MONSANTO

Food and Feed Safety and Nutritional Assessment of Roundup RReady2Yield™ Soybean MON 89788 (OECD Unique Identifier MON-89788-1)

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

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PART I: SUMMARY

Table of Contents

List of Figures

List of Tables

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 that: (i) RReady2Yield soybean, MON 89788, is as safe as conventional varieties of soybean; and (ii) the intended uses of the food and feed derived from RR2Yield soybean MON 89788 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 RReady2Yield soybean MON 89788.

Signature: [redacted]

Date

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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

~	Approximately
AA	Amino acid
AACC	American Association for Clinical Chemistry
ADIE	Acute dietary intake estimate
aadA	Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme from the transposon Tn7
ADF	Acid detergent fiber
AD5	Allergen, gliadin, and glutenin protein sequence database version 5, compiled by Monsanto Company
ALLPEPTIDES	Protein sequence database comprised of GenPept, PIR and Swiss Prot, as curated by Monsanto Company
AOAC	Association of Analytical Communities
AOCS	American Oil Chemists' Society
APS	Analytical protein standard
ASA	American Soybean Association
B-	Border region
BLOSUM	BLOcks SUbstitution Matrix, used to score similarities between pairs of distantly related protein or nucleotide sequences
BSA	Bovine serum albumin
CAPS	3-[cyclohexylamino]-1-propanesulfonic acid
CI	Confidence interval
CFIA	Canadian Food Inspection Agency
CFR	Code of Federal Regulations
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-GMGOX20
CS-*rop*	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli*
CSFII	Continuing Survey of Food Intakes by Individuals
CTAB	Cetyltrimethylammonium bromide
CTP2	Chloroplast transit peptide, isolated from *Arabidopsis thaliana* L. EPSPS
CV	Coefficient of variation
CVol	Column volume
dCTP	Deoxycytidine triphosphate
DATASET	Command used to create a GCG data library from a set of sequences in GCG format
DEEM-FCID	Dietary Exposure Evaluation Model is a dietary exposure analysis system for performing chronic and acute exposure assessments
dNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol

DWCF	Dry weight conversion factor
DW	Dry weight
ECL	Enhanced chemiluminescence
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
E-score	Expectation score
EMBL	A public genetic database maintained by the European Molecular Biology Laboratory at the European Bioinformatics Institute, Hinxton, England
Entrez	The main database searching system of the National Center for Biotechnology Information at the National Institutes of Health, Bethesda, MD
EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase
FA	Fatty acid
FAO/WHO	Food and Agriculture Organization and World Health Organization of the United Nation
FASTA	Algorithm used to find local high scoring alignments between a pair of protein or nucleotide sequences
FDA	United States Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FMV	Figwort mosaic virus
FW	Fresh weight
GCG	Accelrys Genetics Computer Group is a collection of sequence analysis tools, formally known as the Wisconsin Package
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
HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase
IgG	Immunoglobulin G
ILDIS	International Legume Database and Information Service
ILSI-CCD	International Life Sciences Institute Crop Composition Database
IPM	Integrated pest management
I-*Tsf1*	Intron from the *Arabidopsis thaliana Tsf1* gene encoding elongation factor EF-1 alpha
IUIS	International Union of Immunological Societies
IUPAC-IUB	International Union of Pure and Applied Chemistry - International Union of Biochemistry
kb	Kilo base pair
kDa	Kilo dalton
LOQ	Limit of quantitation
LOD	Limit of detection

L-*Tsf1*	Leader (exon 1) from the *Arabidopsis thaliana tsf1* gene encoding elongation factor EF-1 alpha
MAFF	Ministry of Agriculture, Forestry and Fisheries of Japan
MALDI-TOF MS	Matrix assisted laser desorption ionization time of flight mass spectrometry
MES	2-[N-Morpholino]ethanesulfonic acid
MHLW	Ministry of Health, Labor and Welfare of Japan
MOA	Ministry of Agriculture of China
MOE	Margin of exposure
MRL	Maximum residue level
MS	Mass spectrometry
MW	Molecular weight
NDF	Neutral detergent fiber
NRL3D	National Research Laboratory's three-dimensional protein database founded at Brookhaven National Laboratory and maintained by the RCSB
NFDM	Non-fat dried milk
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	Over-season leaf - leaf material collected from different time points during the growing season
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline containing 0.05% (v/v) Tween-20
PCR	Polymerase chain reaction
PDB	Protein Data Bank
PEP	Phosphoenolpyruvate
P-*FMV/Tsf1*	Chimeric promoter containing the *Arabidopsis thaliana Tsf1* gene promoter, encoding elongation factor EF-1 alpha, and enhancer sequences from the Figwort Mosaic virus 35S promoter
PIR	Protein Information Resource
PMSF	Phenylmethylsulfonyl fluoride
PTH	Phenylthiohydantoin
PubMed	A MEDLINE journal citation database maintained by the National Center for Biotechnology Information at the National Institutes of Health, Bethesda, MD
PVDF	Polyvinylidene difluoride
PVPP	Polyvinylpolypyrrolidone
RbcS2	Ribulose-1, 5-bisphosphate carboxylase small subunit

RCSB	Research Collaborator for Structural Bioinformatics
SAM	S-adenosyl methionine
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SE	Standard error
SeqLab	The graphical X Windows-based interface for the GCG Wisconsin Package
SGF	Simulated gastric fluid
sp	Species
STRINGSEARCH	Algorithm used to identify sequence entries by searching for character patterns, such as "toxin", in the annotation section of database flatfiles
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
T-DNA	Transfer(ed) DNA
TE	Tris-EDTA buffer
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
TIU	Trypsin Inhibitor Unit
TOXIN5	Toxin protein sequence database
Tris	Tris(hydroxymethyl)aminomethane
TS-*CTP2*	Targeting sequence of chloroplast transit peptide, isolated from *Arabidopsis thaliana* L. EPSPS
TSSP	Tissue-specific site pool
U	Units
UNIX	A computer operating system originally termed "UNiplexed Information and Computing Service"
USB	United Soybean Board
USDA-APHIS	United States Department of Agriculture – Animal and Plant Health Inspection Service
USDA-ARS	United States Department of Agriculture – Agricultural Research Service
USDA-GRIN	United States Department of Agriculture – Germplasm Resources Information Network
USDA-NASS	United States Department of Agriculture – National Agricultural Statistics Service
v/v	Volume per volume
w/v	Weight per volume

Note: 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 RReady2Yield™ Soybean MON 89788

Roundup Ready® soybean 40-3-2 (herein referred to as Roundup Ready soybean) was the first soybean product containing a biotechnology trait commercialized in the U.S. Roundup Ready soybean was produced by incorporation of the *cp4 epsps* coding sequence derived from the common soil bacterium *Agrobacterium sp*. strain CP4. The *cp4 epsps* coding sequence directs the production of the 5-enolpyruvyl shikimate-3-phosphate synthase (termed CP4 EPSPS) that is less sensitive to inhibition by glyphosate compared to plant endogenous EPSPS. The CP4 EPSPS renders Roundup Ready soybean tolerant to glyphosate, which is the active ingredient in Roundup® agricultural herbicides. The utilization of Roundup agricultural herbicides plus Roundup Ready soybean, collectively referred to as the Roundup Ready soybean system, has provided significant convenience in weed control, encouraged the use of conservation-tillage, and provided positive economic impact to the farmers. In 2005, Roundup Ready soybean was planted on approximately 89% of the U.S. (USDA-NASS, 2005) and 60% of the global soybean areas (James, 2005), which is the most cultivated biotechnology product to date.

Developments in biotechnology and molecular-assisted breeding have enabled Monsanto to develop a second-generation glyphosate-tolerant soybean product, Roundup RReady2Yield or MON 89788. MON 89788 will continue to provide growers flexibility, simplicity, and cost effective weed control options; in addition, MON 89788 and varieties containing the trait have the potential to enhance yield and thereby further benefit farmers and the soybean industry. MON 89788 was developed by introduction of the *cp4 epsps* gene cassette containing a promoter that has been used in other crops such as Roundup Ready Flex cotton (Fincher et al., 2003; FDA, 2005). In addition, the transformation was based on a new technique of *Agrobacterium*-mediated gene delivery to soybean meristem, where cells were induced directly to form shoots and give rise to transgenic plants (Martinell et al., 2002). This new technique allowed direct transformation of the gene cassette into elite soybean germplasm such as the Asgrow soybean variety A3244 (Paschal, 1997), which is known for its superior agronomic characteristics and high yielding property (Tylka and Marett, 1999). Using elite germplasm as the base genetics, the superior agronomic characteristic of A3244 can be introgressed to other soybean varieties through crosses with MON 89788 containing the *cp4 epsps* cassette. In general, MON 89788 has been found to have a 4 to 7% yield advantage compared to Roundup Ready soybeans in the same genetic background while maintaining the weed control and crop safety benefits of the Roundup Ready soybean system. As a result, MON 89788 will be an excellent agronomic base trait for future breeding improvements and multi-trait products.

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The data and information presented in this summary demonstrate that MON 89788 and the foods and feeds derived from it are as safe and nutritious as conventional (commercially available non-transgenic) soybean and the comparable foods and feeds derived from them. This conclusion is based on several lines of evidence. The first is the detailed molecular characterization of the inserted DNA. Results confirm the insertion of an intact *cp4 epsps* cassette integrated at a single locus within the genome. The second is a detailed biochemical characterization of the CP4 EPSPS protein produced in MON 89788. Data demonstrate that the CP4 EPSPS produced in MON 89788 is equivalent to the CP4 EPSPS proteins consumed in foods and feeds derived from other Roundup Ready crops such as Roundup Ready soybean that have an experience of safe use. The third is an updated assessment of the toxicity and allergenicity potential of the CP4 EPSPS protein produced in MON 89788 based on extensive information collected and studies performed on the protein. Results confirm the previous assessment and the safety of the CP4 EPSPS due to the lack of allergenic potential and the lack of acute toxicity when ingested. Finally, the compositional and nutritional assessment of MON 89788 grain and forage confirm that MON 89788 is compositionally equivalent to and as safe as conventional soybeans.

Molecular analyses indicate that MON 89788 contains a single intact *cp4 epsps* expression cassette integrated at a single locus within the soybean genome. DNA sequencing analyses of the MON 89788 insert confirm the expected coding region of the *cp4 epsps* gene cassette, which encodes a CP4 EPSPS protein identical to that in Roundup Ready soybean. No backbone sequences from the transformation plasmid were detected. In addition, no partial genetic elements, linked or unlinked to the inserted expression cassette were detected. Furthermore, the DNA insert and the glyphosate-tolerant trait in MON 89788 were shown to be stably inherited across multiple generations.

The protein characterization studies show that grain derived from MON 89788 contains the CP4 EPSPS protein of the expected molecular weight, amino acid sequence, immunological activity, and functional activity. The CP4 EPSPS protein in MON 89788 has the same functional and enzymatic activity as the CP4 EPSPS in other Roundup Ready crops, and is structurally homologous to EPSPSs naturally present in food crops and in microbial food sources such as baker's yeast. The amino acid sequence of the CP4 EPSPS protein in MON 89788 is identical to that in Roundup Ready soybean, Roundup Ready canola, and Roundup Ready Flex cotton, all of which have completed the FDA consultation process and have been commercialized. In addition, the average CP4 EPSPS protein level in MON 89788 grain was lower than that in Roundup Ready soybean. Taken together, these data and information demonstrate a history of safe use with respect to the family of EPSPS proteins, which naturally occur in crops and microbial-based foods and 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 also support the safety of the CP4 EPSPS protein and demonstrate that this protein is unlikely to be an allergen or toxin. This is based on the assessment of the donor organism, *Agrobacterium sp.* strain CP4, which is not a known human or animal pathogen and there are no reports of allergies derived from the organism. Examination of the CP4 EPSPS amino acid sequence against an updated bioinformatics database showed a lack of significant structural similarity between the CP4 EPSPS protein and known allergens or pharmacologically active proteins. In addition, studies using the purified CP4 EPSPS protein have demonstrated that the protein was digested rapidly in simulated gastric fluid, and ingestion of the protein did not cause acute toxicity in mice. These data are consistent with the conclusion of safety for CP4 EPSPS protein. This conclusion is further supported by the lack of any documented reports of adverse effects from the consumption of other Roundup Ready crops since 1996, all of which contain the same CP4 EPSPS protein as in MON 89788.

Compositional assessment of the grain and forage demonstrated that MON 89788 is nutritionally and biologically equivalence to, and as safe and nutritious as its conventional counterpart, A3244. The composition analyses compared the levels of 63 components between MON 89788 and A3244, each of which were grown at five field sites in the U.S. during 2005. In addition, the same components were analyzed in 12 conventional soybean varieties to establish the 99% tolerance interval for each of the analytes. Results of the compositional analyses indicate that there were no statistically significant differences ($p \leq 0.05$) in 91% of the comparisons made between MON 89788 and A3244. Of the few analytes where statistical differences occurred, differences were not reproducible across sites and the trends of the differences were not consistent. Furthermore, the mean levels of all analytes from MON 89788 grain were within the 99% tolerance intervals for conventional soybeans and within the ranges of the International Life Science Institute Crop Composition Database and published literature. Therefore, the few statistically significant differences between MON 89788 and A3244 were not considered to be biologically relevant. These data support the conclusion that MON 89788 soybean grain and forage are compositionally and nutritionally equivalent to conventional varieties.

Based on the information provided in this summary, we have concluded that MON 89788 and the foods and feeds derived from it are as safe and nutritious as conventional soybeans and the comparable foods and feeds derived from them. This conclusion is based on several lines of evidence including:

1. The similarity of MON 89788 to the previously reviewed and extensively consumed Roundup Ready soybean, including the presence of an identical CP4 EPSPS protein that is the basis for the glyphosate-tolerant phenotype in both products;
2. The detailed molecular characterization of the inserted DNA, which confirmed the presence of an intact *cp4 epsps* cassette stably integrated at a single locus of the genome;
3. The biochemical characterization of the CP4 EPSPS protein produced in MON 89788 confirmed that the CP4 EPSPS protein is structurally and functionally

equivalent to the EPSPS family of proteins present in all plants, fungi and bacteria that have a history of safe use;

4. Compositional and nutritional assessments, which demonstrate that the MON 89788 is compositionally equivalent to and as safe as conventional soybeans.

It is therefore concluded that sales and consumption of MON 89788 soybean and the food and feed derived from it will be fully consistent with FDA's Policy (FDA, 1992) and 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 RReady2Yield soybean MON 89788 is:

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St. Louis, MO 63167

Communications with regard to this submission should be directed to [redacted] Regulatory Affairs Manager, at the Monsanto address, or by telephone at [redacted] or by FAX at [redacted].

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

The subject of this summary is Roundup RReady2Yield soybean (MON 89788). The soybean variety used as the recipient for the DNA insert to generate MON 89788 was A3244, an elite commercial variety developed by Asgrow. Elite varieties were developed by breeding and selected for superior agronomic performance. A3244 is a maturity group III soybean variety, which was superior in yield amongst more than 50 varieties of soybean of the same maturity group (Tylka and Marett, 1999).

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

Roundup RReady2Yield soybean has been designated as MON 89788. Therefore, in subsequent discussions in this submission, Roundup RReady2Yield soybean will be referred to as "MON 89788". In accordance with OECD's "Guidance for the Designation of a Unique Identifier for Transgenic Plants", MON 89788 has been assigned the unique identifier of MON-89788-1.

Section 4. Identity and Sources of the Genetic Material Introduced into Soybean to Produce MON 89788

MON 89788 was generated through *Agrobacterium*-mediated transformation utilizing plasmid vector PV-GMGOX20 (Figure IV-2). The integrated T-DNA is comprised of a *cp4 epsps* gene expression cassette, which contains the following genetic elements: P-*FMV*/*Tsf1* chimeric promoter derived from enhancer sequences of 35S promoter of the Figwort Mosaic virus (Richins et al., 1987) and the promoter from the *Tsf1* gene of *Arabidopsis thaliana* (Axelos et al., 1989); the leader and intron sequences from the *Arabidopsis thaliana Tsf1* gene; the EPSPS chloroplast transit peptide coding sequence

from the *ShkG* gene of *Arabidopsis thaliana* (Klee et al., 1987); the codon optimized coding sequence of *cp4 epsps* from *Agrobacterium sp.* strain CP4 (Padgette et al., 1996; Barry et al., 1997); and the polyadenylation sequence derived from the 3' nontranslated region of the pea (*Pisum sativum*) ribulose-1, 5-bisphosphate carboxylase small subunit (*RbcS2*) *E9* gene. The genetic elements present in MON 89788 are summarized in Table IV-2.

Section 5. The Intended Technical Effect of MON 89788

MON 89788 soybean contains CP4 EPSPS protein (5-enolpyruvyl shikimate-3-phosphate synthase protein from *Agrobacterium sp.* strain CP4) that provides tolerance to the action of glyphosate, which is the active ingredient in 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, aromatic amino acids requirement for growth and development are met by the continued action of the CP4 EPSPS enzyme in the presence of glyphosate. MON 89788 produces the CP4 EPSPS protein, and is therefore tolerant to glyphosate agricultural herbicides applied over the top of soybean during the growing season.

The mechanism of herbicide tolerance using CP4 EPSPS has several advantages over other modes of action to confer glyphosate tolerance in plants. The family of EPSPS proteins has been shown to have a history of safe use and consumption. In addition, glyphosate and its plant-produced metabolites are known to have favorable safety profiles (Codex Alimentarius, 2005). In addition, application of glyphosate on soybean under the current agronomic practices will result in herbicide and plant-produced metabolite residues well within the maximum residue levels (MRLs) for soybeans established by EPA and Codex (EPA, 1996; Codex Alimentarius, 2005).

Section 6. The Applications and Uses of MON 89788

The introduction of MON 89788 is expected to provide enhanced soybean yield potential, and continue to offer growers superb weed control options in addition to economic and environmental benefits currently provided by utilization of the Roundup Ready soybean system. These benefits include:

1. *Effective weed control:* The most critical period of weed control in soybean is the first month after planting, as early-season canopy closure gives soybean a competitive advantage over late-emerging weeds and increases herbicide effectiveness (Mickelson and Renner 1997; Wax et al., 1977; Yelverton and Coble 1991). The Roundup Ready soybean system provides growers improved efficacy in weed control compared to

herbicide programs used in conventional soybeans (non-transgenic commercial soybean varieties), as specific preemergent herbicides that are used for prevention are replaced by a post-emergent herbicide that can be used on an as-needed basis (Roberts et al., 1999). Although soybean growers have many post-emergence herbicide options, none has the broad spectrum of weed control of glyphosate. Further, many conventional herbicides cause injury to the crop, while glyphosate may be applied over Roundup Ready varieties at any stage of growth without causing damage (Carpenter and Gianessi, 2001). Crop injury may not reduce yield, but it can delay canopy closure and increase weed competition with the crop.

2. *Convenience and simplicity:* The Roundup Ready soybean system increases simplicity and flexibility of a weed-control program that relies on glyphosate to control a broad spectrum of weeds without crop injury or crop rotation restrictions, which was a major driver for the adoption of Roundup Ready soybean (Carpenter and Gianessi, 1999). Additionally, the Roundup Ready soybean system has been recognized as affording outstanding flexibility of production system because it presents no herbicide carryover problems (Marra et al., 2002). The introduction of Roundup Ready soybeans in the U.S. has eliminated 19 million herbicide applications per year – a decrease of 12%, even though the total soybean acres increased by 18% from 1996-1999 (Carpenter, 2001). This decrease in herbicide applications means that growers make fewer trips over their fields to apply herbicides, which translates into ease of management and reduced fuel use.

3. *Increased adoption of reduced tillage practices:* Conservation tillage improves water quality and creates habitat for wildlife (CTIC, 2000; Fawcett and Towry, 2002), and control of existing weeds has been a major barrier to the success of conservation tillage systems (Nowak, 1983; Wilson and Worsham, 1988). Success in adoption of conservation tillage has been enhanced with the introduction of Roundup Ready soybean and use of glyphosate in the cropping systems (Marra et al., 2004; Duffy, 2001; Swanton et al., 2000; Krausz et al., 1996). In an survey by the American Soybean Association (ASA), it was found that 48% of the growers have increased no-till soybean acres from 1996 to 2001 due to adoption of Roundup Ready soybeans, and 53% of the growers were making fewer tillage passes in soybean fields. Reduced tillage practices in Roundup Ready soybeans was estimated to save 247 million tons of irreplaceable topsoil and reduce fuel use by 234 million gallons in 2000 (ASA, 2001).

4. *Compatibility with Integrated Pest Management (IPM) and soil conservation techniques:* Roundup Ready soybean is highly compatible with integrated pest management and soil conservation techniques (Keeling et al., 1998; ASA, 2001; Fawcett and Towry, 2002), resulting in a number of important environmental benefits including reduced soil erosion and improved water quality as discussed above, improved soil structure with higher organic matter (Kay, 1995; CTIC, 2000), improved carbon sequestration (Reicosky, 1995; Reicosky and Lindstrom, 1995) and reduced CO_2 emissions (Kern and Johnson, 1993; CTIC, 2000).

5. *Increased income and enhanced value for the growers:* It has been estimated that U.S. soybean growers saved a net of $[redacted] in weed control costs in 1999 compared to 1995, the year before Roundup Ready soybean was introduced (Carpenter, 2001). In addition, there are nonpecuniary values that growers perceive in adopting Roundup Ready soybean according to a survey conducted of 610 growers in the Midwest and the South in 2003, where comparative assessments of all measurable costs and revenue were made between farms that grew conventional soybeans and those that produced Roundup Ready soybeans. This survey considered both financial and nonfinancial aspects of farm management practices, and assigned value judgments (in dollars) to assess costs and benefits of adopting Roundup Ready soybean. Results indicated that farmers perceived up to $[redacted] per acre benefit by adopting Roundup Ready soybean, and the most profound benefits came from reduced herbicide costs, overall convenience, and time saved from reduced tillage (Marra et al., 2004). Also, as noted above, use of MON 89788 is expected to increase soybean yield over use of Roundup Ready soybean. Therefore, adoption of MON 89788 will provide more income per acre and bring added values to the growers.

6. *History of safe use of glyphosate:* The Roundup Ready soybean system utilizes one main ingredient, glyphosate, to control a broad spectrum of weeds. Aside from being one of the most effective herbicides, glyphosate has been shown to have favorable environmental characteristics compared to other herbicides (Nelson and Bullock, 2003). In addition, glyphosate has been shown to have favorable safety profile as concluded by the U.S. EPA (1993) where it indicates that use of Roundup agricultural herbicides does not pose unreasonable risks to humans, birds, mammals, aquatic organisms, bees and invertebrates.

Section 7. Applications for which MON 89788 is not Suitable

Monsanto Company is aware of no food or feed uses of conventional soybean that are not applicable to MON 89788.

PART III: STATUS OF SUBMISSIONS TO OTHER REGULATORY AGENCIES

Section 1. Status of Submission to USDA-APHIS

Monsanto will request a Determination of Nonregulated Status for MON 89788 from USDA-APHIS in mid-2006, and the request will include all progenies derived from MON 89788. Under regulations administered by USDA-APHIS (7 CFR 340), MON 89788 is currently considered a "regulated article". Monsanto will continue to conduct all field tests for MON 89788 in strict compliance with USDA field regulations until a Determination of Nonregulated Status is obtained for MON 89788.

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 proposed label for the use of Roundup WeatherMAX® herbicide (EPA Reg. No. 524-537) on MON 89788 will be submitted in 2007. The resulting glyphosate and plant metabolite residue levels using the proposed label will be consistent with national and international MRLs.

Section 3. Status of Submissions to Other Governmental Agencies

Regulatory submissions will be made to countries that import significant soybean grain or food and feed products derived from U.S. soybeans and have functional regulatory review processes in place. These will include submissions to a number of additional governmental regulatory agencies including, but not limited to, Ministry of Agriculture (MOA) of China, Ministry of Health, Labor and Welfare (MHLW) and Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan, Canadian Food Inspection Agency (CFIA) and Health Canada, and the European Commission of the European Union. As appropriate, notifications will be made to countries that import significant quantities of U.S. soybeans and soybean products and do not have a formal regulatory review process for biotechnology-derived crops.

PART IV: DEVELOPMENT OF MON 89788

Section 1. History and Biology of Soybean

This section summarizes the biology of soybean based on the consensus document for *Glycine max* (L.) Merr. prepared by the Organization for Economic Co-operation and Development (OECD, 2000; OECD, 2001), a summary prepared by USDA-APHIS (USDA-APHIS, 2006), a biology document published by CFIA-PBO (CFIA, 1996), information provided in the USDA petition for Roundup Ready soybean (93-258-01P), as well as recent literature.

Soybean is grown as a commercial crop in over 35 countries. The major producers of soybeans are the U.S., Brazil, Argentina, China, and India, which accounted for approximately 90% of the global soybean production in 2004 (Soya and Oilseed Bluebook, 2005). The soybean produced in China and India are primarily for domestic use, while a significant portion of that produced in U.S., Brazil, and Argentina is traded globally in the form of soybean, soybean meal or soybean oil. Globally, the U.S. is the largest soybean export country, while Argentina led the soybean meal and soybean oil export markets in 2004 (Soy Stats, 2005).

There were 85.5 million metric tons of soybeans produced in the U.S. in 2004, which contributed to greater than $[redacted] of total crop value (Soya and Oilseeds Bluebook, 2005). Approximately half the total soybean supply in the U.S. was crushed to produce soybean meal and oil, and the majority was used domestically, primarily supplying the feed industry for livestock use or the food industry for edible vegetable oil and soy protein isolates. Another one-third of the U.S. soybean supply was exported as grain to other geographies, with China, Japan, Mexico and EU being the top soybean import geographies (Soya and Oilseed Bluebook, 2005). The remainder of the soybean produced was used as seed, feed or stocks.

Soybeans are used in various food products, including tofu, soy sauce, soymilk, energy bars, and meat products. A major food use for soybean in North America is purified oil, for use in margarines, shortenings, and cooking and salad oils. Soybean oil generally has a smaller contribution to soybean's overall value compared to soybean meal because it constitutes just 18 to 19% of the soybean's weight. Nonetheless, soybean oil accounts for approximately two-thirds of all the vegetable oils and animal fats consumed in the U.S., and is still the largest source of vegetable oil worldwide (USDA-NASS, 2006).

Soybean meal is used as a supplement in feed rations for livestock. Soybean meal is the most valuable component obtained from processing the soybean, accounting for roughly 50-75% of its overall value. By far, soybean meal is the world's most important protein feed, accounting for nearly 65% of world supplies. Industrial uses of soybeans range from the production of yeasts via fermentation to the manufacture of soaps, inks, paints and disinfectants. Industrial uses of soybean have been summarized by Cahoon (2003), and United Soybean Board (USB, 2003).

U.S. soybean plantings reached 75.1 million acres in 2004, a 30% increase since 1990. Increased planting flexibility, rising yield improvements from narrow-row seeding practices, a higher rate of corn-soybean rotations, and low production costs favored expansion of soybean acreage in the 1990s. More than 80% of U.S. soybean acreage is concentrated in the upper Midwest, although significant amounts are still planted in historically important areas of the Delta and Southeast. Acreage tends to be concentrated where soybean yields are highest, and the top soybean producing states include Illinois, Iowa, Indiana, Minnesota, Missouri, Nebraska, and Ohio, which accounted for over 65% of U.S. soybean production in 2004 (USDA-NASS, 2005 and 2006).

Convenience in weed management also has encouraged expansion of soybean acreage since the introduction of Roundup Ready soybean in 1996. Because glyphosate agricultural herbicides are highly effective against the majority of annual and perennial grasses and broadleaf weeds, growers planting Roundup Ready soybeans are able to reduce the number of herbicides used to control economically destructive weeds that grow in their fields. Farmers realize savings in weed control costs and enhancement in yield by reduction of crop-weed competition. The benefit of the Roundup Ready system (combining Roundup Ready soybean with Roundup herbicide use) was evidenced from the rapid adoption of Roundup Ready soybean. The U.S. soybean acreage planted with Roundup Ready soybean grew from less than 5% in 1996 to 87% in 2005 (USDA-NASS, 2006). In 2004, Roundup Ready soybean was planted on 56% of the 86 million hectares of soybean grown globally (James, 2004).

1.1. Scientific Name and Taxonomic Classification of Soybean

Cultivated soybean, *Glycine max* (L.) Merr., is a diploidized tetraploid (2n=40), which belongs to the family Leguminosae, the subfamily Papilionoideae, the tribe Phaseoleae, the genus *Glycine* Willd. and the subgenus *Soja* (Moench) F.J. Herm.

Family: Leguminosae
Subfamily: Papilionoideae
Tribe: Phaseoleae
Genus: *Glycine*
Subgenus: *Soja* (Moench) F.J. Herm.
Species: *max*

The genus *Glycine* Willd. is of Asian and Australian origin and is divided into two subgenera, *Glycine* and *Soja* (Moench) F.J. Herm. The subgenus *Glycine* consists of 22 wild perennial species, which are indigenous to Australia, west, central and south Pacific Islands, China, Russia, Japan, Indonesia, Korea, Papua New Guinea, Philippines and Taiwan (Hymowitz, 2004). The subgenus *Soja* includes the cultivated soybean, *G. max* (L.) Merr. and its wild annual relatives from Asia, *G. soja* Sieb. and Zucc.

Glycine soja grows wild in China, Japan, Korea, Russia, and Taiwan, which is commonly found in fields, hedgerows, roadsides, and riverbanks. The plant is an annual, slender in

build with narrow trifoliolate leaves. The purple or very rare white flowers are inserted on short, slender racemes. The pods are short and tawny with hirsute pubescence, which produce oval-oblong seeds (Hermann, 1962).

Glycine max (L.) Merr., the cultivated soybean, is an annual that generally exhibits an erect, sparsely branched, bush-type growth habit with trifoliolate leaves. The leaflets are broadly ovate, and the purple, pink, or white flowers are borne on short axillary racemes or reduced peduncles. The pods are either straight or slightly curved, and one to three ovoid to sub-spherical seeds are produced per pod.

A third and unofficial species named *G. gracilis* is also described within the context of *Soja* subgenus in addition to *G. soja* and *G. max*. The *G. gracilis* is known only from northeast China, is intermediate in morphology between *G. max* and *G. soja*, and is sometimes considered a variant of *G. max*. The three species in the *Soja* subgenus can cross pollinate, and the hybrid seed can germinate normally and subsequently produce fertile pollen and seed (Singh and Hymowitz, 1989). The taxonomic position of *G. gracilis* has been an area of debate, and neither ILDIS (International Legume Database and Information Service) nor USDA-GRIN (USDA Germplasm Resources Information Network) recognizes *G. gracilis* as a distinct species. The wild and weedy relatives (*G. soja* and *G. gracilis*) do not occur in the U.S., and are therefore not likely to contribute to the potential for outcrossing (USDA-APHIS, 2006).

1.2. History of Soybean Development

Domestication of soybean is thought to have taken place during the Shang dynasty (approximately 1500 to 1027 B.C.) or earlier (Hymowitz, 1970). However, historical and geographical evidence could only be traced back to the Zhou dynasty (1027 to 221 B.C.) where the soybean was utilized as a domesticated crop in the northeastern part of China. By the first century A.D., the soybean probably reached central and southern China as well as peninsular Korea. The movement of soybean germplasms was probably associated with the development and consolidation of territories and the degeneration of Chinese dynasties (Ho, 1969; Hymowitz, 1970).

From the first century A.D. to approximately the 15th to 16th centuries, soybeans were introduced into several countries, with land races eventually developing in Japan, Indonesia, Philippines, Vietnam, Thailand, Malaysia, Myanmar, Nepal, and northern India. The movement of the soybean throughout this period was due to the establishment of sea and land trade routes, the migrations of certain tribes from China, and the rapid acceptance of grains as a stable food by other cultures (Hymowitz et al., 1990; Hymowitz and Newell, 1981).

Starting in the late 16th century and throughout the 17th century, soybean was used by the Europeans, and in the 17th century, soy sauce was a common item of the trade from the East to the West.

Soybean was introduced into North America in the 18th century. Samuel Bowen, a former seaman employed by the East India Company, brought soybean to Georgia from

China, and Benjamin Franklin also brought soybean to North America in 1770 (Hymowitz and Harlan, 1983). In 1851, the soybean was introduced in Illinois and subsequently throughout the Corn Belt. In 1853, soybean seeds were deposited into the New York State Agricultural Society, the Massachusetts Horticultural Society, and the Commissioner of Patents. The two societies and the Commissioner of Patents sent soybean seeds to dozens of farmers throughout the U.S., and soybean has been cultivated ever since and subsequently has become a key source of nutrient for food and feed use in the U.S. (Hymowitz, 1987).

1.3. Characteristics of the Recipient Plant

The soybean variety used as the recipient for the DNA insertion to create MON 89788 was A3244, a non-transgenic conventional variety developed by Asgrow Seed Company. The A3244 is an elite maturity group III soybean variety, which was developed and selected based on its superior agronomic performance over other soybean lines (Tylka and Marett, 1999).

In developing the data to support this petition, MON 89788 and appropriate control materials (A3244 or *E. coli*-produced CP4 EPSPS) were used as the comparators. In addition, conventional soybean varieties were also used as reference materials to establish a range of natural variability. In general, the genetic background of MON 89788 was matched with that of the control, so the effect of the genetic insertion and the presence of CP4 EPSPS protein could be assessed in an unbiased manner. Since the MON 89788 was derived from the A3244 conventional variety, it was deemed appropriate to use A3244 as the control variety as its use would minimize the potential bias in subsequent assessments of equivalence. On the other hand, reference varieties were selected based on prevalence and performance of the soybean varieties at each trial location. As a general principle, varieties that were well adapted to the local environments and were commonly used by the local producers would be considered for use as reference soybean varieties.

Section 2. Characterization of the Vector Used in Transformation

MON 89788 was developed through *Agrobacterium*-mediated transformation of soybean meristem tissue using the double-border, binary vector PV-GMGOX20. The schematic of the development of MON 89788 is depicted in Figure IV-1, and the PV-GMGOX20 vector map and the probes used in the molecular characterization is shown in Figure IV-2. This vector is approximately 9.7 kb and contains a *cp4 epsps* gene expression cassette delineated by left and right border regions. The T-DNA that is incorporated into the soybean genome is approximately 4.3 kb, and the DNA backbone region that is not incorporated into the soybean genome is approximately 5.4 kb.

The T-DNA contains, from the right border region, a chimeric transcriptional promoter (P-*FMV/Tsf1*), a leader and an intron sequence derived from *Tsf1* gene (L-*Tsf1* and I-*Tsf1*), a chloroplast transit peptide sequence (TS-*CTP2*), the *cp4 epsps* coding sequence (CS-*cp4 epsps*), and a polyadenylation sequence from *RbcS2* gene (T-*E9*). The *cp4 epsps*

expression cassette used to generate MON 89788 is the same as one of the cassettes present in the current Roundup Ready Flex cotton product.

The backbone region outside of the T-DNA, which is not integrated into the soybean genome during transformation, contains two origins of replication for maintenance of plasmid in bacteria (OR-*oriV,* OR-*ori-PBR322*), as well as a bacterial selectable marker gene (*aadA*). A description of the genetic elements and their prefixes (e.g. P-, L-, I-, TS-, OR-, B-, CS-, and T-) in PV-GMGOX20 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 endogenous 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 relative to endogenous plant EPSPSs (Padgette et al., 1996). The amino acid of the mature CP4 EPSPS protein in MON 89788 is identical to that in Roundup Ready soybean. The deduced full-length amino acid sequence is shown in Figure IV-3.

In conventional plants, glyphosate binds to the endogenous plant EPSPS enzyme and blocks the biosynthesis of the 5-enolpyruvyl shikimate-3-phosphate, thereby depriving plants of essential amino acids that are necessary for growth and development (Steinrücken and Amrhein, 1980; Haslam, 1993). In Roundup Ready plants, the presence of CP4 EPSPS reconstitutes the shikimic acid pathway, and is able to continuously synthesize aromatic amino acids even in the presence of glyphosate (Padgette et al., 1996).

2.1.1. The *Arabidopsis thaliana* EPSPS Transit Peptide

The *cp4 epsps* coding sequence is preceded by a chloroplast transit peptide sequence, *CTP2*, derived from the *Arabidopsis thaliana epsps* gene (Klee et al., 1987). This transit peptide directs the transport of the CP4 EPSPS protein to the chloroplast, which is where the plant EPSPS resides and the site of aromatic amino acid biosynthesis (Klee et al., 1987; Kishore et al., 1988). Transit peptides are typically cleaved from the translated polypeptide following delivery to the plastid (Della-Cioppa et al., 1986). The *CTP2* present in PV-GMGOX20 is identical to the *CTP2* transit peptide sequence in Roundup Ready Flex cotton.

2.1.2. Regulatory Sequences

From the right border region of plasmid PV-GMGOX20, the *CTP2/cp4 epsps* coding sequence is under the regulatory control of the P-*FMV/Tsf1* transcriptional promoter. P-*FMV/Tsf1* is a chimeric promoter containing the *Arabidopsis thaliana Tsf1* gene promoter (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 *Pisum sativum*, containing the 3' nontranslated region of the pea ribulose-1,5-bisphosphate carboxylase, small subunit (*RbcS2*) E9 gene (Coruzzi et al., 1984) for transcriptional termination and polyadenylation of the *CTP2/cp4 epsps* mRNA.

2.2. T-DNA Borders

Plasmid PV-GMGOX20 contains right border and left border regions that delineate the T-DNA to be transferred into soybean and are necessary for the efficient transfer of the T-DNA into the soybean genome. These border regions (Figures IV-2 and Table IV-1) were derived from *Agrobacterium tumefaciens* plasmids (Depicker et al., 1982; Barker et al., 1983).

2.3. Genetic Elements outside of the T-DNA Borders

Four genetic elements exist outside of the T-DNA borders that are essential for the maintenance and selection of the vector PV-GMGOX20 in bacteria. They include: *OR-ori V,* origin of replication for maintenance of plasmid in *Agrobacterium* (Stalker et al., 1981); CS-*rop,* coding sequence for repressor of primer (ROP) protein for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989); *OR-ori-pBR322,* origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1978); and *aadA*, a bacterial promoter and coding sequence of an enzyme from transposon Tn7 that confers spectinomycin and streptomycin resistance for molecular cloning and selection purposes (Fling et al., 1985). As these elements are outside of the border regions, they are not expected to be transferred into the soybean genome. The absence of the backbone sequence in MON 89788 has been confirmed by Southern blot analyses, which are presented in the following section.

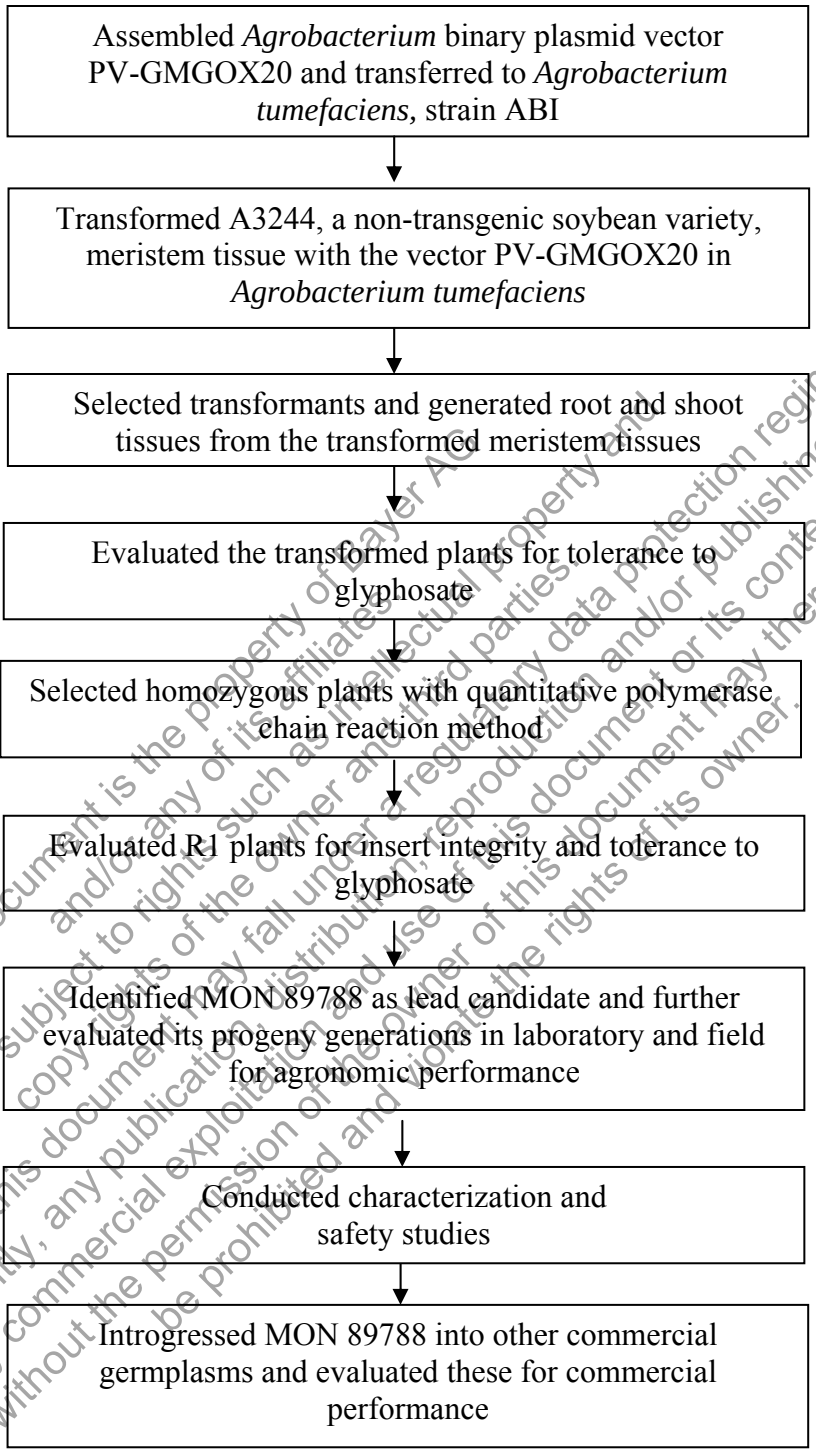
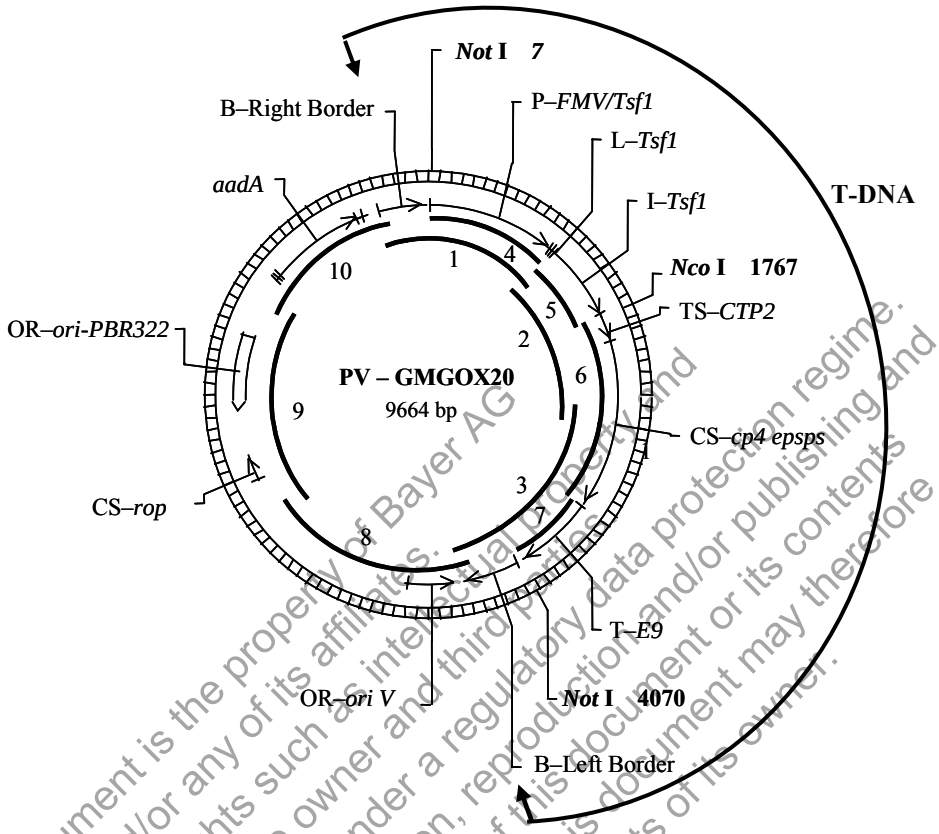


Figure IV-1. Schematic of the Development of MON 89788



Probe	DNA Probe	Start Position	End Position	Total Length (~kb)
1	T–DNA Probe 1	9271	1164	1.6
2	T–DNA Probe 2	1071	2916	1.8
3	T–DNA Probe 3	2784	4583	1.8
4	P–*FMV/Tsf1*/ L–*Tsf1*	28	1153	1.1
5	I–*Tsf1* Probe	1131	1764	0.6
6	TS–*CTP2*/CS–*cp4 epsps* Probe	1769	3364	1.6
7	T–*E9* Probe	3407	4060	0.7
8	Backbone Probe 1	4508	6178	1.7
9	Backbone Probe 2	6041	8187	2.1
10	Backbone Probe 3	8056	9322	1.3

Figure IV-2. Circular Map of Plasmid PV-GMGOX20

Plasmid PV-GMGOX20 containing the T-DNA was used in *Agrobacterium*-mediated transformation to generate MON 89788. Genetic elements and restriction sites for enzymes used in the Southern analyses (with positions relative to the plasmid vector) are shown on the exterior of the map. Probes used in the Southern analyses are detailed in the accompanying table.

Table IV-1. Summary of Genetic Elements in the Plasmid PV-GMGOX20

Genetic Element[1, 2]	Position in Plasmid	Function and Source (Reference)
	T-DNA	
Intervening Sequence	1-51	Sequences used in DNA cloning
P – *FMV/Tsf1*	52-1091	Chimeric promoter consisting of enhancer sequences from the 35S promoter of the Figwort Mosaic virus (Richins et al., 1987) and the promoter from the *Tsf1* gene of *Arabidopsis thaliana* encoding elongation factor EF-1 alpha (Axelos et al., 1989)
L – *Tsf1*	1092-1137	5' nontranslated leader (exon 1) from the *Tsf1* gene of *Arabidopsis thaliana* encoding elongation factor EF-1 alpha (Axelos et al., 1989)
I – *Tsf1*	1138-1759	Intron from the *Tsf1* gene of *Arabidopsis thaliana* encoding elongation factor EF-1 alpha (Axelos et al., 1989)
Intervening Sequence	1760-1768	Sequences used in DNA cloning
TS – *CTP2*	1769-1996	Sequences encoding the chloroplast transit peptide from the *ShkG* gene of *Arabidopsis thaliana* encoding EPSPS (Klee et al., 1987)
CS – *cp4 epsps*	1997-3364	Codon optimized coding sequence of the *aroA* (epsps) gene from the *Agrobacterium sp.* strain CP4 encoding the CP4 EPSPS protein (Padgette et al., 1996; Barry et al., 1997)
Intervening Sequence	3365-3406	Sequences used in DNA cloning
T – *E9*	3407-4049	3' nontranslated sequence from the ribulose-1, 5-bisphosphate carboxylase small subunit (*RbcS2*) *E9* gene of pea (*Pisum sativum*)(Coruzzi et al., 1984)
Intervening Sequence	4050-4092	Sequences used in DNA cloning
B – Left Border	4093-4534	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983)
	Vector Backbone	
Intervening Sequence	4535-4620	Sequences used in DNA cloning
OR – *ori V*	4621-5017	Origin of replication from the broad host range plasmid RK2 for maintenance of plasmid in *Agrobacterium* (Stalker et al., 1981)
Intervening Sequence	5018-6525	Sequences used in DNA cloning
CS – *rop*	6526-6717	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989)

Table IV-1 (Continued). Summary of Genetic Elements in the Plasmid PV-GMGOX20

Intervening Sequence	6718-7134	Sequences used in DNA cloning
OR – *ori-PBR322*	7135-7763	Origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1978)
Intervening Sequence	7764-8263	Sequences used in DNA cloning
aadA	8264-9152	Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme, 3' (9)-O-nucleotidyltransferase from the transposon Tn7 (Fling et al., 1985)
Intervening Sequence	9153-9288	Sequences used in DNA cloning
T-DNA		
B – Right Border	9289-9645	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Depicker et al., 1982)
Intervening Sequence	9646-9664	Sequences used in DNA cloning

[1] Intervening sequences are not regarded as genetic elements.

[2] P – Promoter; L– Leader; I – Intron; TS– Targeting Sequence; CS – Coding Sequence; T – 3' nontranslated transcriptional termination sequence and polyadenylation signal sequences; B – Border; OR – Oigin of Replication.

```
  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-3. Deduced Amino Acid Sequence of the Mature CP4 EPSPS Protein in MON 89788

The amino acid sequence of the mature plant-produced CP4 EPSPS protein in MON 89788 was deduced from the full-length *cp4 epsps* coding sequence present in PV-GMGOX20.

Section 3. Characterization of the Inserted *cp4 epsps* Cassette in MON 89788

This section details the molecular analyses that characterized the integrated DNA insert in MON 89788. The results confirmed the presence of each genetic element at the insertion site and not at any region outside of the insert, confirmed the lack of plasmid backbone elements, and confirmed the insert stability across generations. In addition, DNA sequencing analyses were performed, and results confirmed the expected nucleotide sequence of the insert in MON 89788 as well as the organization of the genetic elements. Furthermore, insert segregation analysis also confirmed that the expected and the observed segregation ratios were identical. This result is consistent with the finding of a single chromosomal insertion of the *cp4 epsps* gene cassette that segregates according to Mendel's laws of genetics.

3.1. Introduction

Genomic DNA from MON 89788 was digested with restriction enzymes and subjected to Southern blot analyses to characterize the DNA that was integrated into the soybean genome. Genomic DNA samples from conventional soybean (A3244) were used as the negative controls on the blots to determine potential nonspecific hybridization signals. The positive controls for Southern blots were generated by digestions of plasmid DNA with different restriction enzymes or enzyme combinations to produce the DNA banding patterns that were most relevant to the molecular assessment of MON 89788. In addition, DNA markers were included to provide size estimation of the hybridized bands on Southern blots. The genetic elements that are expected to be present in MON 89788 are listed in Table IV-2. The probes used in the Southern analyses and the map of the plasmid (PV-GMGOX20) used in the transformation to generate MON 89788 are presented in Figure IV-2. The information and results derived from the molecular analyses were used to construct a linear map of the insert in MON 89788. This linear map depicts restriction sites identified in the T-DNA insert and the flanking soybean genome, and provides information on the expected banding patterns and sizes of the DNA fragments after restriction enzyme digestions. The liner map is shown in Figure IV-4. Based on these two figures and the probes used in the analyses, a table summarizing the expected DNA fragments for Southern analyses is presented in Table IV-3. The materials and methods used in the analyses are presented in Appendix A.

Table IV-2. Summary of Genetic Elements in MON 89788

Genetic Element[1,2]	Position in Sequence[3]	Function (Reference)
Sequence flanking 5′ end of the insert	1-1103	Soybean genomic DNA
B – Right Border	1104-1145	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Depicker et al., 1982)
Intervening Sequence	1146-1215	Sequences used in DNA cloning
P – *FMV/Tsf1*	1216-2255	Chimeric promoter consisting of enhancer sequences from the 35S promoter of the Figwort Mosaic virus (Richins et al., 1987) and the promoter from the *Tsf1* gene of *Arabidopsis thaliana* encoding elongation factor EF-1 alpha (Axelos et al., 1989)
L – *Tsf1*	2256-2301	5′ nontranslated leader (exon 1) from the *Tsf1* gene of *Arabidopsis thaliana* encoding elongation factor EF-1 alpha (Axelos et al., 1989)
I – *Tsf1*	2302-2923	Intron from the *Tsf1* gene of *Arabidopsis thaliana* encoding elongation factor EF-1 alpha (Axelos et al., 1989)
Intervening Sequence	2924-2932	Sequences used in DNA cloning
TS – *CTP2*	2933-3160	Sequences encoding the chloroplast transit peptide from the *ShkG* gene of *Arabidopsis thaliana* encoding EPSPS (Klee et al., 1987)
CS – *cp4 epsps*	3161-4528	Codon optimized coding sequence of the *aroA* (*epsps*) gene from the *Agrobacterium sp.* strain CP4 encoding the CP4 EPSPS protein (Padgette et al., 1996; Barry et al., 1997)
Intervening Sequence	4529-4570	Sequences used in DNA cloning
T – E9	4571-5213	3′ nontranslated sequence from the *RbcS2* gene of *Pisum sativum* encoding the Rubisco small subunit (Coruzzi et al., 1984)
Intervening Sequence	5214-5256	Sequences used in DNA cloning
B – Left Border	5257-5406	DNA region from Agrobacterium tumefaciens containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983)
Sequence flanking 3′ end of the insert	5407-6466	Soybean genomic DNA

[1] Intervening sequences and genomic flanking sequences are not regarded as genetic elements.
[2] B – Border; P – Promoter; L– Leader; I – Intron; TS– Targeting Sequence; CS – Coding Sequence; T – 3' nontranslated transcriptional termination sequence and polyadenylation signal sequences.
[3] Numbers correspond to the sequence in Figure IV-4 that includes the insert in MON 89788 and adjacent genomic DNA.

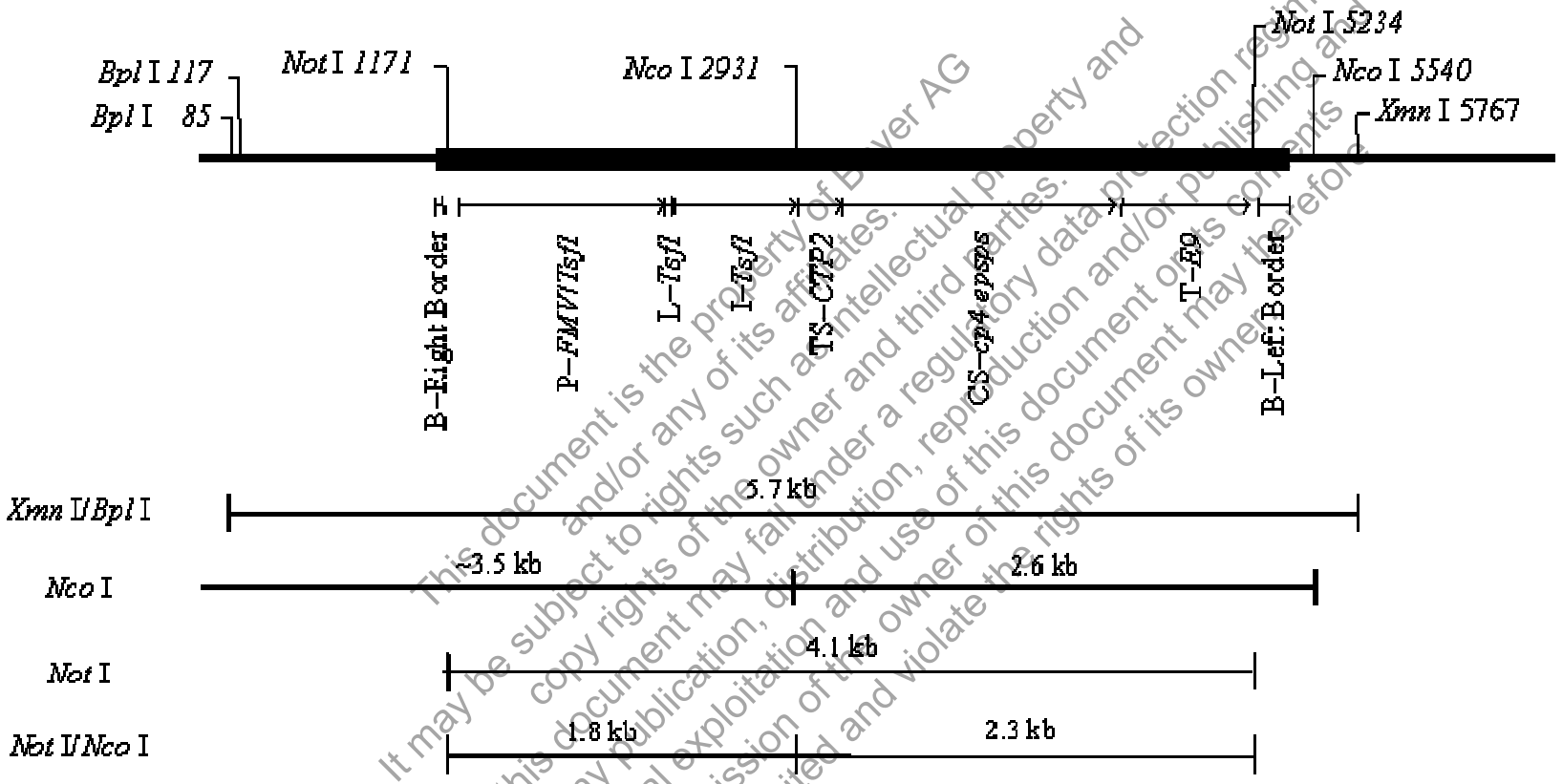


Figure IV-4. Schematic Representation of the Insert and Genomic Flanking Sequences in MON 89788
A linear map of the insert and genomic DNA flanking the insert in MON 89788 is shown. The upper portion of the figure displays genetic elements within the insert (thick rectangular bar), as well as the restriction sites used in Southern blot analyses. The positions of the restriction sites are consistent with the information presented in Table IV-2. Arrows underneath the designated insert indicate the direction of transcription. Shown on the lower portion of the map are the expected sizes of the DNA fragments after digestion with the respective restriction enzyme or combination of enzymes.

Table IV-3. Summary Chart of the Expected DNA Fragments Using Combination of Restriction Enzymes and Probes

	Expected Size of the DNA Fragment (kb)					
Probes used	**1, 2, 3**	**8, 9, 10**	**4**	**5**	**6**	**7**
Southern blot in Figure	IV-5; IV-12[1]	IV-6; IV-13[1]	IV-7	IV-8	IV-9	IV-10
Plasmid						
Not I	5.6 + 4.1	5.6	4.1	--	4.1	4.1
Not I + *Nco* I	--[2]	--	--	1.8	--	--
MON 89788						
Xmn I/*Bpl* I	5.7	ND[3]	--	--	--	--
Nco I	~3.5 + 2.6	ND	--	--	--	--
Not I	--	--	4.1	4.1	4.1	4.1
Not I + *Nco* I	--	--	1.8	1.8	2.3	2.3

[1] In Figures IV-12 and IV-13, MON 89788 DNA samples were only digested with *Nco* I and not with *Xmn* I/*Bpl* I.
[2] '--' indicates that the particular restriction enzyme or the combination of the enzymes was not used in the analysis.
[3] 'ND' indicates that no DNA band was detected.

3.2. Insert and Copy Number Determination

The insert number (the number of integration sites of the T-DNA in the soybean genome) was evaluated by digesting the MON 89788 and A3244 DNA with the combination of restriction enzymes *Bpl* I and *Xmn* I, which do not cleave within the T-DNA. Therefore, these enzymes should release a restriction fragment containing the entire T-DNA and adjacent plant genomic DNA (Figure IV-4). The number of restriction fragments detected should indicate the number of inserts present in MON 89788. The number of copies of the T-DNA integrated at a single locus was determined by digesting the MON 89788 DNA with the restriction enzyme *Nco* I, which cleaves once within the T-DNA (Figure IV-4). If MON 89788 contains one copy of the T-DNA, the Southern blot probed with the entire T-DNA will result in two bands, each representing a portion of the T-DNA along with adjacent plant genomic DNA.

The blot was hybridized with three overlapping ^{32}P-labeled T-DNA probes (probes 1, 2, and 3, Figure IV-2). The results of this analysis are presented in Figure IV-5, and the expected DNA fragments are summarized in Table IV-3. As shown in the figure, the A3244 DNA digested with a combination of *Bpl* I and *Xmn* I (lanes 1 and 7) or *Nco* I alone (lanes 3 and 9) produced no hybridization signal. Plasmid PV-GMGOX20 DNA that was mixed with A3244 DNA and digested with *Not* I (lanes 5 and 6) produced the expected size bands of 4.1 kb and 5.6 kb (refer to Table IV-3). MON 89788 DNA digested with a combination of *Bpl* I and *Xmn* I (lanes 2 and 8) produced a single band of 5.7 kb, indicating that MON 89788 contains one insert located within a 5.7 kb *Bpl* I/*Xmn* I restriction fragment. MON 89788 DNA digested with *Nco* I (lanes 4 and 10) produced two unique bands of 2.6 and ~3.5 kb representing the two expected fragments. This banding pattern indicates that only one single copy of the T-DNA is present in MON 89788.

3.3 Confirmation of the Absence of Plasmid PV-GMGOX20 Backbone

MON 89788 and A3244 DNA were digested with either a combination of the restriction enzymes *Bpl* I and *Xmn* I or the restriction enzyme *Nco* I. Plasmid PV-GMGOX20 DNA digested with *Not* I was used as a positive hybridization control. The blot was hybridized simultaneously with three overlapping probes (probes 8, 9, and 10, Figure IV-2) that spanned the backbone sequence of PV-GMGOX20. The results are shown in Figure IV-6, and the expected DNA fragments are summarized in Table IV-3.

A3244 control DNA digested with a combination of *Bpl* I and *Xmn* I (lanes 1 and 7) or *Nco* I (lanes 3 and 9) showed no detectable hybridization bands, as expected for the negative control. Plasmid PV-GMGOX20 *Not* I restriction fragments mixed with control DNA (lanes 5 and 6) produced the expected size band at 5.6 kb. MON 89788 DNA digested with either a combination of *Bpl* I and *Xmn* I (lanes 2 and 8) or *Nco* I (lanes 4 and 10) showed no detectable hybridization signal. This result indicates that MON 89788 does not contain any detectable backbone sequence from the transformation vector PV-GMGOX20.

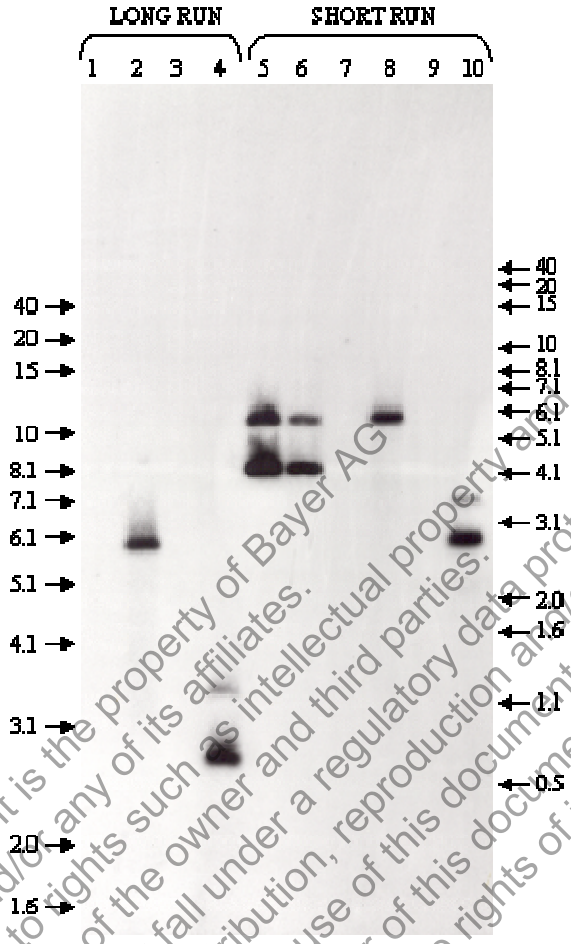


Figure IV-5. Southern Blot Analysis of MON 89788: Insert and Copy Number
The blot was hybridized simultaneously with three overlapping ^{32}P-labeled T-DNA probes (probes 1, 2, and 3, Figure IV-2). Each lane contains ~10 µg of digested genomic DNA isolated from leaf. Lane designations are as follows:

Lane 1: Conventional (*Bpl* I/*Xmn* I)
2: MON 89788 (*Bpl* I/*Xmn* I)
3: Conventional (*Nco* I)
4: MON 89788 (*Nco* I)
5: Conventional mixed with PV-GMGOX20 (*Not* I) [2 copies]
6: Conventional mixed with PV-GMGOX20 (*Not* I) [1 copy]
7: Conventional (*Bpl* I/*Xmn* I)
8: MON 89788 (*Bpl* I/*Xmn* I)
9: Conventional (*Nco* I)
10: MON 89788 (*Nco* I)

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

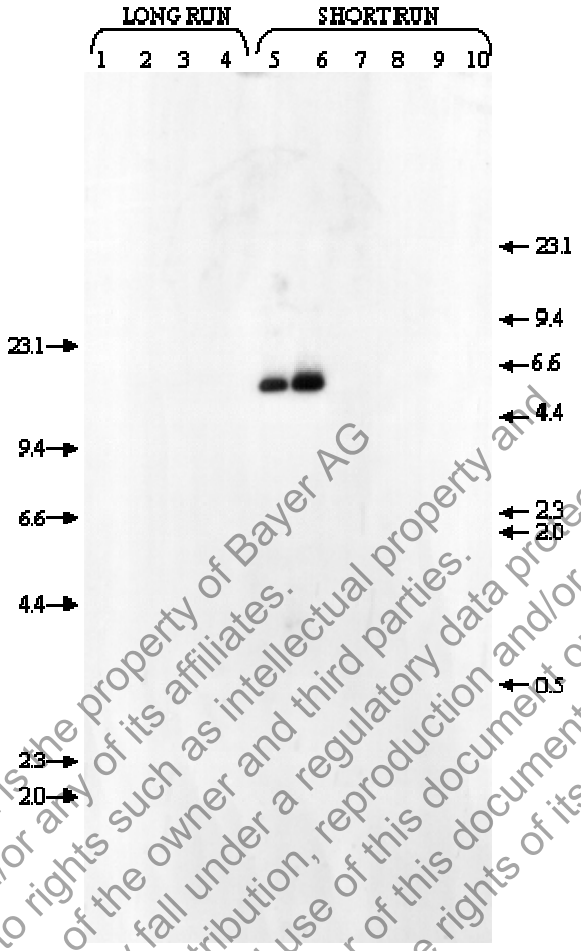


Figure IV-6. Southern Blot Analysis of MON 89788: PV-GMGOX20 Backbone
The blot was hybridized simultaneously with three ^{32}P-labeled probes that span the entire backbone sequence (probes 8, 9, and 10, Figure IV-2) of plasmid PV-GMGOX20. Each lane contains ~10 µg of digested genomic DNA isolated from leaf. Lane designations are as follows:

Lane 1: Conventional (*Bpl* I/*Xmn* I)
2: MON 89788 (*Bpl* I/*Xmn* I)
3: Conventional (*Nco* I)
4: MON 89788 (*Nco* I)
5: Conventional (*Nco* I) mixed with PV-GMGOX20 (*Not* I) [0.5 copy]
6: Conventional (*Nco* I) mixed with PV-GMGOX20 (*Not* I) [1 copy]
7: Conventional (*Bpl* I/*Xmn* I)
8: MON 89788 (*Bpl* I/*Xmn* I)
9: Conventional (*Nco* I)
10: MON 89788 (*Nco* I)

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

3.4. *cp4 epsps* Cassette Integrity

The intactness of the inserted *cp4 epsps* coding sequence and the associated genetic elements was assessed by digesting MON 89788 DNA with *Not* I or a combination of *Not* I and *Nco* I and probing the Southern blots with individual genetic elements in the *cp4 epsps* cassette. Digestion with *Not* I was expected to generate a single 4.1 kb restriction fragment containing the *cp4 epsps* gene cassette, and digestion with the combination of *Not* I and *Nco* I was expected to generate two restriction fragments of 1.8 kb and 2.3 kb (Figure IV-4). The 1.8 kb fragment contains the *FMV/Tsf1* promoter, *Tsf1* leader, and *Tsf1* intron, whereas the 2.3 kb fragment contains the *CTP2* targeting sequence, *cp4 epsps* coding sequence, and the *E9* 3′ nontranslated region. Plasmid PV-GMGOX20 DNA digested with *Not* I or a combination of *Not* I and *Nco* I was used as a positive hybridization control and size estimator. Individual Southern blot was examined with the *FMV/Tsf1* promoter + *Tsf1* leader probe, *Tsf1* intron probe, *CTP2* targeting sequence + *cp4 epsps* coding sequence probe, or *E9* 3′ nontranslated sequence probe (probes 4, 5, 6, and 7 respectively; Figure IV-2). The expected DNA fragments identified by probes 4-7 are summarized in Table IV-3.

3.4.1. *FMV/Tsf1* Promoter + *Tsf1* Leader

The A3244 control DNA digested with *Not* I (Figure IV-7; lanes 1 and 7) or the combination of *Not* I and *Nco* I (lanes 3 and 9) showed no detectable bands when hybridized with the *FMV/Tsf1* promoter + *Tsf1* leader probe (probe 4, Figure IV-2). Plasmid PV-GMGOX20 DNA digested with *Not* I and mixed with control DNA produced the expected size band at 4.1 kb (lanes 5 and 6). MON 89788 DNA digested with *Not* I (lanes 2 and 8) produced the expected band of 4.1 kb, and the DNA digested with the combination of *Not* I and *Nco* I (lanes 4 and 10) produced a single expected size band of 1.8 kb. There were no additional bands detected using the promoter and leader sequence probe. Based on the results presented in Figure IV-7, it is concluded that MON 89788 contains no additional *FMV/Tsf1* promoter or *Tsf1* leader elements other than those associated with the intact *cp4 epsps* cassette.

3.4.2. *Tsf1* Intron

The A3244 control DNA digested with *Not* I (Figure IV-8; lanes 1 and 7) or the combination of *Not* I and *Nco* I (lanes 3 and 9) was hybridized with the *Tsf1* intron probe (probe 5, Figure IV-2). Results indicated that there were no detectable hybridization bands, as expected for the negative control. As positive control, plasmid PV-GMGOX20 DNA digested with the combination of *Not* I and *Nco* I (lanes 5 and 6) produced the expected size band of 1.8 kb. MON 89788 DNA digested with *Not* I (lanes 2 and 8) or with the combination of *Not* I and *Nco* I (lanes 4 and 10) produced the expected bands of 4.1 kb or 1.8 kb, respectively. No additional bands were detected using the *Tsf1* intron probe. These results indicate that MON 89788 contains no additional *Tsf1* intron elements other than that associated with the intact *cp4 epsps* cassette.

3.4.3. *CTP2* Targeting Sequence + *cp4 epsps* Coding Sequence

Hybridization of the *Not* I-digested (Figure IV-9; lanes 1 and 7) or *Not* I- and *Nco* I-digested A3244 DNA (lanes 3 and 9) with the *CTP2* targeting sequence + *cp4 epsps* coding sequence probe (probe 6, Figure IV-2) showed no detectable hybridization bands. Positive control plasmid PV-GMGOX20 DNA digested with *Not* I produced the expected size band of 4.1 kb (lanes 5 and 6). MON 89788 DNA digested with *Not* I (lanes 2 and 8) produced the expected size band of 4.1 kb, and the same source of DNA digested with a combination of *Not* I and *Nco* I (lanes 4 and 10) produced the expected size band of 2.3 kb. As there were no unexpected bands on the Southern blot, the results indicate that MON 89788 contains no additional *CTP2* targeting sequence or *cp4 epsps* coding sequence elements other than those associated with the intact *cp4 epsps* gene cassette.

3.4.4. *E9* 3′ Nontranslated Sequence

The A3244 control DNA digested with *Not* I (Figure IV-10; lanes 1 and 7) or a combination of *Not* I and *Nco* I (lanes 3 and 9) showed no detectable hybridization bands when examined with the *E9* 3′ nontranslated sequence probe (probe 7, Figure IV-2). Positive control plasmid PV-GMGOX20 DNA digested with *Not* I produced the expected size band of 4.1 kb (lanes 5 and 6). MON 89788 DNA digested with *Not* I (lanes 2 and 8) or a combination of *Not* I and *Nco* I (lanes 4 and 10) produced the expected size band of 4.1 kb or 2.3 kb, respectively. There were no additional bands detected using the *E9* 3' nontranslated sequence probe. These results indicate that MON 89788 contains no additional *E9* elements other than those associated with the intact *cp4 epsps* gene cassette.

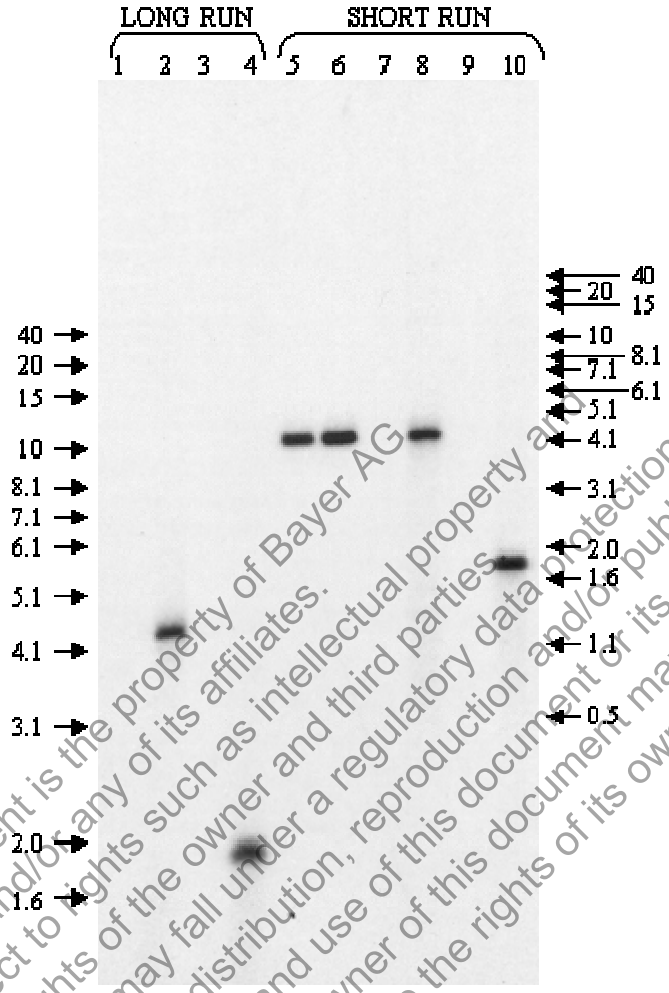


Figure IV-7. Southern Blot Analysis of MON 89788: P-*FMV/Tsf1* + L-*Tsf1*
The blot was hybridized with a ^{32}P-labeled probe that spanned the *FMV/Tsf1* promoter and *Tsf1* leader (probe 4, Figure IV-2). Each lane contains ~10 µg of digested genomic DNA isolated from leaf. Lane designations are as follows:

Lane 1: Conventional (*Not* I)
2: MON 89788 (*Not* I)
3: Conventional (*Not* I/*Nco* I)
4: MON 89788 (*Not* I/*Nco* I)
5: Conventional (*Not* I/*Nco* I) mixed with PV-GMGOX20 (*Not* I) [0.5 copy]
6: Conventional (*Not* I/*Nco* I) mixed with PV-GMGOX20 (*Not* I) [1 copy]
7: Conventional (*Not* I)
8: MON 89788 (*Not* I)
9: Conventional (*Not* I/*Nco* I)
10: MON 89788 (*Not* I/*Nco* I)

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

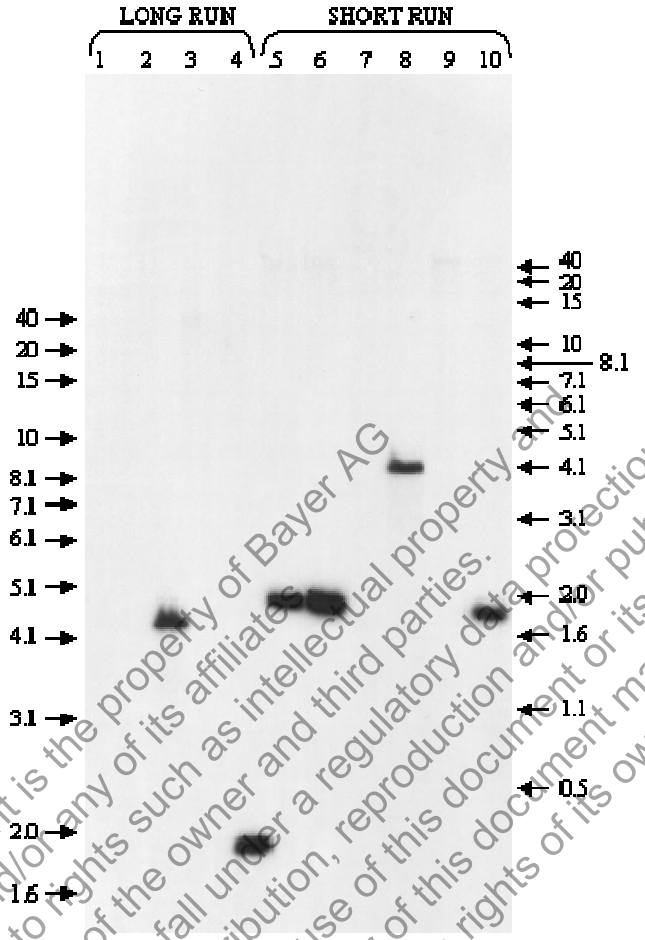


Figure IV-8. Southern Blot Analysis of MON 89788: I-*Tsf1*

The blot was hybridized with a ^{32}P-labeled probe that spanned the *Tsf1* intron (probe 5, Figure IV-2). Each lane contains ~10 µg of digested genomic DNA isolated from leaf. Lane designations are as follows:

Lane 1: Conventional (*Not* I)
2: MON 89788 (*Not* I)
3: Conventional (*Not* I/*Nco* I)
4: MON 89788 (*Not* I/*Nco* I)
5: Conventional mixed with PV-GMGOX20 (*Not* I/*Nco* I) [1 copy]
6: Conventional mixed with PV-GMGOX20 (*Not* I/*Nco* I) [2 copies]
7: Conventional (*Not* I)
8: MON 89788 (*Not* I)
9: Conventional (*Not* I/*Nco* I)
10: MON 89788 (*Not* I/*Nco* I)

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

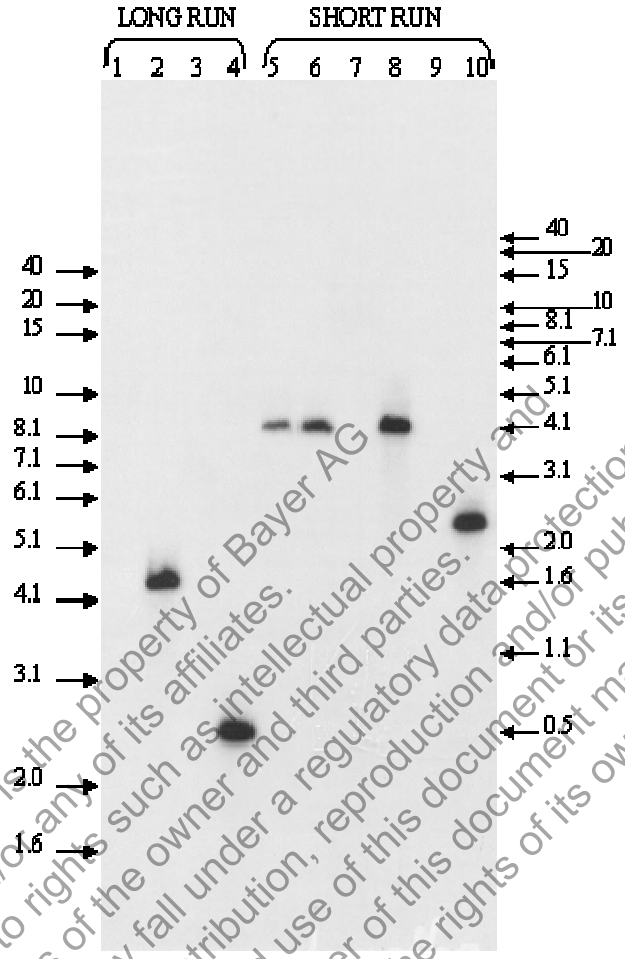


Figure IV-9. Southern Blot Analysis of MON 89788: TS-*CTP2* + CS-*cp4 epsps*
The blot was hybridized with a ^{32}P-labeled probe that spanned the *CTP2* targeting sequence and *cp4 epsps* coding sequence (probe 6, Figure IV-2). Each lane contains ~10 µg of digested genomic DNA isolated from leaf. Lane designations are as follows:

Lane 1: Conventional (*Not* I)
2: MON 89788 (*Not* I)
3: Conventional (*Not* I/*Nco* I)
4: MON 89788 (*Not* I/*Nco* I)
5: Conventional (*Not* I/*Nco* I) mixed with PV-GMGOX20 (*Not* I) [0.5 copy]
6: Conventional (*Not* I/*Nco* I) mixed with PV-GMGOX20 (*Not* I) [1 copy]
7: Conventional (*Not* I)
8: MON 89788 (*Not* I)
9: Conventional (*Not* I/*Nco* I)
10: MON 89788 (*Not* I/*Nco* I)

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

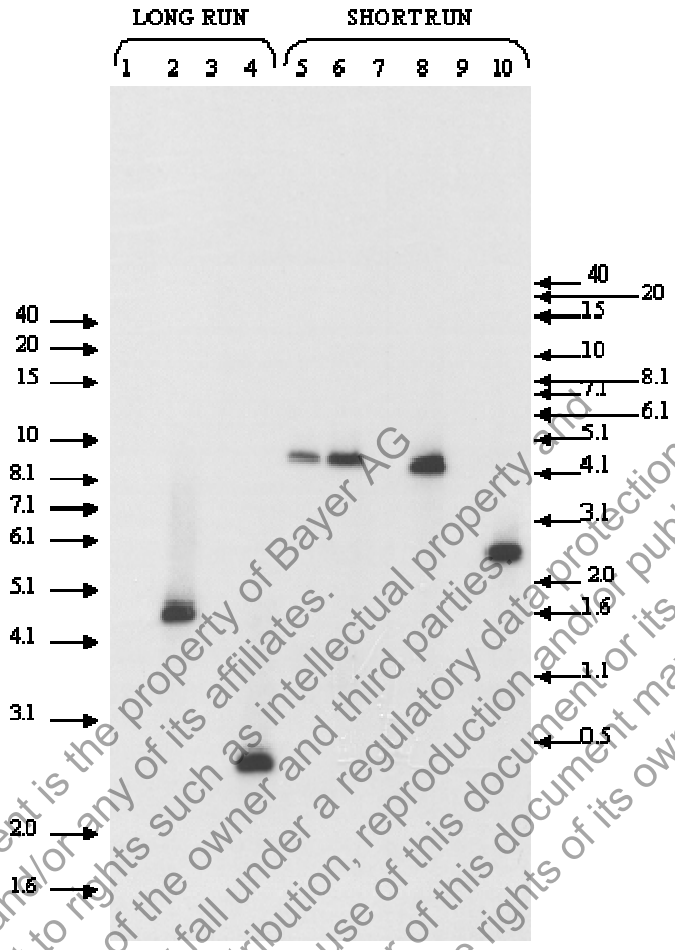


Figure IV-10. Southern Blot Analysis of MON 89788: T-*E9*

The blot was hybridized with a ^{32}P-labeled probe that spanned the E9 3' nontranslated sequence (probe 7, Figure IV-2). Each lane contains ~10 µg of digested genomic DNA isolated from leaf. Lane designations are as follows:

Lane 1: Conventional (*Not* I)
2: MON 89788 (*Not* I)
3: Conventional (*Not* I/*Nco* I)
4: MON 89788 (*Not* I/*Nco* I)
5: Conventional (*Not* I/*Nco* I) mixed with PV-GMGOX20 (*Not* I) [0.5 copy]
6: Conventional (*Not* I/*Nco* I) mixed with PV-GMGOX20 (*Not* I) [1 copy]
7: Conventional (*Not* I)
8: MON 89788 (*Not* I)
9: Conventional (*Not* I/*Nco* I)
10: MON 89788 (*Not* I/*Nco* I)

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

3.5. Southern Blot Analyses of MON 89788 across Multiple Generations

To assess the stability of the T-DNA in MON 89788, Southern blot analysis was performed using MON 89788 DNA across four generations. For reference, the breeding history of MON 89788 is presented in Figure IV-11, and the grain examined included R4 to R7 generations. The expected Southern hybridization DNA banding pattern for these analyses is summarized in Table IV-3.

3.5.1. Generational Stability of the Insert

DNA samples from four generations of MON 89788 were isolated and subjected to digestion with *Nco* I. The blot was hybridized simultaneously with three overlapping probes, which, taken together, span the entire T-DNA region of plasmid PV-GMGOX20 (probes 1, 2, and 3, Figure IV-2).

Hybridization of A3244 control DNA digested with *Nco* I (Figure IV-12; lane 1) showed no detectable hybridization bands, which was as expected for the negative control. Plasmid PV-GMGOX20 DNA digested with *Not* I produced the expected size bands of 4.1 and 5.6 kb (lane 2). Hybridization of MON 89788 DNA digested with *Nco* I produced two bands of 2.6 kb and ~3.5 kb (lanes 3 – 8). This is the same restriction pattern observed for the R5 generation shown in Figure IV-5 (lanes 4 and 10). The results of this analysis establish the stability of the inserted DNA over four generations of MON 89788.

3.5.2. Confirmation of the Absence of PV-GMGOX20 Backbone Sequence

The four generations of MON 89788 material utilized to assess generational stability were also examined for the absence of backbone sequence by Southern blot. MON 89788 and control DNA samples were digested with *Nco* I and the blot was hybridized simultaneously with three overlapping probes, which taken together, span the entire backbone sequence of plasmid PV-GMGOX20 (probes 8, 9, and 10, Figure IV-2).

Hybridization of the A3244 control DNA digested with *Nco* I did not detect any bands (Figure IV-13; lane 1), as expected for the negative control. Hybridization of plasmid PV-GMGOX20 DNA digested with *Not* I produced the expected size band of 5.6 kb (lane 2). MON 89788 DNA from four generations showed no detectable hybridization signal (lanes 3-8). Consistent with the results depicted in Figure IV-6, these results indicate that the generations examined do not contain any detectable backbone sequence from the transformation vector PV-GMGOX20.

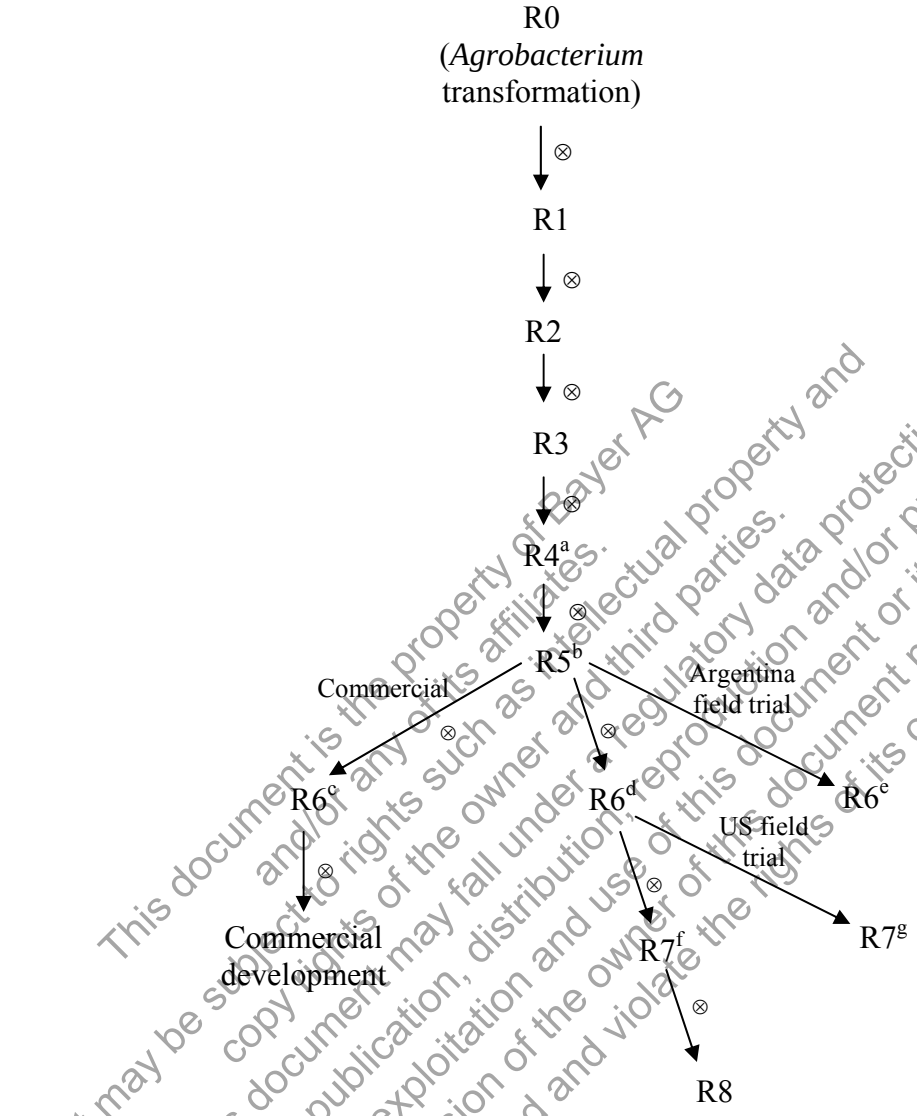


Figure IV-11. MON 89788 Breeding Diagram
All generations were self-pollinated (⊗). R1 generation was used for segregation analysis and the selection of homozygous plants (Section 3.7). $R5^b$ seed material was used either for commercial development (on the left) or for regulated field trials (on the right). Generation $R5^b$ was used in the molecular analyses and was the starting seed for Argentina field trial, and the resulting seed ($R6^e$) was used in the protein characterization studies. $R6^d$ was the seed source for U.S. field trial, and the resulting seed ($R7^g$) was used in the composition and expression analyses. Seed lot $R7^f$ was the seed source for additional field trial. Generation $R6^c$ represents the materials entering commercial development. Seed lots $R4^a$, $R5^b$, $R6^c$, $R6^d$, $R6^e$, and $R7^f$ were used in molecular generation stability analyses.

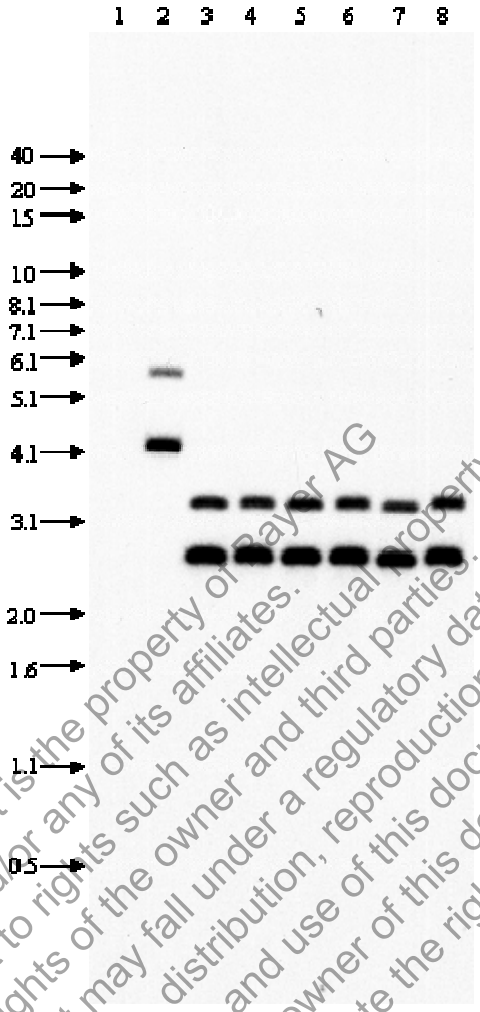


Figure IV-12. Generational Stability Analyses of MON 89788 Using Insert and Copy Number Probes

The blot was hybridized simultaneously with three overlapping ^{32}P-labeled T-DNA probes (probes 1, 2, and 3, Figure IV-2). Each lane contains ~10 µg of digested genomic DNA isolated from leaf material. The breeding history of MON 89788 is illustrated in Figure IV-11. Lane designations are as follows:

Lane 1: Conventional (*Nco* I)
2: Conventional (*Nco* I) mixed with PV-GMGOX20 (*Not* I) [1 copy]
3: MON 89788 – R4[a] (*Nco* I)
4: MON 89788 – R5[b] (*Nco* I)
5: MON 89788 – R6[c] (*Nco* I)
6: MON 89788 – R6[d] (*Nco* I)
7: MON 89788 – R6[e] (*Nco* I)
8: MON 89788 – R7[f] (*Nco* I)

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

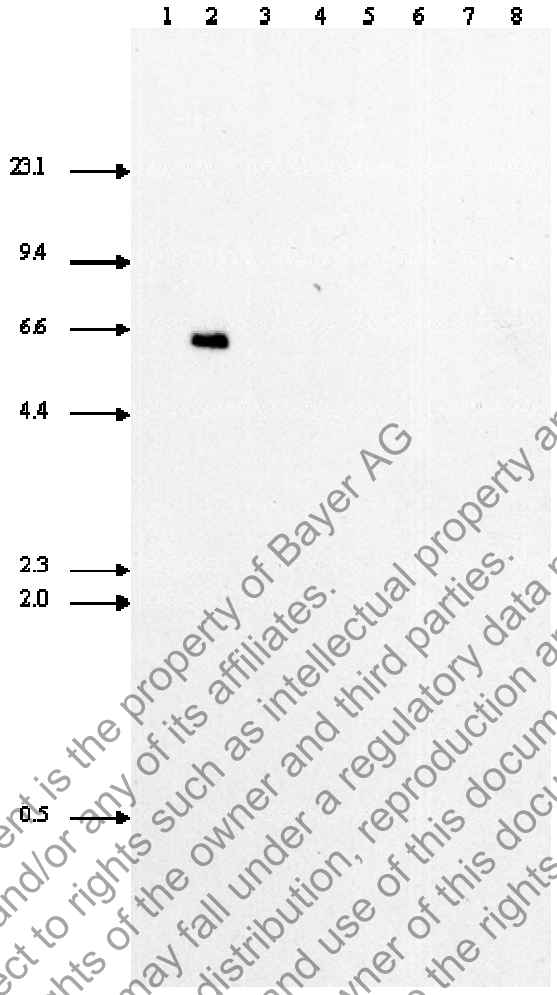


Figure IV-13. Generational Stability of MON 89788 Using PV-GMGOX20 Backbone Probes

The blot was hybridized simultaneously with three ^{32}P-labeled probes that span the entire backbone sequence (probes 8, 9, and 10, Figure IV-2) of plasmid PV-GMGOX20. Each lane contains ~10 µg of digested genomic DNA isolated from leaf material. Lane designations are as follows:

Lane 1: Conventional (*Nco* I)
2: Conventional (*Nco* I) mixed with PV-GMGOX20 (*Not* I) [1 copy]
3: MON 89788 – R4[a] (*Nco* I)
4: MON 89788 – R5[b] (*Nco* I)
5: MON 89788 – R6[c] (*Nco* I)
6: MON 89788 – R6[d] (*Nco* I)
7: MON 89788 – R6[e] (*Nco* I)
8: MON 89788 – R7[f] (*Nco* I)

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

3.6. Organization of the Genetic Elements in MON 89788

The organization of the genetic elements within the insert of MON 89788 was confirmed by DNA sequence analyses. Several PCR primers were designed with the intent to amplify three overlapping DNA fragments spanning the entire length of the inert (Appendix A). The amplified DNA fragments were subjected to DNA sequencing analyses. Results confirm that the arrangement of the genetic elements is identical to that in plasmid PV-GMGOX20 and is as depicted in Figure IV-4.

3.7. Inheritance of the Glyphosate Tolerance Trait in MON 89788

During the development of the MON 89788, phenotypic segregation data were generated and analyzed across several generations. Summaries of these analyses are presented in Tables IV-5, and the expected segregation ratio for each generation is summarized in Table IV-4. The presence and gene copy number of the *cp4 epsps* gene was determined by quantitative PCR or sometimes referred to as TaqMan (Schmidt and Parrott, 2001; Bubner and Baldwin, 2004). The presence of the glyphosate-tolerance trait of individual plants was determined by CP4 EPSPS ELISA and/or by treatment with glyphosate.

After self-pollination of the R0 plant, the R1 seeds were germinated, and the resulting plants were expected to segregate on a 3:1 ratio of positive:negative based on glyphosate-tolerance phenotype (Table IV-4). Selected R1 plants that survived the glyphosate treatment (29 out of 43; Table IV-5) were subjected to quantitative PCR analyses, and a single plant that was homozygous for *cp4 epsps* expression cassette was selected. This homozygous plant was self-pollinated to give rise to a population of R2 plants, and the segregation ratio for R2 and the subsequent generation is expected to maintain a population of 100% positive (1:0 for positive:negative plants) for the glyphosate-tolerance trait (Table IV-4).

Table IV-4. Selection Process and Expected Segregation Ratio during MON 89788 Development

Generation	Expected Ratio and Selection
R0	Plant was self-pollinated to produce R1 seed; no Chi-square analysis
R1	3:1 (positive:negative) based on glyphosate-tolerance phenotype
R1 Homozygous plant selection	Homozygous plant selection was conducted using TaqMan for *cp4 epsps* from the segregating R1 population.
R2	1:0 positive:negative (homozygous progeny, derived from R1 selection)
R3	1:0 positive:negative (homozygous progeny established in field plots, derived from homozygous selection)

Phenotype frequency was compared by means of a Chi-square analysis (Little and Hills, 1978), which was performed on the R1 generation to determine heritability and phenotype stability of the *cp4 epsps* expression cassette in MON 89788. The Chi-square analysis is based on testing the observed to the expected trait segregation ratio according to Mendelian principles, and the Chi-square test was computed as:

$$\chi^2 = \Sigma [(| o - e | - 0.5)^2 / e]$$

where, o = observed frequency of the genotype, e = expected frequency of the genotype, and 0.5 = Yates correction factor for analysis with one degree of freedom (df).

The χ^2 value in the R1 generation indicated no significant differences between the observed and expected phenotypic ratio for MON 89788 as the Chi-square was less than the critical value of 3.84 at $p<0.05$ (Table IV-5). Following the selection of the homozygous event, the subsequent generations were no longer segregating, and the expected and the observed segregation ratios are identical. The results of this analysis are consistent with the finding of a single chromosomal insertion of the *cp4 epsps* gene cassette that segregates according to Mendel's laws of genetics. These results are also consistent with the molecular characterization data indicating a single insertion site of the *cp4 epsps* cassette.

Table IV-5. Glyphosate-Tolerant Trait Segregation Patterns of MON 89788

Generation	# of Plant; (% Germ.) [1]	Expected [2]		Observed [3]		Chi-square
		Positive	Negative	Positive	Negative	
R1	43	32.25	10.75	29	14	1.31[4]
R2	58	58	0	58	0	Fixed
R3	240; (80%)	192	0	192[5]	0	Fixed
R3	240; (85%)	204	0	204[5]	0	Fixed
R3	240; (85%)	204	0	204[5]	0	Fixed

[1] Percent germination based on visual estimation (plant stand, in 5% increments).
[2] Expected number of glyphosate-tolerant plants.
[3] Observed number of glyphosate-tolerant plants by ELISA and glyphosate application.
[4] Not significant at $p \leq 0.05$ (Chi-square = 3.84 at 1df)
[5] Number of plants (observed positives) was calculated based on #seed planted × percent germination

3.8. Conclusions of Molecular Characterization

Molecular analyses were performed to characterize the integrated DNA insert in MON 89788. Southern blot genomic analyses were used to determine the DNA insert

number (number of integration sites within the soybean genome), copy number (the number of copies within one insert), the intactness of the *cp4 epsps* gene expression cassette, 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. In addition, DNA sequencing analyses were performed to confirm the organization of the elements within the DNA insert.

Data show that one intact copy of the *cp4 epsps* expression cassette was integrated at a single chromosomal locus contained within a ~5.7 kb *Xmn* I/*Bpl* I restriction fragment. No additional elements from the transformation vector PV-GMGOX20, linked or unlinked to the intact DNA insert, were detected in the genome of MON 89788. Additionally, backbone sequence from PV-GMGOX20 was not detected. Generational stability analysis demonstrated that the expected Southern blot fingerprint of MON 89788 has been maintained across four 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-GMGOX20. In addition, DNA sequence analyses confirmed the organization of the genetic elements within the *cp4 epsps* expression cassette of MON 89788, which is identical to that in plasmid PV-GMGOX20 and is as depicted in the schematic of Figure IV-4. Finally, heritability and stability of the glyphosate-tolerance phenotype were as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the DNA insert at a single chromosomal locus.

Section 4. Other Data or Information Regarding the Development of MON 89788

All relevant information regarding development of MON 89788 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 marker were inserted into the soybean genome during the development of MON 89788. Molecular characterization data presented in Part IV, Section 3.3 demonstrate the absence of the *aad* antibiotic resistant marker in MON 89788.

PART VI: CHARACTERIZATION OF THE CP4 EPSPS PROTEIN PRODUCED IN MON 89788 SOYBEAN

Section 1. Identity and Characterization Summary of the CP4 EPSPS Protein Present in MON 89788

MON 89788 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 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 the Roundup family of agricultural herbicides, requirements for aromatic amino acids and other metabolites are met by the continued action of the CP4 EPSPS enzyme in the presence of glyphosate (Padgette et al., 1996). The CP4 EPSPS protein expressed in MON 89788 is identical to the CP4 EPSPSs in other Roundup Ready crops including Roundup Ready soybean, Roundup Ready canola, Roundup Ready sugar beet, and Roundup Ready Flex cotton.

In plants, the chloroplast is the primary site of aromatic amino acid biosynthesis and is where the EPSPS enzyme resides. Therefore, the CP4 EPSPS protein produced in MON 89788 is targeted to the chloroplasts via an N-terminal fusion with the CTP2 transit peptide derived from *Arabidopsis thaliana* to form a CTP2-CP4 EPSPS precursor protein. The precursor protein is synthesized in the cytoplasm, and is processed to remove the transit peptide upon translocation into the plant chloroplast to produce the mature protein (Chua and Schmidt, 1978; Highfield and Ellis, 1978; Oblong and Lamppa, 1992). The safety assessment of the CP4 EPSPS protein has been described previously (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).

To assess the safety of the CP4 EPSPS protein expressed in MON 89788, a number of studies similar to those conducted previously by Harrison et al. (1996) were performed. The purposes were to characterize the purified CP4 EPSPS protein produced in MON 89788 and to demonstrate the equivalence between MON 89788- and *E. coli*-produced CP4 EPSPS proteins. As the *E. coli*-produced CP4 EPSPS has been used previously in a number of safety assessment studies, including the simulated gastric fluid (SGF) and acute mouse gavage, demonstration of protein equivalence between *E. coli*- and MON 89788-produced CP4 EPSPS proteins allows utilization of the existing data to confirm the safety of the CP4 EPSPS protein in MON 89788. The analyses employed for characterization or establishment of the identity of MON 89788-produced CP4 EPSPS protein included: (1) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to establish equivalence of the apparent molecular weight between MON 89788-

and *E. coli*-produced proteins, (2) immunoblot analysis to establish immunoreactivity equivalence between MON 89788- and *E. coli*-produced proteins using anti-CP4 EPSPS antibody, (3) N-terminal sequence analysis, (4) matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry to generate tryptic peptide map, (5) CP4 EPSPS enzymatic activity analysis to demonstrate functional equivalence between MON 89788- and *E. coli*-produced proteins, and (6) glycosylation analysis to establish equivalence of the glycosylation status between MON 89788- and *E. coli*-produced proteins. The results from each of these analyses are summarized below. Details on materials and methods can be found in Appendix B.

1.1. CP4 EPSPS Molecular Weight Equivalence

The equivalence in apparent molecular weight of the purified MON 89788- and the *E. coli*-produced CP4 EPSPS proteins was demonstrated using SDS-PAGE and stained with Brilliant Blue G-Colloidal stain (Figure VI-1). The MON 89788-produced CP4 EPSPS protein migrated with a molecular weight indistinguishable to that of the *E. coli*-produced protein standard analyzed concurrently (Figure VI-1, lane 2 vs. lanes 3-8). Based on the comparable electrophoretic mobility, the MON 89788- and *E. coli*-produced CP4 EPSPS proteins were determined to have equivalent apparent molecular weight. The estimated molecular weight is consistent with the calculated molecular weight of 47.6 kDa based on translation of the coding sequence of *cp4 epsps*.

1.2. CP4 EPSPS Immunoreactivity Equivalence

A western blot analysis using goat anti-CP4 EPSPS serum was conducted to determine the relative immunoreactivity of the MON 89788-produced CP4 EPSPS protein and the *E. coli*-produced CP4 EPSPS reference standard. Results indicated that the anti-CP4 EPSPS antibody recognized the mature MON 89788-produced CP4 EPSPS protein that migrated identically to the *E. coli*-produced reference standard protein (Figure VI-2). Moreover, the immunoreactive signal increased with increasing levels of the CP4 EPSPS protein. The observed immunoreactivities between the MON 89788- and *E. coli*-produced proteins were similar based on densitometric analysis of the western blot. Based on the above analysis, the MON 89788- and *E. coli*-produced CP4 EPSPS demonstrated equivalent immunoreactive properties, which confirmed the identity and equivalence of the two proteins.

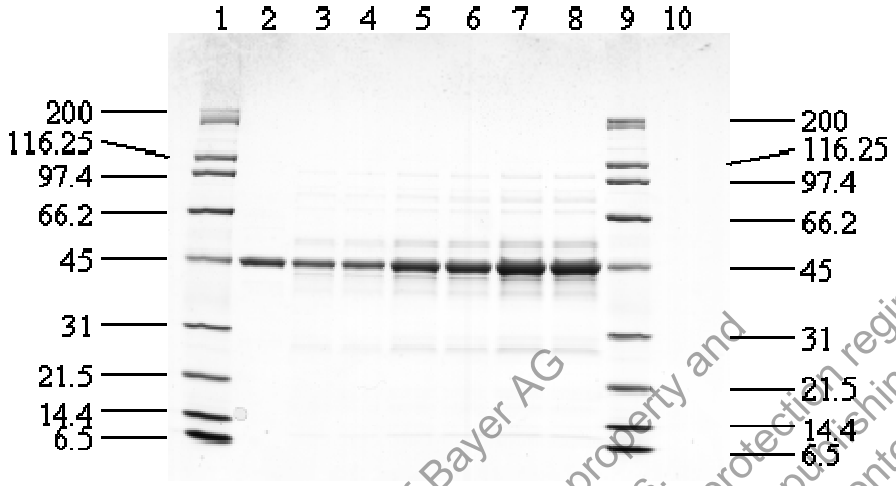


Figure VI-1. SDS-PAGE Molecular Weight Analysis of the CP4 EPSPS Protein Isolated from MON 89788 Grain

Aliquots of the purified MON 89788-produced CP4 EPSPS protein, and the *E. coli*-produced CP4 EPSPS reference standard were separated by denaturing tris-glycine 4→20% PAGE and stained with Brilliant Blue G-Colloidal stain. Amounts correspond to total protein loaded per lane. Approximate molecular weights (kDa) correspond to the markers loaded in Lanes 1 and 9.

Lane	Sample	Amount (µg)
1	MW Markers	4.5
2	*E. coli*-produced CP4 EPSPS reference standard	1
3	MON 89788-produced CP4 EPSPS protein	1
4	MON 89788-produced CP4 EPSPS protein	1
5	MON 89788-produced CP4 EPSPS protein	2
6	MON 89788-produced CP4 EPSPS protein	2
7	MON 89788-produced CP4 EPSPS protein	3
8	MON 89788-produced CP4 EPSPS protein	3
9	MW Markers	4.5
10	Empty lane	N/A

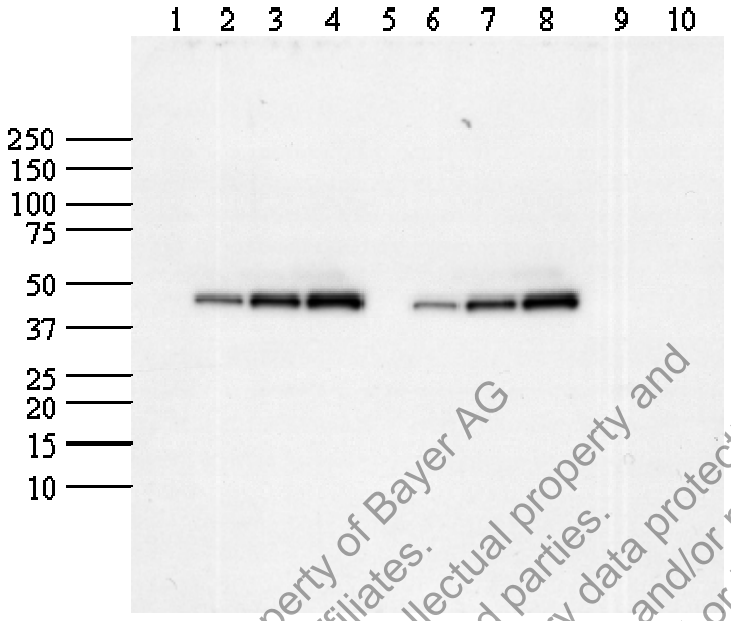


Figure VI-2. Immunoblot Analysis of the CP4 EPSPS Protein Isolated from MON 89788 Grain

Aliquots of the purified MON 89788-produced CP4 EPSPS protein and the *E. coli*-produced CP4 EPSPS reference standard were separated by denaturing tris-glycine 4→20% PAGE, electrotransferred to a PVDF membrane and detected using CP4 EPSPS polyclonal antiserum followed by development using the ECL system (10-second exposure shown). Approximate molecular weights (kDa) correspond to the markers loaded in lane 1.

Lane	Sample	Amount of CP4 EPSPS (ng)
1	MW Markers	N/A
2	*E. coli*-produced CP4 EPSPS reference standard	1
3	*E. coli*-produced CP4 EPSPS reference standard	2
4	*E. coli*-produced CP4 EPSPS reference standard	3
5	Empty Lane	N/A
6	MON 89788-produced CP4 EPSPS protein	1
7	MON 89788-produced CP4 EPSPS protein	2
8	MON 89788-produced CP4 EPSPS protein	3
9	Empty lane	N/A
10	Empty lane	N/A

1.3. N-terminal Sequence Analysis

The N-terminus of the purified MON 89788-produced CP4 EPSPS protein was determined. The resulting sequence matched the predicted CP4 EPSPS N-terminal sequence translated from the *cp4 epsps* coding region (Table VI-1, Observed Sequence-1 and 2). The removal of the N-terminal methionine was observed in a fraction of the purified MON 89788-produced protein. This is likely due to cellular enzyme processing in plant (Schmidt et al., 1992). This result is not unexpected as the initial methionine is frequently removed from proteins in eukaryotic organisms by an endogenous methionine aminopeptidase (Arfin and Bradshaw, 1988). Similar findings have been observed in a number of products that have completed the FDA consultation processes, which include Roundup Ready Flex cotton (FDA, 2005) and Roundup Ready soybean (Harrison, et. al., 1996). This information, therefore, confirms the N-terminal sequence identity of the CP4 EPSPS protein isolated from MON 89788, and that this sequence is consistent with the coding region of the gene.

1.4. MALDI-TOF Mass Spectrometry Analysis

The identity of the CP4 EPSPS protein was established using matrix assisted laser desorption ionization - time of flight (MALDI-TOF) mass spectrometry. With appropriate mass accuracy, four tryptic peptides were found to be sufficient to identify a protein (Jiménez et al., 1998). Observed tryptic peptides were considered a match to the expected tryptic mass when differences in molecular weight of less than one Dalton were found between the observed and predicted fragment masses. Such matches were made without consideration for potential natural amino acid modifications such as glycosylation.

Using the aforementioned criteria, the identity of the MON 89788-produced CP4 EPSPS protein was assessed by MALDI-TOF mass spectrometry of chemically reduced and alkylated tryptic fragments prepared from the MON 89788-produced CP4 EPSPS protein. A total of 23 masses matched the expected tryptic digest mass fragments from the deduced amino acid sequence of the CP4 EPSPS protein. The identified masses were used to assemble a coverage map indicating the matched peptide sequences for the entire CP4 EPSPS protein (Figure VI-3). This analysis confirmed the identity of the MON 89788-produced CP4 EPSPS protein.

1.5. CP4 EPSPS Functional Activity Equivalence

The specific activity of the MON 89788-produced CP4 EPSPS protein was estimated using a phosphate release assay, where one unit (U) of enzyme activity was defined as the amount of enzyme that produced 1 μmole of inorganic phosphate from PEP per minute at 25°C. The *E. coli*- and MON 89788-produced CP4 EPSPS were considered functional equivalent if the specific activity of one protein was within two-fold of the other. Results showed that the estimated specific activity was 3.7 U/mg protein for the MON 89788-produced CP4 EPSPS, and 4.4 U/mg protein for the *E. coli*-produced reference standard. The enzymatic activity assay demonstrated that the MON 89788-produced CP4 EPSPS

protein was as active as the *E. coli*-produced reference standard. These results confirmed that these two proteins are functionally equivalent.

1.6. CP4 EPSPS Glycosylation Equivalence

As many eukaryotic proteins are post-translationally modified with carbohydrate moieties (Rademacher et al., 1988), glycosylation analysis was conducted to further demonstrate the equivalence between *E. coli*- and MON 89788-produced CP4 EPSPS proteins. Since non-virulent *E. coli* strains used for cloning and expression purposes lack the ability to glycosylate endogenous proteins, the *E. coli*-produced CP4 EPSPS was used as the negative control for glycosylation analysis. The positive control was represented by transferrin protein that was known to have multiple covalently linked carbohydrate modifications on each molecule. The transferrin protein, as well as the purified CP4 EPSPS proteins isolated from MON 89788 and *E. coli* were separated on SDS-PAGE, and western blot analysis was performed to detect oxidized carbohydrate moieties on the proteins (method in Appendix B).

Results of this analysis are presented in Figure VI-4. No carbohydrate moieties were detected for CP4 EPSPS protein isolated from either *E. coli* or MON 89788 (lanes 5-6 and lanes 7-8, respectively). As expected, carbohydrate moieties covalently lined to transferrin were detected at the expected transferrin molecular weight of ~75 kDa (lanes 3 and 4). The additional lower molecular weight fragments in lanes 3 and 4 are likely to be the proteolytic fragments of the full-length protein. In addition, a faint band migrating at approximately 44 kDa was observed in lane 5 through lane 8. Since it was established that the *E. coli* strains used in the expression system were non-virulent, and lack the ability to glycosylate recombinant proteins (Letourneur et al., 1995), this faint band observed across *E. coli*- and MON 89788-CP4 EPSPS samples was deemed nonspecific. Taken together, the results demonstrated that, similar to the *E. coli*-produced CP4 EPSPS, the MON 89788-produced CP4 EPSPS protein is not glycosylated. This analysis also confirms the equivalence between the MON 89788- and the *E. coli*-produced CP4 EPSPS reference standard with respect to the status of glycosylation.

1.7. Conclusions

The CP4 EPSPS protein isolated from MON 89788 was purified and characterized, and results confirmed the equivalence between MON 89788- and *E. coli*-produced CP4 EPSPS proteins. The apparent molecular weight was estimated by SDS-PAGE. Since the MON 89788-derived CP4 EPSPS migrated comparably to the *E. coli*-produced protein on SDS-PAGE, the apparent molecular weight of these two proteins was determined to be equivalent. This result is consistent with the deduced amino acid sequence based on the DNA sequence analysis. On the basis of western blot analysis, the electrophoretic mobility and immunoreactive properties of the MON 89788-produced CP4 EPSPS protein were demonstrated to be comparable to those of the *E. coli*-produced CP4 EPSPS reference standard. The N-terminus of the CP4 EPSPS derived from MON 89788 was consistent with the predicted amino acid sequence translated from the *cp4 epsps* coding sequence, and the MALDI-TOF mass spectrometry analysis also yielded peptide masses consistent with the expected peptide masses from the translated *cp4 epsps*

coding sequence. In addition, the MON 89788- and the *E. coli*-produced CP4 EPSPS reference standard were found to be equivalent based on functional activities and the lack of glycosylation. Taken together, these data provide a detailed characterization of the CP4 EPSPS protein isolated from MON 89788 and established its equivalence to the *E. coli*-produced CP4 EPSPS protein standard. Furthermore, since all CP4 EPSPS proteins isolated from other Roundup Ready crops have established equivalence to the *E. coli*-produced protein standard previously, by inference, the MON 89788-derived CP4 EPSPS protein is likely to possess equivalent biochemical and physiological characteristics with the CP4 EPSPSs expressed in other Roundup Ready crops, all of which have completed the consultation processes.

Table VI-1. N-terminal Amino Acid Sequence Analysis of the CP4 EPSPS Protein Purified from Grain Tissue of MON 89788

Amino acid residue # from the N-terminus	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Predicted CP4 EPSPS Sequence[1, 2]	M	L	H	G	A	S	S	R	P	A	T
	|	|	|	|	|	|	|	|	|	|	|
Observed Sequence-1[3,4]	M	X	H	G	A	X	S	(R)	(P)	(A)	(T)
Observed Sequence-2[3,4]		L	H	G	A	S	S	(R)	(P)	X	X

1 The predicted amino acid sequence of the CP4 EPSPS protein was deduced from the coding region of the full length *cp4 epsps* gene present in MON 89788.

2 The single letter IUPAC-IUB amino acid code is **A**, alanine; **G**, glycine; **H**, histidine; **L**, leucine; **M**, methionine; **P**, proline; **R**, arginine; **S**, serine, and **T,** threonine.

3 The amino acids in parentheses ( ) were tentatively designated due to high background noises. The undesignated amino acids are shown as "X" due to interferences from other amino acids.

4 Observed sequence-1 and -2 were identified after comparison to the predicted CP4 EPSPS protein sequence.

```
  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-3. MALDI-TOF Coverage Map of the CP4 EPSPS Protein Isolated from MON 89788 Grain

Tryptic masses identified by MALDI-TOF are boxed. These identified masses yielded a coverage map equal to 50.3% (229 of 455 amino acids) of the full-length CP4 EPSPS protein, which is considered sufficient to confirm the identity of the MON 89788-produced CP4 EPSPS protein.

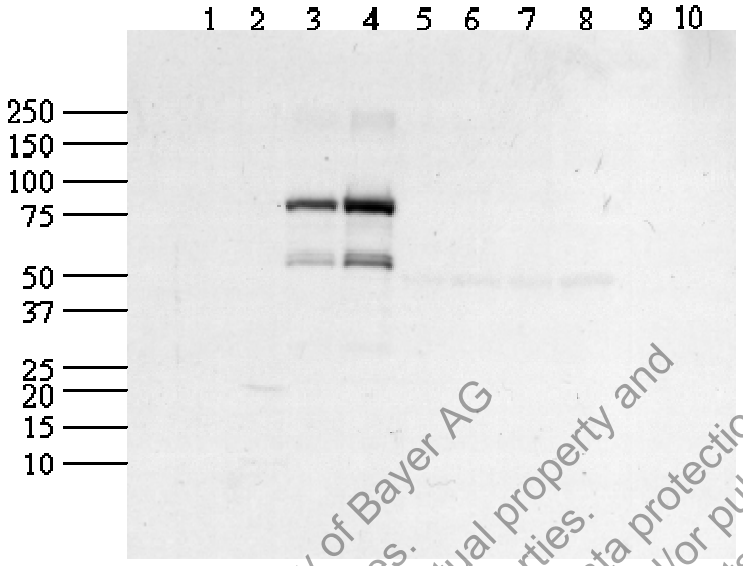


Figure VI-4. Glycosylation Analysis of the CP4 EPSPS Protein Isolated from MON 89788 Grain

Aliquots of the MON 89788-produced CP4 EPSPS protein, *E. coli*-produced CP4 EPSPS reference standard (negative control), and transferrin (positive control) were separated by denaturing tris-glycine 4→20% PAGE and electrotransferred to PVDF membrane. Approximate molecular weights (kDa) in the figure correspond to the markers loaded in lane 2. Amount below refers to total protein loaded per lane for transferrin, and purity-corrected protein values for the *E. coli*- and the MON 89788-produced proteins.

Lane	Sample	Amount (µg)
1	No Protein Control	N/A
2	MW Markers (Precision Plus Dual Color)	N/A
3	Transferrin (positive control)	0.5
4	Transferrin (positive control)	1
5	*E. coli*-produced CP4 EPSPS protein (negative control)	0.5
6	*E. coli*-produced CP4 EPSPS protein (negative control)	1
7	MON 89788-produced CP4 EPSPS protein	0.6
8	MON 89788-produced CP4 EPSPS protein	1.1
9	Empty Lane	N/A
10	Empty Lane	N/A

Section 2. Levels of the CP4 EPSPS Protein in MON 89788

CP4 EPSPS protein levels in tissues derived from MON 89788 were determined by a validated enzyme-linked immunosorbent assay (ELISA). The levels of the CP4 EPSPS protein in over-season leaf (OSL), grain, root, and forage were determined in tissues collected from MON 89788 produced in replicated field trials across five U.S. field locations during 2005. CP4 EPSPS protein levels for all tissue types were calculated on a µg/g fresh weight (FW) basis. Moisture content was determined in each tissue type, and protein levels in these tissues were converted to a dry weight (DW) basis by calculation. Materials and methods are described in detail in Appendix C.

For MON 89788, the mean CP4 EPSPS protein levels across sites for OSL1, OSL2, OSL3, OSL4, grain, root, and forage were 300, 340, 330, 290, 150, 74, and 220 µg/g DW, respectively (Table VI-2). The levels of the CP4 EPSPS protein from the conventional control (A3244) were less than the assay limits of detection (LOD) in all tissue types. The mean CP4 EPSPS expression level in grain from MON 89788 is lower than that from Roundup Ready soybean (Padgette et. al., 1995).

Table VI-2. Summary of CP4 EPSPS Protein Levels in Tissue Collected from MON 89788 Produced in the U.S. During 2005

Tissue Type	CP4 EPSPS µg/g FW (SD)[1]	Range[2] (µg/g FW)	CP4 EPSPS µg/g DW (SD)[3]	Range[2] (µg/g DW)	LOQ / LOD (µg/g FW)
OSL1[4]	54 (7.8)	40 – 66	300 (51)	220 – 380	0.57 / 0.26
OSL2[4]	60 (10)	42 – 80	340 (55)	250 – 440	0.57 / 0.26
OSL3[4]	58 (11)	40 – 79	330 (94)	200 – 520	0.57 / 0.26
OSL4[4]	75 (17)	60 – 110	290 (48)	210 – 390	0.57 / 0.26
Grain	140 (20)	98 – 170	150 (22)	110 – 180	0.34 / 0.26
Root	22 (6.0)	13 – 38	74 (27)	41 – 150	0.57 / 0.11
Forage	59 (14)	41 – 94	220 (51)	140 – 330	0.57 / 0.10

1. Protein quantities are expressed as mean µg of CP4 EPSPS/g tissue on a fresh weight (FW) basis. The mean and standard deviation (SD) were calculated across all sites.
2. Minimum and maximum values across all sites.
3. Protein quantities are expressed as mean µg of CP4 EPSPS/g tissue on a dry weight (DW) basis. The dry weight values were calculated by dividing the fresh weight values by the dry weight conversion factors obtained from moisture analysis data.
4. OSL1 to OSL4 represent over-season leaves collected at the following developmental stages: OSL1: V3-V4 growth stage; OSL2: V6-V8 growth stage; OSL3: V10-V12 growth stage; OSL4: V14-V16 growth stage.

Note: Sample number is 14 for forage, and 15 each for OSL1 to OSL4, grain, and root.

Section 3. Assessment of the Potential for Allergenicity of the CP4 EPSPS Protein Produced in MON 89788

This assessment of the allergenic potential of the CP4 EPSPS protein compares the biochemical characteristics of this protein to characteristics of known allergens. A protein is not likely to be an allergen if:

1. The protein is from a non-allergenic source;
2. The protein does not represent a relatively large portion of the total protein;
3. The protein does not share structural similarities to known allergens based on the amino acid sequence;
4. The protein is unstable to digestion in simulated gastric fluid.

The following sections address each of these questions for the CP4 EPSPS protein produced in MON 89788, and demonstrate that this protein is not likely to be allergenic. General information on the methods used in assessment of the structural similarity to known allergens and toxins, and stability in simulated digestive fluids is provided in Appendix D.

3.1. Source of the CP4 EPSPS Protein

As described earlier, the *cp4 epsps* coding sequence was obtained from a naturally occurring soil bacterium and has been identified by the American Type Culture Collection as an *Agrobacterium* species. As there are no reports of allergies to *Agrobacterium* species (see section 4.1 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.

3.2. CP4 EPSPS as a Proportion of Total Protein

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, soybean grain is the most relevant to the assessment of food allergenicity. Data presented in Section 2 of Part VI show that the mean CP4 EPSPS protein level in MON 89788 grain was 150 µg/g DW (Table VI-2), and the mean percent total protein in MON 89788 grain was 40.32% DW (Table VII-2, combine site). Results indicate that the CP4 EPSPS protein represents only approximately 0.037% of the total protein in MON 89788 grain (150 µg/g ÷ 403,000 µg × 100% = 0.037%). Therefore, the CP4 EPSPS protein represents only a small portion of the total protein in MON 89788 grain.

3.3. Bioinformatic Analyses of Sequence Similarity of the CP4 EPSPS protein produced in MON 89788 to Allergens

In 2003, the Codex Alimentarius Commission published guidelines for the evaluation of the potential allergenicity of novel proteins (Codex Alimentarius, 2003). The guideline is based on the comparison of amino acid sequences between introduced proteins and

allergens, where potential allergenic cross-reactivity may exist if the introduced protein is found to have at least 35% amino acid identity with an allergen over any segment of at least 80 amino acids. The Codex guideline also recommended that a sliding window search with a scientifically justified peptide size could be used to identify immunologically relevant peptides in otherwise unrelated proteins. The extent of sequence similarities between the CP4 EPSPS protein present in MON 89788 and known allergens, gliadins and glutenins was assessed using the FASTA sequence alignment tool and an eight-amino acid sliding window search (Thomas et al., 2004 Codex Alimentarius, 2003). The data generated from these analyses confirm that the CP4 EPSPS protein does not share any amino acid sequence similarities with known allergens, gliadins, or glutenins.

The allergen database 5 (AD5; Monsanto internal database; see Appendix D) was used for the evaluation of sequence similarities shared between the CP4 EPSPS protein and all proteins. Using the FASTA sequence alignment tool, proteins were ranked according to their degree of similarity to CP4 EPSPS. None of the proteins in the AD5 database met or exceeded the threshold of 35% identity over 80 amino acids. One low quality alignment between CP4 EPSPS and the dust mite allergen Der f II (GI number 546852) was identified, where five gaps were needed to align a stretch of 82 amino acids with 30% identity. This alignment revealed that the length of the overlap was very short (18%) when compared to full length (455 amino acids) CP4 EPSPS protein, and the alignment had an *E*-score of 0.66. The *E*-score (expectation score) is a statistical measure of the likelihood that the observed similarity score could have occurred by chance in a search. A larger *E*-score indicates a lower degree of similarity between the query sequence and the sequence from the database. Typically, alignments between two sequences will need to have an *E*-score of 1×10^{-5} or smaller to be considered to have significant homology. This *E*-score of 0.66 is not reflective of homology between CP4 EPSPS and Der f II, as *E*-scores of ~1 are expected to occur for alignments between random, non-homologous sequences (Pearson, 2000). Therefore, this low quality alignment is considered not relevant from an allergenic assessment perspective. Inspection of the remaining 23 alignments also did not show any significant similarities between the CP4 EPSPS protein and other allergens.

A second bioinformatics tool, an eight-amino acid sliding window search, was used to specifically identify short linear polypeptide matches to known or suspected allergens. It is possible that proteins structurally unrelated to allergens, gliadins, and glutenins may still contain smaller immunologically significant epitopes. An amino acid sequence may be considered to have allergenic potential if it has an exact sequence identity of at least eight linearly contiguous amino acids with a potential allergen epitope (Metcalfe et al., 1996; Hileman et al., 2002). Using a sliding window of less than eight amino acids can produce matches containing significant uncertainty depending on the length of the query sequence (Silvanovich et al., 2006) and are not be useful to the allergy assessment process (Thomas et al., 2004).

An eight-mer search was performed using an algorithm (ALLERGENSEARCH) that was developed to identify whether or not a linearly contiguous match of eight amino acids

existed between the CP4 EPSPS sequence and amino acid sequences within the allergen database (AD5). This program compares the CP4 EPSPS sequence to each protein sequence in the allergen database using a sliding-window of eight amino acids; that is, with a seven-amino acid overlap relative to the preceding window. No alignments of eight contiguous amino acid identities were detected when the CP4 EPSPS protein sequence was compared to all sequences in the AD5 database.

Together, these data demonstrate and confirm that the CP4 EPSPS protein does not share any relevant amino acid sequence similarities with known allergens, gliadins, or glutenins.

3.4. Stability of the CP4 EPSPS Protein in Simulated Gastric Fluids

Harrison et al. (1996) demonstrated that the CP4 EPSPS protein is rapidly degraded in simulated digestive fluids. Based on western blot analysis, the half-life of CP4 EPSPS was less than 15 seconds in the gastric system and less than 10 minutes in the intestinal system. Therefore, if any of the CP4 EPSPS protein were to survive in the gastric system, it would be degraded in the intestine. As a comparison, it has been estimated that 50% of solid food was digested in the human stomach within two hours, while 50% of liquid was digested within 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 confirm the *in vitro* digestibility of the CP4 EPSPS protein in simulated gastric fluid (SGF) using a standardized method published by the International Life Science Institute (ILSI; Thomas et al., 2004; Appendix E). As with the previous study by Harrison et al. (1996), the *E. coli*-produced CP4 EPSPS protein was used, and the digestibility was assessed by colloidal blue staining, western blot analysis, and EPSPS enzymatic activity assay.

Results of these experiments confirmed that the *E. coli*-produced CP4 EPSPS protein was rapidly digested after incubation in SGF. The SDS-PAGE colloidal blue gel staining method demonstrated that greater than 98% of the CP4 EPSPS protein was digested within 15 seconds (Figure VI-5). Western blot analysis confirmed that greater than 95% of the *E. coli*-produced CP4 EPSPS protein was digested in SGF within 15 seconds (Figure VI-6). Likewise, the EPSPS activity was reduced to <10% within 15 seconds of incubation of the CP4 EPSPS protein in SGF (Table VI-3). In summary, the results concluded that the *E. coli*-produced CP4 EPSPS protein was rapidly degraded in simulated gastric fluid and is unlikely to pose a human health concern.

3.5. Conclusions

The data and information provided in this section address the questions important to the assessment of allergenic potential of CP4 EPSPS protein that has been introduced into a number of products currently in commerce. These products include Roundup Ready soybean, Roundup Ready canola, and Roundup Ready Flex cotton. As the allergenicity

assessment for the current product, MON 89788, is largely identical to those provided previously for the other Roundup Ready crops, the current assessment focuses on employing latest methodology and updated databases to further confirm the previous conclusion regarding the safety of the CP4 EPSPS protein. To summarize, there are no reports of allergies to the donor organism, the *Agrobacterium* species; thus, the CP4 EPSPS protein is not from a known allergenic source. The CP4 EPSPS protein represents only approximately 0.037% of the total protein in MON 89788 grain. Since the CP4 EPSPS protein represents only a small portion of the total protein in MON 89788 grain, it is not likely to be an allergenic protein. The updated bioinformatic analysis confirmed that the CP4 EPSPS protein did not share significant amino acid sequence similarities with known allergens or gliadins. Therefore, it is unlikely that CP4 EPSPS contains allergenic epitopes. In addition, analyses using *E. coli*-produced CP4 EPSPS protein demonstrated that it was rapidly digested in simulated digestive fluids, a characteristic shared among proteins with a history of safe consumption. As the CP4 EPSPS protein equivalence from MON 89788- and *E. coli*-derived sources has been established, the digestibility of the MON 89788-produced CP4 EPSPS can be inferred. Taken together, the updated assessment on allergenic potential reaffirms the earlier conclusion that the CP4 EPSPS protein expressed in MON 89788, as in other Roundup Ready crops, does not pose a significant allergenic risk.

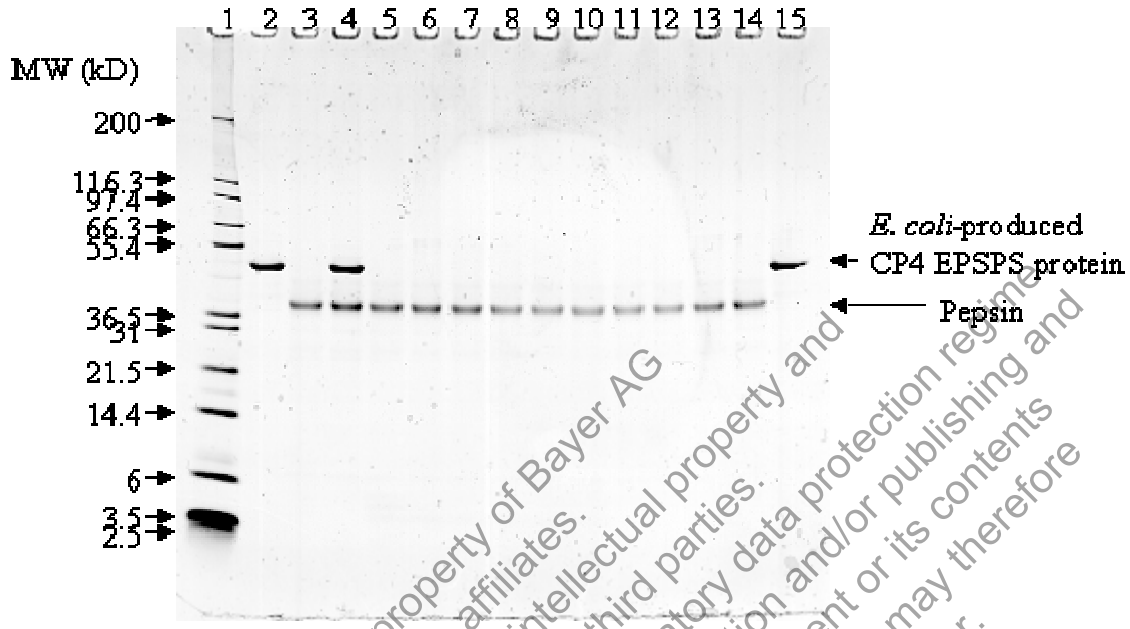


Figure VI-5. Colloidal Blue Stained SDS-PAGE Gel Showing the Digestion of Purified *E. coli*-produced CP4 EPSPS Protein in Simulated Gastric Fluid
Proteins were separated by SDS-PAGE using a 10→20% polyacrylamide gradient in a tricine buffered gel. Proteins were detected by staining with Brilliant Blue 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

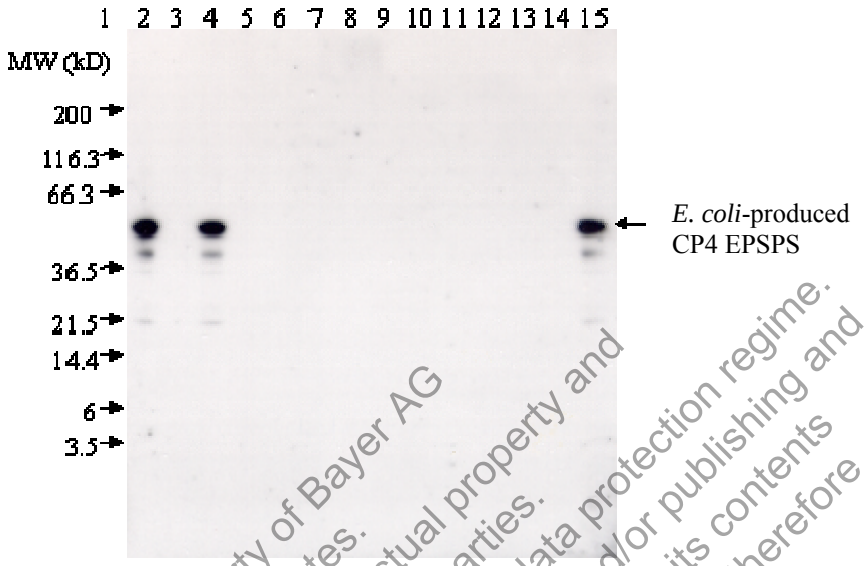


Figure VI-6. Western Blot Showing the Digestion of Purified *E. coli*-produced CP4 EPSPS Protein in Simulated Gastric Fluid
Proteins were separated by SDS-PAGE using a 10→20% polyacrylamide gradient in a tricine buffered gel. *E. coli*-produced CP4 EPSPS protein was loaded at 1 ng per lane based on 90%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

Lane	Description	Incubation Time
1	Molecular weight markers	
2	Experimental control without pepsin (P0)	0 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

Table VI-3. 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

Section 4. Safety Assessment of the CP4 EPSPS Protein in MON 89788

The previous section demonstrated the lack of structural similarity of the CP4 EPSPS protein to known allergens and 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 lack of 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, an evaluation of the similarity of the CP4 EPSPS protein to EPSPSs naturally present in foods, and to the CP4 EPSPS protein in other Roundup Ready crops.

4.1. 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 protein (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 exerts its functions in the shikimate pathway that is 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 found widely in food and feed derived from plant and microbial sources. Genes for numerous EPSPSs have been cloned (Padgette et al., 1996), and the catalytic domains of this group of proteins are conserved. 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, and the CP4 and native plant 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 2 (1996), Roundup Ready sugar beet (1998), and Roundup Ready Flex cotton (2005). 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 (EPA, 1996).

4.2. Structural Similarity of the CP4 EPSPS to Other Proteins

Potential structural similarities shared between the CP4 EPSPS protein and proteins in the ALLPEPTIDES database (Appendix D) 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 of structural similarities (i.e., secondary and tertiary protein structures). Proteins that share a high degree of similarity throughout the entire sequence are often homologous, and homologous proteins usually have common secondary and tertiary structures. Identified proteins were ranked according to their degree of similarity to the CP4 EPSPS. As expected, the most significant alignment was to the CP4 EPSPS protein found in Roundup Ready soybean (Accession No. AY125353), demonstrating 100% identity over a 455 amino acid overlap window with an *E*-score of 1.4×10^{-165}. This result was expected as the CP4 EPSPS proteins in Roundup Ready soybean and MON 89788 are identical. All the remaining alignments with significant *E*-scores (i.e., $<1\times10^{-5}$) were to other members of the EPSPS protein family. These results indicate that CP4 EPSPS does not share significant sequence similarity with any protein that is known to cause adverse biological activity in humans and animals.

Potential structural similarities shared between the CP4 EPSPS protein and proteins in the toxin database (TOXIN5; Appendix D) 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 amino acid overlap window with an *E*-score of 0.26. Since the length and quality of the alignments are low (i.e., *E*-score $>1\times10^{-5}$), these data demonstrate that the CP4 EPSPS protein is highly unlikely to share any structural homology to any known toxins.

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.

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

The CP4 EPSPS protein present in MON 89788 is similar to EPSPSs consumed in a variety of food and feed sources. As shown in Table VI-4, 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*), all of 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. Furthermore, the ubiquitous presence of homologous EPSPS enzymes in food crops and common microorganisms establishes that EPSPS proteins, and their enzyme activity, pose no hazards for human and animal consumption.

Table VI-4. Comparison of the Deduced Amino Acid Sequence of Native CP4 EPSPS to that of other EPSPSs

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

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

CP4 EPSPS-containing crops have been commercialized and consumed as foods and feeds since their initial introduction in 1996. Roundup Ready soybean represents a significant portion of commodity crops containing CP4 EPSPS protein, which was grown on 89% of the U.S. soybean area in 2005. Globally, Roundup Ready soybean was produced on approximately 134 million acres, which represented 60% of global soybean area (James, 2005). By inference, 60% of the soybean and soybean products consumed globally were likely to contain CP4 EPSPS protein.

In addition to soybean, CP4 EPSPS protein is also expressed in other Roundup Ready crops that are used primarily as food and feed sources. These products include Roundup Ready Corn 2, Roundup Ready canola, Roundup Ready sugar beet, Roundup Ready alfalfa, and a small portion of Roundup Ready cotton. These Roundup Ready crops were planted on greater than 42 million acres globally either as Roundup Ready-trait or as combined-trait products in 2005 (James, 2005).

In all, Roundup Ready crops were grown on approximately 25% of the global crop acres, and CP4 EPSPS is likely to be present in one quarter of the foods and feeds derived from these crops. As described above, CP4 EPSPS-containing food and feed products have been consumed since 1996. This demonstrates significant experience with the safe use of these Roundup Ready crops and the CP4 EPSPS protein they contain, and supports the safe use of MON 89788 and the CP4 EPSPS protein it contains.

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

Most known protein toxins act through acute mechanisms to exert toxicity (Sjoblad et al., 1992; Pariza and Johnson, 2001, Hammond and Fuchs, 1998). The exceptions to this rule include certain anti-nutritional proteins such as lectins and protease inhibitors, which typically require a short-term (2-4 week) feeding study to manifest toxicity (Liener, 1983). Since CP4 EPSPS is not similar to any of these antinutritional proteins, an acute oral mouse study was considered appropriate to confirm its lack of mammalian toxicity.

In the acute study conducted by Harrison et al. (1996), CP4 EPSPS protein was administered as a single dose by gavage to groups of 10 male and 10 female CD-1 mice at dose levels up to 572 mg/kg. The CP4 EPSPS protein was obtained from *E. coli* and was demonstrated to be equivalent to that produced by MON 89788 (Part VI, Section I). There were no treatment-related effects on survival, clinical observations, body weight gain, food consumption or gross pathology. Therefore, the No Observable Effect Level

(NOEL) for CP4 EPSPS was considered to be equal to or greater than 572 mg/kg, the highest dose tested.

4.6. Estimate of Dietary and Margin of Exposure

CP4 EPSPS safety assessment involved calculation of the potential exposure of humans to the protein derived from MON 89788 as the primary source of CP4 EPSPS in the diet. Although CP4 EPSPS protein is already produced in a number of commercial Roundup Ready crops, an acute dietary exposure assessment was conducted nonetheless to assess the potential exposure level to CP4 EPSPS protein from consumption of MON 89788 soybeans. The amount of soy-derived foods consumed in the U.S. that could potentially contain CP4 EPSPS protein from MON 89788 soybeans was estimated using the Dietary Exposure Evaluation Model (DEEM-FCID version 2.03, Exponent Inc.) and food consumption data from the 1994-1996 and 1998 USDA Continuing Survey of Food Intakes by Individuals (CSFII). DEEM-FCID differentiates soybean consumption into four fractions: grain, flour, milk and oil. However, since soybean oil contains negligible amounts of protein (Tattrie and Yaguchi, 1973), only the grain, flour and milk fractions are considered potential dietary sources of CP4 EPSPS and were included in this assessment. Furthermore, for the purposes of this assessment, all soybean food items consumed were assumed to be derived from MON 89788 soybeans, i.e., no adjustments were made for anticipated market share.

According to the DEEM-FCID analysis, 95% of the overall U.S. population consumes no more than 0.071 g/kg body weight of soybean grain, flour or milk on any one day. The highest value was for non-nursing infants, for whom the 95th percentile consumption was 2.79 g/kg body weight.

As soybean is a blended commodity, the mean level of CP4 EPSPS protein in each of the relevant food fractions (grain, flour and milk) should be used when estimating total intake of CP4 EPSPS from consumption of MON 89788. However, specific values for each of these fractions are not available. Thus, the concentration of CP4 EPSPS protein for each of these fractions was assumed to be equal to the mean concentration of CP4 EPSPS measured in whole soybean, which was 140 μg/g FW (Table VI-2).

The assumption that the mean concentrations of CP4 EPSPS in the consumed food fractions are comparable to that in the intact grain is highly conservative. Soybeans contain certain factors, such as trypsin inhibitors, which may act as antinutrients if the soybeans are not properly heated during preparation (Rackis, 1974). Thus, virtually all protein-containing soybean fractions are heated during processing prior to consumption by humans and most animals (Liener, 1996). However, as CP4 EPSPS protein is less likely to be detected after heat treatment (Ahmed, 2002), presumably denatured after exposure to heat, the amounts of functionally active CP4 EPSPS protein present in consumed soybean products will be substantially lower than assumed for this evaluation.

Utilizing the above highly conservative estimate of the amount of CP4 EPSPS protein in processed soybean fractions, and the assumption that all soybean grain, flour and milk consumed in one day are derived from MON 89788, the 95th percentile estimate for acute

dietary intake (ADIE) of CP4 EPSPS from consumption of MON 89788 is 9.9 and 391 μg/kg body weight for the overall U.S. population and non-nursing infants, respectively, based on the following calculation:

ADIE of CP4 EPSPS from MON 89788 =
Soybean product consumption (g/kg body weight) × CP4 EPSPS concentration (μg/g)

By using estimates of CP4 EPSPS exposure/intake/consumption and the acute NOEL of 572 mg/kg for CP4 EPSPS, it is possible to calculate the margin of exposure (MOE) for consumption of CP4 EPSPS protein from MON 89788. The margin of exposure is defined as the ratio of the NOEL derived from toxicology test to the estimate of human exposure. The MOE is computed as follows:

MOE = NOEL (mg/kg) × 1000 / ADIE (μg/kg)

Utilizing these assumptions, the MOEs were determined to be approximately 58,000 for the overall U.S. population and 1,500 for non-nursing infants, the most highly exposed subpopulation. These large margins of exposure indicate that there is no meaningful risk to human health from dietary exposure to CP4 EPSPS from consumption of MON 89788 soybeans. In addition, as the CP4 EPSPS expression level in MON 89788 grain is lower than that of current commercial Roundup Ready soybean, the MOEs from consumption of MON 89788 would be greater than those from consumption of current Roundup Ready soybeans.

Table VI-5. Margins of Exposure for Acute Dietary Consumption of CP4 EPSPS Proteins in MON 89788 in the U.S.

Parameter	Adults	Non-nursing Infants
Soybean consumption* (g/kg body wt)	0.071	2.79
ADIE (μg/ kg body wt)	9.9	391
MOE	58,000	1,500

* Oil fraction was not included, and numbers are based on 95th percentile estimation.

4.7. Conclusions

Studies and evaluations were performed to assess the safety of the CP4 EPSPS protein in MON 89788. The donor organism, *Agrobacterium* sp. strain CP4, is not known as a human or animal pathogen, and is not commonly allergenic. Additionally, *Agrobacterium* sp. strain CP4 and the CP4 EPSPS protein it produces have been reviewed previously as a part of the safety assessment of the donor organism for other Roundup Ready crops. Using updated bioinformatics analyses, no biologically relevant

similarities in protein sequences were observed between the CP4 EPSPS protein and pharmacologically active proteins. This result indicated the lack of potential toxicity for CP4 EPSPS based on protein sequence analysis. Additional protein sequence comparisons between EPSPS proteins indicated that CP4 EPSPS shared high degree (~50%) of sequence similarity to other EPSPSs naturally present in foods and feeds that have a history of safe consumptions. Furthermore, since the CP4 EPSPS expressed in MON 89788 is identical to that of Roundup Ready soybean, the safe use of the protein in soybean has been demonstrated.

The lack of acute toxicity was confirmed by the mouse gavage study where the NOEL for CP4 EPSPS protein was determined to be 572 mg/kg. The dietary exposure margins based on the NOEL and the 95th percentile soybean intakes were determined to be at least 58,000- and 1,500-fold for adults and non-nursing infants, respectively, assuming all soybean products were derived from MON 89788. In addition, as MON 89788 expresses a lower level of CP4 EPSPS protein in the grain than Roundup Ready soybean, there is an increased margin of exposure resulting from consumption of MON 89788 compared to consumption of Roundup Ready soybean products.

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 89788. It is concluded that the data and information provided in Section 5 and supported by other data and information in Part VI demonstrate that the CP4 EPSPS protein in MON 89788 is safe for human and animal consumption.

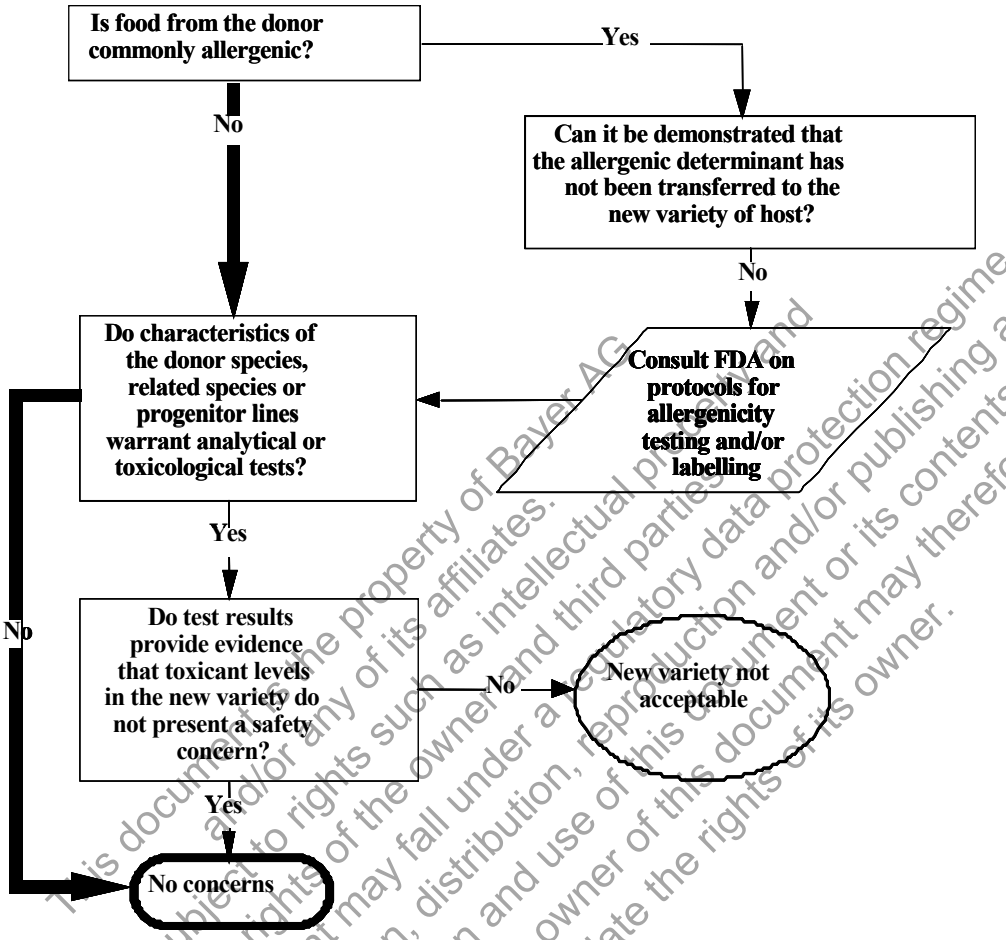


Figure VI-7. Safety Assessment of New Varieties: The Donor

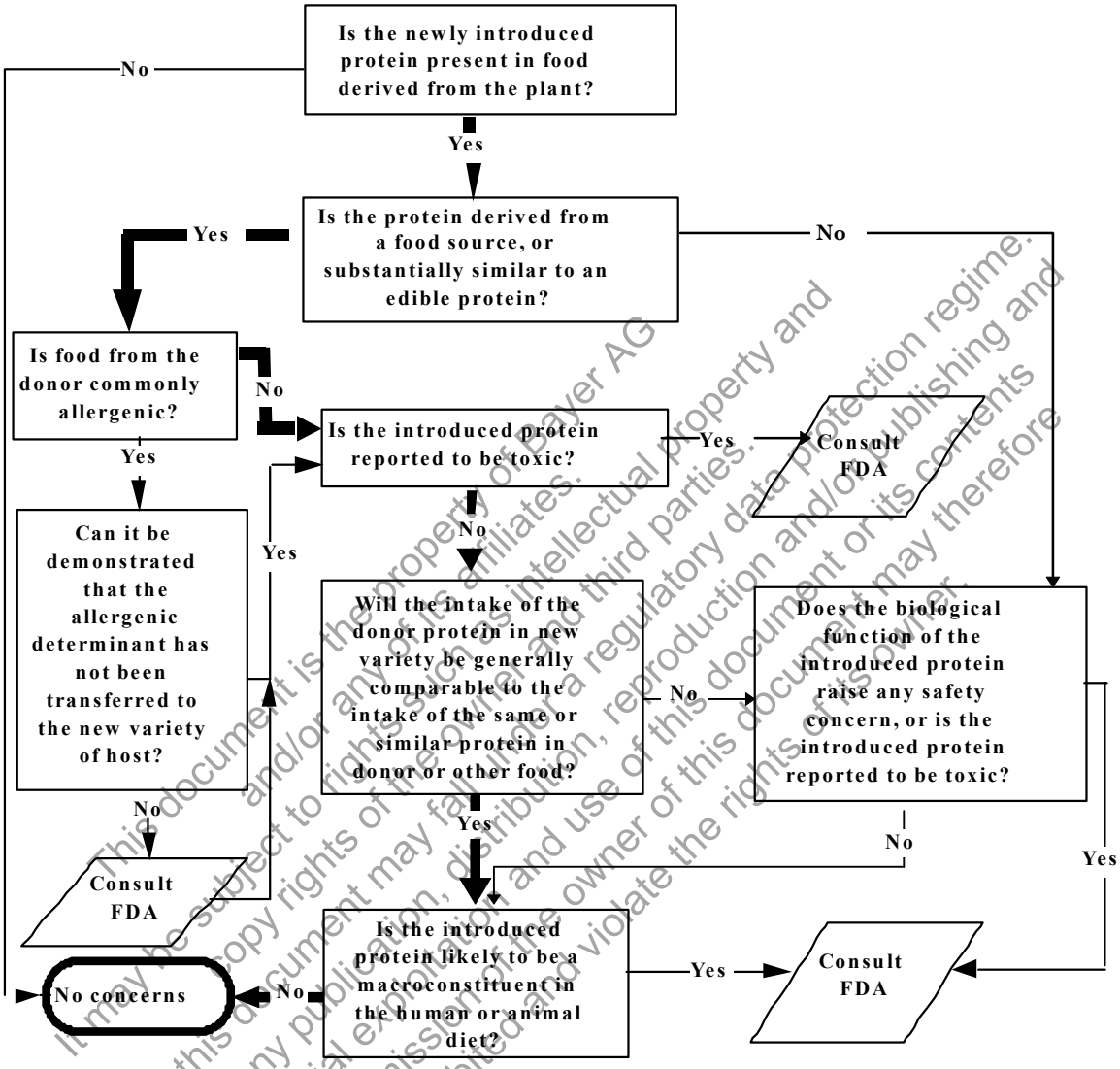


Figure VI-8. Safety Assessment of New Varieties: Proteins Introduced from the Donor

PART VII: FOOD / FEED SAFETY AND NUTRITIONAL ASSESSMENT OF MON 89788

Section 1. Soybean Varieties as the Comparable Food and Feed

Soybeans are used widely for food and feed purposes, and it is intended that MON 89788 will be utilized in the same manner and for the same uses as conventional soybeans. To assess whether the Roundup Ready trait in MON 89788 caused any unintended effects on the composition of the soybean grain, compositional analyses were conducted on MON 89788 soybean grain and forage grown under replicated field conditions at five U.S. locations during the 2005 season. Levels of the various analytes assessed from MON 89788 were compared to that of A3244, a conventional soybean variety, where the A3244 has background genetics representative of MON 89788 but does not contain the *cp4 epsps* gene cassette or produce the CP4 EPSPS protein. Additionally, grain and forage from 12 conventional soybean varieties produced in the same field trials alongside MON 89788 and A3244 were also subjected to compositional analyses. Values derived from these conventional varieties were used as references to generate a 99% tolerance interval for each of the analytes for conventional soybean. MON 89788 was found to be compositionally equivalent to conventional soybeans and thus is as safe as conventional soybeans for uses in food and feed applications.

Section 2. Uses of Soybean

In 2004, soybeans accounted for 59% of the global oilseed production, with the U.S. representing 36% of the world soybean production (Soya and Oilseeds Bluebook, 2005). Soybeans were planted on 75 million acres in the U.S. in 2004, yielding over 80 million metric tons with an approximate crop value of over $[redacted] dollars (Soya and Oilseeds Bluebook, 2005).

Soybean is the main source of plant protein consumed by humans and animals, and is also the leading source of vegetable oil of all crops produced in the world. Utilization of soybean and its derived products is not limited to food and feed, as it has been used for the manufacture of multiple industrial products including soaps, inks, paints, disinfectants and biodiesel (Cahoon, 2003). Although the use of soybean for industrial purposes is economically significant, it consumes only 4% of the total U.S. soybean oil produced (Soy Stats, 2005). Consequently, food and feed uses of soybean and its processed products remain the predominant use of soybeans produced in the U.S. and globally.

2.1. History of Soybean Utilization

In addition to the description below regarding the history and uses of soybeans as foods and feeds, the OECD Consensus document on soybean compositional considerations (OECD, 2001) provides an overview of the uses of whole and processed soybean fractions in food applications.

The history and development of soybean has been discussed previously in Part IV, Section 1.2., where it indicated that soybeans originated in northern China. During the course of soybean domestication, the Chinese began using soybeans for the preparation of various types of soyfoods, including soymilk, tofu, soy sauce, soy paste, and had started to consume soybean sprout (Liu, 2004b). As soybeans were introduced to neighboring countries through migrations and through sea and land trade, the soyfood products and the methods of product preparation were also spread into Korea, Japan, and other regions in Asia (Hymowitz et al., 1990). As a result, additional soyfood products such as tempeh and natto were also developed outside of China (Nout and Kiers, 2004).

The types of traditional soyfoods mentioned above were all made from whole soybeans for human consumption. In the U.S., however, human and animal consumption of soybean are primarily in the forms of processed fractions such as soybean oil and protein ingredients.

2.2. Soybean as a Food Source

Soybean has the remarkable ability to produce more edible protein per acre of land than any other known crop (Liu, 2004a). On average, dry soybean contains roughly 40% protein and 20% oil, and it has the highest protein content among cereals and other legume species, and has the second-highest oil content among all food legumes. Soybean is highly versatile and can be processed into a wide variety of food products. In general, soyfoods can be roughly classified into four major categories:

(a) Traditional soyfoods – as discussed above, traditional soyfoods are primarily made from whole soybeans. The non-fermented traditional soyfoods may include soymilk, tofu, and soybean sprouts, and the fermented soyfoods that include soybean paste (miso), soy sauce, natto, and tempeh.

(b) Soybean oil – soybean oil constitutes ~80% of the total annual consumption of edible fats and oil in the U.S. (Soy Stats, 2005), and is still the largest source of vegetable oil worldwide (USDA, 2006). Refined, bleached, and deodorized soybean can be further processed to produce cooking oils, shortening, margarine, mayonnaise, salad dressings, and a wide variety of products that are either based entirely on fats and oils or contain fat or oil as a principal ingredient.

(c) Soy protein products – soy protein products are made from defatted soy flakes, and they include soy flour, soy protein concentrate, and soy protein isolate. Soy flour has a protein content of ~50%, and is mainly used as an ingredient in the bakery industry. Soy protein concentrate has a protein content of ~70%, and is widely used in the meat industry as a key ingredient of meat alternative products such as soy burgers and meatless "meatballs". Soy protein isolate has a protein content of 90%, and possesses many functional properties such as gelation and emulsification. As a result, it can be used in a wide range of food applications, including soup, sauce bases, energy bars, nutritional beverages, infant formula, and dairy replacements.

(d) Dietary supplements – soybeans are a rich source of certain phytochemicals used as dietary supplements, which include isoflavones, tocopherols. Isoflavones have been shown to inhibit the growth of cancer cells, lower cholesterol levels, and inhibit bone resorption (Messina, 1999; Setchell and Cassidy, 1999). Tocopherols have long been recognized as a classic free radical scavenging antioxidant whose deficiency impairs mammalian fertility. In addition, new biological activities have been reported for the desmethyl tocopherols, such as γ-tocopherol, to possess anti-inflammatory, antineoplastic, and natriuretic functions (Schafer et al., 2003; Hensley et al., 2004; IFIC, 2006). Detailed reviews of soybeans as functional foods can be found at IFIC (2005) and Liu (2004b).

2.3. Soybean as a Feed Source

Soybean meal is the most valuable component obtained from processing the soybean, accounting for roughly 50-75% of its overall value (USDA, 2006). Soybean meal is produced by solvent extraction of the dehulled soybean flakes, and the spent flakes (soybean flakes with the oil removed) are conveyed to a desolventizer-toaster for removal of the hexane. The process involves heating the spent flakes to evaporate the hexane and utilizing steam to carry away hexane vapors. This process also provides toasting of the meal to inactivate enzymes like urease and trypsin inhibitors that may reduce the digestibility and nutritional value of the meal. The meal is dried to about 13 to 14 percent moisture subsequently, and is screened and ground to produce a uniform particle size prior to shipment to the end user. The finished meal from dehulled soybeans will contain less than 1.5% crude fat and approximately 48% protein, and is referred to as high protein meal (SMIC, 2006).

Soybean meal is the premier supplemental protein source in U.S. livestock and poultry rations due to its nutrient composition, availability, and price. Typically, soybean meal is used to meet the animal's requirement for limiting amino acids, as it is the most cost-effective source of amino acids. Soybean meal is also one of the best protein sources for complementing the limiting amino acid profile of corn protein (Kerley and Allee, 2003). Due to the high value and versatility, approximately two-thirds of the total protein meal use in the world is derived from soybeans, with the remainder divided between rapeseed, cottonseed, sunflower, peanut, and other meals (Soy Stats, 2005). Poultry and swine account for most of the soybean meal utilized in the U.S., with poultry consuming 50%, swine 26%, cattle 18%, and 6% for pet foods and other feed uses (Soy Stats, 2005).

Dairy and livestock producers need an inexpensive, readily available, on-farm source of high-quality, high-protein forage adapted to growth during the summer months when other forage legume species typically are restricted in growth. Soybean forage provides livestock and dairy producers with a valuable new source of high-protein feed for their livestock (USDA-ARS, 2006), and it can be used as hay or to produce silage (MAFRI, 2004).

Section 3. Comparison of the Composition and Nutritional Components of MON 89788

3.1. Levels of Significant Nutrients, Antinutrients, and Other Components in Soybean Grain and Forage

Compositional analyses were conducted to assess whether the nutrient and anti-nutrient levels in grain and forage tissues derived from MON 89788 are comparable to those in the conventional soybean variety, A3244, which has background genetics similar to MON 89788 but does not contain the *cp4 epsps* gene cassette. Additional conventional soybean varieties currently in the marketplace were also included in the analysis to establish a range of natural variability for each analyte, where the range of variability is defined by a 99% tolerance interval for that particular analyte. Results of the comparisons indicate that MON 89788 is compositionally and nutritionally equivalent to conventional soybean varieties currently in commerce.

Grain and forage tissues of MON 89788 and A3244 were harvested from soybeans grown in three replicated plots at each of five field sites across the U.S. during 2005. The field sites were located in regions that were conducive to the growth of soybean maturity group III varieties, and were representative of commercial soybean production. In addition, 12 conventional soybean varieties were also included as references where three varieties were grown at each of two sites and two varieties were grown at each of three sites for a total of 12 references. The 12 conventional soybean reference varieties were included to provide data for the development of a 99% tolerance interval for each component analyzed. For each compositional component, 99% tolerance interval was calculated. This interval is expected to contain, with 95% confidence, 99% of the values obtained from the population of commercial references. It is important to establish the 99% tolerance interval from representative conventional soybean varieties for each of the analytes, because such data illustrate the compositional variability naturally occurring in commercially grown varieties. By comparison to the 99% tolerance interval, any statistically significant differences between MON 89788 and the control (A3244) may be put into perspective, and can be assessed for biological relevance in the context of the natural variability in soybean. Additional information on the field design and reference varieties is presented in Appendix F.

A total of 63 components were analyzed in grain and forage samples. Components for forage samples included proximates (protein, fat, ash, and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), and carbohydrates by calculation. Components for grain samples included proximates (protein, fat, ash, and moisture), ADF, NDF, amino acids, fatty acids (C8-C22), phytic acid, trypsin inhibitor, isoflavones, lectins, raffinose, stachyose, Vitamin E, and carbohydrates by calculation. The methods employed for these analyses are presented in Appendix F.

Statistical analyses of the compositional data were conducted using a mixed model analysis of variance with data from each of five sites, and a combination of all five field sites. Each individual analyte for MON 89788 was compared to that of the conventional

control, A3244, for each of the five sites and for the combination of all five sites (i.e., the combined-site). The statistical significance is defined at the level of $p<0.05$. Of the 63 components analyzed, 14 minor fatty acids had greater than 50% of the analytical values that were below the limit of quantitation. These fatty acids are known to occur at low or non-detectable levels in soybean oil (Codex Standard, 2005), and were not included in the statistical analyses.

Statistical analyses of the remaining 49 components (63 minus the 14) between MON 89788 and A3244 were conducted. The overall data set was examined for evidence of biologically relevant changes. Based on this evaluation and the results of statistical analyses, analytes for which the levels were not statistically different were deemed to be present at equivalent levels between MON 89788 and A3244. Analyses using data from the combination of all five sites (combined-site) indicated that there were no statistical differences in the levels of 92% of the analytes (45 of the 49). Statistical analyses for the combined-site data are presented in Table VII-1 for forage and Table VII-2 for grain. Analyses using the five single-site analyses indicated that there were no statistically significant differences in the levels of 91% of the analytes (223 of the 245) between MON 89788 and A3244. Individual site composition data and statistical analyses are presented in Appendix G. In addition, the means of amino acids normalized against total amino acids and total protein are presented in Appendix H-1, and the means of fatty acids normalized against total fatty acids and total fat are presented in Appendix H-2.

For the combined-site analyses, statistical differences between MON 89788 and A3244 were observed for four analytes, which included forage moisture, and grain daidzein, glycitein, and Vitamin E (Table VII-3). The differences observed are generally small (1.6 – 11%), and the mean levels of MON 89788 are well within the 99% tolerance intervals for the conventional soybeans. The mean levels of MON 89788 grain daidzein, glycitein, and Vitamin E are also well within the ranges for conventional soybeans reported in the International Life Science Institute Crop Composition Database (ILSI-CCD; ILSI, 2004) as well as in the literature. The mean levels of forage moisture for both MON 89788 and A3244 are below that of the ILSI-CCD and literature ranges; however, the difference between MON 89788 and A3244 is only 1.6%. Therefore, it was concluded that MON 89788 and A3244 are compositionally and nutritionally equivalent based on analyses of the combined-site data. The reported ILSI-CCD ranges and the published literature ranges for the analytical components present in soybeans are summarized in Table VII-4 for forage and Table VII-5 for grain, respectively.

The reproducibility and trends across sites were also examined, and comparisons to conventional soybean varieties using the 99% tolerance intervals were made. There were no analytes that were consistently and statistically different across sites. Statistically significant differences were observed in as many as two sites for only one analyte, raffinose. Since the differences observed were lower for MON 89788 at one site (AR) while higher at the other (IL-2), and there is no evidence of any trend across sites, it is concluded that the statistical differences are not biologically relevant.

For the remaining 16 analytes where statistically significant differences were observed in only one site, the differences between MON 89788 and A3244 were not reproducible across sites, and no consistent trends were observed. In addition, all mean levels of MON 89788 analytes were well within the 99% tolerance interval for conventional soybeans that were grown concurrently in all sites. It is concluded that these analytes where the statistical differences were observed in only site were not biologically different between MON 89788 and A3244.

Based on the data and information presented above, it was concluded that soybean grain and forage derived from MON 89788 are compositionally and nutritionally equivalent to those of the conventional soybeans. The few statistical differences between MON 89788 and A3244 are likely to reflect the natural variability of the components since the mean levels of analytes for MON 89788 are well within the 99% tolerance intervals for conventional soybeans, and within the ranges in ILSI-CCD and in literature.

Table VII-1. Statistical Summary of Combined-Site Soybean Forage Fiber and Proximate Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Fiber						
Acid Detergent Fiber (% DW)	36.82 (2.35) [30.95 - 45.99]	38.23 (2.37) [31.18 - 50.89]	-1.41 (1.88) [-11.96 - 4.12]	-6.63, 3.81	0.494	(29.64 - 50.69) [19.03, 54.55]
Neutral Detergent Fiber (% DW)	36.37 (0.80) [32.77 - 41.12]	38.25 (0.86) [32.69 - 43.14]	-1.88 (1.17) [-9.45 - 6.95]	-4.29, 0.53	0.121	(31.43 - 43.70) [26.89, 46.89]
Proximate						
Ash (% DW)	6.76 (0.38) [5.20 - 8.45]	6.65 (0.39) [5.28 - 7.95]	0.11 (0.36) [-1.40 - 2.10]	-0.90, 1.12	0.775	(5.36 - 8.36) [3.50, 9.58]
Carbohydrates (% DW)	67.28 (1.06) [61.61 - 71.00]	67.40 (1.08) [64.55 - 72.30]	-0.12 (0.55) [-3.34 - 4.46]	-1.30, 1.07	0.837	(62.57 - 72.28) [55.96, 77.90]
Fat (% DW)	5.87 (0.70) [4.20 - 9.49]	6.11 (0.70) [3.96 - 8.60]	-0.24 (0.17) [-0.93 - 0.88]	-0.60, 0.12	0.176	(3.51 - 9.87) [0, 14.70]
Moisture (% FW)	72.07 (1.25) [67.90 - 77.60]	73.21 (1.25) [69.90 - 77.60]	-1.14 (0.21) [-2.60 - 0]	-1.72, -0.55	0.006	(68.50 - 78.40) [60.84, 83.36]
Protein (% DW)	20.08 (0.51) [18.41 - 23.50]	19.79 (0.52) [17.47 - 22.18]	0.29 (0.47) [-3.75 - 2.34]	-1.00, 1.58	0.572	(16.48 - 22.78) [13.55, 25.95]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, tolerance interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Table VII-2. Statistical Summary of Combined-Site Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	1.77 (0.017) [1.56 - 1.87]	1.77 (0.018) [1.71 - 1.83]	-0.0035 (0.018) [-0.19 - 0.069]	-0.042, 0.035	0.845	(1.62 - 1.89) [1.51, 2.00]
Arginine (% DW)	3.06 (0.082) [2.73 - 3.31]	3.07 (0.083) [2.76 - 3.34]	-0.0095 (0.037) [-0.26 - 0.33]	-0.090, 0.071	0.801	(2.61 - 3.27) [2.27, 3.60]
Aspartic Acid (% DW)	4.73 (0.068) [4.20 - 5.08]	4.72 (0.070) [4.42 - 4.98]	0.0072 (0.045) [-0.41 - 0.33]	-0.090, 0.10	0.875	(4.21 - 5.02) [3.85, 5.44]
Cystine (% DW)	0.62 (0.0084) [0.58 - 0.67]	0.62 (0.0085) [0.59 - 0.65]	-0.00028 (0.0050) [-0.044 - 0.026]	-0.011, 0.010	0.955	(0.57 - 0.65) [0.55, 0.67]
Glutamic Acid (% DW)	7.53 (0.12) [6.69 - 8.20]	7.49 (0.13) [6.97 - 7.90]	0.035 (0.075) [-0.63 - 0.53]	-0.13, 0.20	0.647	(6.62 - 8.19) [5.86, 8.96]
Glycine (% DW)	1.78 (0.020) [1.58 - 1.88]	1.78 (0.021) [1.71 - 1.86]	0.0012 (0.018) [-0.18 - 0.11]	-0.037, 0.040	0.949	(1.62 - 1.90) [1.46, 2.05]
Histidine (% DW)	1.07 (0.014) [0.95 - 1.13]	1.07 (0.015) [1.02 - 1.13]	-0.0035 (0.0099) [-0.10 - 0.057]	-0.025, 0.018	0.729	(0.96 - 1.13) [0.90, 1.21]
Isoleucine (% DW)	1.83 (0.029) [1.65 - 1.97]	1.83 (0.031) [1.70 - 1.99]	-0.0092 (0.030) [-0.22 - 0.26]	-0.071, 0.053	0.760	(1.64 - 2.00) [1.44, 2.16]

Table VII-2 (continued). Statistical Summary of Combined-Site Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	3.18 (0.040) [2.81 - 3.39]	3.18 (0.042) [3.04 - 3.33]	-0.0024 (0.031) [-0.32 - 0.20]	-0.070, 0.065	0.940	(2.89 - 3.42) [2.62, 3.66]
Lysine (% DW)	2.62 (0.025) [2.33 - 2.76]	2.62 (0.026) [2.51 - 2.73]	-0.00003 (0.023) [-0.25 - 0.13]	-0.051, 0.050	0.998	(2.40 - 2.77) [2.22, 2.95]
Methionine (% DW)	0.52 (0.0059) [0.47 - 0.56]	0.53 (0.0062) [0.50 - 0.55]	-0.0081 (0.0060) [-0.040 - 0.032]	-0.021, 0.0049	0.200	(0.45 - 0.56) [0.42, 0.60]
Phenylalanine (% DW)	2.10 (0.030) [1.84 - 2.24]	2.10 (0.031) [2.00 - 2.19]	-0.0011 (0.021) [-0.21 - 0.14]	-0.047, 0.045	0.959	(1.90 - 2.29) [1.70, 2.45]
Proline (% DW)	2.05 (0.029) [1.81 - 2.21]	2.05 (0.029) [1.95 - 2.16]	0.0047 (0.020) [-0.18 - 0.12]	-0.039, 0.048	0.819	(1.86 - 2.23) [1.66, 2.38]
Serine (% DW)	2.23 (0.029) [1.93 - 2.42]	2.21 (0.030) [2.08 - 2.28]	0.019 (0.023) [-0.16 - 0.17]	-0.031, 0.069	0.432	(1.99 - 2.42) [1.84, 2.54]
Threonine (% DW)	1.58 (0.014) [1.42 - 1.68]	1.59 (0.015) [1.51 - 1.66]	-0.0073 (0.013) [-0.13 - 0.062]	-0.035, 0.020	0.573	(1.44 - 1.67) [1.38, 1.76]
Tryptophan (% DW)	0.39 (0.015) [0.34 - 0.44]	0.39 (0.015) [0.33 - 0.46]	-0.0025 (0.015) [-0.10 - 0.064]	-0.044, 0.039	0.875	(0.30 - 0.47) [0.25, 0.54]

Table VII-2 (continued). Statistical Summary of Combined-Site Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	1.41 (0.019) [1.25 - 1.48]	1.42 (0.020) [1.33 - 1.47]	-0.0091 (0.015) [-0.12 - 0.070]	-0.051, 0.033	0.582	(1.28 - 1.51) [1.18, 1.64]
Valine (% DW)	1.91 (0.035) [1.73 - 2.05]	1.93 (0.036) [1.77 - 2.11]	-0.017 (0.032) [-0.24 - 0.28]	-0.084, 0.051	0.615	(1.71 - 2.09) [1.51, 2.27]
Fatty Acid (% DW)						
16:0 Palmitic (% DW)	2.07 (0.094) [1.84 - 2.40]	2.07 (0.094) [1.71 - 2.46]	-0.0027 (0.052) [-0.21 - 0.24]	-0.14, 0.14	0.961	(1.66 - 2.35) [1.32, 2.64]
18:0 Stearic (% DW)	0.78 (0.027) [0.65 - 0.89]	0.77 (0.027) [0.61 - 0.86]	0.012 (0.018) [-0.053 - 0.14]	-0.036, 0.060	0.531	(0.63 - 1.07) [0.37, 1.28]
18:1 Oleic (% DW)	3.53 (0.14) [3.05 - 4.24]	3.54 (0.14) [2.92 - 4.09]	-0.015 (0.10) [-0.40 - 0.51]	-0.29, 0.26	0.890	(2.99 - 5.29) [2.06, 6.43]
18:2 Linoleic (% DW)	9.17 (0.47) [8.00 - 10.42]	9.25 (0.47) [7.42 - 11.29]	-0.079 (0.21) [-0.86 - 0.99]	-0.64, 0.48	0.720	(8.41 - 10.69) [7.75, 11.22]
18:3 Linolenic (% DW)	1.29 (0.063) [1.09 - 1.48]	1.30 (0.063) [1.09 - 1.60]	-0.0059 (0.028) [-0.13 - 0.15]	-0.082, 0.070	0.843	(1.02 - 1.55) [0.84, 1.69]
20:0 Arachidic (% DW)	0.061 (0.0026) [0.049 - 0.071]	0.060 (0.0026) [0.046 - 0.068]	0.0012 (0.0016) [-0.0048 - 0.012]	-0.0031, 0.0055	0.482	(0.046 - 0.076) [0.031, 0.094]

Table VII-2 (continued). Statistical Summary of Combined-Site Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Fatty Acid (% DW)						
20:1 Eicosenoic (% DW)	0.042 (0.0031) [0.032 - 0.050]	0.042 (0.0031) [0.029 - 0.053]	0.00036 (0.0013) [-0.0062 - 0.0073]	-0.0032, 0.0039	0.796	(0.030 - 0.057) [0.021, 0.065]
22:0 Behenic (% DW)	0.063 (0.0030) [0.050 - 0.072]	0.062 (0.0031) [0.046 - 0.071]	0.00094 (0.0014) [-0.0056 - 0.0096]	-0.0029, 0.0048	0.539	(0.046 - 0.073) [0.034, 0.091]
Fiber						
Acid Detergent Fiber (% DW)	18.01 (0.94) [14.64 - 23.94]	17.46 (0.95) [14.39 - 22.44]	0.54 (1.21) [-3.22 - 5.67]	-2.79, 3.88	0.676	(13.30 - 26.26) [9.62, 28.57]
Neutral Detergent Fiber (% DW)	18.18 (0.46) [16.38 - 20.49]	19.11 (0.48) [15.60 - 20.73]	-0.93 (0.60) [-3.35 - 2.77]	-2.34, 0.49	0.165	(14.41 - 23.90) [13.26, 26.33]
Isoflavones						
Daidzein (ug/g DW)	993.67 (114.34) [631.32 - 1571.41]	1073.57 (114.79) [747.53 - 1526.23]	-79.90 (30.47) [-272.18 - 106.63]	-146.14, -13.66	0.021	(274.88 - 1485.52) [0, 1925.63]
Genistein (ug/g DW)	797.90 (49.93) [565.26 - 996.66]	824.83 (50.35) [651.01 - 1003.02]	-26.93 (19.52) [-151.16 - 74.36]	-69.66, 15.81	0.193	(354.09 - 984.29) [0, 1387.95]
Glycitein (ug/g DW)	91.77 (9.88) [53.78 - 162.52]	102.61 (10.01) [72.93 - 148.31]	-10.84 (4.69) [-32.97 - 30.19]	-20.98, -0.70	0.037	(52.72 - 298.57) [0, 287.45]
Proximate						
Ash (% DW)	5.04 (0.12) [4.66 - 5.60]	5.03 (0.12) [4.75 - 5.46]	0.0099 (0.073) [-0.81 - 0.42]	-0.14, 0.16	0.892	(4.61 - 5.57) [4.00, 6.08]

Table VII-2 (continued). Statistical Summary of Combined-Site Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Proximate						
Carbohydrates (% DW)	37.07 (0.54) [35.01 - 40.24]	36.88 (0.56) [35.17 - 40.74]	0.20 (0.55) [-2.38 - 2.95]	-1.30, 1.69	0.738	(32.75 - 40.98) [27.86, 45.79]
Fat (% DW)	17.57 (0.74) [15.35 - 19.98]	17.72 (0.74) [14.40 - 20.91]	-0.15 (0.42) [-1.74 - 1.73]	-1.28, 0.99	0.745	(15.97 - 20.68) [15.38, 21.95]
Moisture (% FW)	7.76 (0.47) [6.41 - 9.35]	7.51 (0.47) [6.51 - 9.63]	0.25 (0.27) [-0.44 - 1.31]	-0.51, 1.01	0.417	(6.24 - 9.11) [4.64, 9.94]
Protein (% DW)	40.32 (0.72) [37.31 - 42.54]	40.38 (0.73) [36.96 - 42.44]	-0.069 (0.31) [-1.72 - 2.44]	-0.74, 0.60	0.828	(36.48 - 43.35) [31.50, 47.45]
Vitamin						
Vitamin E (mg/100g DW)	2.71 (0.22) [1.88 - 3.72]	2.52 (0.22) [1.58 - 3.07]	0.19 (0.065) [-0.23 - 0.66]	0.043, 0.33	0.015	(1.29 - 4.80) [0, 7.00]
Antinutrient						
Lectin (H.U./mg FW)	4.29 (0.97) [0.70 - 9.77]	4.55 (1.01) [1.44 - 10.87]	-0.26 (1.02) [-8.11 - 5.75]	-2.38, 1.86	0.800	(0.45 - 9.95) [0, 9.72]
Phytic Acid (% DW)	0.76 (0.035) [0.58 - 0.93]	0.75 (0.037) [0.51 - 1.07]	0.011 (0.044) [-0.24 - 0.30]	-0.084, 0.11	0.811	(0.41 - 0.96) [0.39, 1.07]
Raffinose (% DW)	0.52 (0.063) [0.40 - 0.71]	0.54 (0.063) [0.31 - 0.83]	-0.014 (0.041) [-0.20 - 0.11]	-0.13, 0.099	0.751	(0.26 - 0.84) [0, 1.01]

Table VII-2 (continued). Statistical Summary of Combined-Site Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Antinutrient						
Stachyose (% DW)	2.36 (0.070)	2.50 (0.073)	-0.15 (0.10)	-0.38, 0.085	0.183	(1.53 - 2.98)
	[2.02 - 2.85]	[2.12 - 3.04]	[-0.59 - 0.53]			[1.19, 3.31]
Trypsin Inhibitor (TIU/mg DW)	33.69 (2.84)	31.44 (2.88)	2.25 (1.56)	-2.32, 6.81	0.231	(20.79 - 55.51)
	[24.59 - 53.85]	[23.43 - 41.91]	[-4.81 - 13.99]			[5.15, 59.34]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Table VII-3. Summary of Statistical Differences between Component Levels of MON 89788, A3244 and Conventional Varieties

Analytical Component (Units)[1]	MON 89788 Mean	A3244 Mean	Difference (MON 89788 minus A3244) % of A3244	p-Value	MON 89788 (Range)	Conventional Tol. Int.[2]
Statistical Differences Observed in Combined-Site Analyses						
Forage Moisture (% FW)	72.07	73.21	-1.55	0.006	[67.90 - 77.60]	[60.84, 83.36]
Daidzein (ug/g DW)	993.67	1073.57	-7.44	0.021	[631.32 - 1571.41]	[0, 1925.63]
Glycitein (ug/g DW)	91.77	102.61	-10.56	0.037	[53.78 - 162.52]	[0, 287.45]
Vitamin E (mg/100g DW)	2.71	2.52	7.41	0.015	[1.88 - 3.72]	[0, 7.00]
Statistical Differences Observed in More Than One Site and Not in the Combined-Site						
Site AR Raffinose (% DW)	0.65	0.81	-20.02	0.024	[0.58 - 0.71]	[0, 1.01]
Site IL-2 Raffinose (% DW)	0.42	0.33	25.45	0.035	[0.40 - 0.43]	[0, 1.01]
Statistical Differences Observed in One Site and Not in the Combined-Site						
Site AR Phenylalanine (% DW)	2.00	2.01	-0.41	0.014	[2.00 - 2.01]	[1.70, 2.45]
Site AR Palmitic (% DW)	2.21	2.40	-7.73	0.004	[2.17 - 2.25]	[1.32, 2.64]
Site AR Stearic (% DW)	0.76	0.81	-5.43	0.024	[0.75 - 0.77]	[0.37, 1.28]
Site AR Oleic (% DW)	3.30	3.68	-10.31	0.001	[3.24 - 3.36]	[2.06, 6.43]
Site AR Linoleic (% DW)	10.27	11.02	-6.86	0.005	[10.06 - 10.42]	[7.75, 11.22]
Site AR Linolenic (% DW)	1.45	1.55	-6.16	0.029	[1.41 - 1.48]	[0.84, 1.69]

Table VII-3 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 89788 vs. A3244 and Conventional Reference Varieties

Analytical Component (Units)[1]	MON 89788 Mean	A3244 Mean	Difference (MON 89788 minus A3244) % of A3244	p-Value	MON 89788 (Range)	Conventional Tol. Int.[2]
Statistical Differences Observed in One Site and Not in the Combined-Site						
Site AR Arachidic (% DW)	0.060	0.064	-6.35	0.021	[0.058 - 0.060]	[0.031, 0.094]
Site AR Eicosenoic (% DW)	0.048	0.053	-8.60	0.032	[0.047 - 0.049]	[0.021, 0.065]
Site AR Behenic (% DW)	0.066	0.070	-5.85	0.034	[0.064 - 0.068]	[0.034, 0.091]
Site AR ADF (% DW)	21.17	16.10	31.47	0.003	[19.28 - 23.94]	[9.62, 28.57]
Site AR Carbohydrates (% DW)	38.13	36.02	5.88	0.048	[37.77 - 38.42]	[27.86, 45.79]
Site AR Fat (% DW)	18.82	20.41	-7.79	0.002	[18.42 - 19.17]	[15.38, 21.95]
Site AR Stachyose (% DW)	2.32	2.83	-18.13	0.010	[2.10 - 2.50]	[1.19, 3.31]
Site IL-2 Genistein (ug/g DW)	762.46	849.88	-10.29	0.032	[721.05 - 797.84]	[0, 1387.95]
Site IL-2 Grain Moisture (% FW)	8.53	7.48	14.04	0.045	[8.19 - 9.13]	[4.64, 9.94]
Site NE Grain NDF (% DW)	17.42	19.91	-12.51	0.023	[16.79 - 18.39]	[13.26, 26.33]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid.
[2]With 95% confidence, tolerance interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Table VII-4. Literature and Historical Ranges for Components in Soybean Forage

Tissue/Component[1]	Literature Range[2]	ILSI Range[3]
Proximates (% DW)		
Ash	8.8-10.5[a]	6.718-10.782
Carbohydrates	not available	59.8-74.7
Fat, total	3.1-5.1[a]	1.302-5.132
Moisture (% FW)	74-79[f]	73.5-81.6
Protein	11.2-17.3[a]	14.38-24.71
Fiber (% DW)		
Acid detergent fiber (ADF)	32-38[a]	not available
Neutral detergent fiber (NDF)	34-40[a]	not available
Crude fiber	not available	13.58-31.73

[1] FW=fresh weight; DW=dry weight;
[2] Literature range references: [a]OECD, 2001.
[3] ILSI Soybean Database, 2004.
Conversions: % DW × 10^4 = µg/g DW; mg/g DW × 10^3 = mg/kg DW; mg/100g DW × 10 = mg/kg DW; g/100g DW × 10 = mg/g DW

Table VII-5. Literature and Historical Ranges for Components in Soybean Grain

Tissue/Component[1]	Literature Range[2]	ILSI Range[3]
Proximates (% DW)		
Ash	4.61-5.94[b]; 4.29-5.88[a]	3.885-6.542
Carbohydrates	29.3-41.3[a]	29.6-50.2
Fat, total	198-277[c] g/kg DW; 160-231[d] g/kg DW	8.104-23.562
Moisture (% FW)	5.3-8.73[a] , 5.18-14.3[b]	5.1-14.9
Protein	329-436[c] g/kg DW; 360-484[d] g/kg DW	33.19-45.48
Fiber (% DW)		
Acid detergent fiber (ADF)	not available	7.81-18.61
Neutral detergent fiber (NDF)	not available	8.53-21.25
Crude fiber	5.74-7.89[a]	4.12-10.93
Amino Acids (mg/g DW = % DW × 10)		
Alanine	16.0-18.6[a,h]	15.13-18.51
Arginine	25.6-34.6[a,h]	22.85-33.58
Aspartic acid	41.8-49.9[a,h]	38.08-51.22
Cystine/Cysteine	5.4-6.6[a,h]	3.70-8.08
Glutamic acid	66.4-81.6[a,h]	58.43-80.93
Glycine	16.0-18.7[a,h]	14.58-18.65
Histidine	9.8-11.6[a,h]	8.78-11.75
Isoleucine	16.5-19.5[a,h]	15.63-20.43
Leucine	28.1-33.7[a,h]	25.90-33.87
Lysine	24.7-28.4[a,h]	22.85-28.39
Methionine	5.1-5.9[a,h]	4.31-6.81
Phenylalanine	17.8-21.9[a,h]	16.32-22.36
Proline	18.6-22.3[a,h]	16.87-22.84
Serine	19.6-22.8[a,h]	16.32-24.84
Threonine	15.1-17.3[a,h]	12.51-16.18
Tryptophan	5.6-6.3[a,h]	3.563-5.016
Tyrosine	13.5-15.9[a,h]	10.16-15.59
Valine	17.1-20.2[a,h]	16.27-22.04

Table VII-5 (continued). Literature and Historical Ranges for Components in Soybean Grain

Tissue/Component[1]	Literature Range[2]	ILSI Range[3]
Fatty Acids (% DW)		
12:0 Lauric	not available	not available
14:0 Myristic	not available	not available
16:0 Palmitic	1.44-2.31[f]	not available
16:1 Palmitoleic	not available	not available
17:0 Heptadecanoic	not available	not available
17:1 Heptadecenoic	not available	not available
18:0 Stearic	0.54-0.91[f]	not available
18:1 Oleic	3.15-8.82[f]	not available
18:2 Linoleic	6.48-11.6[f]	not available
18:3 Linolenic	0.72-2.16[f]	not available
20:0 Arachidic	0.04-0.7[f]	not available
20:1 Eicosenoic	not available	not available
20:2 Eicosadienoic	not available	not available
22:0 Behenic	not available	not available
Vitamins (mg/100g)	**FW[i]**	**DW**
Vitamin E	0.85[g]	0.47-6.17
Anti-Nutrients		
Lectin (H.U./mg FW)	0.8-2.4[a]	0.105-9.038
Trypsin Inhibitor (TIU/mg DW)	33.2-54.5[a]	19.59-118.68
Raffinose	not available	0.212-0.661
Stachyose	not available	1.21-3.50
Isoflavones	**mg/100g FW**	**(mg/kg DW)**
Daidzein	9.88-124.2[e]	60.0-2453.5
Genistein	13-150.1[e]	144.3-2837.2
Glycitein	4.22-20.4[e]	15.3-310.4

[1] FW=fresh weight; DW=dry weight;
[2] Literature range references: [a]Padgette et al., 1996. [b]Taylor et al., 1999. [c]Maestri et al., 1998. [d]Hartwig and Kilen, 1991. [e]USDA-ISU Isoflavone Database, 2002. [f]OECD, 2001. [g]USDA-NND, 2005. [h]Data converted from g/100g DW to mg/g DW. [i]Moisture value = 8.54g/100g.
[3] ILSI Soybean Database, 2004.
Conversions: % DW × 10^4 = µg/g DW; mg/g DW × 10^3 = mg/kg DW; mg/100g DW × 10 = mg/kg DW; g/100g DW × 10 = mg/g DW

3.2. Levels of Naturally Occurring Anti-nutrients

Soybean grain contains several well-described anti-nutritional factors according to OECD (2001), which include: trypsin inhibitors, lections, isoflavones (daidzein, genistein and glycitein), stachyose, raffinose, and phytic acid. The levels of these components were analyzed in MON 89788, and the resulting means were compared to those from the conventional control, A3244.

The overall data set was examined for evidence of biologically relevant changes. In addition, statistical analyses were applied to assess significant differences of each analyte at $p<0.05$. As described previously, greater than 90% of the comparisons showed no statistically significant differences between MON 89788 and A3244. The few observed statistical differences were not considered biologically relevant since these differences were not reproducible across sites and no consistent trends were observed. Furthermore, the mean levels of MON 89788 analytes were all well within the 99% tolerance intervals, and were within the ranges in ILSI-CCD (ILSI, 2004) and literature. The statistical summaries for the anti-nutrients are captured under the grain analyses, where the combined-site data are presented in Table VII-2, and the individual-site data are in Appendix G. The analytes that are statistically different between MON 89788 and A3244 are presented in Table VII-3. Reported literature and ILSI-CCD ranges for the analytical components present in soybean grain is in Table VII-5.

Trypsin inhibitors are heat-labile anti-nutrients that interfere with the digestion of proteins and result in decreased animal growth (Liener, 1994). Lectins are also heat labile, and it can inhibit growth and cause death in animals if raw soybeans are consumed (Liener, 1994). Both trypsin inhibitor and lectins are inactivated during processing of soybean protein products or soybean meal, and if processed appropriately, the final edible soybean fractions should contain minimal levels of these anti-nutrients. Composition analyses of the grain indicated that both trypsin inhibitors and lectins were present at similar levels in MON 89788 and A3244, and no statistical differences were observed for all comparisons.

There are three basic categories of isoflavones in soybean grain, namely, daidzein, genistein, and glycitein. Although they have been reported to possess biochemical activities including estrogenic, anti-estrogenic and hypocholesterolemic effects, it is not universally accepted that the isoflavones are anti-nutrients as they have also been reported to have beneficial anti-carcinogenic effects as described in Part VII, Section 2 and in additional literature (OECD, 2001). Daidzein and glycitein levels were observed to be statistically different between MON 89788 and A3244 in the combined-site analyses. These results are not unexpected as it is well-documented that the soybean isoflavone levels are greatly influenced by many factors, ranging from environmental conditions, variety, and agronomic practices (Messina, 2001; Nelson et al., 2001). As discussed in Part VII, Section 3.1., the statistical differences are not biologically meaningful since the mean levels of daidzein and glycitein in MON 89788 are well within the 99% tolerance intervals for the conventional soybeans, and within the literature and ILSI-CCD ranges. Therefore, these differences do not raise any nutritional,

anti-nutritional or other biological or toxicological concerns and are not considered biologically relevant.

Stachyose and raffinose are low molecular weight carbohydrates present in soybean grain that are considered to be anti-nutrients due to the gas production and resulting flatulence caused by consumption. Although there were statistical differences observed for stachyose and raffinose between MON 89788 and A3244, as discussed in Section 3.1., the differences were not consistent across sites, and no trends were observed. Therefore, the statistically differences for stachyose and raffinose are considered not reproducible and hence not biologically significant.

Phytic acid is present in soybean grain and it chelates mineral nutrients, including calcium, magnesium, potassium, iron and zinc, rendering them biologically unavailable to monogastric animals consuming the grain (Liener, 2000). Unlike trypsin inhibitors, phytic acid is not heat labile, and remains stable through most soybean processing steps. For MON 89788, there were no significant differences observed in phytic acid levels when compared to those of A3244.

Based on the data and information presented above, it was concluded that MON 89788 is compositionally equivalent to conventional soybeans with regards to the anti-nutrients in soybean grain. These data further confirm that MON 89788 soybeans are compositionally and nutritionally equivalent to conventional soybeans.

3.3. 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 89788 compared to other conventional soybean varieties other than the introduced *cp4 epsps* coding sequence and the production of a CP4 EPSPS protein that confers tolerance to glyphosate. The analyses of soybean grain and forage composition (63 components) have shown no biologically meaningful differences between MON 89788 and the control, A3244, or with conventional soybean varieties. Given this extensive compositional characterization, it is concluded that no pleiotropic changes have occurred in MON 89788, and that MON 89788 is compositionally and nutritionally equivalent to conventional soybeans already on the market.

Section 4. Other Information Relevant to the Safety and Nutritional Assessment of MON 89788

Having demonstrated the compositional equivalence of soybean grain and forage derived from MON 89788 to grain and forage derived from conventional soybeans already on the market, and considering the history of safe use of the host organism, no additional information was considered necessary to support the safety and nutritional assessment of MON 89788.

Section 5. Food and Feed Safety Assessment for MON 89788

5.1. Substantial Equivalence of MON 89788 to A3244 and Conventional Soybean Varieties

A detailed compositional assessment of soybean grain and forage was presented where the levels of key nutrients, anti-nutrients and other components in MON 89788 were examined and compared to that of the conventional control, A3244. Additionally, tolerance intervals representing 99% of the analytical values of each analyte from conventional soybean population were established. Results demonstrate that the levels of key nutrients, anti-nutrients and other components of MON 89788 are compositionally and biologically equivalent to, and as safe and nutritious as those of conventional soybeans. The composition analyses compared 63 components between MON 89788 and A3244, each of which were grown at five field sites in the U.S. during 2005. Greater than 90% of the analytical means from MON 89788 are not statistically different from that of A3244. For the few statistical differences between MON 89788 and A3244, it was concluded that those differences were not biologically relevant since the differences were not observed across all sites, and there were no consistent trends regarding the differences. Furthermore, all MON 89788 analytical means are within the 99% tolerance intervals of conventional soybeans for all analytes. These results establish that, with a confidence level of 95%, the levels of key nutrients, anti-nutrients and other components of the soybean grain and forage produced in MON 89788 are within the ranges of those in conventional soybeans. Therefore, the soybean grain and forage derived from MON 89788 are considered compositionally equivalent to those derived from conventional soybeans. Based on the data and information provided, Monsanto has concluded that MON 89788 is substantially equivalent to conventional varieties of soybean, and this conclusion extends to the intended foods and feeds derived from MON 89788.

5.2. Conclusions

Collectively, these data and a history of safe use of soybean as a common source of processed human foods and animal feeds support a conclusion of "no concerns" for every criterion specified in the flowcharts outlined in the FDA's Food Policy document (Figure VII-1). MON 89788 is not materially different in composition, safety or nutrition from conventional soybeans, other than its tolerance to Roundup agricultural herbicides. Sales or consumption of soybean grain or processed products derived from MON 89788 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 soybean varieties and biotechnology traits.

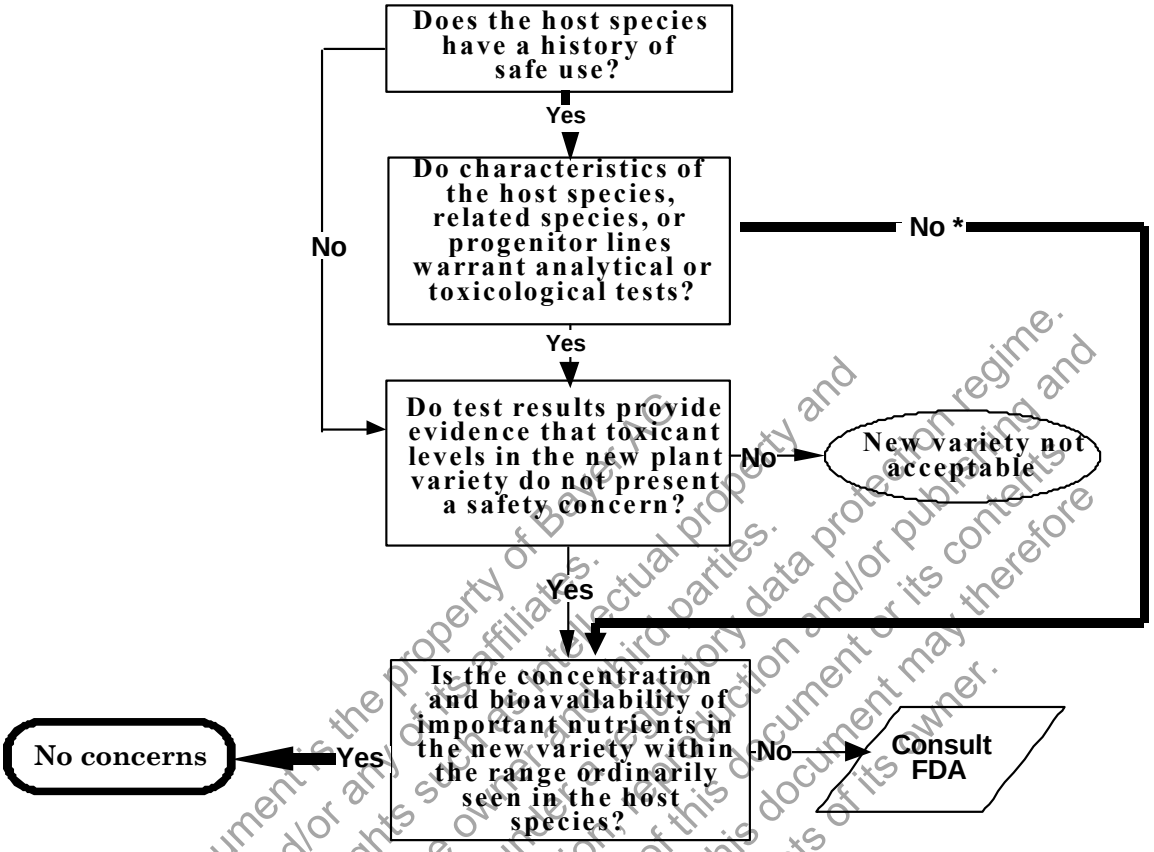


Figure VII-1. Safety Assessment of New Varieties: The Host Plant

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APPENDICES

Appendix A. Materials and Methods Used for Molecular Analyses of MON 89788

Materials

The DNA used in molecular analyses was isolated from leaf tissue of MON 89788 collected in 2005 from seed lot GLP-0405-15118-S. Additional DNA extracted from various generations of leaf tissues were used in generation stability analyses. The control DNA was isolated from the leaf tissue of a conventional soybean variety, A3244. The reference substances included the PV-GMGOX20 plasmid and the size estimation molecular weight standards. As a positive control on Southern blots, PV-GMGOX20 plasmid DNA was digested with a restriction enzyme or combination of enzymes to produce the banding patterns that were most relevant to the assessment of the test substance digested with appropriate enzyme(s). The plasmid DNA was either added to undigested A3244 soybean genomic DNA and digested, or was digested first and then added to pre-digested A3244 soybean genomic DNA. The molecular weight standards include the 1 kb DNA Extension Ladder (Invitrogen) and λ DNA/*Hin*d III fragments (Invitrogen) for size estimations on Southern blots. The 500 bp DNA ladder (Invitrogen) was used for size estimations for the PCR analyses.

Characterization of the Materials

The quality of the source materials from MON 89788 and A3244 were verified by PCR analysis to confirm the presence or absence of MON 89788 except the materials used in the generational stability analyses where the identity of the materials was confirmed by the generation stability Southern blots themselves. The stability of the genomic DNA was confirmed in each Southern analysis by observation of the digested DNA sample on an ethidium bromide-stained agarose gel.

DNA Isolation for Southern Blot and PCR Analyses

Genomic DNA samples from MON 89788 and A3244 used in the insert and copy number, insert integrity, backbone analysis, and PCR analyses were isolated from soybean leaf tissues that were ground to a fine powder in liquid nitrogen using a mortar and pestle. DNA was extracted from the processed leaf tissue using the Sarkosyl DNA isolation method by Fulton et al. (1995) with the following exceptions. Instead of recovering DNA by centrifugation, the DNA was spooled using a glass hook and placed in a microcentrifuge tube containing 70% ethanol. Also, during one of the isolations, RNAse A was added to the extraction buffer to minimize the co-purification of RNA.

Genomic DNA used in the generational stability analysis was isolated using the following method. Leaf tissue was ground to a fine powder in liquid nitrogen using a mortar and pestle. Approximately 2 ml equivalents of fresh leaf tissue powder were transferred to 13 ml conical tubes, and ~10 ml of CTAB extraction buffer [1.5% CTAB, 75 mM Tris pH 8.0, 100 mM EDTA, 1.05 M NaCl, 0.75% PVP (40K)] were added to the tissue. The samples were incubated at 68°C for 45-50 minutes and were mixed halfway through the incubation. Samples were split into 13 ml conical tubes (2/sample) containing 5 ml of chloroform. The suspensions were mixed by inversion for 2 minutes. The two phases were separated by centrifugation at ~10,300 x *g* for 8 minutes at room temperature. The aqueous (upper) layer was transferred to a clean 13 ml tube and the chloroform extraction

was repeated as above with 5 ml of chloroform. The aqueous layer was transferred to a clean 13 ml tube containing 5 ml of 100% ethanol to precipitate the genomic DNA. The genomic DNA of like samples was spooled into a 13 ml tube containing 10 ml of 70% ethanol. Samples were centrifuged at ~5,100 x *g* for 5 minutes at room temperature to pellet the DNA. The pellet was transferred with an inoculating loop to a microcentrifuge tube containing 1 ml of 70% ethanol. The DNA was spun for 1 minute at maximum speed in a microcentrifuge. Ethanol was removed with a pipette tip and the samples were allowed to air dry for 1-2 hours. The DNA was resuspended in TE buffer and stored in a 4°C refrigerator until use.

Quantification of Genomic DNA

Quantification of DNA samples was performed using a Hoefer DyNA Quant 200 Fluorometer with Roche molecular size marker IX as a DNA calibration standard.

Restriction Enzyme Digestion of Genomic DNA

Approximately 10 µg of genomic DNA were used for restriction enzyme digestions. When digesting genomic DNA with *Not* I (Roche), *Nco* I (Roche), or the combination of *Not* I and *Nco* I (Roche), 10X buffer H (Roche) was used. When digesting genomic DNA with the restriction enzyme combination of *Bpl* I (Fermentas) and *Xmn* I (New England Biolabs), buffers 10X Tango buffer and 2.5 mM SAM (Fermentas) were used. Finally, 100X BSA (New England Biolabs) was added to all digests to a final concentration of 1X. Overnight digests were performed at 37°C in a total volume of 500 µl using 100 units of the appropriate restriction enzyme(s).

DNA Probe Preparation for Southern Blot Analyses

Probes were prepared by PCR amplification of the PV-GMGOX20 template using a standard procedure based on Sambrook and Russell (2001). Approximately 25 ng of each template was used to generate the probe labeled with ^{32}P-dCTP (~6000 Ci/mmol) by random priming method (RadPrime DNA Labeling System, Invitrogen) or by PCR. Probe positions relative to the genetic elements in plasmid PV-GMGOX20 are depicted in Figure IV-2.

Southern Blot Analyses of Genomic DNA

Digested DNA was separated using 0.8% (w/v) agarose gel electrophoresis. Except for generational stability analyses, DNA samples were loaded on the gels for a long run and a short run in an effort to provide better resolution of larger DNA fragments while retaining smaller DNA fragments on the gel. After transferring the DNA to the membrane, Southern blots were hybridized at 65^{o}C except when probing with the *Tsf1* intron sequence and the *E9* 3' nontranslated sequence. These elements contain A-T rich sequences; therefore, it is necessary to lower the hybridization temperature to 60^{o}C. Multiple exposures of each blot were then generated using Kodak Biomax MS film in conjunction with one Kodak Biomax MS intensifying screen in a -80°C freezer.

DNA Sequence Analyses of the Insert

The organization of the elements within the T-DNA of MON 89788 was confirmed using DNA sequencing analyses. Several PCR primers were designed with the intent to

amplify three overlapping DNA fragments (Products A, B and C) spanning the entire length of the insert. The PCR for Products A and B were conducted using 50 ng of genomic DNA or 6 ng of plasmid DNA as templates in a 50 µl reaction volume containing a final concentration of 1.5 mM $MgCl_2$, 0.2 µM of each primer, 0.2 mM each dNTP, and 2.5 µl of Platinum Taq DNA polymerase (Invitrogen). The PCR for Product C was conducted using 50 ng of genomic DNA template in a 50 µl reaction volume containing a final concentration of 2 mM $MgSO_4$, 0.2 µM of each primer, 0.2 mM each dNTP, and 1 unit of Accuprime *Taq* (Invitrogen) DNA polymerase mix. The amplification of Product A was performed under the following cycling conditions: 94°C for 3 minutes, 35 cycles at 94°C for 30 seconds, 59°C for 30 seconds, 72°C for 3 minutes, and 1 cycle at 72°C for 10 minutes. The amplification of Product B was performed under the following cycling conditions: 94°C for 3 minutes, 35 cycles at 94°C for 30 seconds, 65°C for 30 seconds, 72°C for 3 minutes, and 1 cycle at 72°C for 10 minutes. The amplification of Product C was performed under the following cycling conditions: 94°C for 3 minutes, 35 cycles at 94°C for 15 seconds, 55°C for 30 seconds, 68°C for 3 minutes, and 1 cycle at 68°C for 5 minutes. Aliquots of each PCR product were separated on 1.0 % (w/v) agarose gels and visualized by ethidium bromide staining to verify that the products were of the expected size prior to sequencing. The PCR products were sequenced with primers used for PCR amplification as well as multiple primers designed internal to the amplified sequences. All sequencing was performed by the Monsanto Genomics Sequencing Center using dye-terminator chemistry.

Appendix B. Materials and Methods Used for Characterization of the CP4 EPSPS Protein Produced in MON 89788

Materials

The MON 89788-produced CP4 EPSPS protein was isolated from grain of MON 89788. The grain used for the isolation of CP4 EPSPS protein was produced in Argentina field production during the 2004-2005 season. The identity of the grain sample containing MON 89788 was confirmed by event-specific PCR. The isolated MON 89788-produced CP4 EPSPS protein was stored in a –80°C freezer in a buffer solution containing 50 mM Tris-HCl pH 7.5, 50 mM KCl, 2 mM DTT, 0.5 mM PMSF, 1 mM benzamidine-HCl, and 25% (v/v) glycerol. Data supporting the extraction and isolation of the CP4 EPSPS protein from the grain of MON 89788 conducted prior to the initiation of this plan are archived under APS lot 60-100085.

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, 2 mM DTT, 1 mM benzamidine-HCl, and 25% (v/v) glycerol] at a total protein concentration of 3.8 mg/mL.

Description of Assay Controls

Protein molecular weight markers were used to calibrate SDS-PAGE gels and verify protein transfer to PVDF membranes. The *E. coli*-produced CP4 EPSPS reference standard protein was used in the generation of the standard curve to estimate the total protein concentration using the Bio-Rad protein assay. It was also used as the positive control in the immunoblot analysis. Beta-lactoglobulin protein and PTH-amino acid standards were used to verify the performance of the amino acid sequencer. A peptide mixture was used to calibrate the MALDI-TOF mass spectrometer for tryptic mass analysis. Transferrin and *E. coli*-produced CP4 EPSPS proteins were used as the positive control and the negative control, respectively, in glycosylation analysis.

Protein Purification

The CP4 EPSPS protein was purified from an extract of ground grain of MON 89788, using a combination of isoelectric precipitation, ammonium sulfate fractionation, hydrophobic interaction chromatography, anion exchange chromatography, and cellulose phosphate affinity chromatography.

Approximately one kilogram of pre-chilled MON 89788 grain material was ground and defatted in hexane, air-dried, and stored in a −80°C freezer prior to protein extraction. The ground and defatted material (100 g) was mixed in Buffer A [1 mM KH_2PO_4, 10 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl, 10% (v/v) glycerol, 2 mM DTT, 1 mM EDTA, 1 mM benzamidine-HCl, 0.5 mM PMSF, and 1% (w/v) PVPP, pH 7.4] at 1:50 sample weight to buffer volume ratio. The sample-buffer suspension was homogenized and the crude homogenate was clarified by centrifugation and filtration. The 11S globulin protein in the extract was removed by lowering the pH of the supernatant to 5.5 by addition of ~50.5 ml of 1 N HCl (Liu, 1999). The protein precipitate was removed by

centrifugation followed by filtration. The resultant 4.55 L supernatant was subjected to 40% ammonium sulfate protein fractionation. The solution was stirred and centrifuged, and the remaining supernatant was subject to a 70% ammonium sulfate fractionation. The pellet was collected by centrifugation and was re-suspended in 500 ml of Buffer B [50 mM Tris-HCl, 10% (v/v) glycerol, 0.5 mM PMSF, 2 mM DTT, 1 mM EDTA, 1 mM benzamidine-HCl, 1.25 M ammonium sulfate, pH 7.56]. The supernatant was filtered and the volume was brought to 740 ml with Buffer B.

The sample was loaded onto a 206 ml (5 cm × 10.5 cm column) Phenyl Sepharose 6 Fast Flow (high sub) hydrophobic resin column, which was equilibrated with 5 column volume (CVol) of Buffer B. The unbound proteins were removed with 2 CVol of Buffer B. The bound CP4 EPSPS protein was eluted with a linear salt gradient of 100-40% of Buffer B in 1 CVol followed by a 40-0% gradient of Buffer B in 8 CVol. Fractions containing the CP4 EPSPS protein, identified based on phosphate release activity assay and immunoblot analysis, were pooled to a final volume of ~500 ml. The pooled sample was concentrated and desalted by diafiltration against Buffer C [50 mM Tris-HCl, 10% (v/v) glycerol, 0.5 mM PMSF, 2 mM DTT, 1 mM EDTA, 1 mM benzamidine-HCl, pH 7.5]. The final volume of the concentrated sample was brought to 125 ml, and it was clarified by centrifugation.

The protein solution of ~125 ml was loaded onto an anion exchange column (Source 15Q resin; 45 ml; 2 cm × 14.2 cm column), which was equilibrated with Buffer C prior to sample loading. The resin was washed with 3 CVol of Buffer C and the bound CP4 EPSPS protein was eluted with a linear salt gradient of 0-25% of 1 M NaCl in 4 CVol of Buffer C followed by 25-100% in 3 CVol. Fractions containing CP4 EPSPS protein were identified using SDS-PAGE, immunoblot analysis, and phosphate release activity assays.

Fractions containing the highest amount of CP4 EPSPS protein were buffer exchanged into Buffer D [50 mM MES, 1 mM DTT, 1 mM benzamidine-HCl, 15% (v/v) glycerol, pH 5.8] and applied to a 7 ml pre-cycled cellulose phosphate cation exchange resin (1.6 cm × 3.5 cm column). Prior to sample loading, the cellulose phosphate column was equilibrated with at least 200 ml of Buffer D and the bound protein was eluted with Buffer D, pH 5.8, containing 0.5 mM phosphoenolpyruvate (PEP) and 0.5 mM shikimate-3-phosphate (S3P). The MON 89788-produced CP4 EPSPS protein found in the flow-through fractions and the column wash were pooled and prepared for anion exchange column chromatography. One of the major contaminant proteins was removed by cellulose phosphate affinity column chromatography.

A pooled sample of ~13 ml containing the CP4 EPSPS protein was buffer exchanged against Buffer E (50 mM bis-tris propane, 0.5 mM PMSF, 2 mM DTT, 1 mM EDTA, and 1 mM benzamidine-HCl, pH 8.5) and concentrated to a final volume of 5 ml prior to loading onto a Mono Q column (Amersham, 5/50 GL; 0.5 cm × 5 cm). Unbound proteins were removed with 5 CVol of Buffer E and the bound CP4 EPSPS protein was eluted with Buffer E containing 1 M NaCl with a linear salt gradient of 0-50% in 12 CVol followed by 50-100% in 8 CVol. Fractions containing CP4 EPSPS protein, identified by SDS-PAGE, were pooled and buffer exchanged against Storage Buffer [50mM Tris-HCl,

50 mM KCl, 25% (v/v) glycerol, 0.5 mM PMSF, 2 mM DTT, 1 mM benzamidine-HCl, pH 7.5]. The volume of the concentrated protein sample was brought to 2.4 ml in Storage Buffer. Prior to the protein characterization, the protein sample was assigned to the APS program as lot 60-100085.

Molecular Weight and Purity Estimation – SDS-PAGE

Aliquots of stock solutions of the MON 89788-produced CP4 EPSPS and reference standard protein were each diluted with 5× loading buffer [312 mM Tris-HCl, 20% (v/v) 2-mercaptoethanol, 10% (w/v) SDS, 0.025% (w/v) bromophenol blue, 50% (v/v) glycerol, pH 6.8)] and water to a final concentration of 0.2 µg/µL. Molecular weight markers (Bio-Rad broad-range) were diluted to a final total protein concentration of 0.9 µg/µL. The MON 89788-produced CP4 EPSPS protein was analyzed in duplicate at 1, 2, and 3 µg total protein per lane. The *E. coli*-produced CP4 EPSPS reference standard (APS lot 20-100015) was analyzed at 1 µg total protein. All samples were heated at 98-99°C for 5 min and 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 150 V for 78 or 90 min. Proteins were fixed by placing the gel in a solution of 40% (v/v) methanol and 7% (v/v) glacial acetic acid for 30 min, stained 16 h with Brilliant Blue G-Colloidal stain (Sigma, St. Louis, MO), destained with a solution containing 10% (v/v) acetic acid and 25% (v/v) methanol followed by 25% (v/v) methanol.

Analysis of the gel was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0, Hercules, CA). Molecular weight markers were used to estimate the apparent molecular weight of each observed band. All visible bands within each lane were quantified using Quantity One software. For the MON 89788-produced CP4 EPSPS protein, purity was estimated as the percent optical density of the 44 kDa band relative to all bands detected in the lane. Apparent molecular weight and purity were reported as an average of all six loadings containing the MON 89788-produced CP4 EPSPS protein.

Immunoblot Analysis – Immunoreactivity

Aliquots of the stock solutions of the MON 89788-produced CP4 EPSPS protein and reference standard were diluted to a final purity-corrected protein concentration of 0.2 ng/µL in water and in 5× loading buffer. Samples were then heated to ~100°C for 5 min and loaded onto a pre-cast tris-glycine 4→20% polyacrylamide gradient 10-well gel. The MON 89788-produced CP4 EPSPS protein and reference standard protein were loaded at three different loadings of 1, 2, and 3 ng per lane. Electrophoresis was performed at a constant voltage of 140 V for 20 min followed by a constant voltage of 200 V for 47 min. Pre-stained molecular weight markers included during electrophoresis (Bio-Rad Precision Plus Dual Color, Hercules, CA) were used to verify electrotransfer of protein to the membrane and to estimate the molecular weight of the immunoreactive bands. Samples were electrotransferred to a 0.45 micron PVDF membrane (Invitrogen, Carlsbad, CA) for one h at a constant current of 300 mA.

The membrane was blocked for one h with 5% (w/v) NFDM in PBST. The membrane was probed with a 1:4000 dilution of goat anti-CP4 EPSPS antibody (lot 6844572) in 2% (w/v) NFDM in PBST for one hour. Excess antibody was removed by three washes with PBST. The membrane was probed with peroxidase-conjugated rabbit anti-goat IgG (Sigma, St. Louis, MO) at a dilution of 1:10,000 in 2% (w/v) NFDM in PBST for one hour. Excess peroxidase-conjugated IgG was removed by three washes with PBST. Immunoreactive bands were visualized using the ECL detection system (Amersham Biosciences, Piscataway, NJ) and exposed (5 s, 10 s, and 3 min) to Hyperfilm ECL film (Amersham Biosciences, Piscataway, NJ). Films were developed using a Konica SRX-101A automated film processor.

Image analysis of immunoreactive bands on blot films was conducted using a Bio-Rad model GS-800 calibrated imaging densitometer (Hercules, CA) equipped with Quantity One software Version 4.4.0. The intensity of signal detected in each lane was measured as band adjusted intensity (average band OD × band area in mm^2). The percent difference between the MON 89788- and *E. coli*-produced CP4 EPSPS proteins was calculated as shown below:

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

N-terminal Sequence Analysis

An aliquot of the MON 89788-produced CP4 EPSPS protein was diluted with 5 × loading buffer to a final purity corrected protein concentration of 272 ng/µL. Pre-stained molecular weight markers included during electrophoresis (Bio-Rad Precision Plus Dual Color, Hercules, CA) were used to verify electrotransfer of protein to the membrane and to estimate MW. The MON 89788-produced CP4 EPSPS protein was loaded in five lanes at 5.4 µg (purity corrected) per lane. The CP4 EPSPS containing samples were heated to ~99°C for 4 min prior to electrophoresis on a pre-cast tris-glycine 4→20% SDS polyacrylamide gel at 125V for 90 min. The gel was then electroblotted to a 0.45 micron PVDF membrane for 90 min at a constant current of 125 mA in a solution containing 10 mM CAPS, 10% (v/v) methanol, pH 11. Protein bands on the membrane were visualized with Ponceau S stain (Sigma).

The protein band that migrated at 44 kDa in each of three lanes was excised individually from the membrane and pooled prior to sequence analysis. N-terminal sequence analysis was performed using automated Edman degradation chemistry (Hunkapillar et al., 1983). An Applied Biosystems 494 Procise Sequencing System with 140C Microgradient system and 785 Programmable Absorbance Detector and Procise™ Control Software (version 2.1) was used. Chromatographic data were collected using Atlas[99] software (version 2003R1.1). A PTH-amino acid standard mixture (Applied Biosystems, Foster City, CA) was used to calibrate the instrument for each analysis. This mixture served to verify system suitability criteria such as peak resolution, peak area and relative amino acid chromatographic retention times. A control protein (β-lactoglobulin, Applied

Biosystems) was analyzed before and after the analysis of the CP4 EPSPS protein to verify that the sequencer met performance criteria for repetitive yield and sequence identity.

<u>*MALDI-TOF Analysis*</u>
MALDI-TOF mass spectrometry was used to confirm the identity of the MON 89788-produced CP4 EPSPS protein. With sufficient mass accuracy, four tryptic peptides were found to be sufficient to identify a protein (Jiménez et al., 1998).

SDS-PAGE Separation of Proteins: Approximately 5.4 μg of the MON 89788-produced CP4 EPSPS protein along with broad Range molecular weight markers (Bio-Rad, Hercules, CA) were heated to 99°C for 4 min prior to electrophoresis on a pre-cast tris-glycine 4→20% polyacrylamide gel. Proteins were fixed by placing the gel in a solution of 40% (v/v) methanol and 7% (v/v) glacial acetic acid for 50 min, stained with Brilliant Blue G-Colloidal stain (Sigma, St. Louis, MO), destained with a solution containing 10% (v/v) acetic acid and 25% (v/v) methanol, followed by 25% (v/v) methanol.

In-gel Protein Digestion: The stained protein band that migrated at 44 kDa was excised from the gel, destained, reduced, alkylated, and subjected to an in-gel trypsin (Promega, Madison, WI) digestion (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. 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 h at 37°C. Proteins were then alkylated by the addition of 100 μL of buffer containing 200 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 min. The ammonium bicarbonate/acetonitrile incubations were repeated two additional times to remove the reducing and alkylating agents and salts from the gel. The gel was dried in a SpeedVac concentrator (Savant, Holbrook, NY), rehydrated with 40 μL 25 mM ammonium bicarbonate containing 33 μg/ml trypsin, and digested for 16 h at 37°C. Digested peptides were extracted with 50 μL 70% (v/v) acetonitrile containing 0.1% (v/v) TFA. Supernatant from each extraction was combined and dried in a SpeedVac concentrator. This process was repeated two more times, and the dried material was reconstituted in 10 μL of 0.1% (v/v) TFA.

Sample Preparation: A portion (5 μL) of the digested sample was desalted (Bagshaw et al., 2000) using Millipore (Bedford, MA) ZipTip® C18 pipette tips. The mixture of tryptic peptides was applied to a ZipTip C18 and eluted with 5 μL of Wash 1 [0.1% (v/v) TFA], followed by 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 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 a 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 (Waters, Milford, MA) on the analysis plate. All samples were analyzed in the 500 to 5000 dalton range using 100 shots at a laser intensity setting of 2603-2960 (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 CP4 EPSPS protein sequence deduced from the nucleotide sequence. Masses were calculated for each theoretical peptide and compared to the raw mass data. Experimental masses (MH+) were assigned to peaks in the 500-1000 Da range if there were two or more isotopically resolved peaks, and in the 1000-5000 Da range if there were three or more isotopically resolved peaks in the spectra. Peaks were not assessed if the 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 of ± 2 daltons from the mass analyzed. Known trypsin autocatalytic fragments were also identified in the raw data. The identity of the CP4 EPSPS protein is confirmed if ≥ 40 % of the protein sequence can be identified by matching experimental masses to the expected masses for the fragments.

Functional Activity Assay

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 containing the isolated CP4 EPSPS enzyme with S3P were initiated by the addition of PEP. The final reagent concentrations in the assay were 50 mM HEPES (pH 7.0), 0.1 mM ammonium molybdate, 2 mM S3P, 1 mM PEP 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; (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 CP4 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. As specified in Monsanto characterization plan, the MON 89788-produced CP4 EPSPS protein was considered equivalent to the *E. coli*-produced CP4 EPSPS protein if the average specific activity was within two-folds of the average specific activity of the *E. coli*-produced protein.

Glycosylation Analysis

Glycosylation analysis was used to determine whether the MON 89788-produced CP4 EPSPS protein was post-translationally modified with covalently bound carbohydrate moieties. Aliquots of the MON 89788-produced CP4 EPSPS and the *E. coli*-produced CP4 EPSPS reference standard (in this instance, a negative control) were diluted in 5 ×

loading buffer and water to a final purity corrected concentration of ~55 ng/µL and 50 ng/µL, respectively. An aliquot of the transferrin protein (positive control) was diluted in 5 × loading buffer and water to a total protein concentration of 50 ng/µL. These samples were heated to ~100.3 °C for five min, and loaded along with Precision Plus Dual Color pre-stained protein molecular weight markers (Bio-Rad, Hercules, CA) and a No Protein Control (loading buffer only) and electrophoresed on a pre-cast tris-glycine 4→20% polyacrylamide gradient 10-well mini-gel. The transferrin and *E. coli*-produced CP4 EPSPS protein were loaded at 0.5 and 1 µg protein per lane, while the MON 89788-produced protein was loaded at 0.6 µg and 1.1 µg protein per lane. Electrophoresis was performed at a constant voltage of 140 V for 20 min followed by a constant voltage of 200 V for 47 min. After electrophoresis, proteins were electrotransferred to a 0.45 micron PVDF membrane for 75 min at a constant current of 300 mA.

Carbohydrate detection was performed directly on the PVDF membrane using the ECL detection system (Amersham Biosciences, Piscataway, NJ). The PVDF membrane was incubated in PBS for 10 min, and transferred to a solution of 100 mM acetate buffer, pH 5.5, containing the oxidation reagent, 10 mM sodium metaperiodate. The membrane was incubated in the dark for 20 min. 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 acetate buffer, pH 5.5, containing 25 nM biotin hydrazide and incubated for 60 min. Biotin hydrazide solution was removed by washing in PBS as previously described. The membrane was blocked with 5% blocking agent (provided with the ECL detection system) in PBS for 60 min. 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). Films were exposed (10 s, 30 s, 1 min, and 3 min) to Hyperfilm ECL film (Amersham Biosciences). Films were developed using a Konica SRX-101A automated film processor.

Appendix C. Materials and Methods Used for the Analysis of the Levels of CP4 EPSPS Protein in MON 89788

<u>Materials</u>

Tissue samples analyzed in this study were produced from five field sites in the U.S. during 2005 season from seed lot GLP-0504-16045-S for MON 89788 and GLP-0504-16046-S for control. The control line was A3244, which is a conventional variety and does not contain the *cp4 epsps* coding region. Samples were stored in a -80°C freezer throughout the study. An *E. coli*-produced CP4 EPSPS protein (Monsanto APS lot # 20-100015) was used as a reference standard for the assay.

<u>Characterization of the Materials</u>

All samples were verified by either the chain-of-custody documentation or an event-specific PCR method. Three MON 89788 grain samples (one each from the IL-1, IL-2, and NE sites) contained less than or equal to 3.05% of the Roundup Ready soybean, and the samples were included for analyses as the low level of impurity would not impact the integrity of the study. However, two control grain samples from the IL-1 site also contained the Roundup Ready soybean, and these two samples along with their associated tissues were not analyzed.

<u>Field Design and Tissue Collection</u>

Field trial was initiated during the 2005 growing season at five locations in the U.S. to generate the MON 89788 and control substances. The field locations were: York County, Nebraska (NE), Clinton County, Illinois (IL-1), Warren County, Illinois (IL-2), Jackson County, Arkansas (AR), and Fayette County, Ohio (OH). The production sites were located within major soybean growing regions, and they provided a range of environmental and agronomic conditions representative of eventual MON 89788 commercial production. At each location, three replicated plots of MON 89788 and control were planted using a randomized complete block field design. Over-season leaf (OSL1, OSL2, OSL3, and OSL4), grain, root, and forage tissues were collected from each replicated plot at all field locations. Samples were tracked throughout the field production using unique sample identifiers and proper chain-of-custody documentation. Upon collection, all samples were placed in uniquely labeled bags or containers. Over-season leaf, root, and forage tissue samples were stored on dry ice and shipped frozen on dry ice to Monsanto's processing facility in Creve Coeur, MO. Grain samples were stored and shipped at ambient temperature.

Over-season leaf tissue samples were collected from the youngest set of fully expanded trifoliate leaves at the following growth stages: OSL1 at the V3-V4 growth stage; OSL2 at the V6-V8 growth stage; OSL3 at the V10-V12 growth stage; and OSL4 at the V14-V16 growth stage. The root and forage tissues were collected at approximately the R6 growth stage, and the above-ground portion of the plant was labeled as the forage, and the below ground portion was washed and labeled as root tissue. Grain samples were collected at the R8 growth stage.

Tissue Processing and Protein Extraction

All samples produced at the field sites were shipped to Monsanto's processing facility in Creve Coeur, MO. During the processing step, dry ice was combined with the individual samples, and vertical cutters or mixers were used to thoroughly grind and mix the tissues. Processed samples were transferred into capped 15 ml tubes and stored in a -80°C freezer until use.

The CP4 EPSPS protein was extracted from all tissues using a Harbil mixer and the appropriate amount of Tris-borate buffer with L-ascorbic acid (TBA) [0.1 M Tris, 0.1 M $Na_2B_4O_7 \cdot 10H_2O$, 0.01 M $MgCl_2$, 0.05% (v/v) Tween-20 at pH 7.8, and 0.2% (w/v) L-ascorbic acid]. Insoluble material was removed from the extracts using a serum filter (Fisher Scientific, Pittsburgh, PA). The clarified extracts were aliquot, and stored frozen in a -80°C freezer until ELISA analysis.

Anti-CP4 EPSPS Antibodies

The capture antibody was mouse monoclonal antibody clone 39B6 (IgG2a isotype, kappa light chain; lot 6199732) specific for CP4 EPSPS protein, and was purified from mouse ascites fluid using Protein-A Sepharose affinity chromatography. The production of the 39B6 IgG2a monoclonal antibody was performed by TSD Bioservices, Inc. (Newark, DE), and the concentration of the purified IgG2a was 3.2 mg/ml. The purified antibody was stored in a buffer containing 0.02 M $Na_2HPO_4 \cdot 7H_2O$, 0.15 M NaCl, and 15 ppm ProClin 300, pH 7.2. The detection reagent was goat anti-CP4 EPSPS polyclonal antibodies (Sigma, St. Louis, MO) conjugated to horseradish peroxidase (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 [0.015 M Na_2CO_3, 0.035 M $NaHCO_3$, and 0.15 M NaCl, pH 9.6] at 1.0 µg/ml and immobilized onto 96-well microtiter plates, followed by incubation in a 4°C refrigerator for $\geq$ 12 h. Plates were washed with phosphate buffered saline (PBS) with 0.05% (v/v) Tween-20 (PBST), followed by the addition of CP4 EPSPS protein standard or sample extract at 100 µl per well, and incubated at 37°C for 1 h. Plates were washed with PBST, followed by the addition of goat anti-CP4 EPSPS peroxidase conjugate at 100 µl per well, and incubated at 37°C for 1 h. Plates were washed with PBST, and developed by adding TMB substrate (3,3',5,5'- tetramethyl-benzidine, Kirkegaard & Perry, Gaithersburg, MD) at 100 µl per well. The enzymatic reaction was terminated by the addition of 100 µl of 6 M H_3PO_4 per well. Quantitation of CP4 EPSPS protein levels was accomplished by interpolation from a CP4 EPSPS protein standard curve that spanned 0.456 - 14.6 ng/ml.

Moisture Analysis

A homogeneous, tissue-specific site pool (TSSP) was prepared by mixing comparable amounts (on a volumetric basis) of at least four test and control samples from each field location. Pools were prepared for all tissue types analyzed in this study. All tissues were analyzed for moisture content using an IR 200 Infrared Moisture Analyzer (Denver Instrument Company, Arvada, CO). The mean percent moisture for each TSSP was

calculated from three analyses of a given pool and used to convert the fresh weight values for the test and control substances at each site to dry weight values. A tissue-specific Dry Weight Conversion Factor (DWCF) was calculated as follows:

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

The DWCF was only applied to samples with protein quantities greater than the assay limits of quantitation (LOQ). All protein values calculated on a fresh weight basis were converted into protein values reported on a dry weight basis using the following calculation.

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

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 analysis was 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 FW basis. This conversion utilized the sample dilution factor and tissue-to-buffer ratio. The protein quantities in µg/g FW were also converted to µg/g DW by applying the DWCF. The arithmetic mean, standard deviation (SD), and range (FW and DW) were calculated for each tissue type across locations. Microsoft Excel 2002 (Version 10.6730.6735 SP3, Microsoft, Redmond, WA) was used to calculate the CP4 EPSPS protein quantities in all tissues from MON 89788.

Appendix D. General Methods used in Assessing Structural Similarity to Known Allergens, Toxins and Peptides

Sequence Database Preparation

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.

To assess potential immunogenic cross-reactivity, bioinformatic analyses were performed on the CP4 EPSPS protein against allergen database that comprised of allergen, gliadin, and glutenin sequences. The allergen, gliadin, and glutenin sequence database (AD5) was assembled from the Entrez-protein search and retrieval system at the Nation Center for Biotechnology Information (www.ncbi.nlm.nih.gov/Entrez), consisting of public domain databases that includes GenBank and EMBL (Benson et al., 1997; Stoesser et al., 1997), PIR (George et al., 1997), the NRL3D version of RCSB PDB (Berman et al., 2000; Bernstein et al., 1977), and SwissProt (Bairoch and Apweiler, 1997). A preliminary list of sequences was compiled from the public databases using STRINGSEARCH (keyword = allerg*, where * is any character). Non-allergen entries were identified by reviewing published information for each entry and removed. Additional sequences were added from a previously published allergen list (Metcalfe et al., 1996), allergens recognized by the International Union of Immunological Societies (IUIS) (ftp://biobase.dk/resourcespub/who-iuis/allergen.list), and from searches of current literature available on PubMed and Entrez (http://www.ncbi.nlm.nih.gov/) (Schuler et al., 1996). Duplicate sequences were identified and removed, but unique isoforms differing by at least one amino acid were retained. The selected allergen, gliadin, and glutenin sequences were compiled in September 2004 into a searchable database (AD5), containing 1,191 protein sequences.

The toxin sequence database (TOXIN5) was assembled from public sequence databases, including Genbank and EMBL release 124 and SwissProt release 1. Protein sequences were retrieved using the STRINGSEARCH function (keyword = toxin) of the GCG Wisconsin Package (version 10). This search was used to identify and retrieve 12,771 separate entries containing the word toxin within the flatfile annotation section. The list data file was loaded into the editor window of SeqLab, selected, and compiled into a sub-database using the DATASET database utility. The actual number of unique toxin sequences is less than 12,771 because of the redundancy of these public databases, and because some entries may contain the word toxin, but are not qualified as protein toxins.

The ALLPEPTIDES sequence database, used to reveal potential similarity towards pharmacologically active proteins, represents all currently known publicly available

protein sequences and consists of sequences from SwissProt release 1 (October 2001) and GenBank release 135.

Sequence Database Searches

All analyses were performed using the UNIX-based Wisconsin Package software, Genetics Computer Group (GCG, version 10.3, Madison, WI) on a personal computer supported with Reflection X Client Manager network software (version 7.20, WRQ, Inc. Seattle, WA). The structural similarity of the CP4 EPSPS protein sequence to sequences in the AD5 database was assessed using the FASTA algorithm (Lipman and Pearson, 1985; Pearson and Lipman, 1988). FASTA comparisons are initiated by aligning the first match of a specific wordsize. The alignment is then extended based on the chosen scoring matrix. Specific FASTA comparison parameters used in this study included a wordsize (k-tuple) of two, a gap creation penalty of 12, a gap extension penalty of two, and an expectation threshold (*E*-score) of ten. FASTA comparisons were performed using the BLOSUM50 scoring matrix (Henikoff and Henikoff, 1992). Multiple alignments are made between the CP4 EPSPS sequence and each sequence in the database with a score calculated for each alignment. Only the best scoring alignment is extensively analyzed for each database sequence. BLOSUM50 works well for identifying sequence similarities that include gaps, and thus recognizes distant evolutionary relationships (Pearson, 2000).

The extent of similarity was evaluated by visual inspection of the aligned sequences, the calculated percent identity, and the *E*-score. The *E*-score reflects the degree of similarity between a pair of sequences, and it can be used to evaluate the significance of an alignment. The calculated *E*-score depends on the overall length of joined (gapped) local sequence alignments, the quality (percent identity, similarity) of the overlap, and the size of the database (Pearson and Lipman, 1988; Baxevanis and Ouellette, 1998). For a pair of sequences, a very small *E*-score may indicate a structurally relevant similarity. Conversely, large *E*-scores are typically associated with alignments that do not represent a biologically relevant structural similarity.

In addition to the FASTA comparisons of the CP4 EPSPS protein sequence to allergens (to assess overall structural similarity), an eight-mer search was performed. An algorithm (ALLERGENSEARCH) was developed to identify whether or not a linearly contiguous match of eight amino acids existed between the query sequence and sequences within the allergen database (AD5). This program compares the query sequence to each protein sequence in the allergen database using a sliding window of eight amino acids; that is, with a seven amino acid overlap relative to the preceding window. While there have been recommendations for using a shorter scanning window (Gendel, 1998; Kleter and Peijnenburg, 2002), only a few studies have actually investigated the ability of six-, seven-, or eight-amino acid search windows to identify allergens (Hileman et al., 2002; Goodman et al., 2002; Stadler and Stadler, 2003). In these studies, randomly or specifically selected protein sequences were used as query sequences in both FASTA, and six- seven- and eight-amino acid window searches against allergen databases. The results demonstrated that searches with six- and seven-amino acid windows led to high rates of false positive matches between non-allergenic query sequences and allergen

database sequences. Additionally, searches with a six- or seven-amino acid window identified apparently random matches between totally unrelated proteins, such that the matched proteins were not likely to share any structural or sequence similarities that could act as cross-reactive epitopes. These studies concluded that six-mer or seven-mer sliding window searches yielded such a high rate of false positive hits that they were of no predictive value. In order to provide the best predictive capability to identify potentially cross-reactive proteins, these reports support the use of eight contiguous amino acids to represent the smallest immunologically significant sequential, or linear IgE binding epitope (Metcalfe et al., 1996).

Significance of the Alignment

An *E*-score of 1×10^{-5} was set as an initial high cut off value for alignment significance. Although all alignments were inspected visually, any sequence whose alignment yielded an *E*-score less than 1×10^{-5} was further analyzed to determine if such an alignment represented a bona fide sequence homology.

Appendix E. General Methods used in Assessing Stability of Proteins 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). *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), and to assess protein quality (Akeson and Stahmann, 1964), study digestion in pigs and poultry, 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).

The previously characterized *E. coli*-produced CP4 EPSPS protein (Lot 5192245) with a total protein concentration of 3.96 mg/ml was used in this study. The protein was diluted to 1.68 mg/ml with PBS before use.

Digestions were initiated by addition of CP4 EPSPS protein to tubes containing simulated gastric fluid (SGF), where 10 units of pepsin activity were used per 1 µg of total protein. Digestions were incubated at 37 ± 2°C in separate tubes for various durations, and the reactions were quenched by addition of a sodium carbonate solution to each tube. Zero incubation time points (T = 0) will be quenched by addition of sodium carbonate solution to SGF prior to addition of the test substance. The SGF was assayed before and after conducting the timed incubations to demonstrate that pepsin remained active throughout the experiment.

Experimental controls were prepared to characterize the stability of the CP4 EPSPS in the system (SGF-p) without pepsin. These controls were incubated for 0 and 60 minutes and were designated with the letter "P". Additionally, experimental controls to characterize the system (SGF) without the CP4 EPSPS were also included. These experimental controls were prepared by substituting Tris-HCl for the CP4 EPSPS, and were designated with the letter "N".

All samples were frozen on dry ice and stored in a -20°C freezer before analyses. The digestibility of purified *E. coli*-produced CP4 EPSPS protein in SGF was assessed using SDS-PAGE gel followed by Brilliant Blue G Colloidal dye (Sigma P/N B-2025) staining or western blotting, and an EPSPS enzyme activity assay. Limits of detection (LOD) were determined for the gel staining and western blot methods.

Appendix F. Materials and Methods Used for Compositional Analysis of MON 89788 Soybean Grain and Forage from Five Replicated Field Sites

Materials

MON 89788, A3244 and conventional reference soybeans were grown at five U.S. locations in 2005. MON 89788 and A3244 were grown from seed lots GLP-0504-16045-S and GLP-0504-16046-S, respectively. The control material, A3244, has background genetics representative of MON 89788 but does not contain the *cp4 epsps* coding sequence or produce the CP4 EPSPS protein. In addition, twelve conventional soybean varieties produced alongside of MON 89788 were included for the generation of 99% tolerance interval. The varieties, locations, and seed lot numbers are listed below:

Variety	Starting Seed Lot Number	Field Site
Stine/ST3600	REF-0409-15515-S	AR
Stine/ST3870	REF-0409-15516-S	AR
Asgrow/A3525	REF-0409-15502-S	IL-1
Asgrow/A3559	REF-0504-16051-S	IL-1
Asgrow/A2553	REF-0504-16052-S	IL-2
Asgrow/A3204	REF-0409-15509-S	IL-2
Stine/ST2788	REF-0409-15512-S	IL-2
Asgrow/A2804	REF-0504-16048-S	NE
Stine/ST3300	REF-0409-15514-S	NE
Asgrow/A2704	REF-0504-16053-S	OH
Stine/ST2800	REF-0409-15513-S	OH
Asgrow/A2833	REF-0504-16056-S	OH

Characterization of the Materials

The identities of the MON 89788, A3244, and reference soybean varieties were verified prior to use by examination of the chain-of-custody documentation. Additionally, the identities of the MON 89788 and A3244 grain samples were confirmed by event-specific PCR analysis to determine the presence or absence of MON 89788.

Field Production of the Samples

The field design and tissue collection process have been described previously in Appendix C with the addition of reference varieties as described above. A total of twelve different conventional soybean varieties were planted at five field locations with two to three different varieties grown at each site. Fields were managed with normal agronomic practices for soybean, and plots containing MON 89788 were treated with a commercial rate of Roundup agricultural herbicide.

Summary of Analytical Methods

Soybean grain and forage samples from MON 89788, A3244, 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.

The following analyses were performed on **forage** samples:

Analyte	**Method Mnemonic**[1]
Proximates	
Moisture	M100
Protein	PGEN
Fat	FAAH
Ash	ASHM
Acid detergent fiber	ADF
Neutral detergent fiber	NDFE

[1]analytical methods were kept on file at Covance Laboratories Inc.

The following analyses were performed on the **grain** samples:

Analyte	**Method Mnemonic**[1]
Proximates	
Moisture	M100
Protein	PGEN
Fat	FSOX
Ash	ASHM
Acid Detergent Fiber	ADF
Neutral Detergent Fiber	NDFE
Amino Acid composition	TAAP
Fatty Acid profile (C8-C22)	FAPM
Trypsin Inhibitor	TRIP
Lectin	LECT
Isoflavones	ISOF
Phytic acid	PHYT
Stachyose/Raffinose	SUGT
Vitamin E (alpha-tocopherol)	LCAT

[1]analytical methods were kept on file at Covance Laboratories Inc.

In addition, carbohydrate (CHO) values were estimated by calculation. The 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 0.100%.

Amino Acid Composition (TAAP) The method used was based on AOAC International (2000a) 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 FW. The reference standards were Beckman, K18, 2.5 µmol/mL per constituent (except cystine 1.25 µmol/mL), Lot Number S504255; Sigma, L-Tryptophan, >99% (used as 100%), Lot Number 063K0382; Fluka, L-Cysteic Acid Monohydrate, 99.9% (used as 100%), Lot Number 1157629; Sigma, L-Methionine Sulfone, >99% (used as 100%), Lot Number 012H3349.

Ash (ASHM) The method used was based on AOAC International (2000b) 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% FW.

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% FW. The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation:

$$\% \text{ carbohydrates} = 100\% - (\% \text{ protein} + \% \text{ fat} + \% \text{ moisture} + \% \text{ ash})$$

Fat by Acid Hydrolysis (FAAH) The method used was based on AOAC International (2000c) method 922.06 and 954.02. The sample was hydrolyzed with hydrochloric acid at an elevated temperature. The fat was extracted using ether and hexane. The extract was washed with a dilute alkali solution, then evaporated under nitrogen, re-dissolved in hexane and filtered through a sodium sulfate column. The hexane extract was then evaporated again under nitrogen, dried, and weighed. The limit of quantitation for this study was 0.100%.

Fat by Soxhlet Extraction (FSOX) The method used was based on AOAC International (2000d) 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% FW.

Fatty Acids (FAPM) The method used was based on AOCS (1997a) method Ce 1-62 that estimates the levels of C8-C22 fatty acids in the samples. 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 for this study was 0.00300%.

Reference Standards:
Nu Chek Prep GLC Reference Standard Hazelton No. 1, used as 100%, Lot AU22-P
Nu Chek Prep GLC Reference Standard Hazelton No. 2, used as 100%, Lot M13-0
Nu Chek Prep GLC Reference Standard Hazelton No. 3, used as 100%, Lot MA13-0
Nu Chek Prep GLC Reference Standard Hazelton No. 4, used as 100%, Lot JA13-P
Nu Chek Prep Methyl Gamma Linolenate, used as 100%, Lot U-63M-J1-P
Sigma Methyl Tridecanoate, used as 100%, Lot 035K1392

Isoflavones Analysis (ISOF) The method is based on Seo and Morr (1984) and Pettersson and Kiessling (1984). The sample was extracted using a solution of hydrochloric acid and reagent alcohol heated on steam baths or hot plates. The extract was brought to volume, diluted, and centrifuged. An aliquot of the supernatant was placed onto a C18 solid-phase extraction column. Unwanted components of the matrix were rinsed off with 20% methanol and then the isoflavones were eluted with 80% methanol. The sample was analyzed on a high-performance liquid chromatography system with ultraviolet spectrophotometric quantitation and was compared against an external standard curve of known standards. The limit of quantitation for each component was 10.0 mcg/g.

Reference Standards:
Indofine, daidzein, 99+%[1], lot number 020508146
Indofine, genistein, 99+%[1], lot number 0103070
Indofine, Glycitein, 99%[1], Lot Number 0310189
Note: [1]Used as 100% in calculations

Lectin (LECT) The method used was based on Klurfeld and Kritchevsky (1987) and Liener (1955). The sample was suspended in phosphate buffered saline (PBS), shaken, and filtered. An aliquot of the resulting extract was serially diluted in 10 cuvettes containing PBS. A 10% hematocrit of lyophilized rabbit blood in PBS was added to each dilution. After 2.5 hours, the absorbance of each dilution of the sample and lectin control was read by a spectrophotometer at 620 nm, using PBS to zero the instrument. One hemagglutinating unit (H.U.) was defined as the level that caused 50% of the standard cell suspension to sediment in 2.5 hours. The limit of quantitation for this study was 0.10 H.U./mg based on a 2 g equivalent sample.

Moisture (M100) The method used was based on AOAC International (2000e) 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% FW.

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% FW.

Phytic Acid (PHYT) The method used was based on Lehrfeld (1989 and 1994). The sample was extracted using 0.5M HCl with ultrasonication. Purification and concentration was done on a silica based anion exchange (SAX) column. Sample analysis was done on a macroporous polymer HPLC column PRP-1, 5µm (150 x 4.1mm) and a refractive index detector. The limit of quantitation for this study was approximately 0.100%. Reference Standard was: Aldrich, Phytic Acid Dodecasodium Salt Hydrate, 95%, Lot Number 01913EC

Protein (PGEN) The method used was based on AOAC International (2000f) 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 0.100%.

Raffinose and Stachyose (SUGT) The method is based on Mason and Slover (1971) and Brobst (1972). After extraction from the sample with deionized water, the sugars were treated with a hydroxylamine hydrochloride solution in pyridine, containing phenyl- β - D-glucoside as the internal standard. The resulting oximes were converted to silyl derivatives with hexamethyldisilazane (HMDS) and trifluoracetic acid (TFA) and analyzed by gas chromatography using a flame ionization detector. The limit of quantitation for this study was calculated out to be a range of 0.179-3.571% for a 4/5 dilution. Reference Standards: Sigma, Raffinose Pentahydrate, 99%/84.0% after correction for degree of hydration, Lot Number 073K0938; Sigma, Stachyose, 99%/95.4% after correction for degree of hydration, Lot Number 103K3776

Trypsin Inhibitor (TRIP) The method is based on AOCS (1997b). The sample was ground and/or defatted with petroleum ether, if necessary. A sample of matrix was extracted for 3 hours with 0.1N sodium hydroxide. Varying aliquots of the sample suspension were exposed to a known amount of trypsin and benzoy1-DL-arginine~p~nitroana1ide hydrochloride. The sample was allowed to react for 10 minutes at 37°C. After 10 minutes, the reaction was halted by the addition of acetic acid. The solution was filtered or centrifuged, then the absorbance was determined at 410 nm. Trypsin inhibitor unit (TIU) was determined by photometrically measuring the inhibition

of trypsin's reaction with benzoyl-DL-arginine-p-nitroanalide hydrochloride. The limit of quantitation for this study was 1.00 Trypsin Inhibitor Unit/mg.

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 0.005 mg/100g. Reference Standard: USP, Alpha Tocopherol, 100%, Lot Number M.

Data Processing and Statistical Analysis

After compositional analyses were performed at Covance Laboratories Inc., data spreadsheets containing individual values for each analysis were sent to Monsanto Company for review. Data were then transferred to Certus International where they were converted into the appropriate units and statistically analyzed. The following formulas were used for re-expression of composition data for statistical analysis:

Component	From (X)	To	Formula[1]
Proximates (excluding Moisture), Fiber, Phytic Acid, Raffinose, Stachyose	% FW	% DW	X/d
Isoflavones	µg/g FW	µg/g DW	X/d
Trypsin Inhibitor	TIU/mg FW	TIU/mg DW	X/d
Vitamin E	mg/100g FW	mg/100g DW	X/d
Amino Acids (AA)	mg/g FW	% DW	X/(10*d)
Fatty Acids (FA)	% FW	% DW	X/d

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

Across samples, analytes with greater than fifty percent of observations below the assay's limit of quantitation (LOQ) were excluded from summaries and analysis. Otherwise, results below the quantitation limit were assigned a value equal to half the quantitation limit. No analytes were assigned values in this study. The following 14 analytes with >50% of observations below the LOQ of the assay were excluded from statistical analysis: 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, 16:1 palmitoleic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma linolenic, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid. Studentized PRESS residuals revealed the absence of outliers. No data was excluded from the statistical analyses. A PRESS residual is the difference between any value and its predicted value from a statistical model that excludes the data point.

Statistical analyses were conducted on the converted values for each component in the soybean grain and forage using a mixed model analysis of variance for the six sets of comparisons: analysis for each of the five replicated trial sites (AR, IL-1, IL-2, NE, OH), and one for the combination of all five sites. There were a total of 49 components

statistically evaluated (the initial 63 analytes minus the 14 for which >50% of the observations were below the LOQ). A total of 294 comparisons were made: 49 components with six statistical analyses each.

At the field sites, the MON 89788, A3244 and references substances were grown in single plots randomly assigned within each of three replication blocks. The compositional components for the test and control substances were statistically analyzed using a mixed model analysis of variance. The five replicated sites were analyzed both separately and combined across sites. 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 = substance 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 = substance effect, L_j = random location effect, $B(L)_{jk}$ = random block within location effect, LT_{ij} = random location by substance interaction effect, and e_{ijk} = residual error. For each compositional component, the values obtained for the forage and grain from the test substance were compared to the conventional control.

A range of observed values from the reference substances was determined for each analytical component. Additionally, the reference substances data were used to develop population tolerance intervals. A tolerance interval is an interval that one can claim, with a specified degree of confidence, contains at least a specified proportion, p, of an entire sampled population for the parameter measured. For each compositional component, 99% tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of commercial references. Each tolerance interval estimate was based upon one observation per unique reference substance. Individual references with multiple observations were averaged within sites to obtain a single estimate for inclusion in tolerance interval calculations. Because negative quantities are not possible, calculated negative lower tolerance bounds were set to zero. SAS® software was used to generate all summary statistics and perform all analyses (SAS Software Release 9.1, 2002-2003). Report tables present p-values from SAS® as either <0.001 or the actual value truncated to three decimal places.

Appendix G. Individual Site Soybean Grain Composition Tables from Five Replicated Field Sites

Table G-1. Statistical Summary of Site AR Soybean Forage Fiber and Proximate Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Fiber						
Acid Detergent Fiber (% DW)	31.91 (0.70) [30.95 - 33.54]	35.04 (0.70) [33.99 - 35.93]	-3.12 (0.87) [-4.68 - -1.66]	-6.88, 0.64	0.070	(29.64 - 50.69) [19.03, 54.55]
Neutral Detergent Fiber (% DW)	35.82 (1.13) [33.21 - 38.16]	37.76 (1.13) [36.79 - 39.11]	-1.94 (0.83) [-3.58 - -0.95]	-5.49, 1.61	0.143	(31.43 - 43.70) [26.89, 46.89]
Proximate						
Ash (% DW)	7.45 (0.41) [6.71 - 8.45]	6.88 (0.41) [6.35 - 7.25]	0.56 (0.59) [-0.33 - 2.10]	-1.96, 3.09	0.437	(5.36 - 8.36) [3.50, 9.58]
Carbohydrates (% DW)	68.92 (1.23) [66.38 - 70.32]	67.88 (1.23) [65.61 - 69.56]	1.04 (1.73) [-2.10 - 4.46]	-6.42, 8.50	0.608	(62.57 - 72.28) [55.96, 77.90]
Fat (% DW)	4.71 (0.20) [4.32 - 5.24]	4.94 (0.20) [4.83 - 5.02]	-0.23 (0.28) [-0.64 - 0.41]	-1.44, 0.98	0.499	(3.51 - 9.87) [0, 14.70]
Moisture (% FW)	72.20 (0.32) [71.70 - 72.90]	73.47 (0.32) [72.90 - 73.80]	-1.27 (0.29) [-1.80 - -0.80]	-2.52, -0.016	0.048	(68.50 - 78.40) [60.84, 83.36]
Protein (% DW)	18.92 (0.85) [18.41 - 19.93]	20.30 (0.85) [18.38 - 22.18]	-1.38 (1.19) [-3.75 - 0.034]	-6.51, 3.75	0.367	(16.48 - 22.78) [13.55, 25.95]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, tolerance interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Table G-2. Statistical Summary of Site AR Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

			Difference (MON 89788 minus A3244)			
Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	1.72 (0.011) [1.70 - 1.74]	1.73 (0.011) [1.71 - 1.74]	-0.0077 (0.016) [-0.041 - 0.015]	-0.077, 0.062	0.681	(1.62 - 1.89) [1.51, 2.00]
Arginine (% DW)	2.77 (0.024) [2.74 - 2.81]	2.79 (0.024) [2.76 - 2.85]	-0.017 (0.0094) [-0.036 - -0.0046]	-0.058, 0.023	0.202	(2.61 - 3.27) [2.27, 3.60]
Aspartic Acid (% DW)	4.49 (0.026) [4.47 - 4.51]	4.49 (0.026) [4.42 - 4.53]	0.0042 (0.020) [-0.022 - 0.043]	-0.082, 0.090	0.852	(4.21 - 5.02) [3.85, 5.44]
Cystine (% DW)	0.60 (0.0087) [0.58 - 0.61]	0.60 (0.0087) [0.59 - 0.61]	-0.0018 (0.010) [-0.020 - 0.016]	-0.046, 0.043	0.879	(0.57 - 0.65) [0.55, 0.67]
Glutamic Acid (% DW)	7.09 (0.047) [7.05 - 7.11]	7.09 (0.047) [6.97 - 7.16]	-0.0056 (0.044) [-0.054 - 0.082]	-0.20, 0.18	0.910	(6.62 - 8.19) [5.86, 8.96]
Glycine (% DW)	1.72 (0.0053) [1.71 - 1.73]	1.71 (0.0053) [1.71 - 1.72]	0.0034 (0.0066) [-0.0083 - 0.014]	-0.025, 0.032	0.659	(1.62 - 1.90) [1.46, 2.05]
Histidine (% DW)	1.03 (0.0037) [1.02 - 1.03]	1.02 (0.0037) [1.02 - 1.03]	0.0046 (0.0021) [0.0016 - 0.0087]	-0.0046, 0.014	0.163	(0.96 - 1.13) [0.90, 1.21]
Isoleucine (% DW)	1.79 (0.020) [1.77 - 1.81]	1.75 (0.020) [1.70 - 1.78]	0.033 (0.028) [-0.018 - 0.10]	-0.087, 0.15	0.361	(1.64 - 2.00) [1.44, 2.16]

Table G-2 (continued). Statistical Summary of Site AR Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	3.06 (0.0092) [3.04 - 3.06]	3.06 (0.0092) [3.04 - 3.08]	-0.0034 (0.0063) [-0.015 - 0.0070]	-0.031, 0.024	0.641	(2.89 - 3.42) [2.62, 3.66]
Lysine (% DW)	2.55 (0.012) [2.53 - 2.56]	2.53 (0.012) [2.51 - 2.56]	0.012 (0.0071) [-0.0014 - 0.022]	-0.018, 0.043	0.220	(2.40 - 2.77) [2.22, 2.95]
Methionine (% DW)	0.52 (0.0099) [0.51 - 0.53]	0.52 (0.0099) [0.50 - 0.54]	-0.00008 (0.014) [-0.020 - 0.032]	-0.060, 0.060	0.996	(0.45 - 0.56) [0.42, 0.60]
Phenylalanine (% DW)	2.00 (0.0040) [2.00 - 2.01]	2.01 (0.0040) [2.00 - 2.02]	-0.0083 (0.0010) [-0.0097 - -0.0064]	-0.013, -0.0040	0.014	(1.90 - 2.29) [1.70, 2.45]
Proline (% DW)	1.96 (0.0081) [1.94 - 1.97]	1.96 (0.0081) [1.95 - 1.97]	0.0028 (0.011) [-0.029 - 0.024]	-0.047, 0.052	0.828	(1.86 - 2.23) [1.66, 2.38]
Serine (% DW)	2.13 (0.024) [2.13 - 2.14]	2.14 (0.024) [2.08 - 2.19]	-0.012 (0.033) [-0.065 - 0.060]	-0.16, 0.13	0.746	(1.99 - 2.42) [1.84, 2.54]
Threonine (% DW)	1.53 (0.010) [1.52 - 1.55]	1.53 (0.010) [1.51 - 1.55]	0.0037 (0.011) [-0.018 - 0.015]	-0.043, 0.050	0.761	(1.44 - 1.67) [1.38, 1.76]
Tryptophan (% DW)	0.36 (0.0091) [0.35 - 0.37]	0.35 (0.0091) [0.33 - 0.37]	0.0087 (0.013) [-0.023 - 0.037]	-0.047, 0.064	0.567	(0.30 - 0.47) [0.25, 0.54]

Table G-2 (continued). Statistical Summary of Site AR Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	1.35 (0.027) [1.28 - 1.38]	1.36 (0.027) [1.33 - 1.39]	-0.014 (0.019) [-0.050 - 0.014]	-0.095, 0.067	0.531	(1.28 - 1.51) [1.18, 1.64]
Valine (% DW)	1.86 (0.022) [1.83 - 1.88]	1.82 (0.022) [1.77 - 1.86]	0.033 (0.031) [-0.029 - 0.11]	-0.10, 0.17	0.407	(1.71 - 2.09) [1.51, 2.27]
Fatty Acid (% DW)						
16:0 Palmitic (% DW)	2.21 (0.028) [2.17 - 2.25]	2.40 (0.028) [2.34 - 2.46]	-0.19 (0.012) [-0.21 - -0.17]	-0.24, -0.13	0.004	(1.66 - 2.35) [1.32, 2.64]
18:0 Stearic (% DW)	0.76 (0.012) [0.75 - 0.77]	0.81 (0.012) [0.78 - 0.82]	-0.044 (0.0070) [-0.053 - -0.030]	-0.074, -0.014	0.024	(0.63 - 1.07) [0.37, 1.28]
18:1 Oleic (% DW)	3.30 (0.032) [3.24 - 3.36]	3.68 (0.032) [3.62 - 3.72]	-0.38 (0.013) [-0.40 - -0.36]	-0.43, -0.32	0.001	(2.99 - 5.29) [2.06, 6.43]
18:2 Linoleic (% DW)	10.27 (0.13) [10.06 - 10.42]	11.02 (0.13) [10.76 - 11.29]	-0.76 (0.054) [-0.86 - -0.70]	-0.99, -0.52	0.005	(8.41 - 10.69) [7.75, 11.22]
18:3 Linolenic (% DW)	1.45 (0.026) [1.41 - 1.48]	1.55 (0.026) [1.50 - 1.60]	-0.095 (0.017) [-0.13 - -0.073]	-0.17, -0.023	0.029	(1.02 - 1.55) [0.84, 1.69]
20:0 Arachidic (% DW)	0.060 (0.00097) [0.058 - 0.060]	0.064 (0.00097) [0.061 - 0.065]	-0.0040 (0.00061) [-0.0048 - -0.0028]	-0.0067, -0.0014	0.021	(0.046 - 0.076) [0.031, 0.094]

Table G-2 (continued). Statistical Summary of Site AR Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Fatty Acid (% DW)						
20:1 Eicosenoic (% DW)	0.048 (0.00059) [0.047 - 0.049]	0.053 (0.00059) [0.051 - 0.053]	-0.0045 (0.00084) [-0.0062 - -0.0030]	-0.0081, -0.00093	0.032	(0.030 - 0.057) [0.021, 0.065]
22:0 Behenic (% DW)	0.066 (0.00098) [0.064 - 0.068]	0.070 (0.00098) [0.068 - 0.071]	-0.0041 (0.00078) [-0.0056 - -0.0031]	-0.0074, -0.00075	0.034	(0.046 - 0.073) [0.034, 0.091]
Fiber						
Acid Detergent Fiber (% DW)	21.17 (1.29) [19.28 - 23.94]	16.10 (1.29) [14.39 - 18.27]	5.07 (0.31) [4.64 - 5.67]	3.74, 6.40	0.003	(13.30 - 26.26) [9.62, 28.57]
Neutral Detergent Fiber (% DW)	18.64 (0.85) [17.30 - 19.97]	17.21 (0.85) [15.60 - 18.82]	1.43 (0.86) [-0.18 - 2.77]	-2.28, 5.13	0.238	(14.41 - 23.90) [13.26, 26.33]
Isoflavones						
Daidzein (ug/g DW)	962.42 (43.72) [908.89 - 999.45]	967.53 (43.72) [872.30 - 1064.27]	-5.11 (55.92) [-64.83 - 106.63]	-245.69, 235.47	0.935	(274.88 - 1485.52) [0, 1925.63]
Genistein (ug/g DW)	814.94 (26.50) [807.73 - 824.05]	803.54 (26.50) [736.66 - 865.07]	11.40 (32.56) [-41.02 - 71.07]	-128.69, 151.49	0.759	(354.09 - 984.29) [0, 1387.95]
Glycitein (ug/g DW)	105.71 (12.10) [79.54 - 131.69]	104.79 (12.10) [88.52 - 113.36]	0.92 (9.15) [-8.98 - 19.21]	-38.47, 40.31	0.928	(52.72 - 298.57) [0, 287.45]
Proximate						
Ash (% DW)	5.51 (0.046) [5.39 - 5.60]	5.33 (0.046) [5.29 - 5.35]	0.18 (0.065) [0.054 - 0.30]	-0.096, 0.46	0.106	(4.61 - 5.57) [4.00, 6.08]

Table G-2 (continued). Statistical Summary of Site AR Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Proximate						
Carbohydrates (% DW)	38.13 (0.34) [37.77 - 38.42]	36.02 (0.34) [35.27 - 36.79]	2.12 (0.48) [0.97 - 2.95]	0.046, 4.19	0.048	(32.75 - 40.98) [27.86, 45.79]
Fat (% DW)	18.82 (0.25) [18.42 - 19.17]	20.41 (0.25) [19.92 - 20.91]	-1.59 (0.078) [-1.74 - -1.50]	-1.93, -1.25	0.002	(15.97 - 20.68) [15.38, 21.95]
Moisture (% FW)	9.15 (0.14) [8.88 - 9.35]	9.36 (0.14) [9.14 - 9.63]	-0.21 (0.20) [-0.44 - 0.21]	-1.07, 0.65	0.405	(6.24 - 9.11) [4.64, 9.94]
Protein (% DW)	37.54 (0.47) [37.31 - 37.68]	38.25 (0.47) [36.96 - 39.04]	-0.71 (0.66) [-1.72 - 0.72]	-3.55, 2.12	0.393	(36.48 - 43.35) [31.50, 47.45]
Vitamin						
Vitamin E (mg/100g DW)	3.08 (0.054) [3.01 - 3.15]	2.94 (0.054) [2.87 - 3.07]	0.14 (0.076) [0.0071 - 0.27]	-0.19, 0.47	0.207	(1.29 - 4.80) [0, 7.00]
Antinutrient						
Lectin (H.U./mg FW)	3.39 (0.63) [1.80 - 4.33]	2.10 (0.63) [1.44 - 2.83]	1.30 (0.56) [0.36 - 2.31]	-1.13, 3.72	0.148	(0.45 - 9.95) [0, 9.72]
Phytic Acid (% DW)	0.77 (0.066) [0.66 - 0.88]	0.79 (0.066) [0.69 - 0.92]	-0.022 (0.056) [-0.11 - 0.080]	-0.26, 0.22	0.729	(0.41 - 0.96) [0.39, 1.07]
Raffinose (% DW)	0.65 (0.029) [0.58 - 0.71]	0.81 (0.029) [0.78 - 0.83]	-0.16 (0.026) [-0.20 - -0.11]	-0.27, -0.052	0.024	(0.26 - 0.84) [0, 1.01]

Table G-2 (continued). Statistical Summary of Site AR Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Antinutrient						
Stachyose (% DW)	2.32 (0.11)	2.83 (0.11)	-0.51 (0.054)	-0.75, -0.28	0.010	(1.53 - 2.98)
	[2.10 - 2.50]	[2.69 - 3.04]	[-0.59 - -0.41]			[1.19, 3.31]
Trypsin Inhibitor (TIU/mg DW)	39.00 (1.88)	40.24 (1.88)	-1.23 (1.88)	-9.34, 6.87	0.580	(20.79 - 55.51)
	[36.62 - 43.30]	[37.09 - 41.91]	[-4.81 - 1.58]			[5.15, 59.34]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, tolerance interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Table G-3. Statistical Summary of Site IL-1 Soybean Forage Fiber and Proximate Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Fiber						
Acid Detergent Fiber (% DW)	35.14 (2.29) [32.03 - 40.50]	32.53 (2.80) [31.18 - 33.89]	2.61 (3.62) [-1.86 - 1.72]	-43.36, 48.58	0.602	(29.64 - 50.69) [19.03, 54.55]
Neutral Detergent Fiber (% DW)	36.15 (2.59) [33.44 - 41.12]	36.94 (3.08) [32.69 - 39.06]	-0.79 (3.23) [-5.18 - 0.75]	-41.85, 40.27	0.847	(31.43 - 43.70) [26.89, 46.89]
Proximate						
Ash (% DW)	5.68 (0.26) [5.40 - 5.86]	5.85 (0.30) [5.28 - 6.33]	-0.17 (0.29) [-0.54 - 0.12]	-3.89, 3.55	0.662	(5.36 - 8.36) [3.50, 9.58]
Carbohydrates (% DW)	64.21 (1.06) [61.61 - 65.53]	64.75 (1.30) [64.55 - 64.95]	-0.54 (1.68) [-3.34 - 0.98]	-21.94, 20.85	0.801	(62.57 - 72.28) [55.96, 77.90]
Fat (% DW)	8.09 (0.57) [7.29 - 9.49]	8.44 (0.70) [8.42 - 8.60]	-0.35 (0.83) [-0.92 - 0.88]	-10.92, 10.23	0.749	(3.51 - 9.87) [0, 14.70]
Moisture (% FW)	68.80 (0.48) [67.90 - 69.60]	69.67 (0.48) [69.90 - 70.30]	-0.87 (0.15) [-1.00 - -0.70]	-2.78, 1.05	0.109	(68.50 - 78.40) [60.84, 83.36]
Protein (% DW)	22.02 (0.61) [21.18 - 23.50]	20.83 (0.74) [20.71 - 21.16]	1.19 (0.82) [0.48 - 2.34]	-9.23, 11.61	0.383	(16.48 - 22.78) [13.55, 25.95]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, tolerance interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Table G-4. Statistical Summary of Site IL-1 Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	1.78 (0.032) [1.71 - 1.84]	1.75 (0.037) [1.72 - 1.77]	0.032 (0.035) [-0.011 - 0.069]	-0.41, 0.48	0.528	(1.62 - 1.89) [1.51, 2.00]
Arginine (% DW)	3.11 (0.090) [2.94 - 3.31]	3.00 (0.11) [2.98 - 3.03]	0.10 (0.14) [-0.086 - 0.33]	-1.70, 1.90	0.598	(2.61 - 3.27) [2.27, 3.60]
Aspartic Acid (% DW)	4.77 (0.081) [4.62 - 4.96]	4.63 (0.10) [4.63 - 4.64]	0.14 (0.13) [-0.021 - 0.33]	-1.50, 1.77	0.476	(4.21 - 5.02) [3.85, 5.44]
Cystine (% DW)	0.62 (0.012) [0.61 - 0.63]	0.63 (0.014) [0.61 - 0.65]	-0.012 (0.018) [-0.044 - 0.023]	-0.25, 0.22	0.628	(0.57 - 0.65) [0.55, 0.67]
Glutamic Acid (% DW)	7.61 (0.13) [7.35 - 7.90]	7.38 (0.16) [7.37 - 7.39]	0.23 (0.21) [-0.032 - 0.53]	-2.39, 2.86	0.463	(6.62 - 8.19) [5.86, 8.96]
Glycine (% DW)	1.79 (0.036) [1.72 - 1.87]	1.75 (0.044) [1.74 - 1.76]	0.045 (0.052) [-0.021 - 0.11]	-0.62, 0.71	0.546	(1.62 - 1.90) [1.46, 2.05]
Histidine (% DW)	1.08 (0.019) [1.04 - 1.12]	1.06 (0.023) [1.06 - 1.06]	0.013 (0.030) [-0.024 - 0.057]	-0.37, 0.39	0.731	(0.96 - 1.13) [0.90, 1.21]
Isoleucine (% DW)	1.81 (0.079) [1.70 - 1.97]	1.79 (0.097) [1.71 - 1.88]	0.013 (0.12) [-0.18 - 0.26]	-1.57, 1.60	0.931	(1.64 - 2.00) [1.44, 2.16]

Table G-4 (continued). Statistical Summary of Site IL-1 Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	3.20 (0.055) [3.09 - 3.32]	3.13 (0.068) [3.12 - 3.15]	0.067 (0.087) [-0.054 - 0.20]	-1.04, 1.18	0.582	(2.89 - 3.42) [2.62, 3.66]
Lysine (% DW)	2.65 (0.034) [2.58 - 2.72]	2.60 (0.042) [2.59 - 2.61]	0.046 (0.054) [-0.032 - 0.13]	-0.64, 0.74	0.554	(2.40 - 2.77) [2.22, 2.95]
Methionine (% DW)	0.52 (0.010) [0.50 - 0.54]	0.54 (0.013) [0.53 - 0.54]	-0.018 (0.016) [-0.040 - 0.0088]	-0.23, 0.19	0.474	(0.45 - 0.56) [0.42, 0.60]
Phenylalanine (% DW)	2.11 (0.037) [2.04 - 2.19]	2.06 (0.045) [2.05 - 2.07]	0.051 (0.058) [-0.032 - 0.14]	-0.69, 0.79	0.539	(1.90 - 2.29) [1.70, 2.45]
Proline (% DW)	2.07 (0.042) [1.99 - 2.16]	2.03 (0.051) [2.02 - 2.04]	0.039 (0.061) [-0.032 - 0.12]	-0.73, 0.81	0.640	(1.86 - 2.23) [1.66, 2.38]
Serine (% DW)	2.28 (0.036) [2.25 - 2.32]	2.20 (0.044) [2.13 - 2.27]	0.083 (0.050) [0.013 - 0.13]	-0.55, 0.71	0.342	(1.99 - 2.42) [1.84, 2.54]
Threonine (% DW)	1.60 (0.0089) [1.58 - 1.61]	1.58 (0.0098) [1.57 - 1.59]	0.019 (0.0073) [0.011 - 0.026]	-0.074, 0.11	0.233	(1.44 - 1.67) [1.38, 1.76]
Tryptophan (% DW)	0.37 (0.021) [0.34 - 0.42]	0.36 (0.025) [0.35 - 0.37]	0.0045 (0.033) [-0.033 - 0.064]	-0.41, 0.42	0.913	(0.30 - 0.47) [0.25, 0.54]

Table G-4 (continued). Statistical Summary of Site IL-1 Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	1.44 (0.018) [1.41 - 1.48]	1.42 (0.022) [1.41 - 1.42]	0.029 (0.029) [-0.011 - 0.070]	-0.34, 0.40	0.503	(1.28 - 1.51) [1.18, 1.64]
Valine (% DW)	1.88 (0.086) [1.77 - 2.05]	1.87 (0.11) [1.77 - 1.98]	0.0078 (0.14) [-0.20 - 0.28]	-1.73, 1.74	0.963	(1.71 - 2.09) [1.51, 2.27]
Fatty Acid (% DW)						
16:0 Palmitic (% DW)	2.27 (0.068) [2.16 - 2.40]	2.26 (0.083) [2.18 - 2.33]	0.018 (0.11) [-0.17 - 0.075]	-1.35, 1.38	0.894	(1.66 - 2.35) [1.32, 2.64]
18:0 Stearic (% DW)	0.85 (0.026) [0.80 - 0.89]	0.86 (0.027) [0.81 - 0.86]	-0.014 (0.0054) [-0.019 - -0.0086]	-0.082, 0.054	0.238	(0.63 - 1.07) [0.37, 1.28]
18:1 Oleic (% DW)	3.99 (0.13) [3.84 - 4.24]	4.06 (0.14) [3.84 - 4.09]	-0.074 (0.11) [-0.21 - 0]	-1.45, 1.30	0.616	(2.99 - 5.29) [2.06, 6.43]
18:2 Linoleic (% DW)	9.85 (0.29) [9.37 - 10.39]	9.82 (0.35) [9.49 - 10.16]	0.028 (0.45) [-0.79 - 0.31]	-5.75, 5.80	0.961	(8.41 - 10.69) [7.75, 11.22]
18:3 Linolenic (% DW)	1.29 (0.031) [1.26 - 1.35]	1.27 (0.038) [1.22 - 1.32]	0.024 (0.049) [-0.059 - 0.054]	-0.60, 0.64	0.713	(1.02 - 1.55) [0.84, 1.69]
20:0 Arachidic (% DW)	0.068 (0.0022) [0.064 - 0.071]	0.068 (0.0022) [0.064 - 0.068]	-0.00002 (0.00009) [-0.00011 - 0.00007]	-0.0012, 0.0011	0.889	(0.046 - 0.076) [0.031, 0.094]

Table G-4 (continued). Statistical Summary of Site IL-1 Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Fatty Acid (% DW)						
20:1 Eicosenoic (% DW)	0.047 (0.0014) [0.046 - 0.050]	0.047 (0.0015) [0.045 - 0.048]	-0.00005 (0.0013) [-0.0017 - 0.00075]	-0.016, 0.016	0.973	(0.030 - 0.057) [0.021, 0.065]
22:0 Behenic (% DW)	0.068 (0.0019) [0.065 - 0.072]	0.068 (0.0019) [0.064 - 0.069]	0.00061 (0.00050) [0.00007 - 0.0011]	-0.0058, 0.0070	0.438	(0.046 - 0.073) [0.034, 0.091]
Fiber						
Acid Detergent Fiber (% DW)	20.34 (1.32) [19.14 - 22.01]	19.63 (1.45) [17.16 - 22.44]	0.72 (1.12) [-0.43 - 1.98]	-13.47, 14.90	0.636	(13.30 - 26.26) [9.62, 28.57]
Neutral Detergent Fiber (% DW)	18.38 (0.92) [16.56 - 19.68]	19.61 (0.93) [20.29 - 20.73]	-1.23 (0.18) [-1.40 - -1.04]	-3.47, 1.01	0.090	(14.41 - 23.90) [13.26, 26.33]
Isoflavones						
Daidzein (ug/g DW)	798.14 (69.24) [631.32 - 906.16]	924.74 (84.31) [903.50 - 935.15]	-126.61 (100.67) [-272.18 - -28.99]	-1405.79, 1152.58	0.427	(274.88 - 1485.52) [0, 1925.63]
Genistein (ug/g DW)	711.35 (48.82) [597.98 - 800.94]	747.13 (59.80) [745.11 - 749.14]	-35.78 (77.20) [-151.16 - 55.83]	-1016.65, 945.10	0.723	(354.09 - 984.29) [0, 1387.95]
Glycitein (ug/g DW)	70.03 (4.57) [66.14 - 73.83]	86.00 (5.48) [76.32 - 94.37]	-15.98 (6.04) [-24.26 - -10.17]	-92.69, 60.74	0.230	(52.72 - 298.57) [0, 287.45]
Proximate						
Ash (% DW)	5.11 (0.22) [4.66 - 5.46]	5.25 (0.27) [5.05 - 5.46]	-0.15 (0.34) [-0.81 - 0.42]	-4.52, 4.23	0.745	(4.61 - 5.57) [4.00, 6.08]

Table G-4 (continued). Statistical Summary of Site IL-1 Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Proximate						
Carbohydrates (% DW)	35.97 (0.68) [35.01 - 37.42]	35.90 (0.69) [35.17 - 37.08]	0.076 (0.24) [-0.16 - 0.33]	-3.02, 3.18	0.808	(32.75 - 40.98) [27.86, 45.79]
Fat (% DW)	18.91 (0.55) [18.18 - 19.98]	18.95 (0.68) [18.25 - 19.65]	-0.039 (0.88) [-1.47 - 0.32]	-11.18, 11.11	0.971	(15.97 - 20.68) [15.38, 21.95]
Moisture (% FW)	6.76 (0.20) [6.41 - 7.02]	6.98 (0.22) [6.86 - 7.36]	-0.22 (0.17) [-0.34 - 0]	-2.41, 1.98	0.429	(6.24 - 9.11) [4.64, 9.94]
Protein (% DW)	40.01 (0.90) [38.54 - 42.16]	39.65 (1.10) [39.62 - 39.72]	0.35 (1.39) [-1.07 - 2.44]	-17.33, 18.04	0.841	(36.48 - 43.35) [31.50, 47.45]
Vitamin						
Vitamin E (mg/100g DW)	2.91 (0.069) [2.74 - 3.00]	2.82 (0.084) [2.80 - 2.82]	0.091 (0.10) [-0.064 - 0.16]	-1.23, 1.41	0.542	(1.29 - 4.80) [0, 7.00]
Antinutrient						
Lectin (H.U./mg FW)	1.54 (0.57) [1.07 - 2.11]	2.77 (0.70) [1.67 - 3.86]	-1.23 (0.91) [-2.79 - -0.23]	-12.73, 10.28	0.405	(0.45 - 9.95) [0, 9.72]
Phytic Acid (% DW)	0.83 (0.14) [0.67 - 0.92]	0.81 (0.16) [0.51 - 1.07]	0.018 (0.14) [-0.15 - 0.16]	-1.72, 1.76	0.917	(0.41 - 0.96) [0.39, 1.07]
Raffinose (% DW)	0.56 (0.025) [0.51 - 0.59]	0.56 (0.026) [0.51 - 0.57]	0.0018 (0.0086) [-0.0075 - 0.0098]	-0.11, 0.11	0.869	(0.26 - 0.84) [0, 1.01]

Table G-4 (continued). Statistical Summary of Site IL-1 Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Antinutrient						
Stachyose (% DW)	2.33 (0.14) [2.02 - 2.55]	2.51 (0.17) [2.43 - 2.60]	-0.19 (0.22) [-0.58 - 0.12]	-2.92, 2.55	0.545	(1.53 - 2.98) [1.19, 3.31]
Trypsin Inhibitor (TIU/mg DW)	41.52 (5.18) [33.50 - 53.85]	31.97 (6.35) [30.12 - 33.82]	9.55 (8.20) [-0.32 - 7.10]	-94.59, 113.70	0.451	(20.79 - 55.51) [5.15, 59.34]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, tolerance interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Table G-5. Statistical Summary of Site IL-2 Soybean Forage Fiber and Proximate Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Fiber						
Acid Detergent Fiber (% DW)	35.92 (1.21) [35.09 - 36.71]	36.10 (1.21) [33.92 - 39.33]	-0.18 (1.61) [-3.38 - 1.68]	-7.10, 6.74	0.921	(29.64 - 50.69) [19.03, 54.55]
Neutral Detergent Fiber (% DW)	38.11 (2.61) [33.19 - 40.57]	39.02 (2.61) [33.61 - 42.64]	-0.92 (3.70) [-9.45 - 6.95]	-16.82, 14.98	0.826	(31.43 - 43.70) [26.89, 46.89]
Proximate						
Ash (% DW)	5.59 (0.26) [5.20 - 6.29]	6.45 (0.26) [6.26 - 6.59]	-0.87 (0.35) [-1.40 - -0.21]	-2.37, 0.64	0.131	(5.36 - 8.36) [3.50, 9.58]
Carbohydrates (% DW)	70.44 (0.82) [69.48 - 71.00]	70.34 (0.82) [68.71 - 72.30]	0.11 (0.70) [-1.30 - 0.85]	-2.92, 3.13	0.894	(62.57 - 72.28) [55.96, 77.90]
Fat (% DW)	4.74 (0.57) [4.20 - 5.73]	5.07 (0.57) [3.96 - 6.18]	-0.32 (0.35) [-0.86 - 0.34]	-1.83, 1.19	0.456	(3.51 - 9.87) [0, 14.70]
Moisture (% FW)	71.80 (0.36) [71.40 - 72.10]	72.93 (0.36) [72.00 - 73.50]	-1.13 (0.27) [-1.40 - -0.60]	-2.28, 0.014	0.051	(68.50 - 78.40) [60.84, 83.36]
Protein (% DW)	19.22 (0.36) [18.50 - 19.75]	18.14 (0.36) [17.47 - 18.61]	1.08 (0.51) [-0.11 - 1.95]	-1.11, 3.27	0.167	(16.48 - 22.78) [13.55, 25.95]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, tolerance interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Table G-6. Statistical Summary of Site IL-2 Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	1.75 (0.069) [1.56 - 1.87]	1.79 (0.069) [1.75 - 1.83]	-0.048 (0.077) [-0.19 - 0.069]	-0.38, 0.28	0.593	(1.62 - 1.89) [1.51, 2.00]
Arginine (% DW)	3.11 (0.15) [2.73 - 3.31]	3.19 (0.15) [2.99 - 3.33]	-0.076 (0.099) [-0.26 - 0.078]	-0.50, 0.35	0.523	(2.61 - 3.27) [2.27, 3.60]
Aspartic Acid (% DW)	4.75 (0.21) [4.20 - 5.08]	4.83 (0.21) [4.61 - 4.98]	-0.086 (0.17) [-0.41 - 0.18]	-0.83, 0.66	0.668	(4.21 - 5.02) [3.85, 5.44]
Cystine (% DW)	0.62 (0.014) [0.59 - 0.64]	0.62 (0.014) [0.59 - 0.64]	0.0034 (0.0014) [0.00075 - 0.0054]	-0.0026, 0.0094	0.133	(0.57 - 0.65) [0.55, 0.67]
Glutamic Acid (% DW)	7.59 (0.35) [6.69 - 8.20]	7.66 (0.35) [7.32 - 7.90]	-0.079 (0.30) [-0.63 - 0.43]	-1.39, 1.23	0.820	(6.62 - 8.19) [5.86, 8.96]
Glycine (% DW)	1.77 (0.070) [1.58 - 1.88]	1.82 (0.070) [1.76 - 1.86]	-0.048 (0.071) [-0.18 - 0.058]	-0.35, 0.26	0.565	(1.62 - 1.90) [1.46, 2.05]
Histidine (% DW)	1.07 (0.046) [0.95 - 1.13]	1.10 (0.046) [1.05 - 1.13]	-0.031 (0.037) [-0.10 - 0.015]	-0.19, 0.13	0.493	(0.96 - 1.13) [0.90, 1.21]
Isoleucine (% DW)	1.83 (0.070) [1.65 - 1.93]	1.92 (0.070) [1.87 - 1.99]	-0.094 (0.068) [-0.22 - 0.0043]	-0.39, 0.20	0.297	(1.64 - 2.00) [1.44, 2.16]

Table G-6 (continued). Statistical Summary of Site IL-2 Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	3.17 (0.13) [2.81 - 3.39]	3.25 (0.13) [3.13 - 3.33]	-0.083 (0.12) [-0.32 - 0.10]	-0.61, 0.44	0.569	(2.89 - 3.42) [2.62, 3.66]
Lysine (% DW)	2.61 (0.10) [2.33 - 2.76]	2.67 (0.10) [2.58 - 2.73]	-0.060 (0.096) [-0.25 - 0.070]	-0.47, 0.35	0.592	(2.40 - 2.77) [2.22, 2.95]
Methionine (% DW)	0.53 (0.015) [0.50 - 0.56]	0.53 (0.015) [0.50 - 0.55]	-0.0014 (0.015) [-0.030 - 0.020]	-0.065, 0.062	0.935	(0.45 - 0.56) [0.42, 0.60]
Phenylalanine (% DW)	2.08 (0.092) [1.84 - 2.24]	2.13 (0.092) [2.05 - 2.19]	-0.048 (0.086) [-0.21 - 0.085]	-0.42, 0.32	0.633	(1.90 - 2.29) [1.70, 2.45]
Proline (% DW)	2.06 (0.095) [1.81 - 2.21]	2.08 (0.095) [1.99 - 2.16]	-0.027 (0.085) [-0.18 - 0.12]	-0.39, 0.34	0.782	(1.86 - 2.23) [1.66, 2.38]
Serine (% DW)	2.19 (0.11) [1.93 - 2.42]	2.18 (0.11) [2.08 - 2.25]	0.011 (0.095) [-0.16 - 0.17]	-0.40, 0.42	0.919	(1.99 - 2.42) [1.84, 2.54]
Threonine (% DW)	1.57 (0.062) [1.42 - 1.68]	1.62 (0.062) [1.55 - 1.66]	-0.043 (0.047) [-0.13 - 0.032]	-0.25, 0.16	0.455	(1.44 - 1.67) [1.38, 1.76]
Tryptophan (% DW)	0.42 (0.013) [0.39 - 0.43]	0.39 (0.013) [0.37 - 0.42]	0.027 (0.015) [0.0016 - 0.055]	-0.039, 0.093	0.220	(0.30 - 0.47) [0.25, 0.54]

Table G-6 (continued). Statistical Summary of Site IL-2 Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	1.37 (0.048) [1.25 - 1.46]	1.43 (0.048) [1.38 - 1.47]	-0.060 (0.035) [-0.12 - -0.0015]	-0.21, 0.090	0.226	(1.28 - 1.51) [1.18, 1.64]
Valine (% DW)	1.93 (0.074) [1.73 - 2.04]	2.04 (0.074) [1.98 - 2.11]	-0.11 (0.071) [-0.24 - -0.0052]	-0.41, 0.20	0.268	(1.71 - 2.09) [1.51, 2.27]
Fatty Acid (% DW)						
16:0 Palmitic (% DW)	1.92 (0.044) [1.86 - 2.02]	1.86 (0.044) [1.80 - 1.93]	0.061 (0.063) [-0.049 - 0.22]	-0.21, 0.33	0.430	(1.66 - 2.35) [1.32, 2.64]
18:0 Stearic (% DW)	0.80 (0.027) [0.77 - 0.85]	0.74 (0.027) [0.71 - 0.80]	0.059 (0.039) [-0.019 - 0.14]	-0.11, 0.22	0.269	(0.63 - 1.07) [0.37, 1.28]
18:1 Oleic (% DW)	3.47 (0.097) [3.35 - 3.65]	3.26 (0.097) [3.14 - 3.46]	0.21 (0.14) [-0.11 - 0.51]	-0.38, 0.80	0.265	(2.99 - 5.29) [2.06, 6.43]
18:2 Linoleic (% DW)	8.39 (0.17) [8.22 - 8.64]	8.30 (0.17) [8.00 - 8.68]	0.088 (0.24) [-0.37 - 0.64]	-0.93, 1.11	0.745	(8.41 - 10.69) [7.75, 11.22]
18:3 Linolenic (% DW)	1.25 (0.024) [1.22 - 1.29]	1.25 (0.024) [1.20 - 1.30]	-0.00019 (0.034) [-0.047 - 0.084]	-0.15, 0.15	0.996	(1.02 - 1.55) [0.84, 1.69]
20:0 Arachidic (% DW)	0.062 (0.0021) [0.060 - 0.066]	0.057 (0.0021) [0.055 - 0.062]	0.0053 (0.0030) [-0.00054 - 0.012]	-0.0078, 0.018	0.221	(0.046 - 0.076) [0.031, 0.094]

Table G-6 (continued). Statistical Summary of Site IL-2 Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Fatty Acid (% DW)						
20:1 Eicosenoic (% DW)	0.040 (0.0014) [0.039 - 0.043]	0.037 (0.0014) [0.036 - 0.040]	0.0028 (0.0020) [-0.0012 - 0.0073]	-0.0058, 0.011	0.301	(0.030 - 0.057) [0.021, 0.065]
22:0 Behenic (% DW)	0.060 (0.0019) [0.058 - 0.064]	0.057 (0.0019) [0.054 - 0.061]	0.0034 (0.0027) [-0.0020 - 0.0096]	-0.0084, 0.015	0.338	(0.046 - 0.073) [0.034, 0.091]
Fiber						
Acid Detergent Fiber (% DW)	16.47 (0.66) [15.03 - 17.28]	17.26 (0.66) [16.12 - 18.07]	-0.79 (0.93) [-3.04 - 0.99]	-4.79, 3.22	0.487	(13.30 - 26.26) [9.62, 28.57]
Neutral Detergent Fiber (% DW)	18.88 (0.96) [17.65 - 20.49]	19.31 (0.96) [17.21 - 20.63]	-0.43 (0.85) [-2.14 - 0.44]	-4.10, 3.23	0.660	(14.41 - 23.90) [13.26, 26.33]
Isoflavones						
Daidzein (ug/g DW)	966.47 (56.27) [889.88 - 1027.90]	1104.43 (56.27) [969.70 - 1192.91]	-137.96 (29.09) [-169.05 - -79.82]	-263.14, -12.78	0.041	(274.88 - 1485.52) [0, 1925.63]
Genistein (ug/g DW)	762.46 (31.26) [721.05 - 797.84]	849.88 (31.26) [775.97 - 903.17]	-87.42 (16.28) [-105.33 - -54.92]	-157.47, -17.38	0.032	(354.09 - 984.29) [0, 1387.95]
Glycitein (ug/g DW)	110.39 (11.21) [97.81 - 131.89]	126.04 (11.21) [109.31 - 148.31]	-15.65 (2.21) [-19.03 - -11.50]	-25.15, -6.14	0.019	(52.72 - 298.57) [0, 287.45]
Proximate						
Ash (% DW)	4.92 (0.083) [4.77 - 5.14]	4.80 (0.083) [4.75 - 4.84]	0.12 (0.10) [-0.034 - 0.31]	-0.31, 0.55	0.352	(4.61 - 5.57) [4.00, 6.08]

Table G-6 (continued). Statistical Summary of Site IL-2 Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Proximate						
Carbohydrates (% DW)	37.54 (1.02) [35.65 - 40.24]	37.63 (1.02) [36.84 - 38.21]	-0.089 (1.22) [-2.19 - 2.04]	-5.34, 5.16	0.948	(32.75 - 40.98) [27.86, 45.79]
Fat (% DW)	16.58 (0.39) [16.18 - 17.21]	16.14 (0.39) [15.48 - 16.98]	0.44 (0.55) [-0.63 - 1.73]	-1.91, 2.79	0.504	(15.97 - 20.68) [15.38, 21.95]
Moisture (% FW)	8.53 (0.29) [8.19 - 9.13]	7.48 (0.29) [6.95 - 7.88]	1.05 (0.23) [0.59 - 1.31]	0.058, 2.04	0.045	(6.24 - 9.11) [4.64, 9.94]
Protein (% DW)	40.96 (1.09) [38.26 - 42.37]	41.44 (1.09) [39.98 - 42.44]	-0.47 (0.65) [-1.72 - 0.49]	-3.28, 2.33	0.544	(36.48 - 43.35) [31.50, 47.45]
Vitamin						
Vitamin E (mg/100g DW)	2.43 (0.093) [2.28 - 2.67]	2.07 (0.093) [2.01 - 2.18]	0.36 (0.13) [0.17 - 0.66]	-0.20, 0.92	0.111	(1.29 - 4.80) [0, 7.00]
Antinutrient						
Lectin (H.U./mg FW)	5.14 (2.06) [0.70 - 7.86]	6.47 (2.06) [4.43 - 10.18]	-1.34 (2.39) [-4.11 - 3.43]	-11.64, 8.96	0.632	(0.45 - 9.95) [0, 9.72]
Phytic Acid (% DW)	0.80 (0.080) [0.67 - 0.93]	0.75 (0.080) [0.64 - 0.91]	0.046 (0.11) [-0.24 - 0.30]	-0.44, 0.53	0.723	(0.41 - 0.96) [0.39, 1.07]
Raffinose (% DW)	0.42 (0.017) [0.40 - 0.43]	0.33 (0.017) [0.31 - 0.38]	0.085 (0.016) [0.055 - 0.11]	0.014, 0.15	0.035	(0.26 - 0.84) [0, 1.01]

Table G-6 (continued). Statistical Summary of Site IL-2 Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Antinutrient						
Stachyose (% DW)	2.47 (0.15) [2.46 - 2.48]	2.41 (0.15) [2.12 - 2.81]	0.059 (0.20) [-0.34 - 0.35]	-0.82, 0.94	0.798	(1.53 - 2.98) [1.19, 3.31]
Trypsin Inhibitor (TIU/mg DW)	29.37 (0.89) [28.39 - 30.41]	28.18 (0.89) [26.22 - 30.09]	1.19 (1.26) [-0.79 - 4.19]	-4.23, 6.61	0.444	(20.79 - 55.51) [5.15, 59.34]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, tolerance interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Table G-7. Statistical Summary of Site NE Soybean Forage Fiber and Proximate Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Fiber						
Acid Detergent Fiber (% DW)	41.40 (1.98) [38.47 - 45.99]	49.11 (1.98) [46.02 - 50.89]	-7.71 (2.80) [-11.96 - -0.026]	-19.74, 4.32	0.110	(29.64 - 50.69) [19.03, 54.55]
Neutral Detergent Fiber (% DW)	37.27 (1.61) [35.19 - 39.29]	39.85 (1.61) [36.42 - 43.14]	-2.59 (2.27) [-7.95 - 0.91]	-12.37, 7.19	0.373	(31.43 - 43.70) [26.89, 46.89]
Proximate						
Ash (% DW)	7.24 (0.29) [6.65 - 7.77]	7.42 (0.29) [7.11 - 7.95]	-0.18 (0.16) [-0.46 - 0.087]	-0.86, 0.50	0.364	(5.36 - 8.36) [3.50, 9.58]
Carbohydrates (% DW)	67.47 (0.23) [67.10 - 68.09]	67.83 (0.23) [67.70 - 67.93]	-0.36 (0.27) [-0.76 - 0.16]	-1.53, 0.81	0.317	(62.57 - 72.28) [55.96, 77.90]
Fat (% DW)	5.00 (0.19) [4.64 - 5.21]	4.89 (0.19) [4.64 - 5.30]	0.11 (0.15) [-0.090 - 0.41]	-0.56, 0.77	0.558	(3.51 - 9.87) [0, 14.70]
Moisture (% FW)	76.77 (0.34) [76.30 - 77.60]	77.27 (0.34) [76.80 - 77.60]	-0.50 (0.32) [-1.10 - 0]	-1.88, 0.88	0.260	(68.50 - 78.40) [60.84, 83.36]
Protein (% DW)	20.29 (0.20) [20.04 - 20.49]	19.85 (0.20) [19.55 - 20.35]	0.44 (0.28) [-0.016 - 0.94]	-0.75, 1.63	0.255	(16.48 - 22.78) [13.55, 25.95]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, tolerance interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Table G-8. Statistical Summary of Site NE Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	1.76 (0.014) [1.75 - 1.77]	1.77 (0.014) [1.73 - 1.79]	-0.0087 (0.019) [-0.042 - 0.022]	-0.089, 0.072	0.687	(1.62 - 1.89) [1.51, 2.00]
Arginine (% DW)	3.06 (0.027) [2.99 - 3.12]	3.07 (0.027) [3.06 - 3.07]	-0.0098 (0.039) [-0.085 - 0.066]	-0.18, 0.16	0.823	(2.61 - 3.27) [2.27, 3.60]
Aspartic Acid (% DW)	4.74 (0.028) [4.69 - 4.81]	4.75 (0.028) [4.73 - 4.78]	-0.0076 (0.039) [-0.095 - 0.067]	-0.18, 0.16	0.863	(4.21 - 5.02) [3.85, 5.44]
Cystine (% DW)	0.65 (0.0087) [0.63 - 0.67]	0.64 (0.0087) [0.64 - 0.64]	0.0063 (0.012) [-0.015 - 0.026]	-0.045, 0.058	0.651	(0.57 - 0.65) [0.55, 0.67]
Glutamic Acid (% DW)	7.52 (0.049) [7.41 - 7.63]	7.50 (0.049) [7.48 - 7.56]	0.019 (0.069) [-0.15 - 0.15]	-0.28, 0.32	0.812	(6.62 - 8.19) [5.86, 8.96]
Glycine (% DW)	1.77 (0.0086) [1.75 - 1.79]	1.78 (0.0086) [1.76 - 1.78]	-0.0015 (0.012) [-0.031 - 0.022]	-0.054, 0.051	0.911	(1.62 - 1.90) [1.46, 2.05]
Histidine (% DW)	1.07 (0.0066) [1.06 - 1.09]	1.08 (0.0066) [1.07 - 1.09]	-0.0027 (0.0094) [-0.025 - 0.017]	-0.043, 0.038	0.799	(0.96 - 1.13) [0.90, 1.21]
Isoleucine (% DW)	1.79 (0.010) [1.77 - 1.82]	1.80 (0.010) [1.80 - 1.81]	-0.012 (0.014) [-0.031 - 0.022]	-0.074, 0.049	0.481	(1.64 - 2.00) [1.44, 2.16]

Table G-8 (continued). Statistical Summary of Site NE Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	3.19 (0.017) [3.15 - 3.23]	3.18 (0.017) [3.17 - 3.20]	0.00087 (0.025) [-0.052 - 0.055]	-0.11, 0.11	0.975	(2.89 - 3.42) [2.62, 3.66]
Lysine (% DW)	2.62 (0.014) [2.59 - 2.65]	2.63 (0.014) [2.62 - 2.65]	-0.013 (0.020) [-0.063 - 0.023]	-0.098, 0.072	0.578	(2.40 - 2.77) [2.22, 2.95]
Methionine (% DW)	0.49 (0.012) [0.47 - 0.52]	0.52 (0.012) [0.51 - 0.52]	-0.023 (0.012) [-0.036 - 0.00035]	-0.073, 0.027	0.189	(0.45 - 0.56) [0.42, 0.60]
Phenylalanine (% DW)	2.10 (0.012) [2.08 - 2.13]	2.10 (0.012) [2.09 - 2.11]	0.0018 (0.017) [-0.031 - 0.033]	-0.072, 0.075	0.925	(1.90 - 2.29) [1.70, 2.45]
Proline (% DW)	2.05 (0.0080) [2.04 - 2.07]	2.06 (0.0080) [2.05 - 2.08]	-0.0053 (0.0045) [-0.0099 - 0.0037]	-0.025, 0.014	0.358	(1.86 - 2.23) [1.66, 2.38]
Serine (% DW)	2.26 (0.018) [2.22 - 2.28]	2.25 (0.018) [2.23 - 2.28]	0.0088 (0.026) [-0.064 - 0.046]	-0.10, 0.12	0.767	(1.99 - 2.42) [1.84, 2.54]
Threonine (% DW)	1.59 (0.0087) [1.58 - 1.61]	1.61 (0.0087) [1.59 - 1.61]	-0.012 (0.0065) [-0.021 - 0.00069]	-0.040, 0.016	0.205	(1.44 - 1.67) [1.38, 1.76]
Tryptophan (% DW)	0.39 (0.019) [0.35 - 0.44]	0.45 (0.019) [0.44 - 0.46]	-0.059 (0.025) [-0.10 - -0.019]	-0.17, 0.047	0.139	(0.30 - 0.47) [0.25, 0.54]

Table G-8 (continued). Statistical Summary of Site NE Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	1.43 (0.010) [1.41 - 1.45]	1.43 (0.010) [1.42 - 1.44]	0.0024 (0.014) [-0.032 - 0.033]	-0.059, 0.064	0.884	(1.28 - 1.51) [1.18, 1.64]
Valine (% DW)	1.88 (0.010) [1.86 - 1.91]	1.90 (0.010) [1.89 - 1.91]	-0.023 (0.014) [-0.042 - 0.012]	-0.085, 0.039	0.248	(1.71 - 2.09) [1.51, 2.27]
Fatty Acid (% DW)						
16:0 Palmitic (% DW)	1.98 (0.092) [1.84 - 2.16]	1.87 (0.092) [1.71 - 2.01]	0.11 (0.088) [-0.060 - 0.24]	-0.27, 0.49	0.340	(1.66 - 2.35) [1.32, 2.64]
18:0 Stearic (% DW)	0.70 (0.034) [0.65 - 0.76]	0.67 (0.034) [0.61 - 0.73]	0.028 (0.034) [-0.030 - 0.086]	-0.12, 0.17	0.496	(0.63 - 1.07) [0.37, 1.28]
18:1 Oleic (% DW)	3.29 (0.15) [3.05 - 3.56]	3.20 (0.15) [2.92 - 3.42]	0.094 (0.15) [-0.19 - 0.33]	-0.57, 0.76	0.604	(2.99 - 5.29) [2.06, 6.43]
18:2 Linoleic (% DW)	8.58 (0.38) [8.00 - 9.32]	8.11 (0.38) [7.42 - 8.71]	0.47 (0.35) [-0.20 - 0.99]	-1.05, 1.98	0.315	(8.41 - 10.69) [7.75, 11.22]
18:3 Linolenic (% DW)	1.34 (0.062) [1.23 - 1.46]	1.27 (0.062) [1.17 - 1.37]	0.071 (0.056) [-0.037 - 0.15]	-0.17, 0.31	0.333	(1.02 - 1.55) [0.84, 1.69]
20:0 Arachidic (% DW)	0.053 (0.0026) [0.049 - 0.058]	0.051 (0.0026) [0.046 - 0.055]	0.0022 (0.0027) [-0.0024 - 0.0068]	-0.0092, 0.014	0.493	(0.046 - 0.076) [0.031, 0.094]

Table G-8 (continued). Statistical Summary of Site NE Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Fatty Acid (% DW)						
20:1 Eicosenoic (% DW)	0.034 (0.0017) [0.032 - 0.037]	0.033 (0.0017) [0.029 - 0.035]	0.0015 (0.0017) [-0.0015 - 0.0042]	-0.0056, 0.0087	0.449	(0.030 - 0.057) [0.021, 0.065]
22:0 Behenic (% DW)	0.054 (0.0026) [0.050 - 0.059]	0.051 (0.0026) [0.046 - 0.055]	0.0029 (0.0025) [-0.0018 - 0.0067]	-0.0078, 0.014	0.360	(0.046 - 0.073) [0.034, 0.091]
Fiber						
Acid Detergent Fiber (% DW)	15.05 (0.50) [14.64 - 15.27]	16.53 (0.50) [15.58 - 17.86]	-1.48 (0.71) [-3.22 - -0.32]	-4.55, 1.59	0.173	(13.30 - 26.26) [9.62, 28.57]
Neutral Detergent Fiber (% DW)	17.42 (0.37) [16.79 - 18.39]	19.91 (0.37) [19.58 - 20.10]	-2.49 (0.39) [-2.97 - -1.71]	-4.18, -0.80	0.023	(14.41 - 23.90) [13.26, 26.33]
Isoflavones						
Daidzein (ug/g DW)	1433.50 (50.67) [1344.09 - 1571.41]	1495.62 (50.67) [1475.98 - 1526.23]	-62.12 (71.66) [-182.14 - 86.74]	-370.44, 246.20	0.477	(274.88 - 1485.52) [0, 1925.63]
Genistein (ug/g DW)	984.78 (7.62) [964.14 - 996.66]	999.96 (7.62) [994.08 - 1003.02]	-15.18 (10.77) [-38.88 - 2.58]	-61.52, 31.16	0.294	(354.09 - 984.29) [0, 1387.95]
Glycitein (ug/g DW)	110.33 (19.95) [71.94 - 162.52]	117.14 (19.95) [104.90 - 132.33]	-6.82 (19.02) [-32.97 - 30.19]	-88.67, 75.04	0.754	(52.72 - 298.57) [0, 287.45]
Proximate						
Ash (% DW)	4.71 (0.033) [4.66 - 4.74]	4.81 (0.033) [4.76 - 4.88]	-0.097 (0.046) [-0.22 - -0.019]	-0.30, 0.10	0.168	(4.61 - 5.57) [4.00, 6.08]

Table G-8 (continued). Statistical Summary of Site NE Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Proximate						
Carbohydrates (% DW)	37.77 (0.83) [36.39 - 38.58]	38.88 (0.83) [37.58 - 40.74]	-1.11 (0.76) [-2.38 - 0.24]	-4.37, 2.16	0.281	(32.75 - 40.98) [27.86, 45.79]
Fat (% DW)	16.49 (0.75) [15.35 - 17.97]	15.75 (0.75) [14.40 - 16.89]	0.74 (0.69) [-0.59 - 1.73]	-2.23, 3.71	0.396	(15.97 - 20.68) [15.38, 21.95]
Moisture (% FW)	6.98 (0.065) [6.86 - 7.09]	7.06 (0.065) [6.96 - 7.18]	-0.080 (0.092) [-0.32 - 0.040]	-0.48, 0.32	0.478	(6.24 - 9.11) [4.64, 9.94]
Protein (% DW)	41.03 (0.23) [40.86 - 41.34]	40.57 (0.23) [39.98 - 40.94]	0.47 (0.22) [0.13 - 0.88]	-0.48, 1.41	0.168	(36.48 - 43.35) [31.50, 47.45]
Vitamin						
Vitamin E (mg/100g DW)	1.99 (0.12) [1.88 - 2.07]	1.89 (0.12) [1.58 - 2.11]	0.099 (0.17) [-0.23 - 0.49]	-0.62, 0.81	0.612	(1.29 - 4.80) [0, 7.00]
Antinutrient						
Lectin (H.U./mg FW)	6.19 (1.84) [1.14 - 9.77]	3.95 (1.84) [3.68 - 4.16]	2.23 (2.60) [-3.02 - 5.75]	-8.96, 13.42	0.480	(0.45 - 9.95) [0, 9.72]
Phytic Acid (% DW)	0.67 (0.062) [0.58 - 0.77]	0.67 (0.062) [0.53 - 0.77]	-0.0019 (0.088) [-0.18 - 0.12]	-0.38, 0.38	0.984	(0.41 - 0.96) [0.39, 1.07]
Raffinose (% DW)	0.45 (0.027) [0.40 - 0.52]	0.45 (0.027) [0.44 - 0.47]	-0.0018 (0.038) [-0.069 - 0.085]	-0.16, 0.16	0.966	(0.26 - 0.84) [0, 1.01]

Table G-8 (continued). Statistical Summary of Site NE Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Antinutrient						
Stachyose (% DW)	2.43 (0.16) [2.18 - 2.85]	2.35 (0.16) [2.26 - 2.46]	0.081 (0.22) [-0.28 - 0.53]	-0.87, 1.03	0.749	(1.53 - 2.98) [1.19, 3.31]
Trypsin Inhibitor (TIU/mg DW)	30.39 (2.69) [24.59 - 37.42]	24.57 (2.69) [23.43 - 25.50]	5.82 (3.80) [-0.19 - 13.99]	-10.54, 22.19	0.265	(20.79 - 55.51) [5.15, 59.34]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, tolerance interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Table G-9. Statistical Summary of Site OH Soybean Forage Fiber and Proximate Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Fiber						
Acid Detergent Fiber (% DW)	39.73 (1.26) [37.54 - 42.47]	37.10 (1.41) [37.37 - 38.35]	2.63 (1.17) [1.81 - 4.12]	-12.27, 17.53	0.266	(29.64 - 50.69) [19.03, 54.55]
Neutral Detergent Fiber (% DW)	34.51 (1.45) [32.77 - 36.52]	37.80 (1.77) [35.37 - 40.23]	-3.29 (2.29) [-7.45 - -1.15]	-32.41, 25.82	0.386	(31.43 - 43.70) [26.89, 46.89]
Proximate						
Ash (% DW)	7.87 (0.25) [7.56 - 8.36]	6.63 (0.31) [6.32 - 6.94]	1.24 (0.40) [0.62 - 2.04]	-3.79, 6.26	0.196	(5.36 - 8.36) [3.50, 9.58]
Carbohydrates (% DW)	65.37 (0.88) [64.03 - 67.08]	66.34 (1.08) [65.30 - 67.37]	-0.97 (1.39) [-2.37 - 1.78]	-18.68, 16.74	0.613	(62.57 - 72.28) [55.96, 77.90]
Fat (% DW)	6.82 (0.38) [6.19 - 7.54]	7.67 (0.39) [7.12 - 7.52]	-0.85 (0.080) [-0.93 - -0.77]	-1.86, 0.17	0.059	(3.51 - 9.87) [0, 14.70]
Moisture (% FW)	70.80 (0.36) [70.70 - 70.90]	72.65 (0.44) [71.90 - 73.40]	-1.85 (0.56) [-2.60 - -1.00]	-9.02, 5.32	0.188	(68.50 - 78.40) [60.84, 83.36]
Protein (% DW)	19.94 (0.57) [19.18 - 20.75]	19.72 (0.70) [18.80 - 20.64]	0.22 (0.90) [-1.47 - 1.10]	-11.27, 11.71	0.846	(16.48 - 22.78) [13.55, 25.95]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, tolerance interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Table G-10. Statistical Summary of Site OH Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	1.83 (0.012) [1.81 - 1.84]	1.81 (0.013) [1.78 - 1.83]	0.021 (0.0091) [0.011 - 0.030]	-0.094, 0.14	0.257	(1.62 - 1.89) [1.51, 2.00]
Arginine (% DW)	3.26 (0.032) [3.20 - 3.29]	3.29 (0.039) [3.24 - 3.34]	-0.031 (0.051) [-0.13 - 0.050]	-0.67, 0.61	0.648	(2.61 - 3.27) [2.27, 3.60]
Aspartic Acid (% DW)	4.89 (0.023) [4.84 - 4.92]	4.87 (0.028) [4.85 - 4.89]	0.019 (0.036) [-0.051 - 0.058]	-0.43, 0.47	0.683	(4.21 - 5.02) [3.85, 5.44]
Cystine (% DW)	0.60 (0.0039) [0.59 - 0.61]	0.60 (0.0043) [0.59 - 0.60]	-0.00028 (0.0031) [-0.0037 - 0.0030]	-0.040, 0.039	0.942	(0.57 - 0.65) [0.55, 0.67]
Glutamic Acid (% DW)	7.83 (0.034) [7.75 - 7.87]	7.78 (0.041) [7.76 - 7.79]	0.048 (0.045) [-0.015 - 0.083]	-0.52, 0.62	0.478	(6.62 - 8.19) [5.86, 8.96]
Glycine (% DW)	1.84 (0.0074) [1.82 - 1.85]	1.82 (0.0091) [1.82 - 1.82]	0.018 (0.012) [-0.0017 - 0.033]	-0.13, 0.17	0.363	(1.62 - 1.90) [1.46, 2.05]
Histidine (% DW)	1.10 (0.0067) [1.09 - 1.11]	1.10 (0.0083) [1.10 - 1.10]	0.0017 (0.011) [-0.016 - 0.013]	-0.13, 0.14	0.899	(0.96 - 1.13) [0.90, 1.21]
Isoleucine (% DW)	1.91 (0.0078) [1.90 - 1.93]	1.90 (0.0085) [1.89 - 1.90]	0.017 (0.0064) [0.0094 - 0.023]	-0.064, 0.098	0.230	(1.64 - 2.00) [1.44, 2.16]

Table G-10 (continued). Statistical Summary of Site OH Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	3.30 (0.014) [3.27 - 3.32]	3.28 (0.017) [3.27 - 3.28]	0.021 (0.019) [-0.0053 - 0.039]	-0.22, 0.26	0.472	(2.89 - 3.42) [2.62, 3.66]
Lysine (% DW)	2.69 (0.011) [2.66 - 2.71]	2.66 (0.013) [2.66 - 2.66]	0.026 (0.016) [0.0024 - 0.043]	-0.18, 0.23	0.354	(2.40 - 2.77) [2.22, 2.95]
Methionine (% DW)	0.53 (0.0037) [0.53 - 0.54]	0.53 (0.0046) [0.53 - 0.54]	-0.0024 (0.0059) [-0.014 - 0.0069]	-0.077, 0.072	0.757	(0.45 - 0.56) [0.42, 0.60]
Phenylalanine (% DW)	2.19 (0.0076) [2.18 - 2.21]	2.18 (0.0086) [2.18 - 2.19]	0.0075 (0.0071) [0.00002 - 0.016]	-0.082, 0.097	0.481	(1.90 - 2.29) [1.70, 2.45]
Proline (% DW)	2.13 (0.012) [2.12 - 2.15]	2.12 (0.015) [2.10 - 2.13]	0.019 (0.019) [-0.011 - 0.058]	-0.22, 0.26	0.504	(1.86 - 2.23) [1.66, 2.38]
Serine (% DW)	2.29 (0.017) [2.26 - 2.32]	2.28 (0.017) [2.25 - 2.28]	0.0086 (0.0027) [0.0057 - 0.011]	-0.026, 0.043	0.195	(1.99 - 2.42) [1.84, 2.54]
Threonine (% DW)	1.59 (0.020) [1.57 - 1.63]	1.60 (0.024) [1.57 - 1.62]	-0.0015 (0.031) [-0.046 - 0.062]	-0.40, 0.40	0.968	(1.44 - 1.67) [1.38, 1.76]
Tryptophan (% DW)	0.41 (0.012) [0.39 - 0.42]	0.40 (0.015) [0.38 - 0.42]	0.0084 (0.019) [-0.027 - 0.032]	-0.24, 0.25	0.737	(0.30 - 0.47) [0.25, 0.54]

Table G-10 (continued). Statistical Summary of Site OH Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	1.46 (0.0071) [1.44 - 1.47]	1.46 (0.0087) [1.45 - 1.46]	0.0045 (0.011) [-0.014 - 0.017]	-0.14, 0.15	0.754	(1.28 - 1.51) [1.18, 1.64]
Valine (% DW)	2.01 (0.011) [1.99 - 2.04]	2.00 (0.011) [1.98 - 2.01]	0.017 (0.0069) [0.0098 - 0.024]	-0.071, 0.10	0.249	(1.71 - 2.09) [1.51, 2.27]
Fatty Acid (% DW)						
16:0 Palmitic (% DW)	1.95 (0.013) [1.94 - 1.97]	1.96 (0.014) [1.94 - 1.97]	-0.0073 (0.012) [-0.023 - 0.0016]	-0.16, 0.15	0.651	(1.66 - 2.35) [1.32, 2.64]
18:0 Stearic (% DW)	0.79 (0.031) [0.75 - 0.85]	0.80 (0.033) [0.74 - 0.81]	-0.0024 (0.021) [-0.027 - 0.014]	-0.27, 0.26	0.925	(0.63 - 1.07) [0.37, 1.28]
18:1 Oleic (% DW)	3.59 (0.18) [3.33 - 3.91]	3.68 (0.19) [3.33 - 3.75]	-0.084 (0.11) [-0.22 - 0.0075]	-1.53, 1.36	0.592	(2.99 - 5.29) [2.06, 6.43]
18:2 Linoleic (% DW)	8.76 (0.024) [8.72 - 8.81]	8.91 (0.030) [8.89 - 8.92]	-0.15 (0.038) [-0.20 - -0.14]	-0.63, 0.34	0.162	(8.41 - 10.69) [7.75, 11.22]
18:3 Linolenic (% DW)	1.11 (0.021) [1.09 - 1.15]	1.12 (0.022) [1.09 - 1.17]	-0.0077 (0.012) [-0.018 - 0.0054]	-0.16, 0.14	0.630	(1.02 - 1.55) [0.84, 1.69]
20:0 Arachidic (% DW)	0.063 (0.0029) [0.059 - 0.068]	0.063 (0.0030) [0.058 - 0.064]	0.00012 (0.0016) [-0.0017 - 0.0014]	-0.020, 0.020	0.952	(0.046 - 0.076) [0.031, 0.094]

Table G-10 (continued). Statistical Summary of Site OH Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Fatty Acid (% DW)						
20:1 Eicosenoic (% DW)	0.040 (0.0014) [0.038 - 0.043]	0.040 (0.0014) [0.038 - 0.040]	0.00038 (0.00007) [0.00030 - 0.00045]	-0.00056, 0.0013	0.122	(0.030 - 0.057) [0.021, 0.065]
22:0 Behenic (% DW)	0.064 (0.0024) [0.060 - 0.068]	0.065 (0.0024) [0.061 - 0.064]	-0.00032 (0.00045) [-0.00079 - 0.00010]	-0.0060, 0.0053	0.605	(0.046 - 0.073) [0.034, 0.091]
Fiber						
Acid Detergent Fiber (% DW)	16.99 (0.70) [16.38 - 17.97]	18.00 (0.85) [16.79 - 19.20]	-1.01 (1.10) [-2.81 - 1.18]	-14.99, 12.98	0.528	(13.30 - 26.26) [9.62, 28.57]
Neutral Detergent Fiber (% DW)	17.60 (0.64) [16.38 - 18.84]	19.17 (0.78) [18.61 - 19.73]	-1.57 (1.01) [-3.35 - 0.22]	-14.34, 11.20	0.362	(14.41 - 23.90) [13.26, 26.33]
Isoflavones						
Daidzein (ug/g DW)	807.81 (115.21) [640.78 - 983.98]	848.94 (123.29) [747.53 - 1091.03]	-41.13 (78.20) [-107.05 - 51.13]	-1034.75, 952.49	0.691	(274.88 - 1485.52) [0, 1925.63]
Genistein (ug/g DW)	715.99 (89.34) [565.26 - 857.33]	678.93 (91.41) [651.01 - 850.36]	37.06 (33.73) [6.97 - 74.36]	-391.57, 465.68	0.470	(354.09 - 984.29) [0, 1387.95]
Glycitein (ug/g DW)	62.41 (4.54) [53.78 - 68.39]	82.07 (4.55) [72.93 - 85.25]	-19.66 (0.52) [-20.19 - -19.15]	-26.30, -13.02	0.016	(52.72 - 298.57) [0, 287.45]
Proximate						
Ash (% DW)	4.96 (0.14) [4.75 - 5.19]	5.12 (0.14) [4.86 - 5.17]	-0.16 (0.070) [-0.24 - -0.10]	-1.05, 0.73	0.257	(4.61 - 5.57) [4.00, 6.08]

Table G-10 (continued). Statistical Summary of Site OH Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Proximate						
Carbohydrates (% DW)	35.95 (0.21) [35.59 - 36.33]	35.76 (0.25) [35.52 - 35.99]	0.19 (0.33) [-0.065 - 0.81]	-3.99, 4.37	0.663	(32.75 - 40.98) [27.86, 45.79]
Fat (% DW)	17.06 (0.22) [16.78 - 17.48]	17.31 (0.24) [16.90 - 17.37]	-0.25 (0.17) [-0.45 - -0.12]	-2.39, 1.90	0.382	(15.97 - 20.68) [15.38, 21.95]
Moisture (% FW)	7.38 (0.12) [7.22 - 7.62]	6.64 (0.14) [6.51 - 6.76]	0.75 (0.18) [0.46 - 1.11]	-1.58, 3.07	0.153	(6.24 - 9.11) [4.64, 9.94]
Protein (% DW)	42.04 (0.22) [41.75 - 42.54]	41.99 (0.24) [41.93 - 42.25]	0.047 (0.20) [-0.12 - 0.29]	-2.45, 2.55	0.851	(36.48 - 43.35) [31.50, 47.45]
Vitamin						
Vitamin E (mg/100g DW)	3.14 (0.33) [2.60 - 3.72]	3.02 (0.33) [2.46 - 2.99]	0.12 (0.013) [0.11 - 0.14]	-0.040, 0.29	0.065	(1.29 - 4.80) [0, 7.00]
Antinutrient						
Lectin (H.U./mg FW)	5.18 (1.99) [2.76 - 8.82]	8.14 (2.43) [5.41 - 10.87]	-2.96 (3.14) [-8.11 - -1.45]	-42.86, 36.94	0.518	(0.45 - 9.95) [0, 9.72]
Phytic Acid (% DW)	0.73 (0.060) [0.60 - 0.81]	0.74 (0.073) [0.68 - 0.80]	-0.0050 (0.095) [-0.19 - 0.13]	-1.21, 1.20	0.966	(0.41 - 0.96) [0.39, 1.07]
Raffinose (% DW)	0.54 (0.017) [0.51 - 0.58]	0.54 (0.021) [0.53 - 0.55]	-0.0048 (0.027) [-0.043 - 0.045]	-0.35, 0.34	0.887	(0.26 - 0.84) [0, 1.01]

Table G-10 (continued). Statistical Summary of Site OH Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Antinutrient						
Stachyose (% DW)	2.24 (0.085)	2.31 (0.086)	-0.072 (0.0086)	-0.18, 0.037	0.075	(1.53 - 2.98)
	[2.09 - 2.39]	[2.31 - 2.46]	[-0.080 - -0.063]			[1.19, 3.31]
Trypsin Inhibitor (TIU/mg DW)	28.15 (2.38)	28.54 (2.78)	-0.40 (2.75)	-35.35, 34.56	0.908	(20.79 - 55.51)
	[25.46 - 30.85]	[24.88 - 33.59]	[-2.74 - 3.25]			[5.15, 59.34]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, tolerance interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Appendix H. Supplemental Summary of Soybean Grain Compositional Analyses

Purpose

The purpose of this report is to provide statistical summaries of re-expressed amino acid and fatty acid soybean grain composition data. The data used were from a SAS® data set (data540.sas7bdat, created 3/15/2006) containing all of the composition data sent to Certus International for statistical analysis of Monsanto study 05-01-30-32.

The fatty acid, total fat, amino acid, protein and moisture data from MON 89788, A3244, and 12 conventional reference varieties were selected from the data set. The reference varieties grown at each site differed. One control replicate and one reference replicate was missing from Site OH due to insufficient quantity. An additional control replicate were missing from Site IL-1 due to presence of Roundup Ready soybean. Analytes with greater than fifty percent of observations below the assays limit of quantitation (LOQ) were excluded from summaries and analysis. Otherwise, results below the quantitation limit were assigned a value equal to half the quantitation limit. No analytes were assigned values in this study.

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 FW	% Total Protein	X/(10*fp)
		% Total AA	$(100)X_j/\Sigma X_j$, for each AA j
Fatty Acid (FA)	% FW	% Total Fat	X/(ff)
		% Total FA	(100)Xj/Σ Xj, for each FA j
[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).			

Statistical Approach

Summary statistics for the analytes of interest were generated using SAS®[1] software.

Results Discussion

Statistical results of re-expression of amino acid and fatty acid components of MON 89788, A3244, and conventional reference varieties are summarized across sites in Table G-1 and Table G-2, respectively. For each amino acid and fatty acid component re-expression, the overall mean, standard error of the mean (S.E.), and the range of observed values are presented. In addition, the overall range of observed values for commercial reference varieties is presented in the desired unit.

Table H-1. Statistical Summary of Combined-Site Soybean Amino Acid Content for MON 89788, A3244 and Conventional Varieties

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Conventional [Range]
Combined-Site Grain Amino Acid (% Total AA)			
Alanine (% Total AA)	4.38 (0.016) [4.29 - 4.51]	4.39 (0.023) [4.27 - 4.52]	[4.31 - 4.55]
Arginine (% Total AA)	7.58 (0.058) [7.11 - 7.81]	7.58 (0.065) [7.17 - 8.00]	[7.17 - 7.66]
Aspartic Acid (% Total AA)	11.71 (0.013) [11.64 - 11.80]	11.69 (0.015) [11.58 - 11.75]	[11.56 - 11.89]
Cystine (% Total AA)	1.53 (0.019) [1.40 - 1.67]	1.53 (0.017) [1.43 - 1.63]	[1.40 - 1.70]
Glutamic Acid (% Total AA)	18.64 (0.042) [18.39 - 19.03]	18.54 (0.033) [18.24 - 18.72]	[18.21 - 19.02]
Glycine (% Total AA)	4.41 (0.0088) [4.37 - 4.49]	4.40 (0.011) [4.35 - 4.47]	[4.32 - 4.48]
Histidine (% Total AA)	2.65 (0.0034) [2.63 - 2.67]	2.66 (0.0029) [2.65 - 2.68]	[2.61 - 2.70]
Isoleucine (% Total AA)	4.52 (0.029) [4.32 - 4.69]	4.55 (0.035) [4.31 - 4.73]	[4.32 - 4.69]
Leucine (% Total AA)	7.88 (0.0095) [7.82 - 7.95]	7.89 (0.011) [7.84 - 7.98]	[7.80 - 7.97]
Lysine (% Total AA)	6.50 (0.019) [6.41 - 6.63]	6.50 (0.019) [6.38 - 6.59]	[6.38 - 6.62]
Methionine (% Total AA)	1.28 (0.017) [1.16 - 1.40]	1.31 (0.012) [1.26 - 1.39]	[1.11 - 1.41]
Phenylalanine (% Total AA)	5.20 (0.0086) [5.12 - 5.25]	5.20 (0.0097) [5.15 - 5.27]	[5.13 - 5.30]
Proline (% Total AA)	5.09 (0.0076) [5.03 - 5.14]	5.08 (0.014) [5.02 - 5.16]	[4.97 - 5.20]
Serine (% Total AA)	5.52 (0.035) [5.25 - 5.75]	5.47 (0.045) [5.18 - 5.72]	[5.31 - 5.73]
Threonine (% Total AA)	3.91 (0.023) [3.75 - 4.02]	3.94 (0.018) [3.78 - 4.03]	[3.50 - 4.07]

Table H-1 (continued). Statistical Summary of Combined-Site Soybean Amino Acid Content for MON 89788, A3244 and Conventional Varieties

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Conventional [Range]
Combined-Site Grain Amino Acid (% Total AA)			
Tryptophan (% Total AA)	0.96 (0.019) [0.85 - 1.10]	0.97 (0.025) [0.85 - 1.14]	[0.78 - 1.21]
Tyrosine (% Total AA)	3.50 (0.024) [3.27 - 3.60]	3.52 (0.012) [3.44 - 3.58]	[3.40 - 3.60]
Valine (% Total AA)	4.73 (0.031) [4.49 - 4.89]	4.78 (0.042) [4.47 - 5.00]	[4.47 - 4.96]
Combined-Site Grain Amino Acid (% Total Protein)			
Alanine (% Total Protein)	4.39 (0.038) [4.07 - 4.62]	4.39 (0.035) [4.25 - 4.70]	[4.21 - 4.66]
Arginine (% Total Protein)	7.59 (0.061) [7.12 - 7.86]	7.57 (0.063) [7.16 - 7.96]	[6.81 - 7.80]
Aspartic Acid (% Total Protein)	11.73 (0.077) [10.97 - 12.09]	11.68 (0.061) [11.42 - 12.22]	[10.98 - 12.41]
Cystine (% Total Protein)	1.53 (0.019) [1.39 - 1.64]	1.53 (0.019) [1.41 - 1.64]	[1.40 - 1.69]
Glutamic Acid (% Total Protein)	18.67 (0.12) [17.49 - 19.40]	18.52 (0.099) [17.98 - 19.37]	[17.25 - 19.63]
Glycine (% Total Protein)	4.41 (0.033) [4.13 - 4.59]	4.40 (0.025) [4.30 - 4.64]	[4.23 - 4.66]
Histidine (% Total Protein)	2.65 (0.018) [2.48 - 2.76]	2.66 (0.012) [2.61 - 2.76]	[2.52 - 2.81]
Isoleucine (% Total Protein)	4.53 (0.041) [4.30 - 4.80]	4.54 (0.037) [4.29 - 4.75]	[4.17 - 4.90]
Leucine (% Total Protein)	7.90 (0.056) [7.35 - 8.21]	7.88 (0.041) [7.75 - 8.29]	[7.54 - 8.35]
Lysine (% Total Protein)	6.51 (0.053) [6.10 - 6.85]	6.49 (0.041) [6.30 - 6.86]	[6.13 - 6.85]
Methionine (% Total Protein)	1.29 (0.018) [1.16 - 1.40]	1.30 (0.013) [1.25 - 1.38]	[1.12 - 1.45]
Phenylalanine (% Total Protein)	5.21 (0.037) [4.81 - 5.38]	5.19 (0.025) [5.09 - 5.45]	[4.96 - 5.46]

Table H-1 (continued). Statistical Summary of Combined-Site Soybean Amino Acid Content for MON 89788, A3244 and Conventional Varieties

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Conventional [Range]
Combined-Site Grain Amino Acid (% Total Protein)			
Proline (% Total Protein)	5.10 (0.035) [4.73 - 5.26]	5.07 (0.026) [4.94 - 5.27]	[4.85 - 5.32]
Serine (% Total Protein)	5.54 (0.060) [5.04 - 5.90]	5.47 (0.057) [5.22 - 5.93]	[5.18 - 5.85]
Threonine (% Total Protein)	3.92 (0.036) [3.70 - 4.15]	3.93 (0.030) [3.72 - 4.19]	[3.42 - 4.23]
Tryptophan (% Total Protein)	0.96 (0.016) [0.85 - 1.06]	0.97 (0.025) [0.85 - 1.12]	[0.80 - 1.23]
Tyrosine (% Total Protein)	3.50 (0.033) [3.28 - 3.71]	3.51 (0.016) [3.44 - 3.60]	[3.36 - 3.77]
Valine (% Total Protein)	4.74 (0.040) [4.52 - 5.00]	4.77 (0.043) [4.46 - 5.04]	[4.36 - 5.13]

[1]DW = dry weight; AA = amino acid; S.E. = standard error

Table H-2. Statistical Summary of Combined-Site Soybean Fatty Acid Content for MON 89788, A3244 and Conventional Varieties

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Conventional [Range]
Combined-Site Grain Fatty Acid (% Total FA)			
16:0 Palmitic (% Total FA)	12.16 (0.051) [11.74 - 12.42]	12.12 (0.051) [11.80 - 12.31]	[9.33 - 12.53]
18:0 Stearic (% Total FA)	4.60 (0.085) [4.18 - 5.12]	4.49 (0.077) [4.04 - 4.92]	[3.72 - 5.62]
18:1 Oleic (% Total FA)	20.78 (0.40) [18.05 - 23.23]	20.62 (0.33) [18.52 - 22.44]	[19.24 - 28.14]
18:2 Linoleic (% Total FA)	53.88 (0.39) [52.00 - 56.56]	54.09 (0.34) [53.12 - 56.23]	[48.34 - 55.20]
18:3 Linolenic (% Total FA)	7.60 (0.16) [6.48 - 8.40]	7.72 (0.17) [6.54 - 8.40]	[5.70 - 8.79]
20:0 Arachidic (% Total FA)	0.36 (0.0075) [0.33 - 0.40]	0.35 (0.0059) [0.32 - 0.38]	[0.29 - 0.42]
20:1 Eicosenoic (% Total FA)	0.25 (0.0050) [0.21 - 0.27]	0.24 (0.0056) [0.21 - 0.27]	[0.19 - 0.29]
22:0 Behenic (% Total FA)	0.37 (0.0054) [0.33 - 0.41]	0.36 (0.0044) [0.33 - 0.38]	[0.29 - 0.40]
Combined-Site Grain Fatty Acid (% Total Fat)			
16:0 Palmitic (% Total Fat)	11.77 (0.065) [11.30 - 12.14]	11.70 (0.057) [11.36 - 11.94]	[9.01 - 12.19]
18:0 Stearic (% Total Fat)	4.45 (0.080) [4.02 - 4.94]	4.34 (0.075) [3.90 - 4.74]	[3.51 - 5.42]
18:1 Oleic (% Total Fat)	20.11 (0.39) [17.44 - 22.35]	19.90 (0.33) [17.78 - 21.60]	[18.53 - 27.13]
18:2 Linoleic (% Total Fat)	52.13 (0.37) [50.19 - 54.65]	52.20 (0.30) [51.14 - 54.03]	[46.61 - 53.20]
18:3 Linolenic (% Total Fat)	7.36 (0.16) [6.23 - 8.20]	7.45 (0.17) [6.30 - 8.13]	[5.40 - 8.47]
20:0 Arachidic (% Total Fat)	0.35 (0.0071) [0.31 - 0.39]	0.34 (0.0057) [0.31 - 0.37]	[0.28 - 0.40]

Table H-2 (continued). Statistical Summary of Combined-Site Soybean Fatty Acid Content for MON 89788, A3244 and Conventional Varieties

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Conventional [Range]
20:1 Eicosenoic (% Total Fat)	0.24 (0.0048) [0.21 - 0.26]	0.23 (0.0053) [0.20 - 0.26]	[0.19 - 0.28]
22:0 Behenic (% Total Fat)	0.36 (0.0050) [0.32 - 0.39]	0.34 (0.0040) [0.32 - 0.37]	[0.27 - 0.38]

[1]DW = dry weight; FA = fatty acid; S.E. = standard error