

Safety Assessment of Roundup Ready® Flex Cotton, MON 88913

Executive Summary

Since the commercialization of the first cotton varieties with biotechnology-derived traits in the mid-1990s, one of the most successful products, in terms of farmer adoption, has been Roundup Ready® cotton. This product, MON 1445, is tolerant to glyphosate, the active ingredient in the Roundup® family of agricultural herbicides. Roundup Ready cotton has been rapidly adopted by U.S. cotton farmers (95% grower satisfaction¹) and has been a significant part of U.S. annual cotton production since its market introduction. Cotton with the Roundup Ready trait is currently cultivated on approximately 59% of the U.S. cotton acres (USDA-NASS, 2003). However, a constraint within that Roundup Ready cotton system was the limitation of in-crop, over-the-top herbicide application to cotton plants after the four true leaf stage. Applications at the fifth true leaf stage and beyond required specialized spray equipment to apply the herbicide between the rows and away from the cotton plant.

Monsanto Company has developed a second-generation glyphosate-tolerant cotton product, Roundup Ready Flex cotton, MON 88913 (OECD Unique Identifier MON-88913-8), that provides increased tolerance to glyphosate during the critical reproductive phases of growth compared to the first generation Roundup Ready cotton product. Use of MON 88913 enables the application of a Roundup agricultural herbicide over the top of the cotton crop at later stages of development than is possible with MON 1445. This allows for effective weed control during crop production, because Roundup agricultural herbicides are highly effective against the majority of annual and perennial weeds that can be problematic during the later stages of crop development, with minimal risk of crop injury.

The data and information presented in this summary demonstrate that MON 88913, and the feeds and foods derived from it, are as safe and nutritious as commercial conventional varieties of cotton and the comparable feeds and foods derived from these varieties. This is based on three categories of analysis. The first is a detailed molecular characterization of the inserted DNA and a detailed biochemical characterization of the CP4 EPSPS protein produced in MON 88913. The second is a direct assessment of the toxicity and allergenic potential of the CP4 EPSPS protein produced in MON 88913, and the potential for environmental interactions. The third is a safety and nutritional assessment that demonstrates MON 88913 is compositionally equivalent to commercial conventional cotton varieties.

MON 88913 produces the same CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium sp.* strain CP4) that provides tolerance to the action of Roundup agricultural herbicides. The CP4 EPSPS protein produced in MON 88913 is derived from the common soil bacterium, *Agrobacterium*.

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¹ Monsanto unpublished survey results.

The amino acid sequence of the CP4 EPSPS protein produced in MON 88913 is identical to the CP4 EPSPS protein made in Roundup Ready cotton 1445. It is also identical to, or greater than 99% identical to the CP4 EPSPS proteins in other Roundup Ready crops, such as soybean, corn (NK603), and canola, which have nearly a decade of safe human and farm animal consumption. The CP4 EPSPS protein produced in MON 88913 is structurally homologous to EPSPSs naturally present in plants, including food crops (*e.g.*, soybean and corn), and in fungal and microbial food sources such as Baker's yeast (*Saccharomyces cerevisiae*), which have a history of safe use in human consumption (Padgett et al., 1996; Harrison et al., 1996). These data taken together demonstrate a long history of safe consumption by humans and farm animals of EPSPS proteins, including CP4 EPSPS protein, that occur in crops and microbially-based foods. Additionally, bioinformatic analyses, feeding studies, and acute toxicity studies demonstrate that CP4 EPSPS protein is unlikely to be an allergen or toxin. Only low levels of CP4 EPSPS protein are present in MON 88913 seed, representing less than 0.12% of the total protein on a dry weight basis, and there is no significant human consumption of cottonseed protein. The only significant sources of food from cotton are oil and linters, neither of which contains significant levels of protein.

An assessment of the nutritional and compositional equivalence of MON 88913 to a negative segregant control and to 16 commercial conventional cotton varieties was performed on 53 components of cottonseed, 41 components of cottonseed meal, and 13 components of refined, bleached cottonseed oil. These analyses included an assessment of protein, ash, moisture, total fat, carbohydrates, calories, minerals, fiber, amino acids, fatty acids, cyclopropenoid fatty acids, and gossypol levels. Results of these extensive compositional analyses demonstrated that the levels of important nutrients, anti-nutrients, and natural toxicants in MON 88913 were comparable to the control, and not outside the expected values for conventional cotton varieties or published ranges for conventional cotton varieties. Based on these data, and the principle of substantial equivalence as articulated by the World Health Organization, Organization for Economic Cooperation and Development as well as the United Nations Food and Agriculture Organization, MON 88913 cottonseed and its processed products are as safe and nutritious as conventional cotton varieties and their processed food and feed products on the market today.

Independent product safety reviews were conducted for Roundup Ready Flex cotton, MON 88913, by governmental regulatory agencies responsible for the safety assessment of products of modern plant biotechnology. Several of these assessments are listed in Table 1. These assessments include environmental, food, and feed safety; the results of all assessments support the safety and nutritive value of Roundup Ready Flex cotton, MON 88913.

Introduction

Four species of the genus *Gossypium* are known as cotton, which is grown primarily for the fiber produced from seed coat trichomes. Cottonseed fiber (lint), the leading plant fiber crop produced in the world and the most important in the United States, is woven into many yarns, fabrics, and textiles. In the U.S., commercial cotton production is located primarily in 17 states across the cottonbelt, which extends across the southern and western U.S. from Virginia to California. In addition to cotton lint, cottonseed, cottonseed meal, and oil are produced as valuable byproducts.

Weeds are a severe constraint that must be managed in the production of cotton. Cotton plants cannot compete effectively in their early growth stages and must be protected from the invasion of aggressive weeds, which compete with the crop for sunlight, water, and nutrients. Failure to control weeds within the crop results in decreased yields and reduced crop quality. In addition, weeds reduce the efficiency of the mechanical harvest of the crop. Weeds can also reduce the quality of the lint because vegetation stains the fibers, reducing potential uses and value. Current crop management systems combine cultural and mechanical practices with herbicides to overcome the competitive effect.

One of the most effective herbicides available to growers is glyphosate, the active ingredient in Roundup agricultural herbicides. Roundup agricultural herbicides are used as foliar-applied, non-selective herbicides, which are effective against the majority of annual and perennial grasses and broad-leaved weeds. Glyphosate has no pre-emergence or residual soil activity (Franz et al., 1997). Furthermore, glyphosate has favorable environmental characteristics, including binding tightly to soil, making it unlikely to move into groundwater, and that it degrades over time into naturally occurring materials. Data generated to support the registration of Roundup agricultural herbicides and over 30 years of use experience with glyphosate, demonstrate that these herbicides pose minimal risk of adverse effects to humans, mammals, and other non-target organisms under normal use conditions (U.S.EPA, 1993; WHO, 1994; Giesy et al., 2000; Williams et al., 2000).

In 1997, Monsanto Company began marketing Roundup Ready cotton, MON 1445, plants that are tolerant to glyphosate, the active ingredient in the Roundup family of agricultural herbicides. The primary mode of action of glyphosate is the competitive inhibition of the enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). MON 1445 produces an EPSPS derived from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). This CP4 EPSPS is naturally less sensitive to inhibition by glyphosate and thus imparts tolerance to Roundup agricultural herbicides. MON 1445 offers growers an additional tool for improved weed control.

Monsanto has now developed a second-generation glyphosate-tolerant cotton product, Roundup Ready Flex cotton, MON 88913, that provides increased tolerance to glyphosate during the critical reproductive phases of growth compared to MON 1445. Use of MON 88913 will enable the application of a Roundup agricultural herbicide over the top of the cotton crop at later stages of development than is possible with the current product. This will allow for effective weed control during crop production because Roundup agricultural herbicides are highly effective against the majority of annual and perennial weeds that can be problematic during the later

stages of crop development, with minimal risk of crop injury. The increased level of glyphosate tolerance in MON 88913 is achieved through the use of improved DNA promoter sequences that regulate the transcription of the *cp4 epsps* coding sequences.

The introduction of MON 88913 is expected to provide the grower with economic and environmental benefits and superior weed control benefits the same as those currently provided by MON 1445. These benefits include improved flexibility in weed control, lowered labor requirements, and high compatibility with Integrated Pest Management and soil conservation techniques.

MON 1445, supports over-the-top applications of a Roundup agricultural herbicide through the fourth leaf (node) stage, and thereafter is limited to directed sprays up to canopy closure. Further, at least 10 days and two nodes of incremental growth must also occur between applications because of limited reproductive tolerance. Although excellent weed control is obtained with the MON 1445 system, MON 88913 provides growers with added benefits including:

- A wider window of glyphosate application with an increased single application rate
- Broader application timing based on weed height versus the crop development stage
- Ability to circumvent adverse weather or field conditions
- Less dependence on selective or directed spray equipment

Further, the expanded over-the-top window allowed by Roundup Ready Flex cotton is expected to increase production efficiency. Growers will have the freedom to schedule a labeled Roundup agricultural herbicide application with insecticide applications and plant growth regulators common to cotton production, allowing reallocation of labor and equipment to other crops or farming operations. In addition, expanded in-crop, over-the-top applications have the potential to provide improved weed control options with an enhanced margin of assured crop safety with Roundup applications, fewer scheduling challenges with irrigation activities, and enhanced flexibility to tailor herbicide applications to weed development stage instead of to the cotton developmental stage.

In conclusion, the introduction of Roundup Ready Flex cotton will continue the weed control benefits currently available in MON 1445, with enhanced flexibility, convenience, and crop safety.

Molecular Characterization of MON 88913

Roundup Ready Flex cotton, MON 88913, was produced using an *Agrobacterium*-mediated transformation system. Disarmed *Agrobacterium tumefaciens* strain ABI, harboring plasmid PV-GHGT35 (Figure 1), was co-cultured with hypocotyl explants of cotton seedlings that were then used to generate somatic embryogenic cotton callus. Desired callus sectors were selected *in vitro* by incorporating glyphosate into the culture medium. The *A. tumefaciens* cells were eliminated from the cultures by incorporating antibiotics into the culture medium. This process generally followed procedures described by Umbeck et al. (1987) except that MON 88913 was selected *in vitro* using glyphosate. No antibiotic-resistant markers were incorporated into MON 88913. Glyphosate-tolerant callus produced somatic embryos that germinated and developed into plants.

The resulting plants were further screened for commercial potential over several years in growth chamber, greenhouse, and replicated field trials. MON 88913 is derived from a single plant from the *Agrobacterium*-mediated transformation and regeneration processes.

The inserted DNA in MON 88913 contains a single, intact insert comprised of two *cp4 epsps* expression cassettes from the T-DNA of plasmid PV-GHGT35 (Figure 1):

(1) the *ctp2/cp4 epsps* coding sequence whose transcription is directed by the FMV/TSF1 chimeric promoter, the leader (exon 1) and intron sequences from the *Arabidopsis thaliana tsf1* gene, and the transcription termination and polyadenylation signal sequence derived from the 3' nontranslated region of the pea (*Pisum sativum*) ribulose-1,5-bisphosphate carboxylase small subunit (*rbcS*) E9 gene;

(2) a second *ctp2/cp4 epsps* coding sequence, identical to the first, whose transcription is directed by the 35S/ACT8 chimeric promoter, and the leader, intron and flanking sequences from the *act8* gene of *Arabidopsis thaliana*, and the transcription termination and polyadenylation signal sequence derived from the 3' nontranslated region of the pea (*Pisum sativum*) ribulose-1,5-bisphosphate carboxylase small subunit (*rbcS*) E9 gene.

The two *cp4 epsps* expression cassettes from the T-DNA of plasmid PV-GHGT35 were inserted into the cotton genome and resulted in the synthesis of a single CP4 EPSPS protein. The *ctp2* chloroplast transit peptide sequence, derived from the *Arabidopsis thaliana epsps* gene, is present to direct the CP4 EPSPS protein to the cotton chloroplast.

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

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

Additionally, Mendelian segregation of the glyphosate-tolerant phenotype across multiple generations and families corroborates the molecular insert stability analysis and establishes the

genetic behavior of the DNA insert as a single locus. These data confirm homozygosity and generational stability of MON 88913 and, thus, the stability of the DNA insert.

CP4 EPSPS Protein Levels in MON 88913

The level of CP4 EPSPS protein in MON 88913 was determined by a validated enzyme-linked immunosorbent assay (ELISA). The levels of CP4 EPSPS protein in young leaf, overseason leaf (OSL), root, seed, and pollen tissues were determined in tissues collected from MON 88913 produced in replicated field trials across four U.S. field locations. With the exception of pollen, CP4 EPSPS protein levels in tissues were converted from fresh weight (fwt) to dry weight (dwt) values. The mean CP4 EPSPS protein levels for young leaf, OSL1, OSL2, OSL3, root, and seed tissues of MON 88913 were 970, 1400, 690, 630, 99, and 340 µg/g dwt, respectively, (Table 2). The mean CP4 EPSPS protein level for pollen was 4.0 µg/g fwt. As expected, the levels of CP4 EPSPS in all tissue types from the control cotton were below the assay limits of quantitation.

Safety Assessment of the CP4 EPSPS Protein Produced in MON 88913

The safety assessment of the CP4 EPSPS protein produced in Roundup Ready Flex cotton, MON 88913, includes protein characterization, *in vitro* digestibility in simulated gastric and intestinal fluids, acute oral toxicity in mice, and amino acid comparison to known toxins and allergens. In addition, functional and structural comparisons were made of the CP4 EPSPS protein produced in MON 88913 to ubiquitous plant and microbial EPSPS proteins with a history of safe consumption.

MON 88913 produces the CP4 EPSPS protein from *Agrobacterium sp.* strain CP4 that provides tolerance to the action of Roundup agricultural herbicides. The CP4 EPSPS protein is structurally and functionally similar to native plant EPSPS proteins, but has a much reduced affinity for glyphosate (Padgett et al., 1996). In conventional plants, glyphosate binds to the plant EPSPS protein, a critical step in the shikimate biosynthetic pathway, and blocks the biosynthesis of aromatic amino acids, thereby depriving plants of these essential components (Steinrücken and Amrhein, 1980; Haslam, 1993). In Roundup Ready plants producing CP4 EPSPS, requirements for growth and development are met by the continued action of the CP4 EPSPS protein in the presence of glyphosate. MON 88913 produces the CP4 EPSPS protein, and is therefore tolerant to Roundup agricultural herbicides applied over the top of cotton during the growing season.

CP4 EPSPS Characterization and History of Safe Use

The *cp4 epsps* coding regions in MON 88913 have been completely sequenced and encode a 47.6 kDa protein consisting of a single polypeptide of 455 amino acids. The CP4 EPSPS protein produced in MON 88913 is functionally similar to a diverse family of EPSPS proteins naturally present in plants, including food crops (*e.g.*, soybean and corn), and fungal and microbial food sources such as Baker's yeast (*Saccharomyces cerevisiae*), which have a history of safe use in human consumption (Padgett et al., 1996; Harrison et al., 1996). The amino acid sequence of the CP4 EPSPS protein produced in MON 88913 is identical to the CP4 EPSPS protein in MON 1445 and identical to, or greater than 99% identical to, the CP4 EPSPS protein in other Roundup

Ready crops with a history of safe human and farm animal consumption, such as soybean, corn (NK603), and canola

The shikimate pathway is not present in mammals, which contributes to the very favorable toxicological profile for glyphosate (Williams et al., 2000). Herbicide-tolerant crops, primarily those carrying the Roundup Ready trait, were planted on 58.6 million hectares globally in 2004 (James, 2004). Roundup Ready cotton represented a modest but increasing portion of this global acreage, comprising about 6% of global biotech acres in 2004 (James, 2004).

Digestion of CP4 EPSPS in Simulated Gastric and Intestinal Fluids

Experiments were performed to assess the *in vitro* digestibility of the CP4 EPSPS protein using a standardized SGF assay. Digestibility was assessed by three methods, including SDS-polyacrylamide gel staining, western blot analysis, and EPSPS enzymatic activity assay. The results of these experiments demonstrate that *E. coli*-produced mature CP4 EPSPS protein was rapidly digested after incubation in SGF. Colloidal blue staining of SDS-polyacrylamide gels demonstrated that at least 98% of the *E. coli*-produced mature CP4 EPSPS protein was digested in SGF within 15 seconds. No degenerative bands due to digestion were observed. Western blot analysis confirmed that greater than 95% of the *E. coli*-produced CP4 EPSPS protein was digested in SGF within 15 seconds. Likewise, EPSPS activity was reduced to <10% within 15 seconds of incubation of CP4 EPSPS in SGF. In summary, and in complement to an earlier study by Harrison et al. (1996), the three methods (SDS-PAGE, western blot, and functional assay) all demonstrate that the *E. coli*-produced CP4 EPSPS protein is rapidly degraded in SGF.

Assessment of Acute Oral Toxicity of the CP4 EPSPS protein in Mice

An oral acute toxicity study was conducted with *E. coli*-produced CP4 EPSPS protein (Harrison et al., 1996). Acute administration is considered appropriate to assess the safety of CP4 EPSPS since proteins that are toxic act via acute mechanisms (Sjoblad et al., 1992; Pariza and Foster, 1983; Jones and Maryanski, 1991). There were no adverse effects of any kind in this study. Therefore, the no-observed effect level (NOEL) for acute oral toxicity in mice was ≥ 572 mg/kg, the highest dose tested. The lack of effects at this dose results in a large (greater than 1000-fold) margin of safety for potential human consumption of the CP4 EPSPS protein (Harrison et al., 1996).

Assessment of Sequence Similarity of CP4 EPSPS to Known Protein Toxins

Potential structural similarities shared between CP4 EPSPS and proteins in the ALLPEPTIDES database were evaluated using the FASTA sequence alignment tool. Although the FASTA program directly compares amino acid sequences (i.e., primary protein structure), the alignment data may be used to infer higher order structural similarities (i.e., secondary and tertiary protein structures). Proteins that share a high degree of similarity throughout the entire length are often homologous. Homologous proteins share secondary structure and common three-dimensional folds. Proteins identified in the sequence alignment with CP4 EPSPS were ranked according to their degree of similarity. Based on these criteria, there were no significant findings for CP4 EPSPS. Potential structural similarities shared between the CP4 EPSPS protein and proteins in

the toxin database were similarly evaluated. The length and quality of the alignments were low, and the data demonstrate that the CP4 EPSPS protein is highly unlikely to share any structural homology to any known toxin proteins.

Assessment of Exposure of Humans to CP4 EPSPS from Cotton

Cottonseed is not generally consumed by humans due to the natural toxicants present in the seed. However, food products containing highly processed and refined fractions of cottonseed are consumed, primarily refined cottonseed oil. Linters are an industrial byproduct of ginning, but some fractions are consumed as viscose, a highly processed food additive composed of nearly pure cellulose (NCPA, 2002). There is virtually no human exposure to CP4 EPSPS through dietary intake of cottonseed oil derived from MON 88913. Analysis of refined cottonseed oil and processed cotton linters derived from conventional cotton and MON 1445, confirmed that there is no detectable protein in either cottonseed oil or processed cotton linters (Sims et al., 1996). Therefore, significant human consumption of the CP4 EPSPS protein present in cotton is extremely unlikely. Furthermore, direct food challenge of individuals allergic to proteins contained in the meal derived from oilseed crops (*e.g.*, soybean, peanut, and sunflower) with the oil from these respective crops has established that refined oil does not elicit an allergenic response (Bush et al., 1985; Halsey et al., 1986; Taylor et al., 1981). This is consistent with the lack of detectable protein in the oil (Tattrie and Yaguchi, 1973). This information provides a strong basis to conclude that cottonseed oil or linter fractions from MON 88913 pose no significant exposure to the CP4 EPSPS protein or allergenic concerns.

Using upper bound estimates of consumption of food and feed products derived from cottonseed, it is possible to calculate the margin of exposure for the CP4 EPSPS protein from MON 88913. The margin of exposure is defined as the ratio of the no observed effect level derived from toxicology tests, to the estimate of human and animal daily dietary exposure. For these calculations, the maximum amount of CP4 EPSPS protein in cottonseed oil was calculated to be 1.6×10^{-3} $\mu\text{g/g}$ cottonseed protein in oil, based on the limit of detection of cottonseed protein in oil ($1.3 \mu\text{g protein/mL oil} \times 0.12\%$ CP4 EPSPS in cottonseed from MON 88913). The exposure calculation also makes the conservative assumption that there is no loss of the introduced protein during the processing of cottonseed into food and feed products. It also assumes that 100% of the cottonseed, or products derived from cottonseed in the marketplace, are derived from MON 88913. These are very conservative estimates, given the number of commercial cotton varieties that exist in the marketplace, and the lack of detectable protein in oil. Based on the information above, cottonseed and cottonseed oil disappearance data, and the NOEL for acute oral toxicity of the CP4 EPSPS protein, large margins of exposure in humans (4.6×10^9) and dairy cows (3.17×10^5) were calculated for the CP4 EPSPS protein from MON 88913. These calculated margins indicate that there is no risk to human and animal health that will be associated with dietary exposure to food and feed products derived from MON 88913.

Assessment of Allergenic Potential of the CP4 EPSPS Protein

Although there are no single predictive bioassays available to assess the allergenic potential of proteins in humans (U.S. FDA, 1992), the physicochemical and human exposure profile of the protein provides a basis for assessing potential allergenicity by comparing it to known protein allergens. Thus, important considerations of the allergenicity of proteins ingested orally include exposure and factors that contribute to exposure, such as stability to digestion, prevalence in the food, and consumption pattern (amount) of the specific food (Metcalf, et al., 1996; Kimber et al., 1999).

A key parameter contributing to the systemic allergenicity of certain food proteins appears to be stability to gastrointestinal digestion, especially stability to acid proteases like pepsin found in the stomach (Astwood et al., 1996; Astwood and Fuchs, 1996; Fuchs and Astwood, 1996; FAO, 1995; Kimber et al., 1999). Important protein allergens tend to be stable to peptic digestion and the acidic conditions of the stomach and reach the intestinal mucosa as intact molecules to elicit an immune response. As noted in the *in vitro* assessment, the CP4 EPSPS protein is readily digested.

Another significant factor contributing to the allergenicity of certain food proteins is their high concentration in foods (Taylor et al., 1987; Taylor, 1992; Fuchs and Astwood, 1996). Most allergens are present as major protein components in the specific food, representing from 2-3% up to 80% of total protein (Fuchs and Astwood, 1996). In contrast, CP4 EPSPS is present at low levels in MON 88913. The CP4 EPSPS protein represents approximately 0.12% of the total protein in cottonseed of MON 88913. It is also important to establish that the protein does not represent a previously described allergen and does not share potentially cross-reactive amino acid sequence segments or structure with a known allergen.

An efficient way to assess a protein's current or potential allergenicity is to compare the amino acid sequence with that of all known allergens. A bioinformatic assessment of CP4 EPSPS, using allergen and public domain protein sequences databases, demonstrated the absence of sequence similarity to proteins known to pose human health risks. No immunologically relevant sequences (eight contiguous amino acid identities) were detected when the amino acid sequence of the CP4 EPSPS protein was compared to the ALLERGEN3 sequence database. Together, these data demonstrate that the CP4 EPSPS protein present in MON 88913 does not share structurally relevant or immunologically relevant amino acid sequence similarities with allergens or gliadins. Therefore, it is highly unlikely that this protein may contain immunologically cross-reactive allergenic epitopes.

The *cp4 epsps* coding sequence was obtained from a naturally occurring bacterium and has been identified by the American Type Culture Collection as an *Agrobacterium* species. Because there are no reports of allergies to *Agrobacterium* species, it can be concluded that CP4 EPSPS is not from a known allergenic source. *Agrobacterium* sp. strain CP4 has been reviewed previously as a part of the safety assessment of the donor organism during the regulatory processes for Roundup Ready soybean (*Glycine max*), canola (*Brassica napus*), cotton (*Gossypium spp.*), NK603 corn (*Zea mays*), and sugarbeet (*Beta vulgaris*).

In summary, these data and analyses demonstrate that the CP4 EPSPS protein is not detectable in cotton products used for human food, is not derived from an allergenic source, does not possess immunologically relevant sequence similarity with known allergens, and does not possess the characteristics of known protein allergens as summarized below; all of which support the conclusion that the CP4 EPSPS protein does not pose a significant allergenic risk.

Characteristics of known allergenic proteins

<i>Characteristic</i>	<i>Allergens</i>	CP4 EPSPS
Stable to digestion	yes	no
Stable to processing	yes	no
Similarity to known allergens	yes	no
Prevalent protein in food	yes	no

As described in Taylor (1992) and Taylor et al. (1987)

Compositional Analysis and Nutritional Assessment of MON 88913

The design of a food and feed safety assessment program for a genetically engineered crop requires detailed understanding of the uses of the crop and crop products in animal and human nutrition. Cotton is the leading plant fiber crop produced in the world and is grown primarily for its fiber. Cottonseed is fed directly to animals or further processed to produce feed ingredients. Cottonseed meal is primarily used as cattle feed, with smaller proportions of meal fractions used in feed for poultry, sheep, catfish, and swine. Cottonseed serves as an excellent source of fiber and protein in animal feed, particularly due to its high lysine content. Oil is the main food ingredient derived from cottonseed and is used for frying oil and in salad dressings.

Compositional Analysis of Cottonseed, Cottonseed Oil, and Meal

Analyses were performed on the cottonseed from MON 88913 to assess if the inserted DNA or glyphosate-tolerant trait (CP4 EPSPS) in MON 88913 caused any unintended effects on the composition of the cottonseed. A compositional analysis was conducted of delinted cottonseed collected from MON 88913 grown under replicated field conditions at four diverse U.S. locations. MON 88913 was compared to its control, MON 88913(-), which has background genetics representative of MON 88913, but does not contain the inserted DNA or produce the CP4 EPSPS protein. Additionally, 16 commercial conventional cotton varieties were produced in the same field trial. Values derived from these conventional varieties were used as references to produce a 99% tolerance interval for commercial conventional cotton (Table 3).

Analyses were conducted on the cottonseed to measure:

- Amino acid composition (levels of individual amino acids)
- Fatty acid composition (C8-C22) and vitamin E
- Fiber [acid detergent fiber (ADF), neutral detergent fiber (NDF), crude fiber, and total dietary fiber (TDF)]
- Minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc)
- Proximates [protein, total fat, ash, moisture; carbohydrates and calories (determined by calculation)]
- Natural antinutrients and toxins [cyclopropenoid fatty acids (malvalic acid, sterculic acid, and dihydrosterculic acid)], gossypol (free and total), and aflatoxins (B1, B2, G1, and G2)

In all, 69 different components were evaluated in cottonseed of MON 88913. Of the 69 components evaluated, 50% of the observations for 16 of the components were below the assay detection limit and were excluded from the statistical analysis. As a result, 53 components were statistically analysed. Statistical analyses of the compositional data were conducted using a mixed model analysis of variance (ANOVA). MON 88913 was compared to the negative control, MON 88913(-), to determine statistically significant differences at a significance level of $p < 0.05$. In addition, for those comparisons in which MON 88913 was statistically different from the control, the range of values for MON 88913 was compared to the 99% tolerance interval (with 95% confidence) for the conventional reference varieties to determine if the values obtained from MON 88913 were within the population of commercial conventional cotton.

Results across production locations showed six statistically significant differences between MON 88913 and MON 88913(-): phenylalanine, tryptophan, oleic acid (18:1), linoleic acid (18:2), manganese, and moisture levels (Table 3). In all of these cases, the differences between values were small and the values for MON 88913 were not outside the range of expected values for these components in conventional cotton (Tables 3 and 4). These observed differences are unlikely to be biologically meaningful because the range of values for all components that were statistically different from the control were found to be within the 99% tolerance interval for the commercial conventional varieties planted in the same field trials as MON 88913 and MON 88913(-), with the exception of moisture. The range of values for moisture in MON 88913 cottonseed was, however, within published ranges for conventional cottonseed (Table 4).

In addition to whole cottonseed, compositional analyses were conducted on refined, bleached, deodorized cottonseed oil and raw (untoasted) cottonseed meal. Delinted cottonseed for the processed fractions was collected from MON 88913 and MON 88913(-) grown under two diverse U.S. field locations. As a control, whole cottonseed from this production was also analyzed as well as six commercial conventional cotton reference varieties. Statistical analyses of the compositional data were conducted using a mixed model ANOVA. For each matrix, MON 88913 was compared to the negative control, MON 88913(-), to determine statistically significant differences at a significance level of $p < 0.05$.

Statistical differences were found in total gossypol in cottonseed meal, and 14:0 myristic acid and 22:0 behenic acid in cottonseed oil (Tables 5 & 7). The range of values for those

components that were statistically different from controls were all within the 99% tolerance interval for commercial conventional cotton, and are comparable to literature values for these components, where available (Tables 6 & 8). A single statistical difference was observed for phenylalanine in the whole cottonseed from this study, but the difference in value was small: mean of 5.53 in MON 88913 versus 5.49 in MON 88913(-) as a percentage total amino acids. This difference is unlikely to be biologically meaningful because the range of values for phenylalanine in MON 88913 was within the 99% tolerance interval for the commercial conventional varieties planted in the same field trials (5.10 – 6.02 % total amino acids).

With the exception of the moisture level value discussed above, these results demonstrated that with 95% confidence, the levels of key nutrients and other components of cottonseed, raw cottonseed meal, and cottonseed oil derived from MON 88913 are within the values expected for commercial conventional cotton. The range of values for moisture in MON 88913 cottonseed were within published ranges for conventional cottonseed. Therefore, the cottonseed, raw cottonseed meal, and cottonseed oil derived from MON 88913, are considered to be compositionally equivalent to those derived from conventional cotton.

Based on these data, and the principle of substantial equivalence as articulated by the World Health Organization, Organization for Economic Cooperation and Development, and the United Nations Food and Agriculture Organization, these data support the conclusion that MON 88913 is as safe and nutritious as conventional cotton on the market today.

Nutritional Assessment and Toxicological Assessment of Cottonseed

The detailed nutritional assessment of cottonseed, cottonseed oil, and cottonseed meal by composition analyses established that the levels of key nutrients and other components of MON 88913 are compositionally equivalent to those of the control and conventional cotton. In the analysis of cottonseed, the majority of statistical differences identified did not occur across all sites, and for the few statistically different components that occurred across sites, the values for MON 88913 fell within the 99% tolerance interval for commercial cotton. In the analysis of control cottonseed in the processed fractions, only four statistical differences were observed between MON 88913 and MON 88913(-). These values also fell within the 99% tolerance interval for conventional cotton and within literature ranges for the components where available.

In addition to the compositional analyses of cottonseed from MON 88913, the nutritional wholesomeness of seed and grain from a variety of glyphosate-tolerant crops that produce CP4 EPSPS, including maize, soybean, and cotton was demonstrated by feeding seed or grain of these products to farm animals such as chickens and dairy cows (Clark and Ipharraguerre, 2001, 2004; Castillo et al., 2001, 2004). Results of a cow feeding study showed that cottonseed from Roundup Ready cotton, 1445, was as wholesome and nutritious as control cottonseed for cows based on similar feed intakes, general health, milk production, and milk composition (Castillo et al., 2001, 2004). Results of these studies further confirm the food and feed safety of seed and grain of Roundup Ready crops and their substantial equivalence to that of conventional crops.

Assessment of Marker Genes

MON 88913 does not contain an antibiotic resistance marker. MON 88913 was produced by selecting plant cells, tissues, and plants with glyphosate.

Environmental Assessment of MON 88913

Cotton belongs to the genus *Gossypium* of the tribe Gossypieae of the family Malvaceae of the order Malvales (Fryxell, 1979; Munro, 1987). However, some authors have grouped *Gossypium* in the tribe Hibisceae (Smith, 1977). The genus *Gossypium* is currently comprised of approximately 49 species that are widely distributed and occur predominately in tropical and subtropical regions around the world (Percival et al., 1999).

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

In the U.S. and Australia, *G. barbadense* is known as “Pima” or “extra long staple” cotton and is produced in regions overlapping with *G. hirsutum*, commonly known as “upland cotton”. *G. barbadense* has a number of other common names in different regions of the world, for example: ‘Egyptian’ cotton in Egypt, ‘Suvin’ cotton in India, or ‘Tanguis’ cotton in Peru. The close taxonomic and genetic relationship between *G. barbadense* and *G. hirsutum* (of similar parentage, genomic complement, evolutionary history, domestication, and historical cropping practices) indicates a similar environmental impact between these two cultivated cotton species. *G. hirsutum* is the primary cultivated species worldwide, however, *G. barbadense* produces a grade of fiber that sells for a premium above that of upland cotton types. Different ginning operations are required for the extra long staple fibers of *G. barbadense* and for lint grading and classification (USDA, 2001). Although the fiber is segregated at the gin, cottonseed derived from either tetraploid species is morphologically similar and is not segregated for use in food or feed.

Assessment of Agronomic Performance

An evaluation of MON 88913 was conducted to assess the phenotypic equivalence to the negative control that possesses similar background genetics to MON 88913, and is designated MON 88913(-). The phenotypic evaluation is based on laboratory and greenhouse experiments and replicated, multi-site field trials. Data were collected on phenotypic characteristics in five general categories: 1) dormancy, germination and emergence; 2) vegetative growth; 3) reproductive growth; 4) seed retention on plant; and 5) plant interactions with disease, insect, and abiotic stressors.

Field trials were conducted in 2002 at 14 locations across the U.S. cottonbelt to thoroughly evaluate phenotypic characteristics. These 14 locations provided a diverse range of environmental and agronomic conditions representative of cotton production regions. A total of

41 different phenotypic characteristics were evaluated including 11 characteristics during plant growth and development, 20 characteristics from plant mapping, four characteristics from boll/seed measurements, and six boll and fiber quality characteristics.

Out of a total of 458 comparisons between MON 88913 and MON 88913(-) by field location, 19 differences were detected at $p \leq 0.05$. Most observed differences occurred for a single characteristic at a single field location. When all data were pooled across locations, a single difference in the growth and development characteristics was observed. The date until 50% flowering was later for MON 88913 compared to the control (64 vs. 63 days after planting, respectively). This difference was one day at most sites, has little biological meaning in terms of plant pest potential. No differences between MON 88913 and MON 88913(-) were detected for any of the measured plant map characteristics. A single difference was observed in the boll/seed measurements. Seed index of MON 88913 was lower than MON 88913(-) (9.56 vs. 9.83g per 100 fuzzy seed, respectively). This difference was approximately 0.3 g per 100 fuzzy seed, and likely has little biological meaning in terms of plant weed potential.

Each field site also was rated at four times during the season for specific insect pests, diseases and abiotic stressors. Fourteen insect categories (species or group), four disease categories and 10 abiotic stressors were evaluated. Out of 106 insect observations, only one site reported a difference in susceptibility between MON 88913 and the control. Beet armyworm was a severe stressor on the first observation date in one of the four replications at one location. The MON 88913 plot had more damage than the control plot. This was not observed in other replications, or at the other observations times or locations, suggesting that this was due to the location of the field plot or a localized infestation. Out of seven disease and 38 abiotic stressor observations, no differences were detected between MON 88913 and the control. These results support the conclusion that environmental interactions of MON 88913 are not expected to be different than that of other cotton.

In addition, phenotypic assessments from product evaluation field trials were conducted over multiple growing seasons, confirming the quantitative agronomic characterization data discussed above. Results of some of these trials were presented at Beltwide Cotton Conferences (Subramani et al., 2002; May et al., 2003; Keeling et al., 2003; Martens et al., 2002; 2003; Croon et al., 2003).

Seed dormancy is an important characteristic that is often associated with plants that are weeds (Anderson, 1996). Changes were not expected in the dormancy and germination characteristics of MON 88913 because of the ubiquitous nature of EPSPSs in plants, the mechanism of EPSPS, and the commercial history of MON 1445. Standardized germination assays of the Association of Official Seed Analysts (AOSA, 1998) were used as a baseline to measure the germination potential of cottonseed of MON 88913. The tested seed were produced during 2002 at three field locations within the U.S., representing environmentally relevant conditions for cotton production.

Out of 87 comparisons between MON 88913 and the control, 75 were not statistically significant at $p \leq 0.05$. Of the 12 significant differences detected, 10 occurred in seed from the AL and GA locations and two in seed from the CA location. Seed produced at AL & GA had reduced seed

quality. This is not uncommon as cotton is usually grown for lint and not seed in the southeastern U.S.. Humid conditions, typical of AL and GA can degrade seed quality. Although seed quality at AL and GA was poor by seed production standards, it is representative of areas where MON 88913 will be grown for lint. Specifically, one significant difference was observed under the optimal temperature regime (20/30°C); cottonseed from the AL location showed reduced germination in MON 88913 relative to the control. The difference was not detected in cottonseed from the GA or CA locations. With the exception of the AL location at 40°C, the percent germination for MON 88913 was within the range of values generated for the commercial conventional cottonseed produced in the same field trial as MON 88913. Therefore, these differences are unlikely to be biologically meaningful in terms of weediness potential. No differences were detected for seed dormancy-related characteristics, such as hard seed, with seed from any location.

Assessment of MON 88913 Tolerance to Glyphosate

MON 88913 was designed to enhance the reproductive tolerance to glyphosate compared to MON 1445. Data were generated to determine the effects on pollen and floral morphology from over-the-top, sequential applications of glyphosate at 1.5 lb. ae/A per application, applied over the top at three different plant growth stages. Anther dehiscence, anther height, stamen length (anther + filament), staminal column height, pollen grains on stigmatic lobe, pollen deposition, and pollen viability were evaluated.

Untreated MON 88913 anther height as a percent of pistil length was greater than untreated MON 88913(-). This small percentage difference (4%) has little biological meaning in terms of flower morphology or function; this was corroborated by the field plant mapping data in the field phenotypic analyses. No differences were detected in anther dehiscence, stamen length, staminal column height, the number of pollen grains attached to a stigmatic lobe, pollen deposition rating or percent pollen viability between untreated MON 88913 and untreated MON 88913(-).

MON 88913 demonstrated significantly increased reproductive tolerance and pollen viability compared to MON 1445 under these herbicide treatments. Percent pollen viability was significantly greater in treated MON 88913 compared to treated MON 1445. Furthermore, the number of pollen grains attached to the stigmatic lobe was markedly increased in treated MON 88913 over treated MON 1445.

Impact on the Environment and Biodiversity

Assessment of Effects on Other Organisms in the Environment

The CP4 EPSPS protein produced in Roundup Ready crops, including MON 88913, is functionally equivalent to native EPSPS proteins ubiquitous in plants and microbes in the environment. Based on this widespread prevalence of the EPSPS protein in the environment, there is no *a priori* reason to suspect that the CP4 EPSPS protein will possess biological activity towards organisms in the environment. The absence of a plausible mechanism for biological activity in animals minimizes the need for extensive toxicity studies to characterize hazard.

Although minimal ecological risk is predicted, several field monitoring studies have been conducted for Roundup Ready crops, including MON 88913. Field investigations on potential adverse effects of Roundup Ready crops producing CP4 EPSPS have indicated no effects in abundance or population dynamics to field insects (Jasinski et al., 2003; Jackson and Pitre, 2004; Yoshimura et al., 2004; Bitzer et al., 2002; Buckelew et al., 2000; McPherson et al., 2003; Rosca, 2004), or microbial populations (Siciliano and Germida, 1999; Dunfield and Germida, 2003, 2004; Kim et al., 2004; Shin et al., 2004).

In addition, laboratory studies investigating potential effects to organisms exposed to Roundup Ready crops producing CP4 EPSPS, have shown no adverse effects to pollinators (honey bee), soil organisms (Collembola), beneficial insects (lacewing, green cloverworm), or various pest insect species (Boongird et al., 2003; Jamornman et al., 2003; Goldstein, 2003; Michigan Farm News, 2002; Morjan and Pedigo, 2002; Harvey et al., 2004).

Adverse effects on other field organisms from MON 88913 are not expected because EPSPS proteins are ubiquitous in plants, the mechanism of EPSPS, and the extensive commercial production history of Roundup Ready crops, including the first generation Roundup Ready cotton. During regulatory field trials, MON 88913 was grown under agronomic and cultural practices that are typical of cotton production, and disease problems were not observed at any of the sites and there were no meaningful observed differences among the test, control, and commercial conventional reference cotton plots with respect to arthropod damage. Additional observations during multiple years of product development trials have not indicated adverse environmental effects to other field organisms.

Pollen-Mediated Gene Flow

Cotton is normally considered a self-pollinating crop but can be cross-pollinated by certain insects. Sexual transfer of the inserted DNA in MON 88913 to other *Gossypium* species outside of cotton production fields, or to other genera, is considered very unlikely for the following reasons:

- *Gossypium hirsutum* and *G. barbadense* are allotetraploids and are sexually incompatible with diploid cotton species (cultivated or wild). Therefore, these species cannot successfully cross with diploid *Gossypium* spp. to produce fertile offspring.
- Although the potential for outcrossing to wild or feral tetraploid cotton exists, cotton production generally does not occur in the same geographical locations as the wild relatives. For example, outcrossing to *G. tomentosum* in Hawaii is possible, but cotton is not commercially cultivated in Hawaii.
- There are no known plant species outside of the *Gossypium* genus that are sexually compatible with cultivated cotton.

Equally germane, the environmental consequences of pollen transfer from MON 88913 to other cotton or other related *Gossypium* species is considered to be negligible. This is because of limited movement of cotton pollen, the safety of the introduced protein, and the lack of any selective advantage that would be conferred to recipient feral commercial cotton or wild relatives in unmanaged environments if pollen transfer were to occur. In addition, agronomic

consequences of volunteer cotton plants of MON 88913 would be minimal as these plants are easily controlled by mechanical means or by one of a number of non-glyphosate herbicides currently registered for control of cotton.

Horizontal DNA Transfer

Monsanto is not aware of any reports verifying the unaided transfer of genetic material from one cotton species to another species with which cotton cannot sexually interbreed. The DNA that was transferred into the cotton genome to produce MON 88913 does not contain microbial replication or genetic transfer functions. Additionally, MON 88913 does not contain an antibiotic resistance marker, and was produced by selecting plant cells, tissues, and plants with glyphosate.

Weed Resistance to Glyphosate

As leaders in the development and stewardship of glyphosate products for almost 30 years, Monsanto invests considerably in research to understand the proper uses and stewardship of the glyphosate molecule. This research includes an evaluation of the factors that can contribute to the development of weed resistance. Managing the potential for weed resistance is an important issue with all herbicides because it can adversely impact the utility and life cycle of products, if it is not managed properly.

As part of our product stewardship and customer service policy, Monsanto investigates cases of unsatisfactory weed control to determine the cause. Weed control failures following application of Roundup agricultural herbicides are most often the result of management and/or environmental issues and are only rarely the result of herbicide resistance. The procedures included in Monsanto's performance evaluation program provide early detection of potential resistance, field and greenhouse protocols to investigate suspected cases, and mitigation procedures to respond to confirmed cases of glyphosate resistance.

As of October, 2005, some 182 herbicide-resistant species and 304 biotypes within those species have been identified (Heap, 2005). Most of these are resistant to the ALS inhibitor and photosystem II inhibitor herbicide groups, however, eight species have been confirmed as glyphosate resistant (Heap, 2005). The question has been raised as to whether the widespread introduction of crops tolerant to a specific herbicide, such as glyphosate, may increase the occurrence of weeds resistant to that particular herbicide.

It is important to recognize that weed resistance is a herbicide-related issue, not a crop-related issue. The use of a specific herbicide with a herbicide-tolerant crop is no different than the use of a selective herbicide over a conventional crop from a weed resistance standpoint. While the incidence of weed resistance is often associated with repeated applications of an herbicide product, its development depends very much on the chemical characteristics of the specific herbicide in question as well as the plant's ability to inactivate them. Some herbicide products are much more prone to develop herbicide resistance than others. Glyphosate is a unique herbicide as it is a transition state inhibitor of EPSPS, has very limited metabolism in plants, and has a lack of soil residual activity. Extensive research has led to the hypothesis that the most

effective way to minimize the potential for glyphosate weed resistance is to utilize a high dose strategy. Briefly, higher herbicide doses result in higher weed mortality and less diversity of resistance genes in the surviving population (Matthews, 1994). The glyphosate rates recommended in Roundup agricultural product labelling have been evaluated for the effective control of the target weed populations, and are consistent with the high dose strategy. Glyphosate has been used extensively for three decades with relatively few cases of weed resistance development in relation to many other herbicides. In the instances of confirmed glyphosate resistant weeds in cotton cropping production systems, recommendations have been developed for control of these weeds. Monsanto's control recommendations for specific glyphosate resistant weeds in glyphosate-tolerant cotton, include use of a tank mix of glyphosate with an additional herbicide employing a different mode of action and the use of residual herbicides. Monsanto promotes and distributes weed resistance management guidelines in grower presentations, technical bulletins and advertising.

Conclusions of Environmental Assessment

Phenotypic data were collected for MON 88913 from a broad range of environmental conditions and agronomic practices. These data include observations that are typically recorded by plant breeders and agronomists to evaluate the qualities of cotton. No biologically meaningful differences between MON 88913, the control, and conventional cotton have been detected, indicating MON 88913 possesses no fitness advantage comparable to other cotton that would result in increased weediness potential or altered environmental interactions. Additionally, the potential for environmentally-relevant outcrossing is limited due to genetic barriers to most non-commercial or wild cotton. The environmental consequences of successful outcrossing events would be negligible because the inserted DNA in MON 88913 would confer no selective advantage to the recipient cotton in the absence of glyphosate. Effective weed control system recommendations have been developed for the few cotton production areas encountering glyphosate-resistant weeds.

Summary

Monsanto Company has developed a second-generation glyphosate-tolerant cotton product, Roundup Ready Flex cotton, MON 88913, that provides increased tolerance to glyphosate during the critical reproductive phases of growth. Use of MON 88913 enables the application of a Roundup agricultural herbicide over the top of the cotton crop at later stages of development than is possible with MON 1445, and is expected to provide significant benefits to cotton production. These include a wider window of glyphosate herbicide application (based on weed height versus the crop development stage), reduced production risk with glyphosate applications beyond the fifth node stage, and increased crop management flexibility.

Detailed food, feed, and environmental safety assessments confirm the safety of this product. This is based on three categories of analysis. The first is the detailed molecular characterization of the inserted DNA and a detailed biochemical characterization of the CP4 EPSPS protein produced in MON 88913. The second is a direct assessment of the toxicity and allergenicity potential of the CP4 EPSPS protein produced in MON 88913, and the potential for

environmental interactions. The third is a safety and nutritional assessment that demonstrates that MON 88913 is compositionally equivalent to commercial conventional cotton.

The data and information presented in this summary demonstrate that Roundup Ready Flex cotton, MON 88913, and the feeds and foods derived from it, are as safe and nutritious as commercial conventional varieties of cotton and the comparable feeds and foods derived from them. This conclusion has been supported by all regulatory agencies in countries where reviews have been completed.

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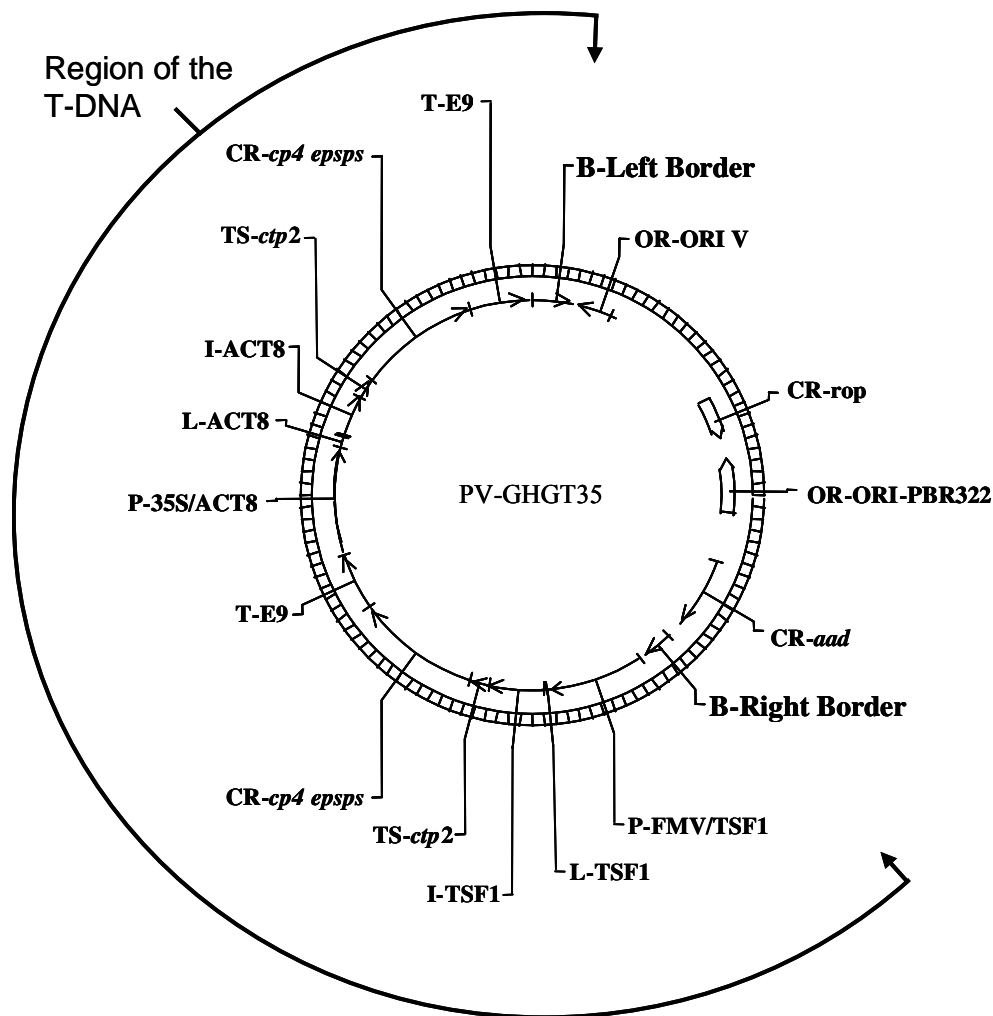


Figure 1. Plasmid Map of PV-GHGT35 Used to Produce Roundup Ready Flex cotton, MON 88913.

Key to genetic elements within the T-DNA:

B-Left Border / B-Right Border: DNA sequences derived from *Agrobacterium* containing the left border or right border sequence, for efficient T-DNA transfer

P-FMV/TSF1: Chimeric promoter containing the *Arabidopsis thaliana tsf1* gene promoter and enhancer sequences from the Figwort Mosaic virus 35S promoter

L-TSF1: Leader (exon 1) from the *Arabidopsis thaliana tsf1* gene

I-TSF1: Intron from the *Arabidopsis thaliana tsf1* gene

TS-ctp2: Coding sequence for the chloroplast transit peptide derived from the *Arabidopsis thaliana epsps* gene

CR-cp4 epsps: Synthetic coding sequence for CP4 EPSPS from *Agrobacterium sp.* strain CP4

T-E9: DNA sequences containing the 3' nontranslated region of the pea ribulose-1,5-bisphosphate carboxylase small subunit (*rbcS*) E9 gene

P-35S/ACT8: Chimeric promoter containing the promoter of the *act8* gene of *Arabidopsis thaliana* combined with the enhancer sequences of the Cauliflower Mosaic Virus 35S promoter

L-ACT8: Leader sequence from the *act8* gene of *Arabidopsis thaliana*

I-ACT8: Intron and flanking exon sequence from the *act8* gene of *Arabidopsis thaliana*

Table 1. List of Published Agency Assessments as of 11-30-05

<u>United States Department of Agriculture</u> , final EA and determination: 04-086-01p com. http://www.aphis.usda.gov/brs/not_reg.html ,
<u>United States Food and Drug Administration</u> , Completed consultation, BNF No. 98. http://www.cfsan.fda.gov/~lrd/biocon.html
<u>Japanese Ministry of Health, Labor and Welfare</u> , MHLW notice 217, April 7, 2005. http://www.mhlw.go.jp/english/topics/food/index.html

Table 2. Levels of CP4 EPSPS in Leaf, Root, Seed, and Pollen of MON 88913

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

OSL = Over-season leaves collected at different time points throughout the growing season
n/a = Moisture levels were not determined in this tissue.

Levels of 5-enolpyruvylshikimate-3-phosphate synthase in MON 88913, derived from *Agrobacterium sp.* strain CP4 (CP4 EPSPS), were determined in replicated field-produced tissues in 2002 from Alabama, California, Georgia, and Texas.

Protein levels are expressed as micrograms (µg) of protein per gram (g) of tissue on a fresh weight (fwt) or dry weight (dwt) basis. The dry weight values were calculated from fresh weight values using moisture analysis data. The arithmetic mean and standard deviation (SD) were calculated, and the range determined, for each tissue type across sites. The limit of quantitation (LOQ) and limit of detection (LOD) was 0.23 and 0.069 µg/g fwt, respectively, in leaf tissue; 0.23 and 0.073 µg/g fwt in root; 2.7 and 1.7 µg/g fwt in seed; and 0.23 and 0.11 µg/g fwt in pollen.

Table 3. Statistical Summary¹ of Combined Site Cottonseed Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, and Gossypol Content for MON 88913 Versus MON 88913(-)

Analytical Component	MON 88913 Mean ± S.E. (Range)	MON 88913(-) Mean ± S.E. (Range)	Commercial (Range) [99% Tolerance Interval²]
Amino Acid (% Total AA)			
Alanine	4.28 ± 0.056 (4.09 - 4.51)	4.30 ± 0.056 (4.15 - 4.46)	(4.08 - 4.46) [4.01,4.58]
Arginine	11.78 ± 0.17 (11.19 - 12.25)	11.77 ± 0.17 (11.11 - 12.27)	(11.08 - 12.77) [10.57,12.96]
Aspartic Acid	9.82 ± 0.064 (9.59 - 10.08)	9.80 ± 0.064 (9.59 - 9.99)	(9.70 - 10.38) [9.48,10.35]
Cystine	1.89 ± 0.042 (1.69 - 2.10)	1.92 ± 0.042 (1.76 - 2.10)	(1.62 - 2.35) [1.60,2.14]
Glutamic Acid	21.66 ± 0.13 (21.08 - 22.14)	21.55 ± 0.13 (21.10 - 21.96)	(20.92 - 22.18) [20.88,22.49]
Glycine	4.42 ± 0.029 (4.33 - 4.56)	4.45 ± 0.029 (4.33 - 4.64)	(4.29 - 4.66) [4.21,4.64]
Histidine	3.15 ± 0.0079 (3.09 - 3.21)	3.14 ± 0.0079 (3.11 - 3.20)	(3.01 - 3.22) [3.04,3.23]
Isoleucine	3.43 ± 0.020 (3.31 - 3.54)	3.43 ± 0.020 (3.34 - 3.56)	(3.19 - 3.59) [3.13,3.65]

* Values for MON 88913 and MON 88913(-) are statistically different at $p < 0.05$.

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

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

Table 3 (Continued). Statistical Summary¹ of Combined Site Cottonseed Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, and Gossypol Content for MON 88913 Versus MON 88913(-)

Analytical Component	MON 88913 Mean \pm S.E. (Range)	MON 88913(-) Mean \pm S.E. (Range)	Commercial (Range) [99% Tolerance Interval ²]
Amino Acid (% Total AA)			
Leucine	6.31 \pm 0.048 (6.14 - 6.52)	6.27 \pm 0.048 (6.10 - 6.48)	(6.03 - 6.48) [5.84,6.66]
Lysine	4.99 \pm 0.052 (4.77 - 5.23)	5.09 \pm 0.052 (4.89 - 5.48)	(4.72 - 5.38) [4.53,5.43]
Methionine	1.65 \pm 0.040 (1.47 - 1.90)	1.69 \pm 0.040 (1.49 - 1.95)	(1.27 - 1.94) [1.30,1.93]
Phenylalanine	*5.64 \pm 0.014 (5.53 - 5.75)	5.60 \pm 0.014 (5.45 - 5.72)	(5.44 - 5.82) [5.43,5.82]
Proline	4.17 \pm 0.045 (3.92 - 4.39)	4.16 \pm 0.045 (3.93 - 4.25)	(3.97 - 4.49) [3.91,4.43]
Serine	4.88 \pm 0.096 (4.35 - 5.32)	4.90 \pm 0.096 (4.65 - 5.32)	(4.53 - 5.31) [4.55,5.42]
Threonine	3.19 \pm 0.094 (2.61 - 3.49)	3.20 \pm 0.094 (2.70 - 3.45)	(2.67 - 3.50) [2.73,3.74]
Tryptophan	*1.10 \pm 0.012 (1.03 - 1.23)	1.14 \pm 0.012 (1.09 - 1.25)	(0.97 - 1.31) [0.94,1.26]

* Values for MON 88913 and MON 88913(-) are statistically different at $p < 0.05$.

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

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

Table 3 (Continued). Statistical Summary¹ of Combined Site Cottonseed Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, and Gossypol Content for MON 88913 Versus MON 88913(-)

Analytical Component	MON 88913 Mean ± S.E. (Range)	MON 88913(-) Mean ± S.E. (Range)	Commercial (Range) [99% Tolerance Interval ²]
Amino Acid (% Total AA)			
Tyrosine	2.79 ± 0.033 (2.70 - 2.90)	2.78 ± 0.033 (2.62 - 2.89)	(2.63 - 2.93) [2.61,3.00]
Valine	4.84 ± 0.028 (4.68 - 5.00)	4.81 ± 0.028 (4.68 - 4.96)	(4.57 - 5.02) [4.48,5.02]
Fatty Acid (% Total FA)			
14:0 Myristic	0.76 ± 0.040 (0.66 - 0.90)	0.75 ± 0.040 (0.65 - 0.90)	(0.64 - 1.03) [0.44,1.14]
16:0 Palmitic	23.55 ± 0.40 (22.09 - 24.69)	23.09 ± 0.40 (21.26 - 24.17)	(21.47 - 25.36) [20.76,26.19]
16:1 Palmitoleic	0.54 ± 0.0066 (0.51 - 0.59)	0.53 ± 0.0066 (0.50 - 0.59)	(0.46 - 0.77) [0.37,0.80]
18:0 Stearic	2.64 ± 0.073 (2.32 - 2.85)	2.65 ± 0.073 (2.33 - 2.94)	(2.38 - 3.03) [2.18,3.17]
18:1 Oleic	*18.61 ± 0.75 (16.35 - 20.72)	20.94 ± 0.75 (18.34 - 23.29)	(13.29 - 18.60) [10.59,21.29]
18:2 Linoleic	*52.36 ± 0.76 (49.66 - 54.32)	50.42 ± 0.76 (47.89 - 53.27)	(51.51 - 59.40) [48.89,61.11]

* Values for MON 88913 and MON 88913(-) are statistically different at $p < 0.05$.

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

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

Table 3 (Continued). Statistical Summary¹ of Combined Site Cottonseed Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, and Gossypol Content for MON 88913 Versus MON 88913(-)

Analytical Component	MON 88913 Mean \pm S.E. (Range)	MON 88913(-) Mean \pm S.E. (Range)	Commercial (Range) [99% Tolerance Interval ²]
Fatty Acid (% Total FA)			
18:3 Gamma Linolenic	0.12 \pm 0.023 (0.045 - 0.28)	0.13 \pm 0.023 (0.049 - 0.20)	(0.043 - 0.23) [0,0.24]
18:3 Linolenic	0.18 \pm 0.025 (0.11 - 0.26)	0.17 \pm 0.025 (0.12 - 0.24)	(0.11 - 0.27) [0.031,0.31]
20:0 Arachidic	0.27 \pm 0.0057 (0.25 - 0.31)	0.28 \pm 0.0057 (0.24 - 0.30)	(0.22 - 0.33) [0.21,0.34]
22:0 Behenic	0.15 \pm 0.0048 (0.13 - 0.17)	0.15 \pm 0.0048 (0.12 - 0.17)	(0.12 - 0.18) [0.099,0.19]
Dihydrosterculic	0.15 \pm 0.0081 (0.12 - 0.18)	0.17 \pm 0.0081 (0.10 - 0.21)	(0.075 - 0.24) [0.056,0.25]
Malvalic	0.36 \pm 0.040 (0.24 - 0.56)	0.39 \pm 0.040 (0.23 - 0.55)	(0.23 - 0.56) [0.16,0.58]
Sterculic	0.31 \pm 0.025 (0.24 - 0.41)	0.33 \pm 0.025 (0.21 - 0.44)	(0.19 - 0.41) [0.18,0.40]

* Values for MON 88913 and MON 88913(-) are statistically different at $p < 0.05$.

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

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

Table 3 (Continued). Statistical Summary¹ of Combined Site Cottonseed Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, and Gossypol Content for MON 88913 Versus MON 88913(-)

Analytical Component	MON 88913 Mean ± S.E. (Range)	MON 88913(-) Mean ± S.E. (Range)	Commercial (Range) [99% Tolerance Interval²]
Fiber (% dwt)			
Acid Detergent Fiber	31.31 ± 0.50 (27.72 - 34.98)	30.78 ± 0.50 (28.08 - 34.42)	(26.32 - 38.97) [25.48,38.48]
Crude Fiber	17.76 ± 0.68 (14.96 - 20.41)	17.97 ± 0.68 (16.04 - 20.39)	(15.96 - 23.10) [13.34,24.17]
Neutral Detergent Fiber	42.26 ± 1.07 (33.91 - 47.36)	42.56 ± 1.07 (38.00 - 46.92)	(38.49 - 51.84) [34.51,53.25]
Total Dietary Fiber	40.23 ± 0.53 (37.85 - 43.17)	39.60 ± 0.53 (36.55 - 43.27)	(36.47 - 47.54) [36.13,48.96]
Mineral			
Calcium (% dwt)	0.16 ± 0.012 (0.13 - 0.19)	0.16 ± 0.012 (0.11 - 0.19)	(0.10 - 0.19) [0.074,0.22]
Copper (mg/kg dwt)	6.72 ± 0.61 (5.15 - 8.51)	6.54 ± 0.61 (4.53 - 9.47)	(4.92 - 12.47) [2.01,12.94]
Iron (mg/kg dwt)	52.65 ± 1.68 (41.27 - 58.87)	52.20 ± 1.68 (46.77 - 62.47)	(36.71 - 67.75) [33.44,68.99]
Magnesium (% dwt)	0.41 ± 0.011 (0.38 - 0.45)	0.42 ± 0.011 (0.37 - 0.46)	(0.35 - 0.47) [0.31,0.51]
Manganese (mg/kg dwt)	*15.34 ± 1.29 (12.37 - 19.98)	14.64 ± 1.29 (11.91 - 18.23)	(10.68 - 21.96) [4.69,26.45]

* Values for MON 88913 and MON 88913(-) are statistically different at $p < 0.05$.

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

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

Table 3 (Continued). Statistical Summary¹ of Combined Site Cottonseed Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, and Gossypol Content for MON 88913 Versus MON 88913(-)

Analytical Component	MON 88913 Mean ± S.E. (Range)	MON 88913(-) Mean ± S.E. (Range)	Commercial (Range) [99% Tolerance Interval ²]
Mineral			
Phosphorus (% dwt)	0.68 ± 0.052 (0.54 - 0.82)	0.70 ± 0.052 (0.53 - 0.93)	(0.48 - 0.99) [0.31,1.08]
Potassium (% dwt)	1.21 ± 0.030 (1.12 - 1.34)	1.23 ± 0.030 (1.12 - 1.43)	(1.07 - 1.39) [0.96,1.46]
Sodium (% dwt)	0.062 ± 0.015 (0.027 - 0.12)	0.068 ± 0.015 (0.033 - 0.11)	(0.032 - 0.14) [0,0.17]
Zinc (mg/kg dwt)	40.87 ± 3.72 (29.30 - 52.16)	39.42 ± 3.72 (27.60 - 52.16)	(30.11 - 59.51) [17.12,58.50]
Proximate			
Ash (% dwt)	4.33 ± 0.17 (3.94 - 4.81)	4.37 ± 0.17 (3.76 - 5.19)	(3.76 - 5.34) [2.96,5.62]
Calories (Kcal/100g dwt)	460.31 ± 5.33 (424.36 - 481.93)	455.51 ± 5.33 (415.74 - 475.23)	(407.45 - 471.46) [409.12,496.45]
Carbohydrates (% dwt)	44.74 ± 0.49 (42.61 - 47.67)	45.57 ± 0.49 (42.07 - 49.32)	(40.06 - 52.01) [38.23,56.70]
Moisture (% fwt)	*6.39 ± 0.26 (5.65 - 7.34)	6.22 ± 0.26 (5.32 - 7.12)	(5.06 - 6.49) [4.51,7.21]

* Values for MON 88913 and MON 88913(-) are statistically different at $p < 0.05$.

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

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

Table 3 (Continued). Statistical Summary¹ of Combined Site Cottonseed Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, and Gossypol Content for MON 88913 Versus MON 88913(-)

Analytical Component	MON 88913 Mean \pm S.E. (Range)	MON 88913(-) Mean \pm S.E. (Range)	Commercial (Range) [99% Tolerance Interval ²]
Proximate			
Protein (% dwt)	28.23 \pm 0.60 (24.08 - 31.13)	27.41 \pm 0.60 (21.64 - 29.53)	(21.48 - 32.03) [20.19,32.70]
Total Fat (% dwt)	22.70 \pm 0.52 (21.00 - 25.25)	22.66 \pm 0.52 (19.99 - 24.82)	(17.60 - 27.29) [15.16,28.44]
Vitamin			
Vitamin E (mg/kg dwt)	150.85 \pm 14.02 (103.60 - 179.33)	148.79 \pm 14.02 (107.81 - 182.23)	(70.79 - 197.22) [9.30,263.66]
Gossypol			
Free Gossypol (% dwt)	0.65 \pm 0.032 (0.51 - 0.77)	0.68 \pm 0.032 (0.51 - 0.86)	(0.53 - 1.05) [0.43,1.06]
Total Gossypol (% dwt)	0.81 \pm 0.034 (0.70 - 0.91)	0.82 \pm 0.034 (0.69 - 0.96)	(0.78 - 1.19) [0.61,1.25]

* Values for MON 88913 and MON 88913(-) are statistically different at $p < 0.05$.

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

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

Table 4. Literature Values for Cottonseed Compositional Analytes

Component	Literature Ranges ^a
<i>Proximates, Fibers (% dwt)</i>	
Protein	21.2 ¹ – 29.5 ²
Fat	16.9 ³ – 26.8 ²
Ash	3.8 ³ – 4.5 ⁴
Moisture	5.4 ² – 10.1 ²
Carbohydrates	Not Available
Calories (kcal/100g)	Not Available
Acid Detergent Fiber	29.0 ⁵ – 40.1 ⁶
Crude Fiber	20.8 ⁵
Neutral Detergent Fiber	48.7 ³ – 50.3 ⁶
Total Dietary Fiber	Not Available
<i>Amino Acids (% Total AA)</i>	
Alanine	3.6 ¹ – 4.2 ¹
Arginine	10.9 ¹ – 13.2 ¹
Aspartic Acid	8.8 ¹ – 9.5 ¹
Cystine	1.76 ⁶ – 3.4 ¹
Glutamic Acid	19.9 ¹ – 22.4 ¹
Glycine	3.7 ¹ – 4.6 ¹
Histidine	2.6 ¹ – 3.11 ⁶
Isoleucine	2.8 ¹ – 3.4 ¹
Leucine	5.3 ¹ – 6.1 ¹
Lysine	4.2 ¹ – 4.6 ¹

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

Table 4 (Continued). Literature Values for Cottonseed Compositional Analytes

Component	Literature Ranges^a
<i>Amino Acids (% Total AA)</i>	
Methionine	1.2 ¹ – 1.8 ¹
Phenylalanine	5.0 ¹ – 6.2 ¹
Proline	3.1 ¹ – 4.0 ¹
Serine	3.9 ¹ – 4.4 ¹
Threonine	2.8 ¹ – 3.46 ⁶
Tryptophan	1.0 ¹ – 1.4 ¹
Tyrosine	1.6 ¹ – 3.3 ¹
Valine	4.1 ¹ – 4.8 ¹
<i>Fatty Acids (% Total FA)</i>	
14:0 Myristic	0.56 ⁷ – 1.16 ²
16:0 Palmitic	18.4 ⁷ – 26.18 ²
16:1 Palmitoleic	0.56 ² – 1.00 ⁷
18:0 Stearic	2.2 ⁷ – 2.88 ²
18:1 Oleic	15.17 ² – 19.94 ²
18:2 Linoleic	49.07 ² – 59.1 ⁷
18:2 Gamma Linoleic	Not Available
18:3 Linolenic	0.23 ⁷
20:0 Arachidic	0.41 ⁷
22:0 Behenic	Not Available

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

Table 4 (Continued). Literature Values for Cottonseed Compositional Analytes

Component	Literature Ranges^b
<i>Fatty Acids (% Total FA)</i>	
Dihydrosterculic	Not Available
Malvalic	0.7 ⁸ – 1.5 ⁸
Sterculic	0.3 ⁸ – 0.5 ⁸
<i>Minerals</i>	
Calcium (% dwt)	0.1 ³ – 0.17 ⁶
Copper (ppm dwt)	9.9 ³ – 54 ⁵
Iron (ppm dwt)	67.0 ³ – 151 ⁵
Magnesium (% dwt)	0.34 ³ – 0.37 ⁶
Manganese (ppm dwt)	10 ⁵ – 20.1 ³
Phosphorus (% dwt)	0.56 ⁹ – 0.75 ⁵
Potassium (% dwt)	0.96 ³ – 1.21 ⁵
Sodium (% dwt)	0.03 ³ – 0.31 ⁵
Zinc (ppm dwt)	28.9 ³ – 37 ⁶
<i>Miscellaneous</i>	
Gossypol, Free (% dwt)	0.59 ¹⁰ – 2.35 ¹⁰
Gossypol, Total (% dwt)	0.80 ⁷ – 1.09 ⁷
<i>Vitamin (ppm)</i>	
Vitamin E	99 ¹¹ – 224 ¹¹

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

Table 5. Statistical Summary of Combined Site Cottonseed Oil Fatty Acid and Vitamin E Content for MON 88913 Versus MON 88913(-)

Analytical Component	MON 88913 Mean ± S.E. (Range)	MON 88913(-) Mean ± S.E. (Range)	Commercial (Range) [99% Tolerance Int.²]
Fatty Acid (% Total FA)			
14:0 Myristic	*0.61 ± 0.026 (0.57 - 0.64)	0.62 ± 0.026 (0.58 - 0.66)	(0.52 - 0.74) [0.20,1.13]
16:0 Palmitic	23.21 ± 0.83 (22.33 - 24.10)	23.23 ± 0.83 (22.32 - 24.27)	(22.51 - 25.61) [16.41,30.45]
16:1 Palmitoleic	0.53 ± 0.020 (0.51 - 0.55)	0.52 ± 0.020 (0.50 - 0.54)	(0.49 - 0.78) [0,1.24]
18:0 Stearic	2.53 ± 0.19 (2.29 - 2.78)	2.53 ± 0.19 (2.34 - 2.73)	(2.26 - 2.59) [1.69,3.07]
18:1 Oleic	20.18 ± 0.63 (19.41 - 20.82)	20.15 ± 0.63 (19.46 - 20.83)	(13.10 - 15.83) [8.44,20.60]
18:2 Linoleic	51.67 ± 1.66 (50.00 - 53.53)	51.72 ± 1.66 (49.84 - 53.55)	(54.70 - 59.69) [46.72,67.80]
18:3 Linolenic	0.15 ± 0.0067 (0.13 - 0.16)	0.16 ± 0.0067 (0.14 - 0.17)	(0.13 - 0.17) [0.048,0.24]
20:0 Arachidic	0.24 ± 0.0046 (0.24 - 0.26)	0.25 ± 0.0046 (0.24 - 0.25)	(0.23 - 0.25) [0.19,0.30]
22:0 Behenic	*0.11 ± 0.0016 (0.11 - 0.12)	0.12 ± 0.0016 (0.12 - 0.12)	(0.11 - 0.13) [0.080,0.16]

* Values for MON 88913 and MON 88913(-) are statistically different at $p < 0.05$.

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

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

Table 5 (Continued). Statistical Summary¹ of Combined Site Cottonseed Oil Fatty Acid and Vitamin E Content for MON 88913 Versus MON 88913(-)

Analytical Component	MON 88913 Mean ± S.E. (Range)	MON 88913(-) Mean ± S.E. (Range)	Commercial (Range) [99% Tolerance Int.²]
Fatty Acid (% Total FA)			
Dihydrosterculic	0.19 ± 0.016 (0.17 - 0.21)	0.16 ± 0.016 (0.14 - 0.18)	(0.12 - 0.16) [0.058,0.23]
Malvalic	0.31 ± 0.031 (0.26 - 0.37)	0.28 ± 0.031 (0.26 - 0.30)	(0.27 - 0.30) [0.21,0.38]
Sterculic	0.26 ± 0.020 (0.22 - 0.29)	0.25 ± 0.020 (0.22 - 0.29)	(0.17 - 0.23) [0.069,0.34]
Vitamin			
Vitamin E (mg/kg FW)	464.38 ± 25.92 (406.00 - 507.50)	498.50 ± 25.92 (454.50 - 532.00)	(444.00 - 652.00) [0,1089.43]

* Values for MON 88913 and MON 88913(-) are statistically different at $p < 0.05$.

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

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

Table 6. Literature Values for Cottonseed Oil Compositional Analytes

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

^a Range of values found in published literature for cottonseed meal.

¹ Hui, 1996; ² Rossell, 1991; ³Cherry et. al., 1986; ⁴ USDA, 2003.

Table 7. Statistical Summary¹ of Combined Site Cottonseed Meal Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, and Gossypol Content for MON 88913 versus MON 88913(-)

Analytical Component	MON 88913 Mean ± S.E. (Range)	MON 88913(-) Mean ± S.E. (Range)	Commercial (Range) [99% Tolerance Int.²]
Amino Acid (% Total AA)			
Alanine	4.44 ± 0.13 (4.27 - 4.54)	4.42 ± 0.13 (4.25 - 4.62)	(4.17 - 4.40) [3.84,4.77]
Arginine	11.77 ± 0.17 (11.57 - 11.90)	11.94 ± 0.17 (11.67 - 12.23)	(11.91 - 12.70) [10.31,14.04]
Aspartic Acid	9.69 ± 0.032 (9.64 - 9.77)	9.65 ± 0.032 (9.59 - 9.67)	(9.64 - 9.85) [9.28,10.22]
Cystine	1.81 ± 0.022 (1.78 - 1.85)	1.79 ± 0.022 (1.75 - 1.85)	(1.75 - 1.90) [1.53,2.12]
Glutamic Acid	20.65 ± 0.18 (20.11 - 21.13)	20.74 ± 0.18 (20.55 - 20.98)	(20.49 - 20.97) [19.75,21.63]
Glycine	4.64 ± 0.041 (4.56 - 4.73)	4.61 ± 0.041 (4.56 - 4.66)	(4.54 - 4.61) [4.39,4.75]
Histidine	3.20 ± 0.036 (3.13 - 3.26)	3.23 ± 0.036 (3.17 - 3.29)	(3.15 - 3.25) [2.93,3.46]
Isoleucine	3.37 ± 0.076 (3.10 - 3.52)	3.45 ± 0.076 (3.42 - 3.49)	(3.10 - 3.53) [2.26,4.50]

* Values for MON 88913 and MON 88913(-) are statistically different at $p < 0.05$.

¹Data were averaged across duplicates for each sample prior to statistical analysis and summary. Means in the table are least square means from SAS.

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

Table 7 (Continued). Statistical Summary¹ of Combined Site Cottonseed Meal Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, and Gossypol Content for MON 88913 versus MON 88913(-)

Analytical Component	MON 88913 Mean ± S.E. (Range)	MON 88913(-) Mean ± S.E. (Range)	Commercial (Range) [99% Tolerance Int.²]
Amino Acid (% Total AA)			
Leucine	6.65 ± 0.11 (6.44 - 7.09)	6.48 ± 0.11 (6.42 - 6.55)	(6.37 - 7.07) [4.93,8.34]
Lysine	5.19 ± 0.080 (5.10 - 5.28)	5.18 ± 0.080 (5.02 - 5.32)	(4.98 - 5.23) [4.50,5.62]
Methionine	1.68 ± 0.019 (1.64 - 1.72)	1.65 ± 0.019 (1.61 - 1.70)	(1.53 - 1.69) [1.30,1.96]
Phenylalanine	5.57 ± 0.059 (5.52 - 5.64)	5.63 ± 0.059 (5.54 - 5.73)	(5.62 - 5.75) [5.36,5.99]
Proline	4.10 ± 0.039 (4.01 - 4.20)	4.07 ± 0.039 (4.00 - 4.09)	(3.94 - 4.18) [3.53,4.55]
Serine	4.96 ± 0.043 (4.89 - 5.01)	4.91 ± 0.043 (4.80 - 5.00)	(4.72 - 4.83) [4.51,5.01]
Threonine	3.54 ± 0.069 (3.41 - 3.64)	3.53 ± 0.069 (3.49 - 3.58)	(3.47 - 3.56) [3.34,3.69]
Tryptophan	1.15 ± 0.018 (1.12 - 1.18)	1.18 ± 0.018 (1.17 - 1.19)	(1.08 - 1.28) [0.75,1.57]

* Values for MON 88913 and MON 88913(-) are statistically different at $p < 0.05$.

¹Data were averaged across duplicates for each sample prior to statistical analysis and summary. Means in the table are least square means from SAS.

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

Table 7 (Continued). Statistical Summary¹ of Combined Site Cottonseed Meal Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, and Gossypol Content for MON 88913 versus MON 88913(-)

Analytical Component	MON 88913 Mean ± S.E. (Range)	MON 88913(-) Mean ± S.E. (Range)	Commercial (Range) [99% Tolerance Int.²]
Amino Acid (% Total AA)			
Tyrosine	2.79 ± 0.062 (2.69 - 2.89)	2.83 ± 0.062 (2.72 - 2.91)	(2.70 - 2.88) [2.47,3.16]
Valine	4.82 ± 0.064 (4.68 - 5.06)	4.73 ± 0.064 (4.69 - 4.80)	(4.77 - 4.86) [4.62,5.04]
Fatty Acid (µg/g dwt)			
Dihydrosterculic	59.05 ± 14.69 (35.71 - 87.02)	50.60 ± 14.69 (36.25 - 61.90)	(29.62 - 51.13) [0,92.65]
Malvalic	112.95 ± 35.40 (61.16 - 175.47)	97.20 ± 35.40 (76.93 - 136.04)	(76.12 - 131.19) [0,220.73]
Sterculic	100.79 ± 21.19 (61.99 - 143.93)	88.33 ± 21.19 (70.63 - 104.24)	(55.12 - 88.00) [3.67,147.63]
Fiber (% dwt)			
Acid Detergent Fiber	18.87 ± 2.10 (17.95 - 19.71)	16.17 ± 2.10 (11.70 - 20.59)	(14.42 - 21.22) [2.86,31.68]
Crude Fiber	13.46 ± 1.52 (12.87 - 13.97)	11.21 ± 1.52 (8.36 - 13.79)	(10.51 - 15.54) [1.66,23.85]
Neutral Detergent Fiber	25.28 ± 2.75 (24.86 - 25.76)	21.15 ± 2.75 (15.07 - 26.08)	(19.08 - 29.04) [2.46,44.39]

* Values for MON 88913 and MON 88913(-) are statistically different at $p < 0.05$.

¹Data were averaged across duplicates for each sample prior to statistical analysis and summary. Means in the table are least square means from SAS.

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

Table 7 (Continued). Statistical Summary¹ of Combined Site Cottonseed Meal Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, and Gossypol Content for MON 88913 versus MON 88913(-)

Analytical Component	MON 88913 Mean ± S.E. (Range)	MON 88913(-) Mean ± S.E. (Range)	Commercial (Range) [99% Tolerance Int.²]
Total Dietary Fiber	35.44 ± 3.25 (34.58 - 36.65)	32.12 ± 3.25 (27.00 - 37.44)	(31.70 - 37.78) [22.75,45.80]
Mineral			
Calcium (% dwt)	0.24 ± 0.0048 (0.24 - 0.25)	0.26 ± 0.0048 (0.24 - 0.27)	(0.19 - 0.22) [0.14,0.27]
Copper (mg/kg dwt)	13.59 ± 1.56 (11.72 - 14.86)	14.88 ± 1.56 (12.50 - 16.84)	(11.12 - 15.02) [5.89,21.52]
Iron (mg/kg dwt)	88.91 ± 7.70 (76.11 - 100.01)	101.80 ± 7.70 (84.24 - 113.57)	(78.14 - 96.00) [51.88,123.28]
Magnesium (% dwt)	0.75 ± 0.022 (0.71 - 0.79)	0.78 ± 0.022 (0.76 - 0.79)	(0.70 - 0.81) [0.52,1.01]
Manganese (mg/kg dwt)	20.56 ± 1.21 (18.93 - 22.52)	20.52 ± 1.21 (18.74 - 22.08)	(18.63 - 21.05) [14.52,24.91]
Phosphorus (% dwt)	1.49 ± 0.040 (1.45 - 1.51)	1.56 ± 0.040 (1.45 - 1.65)	(1.40 - 1.59) [1.11,1.89]
Potassium (% dwt)	1.87 ± 0.058 (1.82 - 1.93)	1.92 ± 0.058 (1.79 - 1.99)	(1.83 - 1.97) [1.57,2.17]

* Values for MON 88913 and MON 88913(-) are statistically different at $p < 0.05$.

¹Data were averaged across duplicates for each sample prior to statistical analysis and summary. Means in the table are least square means from SAS.

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

Table 7 (Continued). Statistical Summary¹ of Combined Site Cottonseed Meal Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, and Gossypol Content for MON 88913 versus MON 88913(-)

Analytical Component	MON 88913 Mean ± S.E. (Range)	MON 88913(-) Mean ± S.E. (Range)	Commercial (Range) [99% Tolerance Int.²]
Mineral			
Zinc (mg/kg dwt)	65.97 ± 12.70 (55.15 - 76.85)	71.17 ± 12.70 (56.03 - 87.00)	(58.64 - 80.02) [15.87,118.52]
Proximate			
Ash (% dwt)	7.39 ± 0.17 (7.25 - 7.57)	7.79 ± 0.17 (7.11 - 8.11)	(6.97 - 7.53) [6.19,8.45]
Calories (Kcal/100g dwt)	381.57 ± 1.33 (378.52 - 385.01)	382.04 ± 1.33 (380.11 - 383.13)	(379.12 - 383.85) [371.50,391.85]
Carbohydrates (% dwt)	45.49 ± 2.92 (43.97 - 46.09)	41.61 ± 2.92 (37.04 - 45.95)	(41.31 - 48.64) [28.34,59.69]
Moisture (% fwt)	2.25 ± 1.93 (1.46 - 4.10)	4.82 ± 1.93 (0.76 - 9.43)	(1.53 - 5.84) [0,13.79]
Protein (% dwt)	44.94 ± 2.62 (43.84 - 46.47)	47.95 ± 2.62 (43.92 - 51.78)	(42.09 - 49.43) [31.32,61.57]
Total Fat (% dwt)	2.20 ± 0.32 (1.48 - 2.85)	2.65 ± 0.32 (2.20 - 3.09)	(1.82 - 2.48) [0.65,3.76]

* Values for MON 88913 and MON 88913(-) are statistically different at $p < 0.05$.

¹Data were averaged across duplicates for each sample prior to statistical analysis and summary. Means in the table are least square means from SAS.

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

Table 7 (Continued). Statistical Summary¹ of Combined Site Cottonseed Meal Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, and Gossypol Content for MON 88913 versus MON 88913(-)

Analytical Component	MON 88913 Mean ± S.E. (Range)	MON 88913(-) Mean ± S.E. (Range)	Commercial (Range) [99% Tolerance Int.²]
Gossypol			
Free Gossypol (% dwt)	0.18 ± 0.042 (0.13 - 0.23)	0.25 ± 0.042 (0.19 - 0.31)	(0.17 - 0.39) [0,0.76]
Total Gossypol (% dwt)	*1.32 ± 0.027 (1.26 - 1.40)	1.48 ± 0.027 (1.44 - 1.55)	(1.10 - 2.02) [0,3.35]

* Values for MON 88913 and MON 88913(-) are statistically different at $p < 0.05$.

¹Data were averaged across duplicates for each sample prior to statistical analysis and summary. Means in the table are least square means from SAS.

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

Table 8. Literature Values for Cottonseed Meal Compositional Analytes

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

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

Table 8 (Continued). Literature Values for Cottonseed Meal Compositional Analytes

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

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