue samples, with the exception of seed (which was stored and shipped at ambient temperature), were stored on dry ice, shipped frozen on dry ice, and stored at -80°C.

Young leaf samples were collected at the first true leaf growth stage from all field locations. The first fully expanded true leaves were nonsystematically collected from each MON 88913 and MON 88913(-) plot and all leaves from a given plot were pooled. Overseason leaf (OSL) samples were collected from the newest fully expanded leaf from each MON 88913 and MON 88913(-) plot from all field locations at the following time-points: OSL1 at approximately 4th node; OSL2 at approximately 50% white flower; and OSL3 at approximately cut-out. Root samples were collected from each MON 88913 and MON 88913(-) plot at all field locations. The root was removed at the soil line and thoroughly washed with water to remove excess soil. The root samples were collected at approximately 50% white flower growth stage. Pollen samples were collected from each MON 88913 and MON 88913(-) plot at all field locations. Pollen was collected at approximately 50% white flower stage. Because of the limited quantity of cotton pollen, MON 88913 and MON 88913(-) pollen were collected and pooled across replicates at each site to generate sufficient quantities of samples. Seed samples were collected from

each MON 88913 and MON 88913(-) plot at all field locations. The seed was harvested at crop maturity and all seed was ginned and delinted prior to sample processing.

Tissue Processing and Protein Extraction

During the processing step, dry ice was combined with the samples (except pollen) and vertical cutters or mixers were used to thoroughly grind and mix the tissues. Processed tissue samples were transferred into 15 ml tubes. All tissue samples were stored in a -80 °C freezer prior analysis. Extraction parameters and ELISA validation information for each tissue type are described below. All tissues were extracted using a Harbil Mixer and insoluble material was removed from leaf, root, and pollen extracts by a Serum Filter System (Fisher Scientific, Pittsburgh, PA). Insoluble material was removed from seed extracts by centrifugation. The clarified extracts were aliquotted and stored frozen in a -80 °C freezer until ELISA analyses. During validation, the extraction efficiency for each tissue type was determined by successive extraction of three replicates, where the last extraction employed a harsh buffer (e.g., 2X Laemmli buffer). To evaluate the analytical accuracy of the ELISA, extracts prepared from each tissue type of conventional cotton plants were spiked with known quantities of CP4 EPSPS protein at three concentrations spanning the range of the standard curve. The intra- and inter-assay precision were assessed by determining the coefficient of variation (CV) of the concentration of CP4 EPSPS protein measured for the positive control sample from 10 or more independent ELISAs using one-way analysis of variance (ANOVA). The limits of quantitation (LOQ) were calculated based on the lowest standard concentration. The ng/ml value was converted to µg/g fwt using the respective dilution factor and tissue-to-buffer ratio. The limits of detection (LOD) were calculated as the mean value using the data generated on conventional sample extracts for each tissue type plus three standard deviations. The LOD value in ng/ml was converted to µg/g fwt using the respective dilution factor and tissue-to-buffer ratio. The CP4 EPSPS protein was extracted from each tissue by adding the appropriate volume of CP4 EPSPS extraction buffer (TBA) and shaking in a Harbil mixer. The TBA buffer consisted of 100 mM Tris-base, 100 mM $Na_2B_4O_7 \cdot 10H_2O$, 10 mM $MgCl_2$, 0.05% (v/v) Tween-20 at pH 7.8, and 0.2% (w/v) L-ascorbic acid.

The positive quality control (QC) sample was prepared from cotton tissue that contained the CP4 EPSPS protein. The negative quality control sample was prepared from cotton tissue that does not contain the *cp4 epsps* coding sequence and therefore does not produce the CP4 EPSPS protein. Extracts of the positive and negative QC samples were analyzed on every plate in triplicate wells. All positive QC samples fell within the range established during method validation and all negative QC samples were less than the assay LOQ, as expected. Validation of the ELISA method establishes the specificity of the antibodies used to detect the CP4 EPSPS protein.

ELISA Reagents

CP4 EPSPS protein standard (antigen) was produced by fermentation in *E. coli.* The protein was purified by a combination of cell extraction, ammonium sulfate precipitation, hydrophobic and anion exchange chromatography. The purity-corrected total protein concentration of the purified standard was 3.7 mg/ml by amino acid composition

analysis. The purity was 97% as determined by sodium dodecyl-sulfate polyacrylamide gel electrophoresis and densitometric analysis. Mouse monoclonal antibody clone 39B6 (IgG2a isotype, kappa light chain; lot # 6199732) specific for the CP4 EPSPS protein was purified from mouse ascites fluid using Protein-A Sepharose affinity chromatography. The concentration of the purified IgG2a was determined to be 3.2 mg/ml by spectrophotometric methods. Production of the 39B6 monoclonal antibody was performed by TSD Bioservices, Inc. (Newark, DE). The purified antibody was stored in a buffer (pH 7.2) containing 0.02 M $Na_2HPO_4 \cdot 7H_2O$, 0.15 M NaCl, and 15 ppm ProClin 300 (Sigma Chemical Company, St. Louis, MO). The detection reagent was goat anti-CP4 EPSPS antibody (Sigma Chemical Company, St. Louis, MO) conjugated to HRP.

CP4 EPSPS ELISA Method

The CP4 EPSPS ELISA was performed using an automated robotic workstation (Tecan, Research Triangle Park, NC). Mouse anti-CP4 EPSPS antibody was diluted in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, and 150 mM NaCl, pH 9.6) and immobilized onto 96-well microtiter plates at 1.0 µg/ml followed by incubation in a 4 °C refrigerator for $\geq$ 8 h. Plates were washed in 1X PBS with 0.05% (v/v) Tween-20 (1X PBST) and blocked with the addition of 10% (w/v) non-fat dry milk in TBA. Plates were washed as before followed by the addition of 100 µl per well of CP4 EPSPS protein standard or sample extract and incubated at 37 °C for 1 h. Plates were washed as before followed by the addition of 100 µl per well of goat anti-CP4 EPSPS peroxidase conjugate and incubated at 37 °C for 1 h. Plates were developed by adding 100 µl per well of HRP substrate, 3,3',5,5'- tetramethyl-benzidine (Kirkegaard & Perry, Gaithersburg, MD). The enzymatic reaction was terminated by the addition of 100 µl per well of 6 M H_3PO_4. Quantitation of CP4 EPSPS protein levels was accomplished by interpolation from a CP4 EPSPS protein standard curve that ranged in concentration from 0.456 - 14.6 ng/ml.

Moisture Analysis

Young leaf, overseason leaf, and root tissues were analyzed for moisture content using an IR 200 Moisture Analyzer (Denver Instrument Company, Arvada, CO). Covance Laboratories Inc. (Madison, WI) analyzed seed tissue for moisture content. Because of limited sample quantity, moisture was not determined for pollen. A homogeneous tissue specific site pool (TSSP) was prepared by mixing approximately equal portions of the respective tissue type from each MON 88913 and MON 88913(-) plot within each field site. These pools were prepared for all tissues analyzed in this study (except pollen). The mean percent moisture for each TSSP was calculated from three analyses of a given pool and used to convert the fwt protein levels at each site to dwt protein levels. A tissue-specific DWCF was calculated for each site as follows:

DWCF = 1 - [Mean Percent TSSP Moisture / 100]

The DWCF was only applied to samples with protein levels greater than the assay LOQ. All protein levels calculated on a fwt basis were converted into protein levels reported on a dwt basis using the following calculation:

$$Protein\ Level\ in\ Dry\ Weight = \frac{\left(Protein\ Level\ in\ Fresh\ Weight\right)}{\left(DWCF\right)}$$

Data Analyses

All ELISA plates were analyzed on a SPECTRAFluor Plus microplate reader (Tecan, Research Triangle Park, NC) using dual wavelengths. The CP4 EPSPS protein absorbance readings were determined at a wavelength of 450 nm with a simultaneous reference reading of 620 nm that was subtracted from the 450 nm reading. Data reduction analyses were performed using Molecular Devices SOFTmax PRO version 2.4.1. Absorbance readings and protein standard concentrations were fitted with a four-parameter logistic curve fit. Following the interpolation from the standard curve, the amount of protein (ng/ml) in the tissue was reported on a "µg/g fwt" basis. This conversion utilized the sample dilution factor and tissue-to-buffer ratio. The protein values in µg/g fwt were also converted to "µg/g dwt" by applying the DWCF. The arithmetic mean, SD, and range (fwt and dwt) were calculated for each tissue type across sites. Microsoft Excel 2000 (Version 9.0.4402 SR-1, Microsoft, Redmond, WA) was used to calculate the CP4 EPSPS protein levels in MON 88913 tissues.

APPENDIX D

General Methods used in Assessing Structural Similarity to Known Allergens and Stability of Proteins in Simulated Digestive Fluids

Appendix D

General Methods used in Assessing Structural Similarity to Known Allergens and Stability of Proteins in Simulated Digestive Fluids

Structural Similarity to Known Allergens

In order to assess potential similarity to allergens, bioinformatic analyses were performed on the CP4 EPSPS protein produced in MON 88913. The comparisons were performed using the allergen (AD4) database.

Exposure to allergens in foods may cause sudden, severe, life-threatening reactions in susceptible individuals. Gliadins are suspected to cause celiac disease (gluten-sensitive enteropathy) and are also considered important immunologically active proteins. Screening the amino acid sequences of proteins introduced into plants by modern biotechnology for similarity to sequences of known allergens and gliadins is one of many assessments performed to evaluate product safety. Similarly, the amino acid sequences of introduced proteins are also screened against known toxins as well as all known proteins in publicly available genetic databases (see Section 5 of Part VI).

The FASTA algorithm can be used to evaluate the extent of sequence similarity between a query protein sequence and a database sequence. In principle, if two proteins share sufficient linear sequence similarity, they also will share three-dimensional structure and, therefore, functional homology.

The bioinformatics assessment is used to identify similarities between the query protein and known or clinically cross-reactive allergens. While related (homologous) proteins may share only 25% amino acid identity in a 200 amino acid overlap (Pearson, 2000), this is not generally sufficient to indicate IgE-mediated cross-reactivity (Aalberse et al., 2001). Indeed, allergenic cross-reactivity caused by proteins sharing conformational or linear epitopes with known allergens is rare at 50% identity and typically requires >70% amino acid identity across the full length of the protein sequences (Aalberse, 2000). Such high levels of identity are readily detected using FASTA. Additionally, proteins closely related to gliadins or glutenins (the proteins that trigger celiac disease, a non-IgE mediated allergic disorder) can be easily identified using FASTA. It is possible that proteins structurally unrelated to allergens and gliadins may still contain smaller immunologically significant epitopes. For this comparison an immunologically relevant sequence was defined as eight linearly contiguous, identical amino acids (Metcalfe et al., 1996).

Protein Stability in Simulated Digestive Fluids

Protein allergens tend to be stable to the peptic and acidic conditions of the digestive systems if they are to reach and pass through the intestinal mucosa to elicit an allergenic response (Kimber et al., 1999; Astwood et al., 1996; Metcalfe et al., 1996). Previous studies have shown how simulated mammalian gastric and intestinal fluids were prepared

and used to assess the susceptibility of the CP4 EPSPS protein to proteolytic digestion *in vitro*. The method of preparation of the simulated mammalian gastric and intestinal digestive solutions used is described in the U.S. Pharmacopeia (1990). *In vitro* studies with simulated digestive solutions are widely used as models of animal digestion. These models have been used to investigate the digestibility of plant proteins (Nielson, 1988; Marquez and Lajolo, 1981), animal proteins (Zikakis et al., 1977) and food additives (Tilch and Elias, 1984), to assess protein quality (Akeson and Stahmann, 1964), to study digestion in pigs and poultry, to measure tablet dissolution rates to monitor biodegradation for pharmaceutical applications (Akeson and Stahmann, 1964), and to investigate the controlled-release of experimental pharmaceuticals (Doherty et al., 1991).

APPENDIX E

Materials and Methods Used for Compositional Analysis of MON 88913 Cottonseed from Four Replicated Field Sites

Appendix E

Materials and Methods Used for Compositional Analysis of MON 88913 Cottonseed from Four Replicated Field Sites

Materials

MON 88913, MON 88913(-) and conventional reference cottonseed were grown at four U.S. locations in 2002. MON 88913 and MON 88913(-) were grown from seed lots GLP-0203-12170-S and GLP-0203-12171-S, respectively. The control material, MON 88913(-), has background genetics representative of MON 88913 but does not contain the *cp4 epsps* coding sequence or produce the CP4 EPSPS protein. All cottonseed samples were ginned at the production locations and acid-delinted at the Food and Protein Research and Development Center at Texas A&M University prior to compositional analyses. Cottonseed of sixteen commercial conventional cotton varieties produced alongside of MON 88913 was used as reference material. The varieties, locations, and seed lot numbers are listed below.

Reference	Location	Seed Lot Number
Stoneville 474	California	REF-0203-12254-S
Stoneville 580	California	REF-0203-12255-S
DP 90	Alabama	REF-0203-12256-S
DP 51	Alabama	REF-0203-12257-S
DP 5690	Alabama	REF-0203-12258-S
DP 5415	Alabama	REF-0203-12259-S
GTO-Maxx A	California	REF-0203-12260-S
Phytogen 72	California	REF-0203-12261-S
Fibermax 989	Georgia	REF-0203-12264-S
PSC 355	Georgia	REF-0203-12265-S
GA 161	Georgia	REF-0203-12266-S
HS 12	Georgia	REF-0203-12267-S
Paymaster 330	Texas	REF-0203-12268-S
Paymaster 2379	Texas	REF-0203-12269-S
AFD Rocket	Texas	REF-0203-12270-S
All-Tex Atlas	Texas	REF-0203-12271-S

Analytical reference standards were used as appropriate for each analytical procedure.

Characterization of the Materials

The identities of the MON 88913, MON 88913(-), and reference cottonseed were verified prior to use by confirming the chain-of-custody documentation supplied with the samples collected from the field. Additionally, the identities of the field-produced cottonseed were confirmed by PCR analysis by determining the presence or absence of MON 88913, as appropriate.

Field Trials

The analyzed cottonseed were produced in U.S. field trials in 2002 at four replicated sites. The randomized block trials were conducted in Alabama, California, Georgia, and Texas. These sites provided a variety of environmental conditions representative of regions where MON 88913 is expected to be grown commercially. At each site, MON 88913, MON 88913(-) and conventional reference cottonseed were planted in approximately 200 ft^2 plots in each of four replicated blocks. Sixteen different commercial conventional cotton varieties were planted, four per site. Each plot was clearly marked with a unique lot number and plot number for identification. In accordance with commercial practice, all plants were allowed to pollinate openly within a plot. Cottonseed samples were collected from all plots at seed maturity. The seed cotton was ginned and acid-delinted. Plots were harvested and seeds were ginned and delinted in the following order: MON 88913(-), conventional references, and MON 88913. The seed was stored at ambient temperatures until it was homogenized with dry ice. After homogenization, the cottonseed was stored in a –20 °C freezer until shipment to the analytical laboratory facility on dry ice. At the analytical facility, the samples were stored in a –20 °C freezer until analysis.

Summary of Analytical Methods

Cottonseed samples from MON 88913, MON 88913(-), and conventional reference materials were shipped overnight on dry ice to Covance Laboratories Inc., Madison, Wisconsin, for compositional analyses. Analyses were performed using methods that are currently used to evaluate the nutritional quality of food and feed. Samples were analyzed for proximates (protein, fat, ash, and moisture), ADF, NDF, crude fiber, TDF, amino acids, fatty acids, cyclopropenoid fatty acids, vitamin E, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), gossypol (free and total), and aflatoxins. Carbohydrate and caloric levels were determined by calculation. These methods are described below.

Acid Detergent Fiber (ADF). The method was based on a USDA Agriculture Handbook No. 379 (1970) method. 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 quantitation for this study was 0.1% fwt.

Aflatoxins (AHMF). The method used was based on published AOAC International (2000) methods 991.31 and 990.33. The sample was extracted with a mixture of methanol:water. The extract was diluted with water and a portion was applied to an antibody affinity column. The column was washed first with water to remove major interferences present in feeds, then the aflatoxins were eluted with acetonitrile and the sample was dried with a stream of nitrogen. The aflatoxins were derivatized with acid to form the more highly fluorescent hemi-acetal compounds of B_1 and G_1 called B_{2a} and G_{2a} respectively. A portion of the extract was injected on a high-performance liquid chromatography (HPLC) system and the aflatoxins in the sample were compared with a standard of known concentration. The reference standards were from Romer Laboratories and included: Aflatoxin B1, lot number 021210A; Aflatoxin B2, lot number

020521A; Aflatoxin G1, lot number 020826A; and Aflatoxin G2, lot number 021021A. All reference standards were 100%. The limit of quantitation for aflatoxins was 1.0 ppb.

Amino Acid Composition (TAAP). The method used was based on AOAC International (2000) method 982.30 that 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, methoinine, 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 then quantitated using an automated amino acid analyzer. The limit of quantitation for this study was 0.1 mg/g fwt. The reference standards were Beckman, K18, 2.5 µmol/mL per constituent except Cystine (1.25 µmol/mL); Aspartic Acid (2.62 µmol/mL), and Glutamic Acid (2.62 µmol/mL), Lot Number S207070; Pierce, K18, 2.5 µmol/mL per constituent except Cystine (1.25 µmol/mL), Lot Number DJ58806; Sigma-Aldrich, L-Tryptophan, 100%, Lot Number 88HO4391; Sigma-Aldrich, L-Cysteic Acid Monohydrate, 100%, Lot Number 111K2608; and Sigma-Aldrich, L-Methionine Sulfone, 100%, Lot Number 12H3349.

Ash (ASHM). The method used was based on AOAC International (2000) method 923.03. 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 quantitation for this study was 0.1% fwt.

Carbohydrates (CHO). The method used was based on an USDA Agriculture Handbook No. 74 (1973) method. The limit of quantitation for this study was 0.1% fwt. The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation:

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

Crude Fiber (CFIB). The method used was based on AOAC International (2000) method 962.09. 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 quantitation for this study was 0.1% fwt.

Fat by Soxhlet Extraction (FSOX). The method used was based on AOAC International (2000) method 960.39. 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 quantitation for this study was 0.1% fwt.

Cyclopropenoid Fatty Acids (CPFQ). The method used was based on a literature method (Wood, 1986). The total lipid fraction was extracted from the sample using chloroform and methanol and quantitated gravimetrically. A portion of the lipid fraction was then saponified with a mild alkaline hydrolysis. The free fatty acids were extracted with ethyl ether and hexane. The free fatty acids were then converted to their phenacyl derivatives with 2-bromoacetophenone. The derivatives were quantitated on an HPLC system equipped with an ultraviolet detector. The reference standards included: Monsanto Company, Dihydrosterculic Acid, 94%, Lot Number GLP-0210-13065-A; Monsanto Company, Sterculic Acid, 99%, Lot Number GLP-0208-12963-A; Monsanto Company, Malvalic Acid, 100%, Lot Number GLP-0208-12964-A. The limit of quantitation for this study was 50 µg/g for each acid.

Fatty Acids (FAPM). The method used was based on AOCS (1997) method Ce 1-62 that 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 heptadeconoic 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 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 quantitation was 0.02% fwt. The reference standards were Nu Chek Prep GLC Reference Standard Hazelton No. 1, used as 100%, Lot Number O2-M; Nu Chek Prep GLC Reference Standard Hazelton No. 2, used as 100%, Lot Number JA10-H; Nu Chek Prep GLC Reference Standard Hazelton No. 3, used as 100%, Lot Number M18-L; Nu Chek Prep GLC Reference Standard Hazelton No. 4, used as 100%, Lot Number O2-M; and Nu Chek Prep Methyl Gamma Linolenate, used as 100%, Lot Number U-63M-AU24-K.

Free and Total Gossypol (GOSF and GOSS). The method used was based on AOCS (1998) methods Ba 7-58 and Ba 8-78. For free gossypol, the sample was extracted with aqueous acetone (700 parts acetone plus 300 parts distilled water). The solution was then filtered and the free gossypol was reacted with aniline. For total gossypol analysis, the sample was extracted using a complexing reagent containing acetic acid, 3-amino-1-propanol, and dimethlyformamide. The solution was then filtered and the total gossypol was reacted with analine. For both analyses, the dianilinogossypol was quantitated spectrophotometrically against a standard curve. The limit of quantitation for this study was 0.02% fwt. The reference standard was Sigma Gossypol, 98%, Lot Number 91K4050.

ICP Emission Spectrometry (ICPS). The method used was based on AOAC International (2000) methods 984.27 and 985.01 and a literature method (Dahlquist and Knoll, 1978). Samples were 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. The limits of quantitation of this method and Spex CertiPrep reference standards are listed below.

Mineral	Lot Numbers	Concentration (ppm)	Limit of Quantitation (ppm)
Calcium	O8-67CA	10,000	20.0
Copper	9-38CU	1,000	0.50
Iron	9-02FE	1,000	2.00
Magnesium	S8-87MG	10,000	20.0
Manganese	9-16MN	10,000	0.300
Phosphorus	R8-164P	10,000	20.0
Potassium	Q8-108K	10,000	100
Sodium	Q8-116NA	10,000	100
Zinc	9-34ZN	1,000	0.400

Moisture (M100). The method used was based on AOAC International (2000) methods 926.08 and 925.09. 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 quantitation for this study was 0.1% fwt.

Neutral Detergent Fiber, Enzyme Method (NDFE). The method used was based on AACC (1998) methods 32.20 and a USDA Agriculture Handbook No. 379 (1970) method. Samples were 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 quantitation for this study was 0.1% fwt.

Protein (PGEN). The method used was based on AOAC International (2000) methods 955.04 and 979.09 and two literature methods (Bradstreet, 1965; Kalthoff and Sandell, 1948). 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 quantitation for this study was 0.1% fwt.

Total Dietary Fiber (TDF). The method used was based on AOAC International (2000) method 985.29. Duplicate samples were gelatinized with alpha-amylase and digested with enzymes to break down starch and protein. Ethanol was added to each sample to precipitate the soluble fiber. The samples were filtered, and the residue was rinsed with ethanol and acetone to remove starch and protein degradation products and moisture. Protein content was determined for one of the duplicates; ash content was determined for

the other. The total dietary fiber in the sample was calculated using the protein and ash values. The limit of quantitation for this study was 1.0% fwt.

Vitamin E (LCAT). The method used was based on three literature methods (Cort et al., 1983; Speek et al., 1985; McMurray et al., 1980). The sample was saponified to break down any fat and release any vitamin E. The saponified mixture was extracted with ethyl ether and then quantitated directly by high-performance liquid chromatography on a silica column. The limit of quantitation for this study was approximately 0.003 mg/100g fwt. The reference standard was USP, Alpha Tocopherol, 100%, Lot Number M.

Control of Bias

The cottonseed was subjected to identical conditions at the field sites with respect to environmental conditions, harvesting, storage, and shipment. Cottonseed was ground thoroughly before use to minimize tissue bias. The order of compositional analyses of the samples was randomized to minimize assay bias.

Data Reduction and Statistical Analysis

Composition data from Covance Laboratories Inc., containing individual values for each analysis, were reviewed at Monsanto Company. They then were transferred to Certus International where they were converted into the appropriate units and statistically analyzed. The formulas used for re-expression of cottonseed composition data for statistical analysis are presented below.

Component	From (X)	To	Formula[1]
Proximates (excluding Moisture and Calories), Fiber, Gossypol	% fwt	% dwt	X/d
Copper, Iron, Manganese, Zinc	ppm fwt	mg/kg dwt	X/d
Calcium, Magnesium, Phosphorus, Potassium, Sodium	ppm fwt	% dwt	$X/(10^4*d)$
Vitamin E	mg/100g fwt	mg/kg dwt	10 (X/d)
Amino Acids (AA)	mg/g fwt	% Total AA	$(100)X_j/\Sigma X_j$, for each AA j
Fatty Acids (FA)	% fwt	% Total FA	$(100)X_j/\Sigma X_j$, for each FA j

[1] d is the fraction of the sample that is dry matter.

Analytes with >50% of observations below the LOQ of the assay were excluded from statistical analysis. The SAS® software GLM procedure (SAS Institute Inc., Cary, NC, USA) was applied to all data [MON 88913, MON 88913(-) and references] to detect potential outliers in the dataset by screening studentized PRESS residuals. Cottonseed material, site and replication effects were included in the model.

All component values, except moisture, were converted from a fresh weight basis into their respective units. Statistical analyses were conducted on the converted values for each component in the cottonseed using a mixed model analysis of variance for the five sets of comparisons: analysis for each of the four replicated trial sites (AL, CA, GA, and TX), and one for the combination of all four sites. A total of 53 components statistically were evaluated (the initial 69 analytes minus the 16 for which >50% of the observations were below the LOQ). A total of 265 comparisons were made, as there were 53 components with five statistical analyses each.

Individual replicated site analyses used the model:

$$Y_{ij} = U + T_i + B_j + e_{ij},$$

where Y_{ij} = unique individual observation, U = overall mean, T_i = plant material effect, B_j = random block effect, and e_{ij} = residual error.

Combined site analyses used the model:

$$Y_{ijk} = U + T_i + L_j + B(L)_{jk} + LT_{ij} + e_{ijk},$$

where Y_{ijk} = unique individual observation, U = overall mean, T_i = plant material effect, L_j = random location effect, $B(L)_{jk}$ = random block within location effect, LT_{ij} = random location by plant material interaction effect, and e_{ijk} = residual error. MON 88913 was compared to MON 88913(-) to determine statistically significant differences at $p \leq 0.05$.

Compositional analysis data from the conventional references were used to determine a range of the reference values for each compositional analysis component. Additionally, the reference data were used to develop population tolerance intervals. A tolerance interval is an interval with a specified degree of confidence that contains at least a specified proportion, p, of an entire sampled population for the parameter measured. For each component, tolerance intervals were calculated that were expected to contain, with 95% confidence, 99% of the values expressed in the population of conventional cotton. Because negative quantities are not possible, calculated lower tolerance bounds that were negative were set to zero. SAS software (SAS Institute Inc., Cary, NC, USA) was used to generate all summary statistics and perform all analyses.

APPENDIX F

Materials and Methods Used for Compositional Analysis of MON 88913 Cottonseed Oil and Cottonseed Meal

Appendix F

Materials and Methods Used for Compositional Analysis of MON 88913 Cottonseed Oil and Cottonseed Meal

Compositional analyses were conducted on mechanically delinted cottonseed, raw (untoasted) cottonseed meal and cottonseed oil derived from MON 88913.

Materials

The cottonseed and processed fractions analyzed in this study were derived from MON 88913, MON 88913(-), and six conventional cotton reference varieties grown in 2002. MON 88913 was grown in Arkansas and Arizona in 2002 from seed lot GLP-0203-12176-S. MON 88913(-) was also grown in Arkansas and Arizona in 2002 in the same field as MON 88913, using seed lot GPL-0203-12177-S. The references were six commercial conventional cotton varieties grown in the same fields in Arkansas, Arizona as well as an additional site in Georgia. The GA site was added to provide additional reference material. These sites are representative of regions where MON 88913 is expected to be grown as a commercial product. Cottonseed samples were collected from all plots at seed maturity. The seed cotton was ginned at the production locations and mechanically delinted at the Food and Protein Research and Development Center at Texas A&M University. The references, production locations, and seed lot numbers are listed below.

Reference	Location	Seed Lot Number
PSC 355	Arkansas	REF-0203-12235-S
SG 125	Arkansas	REF-0203-12236-S
DP 565	Arizona	REF-0203-12239-S
ST 580	Arizona	REF-0203-12240-S
DPL Acala 90	Georgia	REF-0203-12272-S
HS12	Georgia	REF-0203-12273-S

All cottonseed samples were mechanically delinted prior to processing at the Food and Protein Research and Development Center at Texas A&M University. They were processed under conditions simulating commercial processing into raw (untoasted) cottonseed meal and refined, bleached, and deodorized cottonseed oil.

All processed fractions were generated at the Food and Protein Research and Development Center at Texas A&M University in 2003. The materials analyzed are listed below.

Commodity	Description
Cottonseed	Mechanically delinted cottonseed.
Raw cottonseed meal	De-hulled, flaked cottonseed remaining after solvent extraction, air-dried to remove residual solvent.
Cottonseed oil	Solvent-extracted cottonseed oil following removal of soapstock, bleaching with clay and treatment with steam to remove odors (refined, bleached and deodorized oil).

Appropriate reference standards were used in each assay for the analytical procedures. The analytical standards used for compositional analyses are described later in this Appendix.

Characterization of the Materials

The identities of the MON 88913, MON 88913(-), and reference cottonseed were verified prior to use by chain-of-custody documentation and confirming the results of PCR analyses specifically designed to detect MON 88913, as appropriate, in the delinted cottonseed. The identities of the raw cottonseed samples were verified with an immunological assay. The identity of the cottonseed oil was characterized by chain-of-custody documentation.

Processing Design

The delinted cottonseed was processed into raw cottonseed meal and refined, bleached, and deodorized cottonseed oil at the Food and Protein Research and Development Center at Texas A&M University. The cottonseed was dehulled, moisture adjusted, and heated at approximately 195 °F for 30 minutes to bind gossypol. Following this, the cottonseed was flaked, extruded, dried, and milled. The oil was removed by multiple hexane extractions. The solvent was removed and the remaining cottonseed oil was refined, bleached, and deodorized. The oil was then stored at –20 °C until compositional analyses were performed later as described later in this Appendix. The raw cottonseed meal, which remained after the oil extraction, was air dried to remove residual solvent and frozen in a –20 °C freezer until compositional analyses. Both the raw cottonseed meal and the cottonseed oil were shipped frozen and maintained in a -20 °C freezer until shipped frozen to the analytical laboratory facility.

Analytical Methods

Cottonseed, cottonseed oil, and raw cottonseed meal samples of MON 88913, MON 88913(-), and reference cotton were shipped frozen to Covance Laboratories Inc., Madison, Wisconsin for compositional analyses. Analyses were performed by established methods that are currently used to evaluate the nutritional quality of food and feed. Cottonseed samples were analyzed for proximates (protein, fat, ash, and moisture),

ADF, NDF, crude fiber, TDF, amino acids, fatty acids, cyclopropenoid fatty acids, vitamin E, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and gossypol (free and total). Carbohydrate and caloric values were determined by calculation. The raw cottonseed meal samples were analyzed for proximates (protein, fat, ash, and moisture), ADF, NDF, crude fiber, TDF, amino acids, cyclopropenoid fatty acids, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and gossypol (free and total). Carbohydrate and caloric values were determined by calculation. Cottonseed oil was analyzed for fatty acids, cyclopropenoid fatty acids, vitamin E, and gossypol (free and total).

Analytical methods and reference standards were as previously described for whole cottonseed (Appendix E), with several exceptions. The limit of quantitation for the cyclopropenoid fatty acids (CPFQ) for each acid was 25.0 µg/g for cottonseed meal and 250 µg/g for cottonseed oil; the limit of quantitation for fatty acids (FAPM) was 0.100% for cottonseed oil; the limit of quantitation for free and total gossypol (GOSF, GOSS) was 0.100% for cottonseed meal, and 0.002% for cottonseed oil; and the limit of quantitation for vitamin E (LCAT) was 0.003 mg/g for cottonseed and cottonseed oil.

Control of Bias

MON 88913, MON 88913(-), and four of the reference materials were subjected to identical conditions at the field sites with respect to environmental conditions, harvesting, storage, and shipment. The remaining two references were grown at a separate site, but were harvested, stored and shipped in a manner consistent with the cottonseed of MON 88913, MON 88913(-), and other four references. Cottonseed was ground thoroughly before use to minimize tissue bias. The cottonseed oil and cottonseed meal were produced in the same facility under the same processing conditions. The order of compositional analyses was randomized within a matrix to minimize assay bias.

Data Reduction and Statistical Analysis

Composition data from Covance Laboratories Inc., were transferred to Certus International where they were converted into their respective units and statistically analyzed. Analytes with >50% of observations below the LOQ of the assay were excluded from statistical analysis. Studentized PRESS residuals identifed twelve results as outliers. SAS software (SAS Institute, 1999-2000) was used to generate all summary statistics and perform all statistical analyses.

Statistical analyses were conducted using a mixed model analysis of variance for the comparison of each component in the cottonseed, raw cottonseed meal, and cottonseed oil. The data from both field sites were combined and the combined site analyses used the model previously described for the combined site cottonseed analysis in Appendix E. MON 88913 was compared to MON 88913(-) to determine statistically significant differences at $p<0.05$. For cottonseed, there were 52 components statistically evaluated. The 52 components resulted from the difference between the initial 65 analytes minus the 13 for which >50% of the observations were below the LOQ. For raw cottonseed meal there were 41 components statistically evaluated (42 minus 1 below the LOQ). For

cottonseed oil, there were 13 components statistically evaluated (28 minus 15 below the LOQ).

Conventional references were used to determine a range of the reference values for each compositional analysis component and develop population tolerance intervals following the methods used for the cottonseed analysis from four replicated field sites (Appendix E).

APPENDIX G

Individual Site Cottonseed Composition Tables
From Four Replicated Field Sites

Table G-1. Statistical Summary[1] of Site AL Cottonseed Amino Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Alanine	4.47 ± 0.013	4.43 ± 0.013	0.037 ± 0.018	-0.021,0.096	0.133	(4.08 - 4.46)
	(4.44 - 4.51)	(4.40 - 4.46)	(-0.013 - 0.074)			[4.01,4.58]
Arginine	11.28 ± 0.055	11.42 ± 0.055	-0.14 ± 0.078	-0.38,0.11	0.173	(11.08 - 12.77)
	(11.19 - 11.34)	(11.21 - 11.54)	(-0.30 - 0.069)			[10.57,12.96]
Aspartic Acid	9.88 ± 0.027	9.86 ± 0.027	0.018 ± 0.037	-0.10,0.14	0.672	(9.70 - 10.38)
	(9.80 - 9.93)	(9.83 - 9.93)	(-0.13 - 0.096)			[9.48,10.35]
Cystine	1.89 ± 0.032	1.94 ± 0.032	-0.053 ± 0.045	-0.20,0.089	0.320	(1.62 - 2.35)
	(1.80 - 1.97)	(1.91 - 1.96)	(-0.13 - 0.033)			[1.60,2.14]
Glutamic Acid	21.25 ± 0.085	21.24 ± 0.085	0.0081 ± 0.094	-0.29,0.31	0.936	(20.92 - 22.18)
	(21.08 - 21.55)	(21.10 - 21.37)	(-0.25 - 0.18)			[20.88,22.49]
Glycine	4.47 ± 0.011	4.49 ± 0.011	-0.010 ± 0.015	-0.057,0.036	0.532	(4.29 - 4.66)
	(4.45 - 4.49)	(4.46 - 4.52)	(-0.037 - 0.028)			[4.21,4.64]
Histidine	3.15 ± 0.0070	3.13 ± 0.0070	0.019 ± 0.0081	-0.0068,0.045	0.100	(3.01 - 3.22)
	(3.13 - 3.17)	(3.12 - 3.14)	(-0.0052 - 0.030)			[3.04,3.23]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-1 (Continued). Statistical Summary[1] of Site AL Cottonseed Amino Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Isoleucine	3.44 ± 0.019	3.42 ± 0.019	0.023 ± 0.027	-0.062,0.11	0.445	(3.19 - 3.59)
	(3.41 - 3.48)	(3.36 - 3.46)	(-0.051 - 0.084)			[3.13,3.65]
Leucine	6.37 ± 0.027	6.31 ± 0.027	0.062 ± 0.032	-0.040,0.16	0.147	(6.03 - 6.48)
	(6.33 - 6.45)	(6.23 - 6.37)	(-0.0059 - 0.12)			[5.84,6.66]
Lysine	4.99 ± 0.043	4.96 ± 0.043	0.031 ± 0.027	-0.055,0.12	0.335	(4.72 - 5.38)
	(4.91 - 5.14)	(4.89 - 5.04)	(-0.021 - 0.099)			[4.53,5.43]
Methionine	1.70 ± 0.063	1.78 ± 0.063	-0.076 ± 0.089	-0.36,0.21	0.458	(1.27 - 1.94)
	(1.51 - 1.84)	(1.64 - 1.84)	(-0.33 - 0.20)			[1.30,1.93]
Phenylalanine	5.64 ± 0.020	5.58 ± 0.020	0.054 ± 0.029	-0.038,0.15	0.158	(5.44 - 5.82)
	(5.60 - 5.69)	(5.54 - 5.62)	(0.0098 - 0.15)			[5.43,5.82]
Proline	4.24 ± 0.020	4.20 ± 0.020	0.036 ± 0.029	-0.057,0.13	0.307	(3.97 - 4.49)
	(4.20 - 4.28)	(4.16 - 4.24)	(-0.048 - 0.12)			[3.91,4.43]
Serine	5.13 ± 0.070	5.10 ± 0.070	0.029 ± 0.10	-0.29,0.35	0.788	(4.53 - 5.31)
	(5.00 - 5.32)	(5.01 - 5.32)	(-0.21 - 0.29)			[4.55,5.42]

Table G-1 (Continued). Statistical Summary[1] of Site AL Cottonseed Amino Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Threonine	3.38 ± 0.060	3.37 ± 0.060	0.015 ± 0.085	-0.26,0.29	0.871	(2.67 - 3.50)
	(3.21 - 3.49)	(3.19 - 3.45)	(-0.21 - 0.22)			[2.73,3.74]
Tryptophan	1.10 ± 0.019	1.16 ± 0.019	-0.054 ± 0.027	-0.14,0.032	0.138	(0.97 - 1.31)
	(1.03 - 1.15)	(1.15 - 1.17)	(-0.12 - 0.0016)			[0.94,1.26]
Tyrosine	2.84 ± 0.019	2.85 ± 0.019	-0.013 ± 0.017	-0.067,0.042	0.512	(2.63 - 2.93)
	(2.79 - 2.90)	(2.81 - 2.87)	(-0.048 - 0.034)			[2.61,3.00]
Valine	4.78 ± 0.024	4.77 ± 0.024	0.012 ± 0.034	-0.098,0.12	0.758	(4.57 - 5.02)
	(4.70 - 4.83)	(4.73 - 4.80)	(-0.095 - 0.097)			[4.48,5.02]

Table G-2. Statistical Summary[1] of Site CA Cottonseed Amino Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Alanine	4.18 ± 0.064	4.27 ± 0.064	-0.089 ± 0.091	-0.38,0.20	0.398	(4.08 - 4.46)
	(4.09 - 4.39)	(4.15 - 4.39)	(-0.27 - 0.24)			[4.01,4.58]
Arginine	11.95 ± 0.24	11.58 ± 0.24	0.37 ± 0.34	-0.70,1.45	0.347	(11.08 - 12.77)
	(11.22 - 12.25)	(11.11 - 12.03)	(-0.81 - 0.99)			[10.57,12.96]
Aspartic Acid	9.63 ± 0.021	9.63 ± 0.021	-0.0013 ± 0.029	-0.095,0.092	0.967	(9.70 - 10.38)
	(9.59 - 9.69)	(9.59 - 9.69)	(-0.095 - 0.093)			[9.48,10.35]
Cystine	1.87 ± 0.065	1.94 ± 0.065	-0.065 ± 0.087	-0.34,0.21	0.508	(1.62 - 2.35)
	(1.72 - 2.07)	(1.85 - 2.10)	(-0.25 - 0.16)			[1.60,2.14]
Glutamic Acid	21.88 ± 0.22	21.51 ± 0.22	0.37 ± 0.32	-0.64,1.38	0.327	(20.92 - 22.18)
	(21.16 - 22.14)	(21.10 - 21.94)	(-0.63 - 1.03)			[20.88,22.49]
Glycine	4.44 ± 0.047	4.50 ± 0.047	-0.059 ± 0.067	-0.27,0.15	0.442	(4.29 - 4.66)
	(4.39 - 4.56)	(4.40 - 4.64)	(-0.24 - 0.13)			[4.21,4.64]
Histidine	3.18 ± 0.021	3.15 ± 0.021	0.030 ± 0.029	-0.063,0.12	0.374	(3.01 - 3.22)
	(3.12 - 3.21)	(3.11 - 3.20)	(-0.028 - 0.10)			[3.04,3.23]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-2 (Continued). Statistical Summary[1] of Site CA Cottonseed Amino Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Isoleucine	3.36 ± 0.038	3.44 ± 0.038	-0.080 ± 0.054	-0.25,0.093	0.237	(3.19 - 3.59)
	(3.31 - 3.47)	(3.38 - 3.56)	(-0.25 - 0.076)			[3.13,3.65]
Leucine	6.27 ± 0.052	6.29 ± 0.052	-0.019 ± 0.074	-0.25,0.22	0.816	(6.03 - 6.48)
	(6.26 - 6.29)	(6.17 - 6.48)	(-0.20 - 0.11)			[5.84,6.66]
Lysine	5.10 ± 0.12	5.19 ± 0.12	-0.090 ± 0.17	-0.62,0.44	0.623	(4.72 - 5.38)
	(5.01 - 5.23)	(4.91 - 5.48)	(-0.48 - 0.30)			[4.53,5.43]
Methionine	1.63 ± 0.079	1.77 ± 0.079	-0.13 ± 0.11	-0.49,0.22	0.320	(1.27 - 1.94)
	(1.47 - 1.90)	(1.68 - 1.95)	(-0.34 - 0.22)			[1.30,1.93]
Phenylalanine	5.63 ± 0.046	5.61 ± 0.046	0.020 ± 0.066	-0.19,0.23	0.776	(5.44 - 5.82)
	(5.53 - 5.66)	(5.45 - 5.72)	(-0.19 - 0.21)			[5.43,5.82]
Proline	4.07 ± 0.059	4.13 ± 0.059	-0.057 ± 0.061	-0.25,0.14	0.415	(3.97 - 4.49)
	(3.97 - 4.19)	(3.93 - 4.25)	(-0.18 - 0.050)			[3.91,4.43]
Serine	4.94 ± 0.036	4.94 ± 0.036	-0.0049 ± 0.051	-0.17,0.16	0.929	(4.53 - 5.31)
	(4.85 - 5.01)	(4.89 - 5.06)	(-0.21 - 0.082)			[4.55,5.42]

Table G-2 (Continued). Statistical Summary[1] of Site CA Cottonseed Amino Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p*-Value**	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Threonine	3.14 ± 0.080	3.29 ± 0.080	-0.15 ± 0.11	-0.52,0.21	0.267	(2.67 - 3.50)
	(2.95 - 3.45)	(3.23 - 3.33)	(-0.34 - 0.22)			[2.73,3.74]
Tryptophan	1.11 ± 0.034	1.16 ± 0.034	-0.047 ± 0.046	-0.19,0.099	0.381	(0.97 - 1.31)
	(1.03 - 1.23)	(1.13 - 1.21)	(-0.10 - 0.089)			[0.94,1.26]
Tyrosine	2.81 ± 0.021	2.85 ± 0.021	-0.042 ± 0.025	-0.12,0.039	0.199	(2.63 - 2.93)
	(2.77 - 2.87)	(2.80 - 2.89)	(-0.085 - 0.015)			[2.61,3.00]
Valine	4.81 ± 0.030	4.76 ± 0.030	0.046 ± 0.042	-0.087,0.18	0.350	(4.57 - 5.02)
	(4.80 - 4.81)	(4.68 - 4.85)	(-0.037 - 0.12)			[4.48,5.02]

Table G-3. Statistical Summary[1] of Site GA Cottonseed Amino Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Alanine	4.26 ± 0.024	4.23 ± 0.024	0.030 ± 0.027	-0.056,0.12	0.346	(4.08 - 4.46)
	(4.21 - 4.33)	(4.19 - 4.25)	(-0.037 - 0.091)			[4.01,4.58]
Arginine	11.85 ± 0.081	11.99 ± 0.081	-0.14 ± 0.054	-0.31,0.032	0.080	(11.08 - 12.77)
	(11.61 - 11.95)	(11.80 - 12.19)	(-0.24 - 0.0067)			[10.57,12.96]
Aspartic Acid	9.86 ± 0.049	9.75 ± 0.049	0.11 ± 0.070	-0.12,0.33	0.223	(9.70 - 10.38)
	(9.76 - 10.01)	(9.67 - 9.82)	(-0.049 - 0.29)			[9.48,10.35]
Cystine	1.77 ± 0.047	1.84 ± 0.047	-0.071 ± 0.035	-0.18,0.040	0.135	(1.62 - 2.35)
	(1.69 - 1.85)	(1.76 - 2.00)	(-0.16 - 0.015)			[1.60,2.14]
Glutamic Acid	21.78 ± 0.065	21.67 ± 0.065	0.11 ± 0.043	-0.025,0.25	0.079	(20.92 - 22.18)
	(21.72 - 21.91)	(21.54 - 21.91)	(0.00085 - 0.21)			[20.88,22.49]
Glycine	4.34 ± 0.014	4.37 ± 0.014	-0.028 ± 0.0082	-0.054,-0.0023	0.040	(4.29 - 4.66)
	(4.33 - 4.38)	(4.33 - 4.41)	(-0.040 - -0.0041)			[4.21,4.64]
Histidine	3.13 ± 0.014	3.15 ± 0.014	-0.025 ± 0.020	-0.090,0.040	0.305	(3.01 - 3.22)
	(3.09 - 3.17)	(3.14 - 3.18)	(-0.070 - 0.034)			[3.04,3.23]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-3 (Continued). Statistical Summary[1] of Site GA Cottonseed Amino Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Isoleucine	3.48 ± 0.033	3.47 ± 0.033	0.0087 ± 0.047	-0.14,0.16	0.865	(3.19 - 3.59)
	(3.41 - 3.54)	(3.41 - 3.54)	(-0.13 - 0.12)			[3.13,3.65]
Leucine	6.42 ± 0.025	6.32 ± 0.025	0.10 ± 0.033	-0.00088,0.21	0.051	(6.03 - 6.48)
	(6.35 - 6.52)	(6.31 - 6.33)	(0.044 - 0.20)			[5.84,6.66]
Lysine	4.99 ± 0.062	5.17 ± 0.062	-0.18 ± 0.062	-0.37,0.020	0.064	(4.72 - 5.38)
	(4.82 - 5.16)	(5.05 - 5.24)	(-0.30 - -0.012)			[4.53,5.43]
Methionine	1.57 ± 0.040	1.60 ± 0.040	-0.029 ± 0.040	-0.16,0.096	0.511	(1.27 - 1.94)
	(1.50 - 1.63)	(1.49 - 1.70)	(-0.12 - 0.056)			[1.30,1.93]
Phenylalanine	5.67 ± 0.023	5.62 ± 0.023	0.047 ± 0.033	-0.058,0.15	0.250	(5.44 - 5.82)
	(5.60 - 5.75)	(5.60 - 5.65)	(-0.037 - 0.15)			[5.43,5.82]
Proline	4.09 ± 0.052	4.10 ± 0.052	-0.0096 ± 0.073	-0.24,0.22	0.903	(3.97 - 4.49)
	(3.92 - 4.21)	(4.01 - 4.17)	(-0.16 - 0.20)			[3.91,4.43]
Serine	4.85 ± 0.087	4.77 ± 0.087	0.078 ± 0.12	-0.31,0.47	0.572	(4.53 - 5.31)
	(4.65 - 5.16)	(4.66 - 4.84)	(-0.15 - 0.50)			[4.55,5.42]

Table G-3 (Continued). Statistical Summary[1] of Site GA Cottonseed Amino Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Threonine	3.22 ± 0.10	3.28 ± 0.10	-0.058 ± 0.15	-0.53,0.41	0.722	(2.67 - 3.50)
	(2.79 - 3.45)	(3.25 - 3.29)	(-0.49 - 0.20)			[2.73,3.74]
Tryptophan	1.08 ± 0.015	1.11 ± 0.015	-0.031 ± 0.021	-0.10,0.037	0.241	(0.97 - 1.31)
	(1.03 - 1.11)	(1.09 - 1.13)	(-0.099 - 0.014)			[0.94,1.26]
Tyrosine	2.77 ± 0.019	2.73 ± 0.019	0.040 ± 0.027	-0.046,0.13	0.234	(2.63 - 2.93)
	(2.70 - 2.82)	(2.71 - 2.75)	(-0.050 - 0.082)			[2.61,3.00]
Valine	4.88 ± 0.040	4.84 ± 0.040	0.041 ± 0.056	-0.14,0.22	0.515	(4.57 - 5.02)
	(4.79 - 4.98)	(4.76 - 4.91)	(-0.12 - 0.22)			[4.48,5.02]

Table G-4. Statistical Summary[1] of Site TX Cottonseed Amino Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Alanine	4.23 ± 0.022	4.26 ± 0.022	-0.030 ± 0.032	-0.13,0.071	0.418	(4.08 - 4.46)
	(4.19 - 4.29)	(4.22 - 4.31)	(-0.12 - 0.067)			[4.01,4.58]
Arginine	12.02 ± 0.052	12.10 ± 0.052	-0.082 ± 0.074	-0.32,0.15	0.347	(11.08 - 12.77)
	(11.95 - 12.14)	(12.01 - 12.27)	(-0.32 - 0.13)			[10.57,12.96]
Aspartic Acid	9.89 ± 0.048	9.94 ± 0.048	-0.042 ± 0.046	-0.19,0.10	0.422	(9.70 - 10.38)
	(9.80 - 10.08)	(9.88 - 9.99)	(-0.099 - 0.094)			[9.48,10.35]
Cystine	2.02 ± 0.032	1.97 ± 0.032	0.049 ± 0.046	-0.097,0.19	0.365	(1.62 - 2.35)
	(1.94 - 2.10)	(1.90 - 2.04)	(-0.055 - 0.16)			[1.60,2.14]
Glutamic Acid	21.72 ± 0.12	21.76 ± 0.12	-0.042 ± 0.11	-0.41,0.32	0.739	(20.92 - 22.18)
	(21.58 - 22.04)	(21.45 - 21.96)	(-0.37 - 0.14)			[20.88,22.49]
Glycine	4.44 ± 0.016	4.45 ± 0.016	-0.0039 ± 0.021	-0.069,0.062	0.862	(4.29 - 4.66)
	(4.40 - 4.51)	(4.43 - 4.46)	(-0.032 - 0.056)			[4.21,4.64]
Histidine	3.15 ± 0.013	3.15 ± 0.013	-0.0010 ± 0.018	-0.058,0.056	0.958	(3.01 - 3.22)
	(3.13 - 3.18)	(3.12 - 3.19)	(-0.055 - 0.021)			[3.04,3.23]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-4 (Continued). Statistical Summary[1] of Site TX Cottonseed Amino Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Isoleucine	3.43 ± 0.028	3.40 ± 0.028	0.032 ± 0.020	-0.031,0.095	0.204	(3.19 - 3.59)
	(3.36 - 3.47)	(3.34 - 3.46)	(0.0094 - 0.091)			[3.13,3.65]
Leucine	6.18 ± 0.029	6.14 ± 0.029	0.038 ± 0.041	-0.092,0.17	0.424	(6.03 - 6.48)
	(6.14 - 6.29)	(6.10 - 6.18)	(-0.031 - 0.19)			[5.84,6.66]
Lysine	4.86 ± 0.060	5.05 ± 0.060	-0.19 ± 0.085	-0.47,0.077	0.106	(4.72 - 5.38)
	(4.77 - 4.95)	(4.91 - 5.23)	(-0.46 - -0.049)			[4.53,5.43]
Methionine	1.70 ± 0.030	1.63 ± 0.030	0.069 ± 0.037	-0.048,0.19	0.157	(1.27 - 1.94)
	(1.64 - 1.80)	(1.57 - 1.67)	(-0.0032 - 0.14)			[1.30,1.93]
Phenylalanine	5.65 ± 0.016	5.59 ± 0.016	0.053 ± 0.012	0.014,0.093	0.023	(5.44 - 5.82)
	(5.61 - 5.67)	(5.55 - 5.64)	(0.029 - 0.086)			[5.43,5.82]
Proline	4.30 ± 0.022	4.23 ± 0.022	0.072 ± 0.030	-0.023,0.17	0.095	(3.97 - 4.49)
	(4.24 - 4.39)	(4.21 - 4.24)	(0.027 - 0.16)			[3.91,4.43]
Serine	4.60 ± 0.069	4.77 ± 0.069	-0.17 ± 0.097	-0.48,0.14	0.179	(4.53 - 5.31)
	(4.35 - 4.71)	(4.65 - 4.87)	(-0.48 - 0.041)			[4.55,5.42]

Table G-4 (Continued). Statistical Summary[1] of Site TX Cottonseed Amino Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Amino Acid (% Total AA)						
Threonine	3.04 ± 0.12	2.87 ± 0.12	0.16 ± 0.14	-0.29,0.62	0.336	(2.67 - 3.50)
	(2.61 - 3.19)	(2.70 - 3.17)	(-0.14 - 0.48)			[2.73,3.74]
Tryptophan	1.12 ± 0.029	1.14 ± 0.029	-0.025 ± 0.041	-0.16,0.10	0.579	(0.97 - 1.31)
	(1.08 - 1.17)	(1.10 - 1.25)	(-0.14 - 0.058)			[0.94,1.26]
Tyrosine	2.77 ± 0.027	2.68 ± 0.027	0.084 ± 0.038	-0.037,0.20	0.113	(2.63 - 2.93)
	(2.72 - 2.80)	(2.62 - 2.76)	(-0.0043 - 0.18)			[2.61,3.00]
Valine	4.89 ± 0.061	4.86 ± 0.061	0.030 ± 0.038	-0.091,0.15	0.490	(4.57 - 5.02)
	(4.68 - 5.00)	(4.74 - 4.96)	(-0.052 - 0.11)			[4.48,5.02]

Table G-5. Statistical Summary[1] of Site AL Cottonseed Fatty Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Fatty Acid (% Total FA)						
14:0 Myristic	0.67 ± 0.0058	0.67 ± 0.0058	-0.00063 ± 0.0082	-0.027,0.025	0.943	(0.64 - 1.03)
	(0.66 - 0.68)	(0.65 - 0.68)	(-0.016 - 0.025)			[0.44,1.14]
16:0 Palmitic	23.85 ± 0.090	23.68 ± 0.090	0.17 ± 0.12	-0.21,0.55	0.253	(21.47 - 25.36)
	(23.58 - 24.15)	(23.56 - 23.77)	(-0.15 - 0.38)			[20.76,26.19]
16:1 Palmitoleic	0.53 ± 0.0036	0.52 ± 0.0036	0.011 ± 0.0051	-0.0055,0.027	0.127	(0.46 - 0.77)
	(0.53 - 0.55)	(0.52 - 0.53)	(-0.00058 - 0.025)			[0.37,0.80]
18:0 Stearic	2.68 ± 0.018	2.72 ± 0.018	-0.040 ± 0.022	-0.11,0.031	0.172	(2.38 - 3.03)
	(2.63 - 2.72)	(2.69 - 2.76)	(-0.10 - -0.0049)			[2.18,3.17]
18:1 Oleic	16.60 ± 0.19	18.89 ± 0.19	-2.29 ± 0.24	-3.04,-1.55	0.002	(13.29 - 18.60)
	(16.35 - 17.01)	(18.34 - 19.37)	(-2.98 - -1.99)			[10.59,21.29]
18:2 Linoleic	53.88 ± 0.22	51.69 ± 0.22	2.19 ± 0.14	1.74,2.65	<0.001	(51.51 - 59.40)
	(53.54 - 54.32)	(51.12 - 52.32)	(1.90 - 2.50)			[48.89,61.11]
18:3 Gamma Linolenic	0.12 ± 0.017	0.18 ± 0.017	-0.066 ± 0.022	-0.13,0.0026	0.055	(0.043 - 0.23)
	(0.052 - 0.16)	(0.17 - 0.20)	(-0.13 - -0.028)			[0,0.24]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-5 (Continued). Statistical Summary[1] of Site AL Cottonseed Fatty Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Fatty Acid (% Total FA)						
18:3 Linolenic	0.22 ± 0.014	0.21 ± 0.014	0.013 ± 0.0098	-0.018,0.044	0.284	(0.11 - 0.27)
	(0.20 - 0.25)	(0.16 - 0.23)	(-0.0035 - 0.040)			[0.031,0.31]
20:0 Arachidic	0.28 ± 0.0034	0.29 ± 0.0034	-0.010 ± 0.0045	-0.024,0.0043	0.111	(0.22 - 0.33)
	(0.27 - 0.29)	(0.29 - 0.29)	(-0.019 - 0.00071)			[0.21,0.34]
22:0 Behenic	0.14 ± 0.0045	0.15 ± 0.0045	-0.0099 ± 0.0062	-0.030,0.0099	0.209	(0.12 - 0.18)
	(0.13 - 0.15)	(0.15 - 0.16)	(-0.024 - 0.00086)			[0.099,0.19]
Dihydrosterculic	0.16 ± 0.0053	0.16 ± 0.0053	-0.0024 ± 0.0075	-0.026,0.021	0.766	(0.075 - 0.24)
	(0.15 - 0.17)	(0.15 - 0.17)	(-0.017 - 0.024)			[0.056,0.25]
Malvalic	0.49 ± 0.022	0.46 ± 0.022	0.038 ± 0.032	-0.063,0.14	0.319	(0.23 - 0.56)
	(0.45 - 0.56)	(0.42 - 0.50)	(-0.029 - 0.13)			[0.16,0.58]
Sterculic	0.38 ± 0.015	0.38 ± 0.015	0.00098 ± 0.021	-0.064,0.066	0.964	(0.19 - 0.41)
	(0.34 - 0.41)	(0.35 - 0.41)	(-0.067 - 0.061)			[0.18,0.40]

Table G-6. Statistical Summary[1] of Site CA Cottonseed Fatty Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Fatty Acid (% Total FA)						
14:0 Myristic	0.83 ± 0.041	0.76 ± 0.041	0.073 ± 0.059	-0.11,0.26	0.301	(0.64 - 1.03)
	(0.70 - 0.88)	(0.68 - 0.86)	(-0.065 - 0.20)			[0.44,1.14]
16:0 Palmitic	24.33 ± 0.22	23.70 ± 0.22	0.62 ± 0.16	0.11,1.14	0.031	(21.47 - 25.36)
	(23.56 - 24.69)	(23.34 - 24.17)	(0.22 - 0.95)			[20.76,26.19]
16:1 Palmitoleic	0.56 ± 0.020	0.54 ± 0.020	0.024 ± 0.028	-0.066,0.11	0.451	(0.46 - 0.77)
	(0.51 - 0.59)	(0.50 - 0.59)	(-0.048 - 0.090)			[0.37,0.80]
18:0 Stearic	2.75 ± 0.070	2.74 ± 0.070	0.012 ± 0.082	-0.25,0.27	0.892	(2.38 - 3.03)
	(2.66 - 2.85)	(2.52 - 2.94)	(-0.16 - 0.16)			[2.18,3.17]
18:1 Oleic	19.77 ± 0.32	21.61 ± 0.32	-1.84 ± 0.45	-3.28,-0.40	0.026	(13.29 - 18.60)
	(19.05 - 20.35)	(20.92 - 22.24)	(-3.15 - -0.56)			[10.59,21.29]
18:2 Linoleic	50.48 ± 0.55	49.21 ± 0.55	1.27 ± 0.78	-1.23,3.76	0.204	(51.51 - 59.40)
	(49.66 - 52.31)	(47.89 - 50.02)	(-0.36 - 3.14)			[48.89,61.11]
18:3 Gamma Linolenic	0.087 ± 0.023	0.11 ± 0.023	-0.020 ± 0.033	-0.12,0.084	0.584	(0.043 - 0.23)
	(0.045 - 0.16)	(0.049 - 0.14)	(-0.089 - 0.11)			[0,0.24]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-6 (Continued). Statistical Summary[1] of Site CA Cottonseed Fatty Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Fatty Acid (% Total FA)						
18:3 Linolenic	0.12 ± 0.0037	0.13 ± 0.0037	-0.013 ± 0.0053	-0.030,0.0039	0.091	(0.11 - 0.27)
	(0.11 - 0.13)	(0.12 - 0.14)	(-0.030 - 0.0043)			[0.031,0.31]
20:0 Arachidic	0.29 ± 0.011	0.28 ± 0.011	0.012 ± 0.012	-0.026,0.050	0.392	(0.22 - 0.33)
	(0.25 - 0.31)	(0.26 - 0.30)	(-0.0096 - 0.043)			[0.21,0.34]
22:0 Behenic	0.14 ± 0.0041	0.14 ± 0.0041	0.0055 ± 0.0057	-0.013,0.024	0.406	(0.12 - 0.18)
	(0.13 - 0.15)	(0.13 - 0.14)	(-0.010 - 0.015)			[0.099,0.19]
Dihydrosterculic	0.13 ± 0.0085	0.16 ± 0.0085	-0.034 ± 0.012	-0.073,0.0039	0.064	(0.075 - 0.24)
	(0.12 - 0.15)	(0.13 - 0.18)	(-0.057 - -0.0034)			[0.056,0.25]
Malvalic	0.26 ± 0.0072	0.34 ± 0.0072	-0.087 ± 0.010	-0.12,-0.054	0.003	(0.23 - 0.56)
	(0.24 - 0.27)	(0.32 - 0.36)	(-0.11 - -0.058)			[0.16,0.58]
Sterculic	0.26 ± 0.0061	0.28 ± 0.0061	-0.022 ± 0.0049	-0.038,-0.0066	0.020	(0.19 - 0.41)
	(0.25 - 0.28)	(0.26 - 0.29)	(-0.031 - -0.012)			[0.18,0.40]

Table G-7. Statistical Summary[1] of Site GA Cottonseed Fatty Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Fatty Acid (% Total FA)						
14:0 Myristic	0.71 ± 0.024	0.72 ± 0.024	-0.0033 ± 0.021	-0.069,0.062	0.883	(0.64 - 1.03)
	(0.69 - 0.76)	(0.65 - 0.79)	(-0.044 - 0.050)			[0.44,1.14]
16:0 Palmitic	22.32 ± 0.25	22.18 ± 0.25	0.14 ± 0.26	-0.68,0.96	0.619	(21.47 - 25.36)
	(22.09 - 22.70)	(21.26 - 22.67)	(-0.41 - 0.83)			[20.76,26.19]
16:1 Palmitoleic	0.55 ± 0.0086	0.55 ± 0.0086	0.0025 ± 0.012	-0.036,0.041	0.848	(0.46 - 0.77)
	(0.53 - 0.56)	(0.52 - 0.57)	(-0.018 - 0.036)			[0.37,0.80]
18:0 Stearic	2.73 ± 0.020	2.64 ± 0.020	0.092 ± 0.028	0.0033,0.18	0.045	(2.38 - 3.03)
	(2.68 - 2.77)	(2.61 - 2.66)	(0.035 - 0.17)			[2.18,3.17]
18:1 Oleic	18.57 ± 0.30	20.67 ± 0.30	-2.10 ± 0.42	-3.45,-0.76	0.015	(13.29 - 18.60)
	(17.93 - 19.14)	(19.77 - 21.30)	(-3.37 - -0.89)			[10.59,21.29]
18:2 Linoleic	53.45 ± 0.50	51.63 ± 0.50	1.82 ± 0.62	-0.15,3.80	0.060	(51.51 - 59.40)
	(52.27 - 54.28)	(50.69 - 53.27)	(0.28 - 3.25)			[48.89,61.11]
18:3 Gamma Linolenic	0.20 ± 0.034	0.12 ± 0.034	0.080 ± 0.048	-0.074,0.23	0.197	(0.043 - 0.23)
	(0.14 - 0.28)	(0.049 - 0.20)	(-0.023 - 0.23)			[0,0.24]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-7 (Continued). Statistical Summary[1] of Site GA Cottonseed Fatty Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Fatty Acid (% Total FA)						
18:3 Linolenic	0.23 ± 0.012	0.21 ± 0.012	0.021 ± 0.011	-0.013,0.055	0.146	(0.11 - 0.27)
	(0.20 - 0.26)	(0.19 - 0.24)	(-0.0060 - 0.042)			[0.031,0.31]
20:0 Arachidic	0.27 ± 0.0090	0.27 ± 0.0090	0.0045 ± 0.0085	-0.023,0.032	0.632	(0.22 - 0.33)
	(0.26 - 0.29)	(0.24 - 0.29)	(-0.014 - 0.024)			[0.21,0.34]
22:0 Behenic	0.15 ± 0.0080	0.14 ± 0.0080	0.0019 ± 0.011	-0.032,0.036	0.872	(0.12 - 0.18)
	(0.14 - 0.15)	(0.12 - 0.17)	(-0.019 - 0.023)			[0.099,0.19]
Dihydrosterculic	0.13 ± 0.013	0.15 ± 0.013	-0.015 ± 0.011	-0.049,0.018	0.241	(0.075 - 0.24)
	(0.12 - 0.14)	(0.10 - 0.17)	(-0.032 - 0.014)			[0.056,0.25]
Malvalic	0.38 ± 0.051	0.39 ± 0.051	-0.016 ± 0.072	-0.25,0.21	0.836	(0.23 - 0.56)
	(0.33 - 0.44)	(0.23 - 0.55)	(-0.22 - 0.12)			[0.16,0.58]
Sterculic	0.32 ± 0.040	0.34 ± 0.040	-0.026 ± 0.056	-0.20,0.15	0.674	(0.19 - 0.41)
	(0.27 - 0.36)	(0.21 - 0.44)	(-0.17 - 0.094)			[0.18,0.40]

Table G-8. Statistical Summary[1] of Site TX Cottonseed Fatty Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Fatty Acid (% Total FA)						
14:0 Myristic	0.84 ± 0.028	0.85 ± 0.028	-0.0047 ± 0.032	-0.11,0.096	0.891	(0.64 - 1.03)
	(0.79 - 0.90)	(0.78 - 0.90)	(-0.092 - 0.059)			[0.44,1.14]
16:0 Palmitic	23.71 ± 0.32	22.80 ± 0.32	0.91 ± 0.36	-0.23,2.05	0.084	(21.47 - 25.36)
	(22.97 - 24.47)	(22.17 - 23.38)	(-0.023 - 1.67)			[20.76,26.19]
16:1 Palmitoleic	0.54 ± 0.012	0.54 ± 0.012	0.0016 ± 0.011	-0.034,0.037	0.894	(0.46 - 0.77)
	(0.51 - 0.55)	(0.50 - 0.56)	(-0.020 - 0.033)			[0.37,0.80]
18:0 Stearic	2.38 ± 0.055	2.49 ± 0.055	-0.10 ± 0.078	-0.35,0.15	0.281	(2.38 - 3.03)
	(2.32 - 2.42)	(2.33 - 2.66)	(-0.28 - 0.083)			[2.18,3.17]
18:1 Oleic	19.51 ± 0.41	22.59 ± 0.41	-3.08 ± 0.57	-4.90,-1.27	0.012	(13.29 - 18.60)
	(18.71 - 20.72)	(21.68 - 23.29)	(-4.07 - -1.53)			[10.59,21.29]
18:2 Linoleic	51.63 ± 0.27	49.17 ± 0.27	2.46 ± 0.34	1.37,3.55	0.005	(51.51 - 59.40)
	(50.80 - 52.61)	(49.03 - 49.24)	(1.76 - 3.40)			[48.89,61.11]
18:3 Gamma Linolenic	0.088 ± 0.028	0.10 ± 0.028	-0.017 ± 0.039	-0.14,0.11	0.695	(0.043 - 0.23)
	(0.049 - 0.14)	(0.052 - 0.18)	(-0.13 - 0.059)			[0,0.24]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-8 (Continued). Statistical Summary[1] of Site TX Cottonseed Fatty Acid Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Fatty Acid (% Total FA)						
18:3 Linolenic	0.14 ± 0.0073	0.14 ± 0.0073	-0.0029 ± 0.010	-0.036,0.030	0.797	(0.11 - 0.27)
	(0.11 - 0.16)	(0.13 - 0.15)	(-0.031 - 0.030)			[0.031,0.31]
20:0 Arachidic	0.26 ± 0.0063	0.27 ± 0.0063	-0.0090 ± 0.0079	-0.034,0.016	0.336	(0.22 - 0.33)
	(0.25 - 0.27)	(0.26 - 0.28)	(-0.031 - 0.0076)			[0.21,0.34]
22:0 Behenic	0.16 ± 0.0038	0.16 ± 0.0038	0.0063 ± 0.0054	-0.011,0.024	0.330	(0.12 - 0.18)
	(0.16 - 0.17)	(0.15 - 0.17)	(-0.0083 - 0.018)			[0.099,0.19]
Dihydrosterculic	0.16 ± 0.013	0.19 ± 0.013	-0.032 ± 0.018	-0.089,0.024	0.166	(0.075 - 0.24)
	(0.13 - 0.18)	(0.15 - 0.21)	(-0.062 - 0.031)			[0.056,0.25]
Malvalic	0.31 ± 0.031	0.38 ± 0.031	-0.074 ± 0.044	-0.22,0.067	0.191	(0.23 - 0.56)
	(0.24 - 0.36)	(0.28 - 0.44)	(-0.14 - 0.075)			[0.16,0.58]
Sterculic	0.27 ± 0.022	0.32 ± 0.022	-0.048 ± 0.031	-0.14,0.050	0.216	(0.19 - 0.41)
	(0.24 - 0.32)	(0.25 - 0.37)	(-0.090 - 0.066)			[0.18,0.40]

Table G-9. Statistical Summary[1] of Site AL Cottonseed Fiber Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Fiber (% dwt)						
Acid Detergent Fiber	31.26 ± 0.79	30.62 ± 0.79	0.64 ± 1.12	-2.92,4.19	0.608	(26.32 - 38.97)
	(29.31 - 32.99)	(28.64 - 32.28)	(-2.97 - 3.56)			[25.48,38.48]
Crude Fiber	19.34 ± 0.31	18.49 ± 0.31	0.85 ± 0.44	-0.56,2.26	0.151	(15.96 - 23.10)
	(18.36 - 20.41)	(18.32 - 18.66)	(-0.30 - 1.94)			[13.34,24.17]
Neutral Detergent Fiber	44.74 ± 1.12	44.20 ± 1.12	0.53 ± 0.74	-1.83,2.90	0.524	(38.49 - 51.84)
	(41.55 - 46.95)	(41.93 - 46.92)	(-0.72 - 2.58)			[34.51,53.25]
Total Dietary Fiber	42.02 ± 0.50	40.53 ± 0.50	1.49 ± 0.62	-0.49,3.48	0.095	(36.47 - 47.54)
	(40.61 - 43.17)	(39.86 - 41.07)	(0.16 - 2.81)			[36.13,48.96]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-10. Statistical Summary[1] of Site CA Cottonseed Fiber Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Fiber (% dwt						
Acid Detergent Fiber	30.14 ± 0.60	30.09 ± 0.60	0.055 ± 0.84	-2.63,2.74	0.951	(26.32 - 38.97)
	(28.37 - 31.80)	(28.91 - 31.16)	(-2.78 - 1.46)			[25.48,38.48]
Crude Fiber	16.06 ± 0.35	16.87 ± 0.35	-0.82 ± 0.18	-1.40,-0.24	0.020	(15.96 - 23.10)
	(14.96 - 16.79)	(16.04 - 17.32)	(-1.18 - -0.48)			[13.34,24.17]
Neutral Detergent Fiber	41.21 ± 1.20	41.32 ± 1.20	-0.11 ± 1.70	-5.51,5.30	0.954	(38.49 - 51.84)
	(39.22 - 42.51)	(38.35 - 44.97)	(-5.75 - 3.00)			[34.51,53.25]
Total Dietary Fiber	39.01 ± 1.04	39.10 ± 1.04	-0.086 ± 1.47	-4.78,4.60	0.956	(36.47 - 47.54)
	(38.37 - 39.57)	(36.55 - 43.27)	(-4.90 - 3.03)			[36.13,48.96]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-11. Statistical Summary[1] of Site GA Cottonseed Fiber Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Fiber (% dwt)						
Acid Detergent Fiber	32.05 ± 0.54	31.99 ± 0.54	0.065 ± 0.68	-2.09,2.22	0.929	(26.32 - 38.97)
	(30.85 - 33.91)	(30.85 - 32.64)	(-1.44 - 1.27)			[25.48,38.48]
Crude Fiber	18.96 ± 0.50	19.14 ± 0.50	-0.18 ± 0.71	-2.43,2.08	0.820	(15.96 - 23.10)
	(18.13 - 19.41)	(17.84 - 20.39)	(-2.25 - 1.14)			[13.34,24.17]
Neutral Detergent Fiber	42.40 ± 0.81	44.73 ± 0.81	-2.33 ± 1.14	-5.95,1.28	0.132	(38.49 - 51.84)
	(40.78 - 44.03)	(42.48 - 46.40)	(-4.71 - 0.61)			[34.51,53.25]
Total Dietary Fiber	40.27 ± 0.54	39.01 ± 0.54	1.25 ± 0.51	-0.36,2.86	0.089	(36.47 - 47.54)
	(38.64 - 41.27)	(38.00 - 40.08)	(-0.15 - 2.09)			[36.13,48.96]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-12. Statistical Summary[1] of Site TX Cottonseed Fiber Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Fiber (% dwt)						
Acid Detergent Fiber	31.78 ± 1.50	30.41 ± 1.50	1.37 ± 2.13	-5.40,8.13	0.565	(26.32 - 38.97)
	(27.72 - 34.98)	(28.08 - 34.42)	(-6.70 - 5.74)			[25.48,38.48]
Crude Fiber	16.68 ± 0.29	17.38 ± 0.29	-0.71 ± 0.27	-1.56,0.15	0.078	(15.96 - 23.10)
	(16.27 - 17.00)	(16.41 - 18.25)	(-1.35 - -0.15)			[13.34,24.17]
Neutral Detergent Fiber	40.70 ± 2.01	39.97 ± 2.01	0.73 ± 2.85	-8.32,9.79	0.813	(38.49 - 51.84)
	(33.91 - 47.36)	(38.00 - 41.35)	(-6.11 - 9.36)			[34.51,53.25]
Total Dietary Fiber	39.63 ± 0.51	39.78 ± 0.51	-0.15 ± 0.72	-2.44,2.14	0.848	(36.47 - 47.54)
	(37.85 - 40.45)	(38.63 - 40.41)	(-2.18 - 1.37)			[36.13,48.96]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-13. Statistical Summary[1] of Site AL Cottonseed Mineral Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Mineral						
Calcium (% dwt)	0.16 ± 0.0027	0.16 ± 0.0027	-0.0037 ± 0.0022	-0.011,0.0035	0.200	(0.10 - 0.19)
	(0.15 - 0.16)	(0.16 - 0.17)	(-0.0084 - 0.0022)			[0.074,0.22]
Copper (mg/kg dwt)	5.36 ± 0.16	5.07 ± 0.16	0.29 ± 0.21	-0.39,0.96	0.272	(4.92 - 12.47)
	(5.15 - 5.61)	(4.53 - 5.46)	(-0.037 - 0.91)			[2.01,12.94]
Iron (mg/kg dwt)	55.90 ± 1.02	55.61 ± 1.02	0.29 ± 1.38	-4.10,4.68	0.845	(36.71 - 67.75)
	(51.85 - 57.63)	(54.37 - 56.45)	(-3.80 - 2.17)			[33.44,68.99]
Magnesium (% dwt)	0.44 ± 0.0046	0.45 ± 0.0046	-0.0076 ± 0.0065	-0.028,0.013	0.326	(0.35 - 0.47)
	(0.43 - 0.45)	(0.44 - 0.45)	(-0.026 - 0.0055)			[0.31,0.51]
Manganese (mg/kg dwt)	19.31 ± 0.29	17.77 ± 0.29	1.55 ± 0.40	0.26,2.84	0.031	(10.68 - 21.96)
	(18.45 - 19.98)	(17.23 - 18.23)	(0.46 - 2.48)			[4.69,26.45]
Phosphorus (% dwt)	0.72 ± 0.0096	0.74 ± 0.0096	-0.018 ± 0.014	-0.061,0.025	0.277	(0.48 - 0.99)
	(0.69 - 0.75)	(0.73 - 0.75)	(-0.054 - 0.019)			[0.31,1.08]
Potassium (% dwt)	1.26 ± 0.0081	1.26 ± 0.0081	-0.0025 ± 0.011	-0.036,0.031	0.830	(1.07 - 1.39)
	(1.23 - 1.28)	(1.25 - 1.27)	(-0.031 - 0.020)			[0.96,1.46]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-13 (Continued). Statistical Summary[1] of Site AL Cottonseed Mineral Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Mineral						
Sodium (% dwt)	0.10 ± 0.0067	0.080 ± 0.0067	0.025 ± 0.0052	0.0081,0.041	0.017	(0.032 - 0.14)
	(0.087 - 0.12)	(0.069 - 0.096)	(0.0098 - 0.034)			[0,0.17]
Zinc (mg/kg dwt)	43.39 ± 0.62	40.96 ± 0.62	2.43 ± 0.65	0.38,4.49	0.032	(30.11 - 59.51)
	(41.22 - 44.70)	(40.32 - 41.88)	(0.91 - 3.98)			[17.12,58.50]

Table G-14. Statistical Summary[1] of Site CA Cottonseed Mineral Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Mineral						
Calcium (% dwt)	0.16 ± 0.0030	0.18 ± 0.0030	-0.017 ± 0.0027	-0.025,-0.0081	0.008	(0.10 - 0.19)
	(0.16 - 0.17)	(0.17 - 0.19)	(-0.022 - -0.0098)			[0.074,0.22]
Copper (mg/kg dwt)	7.93 ± 0.36	8.33 ± 0.36	-0.40 ± 0.51	-2.01,1.21	0.489	(4.92 - 12.47)
	(7.40 - 8.51)	(7.63 - 9.47)	(-1.83 - 0.55)			[2.01,12.94]
Iron (mg/kg dwt)	52.92 ± 3.69	54.97 ± 3.69	-2.05 ± 5.22	-18.67,14.58	0.721	(36.71 - 67.75)
	(41.27 - 58.87)	(46.77 - 62.47)	(-21.20 - 8.45)			[33.44,68.99]
Magnesium (% dwt)	0.41 ± 0.011	0.43 ± 0.011	-0.027 ± 0.014	-0.072,0.019	0.160	(0.35 - 0.47)
	(0.40 - 0.43)	(0.40 - 0.46)	(-0.062 - -0.00082)			[0.31,0.51]
Manganese (mg/kg dwt)	15.44 ± 0.62	14.91 ± 0.62	0.53 ± 0.88	-2.26,3.32	0.586	(10.68 - 21.96)
	(13.37 - 16.47)	(13.36 - 15.62)	(-2.25 - 2.64)			[4.69,26.45]
Phosphorus (% dwt)	0.77 ± 0.028	0.85 ± 0.028	-0.084 ± 0.035	-0.20,0.029	0.098	(0.48 - 0.99)
	(0.75 - 0.82)	(0.76 - 0.93)	(-0.17 - -0.011)			[0.31,1.08]
Potassium (% dwt)	1.23 ± 0.048	1.30 ± 0.048	-0.067 ± 0.067	-0.28,0.15	0.395	(1.07 - 1.39)
	(1.19 - 1.34)	(1.16 - 1.43)	(-0.24 - 0.083)			[0.96,1.46]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-14 (Continued). Statistical Summary[1] of Site CA Cottonseed Mineral Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Mineral						
Sodium (% dwt)	0.060 ± 0.0027	0.037 ± 0.0027	0.023 ± 0.0025	0.015,0.031	0.002	(0.032 - 0.14)
	(0.053 - 0.068)	(0.033 - 0.039)	(0.018 - 0.029)			[0,0.17]
Zinc (mg/kg dwt)	48.62 ± 2.27	49.29 ± 2.27	-0.68 ± 3.22	-10.91,9.56	0.847	(30.11 - 59.51)
	(41.06 - 52.16)	(43.59 - 52.16)	(-11.11 - 6.23)			[17.12,58.50]

Table G-15. Statistical Summary[1] of Site GA Cottonseed Mineral Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Mineral						
Calcium (% dwt)	0.14 ± 0.0043	0.12 ± 0.0043	0.014 ± 0.0027	0.0052,0.022	0.014	(0.10 - 0.19)
	(0.13 - 0.15)	(0.11 - 0.13)	(0.0066 - 0.020)			[0.074,0.22]
Copper (mg/kg dwt)	6.81 ± 0.34	6.49 ± 0.34	0.32 ± 0.43	-1.06,1.70	0.515	(4.92 - 12.47)
	(6.30 - 7.46)	(5.48 - 7.48)	(-0.88 - 1.06)			[2.01,12.94]
Iron (mg/kg dwt)	52.48 ± 1.42	49.79 ± 1.42	2.69 ± 1.40	-1.78,7.16	0.151	(36.71 - 67.75)
	(48.19 - 54.65)	(46.96 - 53.39)	(-0.39 - 5.94)			[33.44,68.99]
Magnesium (% dwt)	0.40 ± 0.0094	0.40 ± 0.0094	0.0022 ± 0.0077	-0.022,0.027	0.796	(0.35 - 0.47)
	(0.38 - 0.42)	(0.37 - 0.42)	(-0.021 - 0.013)			[0.31,0.51]
Manganese (mg/kg dwt)	13.39 ± 0.44	13.12 ± 0.44	0.27 ± 0.28	-0.61,1.15	0.405	(10.68 - 21.96)
	(12.37 - 14.08)	(11.91 - 14.33)	(-0.47 - 0.84)			[4.69,26.45]
Phosphorus (% dwt)	0.58 ± 0.015	0.58 ± 0.015	0.00092 ± 0.010	-0.032,0.034	0.935	(0.48 - 0.99)
	(0.54 - 0.61)	(0.53 - 0.61)	(-0.029 - 0.018)			[0.31,1.08]
Potassium (% dwt)	1.24 ± 0.0082	1.22 ± 0.0082	0.018 ± 0.012	-0.019,0.055	0.211	(1.07 - 1.39)
	(1.23 - 1.25)	(1.20 - 1.25)	(-0.019 - 0.035)			[0.96,1.46]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-15 (Continued). Statistical Summary[1] of Site GA Cottonseed Mineral Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Mineral						
Sodium (% dwt)	0.045 ± 0.0035	0.10 ± 0.0035	-0.057 ± 0.0049	-0.073,-0.042	0.001	(0.032 - 0.14)
	(0.037 - 0.051)	(0.094 - 0.11)	(-0.075 - -0.043)			[0,0.17]
Zinc (mg/kg dwt)	39.17 ± 1.14	36.97 ± 1.14	2.21 ± 1.01	-1.00,5.41	0.116	(30.11 - 59.51)
	(37.96 - 40.56)	(33.17 - 40.60)	(-0.037 - 4.79)			[17.12,58.50]

Table G-16. Statistical Summary[1] of Site TX Cottonseed Mineral Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Mineral						
Calcium (% dwt)	0.18 ± 0.0015	0.19 ± 0.0015	-0.0033 ± 0.0022	-0.010,0.0036	0.225	(0.10 - 0.19)
	(0.18 - 0.19)	(0.19 - 0.19)	(-0.0093 - 0.00078)			[0.074,0.22]
Copper (mg/kg dwt)	6.78 ± 0.097	6.28 ± 0.097	0.50 ± 0.14	0.059,0.94	0.036	(4.92 - 12.47)
	(6.59 - 6.96)	(6.09 - 6.61)	(-0.021 - 0.80)			[2.01,12.94]
Iron (mg/kg dwt)	49.31 ± 0.59	48.43 ± 0.59	0.88 ± 0.60	-1.02,2.77	0.238	(36.71 - 67.75)
	(48.33 - 50.91)	(47.21 - 49.83)	(-0.67 - 2.23)			[33.44,68.99]
Magnesium (% dwt)	0.40 ± 0.0063	0.39 ± 0.0063	0.016 ± 0.0088	-0.012,0.044	0.170	(0.35 - 0.47)
	(0.40 - 0.42)	(0.37 - 0.40)	(-0.0012 - 0.050)			[0.31,0.51]
Manganese (mg/kg dwt)	13.20 ± 0.16	12.76 ± 0.16	0.44 ± 0.21	-0.22,1.10	0.123	(10.68 - 21.96)
	(12.83 - 13.66)	(12.49 - 13.13)	(-0.076 - 0.91)			[4.69,26.45]
Phosphorus (% dwt)	0.67 ± 0.013	0.62 ± 0.013	0.049 ± 0.018	-0.0077,0.11	0.070	(0.48 - 0.99)
	(0.66 - 0.68)	(0.57 - 0.64)	(0.021 - 0.11)			[0.31,1.08]
Potassium (% dwt)	1.14 ± 0.011	1.14 ± 0.011	-0.0069 ± 0.016	-0.058,0.044	0.696	(1.07 - 1.39)
	(1.12 - 1.15)	(1.12 - 1.18)	(-0.062 - 0.027)			[0.96,1.46]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-16 (Continued). Statistical Summary[1] of Site TX Cottonseed Mineral Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Mineral						
Sodium (% dwt)	0.038 ± 0.0066	0.053 ± 0.0066	-0.015 ± 0.0072	-0.038,0.0083	0.133	(0.032 - 0.14)
	(0.027 - 0.052)	(0.040 - 0.067)	(-0.034 - 0.00058)			[0,0.17]
Zinc (mg/kg dwt)	32.29 ± 1.19	30.47 ± 1.19	1.82 ± 1.69	-3.56,7.19	0.360	(30.11 - 59.51)
	(29.30 - 35.10)	(27.60 - 33.23)	(-1.87 - 7.50)			[17.12,58.50]

Table G-17. Statistical Summary[1] of Site AL Cottonseed Proximate Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Proximates						
Ash (% dwt)	4.70 ± 0.041	4.73 ± 0.041	-0.030 ± 0.027	-0.11,0.055	0.346	(3.76 - 5.34)
	(4.62 - 4.78)	(4.63 - 4.81)	(-0.074 - 0.046)			[2.96,5.62]
Calories (Kcal/100g dwt)	463.24 ± 2.55	457.36 ± 2.55	5.88 ± 3.01	-3.71,15.47	0.145	(407.45 - 471.46)
	(458.40 - 468.50)	(448.80 - 461.89)	(-1.60 - 13.10)			[409.12,496.45]
Carbohydrates (% dwt)	46.51 ± 0.38	45.60 ± 0.38	0.91 ± 0.54	-0.80,2.62	0.188	(40.06 - 52.01)
	(45.97 - 47.67)	(44.68 - 46.49)	(-0.51 - 1.99)			[38.23,56.70]
Moisture (% fwt)	7.07 ± 0.11	6.86 ± 0.11	0.21 ± 0.15	-0.27,0.70	0.259	(5.06 - 6.49)
	(6.85 - 7.34)	(6.64 - 7.12)	(0.010 - 0.70)			[4.51,7.21]
Protein (% dwt)	27.44 ± 0.39	27.24 ± 0.39	0.20 ± 0.50	-1.38,1.77	0.718	(21.48 - 32.03)
	(26.52 - 28.22)	(26.03 - 27.99)	(-1.04 - 1.38)			[20.19,32.70]
Total Fat (% dwt)	21.33 ± 0.19	22.41 ± 0.19	-1.08 ± 0.14	-1.53,-0.63	0.004	(17.60 - 27.29)
	(21.04 - 21.91)	(21.98 - 22.81)	(-1.50 - -0.91)			[15.16,28.44]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-18. Statistical Summary[1] of Site CA Cottonseed Proximate Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Proximates						
Ash (% dwt)	4.43 ± 0.16	4.63 ± 0.16	-0.20 ± 0.23	-0.94,0.54	0.453	(3.76 - 5.34)
	(4.18 - 4.81)	(4.33 - 5.19)	(-0.82 - 0.30)			[2.96,5.62]
Calories (Kcal/100g dwt)	448.21 ± 9.56	438.65 ± 9.56	9.56 ± 13.51	-33.45,52.57	0.530	(407.45 - 471.46)
	(424.36 - 458.94)	(415.74 - 458.94)	(-34.58 - 36.83)			[409.12,496.45]
Carbohydrates (% dwt)	44.02 ± 1.26	45.37 ± 1.26	-1.35 ± 1.78	-7.03,4.33	0.505	(40.06 - 52.01)
	(42.61 - 45.83)	(42.07 - 49.32)	(-5.22 - 3.76)			[38.23,56.70]
Moisture (% fwt)	5.79 ± 0.092	5.62 ± 0.092	0.18 ± 0.13	-0.24,0.59	0.272	(5.06 - 6.49)
	(5.65 - 5.90)	(5.32 - 5.87)	(-0.13 - 0.58)			[4.51,7.21]
Protein (% dwt)	27.49 ± 1.58	25.96 ± 1.58	1.53 ± 2.24	-5.59,8.65	0.542	(21.48 - 32.03)
	(24.08 - 29.01)	(21.64 - 29.53)	(-5.45 - 6.35)			[20.19,32.70]
Total Fat (% dwt)	24.04 ± 0.35	24.05 ± 0.35	-0.0078 ± 0.47	-1.50,1.48	0.987	(17.60 - 27.29)
	(23.37 - 25.25)	(23.61 - 24.82)	(-0.80 - 1.35)			[15.16,28.44]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-19. Statistical Summary[1] of Site GA Cottonseed Proximate Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Proximates						
Ash (% dwt)	4.05 ± 0.060	4.16 ± 0.060	-0.11 ± 0.085	-0.38,0.16	0.288	(3.76 - 5.34)
	(3.94 - 4.24)	(4.07 - 4.29)	(-0.34 - 0.061)			[2.96,5.62]
Calories (Kcal/100g dwt)	469.05 ± 4.62	463.05 ± 4.62	6.00 ± 2.84	-3.03,15.03	0.124	(407.45 - 471.46)
	(461.14 - 481.93)	(453.39 - 475.23)	(0.72 - 13.66)			[409.12,496.45]
Carbohydrates (% dwt)	43.79 ± 0.54	44.87 ± 0.54	-1.08 ± 0.75	-3.46,1.30	0.244	(40.06 - 52.01)
	(43.61 - 43.93)	(43.16 - 46.67)	(-2.74 - 0.61)			[38.23,56.70]
Moisture (% fwt)	6.25 ± 0.080	6.06 ± 0.080	0.19 ± 0.10	-0.13,0.51	0.152	(5.06 - 6.49)
	(6.21 - 6.32)	(5.82 - 6.31)	(-0.070 - 0.40)			[4.51,7.21]
Protein (% dwt)	29.71 ± 0.46	28.64 ± 0.46	1.07 ± 0.55	-0.68,2.82	0.147	(21.48 - 32.03)
	(28.82 - 31.13)	(27.39 - 29.24)	(0.049 - 2.14)			[20.19,32.70]
Total Fat (% dwt)	22.48 ± 0.69	22.33 ± 0.69	0.15 ± 0.38	-1.07,1.37	0.718	(17.60 - 27.29)
	(21.00 - 23.16)	(19.99 - 23.87)	(-0.71 - 1.02)			[15.16,28.44]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-20. Statistical Summary[1] of Site TX Cottonseed Proximate Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Proximates						
Ash (% dwt)	4.13 ± 0.062	3.94 ± 0.062	0.19 ± 0.086	-0.079,0.47	0.108	(3.76 - 5.34)
	(4.02 - 4.28)	(3.76 - 4.05)	(-0.026 - 0.39)			[2.96,5.62]
Calories (Kcal/100g dwt)	460.75 ± 2.13	462.99 ± 2.13	-2.23 ± 2.26	-9.41,4.95	0.395	(407.45 - 471.46)
	(456.54 - 465.49)	(456.87 - 468.38)	(-7.34 - 3.00)			[409.12,496.45]
Carbohydrates (% dwt)	44.63 ± 0.49	46.43 ± 0.49	-1.80 ± 0.60	-3.72,0.12	0.058	(40.06 - 52.01)
	(42.91 - 45.65)	(45.67 - 47.29)	(-3.55 - -0.96)			[38.23,56.70]
Moisture (% fwt)	6.46 ± 0.091	6.37 ± 0.091	0.090 ± 0.13	-0.32,0.50	0.534	(5.06 - 6.49)
	(6.30 - 6.55)	(6.16 - 6.70)	(-0.40 - 0.39)			[4.51,7.21]
Protein (% dwt)	28.28 ± 0.41	27.80 ± 0.41	0.48 ± 0.45	-0.96,1.92	0.364	(21.48 - 32.03)
	(27.37 - 29.53)	(26.79 - 28.30)	(-0.23 - 1.72)			[20.19,32.70]
Total Fat (% dwt)	22.96 ± 0.19	21.84 ± 0.19	1.12 ± 0.27	0.27,1.96	0.024	(17.60 - 27.29)
	(22.52 - 23.54)	(21.63 - 22.20)	(0.78 - 1.91)			[15.16,28.44]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-21. Statistical Summary[1] of Site AL Cottonseed Vitamin and Gossypol Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Vitamin						
Vitamin E (mg/kg dwt)	172.44 ± 1.16	173.38 ± 1.16	-0.95 ± 0.97	-4.04,2.15	0.402	(70.79 - 197.22)
	(169.09 - 173.91)	(171.19 - 176.74)	(-2.98 - 1.28)			[9.30,263.66]
Gossypol						
Free Gossypol (% dwt)	0.59 ± 0.024	0.66 ± 0.024	-0.073 ± 0.034	-0.18,0.034	0.118	(0.53 - 1.05)
	(0.51 - 0.62)	(0.62 - 0.72)	(-0.15 - -0.0014)			[0.43,1.06]
Total Gossypol (% dwt)	0.87 ± 0.0095	0.87 ± 0.0095	-0.0036 ± 0.010	-0.037,0.030	0.750	(0.78 - 1.19)
	(0.84 - 0.89)	(0.85 - 0.90)	(-0.032 - 0.015)			[0.61,1.25]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-22. Statistical Summary[1] of Site CA Cottonseed Vitamin and Gossypol Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Vitamin						
Vitamin E (mg/kg dwt)	145.68 ± 5.43	137.99 ± 5.43	7.69 ± 2.60	-0.57,15.95	0.059	(70.79 - 197.22)
	(129.43 - 160.04)	(126.42 - 145.30)	(3.01 - 14.75)			[9.30,263.66]
Gossypol						
Free Gossypol (% dwt)	0.73 ± 0.039	0.75 ± 0.039	-0.019 ± 0.055	-0.19,0.15	0.750	(0.53 - 1.05)
	(0.69 - 0.77)	(0.65 - 0.86)	(-0.12 - 0.11)			[0.43,1.06]
Total Gossypol (% dwt)	0.79 ± 0.031	0.85 ± 0.031	-0.055 ± 0.044	-0.19,0.084	0.297	(0.78 - 1.19)
	(0.75 - 0.87)	(0.78 - 0.93)	(-0.16 - 0.084)			[0.61,1.25]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-23. Statistical Summary[1] of Site GA Cottonseed Vitamin and Gossypol Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Vitamin						
Vitamin E (mg/kg dwt)	171.46 ± 4.22	170.32 ± 4.22	1.14 ± 2.52	-6.87,9.15	0.681	(70.79 - 197.22)
	(163.13 - 179.33)	(162.66 - 182.23)	(-2.89 - 8.41)			[9.30,263.66]
Gossypol						
Free Gossypol (% dwt)	0.62 ± 0.043	0.72 ± 0.043	-0.093 ± 0.032	-0.20,0.010	0.063	(0.53 - 1.05)
	(0.54 - 0.72)	(0.58 - 0.78)	(-0.19 - -0.038)			[0.43,1.06]
Total Gossypol (% dwt)	0.86 ± 0.046	0.85 ± 0.046	0.010 ± 0.028	-0.079,0.099	0.738	(0.78 - 1.19)
	(0.76 - 0.91)	(0.70 - 0.96)	(-0.057 - 0.060)			[0.61,1.25]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

Table G-24. Statistical Summary[1] of Site TX Cottonseed Vitamin and Gossypol Content for MON 88913 vs. MON 88913(-)

			Difference [MON 88913 minus MON 88913(-)]			
Analytical Component	**MON 88913 Mean ± S.E. (Range)**	**MON 88913(-) Mean ± S.E. (Range)**	**Mean ± S.E. (Range)**	**95% CI (Lower,Upper)**	***p-Value***	**Commercial (Range) [99% Tolerance Int.[2]]**
Vitamin						
Vitamin E (mg/kg dwt)	113.83 ± 4.37	113.48 ± 4.37	0.35 ± 3.51	-10.81,11.52	0.925	(70.79 - 197.22)
	(103.60 - 128.36)	(107.81 - 120.58)	(-6.89 - 7.77)			[9.30,263.66]
Gossypol						
Free Gossypol (% dwt)	0.66 ± 0.029	0.59 ± 0.029	0.069 ± 0.042	-0.063,0.20	0.196	(0.53 - 1.05)
	(0.60 - 0.72)	(0.51 - 0.66)	(-0.063 - 0.16)			[0.43,1.06]
Total Gossypol (% dwt)	0.74 ± 0.018	0.71 ± 0.018	0.029 ± 0.026	-0.053,0.11	0.348	(0.78 - 1.19)
	(0.70 - 0.79)	(0.69 - 0.76)	(-0.040 - 0.099)			[0.61,1.25]

[1]Means in the table are least square means from SAS.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional varieties. Negative limits were set to zero.

APPENDIX H

Supplemental Summary of Compositional Analyses for Cottonseed and Cottonseed Meal.

Amino Acids and Fatty Acids from MON 88913 and MON 88913(-) Calculated as Percent of Dry Weight, Percent of Total Fat, and Percent of Total Protein

Appendix H

Supplemental Summary of Compositional Analyses for Cottonseed and Cottonseed Meal

Purpose

In response to a request from FDA for the expression of the cottonseed composition values in additional units, data were summarized in this Appendix using these alternative units. Amino acid and fatty acid components from MON 88913, MON 88913(-), and reference cottonseed, plus cottonseed meal, were re-expressed as percent of dry weight, and as percent of total protein (amino acids) and total fat (fatty acids).

Section A. Cottonseed from Four Replicated Field Sites

The cottonseed data from MON 88913, MON 88913(-), and sixteen conventional reference cotton varieties were as described in Appendix E.

The following formulas were used for re-expression of amino acid and fatty acid data:

Component	From (X)	To	Formula[1]
Amino Acids (AA)	mg/g fwt	% Total Protein	X/(10*fp)
		% dwt	X/(10*d)
Sterculic Acid, Malvalic Acid, Dihydrosterculic Acid	µg/g fwt	% dwt	$X/(10^4*d)$
		% Total Fat	$X/(10^4*ff)$
Fatty Acid (FA)[2]	% fwt	% Total Fat	X/(ff)
		% dwt	X/(d)

[1] fp is the protein fraction of fresh weight obtained by proximate analysis = (% protein /100); ff is the total fat fraction of fresh weight obtained by proximate analysis = (% total fat /100); d is the fraction of the sample that is dry matter.

[2] Excluding Sterculic Acid, Malvalic Acid and Dihydrosterculic Acid

Statistical Approach

Means and ranges for MON 88913, MON 88913(-) and reference varieties were generated for the above listed analytes in the prescribed units, using SAS software for each site separately and combined.

Results Discussion

Statistical results of re-expression of amino acid and fatty components of MON 88913 and MON 88913(-) are summarized in Tables H-1 through H-4. For each amino acid and fatty acid component re-expression, the mean and the range of observed values are presented by site and overall. In addition, the overall range of observed values for commercial conventional reference varieties is presented in the desired unit.

Table H-1. Statistical Summary of Cottonseed Amino Acid (% dwt) Content for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Component	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Amino Acid (% dwt)				
Alanine	Combined Sites	1.06 (0.92 - 1.15)	1.02 (0.83 - 1.14)	(0.80 - 1.14)
	Site AL	1.04 (1.02 - 1.07)	1.01 (1.00 - 1.03)	
	Site CA	1.05 (0.92 - 1.15)	0.96 (0.83 - 1.06)	
	Site GA	1.12 (1.11 - 1.13)	1.09 (1.06 - 1.14)	
	Site TX	1.03 (1.03 - 1.04)	1.01 (0.97 - 1.05)	
Arginine	Combined Sites	2.93 (2.34 - 3.43)	2.80 (2.11 - 3.26)	(2.06 - 3.57)
	Site AL	2.63 (2.60 - 2.70)	2.61 (2.53 - 2.69)	
	Site CA	3.03 (2.34 - 3.43)	2.63 (2.11 - 3.06)	
	Site GA	3.11 (3.03 - 3.21)	3.08 (2.96 - 3.26)	
	Site TX	2.94 (2.90 - 2.97)	2.87 (2.74 - 3.04)	
Aspartic Acid	Combined Sites	2.44 (2.01 - 2.70)	2.32 (1.82 - 2.63)	(1.83 - 2.75)
	Site AL	2.31 (2.27 - 2.35)	2.25 (2.23 - 2.29)	
	Site CA	2.43 (2.01 - 2.70)	2.18 (1.82 - 2.46)	
	Site GA	2.59 (2.56 - 2.62)	2.51 (2.44 - 2.63)	
	Site TX	2.42 (2.41 - 2.44)	2.36 (2.28 - 2.47)	
Cystine	Combined Sites	0.47 (0.42 - 0.52)	0.45 (0.39 - 0.51)	(0.35 - 0.49)
	Site AL	0.44 (0.42 - 0.46)	0.44 (0.44 - 0.46)	
	Site CA	0.47 (0.43 - 0.52)	0.44 (0.39 - 0.49)	
	Site GA	0.46 (0.44 - 0.50)	0.47 (0.45 - 0.51)	
	Site TX	0.49 (0.47 - 0.52)	0.47 (0.46 - 0.47)	

Table H-1 (Continued). Statistical Summary of Cottonseed Amino Acid (% dwt) Content for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Component	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Amino Acid (% dwt)				
Glutamic Acid	Combined Sites	5.38 (4.42 - 6.19)	5.12 (4.01 - 5.78)	(3.91 - 6.14)
	Site AL	4.96 (4.90 - 5.05)	4.85 (4.80 - 4.98)	
	Site CA	5.54 (4.42 - 6.19)	4.88 (4.01 - 5.55)	
	Site GA	5.73 (5.62 - 5.83)	5.57 (5.49 - 5.78)	
	Site TX	5.32 (5.27 - 5.39)	5.16 (5.02 - 5.44)	
Glycine	Combined Sites	1.10 (0.95 - 1.23)	1.05 (0.88 - 1.17)	(0.83 - 1.21)
	Site AL	1.04 (1.03 - 1.07)	1.02 (1.01 - 1.05)	
	Site CA	1.12 (0.95 - 1.23)	1.01 (0.88 - 1.13)	
	Site GA	1.14 (1.13 - 1.16)	1.12 (1.10 - 1.17)	
	Site TX	1.09 (1.08 - 1.10)	1.06 (1.02 - 1.10)	
Histidine	Combined Sites	0.78 (0.65 - 0.89)	0.75 (0.61 - 0.84)	(0.57 - 0.87)
	Site AL	0.74 (0.72 - 0.76)	0.72 (0.70 - 0.73)	
	Site CA	0.80 (0.65 - 0.89)	0.71 (0.61 - 0.80)	
	Site GA	0.82 (0.81 - 0.83)	0.81 (0.80 - 0.84)	
	Site TX	0.77 (0.76 - 0.78)	0.75 (0.72 - 0.79)	
Isoleucine	Combined Sites	0.85 (0.72 - 0.95)	0.81 (0.65 - 0.91)	(0.62 - 0.93)
	Site AL	0.80 (0.79 - 0.83)	0.78 (0.78 - 0.78)	
	Site CA	0.85 (0.72 - 0.93)	0.78 (0.65 - 0.86)	
	Site GA	0.91 (0.88 - 0.95)	0.89 (0.87 - 0.91)	
	Site TX	0.84 (0.80 - 0.86)	0.81 (0.76 - 0.83)	

Table H-1 (Continued). Statistical Summary of Cottonseed Amino Acid (% dwt) Content for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Component	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Amino Acid (% dwt)				
Leucine	Combined Sites	1.57 (1.32 - 1.75)	1.49 (1.23 - 1.69)	(1.14 - 1.69)
	Site AL	1.49 (1.45 - 1.54)	1.44 (1.44 - 1.45)	
	Site CA	1.58 (1.32 - 1.75)	1.42 (1.23 - 1.57)	
	Site GA	1.69 (1.68 - 1.71)	1.62 (1.59 - 1.69)	
	Site TX	1.51 (1.50 - 1.53)	1.46 (1.39 - 1.53)	
Lysine	Combined Sites	1.24 (1.09 - 1.43)	1.21 (1.04 - 1.39)	(0.94 - 1.36)
	Site AL	1.16 (1.13 - 1.20)	1.13 (1.12 - 1.15)	
	Site CA	1.29 (1.09 - 1.43)	1.17 (1.04 - 1.25)	
	Site GA	1.31 (1.26 - 1.39)	1.33 (1.27 - 1.39)	
	Site TX	1.19 (1.18 - 1.21)	1.20 (1.17 - 1.24)	
Methionine	Combined Sites	0.41 (0.34 - 0.45)	0.40 (0.36 - 0.43)	(0.30 - 0.43)
	Site AL	0.40 (0.34 - 0.43)	0.41 (0.37 - 0.43)	
	Site CA	0.41 (0.38 - 0.44)	0.40 (0.36 - 0.43)	
	Site GA	0.41 (0.39 - 0.44)	0.41 (0.39 - 0.43)	
	Site TX	0.42 (0.39 - 0.45)	0.39 (0.37 - 0.39)	
Phenylalanine	Combined Sites	1.40 (1.16 - 1.58)	1.33 (1.04 - 1.50)	(1.02 - 1.63)
	Site AL	1.32 (1.30 - 1.34)	1.27 (1.26 - 1.29)	
	Site CA	1.42 (1.16 - 1.58)	1.27 (1.04 - 1.46)	
	Site GA	1.49 (1.46 - 1.51)	1.45 (1.42 - 1.50)	
	Site TX	1.38 (1.36 - 1.40)	1.33 (1.28 - 1.40)	

Table H-1 (Continued). Statistical Summary of Cottonseed Amino Acid (% dwt) Content for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Component	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Amino Acid (% dwt)				
Proline	Combined Sites	1.04 (0.88 - 1.14)	0.99 (0.75 - 1.12)	(0.75 - 1.18)
	Site AL	0.99 (0.96 - 1.00)	0.96 (0.94 - 0.98)	
	Site CA	1.03 (0.88 - 1.14)	0.94 (0.75 - 1.06)	
	Site GA	1.07 (1.02 - 1.10)	1.05 (1.02 - 1.12)	
	Site TX	1.05 (1.05 - 1.06)	1.00 (0.97 - 1.05)	
Serine	Combined Sites	1.21 (1.03 - 1.39)	1.16 (0.96 - 1.30)	(0.91 - 1.34)
	Site AL	1.20 (1.17 - 1.22)	1.16 (1.14 - 1.20)	
	Site CA	1.25 (1.03 - 1.39)	1.12 (0.96 - 1.24)	
	Site GA	1.27 (1.24 - 1.33)	1.23 (1.19 - 1.30)	
	Site TX	1.13 (1.04 - 1.17)	1.13 (1.10 - 1.21)	
Threonine	Combined Sites	0.79 (0.63 - 0.90)	0.76 (0.63 - 0.88)	(0.55 - 0.87)
	Site AL	0.79 (0.73 - 0.83)	0.77 (0.72 - 0.80)	
	Site CA	0.79 (0.72 - 0.83)	0.74 (0.63 - 0.83)	
	Site GA	0.85 (0.72 - 0.90)	0.84 (0.82 - 0.88)	
	Site TX	0.74 (0.63 - 0.79)	0.68 (0.63 - 0.75)	
Tryptophan	Combined Sites	0.27 (0.25 - 0.29)	0.27 (0.22 - 0.29)	(0.19 - 0.32)
	Site AL	0.26 (0.25 - 0.27)	0.26 (0.26 - 0.27)	
	Site CA	0.28 (0.26 - 0.29)	0.26 (0.22 - 0.29)	
	Site GA	0.28 (0.27 - 0.29)	0.29 (0.28 - 0.29)	
	Site TX	0.27 (0.27 - 0.29)	0.27 (0.26 - 0.29)	

Table H-1 (Continued). Statistical Summary of Cottonseed Amino Acid (% dwt) Content for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Component	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Amino Acid (% dwt)				
Tyrosine	Combined Sites	0.69 (0.60 - 0.78)	0.66 (0.53 - 0.73)	(0.53 - 0.82)
	Site AL	0.66 (0.64 - 0.69)	0.65 (0.64 - 0.67)	
	Site CA	0.71 (0.60 - 0.78)	0.65 (0.53 - 0.73)	
	Site GA	0.73 (0.70 - 0.74)	0.70 (0.69 - 0.73)	
	Site TX	0.68 (0.65 - 0.70)	0.64 (0.60 - 0.67)	
Valine	Combined Sites	1.20 (1.00 - 1.35)	1.14 (0.92 - 1.28)	(0.87 - 1.31)
	Site AL	1.12 (1.08 - 1.15)	1.09 (1.07 - 1.12)	
	Site CA	1.21 (1.00 - 1.35)	1.07 (0.92 - 1.19)	
	Site GA	1.28 (1.24 - 1.32)	1.24 (1.22 - 1.28)	
	Site TX	1.20 (1.12 - 1.23)	1.15 (1.08 - 1.20)	

Table H-2. Statistical Summary of Cottonseed Amino Acid (% Total Protein) Content for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Component	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Amino Acid (% Total Protein)				
Alanine	Combined Sites	3.77 (3.53 - 3.97)	3.72 (3.41 - 3.95)	(3.24 - 4.08)
	Site AL	3.80 (3.61 - 3.95)	3.72 (3.66 - 3.86)	
	Site CA	3.83 (3.70 - 3.97)	3.72 (3.58 - 3.85)	
	Site GA	3.77 (3.56 - 3.89)	3.80 (3.65 - 3.95)	
	Site TX	3.66 (3.53 - 3.80)	3.64 (3.41 - 3.77)	
Arginine	Combined Sites	10.37 (9.20 - 11.88)	10.19 (9.49 - 11.20)	(8.94 - 11.30)
	Site AL	9.60 (9.20 - 9.88)	9.58 (9.49 - 9.71)	
	Site CA	10.97 (9.74 - 11.88)	10.09 (9.75 - 10.44)	
	Site GA	10.49 (10.10 - 10.95)	10.77 (10.30 - 11.20)	
	Site TX	10.41 (10.07 - 10.82)	10.34 (9.70 - 10.75)	
Aspartic Acid	Combined Sites	8.64 (8.05 - 9.34)	8.48 (8.07 - 9.15)	(7.69 - 9.47)
	Site AL	8.41 (8.05 - 8.74)	8.27 (8.13 - 8.60)	
	Site CA	8.83 (8.33 - 9.34)	8.40 (8.35 - 8.46)	
	Site GA	8.73 (8.25 - 9.07)	8.76 (8.42 - 9.15)	
	Site TX	8.57 (8.26 - 8.83)	8.49 (8.07 - 8.76)	
Cystine	Combined Sites	1.66 (1.43 - 1.80)	1.66 (1.57 - 1.85)	(1.45 - 1.81)
	Site AL	1.61 (1.48 - 1.72)	1.63 (1.58 - 1.70)	
	Site CA	1.71 (1.55 - 1.80)	1.69 (1.62 - 1.85)	
	Site GA	1.57 (1.43 - 1.69)	1.65 (1.57 - 1.74)	
	Site TX	1.75 (1.69 - 1.79)	1.68 (1.65 - 1.73)	

Table H-2 (Continued). Statistical Summary of Cottonseed Amino Acid (% Total Protein) Content for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Component	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Amino Acid (% Total Protein)				
Glutamic Acid	Combined Sites	19.07 (17.48 - 21.43)	18.65 (17.43 - 20.08)	(16.90 - 20.68)
	Site AL	18.09 (17.48 - 18.70)	17.82 (17.43 - 18.48)	
	Site CA	20.07 (18.37 - 21.43)	18.76 (18.50 - 19.23)	
	Site GA	19.29 (18.39 - 19.89)	19.45 (18.83 - 20.08)	
	Site TX	18.81 (18.26 - 19.38)	18.58 (17.73 - 19.25)	
Glycine	Combined Sites	3.89 (3.63 - 4.26)	3.85 (3.59 - 4.11)	(3.38 - 4.22)
	Site AL	3.81 (3.63 - 3.96)	3.76 (3.70 - 3.88)	
	Site CA	4.07 (3.96 - 4.26)	3.92 (3.81 - 4.07)	
	Site GA	3.85 (3.66 - 3.96)	3.92 (3.77 - 4.11)	
	Site TX	3.85 (3.73 - 3.98)	3.80 (3.59 - 3.92)	
Histidine	Combined Sites	2.77 (2.54 - 3.09)	2.72 (2.55 - 2.93)	(2.46 - 2.99)
	Site AL	2.68 (2.54 - 2.78)	2.63 (2.58 - 2.70)	
	Site CA	2.91 (2.70 - 3.09)	2.74 (2.71 - 2.81)	
	Site GA	2.77 (2.68 - 2.83)	2.83 (2.74 - 2.93)	
	Site TX	2.73 (2.64 - 2.82)	2.69 (2.55 - 2.80)	
Isoleucine	Combined Sites	3.02 (2.79 - 3.24)	2.97 (2.70 - 3.30)	(2.49 - 3.25)
	Site AL	2.93 (2.79 - 3.02)	2.87 (2.80 - 3.00)	
	Site CA	3.08 (2.98 - 3.23)	3.00 (2.92 - 3.10)	
	Site GA	3.08 (2.98 - 3.24)	3.12 (2.98 - 3.30)	
	Site TX	2.97 (2.86 - 3.12)	2.90 (2.70 - 3.06)	

Table H-2 (Continued). Statistical Summary of Cottonseed Amino Acid (% Total Protein) Content for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Component	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Amino Acid (% Total Protein)				
Leucine	Combined Sites	5.55 (5.15 - 6.07)	5.42 (4.92 - 5.89)	(4.74 - 6.08)
	Site AL	5.42 (5.15 - 5.59)	5.29 (5.19 - 5.51)	
	Site CA	5.75 (5.46 - 6.07)	5.49 (5.32 - 5.69)	
	Site GA	5.69 (5.41 - 5.82)	5.67 (5.49 - 5.89)	
	Site TX	5.35 (5.18 - 5.51)	5.25 (4.92 - 5.42)	
Lysine	Combined Sites	4.39 (4.01 - 4.96)	4.41 (4.08 - 4.88)	(3.70 - 4.88)
	Site AL	4.25 (4.01 - 4.53)	4.16 (4.08 - 4.32)	
	Site CA	4.68 (4.51 - 4.96)	4.53 (4.24 - 4.78)	
	Site GA	4.42 (4.14 - 4.73)	4.64 (4.41 - 4.88)	
	Site TX	4.21 (4.02 - 4.41)	4.31 (4.13 - 4.44)	
Methionine	Combined Sites	1.45 (1.22 - 1.65)	1.46 (1.31 - 1.72)	(1.14 - 1.67)
	Site AL	1.45 (1.22 - 1.59)	1.49 (1.42 - 1.53)	
	Site CA	1.50 (1.33 - 1.65)	1.54 (1.45 - 1.72)	
	Site GA	1.39 (1.27 - 1.49)	1.43 (1.36 - 1.52)	
	Site TX	1.47 (1.40 - 1.51)	1.39 (1.31 - 1.45)	
Phenylalanine	Combined Sites	4.97 (4.62 - 5.48)	4.85 (4.51 - 5.23)	(4.25 - 5.38)
	Site AL	4.80 (4.62 - 4.94)	4.68 (4.59 - 4.86)	
	Site CA	5.16 (4.80 - 5.48)	4.89 (4.79 - 4.96)	
	Site GA	5.02 (4.86 - 5.15)	5.05 (4.91 - 5.23)	
	Site TX	4.89 (4.75 - 5.04)	4.78 (4.51 - 4.94)	

Table H-2 (Continued). Statistical Summary of Cottonseed Amino Acid (% Total Protein) Content for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Component	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Amino Acid (% Total Protein)				
Proline	Combined Sites	3.67 (3.42 - 3.93)	3.60 (3.41 - 3.82)	(3.07 - 4.06)
	Site AL	3.61 (3.42 - 3.77)	3.53 (3.48 - 3.60)	
	Site CA	3.73 (3.64 - 3.93)	3.60 (3.45 - 3.71)	
	Site GA	3.62 (3.53 - 3.71)	3.68 (3.56 - 3.82)	
	Site TX	3.72 (3.57 - 3.85)	3.61 (3.41 - 3.74)	
Serine	Combined Sites	4.29 (3.71 - 4.82)	4.24 (3.90 - 4.61)	(3.74 - 4.88)
	Site AL	4.36 (4.31 - 4.41)	4.28 (4.16 - 4.61)	
	Site CA	4.53 (4.28 - 4.82)	4.31 (4.21 - 4.44)	
	Site GA	4.30 (3.97 - 4.51)	4.28 (4.18 - 4.44)	
	Site TX	3.99 (3.71 - 4.18)	4.07 (3.90 - 4.26)	
Threonine	Combined Sites	2.81 (2.23 - 3.13)	2.77 (2.23 - 3.05)	(2.10 - 3.09)
	Site AL	2.88 (2.60 - 3.01)	2.82 (2.76 - 2.86)	
	Site CA	2.87 (2.71 - 3.00)	2.87 (2.78 - 2.91)	
	Site GA	2.85 (2.44 - 3.13)	2.94 (2.84 - 3.05)	
	Site TX	2.63 (2.23 - 2.84)	2.46 (2.23 - 2.80)	
Tryptophan	Combined Sites	0.97 (0.89 - 1.07)	0.99 (0.95 - 1.06)	(0.87 - 1.09)
	Site AL	0.94 (0.89 - 0.98)	0.97 (0.96 - 1.00)	
	Site CA	1.01 (0.98 - 1.07)	1.01 (0.99 - 1.06)	
	Site GA	0.95 (0.93 - 1.00)	1.00 (0.98 - 1.01)	
	Site TX	0.97 (0.91 - 1.02)	0.97 (0.95 - 1.01)	

Table H-2 (Continued). Statistical Summary of Cottonseed Amino Acid (% Total Protein) Content for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Component	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Amino Acid (% Total Protein)				
Tyrosine	Combined Sites	2.46 (2.27 - 2.68)	2.40 (2.12 - 2.56)	(2.02 - 2.76)
	Site AL	2.41 (2.27 - 2.50)	2.39 (2.32 - 2.49)	
	Site CA	2.57 (2.49 - 2.68)	2.49 (2.46 - 2.50)	
	Site GA	2.45 (2.36 - 2.55)	2.45 (2.36 - 2.56)	
	Site TX	2.40 (2.32 - 2.47)	2.29 (2.12 - 2.43)	
Valine	Combined Sites	4.26 (3.82 - 4.67)	4.16 (3.83 - 4.57)	(3.61 - 4.52)
	Site AL	4.07 (3.82 - 4.21)	4.00 (3.92 - 4.12)	
	Site CA	4.41 (4.16 - 4.67)	4.15 (4.03 - 4.25)	
	Site GA	4.32 (4.19 - 4.51)	4.34 (4.18 - 4.57)	
	Site TX	4.24 (3.99 - 4.49)	4.15 (3.83 - 4.34)	

Table H-3. Statistical Summary of Cottonseed Fatty Acid (% dwt) Content for MON 88913, MON 88913(-) and Conventional Commercial Conventional Varieties

Component Fatty Acid (% dwt)	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
14:0 Myristic	Combined Sites	0.16 (0.13 - 0.20)	0.16 (0.14 - 0.19)	(0.11 - 0.20)
	Site AL	0.14 (0.13 - 0.14)	0.14 (0.14 - 0.15)	
	Site CA	0.19 (0.17 - 0.20)	0.17 (0.15 - 0.19)	
	Site GA	0.15 (0.15 - 0.16)	0.15 (0.14 - 0.16)	
	Site TX	0.18 (0.17 - 0.20)	0.17 (0.15 - 0.18)	
16:0 Palmitic	Combined Sites	5.08 (4.55 - 5.59)	4.93 (4.20 - 5.65)	(4.07 - 5.69)
	Site AL	4.91 (4.80 - 5.10)	5.05 (4.98 - 5.18)	
	Site CA	5.49 (5.33 - 5.59)	5.37 (5.18 - 5.65)	
	Site GA	4.77 (4.55 - 4.93)	4.72 (4.20 - 5.05)	
	Site TX	5.14 (4.85 - 5.37)	4.57 (4.42 - 4.66)	
16:1 Palmitoleic	Combined Sites	0.12 (0.11 - 0.13)	0.11 (0.10 - 0.13)	(0.095 - 0.14)
	Site AL	0.11 (0.11 - 0.11)	0.11 (0.11 - 0.11)	
	Site CA	0.13 (0.12 - 0.13)	0.12 (0.11 - 0.13)	
	Site GA	0.12 (0.11 - 0.12)	0.12 (0.11 - 0.12)	
	Site TX	0.12 (0.11 - 0.12)	0.11 (0.10 - 0.11)	
18:0 Stearic	Combined Sites	0.57 (0.50 - 0.64)	0.57 (0.47 - 0.66)	(0.43 - 0.75)
	Site AL	0.55 (0.53 - 0.58)	0.58 (0.57 - 0.60)	
	Site CA	0.62 (0.61 - 0.64)	0.62 (0.58 - 0.66)	
	Site GA	0.58 (0.55 - 0.60)	0.56 (0.49 - 0.61)	
	Site TX	0.52 (0.50 - 0.54)	0.50 (0.47 - 0.53)	

Table H-3 (Continued). Statistical Summary of Cottonseed Fatty Acid (% dwt) Content for MON 88913, MON 88913(-) and Conventional Commercial Conventional Varieties

Component	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Fatty Acid (% dwt)				
18:1 Oleic	Combined Sites	4.02 (3.33 - 4.52)	4.46 (3.86 - 5.13)	(2.24 - 4.30)
	Site AL	3.41 (3.33 - 3.48)	4.03 (3.87 - 4.12)	
	Site CA	4.46 (4.40 - 4.52)	4.89 (4.66 - 5.13)	
	Site GA	3.97 (3.84 - 4.21)	4.40 (3.86 - 4.73)	
	Site TX	4.23 (4.06 - 4.44)	4.53 (4.34 - 4.74)	
18:2 Linoleic	Combined Sites	11.28 (10.48 - 12.41)	10.76 (9.39 - 11.83)	(9.58 - 13.52)
	Site AL	11.08 (10.96 - 11.33)	11.03 (10.71 - 11.30)	
	Site CA	11.41 (10.81 - 12.41)	11.15 (10.26 - 11.83)	
	Site GA	11.42 (10.48 - 11.94)	11.00 (9.39 - 11.79)	
	Site TX	11.20 (10.89 - 11.45)	9.87 (9.71 - 10.08)	
18:3 Gamma Linolenic	Combined Sites	0.026 (0.011 - 0.060)	0.028 (0.011 - 0.044)	(0.011 - 0.051)
	Site AL	0.024 (0.011 - 0.034)	0.039 (0.037 - 0.043)	
	Site CA	0.020 (0.011 - 0.035)	0.024 (0.011 - 0.031)	
	Site GA	0.042 (0.031 - 0.060)	0.026 (0.011 - 0.044)	
	Site TX	0.019 (0.011 - 0.030)	0.021 (0.011 - 0.037)	
18:3 Linolenic	Combined Sites	0.038 (0.024 - 0.053)	0.036 (0.026 - 0.049)	(0.025 - 0.050)
	Site AL	0.045 (0.041 - 0.052)	0.044 (0.034 - 0.049)	
	Site CA	0.026 (0.024 - 0.030)	0.029 (0.027 - 0.033)	
	Site GA	0.049 (0.042 - 0.053)	0.044 (0.043 - 0.046)	
	Site TX	0.030 (0.025 - 0.034)	0.028 (0.026 - 0.030)	

Table H-3 (Continued). Statistical Summary of Cottonseed Fatty Acid (% dwt) Content for MON 88913, MON 88913(-) and Conventional Commercial Conventional Varieties

Component	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Fatty Acid (% dwt)				
20:0 Arachidic	Combined Sites	0.059 (0.053 - 0.069)	0.059 (0.051 - 0.065)	(0.044 - 0.069)
	Site AL	0.057 (0.055 - 0.061)	0.062 (0.060 - 0.063)	
	Site CA	0.065 (0.060 - 0.069)	0.063 (0.059 - 0.065)	
	Site GA	0.058 (0.055 - 0.059)	0.056 (0.054 - 0.059)	
	Site TX	0.056 (0.053 - 0.060)	0.054 (0.051 - 0.056)	
22:0 Behenic	Combined Sites	0.032 (0.026 - 0.037)	0.031 (0.027 - 0.034)	(0.022 - 0.038)
	Site AL	0.029 (0.026 - 0.032)	0.032 (0.031 - 0.033)	
	Site CA	0.032 (0.031 - 0.034)	0.031 (0.028 - 0.034)	
	Site GA	0.031 (0.029 - 0.033)	0.030 (0.027 - 0.032)	
	Site TX	0.036 (0.034 - 0.037)	0.032 (0.030 - 0.034)	
Dihydrosterculic	Combined Sites	0.031 (0.025 - 0.038)	0.035 (0.022 - 0.043)	(0.015 - 0.051)
	Site AL	0.033 (0.030 - 0.035)	0.035 (0.032 - 0.037)	
	Site CA	0.029 (0.028 - 0.033)	0.037 (0.030 - 0.040)	
	Site GA	0.029 (0.025 - 0.032)	0.032 (0.022 - 0.038)	
	Site TX	0.034 (0.029 - 0.038)	0.038 (0.029 - 0.043)	
Malvalic	Combined Sites	0.077 (0.054 - 0.11)	0.084 (0.049 - 0.12)	(0.044 - 0.10)
	Site AL	0.10 (0.091 - 0.11)	0.097 (0.087 - 0.11)	
	Site CA	0.058 (0.055 - 0.064)	0.078 (0.072 - 0.085)	
	Site GA	0.080 (0.073 - 0.088)	0.084 (0.049 - 0.12)	
	Site TX	0.066 (0.054 - 0.077)	0.076 (0.056 - 0.088)	

Table H-3 (Continued). Statistical Summary of Cottonseed Fatty Acid (% dwt) Content for MON 88913, MON 88913(-) and Conventional Commercial Conventional Varieties

Component	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Fatty Acid (% dwt)				
Sterculic	Combined Sites	0.066 (0.053 - 0.088)	0.070 (0.046 - 0.097)	(0.039 - 0.080)
	Site AL	0.078 (0.069 - 0.088)	0.081 (0.074 - 0.089)	
	Site CA	0.059 (0.054 - 0.062)	0.064 (0.058 - 0.068)	
	Site GA	0.067 (0.059 - 0.073)	0.072 (0.046 - 0.097)	
	Site TX	0.059 (0.053 - 0.069)	0.065 (0.050 - 0.074)	

Table H-4. Statistical Summary of Cottonseed Fatty Acid (% Total Fat) Content for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Component	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Fatty Acid (% Total Fat)				
14:0 Myristic	Combined Sites	0.72 (0.63 - 0.85)	0.70 (0.60 - 0.83)	(0.60 - 0.96)
	Site AL	0.65 (0.63 - 0.66)	0.64 (0.60 - 0.66)	
	Site CA	0.78 (0.66 - 0.83)	0.71 (0.64 - 0.78)	
	Site GA	0.68 (0.64 - 0.73)	0.68 (0.62 - 0.73)	
	Site TX	0.80 (0.74 - 0.85)	0.78 (0.71 - 0.83)	
16:0 Palmitic	Combined Sites	22.37 (20.70 - 23.68)	21.73 (20.20 - 22.90)	(20.21 - 23.89)
	Site AL	23.00 (22.81 - 23.30)	22.56 (21.83 - 22.90)	
	Site CA	22.86 (22.14 - 23.68)	22.31 (21.93 - 22.77)	
	Site GA	21.21 (20.70 - 21.68)	21.12 (20.41 - 21.90)	
	Site TX	22.40 (21.42 - 23.24)	20.94 (20.20 - 21.39)	
16:1 Palmitoleic	Combined Sites	0.52 (0.47 - 0.55)	0.50 (0.46 - 0.54)	(0.43 - 0.70)
	Site AL	0.51 (0.51 - 0.53)	0.50 (0.48 - 0.50)	
	Site CA	0.53 (0.48 - 0.55)	0.50 (0.47 - 0.54)	
	Site GA	0.52 (0.50 - 0.54)	0.52 (0.50 - 0.53)	
	Site TX	0.51 (0.47 - 0.52)	0.49 (0.46 - 0.51)	
18:0 Stearic	Combined Sites	2.50 (2.21 - 2.67)	2.49 (2.13 - 2.75)	(2.13 - 2.90)
	Site AL	2.59 (2.53 - 2.63)	2.59 (2.54 - 2.64)	
	Site CA	2.59 (2.50 - 2.67)	2.58 (2.44 - 2.75)	
	Site GA	2.59 (2.53 - 2.64)	2.51 (2.46 - 2.54)	
	Site TX	2.25 (2.21 - 2.28)	2.28 (2.13 - 2.40)	

Table H-4 (Continued). Statistical Summary of Cottonseed Fatty Acid (% Total Fat) Content for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Component	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Fatty Acid (% Total Fat)				
18:1 Oleic	Combined Sites	17.67 (15.81 - 19.72)	19.70 (17.61 - 21.92)	(12.12 - 17.45)
	Site AL	16.00 (15.81 - 16.36)	17.99 (17.61 - 18.31)	
	Site CA	18.58 (17.90 - 18.83)	20.34 (19.51 - 21.47)	
	Site GA	17.65 (16.74 - 18.46)	19.70 (18.99 - 20.67)	
	Site TX	18.43 (17.78 - 19.72)	20.76 (19.57 - 21.92)	
18:2 Linoleic	Combined Sites	49.74 (45.95 - 52.55)	47.48 (43.45 - 51.15)	(48.03 - 56.28)
	Site AL	51.96 (51.52 - 52.55)	49.24 (46.95 - 50.24)	
	Site CA	47.44 (45.95 - 49.16)	46.33 (43.45 - 47.66)	
	Site GA	50.79 (49.90 - 52.34)	49.19 (46.97 - 51.15)	
	Site TX	48.78 (48.34 - 49.07)	45.18 (44.42 - 46.60)	
18:3 Gamma Linolenic	Combined Sites	0.12 (0.042 - 0.26)	0.12 (0.045 - 0.19)	(0.039 - 0.23)
	Site AL	0.11 (0.051 - 0.15)	0.18 (0.17 - 0.19)	
	Site CA	0.082 (0.042 - 0.15)	0.10 (0.045 - 0.13)	
	Site GA	0.19 (0.13 - 0.26)	0.11 (0.048 - 0.19)	
	Site TX	0.082 (0.046 - 0.13)	0.095 (0.049 - 0.17)	
18:3 Linolenic	Combined Sites	0.17 (0.10 - 0.24)	0.16 (0.12 - 0.22)	(0.10 - 0.26)
	Site AL	0.21 (0.19 - 0.24)	0.20 (0.16 - 0.22)	
	Site CA	0.11 (0.10 - 0.12)	0.12 (0.12 - 0.13)	
	Site GA	0.22 (0.18 - 0.24)	0.20 (0.19 - 0.22)	
	Site TX	0.13 (0.11 - 0.15)	0.13 (0.12 - 0.14)	

Table H-4 (Continued). Statistical Summary of Cottonseed Fatty Acid (% Total Fat) Content for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Component	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Fatty Acid (% Total Fat)				
20:0 Arachidic	Combined Sites	0.26 (0.23 - 0.29)	0.26 (0.23 - 0.28)	(0.21 - 0.31)
	Site AL	0.27 (0.26 - 0.28)	0.27 (0.27 - 0.28)	
	Site CA	0.27 (0.24 - 0.29)	0.26 (0.25 - 0.27)	
	Site GA	0.26 (0.24 - 0.28)	0.25 (0.23 - 0.27)	
	Site TX	0.24 (0.23 - 0.26)	0.25 (0.23 - 0.26)	
22:0 Behenic	Combined Sites	0.14 (0.13 - 0.16)	0.14 (0.12 - 0.16)	(0.11 - 0.17)
	Site AL	0.14 (0.13 - 0.15)	0.14 (0.14 - 0.15)	
	Site CA	0.14 (0.13 - 0.15)	0.13 (0.12 - 0.14)	
	Site GA	0.14 (0.13 - 0.14)	0.14 (0.12 - 0.16)	
	Site TX	0.16 (0.15 - 0.16)	0.14 (0.13 - 0.15)	
Dihydrosterculic	Combined Sites	0.14 (0.11 - 0.17)	0.16 (0.098 - 0.20)	(0.070 - 0.22)
	Site AL	0.15 (0.14 - 0.17)	0.15 (0.14 - 0.17)	
	Site CA	0.12 (0.11 - 0.14)	0.15 (0.12 - 0.17)	
	Site GA	0.13 (0.11 - 0.14)	0.14 (0.098 - 0.17)	
	Site TX	0.15 (0.13 - 0.17)	0.17 (0.13 - 0.20)	
Malvalic	Combined Sites	0.34 (0.23 - 0.55)	0.37 (0.22 - 0.53)	(0.22 - 0.54)
	Site AL	0.48 (0.43 - 0.55)	0.43 (0.38 - 0.48)	
	Site CA	0.24 (0.23 - 0.25)	0.32 (0.30 - 0.34)	
	Site GA	0.36 (0.32 - 0.42)	0.37 (0.22 - 0.53)	
	Site TX	0.29 (0.23 - 0.34)	0.35 (0.26 - 0.40)	

Table H-4 (Continued). Statistical Summary of Cottonseed Fatty Acid (% Total Fat) Content for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Component	Location	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Fatty Acid (% Total Fat)				
Sterculic	Combined Sites	0.29 (0.22 - 0.40)	0.31 (0.21 - 0.42)	(0.18 - 0.39)
	Site AL	0.37 (0.33 - 0.40)	0.36 (0.32 - 0.39)	
	Site CA	0.24 (0.23 - 0.26)	0.26 (0.24 - 0.28)	
	Site GA	0.30 (0.26 - 0.34)	0.33 (0.21 - 0.42)	
	Site TX	0.26 (0.22 - 0.30)	0.30 (0.23 - 0.34)	

Section B. Cottonseed and Cottonseed Meal from Processing Analysis

The seed fatty acid and total fat data and the seed and meal amino acid, protein and moisture data from MON 88913, MON 88913(-), and six commercial conventional reference varieties were produced as described in Appendix F. Prior to analyte statistical analysis, all composition data was averaged across duplicates for each sample. The formulas used for re-expression of cottonseed fraction composition data were as described for whole cottonseed in Section A of this appendix.

Statistical Approach

Means and ranges for MON 88913, MON 88913(-), and reference cotton were generated for the above listed analytes in the prescribed units using SAS® software for each site separately and combined.

Results Discussion

Statistical results of re-expression of amino acid and fatty component of MON 88913 and MON 88913(-) are summarized in Tables H-5 through H-8. For each amino acid and fatty acid component, the overall mean and the range of observed values are presented. In addition, the overall range of observed values for commercial conventional reference varieties is presented in the desired unit.

Table H-5. Statistical Summary[1] of Amino Acid (% dwt) Levels in Cotton Fractions for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Fraction	Component	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Amino Acid (% dwt)				
Meal	Alanine	1.81 (1.80 - 1.83)	1.89 (1.81 - 2.00)	(1.73 - 1.90)
	Arginine	4.81 (4.64 - 5.01)	5.14 (4.61 - 5.70)	(4.74 - 5.58)
	Aspartic Acid	3.96 (3.87 - 4.07)	4.15 (3.80 - 4.51)	(3.84 - 4.38)
	Cystine	0.74 (0.71 - 0.78)	0.77 (0.70 - 0.86)	(0.73 - 0.83)
	Glutamic Acid	8.44 (8.04 - 8.89)	8.92 (8.07 - 9.66)	(8.17 - 9.20)
	Glycine	1.90 (1.86 - 1.92)	1.98 (1.83 - 2.14)	(1.80 - 2.04)
	Histidine	1.31 (1.28 - 1.34)	1.39 (1.24 - 1.53)	(1.28 - 1.43)
	Isoleucine	1.38 (1.28 - 1.44)	1.48 (1.37 - 1.61)	(1.29 - 1.52)
	Leucine	2.72 (2.61 - 2.93)	2.78 (2.57 - 3.00)	(2.58 - 3.04)
	Lysine	2.12 (2.11 - 2.15)	2.22 (2.04 - 2.39)	(2.04 - 2.22)
	Methionine	0.69 (0.65 - 0.72)	0.71 (0.64 - 0.79)	(0.66 - 0.71)
	Phenylalanine	2.28 (2.21 - 2.34)	2.42 (2.18 - 2.67)	(2.24 - 2.56)
	Proline	1.68 (1.60 - 1.77)	1.75 (1.60 - 1.88)	(1.61 - 1.79)
	Serine	2.03 (1.95 - 2.08)	2.11 (1.94 - 2.29)	(1.90 - 2.15)

[1]Data were averaged across duplicates for each sample prior to statistical analysis and summary.

Table H-5 (Continued). Statistical Summary[1] of Amino Acid (% dwt) Levels in Cotton Fractions for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Fraction	Component	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Amino Acid (% dwt)				
Meal	Threonine	1.45 (1.44 - 1.46)	1.51 (1.41 - 1.63)	(1.39 - 1.57)
	Tryptophan	0.47 (0.46 - 0.47)	0.51 (0.46 - 0.55)	(0.46 - 0.52)
	Tyrosine	1.14 (1.11 - 1.18)	1.22 (1.09 - 1.36)	(1.07 - 1.28)
	Valine	1.97 (1.89 - 2.09)	2.03 (1.87 - 2.21)	(1.92 - 2.12)
Cottonseed	Alanine	1.03 (0.94 - 1.11)	0.99 (0.86 - 1.04)	(0.91 - 0.97)
	Arginine	2.66 (2.32 - 3.03)	2.56 (2.14 - 2.82)	(2.34 - 2.69)
	Aspartic Acid	2.28 (2.00 - 2.54)	2.17 (1.84 - 2.37)	(2.03 - 2.28)
	Cystine	0.45 (0.40 - 0.51)	0.43 (0.40 - 0.48)	(0.38 - 0.45)
	Glutamic Acid	4.96 (4.29 - 5.61)	4.72 (3.96 - 5.21)	(4.35 - 5.04)
	Glycine	1.06 (0.95 - 1.16)	1.02 (0.87 - 1.11)	(0.93 - 1.01)
	Histidine	0.73 (0.65 - 0.81)	0.71 (0.60 - 0.78)	(0.65 - 0.72)
	Isoleucine	0.79 (0.72 - 0.87)	0.76 (0.65 - 0.84)	(0.71 - 0.77)
	Leucine	1.47 (1.33 - 1.62)	1.42 (1.21 - 1.56)	(1.30 - 1.41)
	Lysine	1.18 (1.10 - 1.27)	1.14 (1.00 - 1.23)	(1.05 - 1.14)
	Methionine	0.40 (0.35 - 0.44)	0.38 (0.35 - 0.41)	(0.33 - 0.38)

Table H-5 (Continued). Statistical Summary[1] of Amino Acid (% dwt) Levels in Cotton Fractions for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Fraction	Component	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Amino Acid (% dwt)				
Cottonseed	Phenylalanine	1.29 (1.14 - 1.43)	1.23 (1.03 - 1.34)	(1.13 - 1.28)
	Proline	0.97 (0.88 - 1.06)	0.94 (0.80 - 1.04)	(0.87 - 0.96)
	Serine	1.17 (1.04 - 1.29)	1.12 (0.98 - 1.21)	(1.03 - 1.15)
	Threonine	0.78 (0.71 - 0.86)	0.74 (0.63 - 0.82)	(0.69 - 0.74)
	Tryptophan	0.26 (0.24 - 0.30)	0.26 (0.25 - 0.28)	(0.23 - 0.25)
	Tyrosine	0.67 (0.60 - 0.76)	0.63 (0.53 - 0.69)	(0.57 - 0.64)
	Valine	1.09 (0.97 - 1.21)	1.04 (0.89 - 1.15)	(0.97 - 1.06)

Table H-6. Statistical Summary[1] of Amino Acid (% Total Protein) Levels in Cotton Fractions for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Fraction	Component	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Amino Acid (% Total Protein)				
Meal	Alanine	4.04 (3.87 - 4.13)	3.96 (3.81 - 4.13)	(3.71 - 4.10)
	Arginine	10.70 (10.49 - 10.85)	10.70 (10.42 - 11.01)	(10.52 - 11.43)
	Aspartic Acid	8.81 (8.74 - 8.93)	8.64 (8.61 - 8.71)	(8.55 - 9.13)
	Cystine	1.64 (1.61 - 1.68)	1.60 (1.57 - 1.66)	(1.58 - 1.74)
	Glutamic Acid	18.78 (18.33 - 19.13)	18.59 (18.38 - 18.82)	(18.20 - 19.42)
	Glycine	4.22 (4.13 - 4.32)	4.13 (4.09 - 4.17)	(4.03 - 4.28)
	Histidine	2.91 (2.86 - 2.96)	2.90 (2.83 - 2.96)	(2.77 - 3.06)
	Isoleucine	3.06 (2.84 - 3.21)	3.09 (3.07 - 3.12)	(2.75 - 3.32)
	Leucine	6.04 (5.83 - 6.48)	5.81 (5.76 - 5.86)	(5.66 - 6.33)
	Lysine	4.72 (4.63 - 4.81)	4.64 (4.52 - 4.75)	(4.43 - 4.92)
	Methionine	1.52 (1.49 - 1.56)	1.48 (1.44 - 1.53)	(1.41 - 1.58)
	Phenylalanine	5.06 (5.00 - 5.14)	5.05 (4.95 - 5.16)	(4.94 - 5.32)
	Proline	3.73 (3.65 - 3.80)	3.64 (3.60 - 3.67)	(3.50 - 3.84)
	Serine	4.51 (4.45 - 4.56)	4.40 (4.31 - 4.47)	(4.18 - 4.51)

[1]Data were averaged across duplicates for each sample prior to statistical analysis and summary.

Table H-6 (Continued). Statistical Summary[1] of Amino Acid (% Total Protein) Levels in Cotton Fractions for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Fraction	Component	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Amino Acid (% Total Protein)				
Meal	Threonine	3.22 (3.09 - 3.32)	3.16 (3.14 - 3.20)	(3.08 - 3.31)
	Tryptophan	1.04 (1.02 - 1.08)	1.06 (1.05 - 1.06)	(0.98 - 1.13)
	Tyrosine	2.54 (2.46 - 2.63)	2.53 (2.43 - 2.62)	(2.47 - 2.65)
	Valine	4.38 (4.24 - 4.63)	4.24 (4.18 - 4.29)	(4.26 - 4.57)
Cottonseed	Alanine	3.89 (3.72 - 4.07)	3.85 (3.55 - 4.12)	(3.66 - 4.08)
	Arginine	10.04 (9.86 - 10.39)	9.99 (8.84 - 10.54)	(9.59 - 10.86)
	Aspartic Acid	8.59 (8.53 - 8.65)	8.46 (7.61 - 8.81)	(8.39 - 9.24)
	Cystine	1.71 (1.71 - 1.72)	1.69 (1.62 - 1.79)	(1.55 - 1.83)
	Glutamic Acid	18.66 (18.22 - 19.03)	18.39 (16.35 - 19.46)	(18.09 - 20.17)
	Glycine	3.99 (3.84 - 4.11)	3.96 (3.57 - 4.12)	(3.78 - 4.21)
	Histidine	2.76 (2.70 - 2.81)	2.76 (2.47 - 2.87)	(2.69 - 2.99)
	Isoleucine	2.98 (2.85 - 3.11)	2.98 (2.68 - 3.11)	(2.79 - 3.29)
	Leucine	5.55 (5.34 - 5.73)	5.55 (4.99 - 5.77)	(5.24 - 6.04)
	Lysine	4.48 (4.17 - 4.82)	4.45 (4.13 - 4.66)	(4.27 - 4.83)

Table H-6 (Continued). Statistical Summary[1] of Amino Acid (% Total Protein) Levels in Cotton Fractions for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Fraction	Component	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Amino Acid (% Total Protein)				
Cottonseed	Methionine	1.50 (1.41 - 1.55)	1.48 (1.41 - 1.55)	(1.34 - 1.58)
	Phenylalanine	4.84 (4.74 - 4.93)	4.77 (4.23 - 5.02)	(4.68 - 5.25)
	Proline	3.66 (3.54 - 3.81)	3.67 (3.32 - 3.84)	(3.58 - 4.08)
	Serine	4.41 (4.33 - 4.47)	4.38 (4.04 - 4.54)	(4.27 - 4.69)
	Threonine	2.94 (2.79 - 3.07)	2.90 (2.60 - 3.05)	(2.75 - 3.10)
	Tryptophan	0.99 (0.96 - 1.04)	1.01 (0.99 - 1.04)	(0.92 - 1.02)
	Tyrosine	2.53 (2.47 - 2.59)	2.45 (2.18 - 2.57)	(2.38 - 2.60)
	Valine	4.11 (3.99 - 4.21)	4.07 (3.65 - 4.27)	(3.97 - 4.45)

Table H-7. Statistical Summary[1] of Cottonseed Fraction Fatty Acid (% dwt) Levels for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Fraction	Component	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Fatty Acid (% dwt)				
Cottonseed	14:0 Myristic	0.14 (0.13 - 0.15)	0.14 (0.13 - 0.15)	(0.098 - 0.16)
	16:0 Palmitic	5.41 (4.99 - 5.82)	5.24 (5.01 - 5.50)	(4.32 - 5.38)
	16:1 Palmitoleic	0.12 (0.11 - 0.13)	0.12 (0.11 - 0.13)	(0.099 - 0.16)
	18:0 Stearic	0.64 (0.57 - 0.71)	0.61 (0.56 - 0.65)	(0.49 - 0.59)
	18:1 Oleic	4.65 (4.28 - 5.00)	4.49 (4.32 - 4.68)	(2.75 - 3.29)
	18:2 Linoleic	11.95 (11.82 - 12.02)	11.42 (11.02 - 11.83)	(10.83 - 12.96)
	18:3 Gamma Linolenic	0.032 (0.028 - 0.036)	0.021 (0.011 - 0.028)	(0.019 - 0.033)
	18:3 Linolenic	0.039 (0.034 - 0.043)	0.037 (0.032 - 0.042)	(0.030 - 0.041)
	20:0 Arachidic	0.058 (0.053 - 0.060)	0.056 (0.053 - 0.058)	(0.043 - 0.057)
	22:0 Behenic	0.023 (0.019 - 0.028)	0.019 (0.018 - 0.021)	(0.017 - 0.027)
	Dihydrosterculic	0.043 (0.042 - 0.045)	0.040 (0.037 - 0.041)	(0.027 - 0.042)
	Malvalic	0.11 (0.093 - 0.12)	0.098 (0.095 - 0.10)	(0.077 - 0.11)
	Sterculic	0.084 (0.076 - 0.093)	0.077 (0.072 - 0.081)	(0.054 - 0.084)

[1]Data were averaged across duplicates for each sample prior to statistical analysis and summary.

Table H-8. Statistical Summary[1] of Cottonseed Fraction Fatty Acid (% Total Fat) Levels for MON 88913, MON 88913(-) and Commercial Conventional Varieties

Fraction	Component	MON 88913 Mean (Range)	MON 88913(-) Mean (Range)	Commercial (Range)
Fatty Acid (% Total Fat)				
Cottonseed	14:0 Myristic	0.57 (0.54 - 0.60)	0.59 (0.54 - 0.63)	(0.48 - 0.70)
	16:0 Palmitic	22.01 (21.21 - 22.92)	22.05 (21.07 - 22.99)	(21.08 - 24.23)
	16:1 Palmitoleic	0.49 (0.47 - 0.52)	0.50 (0.47 - 0.53)	(0.45 - 0.72)
	18:0 Stearic	2.59 (2.42 - 2.78)	2.55 (2.37 - 2.72)	(2.28 - 2.51)
	18:1 Oleic	18.90 (18.20 - 19.68)	18.91 (18.16 - 19.57)	(12.58 - 14.34)
	18:2 Linoleic	48.71 (47.25 - 50.25)	48.06 (46.03 - 50.12)	(50.85 - 55.50)
	18:3 Gamma Linolenic	0.13 (0.12 - 0.14)	0.089 (0.045 - 0.12)	(0.084 - 0.15)
	18:3 Linolenic	0.16 (0.13 - 0.18)	0.16 (0.13 - 0.18)	(0.14 - 0.19)
	20:0 Arachidic	0.23 (0.23 - 0.24)	0.24 (0.22 - 0.24)	(0.21 - 0.24)
	22:0 Behenic	0.093 (0.076 - 0.11)	0.081 (0.074 - 0.087)	(0.084 - 0.12)
	Dihydrosterculic	0.18 (0.17 - 0.18)	0.17 (0.16 - 0.17)	(0.13 - 0.18)
	Malvalic	0.43 (0.37 - 0.49)	0.41 (0.40 - 0.42)	(0.38 - 0.52)
	Sterculic	0.34 (0.30 - 0.39)	0.32 (0.31 - 0.34)	(0.26 - 0.36)

[1]Data were averaged across duplicates for each sample prior to statistical analysis and summary.