Application to Food Standards Australia New Zealand for the Inclusion of Soybean MON 87705 in Standard 1.5.2 - Food Derived from Gene Technology

Submitted by:

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UNPUBLISHED REPORTS BEING SUBMITTED

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GM CHECKLIST

	Data Provided	Part No.	Data Not Provided	Omission Explained
Executive Summary				
Separately bound document	☒		☐	☐
Part 1: General Information				
1.1 Applicant				
(a) Company / Organisation Name	☒	1.1	☐	☐
(b) Address	☒	1.1	☐	☐
(c) Contact	☒	1.1	☐	☐
(d) Nature of business	☒	1.1	☐	☐
(e) Sole or joint application	☒	1.2	☐	☐
(f) Co-applicants	☐	1.2	☐	☒
Part 2: Specific Data Requirements				
2.1 General Details				
(a) Description of GM organism	☒	2.1.a.	☐	☐
(b) Name / Number of new line / strain	☒	2.1.b.	☐	☐
(c) Marketing name	☒	2.1.c.	☐	☐
(d) Product list	☒	2.1.d,e.	☐	☐
2.2 History of Use				
(a) Donor	☒	2.2.a.	☐	☐
(b) Host	☒	2.2.b.	☐	☐
2.3 Nature of Genetic Modification				
(a) Method used	☒	2.3.a.	☐	☐
(b) Bacteria used	☒	2.3.a.	☐	☐
(c) Gene Construct and transformation event	☒	2.3.b.	☐	☐
(d) Molecular characterisation	☒	2.3.c.	☐	☐
(e) Derivation of line or strain	☒	2.3.d.	☐	☐
(f) Evidence of stability	☒	2.3.d.	☐	☐
2.4 Antibiotic Resistance Genes				
(a) Clinical / veterinary importance	☐	2.4.a.(NA)	☒	☐
(b) Viability	☐	2.4.b.(NA)	☒	☐
(c) Presence in food	☐	2.4.c.(NA)	☒	☐
2.5 Characterisation of Novel Protein				
(a) Description	☒	2.5.b.	☐	☐
(b) Site of expression	☒	2.5.c.	☐	☐
(c) Non-expression	☐	2.5.d	☒	☐
(d) History of human consumption	☒	2.5.e.	☐	☐
(e) Oral toxicological studies	☒	2.5.e.	☐	☐
(f) Amino acid sequence	☒	2.5.e.	☐	☐
(g) Known allergenicity of source	☐	NA	☒	☐
(h) Unknown allergenicity information	☒	2.5.f.	☐	☐

GM CHECKLIST (cont'd.)

	Data Provided	Part No.	Data Not Provided	Omission Explained
2.6 Characterisation of Other Novel Substances				
(a) Identification	☐	2.6.a.(NA)	☒	☐
(b) Toxicity	☐	2.6.b.(NA)	☒	☐
2.7 Comparative Analyses				
(a) Key nutrients etc.	☒	2.7.a-d	☐	☐
(b) Other constituents	☒	2.7.e.	☐	☐
(c) Allergenic proteins	☐	2.7.h.(NA)	☒	☐
2.8 Nutritional Impact				
(a) Animal feeding studies	☒	2.8	☐	☐
(b) Nutritional changes	☒	2.8	☐	☐
2.9 Other Technical Information				
(a) Detection methodology	☒	2.9.a.	☐	☐
(b) Market penetration	☒	2.9.b.	☐	☐
Part 3: Regulatory / Legislative Implications				
3.1 Other approvals				
(a) Relevant overseas approvals	☒	3.1.a.	☐	☐
(b) Approval refusal	☒	3.1.b.	☐	☐
Part 4: Statutory Declaration	☒	4.0	☐	☐

PART 1 GENERAL INFORMATION

1.1 Applicant Details

Company Name:	Monsanto Australia Limited
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Contact:	Amanda Forster, +61 3 9522 7109
Nature of Your Business:	Technology Provider to the Agricultural and Food Industries.

1.2 Nature of Application

This application is submitted to Food Standards Australia New Zealand by Monsanto Australia Limited and is not made on behalf of any other party.

The purpose of this submission is to make an application to vary **Standard 1.5.2** – *Food Produced Using Gene Technology* to seek the addition of soybean MON 87705 that produces improved fatty acid profile and products containing soybean MON 87705 (hereafter referred to as MON 87705) to the Table to Clause 2 (see below).

Column 1 **Food derived from gene technology**	**Column 2** **Special requirements**
Food derived from Soybean MON 87705	None

PART 2 SPECIFIC DATA REQUIREMENTS FOR SAFETY ASSESSMENT

2.1 General Details

a) Description of the Nature and Purpose of the Introduced Trait

Monsanto Company has developed biotechnology-derived soybean MON 87705 with an improved fatty acid profile that results in enhanced nutritional characteristics. Given its high proportion of polyunsaturated fatty acids, commodity soybean oil requires hydrogenation to improve its stability for use in many foods. Plant oils such as canola and olive oil are relatively high in monounsaturated 18:1 oleic acid and low in polyunsaturated fatty acids providing stability advantages over commodity soybean oil. MON 87705 was developed to selectively down-regulate two key enzymes, FATB and FAD2, involved in the soybean seed fatty acid biosynthetic pathway. As a result, MON 87705 soybean oil contains lower levels of saturated fatty acids (6% vs. 15% of total fatty acids [FA]) and higher levels of monounsaturated 18:1 oleic acid (76% vs. 23% of total FA), with an associated decrease in polyunsaturated 18:2 linoleic acid levels (10% vs. 53% of total FA) relative to commodity soybean oil. Consequently, MON 87705 soybean oil is expected to have improved oxidative stability and lower saturated fats than currently available commodity soybean oil, and is suitable for a range of food applications. The reduced saturated fat levels in MON 87705 soybean oil, particularly palmitic acid, can positively impact the goal of limiting dietary saturated fat intake. MON 87705 also contains the 5-enolpyruvylshikimate-3-phosphate synthase gene derived from *Agrobacterium sp*. strain CP4 (*cp4 epsps*), which encodes the CP4 EPSPS protein.

MON 87705 contains the same five major fatty acids that are found in conventional soybean: 16:0 palmitic and 18:0 stearic (saturated); 18:1 oleic (monounsaturated); and 18:2 linoleic, and 18:3 linolenic acids (polyunsaturated), but in different proportions. MON 87705 has a fatty acid profile comparable to other widely consumed vegetable oils including olive oil and canola oil.

b) Identity and Intended Function of the Genetic Modification

The intended effect of the genetic modification in MON 87705 is to produce soybean seeds with decreased levels of saturated fatty acids and increased level of monounsaturated fatty acid (18:1 oleic acid) with an associated decrease in the level of the polyunsaturated 18:2 linoleic acid (Figure 1). The improved fatty acids profile in MON 87705 soybean oil was achieved through the use of RNA-based suppression of two key enzymes, FATB and FAD2, involved in the soybean seed fatty acid biosynthetic pathway (Figure 1). The acyl-acyl carrier protein (ACP) thioesterases (referred to herein as FATB enzymes) are localized in plastids and hydrolyze saturated fatty acids from the ACP-fatty acid moiety. The RNA-based suppression of *FATB* results in a decrease in transport of saturated fatty acids out of the plastid, thus increasing their availability for desaturation to 18:1 oleic acid (Figure 1). Therefore, suppression of *FATB* decreases the levels of saturated fatty acids and increases the levels of 18:1 oleic acid in the plastids that are then delivered to the oil body or to the endoplasmic reticulum for further desaturation. The delta-12 desaturases (referred to as FAD2 enzymes) desaturate 18:1 oleic acid to 18:2 linoleic acid. The RNA-based suppression of *FAD2* in MON 87705 soybean seed causes reduced desaturation of 18:1 oleic acid to 18:2 linoleic acid resulting in an increase in 18:1 oleic acid levels and a decrease in 18:2 linoleic acid levels that are available for

transport from the endoplasmic reticulum to the oil body. Therefore, the net effect of the RNA-based suppression of *FATB* and *FAD2* is a reduction in saturated 16:0 palmitic and 18:0 stearic acid levels, an increase in monounsaturated 18:1 oleic acid levels, and a decrease in polyunsaturated 18:2 linoleic acid levels in MON 87705 relative to commodity soybean oil. As a result, MON 87705 soybean oil contains lower levels of saturated fatty acids (6% vs. 15%) than currently available commodity soybean oil, and is suitable for a range of food applications. In addition, soybean meal derived from MON 87705, which contains very low levels of residual oil is compositionally similar to other commodity soybean meal. MON 87705 also contains the 5-enolpyruvylshikimate-3-phosphate synthase gene derived from *Agrobacterium sp*. strain CP4 (*cp4 epsps*), which encodes the CP4 EPSPS protein. This gene was used as a selectable marker during transformation. The suppression of the FATB and FAD2 genes is described in further detail in Appendix 1.

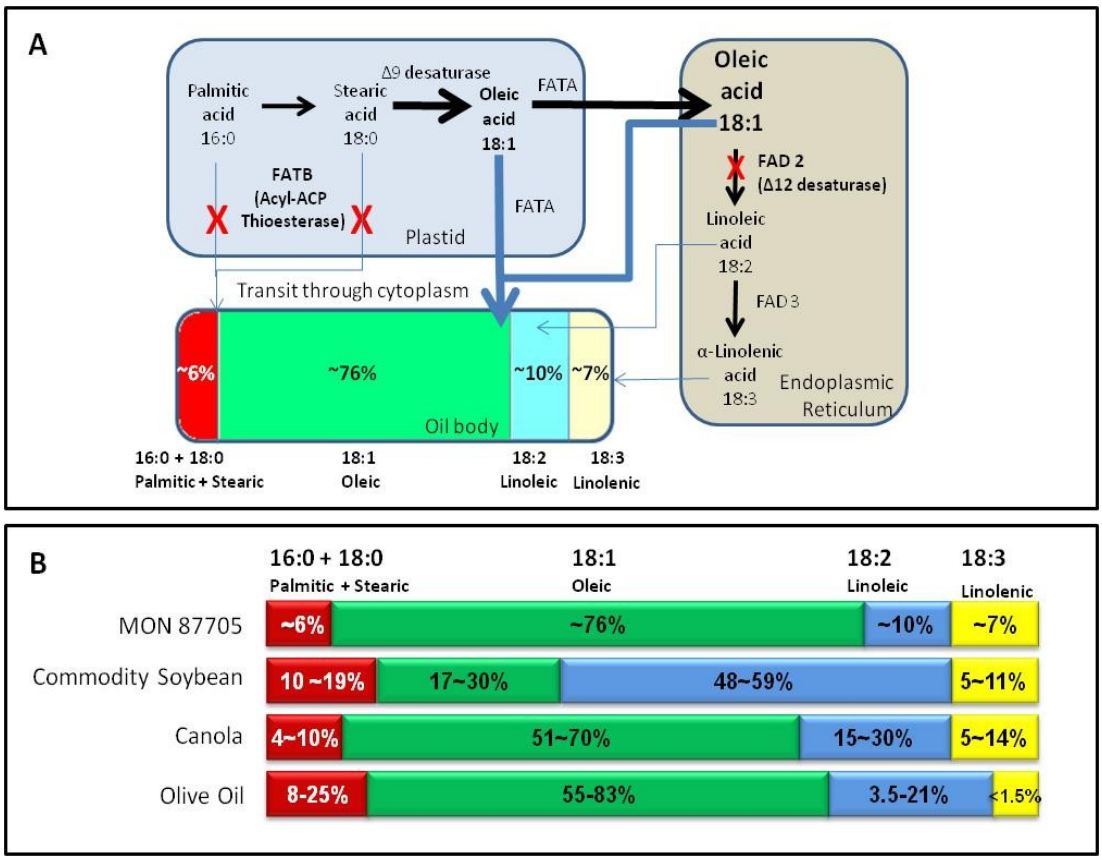


Figure 1. Schematic of Soybean Fatty Acid Biosynthetic Pathway and Comparison of the Fatty Acid Content of MON 87705 Soybean Oil with Other Vegetable Oils

Panel A: Schematic of the soybean fatty acid biosynthetic pathway.
X indicates suppression of endogenous *FATB* and *FAD2* RNAs in MON 87705 seeds.
Panel B: MON 87705 soybean oil compared to commodity soybean oil and other vegetable oils.

c) Application and Proposed Product Name

Monsanto Company has developed biotechnology-derived soybean MON 87705 with an improved fatty acid profile to enhance the suitability of soybean oil for food and industrial uses. The fatty acid levels in MON 87705 soybean oil are lower for saturated fats (6% vs. 15% of total FA) and higher for oleic acid (76% vs. 23% of total FA). The increase in monounsaturated fatty acid (oleic) in MON 87705 is accompanied by an overall decrease in polyunsaturated fatty acids (PUFAs) (17% vs 60% of total FA). Commercial soybean oil typically contains 60-65% PUFAs, mostly in the form of 18:2 linoleic acid. This high PUFA content makes soybean oil unsuitable for many food applications since the high concentrations of PUFAs in the oil are susceptible to oxidation and degradation at high temperature.

To improve the stability of commodity soybean oil, the polyunsaturated fatty acids in the oil can be decreased through a process called hydrogenation that reduces the number of unstable double bonds found in fatty acids such as linolenic and linoleic, and converts them to saturated fatty acids. Although hydrogenation produces oil with excellent thermal and oxidative stability, it also results in the production of significant levels of *trans* fatty acids in the oil. *Trans* fatty acids contribute to cardiovascular risk by elevating LDL (bad cholesterol) and reducing HDL (good cholesterol) (Hu et al., 1997; Kris-Etherton, 1995).

Soybean oil oxidative stability can be improved because the stability of vegetable oils is drastically influenced by the proportion of monounsaturates to polyunsaturates. High oleic soybean oils are estimated to have improved oxidative stability about 17 times greater than conventional soybean oil (Frankel, 2005). Oil from MON 87705 is anticipated to have enhanced oxidative stability relative to conventional soybean oil due to its increase in monounsaturated fatty acid and decrease in PUFAs. Therefore, MON 87705 soybean oil provides more stable oil for use in food formulation.

Numerous global health authorities recognize that diets high in total fat and saturated fat are associated with increased risk of chronic disease (IOM/NAS, 2002; Lichtenstein et al., 2006; U.S. Department of Health and Human Services Public Health Service, 1988; WHO, 2003), and health experts, including the American Heart Association (Eckel et al., 2007), have recognized that an unintended consequence of a shift to oils with no or lower *trans* fat levels, may be an increase in levels of saturated fats in foods. With a fatty acid profile lower in saturated fats than commercial soybean oil, MON 87705 soybean oil can help address this concern as MON 87705 soybean oil contains less than 7% saturated fatty acids (palmitic + stearic acid). Based on U.S. Food and Drug Administration (FDA) guidance, a low saturated fat food contains less than 1 g of fat per serving (www.fda.gov) and the typical serving size for soybean oil is 14 g (www.thumboilseed.com/soy-oil.htm). As a result, to qualify as a low saturate soybean oil the maximum amount of saturated fats allowed is 7% (0.98 g per serving). Therefore, under FDA guidance MON 87705 soybean oil can be classified as a low saturate oil.

The fatty acid profile of MON 87705 soybean oil is also well suited for industrial applications. Soybean oils have very good lubricating properties, and are highly biodegradable compared to mineral oils, but typically lack the stability needed to meet industrial requirements. Hydrogenation of soybean oil is not acceptable for most industrial uses because it leads to formation of saturated and *trans* fatty acids which can cause the oil to be solid at lower temperatures, resulting in excessive wear and tear of

machinery (Kinney, 1998). The fatty acid profile of MON 87705 provides an industrial oil with improved stability that could serve as a lubricant without needing hydrogenation. In addition, soybean oil with elevated oleic acid is an attractive source for other industrial applications, such as a replacement for petrochemical-derived plasticizers (Kinney, 1998). The higher oleic acid and lower saturated fat levels of MON 87705 also make it much more suitable for use in biodiesel due to its greater stability, improved cold weather performance, and reduced nitrous oxide emissions (Bringe, 2005; Graef et al., 2009; Knothe, 2005).

As with all new biotechnology-derived traits, MON 87705 will be bred into soybean varieties with diverse genetic backgrounds. These varieties will include commercial varieties with low linolenic acid levels which can further enhance the oxidative stability of the soybean oil. In addition, MON 87705 will be combined using traditional breeding methods with other biotechnology-derived traits, including glyphosate tolerance, to deliver the best agronomic platform to growers.

To summarize, MON 87705 was developed to improve soybean oil's oxidative stability profile and lower the saturated fat content of the oil. Due to the compositional improvement in MON 87705 soybean oil, MON 87705 could expand the food market applicability of soybean oil, without contributing further to known dietary health risks or sacrificing food functionality. Similarly, MON 87705 soybean oil could also serve as an improved source for industrial and biofuel products.

There are currently no plans to produce in Australia and New Zealand. A commercial trade name for the product has not been determined at the time of this submission and will be available prior to commercial launch of the product.

d) Soybean as a Food Source

Soybean has the ability to produce more edible protein per acre of land than any other known crop (Liu, 2004). On average, dry soybean contains roughly 40% protein and 20% oil. 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 (Liu, 2004). :

1. Traditional soyfoods: As discussed above, traditional soyfoods are primarily made from whole soybean. The nonfermented traditional soyfoods include soymilk, tofu, and soybean sprouts, whereas the fermented soyfoods include soybean paste (miso), soy sauce, natto, and tempeh.

2. Soybean oil: Soybean oil constitutes approximately 71% of global consumption of edible fats and oil (ASA, 2008), and is the second largest source of vegetable oil worldwide (Soyatech, 2009). 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. Soybean oil is comprised primarily of five major fatty acids: saturated fatty acids 16:0 palmitic and 18:0 stearic acids, monounsaturated 18:1 oleic acid, and the polyunsaturated fatty acids, 18:2 linoleic and 18:3 linolenic acids. These five major fatty acids have very different oxidative stabilities and chemical functionalities. Conventional soybean oil typically contains 60-65% polyunsaturated

fatty acids, mostly in the form of 18:2 linoleic acid. This composition makes soybean oil unsuitable for certain food applications since the high concentrations of polyunsaturated fatty acids in the oil are susceptible to oxidation and degradation at high temperature resulting in off-flavors and odors. Therefore, hydrogenation of soybean oil is necessary to reduce levels of polyunsaturated fatty acids by converting them to saturated fatty acids resulting in higher stability oil suitable for a range of food uses. However, the hydrogenation process produces *trans* fatty acids that are linked to increased cardiovascular risk.

3. Soybean protein products: Soybean protein products are made from defatted soybean flakes, and include soybean flour, soybean protein concentrate, and soybean protein isolate. Soybean flour has a protein content of ~50% and is used mainly as an ingredient in the bakery industry. Soybean protein concentrate has a protein content of ~70% and is used widely in the meat industry as a key ingredient of meat alternative products such as soybean burgers and meatless "meatballs." Soybean 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 soups, sauce bases, energy bars, nutritional beverages, infant formula, and dairy replacements.

4. Dietary supplements: Soybean is a rich source of certain phytochemicals used as dietary supplements, which include isoflavones and tocopherols. Isoflavones have been shown to inhibit the growth of cancer cells, lower cholesterol levels, and inhibit bone resorption (Messina, 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 (Hensley et al., 2004; IFIC, 2005; ILSI-CCD, 2006; Schafer et al., 2003). Detailed reviews of soybean as functional foods have been recently reviewed in detail and can be found at IFIC (IFIC, 2005; Liu, 2004).

e) 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-ERS, 2005). 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. Subsequently, the meal is dried to about 13 to 14 percent moisture, and is screened and ground to produce a uniform particle size prior to shipment to the end user. The finished meal from dehulled soybean 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 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 soybean, with the remainder divided between rapeseed, cottonseed, sunflower, peanut, and other meals (ASA, 2008). Poultry and swine account for most of the soybean meal utilized in the U.S., with poultry consuming 50%, swine 27%, cattle 17%, and 3% for companion animals and the reminder to other feed uses (ASA, 2008).

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 can provide livestock and dairy producers with a source of high-protein feed for their livestock (USDA-ARS, 2006). Harvested forage can be used as hay or to produce silage (MAFRI, 2004).

2.2 History of Use

a) Donor Organism

Safety of DNA and RNA from Donor Organisms
The donor organism, *Agrobacterium* sp. strain CP4, was isolated based on its tolerance to glyphosate brought about by the production of a naturally glyphosate-tolerant EPSPS protein (Padgette et al., 1996a). The bacterial isolate, CP4, was identified by the American Type Culture Collection (ATCC) as an *Agrobacterium* species. This identification was made based on morphological and biochemical characteristics of the isolate and its similarity to a reference strain of *Agrobacterium*.

Agrobacterium sp. strain CP4 is related to microbes commonly present in the soil and in the rhizosphere of plants. *Agrobacterium* species are not known for human or animal pathogenicity, and are not commonly allergenic (FAO/WHO, 1991). Furthermore, according to FAO/WHO (FAO/WHO, 2001), there is no known population of individuals sensitized to bacterial proteins.

b) Host Organism

Taxonomic Classification
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, the 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. The list of species in the genus *Glycine* Willd. is presented in Table 1.

Table 1. List of Species in the Genus Glycine Willd., 2n Chromosome Number, Genome Symbol and Distribution

Genus		2n	Genome[1]	Distribution
Subgenus *Glycine*				
1.	*G. albicans* Tind. & Craven	40	I1	Australia
2.	*G. aphyonota* B. Pfeil	40	--[2]	Australia
3.	*G. arenaria* Tind.	40	HH	Australia
4.	*G. argyrea* Tind.	40	A2A2	Australia
5.	*G. canescens* F.J. Herm.	40	AA	Australia
6.	*G. clandestina* Wendl.	40	A1A1	Australia
7.	*G. curvata* Tind.	40	C1C1	Australia
8.	*G. cyrtoloba* Tind.	40	CC	Australia
9.	*G. dolichocarpa* Tateishi and Ohashi	80	--	(Taiwan)
10.	*G. falcate* Benth.	40	FF	Australia
11.	*G. hirticaulis* Tind. & Craven	40	H1H1	Australia
		80	--	Australia
12.	*G. lactovirens* Tind. & Craven.	40	I1I1	Australia
13.	*G. latifolia* (Benth.) Newell & Hymowitz	40	B1B1	Australia
14.	*G. latrobeana* (meissn.) Benth.	40	A3A3	Australia
15.	*G. microphylla* (Benth.) Tind.	40	BB	Australia
16.	*G. peratosa* B. Pfeil & Tind.	40	--	Australia
17.	*G. pindanica* Tind. & Craven	40	H3H2	Australia
18.	*G. pullenii* B. Pfeil, Tind. & Craven	40	--	Australia
19.	*G. rubiginosa* Tind. & B. Pfeil	40	--	Australia
20.	*G. stenophita* B. Pfeil & Tind.	40	B3B3	Australia
21.	*G. tabacina* (Labill.) Benth.	40	B2B2	Australia
		80	Complex[3]	Australia, West Central and South Pacific Islands
22.	*G. tomentella* Hayata	38	EE	Australia
		40	DD	Australia, Papua New Guinea
		78	Complex[4]	Australia, Papua New Guinea
		80	Complex[5]	Australia, Papua New Guinea, Indonesia, Philippines, Taiwan
Subgenus *Soja* (Moench) F.J. Herm.				
23.	*G. soja* Sieb. & Zucc.	40	GG	China, Russia, Taiwan, Japan, Korea (Wild Soybean)
24.	*G. max* (L.) Merr.	40	GG	Cultigen (Soybean)

[1] Genomically similar species carry the same letter symbols.
[2] Genome designation has not been assigned to the species.
[3] Allopolyploids (A and B genomes) and segmental allopolyploids (B genomes).
[4] Allopolyploids (D and E, A and E, or any other unknown combination).
[5] Allopolyploids (A and D genomes, or any other unknown combination).
Note: Table is adapted from Hymowitz (Hymowitz, 2004).

Glycine soja grows wild in China, Japan, Korea, the Russian Far East, and Taiwan, and is commonly found in fields, hedgerows, roadsides, and riverbanks (Lu, 2004). The plant is an annual, slender in build with narrow trifoliolate leaves. The purple or very rarely white flowers are inserted on short, slender racemes. The pods are short and tawny with hirsute pubescence, producing 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 subspherical seeds are produced per pod.

A third and unofficial species named *G. gracilis* is also described within the context of the *Soja* subgenus in addition to *G. soja* and *G. max*. The *G. gracilis* is only found in 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.

Reproduction and Life Cycle of Soybean

Glycine max (L.) Merr, the cultivated soybean, is an annual crop that is planted in late spring from April to May in the north hemisphere, and from November to February in the southern hemisphere. Soybean seed germinates when the soil temperature reaches 10°C and emerges in a 5-7 day period under favourable conditions (OECD, 2000). The system of soybean growth stages divides plant development into vegetative (V) and reproductive (R) stages (Pedersen, 2008). The vegetative stages begin with VE, which designates emergence. V stages continue and are numbered according to how many fully-developed trifoliate leaves are present (i.e., V1, V2, etc.). The reproductive (R) stages begin at flowering (R1) and include pod development and plant maturation. Full maturity is designated R8. The vegetative development phase lasts about 40 days, during which time the root nodules develop slowly, but do not become fully functional. Soybeans grow most rapidly when air temperatures are between 25 and 35°C (Beversdorf, 1993). Pods typically develop in late summer, and harvest occurs in the autumn. The life cycle of soybean is approximately 100 to 160 days, depending on the variety and the region it is cultivated. Harvesting may begin when the plants are completely dry and the seeds are liberated within the pods.

Soybean is a self-pollinated species, propagated by seed (OECD, 2000). The papilionaceous flower consists of a tubular calyx of five sepals, a corolla of five petals, one pistil, and nine fused stamens with a single separate posterior stamen. The stamens form a ring at the base of the stigma and elongate one day before pollination, at which time the elevated anthers form a ring around the stigma (OECD, 2000). The soybean flower stigma is receptive to pollen

approximately 24 hours before anthesis and remains receptive for 48 hours after anthesis. The anthers mature in the bud and directly pollinate the stigma of the same flower. Pollination typically takes place on the day the flower opens. The pollen naturally comes in contact with the stigma during the process of anthesis. Anthesis normally occurs in late morning, depending on the environmental conditions. The pollen usually remains viable for two to four hours, and no viable pollen can be detected by late afternoon. Natural or artificial cross-pollination only can take place during the short time when the pollen is viable. As a result, soybean is considered to be a highly self-pollincated species, with cross-pollincation to adjacent plants of other soybean varieties occurring at very low frequency (0 to 6.3%) in adjacent plants (Caviness, 1966; Ray et al, 2003; Yoshimura et al., 2006).

The soybean variety used as the recipient for the DNA insertion to create MON 87705 was A3525, a non-transgenic elite commercial variety developed by Asgrow Seed Company. A3525 is a mid maturity group III soybean variety with very high yield potential. It has superior yields to lines of similar maturity and has excellent agronomic characteristic (Monsanto Technology, 2006).

History of Soybean

Domestication of soybean is thought to have taken place in China 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 and 16th centuries, soybean was introduced into several countries, with land races eventually developing in Japan, Indonesia, Philippines, Vietnam, Thailand, Malaysia, Myanmar, Nepal, and Northern India. The movement of soybean throughout this period was due to the establishment of sea and land trade routes, the migration of certain tribes from China, and the rapid acceptance of seeds as a staple food by other cultures (Hymowitz and Newell, 1981; Hymowitz et al., 1990).

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

Soybean was introduced into North America in the 18th century. In 1851, soybean was introduced in Illinois and subsequently throughout the Corn Belt. In 1853, soybean seed were deposited at 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 seed to dozens of farmers throughout the U.S. Soybean has been extensively cultivated and improved through conventional breeding programs following its introduction in the U.S. and has become a key source of nutrients for food and feed use in the U.S. (Hymowitz and Singh, 1987).

Soybean Processing

Soybeans are grown primarily for meal, and oil is a secondary product. There is no food use for unprocessed soybeans, since they contain anti-nutrient factors, such as trypsin inhibitors and lectins (OECD, 2001). Adequate heat processing inactivates these factors.

There are three main methods for processing soybeans; these are hydraulic processing, expeller processing and solvent extraction (SMIC, 2006). The description below on the soybean processing is adopted from Snyder and Wilson (2003).

Dehulling and flaking soybean

Soybeans are cleaned and cracked into several pieces (meats). The hulls are removed by aspiration and the meats are conditioned by warming and by adding moisture. Conditioning is necessary to make a cohesive flake. The conditioned meats are put through smooth rollers that make flakes of approximately 0.025 cm thickness. Making flakes is advantageous for uniform penetration of solvent in deep beds (minimal channelling) and for disruption of the soybean tissue, so that solvent can penetrate and dissolve the oil. Additionally, crushers may put the flakes through a cooking extruder to yield collets. This process gives a porous, but still high-density collet, that extracts more readily than flakes. Also, the collet holds less solvent than flake, thereby minimizing the energy needed for removing the solvent.

Oil extraction

The full-fat flakes (or collets) are loaded into the extractor to make beds over which the solvent flows countercurrently to the movement of the beds. The temperature of extraction is about 60ºC to speed up diffusion of solvent and to lower the miscella viscosity, both of which enhance the extraction of oil. Solvent extraction is capable of reducing the residual oil in the soybean flakes to less than 1%.

Removal of solvent and generation of crude oil and meal

Upon completion of the extraction, the solvent will be removed from both the oil and flakes. The full-fat miscella contains 25-30% oil and solvent is removed by two stages of rising-film vacuum evaporators followed by a third-stage stripping column. The flakes are treated in a desolventizer-toaster by direct contact with steam first to remove the solvent and second to heat treat the flakes for trypsin inhibitor destruction.

After leaving the desolventizer-toaster, the flakes are cooled and ground to a meal for use as a high-protein feed ingredient. The protein content is 44% with hulls added or 47.5-49% protein without hulls. If flakes are to be used to produce soybean food products, the solvent has to be removed with minimal heat to maintain protein solubility. Flash desolventizers are available in which superheated solvent is used as the heat-transfer medium to evaporate the solvent. With this system, the flakes are kept dry and protein solubility is preserved.

Oil refining

After extraction and removal of solvent, the crude soybean oil needs to be refined to convert it to edible products. Before going to refining, crude oil may undergo alkali refining (to remove free fatty acids), degumming (to remove phospholipids) or bleaching (to lighten the

colour of the oil). Hydrogenation and deodorisation made it possible to substitute vegetable oils for animal fats in human diets in the early 1900s. Hydrogenation controlled the texture and stability of the oil, while deodorization improves the flavour of vegetable oils.

The gummy material that is removed during the oil refinement is further processed into lecithin, which is typically used as an emulsifier to keep water and fats from separating in foods such as margarine, peanut butter, chocolate candies, ice cream, and infant formulas.

Soy protein isolate

Defatted white flakes are extracted first with sodium hydroxide at ~pH 9 to remove fibre, and the remaining solution is acidified to ~pH 4 to precipitate the protein. The precipitated protein in slurry form can be spray-dried directly to produce an isoelectric soybean protein isolate (pH 4), or can be neutralized and spray-dried to produce soy proteinate (~pH 7).

Resulting soybean products

- Soybean oil products: after sufficient refining, soybean oil is used to produce shortening, margarines, salad dressings and cooking oils of various types.
- Soybean meal: the majority of the defatted soybean flakes is heated to produce soybean meal as animal feeds.
- Soybean protein products: a variety of food products such as soybean flour, soybean protein concentrates and isolates can be used in a variety of food products including simulating meats, nutrition bars and protein-fortified drinks (Figure 2).

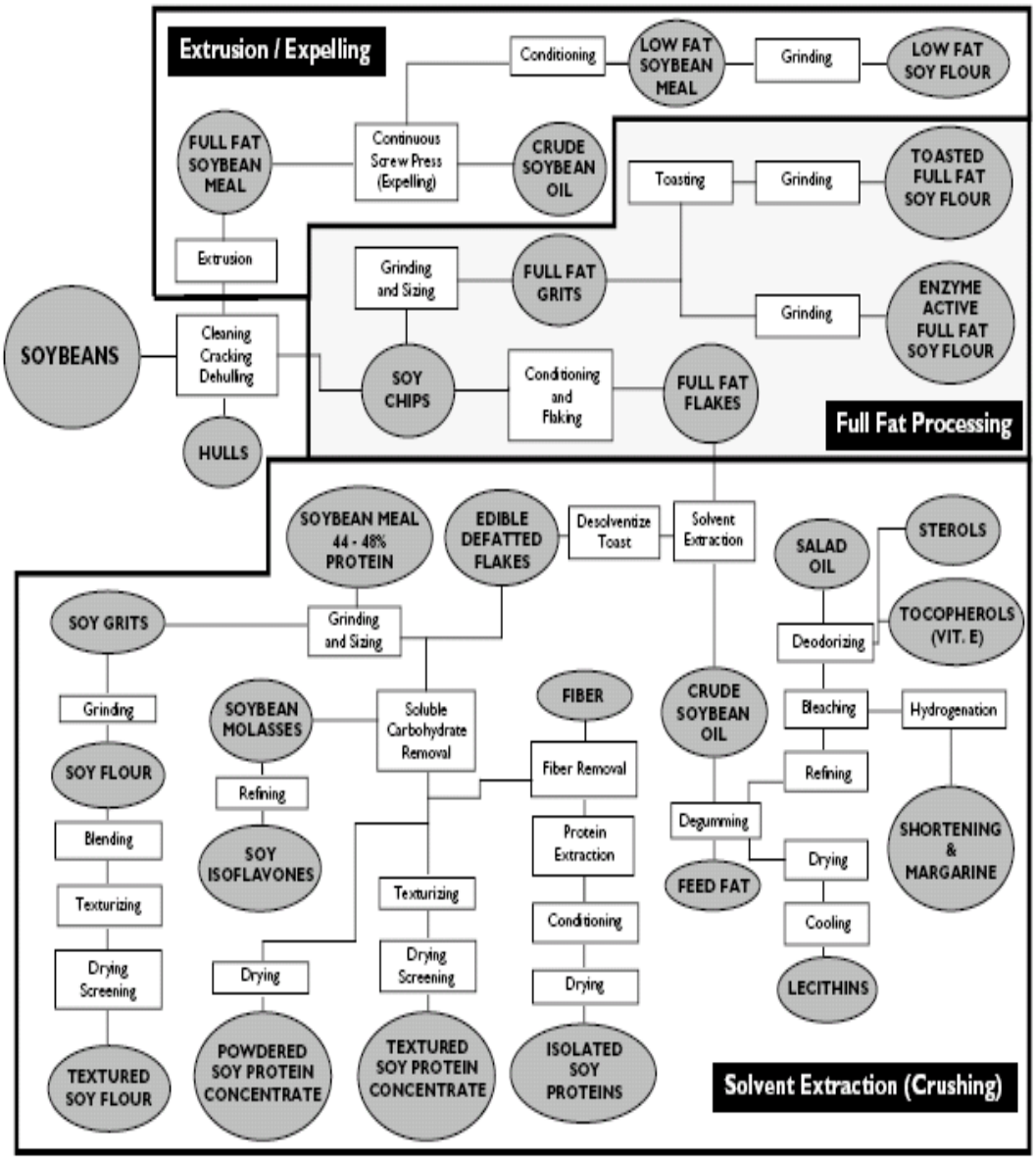


Figure 2. Soybean Processing Using Extrusion or Solvent Extraction

Figure is adapted from Soya and Oilseeds Bluebook, 2004.

2.3 Nature of Genetic Modification

a) Transformation Method

MON 87705 was developed through *Agrobacterium*-mediated transformation of soybean meristem tissue using the double-border, binary vector PV-GMPQ/HT4404 (Figure 3). *Agrobacterium*-mediated transformation is a well-documented process for the transfer and integration of exogenous DNA into a plant's nuclear genome (Bevan, 1984). PV-GMPQ/HT4404, is approximately 13.1 kb and contains two T-DNAs, each delineated by left and right border regions. The first T-DNA, designated T-DNA I, contains a *cp4 epsps* expression cassette and a partial[1] suppression cassette. The *cp4 epsps* expression cassette is under the regulation of *FMV/Tsf1* chimeric promoter and *E9* polyadenylation sequence. The partial suppression cassette in T-DNA I contains the sense segments of the *FAD2-1A* intron and *FATB1-A*[2] 5' UTR and the plastid targeting sequence, that are under the regulation of the seed *7Sα*' promoter from soybean. The second T-DNA, designated T-DNA II, contains a partial suppression cassette that consists of the antisense segment of *FAD2-1A* intron and *FATB1-A 5'* UTR and the plastid targeting sequence, which is flanked by the *H6* untranslated sequence. During plant transformation, the two T-DNAs co-integrated in MON 87705 at one locus in the soybean genome, creating a DNA insert that contains a *cp4 epsps* cassette and a single *FAD2-1A/FATB1-A* suppression cassette.

The *Agrobacterium*-mediated soybean transformation to produce MON 87705 was based on the method described by Martinell et al (2002), which allows the generation of transformed plants without utilization of callus. *Agrobacterium tumefaciens* strain ABI contains a disarmed Ti plasmid that is incapable of inducing tumor formation due to the deletion of the phytohormone genes originally present in the *Agrobacterium* plasmid (Koncz and Schell, 1986). The vector, PV-GMPQ/HT4404, contains two sets of left and right border sequences flanking each of the two transfer DNAs (T-DNA I and T-DNA II) to facilitate transformation. Briefly, meristem tissues were excised from the embryos of germinated A3525 seed. After co-culturing with *Agrobacterium* carrying the vector, the meristems were placed on selection medium containing glyphosate to inhibit the growth of untransformed plant cells, and carbenicillin and claforan to inhibit excess *Agrobacterium*. The meristems then were placed in media conducive to shoot and root development, and only rooted plants with normal phenotypic characteristics were selected and transferred to soil for growth and further assessment. The R0 plants generated through this transformation were self-pollinated, and the subsequent R1 plants were screened for the zygosity of the inserted gene. Only the R1 plants that were homozygous for the inserted gene, as determined by Invader analysis, and produced seeds with the desired fatty acid composition were advanced for development. Their progeny were subjected to further phenotypic assessments. MON 87705 was selected as the lead event based on superior phenotypic characteristics, agronomics, and molecular profile. Regulatory studies on MON 87705 were initiated to further characterize

[1] When referring to the suppression cassette, the term 'partial' refers to either the sense or antisense portion. When referring to either the *FATB1-A* or *FAD2-1A* elements within the suppression cassette, the term 'partial' refers to the fact that these are segments derived from the entire endogenous soybean genes.

[2] Whereever reference is made to FATB1-A, FATB, FAD2-1A, or FAD2 genes, RNAs or enzymes, either from MON 87705 or conventional soybean, the source of these genetic elements is *Glycine max* L.

the genetic insertion and the expressed protein, and to establish the food, feed, and environmental safety relative to conventional soybean. The major steps involving the development of MON 87705 are depicted in Figure 4.

b) Gene Construct and Transformation Event

The PV-GMPQ/HT4404 vector used for the transformation of soybean to produce MON 87705 is shown in Figure 3 and its elements described in Table 2. This vector is approximately 13.1 kb and contains two T-DNAs, each delineated by left and right border regions. T-DNA I contains a *cp4 epsps* expression cassette and a partial suppression cassette. The *cp4 epsps* expression cassette is under the regulation of *FMV/Tsf1* chimeric promoter and *E9* polyadenylation sequence. The partial suppression cassette in T-DNA I contains the sense segments of the *FAD2-1A* intron and *FATB1-A* 5' UTR and the plastid targeting sequence, which are under the regulation of the seed *7Sα'* promoter from soybean. T-DNA II contains a partial suppression cassette, which consists of the antisense segment of *FAD2-1A* intron and *FATB1-A* 5′ UTR that is flanked by the *H6* untranslated sequence. During plant transformation, a portion of the plants that were generated contained the two T-DNAs co-integrated at one locus in the soybean genome creating a DNA insert that contains a *cp4 epsps* cassette and a single *FAD2-1A/FATB1-A* suppression cassette. An expression cassette is comprised of sequences to be transcribed and the regulatory elements necessary for the expression of those sequences. The suppression cassette refers to the sequences and regulatory elements necessary for the suppression of the endogenous *FAD2* and *FATB* RNA transcripts.

The vector backbone region outside of the T-DNA contains two origins of replication for maintenance of plasmid in bacteria (*OR-oriV*, *OR-ori-pBR322*), a bacterial selectable marker gene (*aadA*), and a coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli* (*rop*). A description of the genetic elements and their prefixes (e.g., P-, L-, I-, TS-, OR-, B-, CS-, and T-) in PV-GMPQ/HT4404 is provided in Table 2.

i) The *cp4 epsps* Expression Cassette (T-DNA I)

This section describes the *cp4 epsps* expression cassette genetic elements contained in T-DNA I that were integrated into the soybean genome to produce MON 87705. T-DNA I contains the full *cp4 epsps* expression cassette and a partial *FAD2-1A/FATB1-A* suppression cassette, i.e. the sense segment. The *FAD2-1A/FATB1-A* suppression cassette is discussed in Section 2.3b ii.

The *cp4 epsps* Coding Sequence and CP4 EPSPS Protein

A *cp4 epsps* gene expression cassette is present in MON 87705. The *cp4 epsps* gene in MON 87705 is derived from *Agrobacterium* sp. strain CP4. The *cp4 epsps* coding sequence encodes a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids (Barry et al., 1992; Padgette et al., 1996a). The CP4 EPSPS protein is similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate, the active ingredient in the Roundup family of agricultural herbicides, relative to endogenous plant EPSPSs (Padgette et al., 1996a). 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 (Haslam, 1993; Steinrücken and Amrhein, 1980). 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., 1996a). The CP4 EPSPS protein expressed in MON 87705 is identical to the CP4 EPSPS protein in other Roundup Ready crops including Roundup Ready soybean (40-3-2), Roundup Ready 2 Yield soybean (MON 89788), Roundup Ready Corn 2, Roundup Ready canola, Roundup Ready sugar beet, and Roundup Ready cotton. The deduced CP4 EPSPS full-length amino acid sequence is shown in Figure 5.

The *cp4 epsps* Regulatory Sequences (T-DNA I)

The *cp4 epsps* coding sequence that is located in T-DNA I, is under the regulation of the *FMV/Tsf1* promoter, the *Tsf1* leader and intron, the CTP2 targeting sequence and the *E9* 3' untranslated sequence. The *FMV/Tsf1* is a chimeric promoter consisting of enhancer sequences from the promoter of the Figwort Mosaic virus 35S RNA (Richins et al., 1987) combined with the promoter from the *Tsf1* gene of *Arabidopsis thaliana* that encodes elongation factor EF-1 alpha (Axelos et al., 1989). The *Tsf1* leader is the 5' untranslated region from the *Arabidopsis thaliana Tsf1* gene (Axelos et al., 1989) that encodes the elongation factor EF-1 alpha. The CTP2 targeting sequence is the sequence encoding the transit peptide from the *ShkG* gene of *Arabidopsis thaliana* (Klee et al., 1987) and is present to direct the CP4 EPSPS protein to the chloroplast. The *E9* 3′ untranslated region is the 3' untranslated sequence from the *RbcS2* gene of *Pisum sativum* (Coruzzi et al., 1984) and is present to direct polyadenylation of the *cp4 epsps* transcript.

ii) The *FAD2-1A/FATB1-A* Suppression Cassette (T-DNA I and II)

This section describes the genetic elements contained in T-DNA I and T-DNA II that were integrated into the soybean genome to produce MON 87705. T-DNA II contains a partial *FAD2-1A/FATB1-A* suppression cassette. The partial *FAD2-1A/FATB1-A* suppression cassette, when integrated into the genome adjacent to the partial *FAD2-1A/FATB1-A* suppression cassette present in T-DNA I, comprise the complete *FAD2-1A/FATB1-A* suppression cassette in MON 87705.

The *FAD2-1A* and *FATB1-A* Segments Sequence (T-DNA I and II)

MON 87705 contains a partial sequence of the soybean (*Glycine max*) *FAD2-1A* gene and *FATB1-A* gene. The *FAD2-1A* and *FATB1-A* gene segments are comprised of ~ 0.6 kb of sequence from the *FAD2-1A* intron and the *FATB1-A* 5′ UTR and the plastid targeting sequence, and form the MON 87705 suppression cassette. This suppression cassette expresses an RNA that contains an inverted repeat of the *FAD2-1A* and *FATB1-A* gene segments. The gene transcript with an inverted repeat produces double stranded RNA (dsRNA) that, via the RNA interference (RNAi) pathway (Siomi and Siomi, 2009), suppresses endogenous *FATB* and *FAD2* RNA levels, and, ultimately, leads to improved fatty acid composition in the seed.

The Regulatory Sequences of the *FAD2-1A/FATB1-A* Suppression Cassette (T-DNA I and II)

Located in T-DNA I is a partial suppression cassette with a portion of the *FAD2-1A* intron and a portion of the *FATB1-A* 5' UTR and the plastid targeting sequence, under the regulation of the *Glycine max 7Sα'* seed storage gene promoter (designated *Sphas* 1) which drive expression in immature seeds (Doyle et al., 1986).

T-DNA II contains another partial suppression cassette with the *FAD2-1A/FATB1-A* gene segments followed by the *H6* 3' untranslated sequence. The *H6* 3' untranslated region is from the *Gossypium barbadense* cotton fiber protein gene and is present to terminate transcription (John and Keller, 1995).

T-DNA Borders

Plasmid PV-GMPQ/HT4404 contains right border and left border regions (Figure 3 and Table 2) that were derived from *Agrobacterium tumefaciens* plasmids (Barker et al., 1983; Depicker et al., 1982). The border regions each contain a 24-25 bp nick site that is the site of DNA exchange during transformation. The border regions delineate the T-DNA and are involved in their efficient transfer into the soybean genome. Because PV-GMPQ/HT4404 is a two T-DNA vector, it contains two right border regions and two left border regions, where one set flanks T-DNA I and the other set flanks T-DNA II.

iii) Genetic Elements Outside the T-DNA Borders

Genetic elements that exist outside of the T-DNA borders are those that are essential for the maintenance and selection of the vector PV-GMPQ/HT4404 in bacteria. The origin of replication *OR-ori V* is required for the maintenance of the plasmid in *Agrobacterium* (Stalker et al., 1981) and is derived from the broad host plasmid RK2. The origin of replication OR-*ori-pBR322* is required for the maintenance of the plasmid in *E. coli* and is derived from the plasmid pBR322 (Sutcliffe, 1979). CS-*rop* is the coding sequence of the repressor of primer (ROP) protein and is necessary for the maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989). The selectable marker *aadA* is a bacterial promoter, coding sequence, and 3' UTR for an enzyme from transposon *Tn7* that confers spectinomycin and streptomycin resistance (Fling et al., 1985) in *E. coli* and *Agrobacterium* during molecular cloning. Because these elements are outside the border regions, they are not expected to be transferred into the soybean genome. The absence of the backbone sequence in MON 87705 has been confirmed by Southern blot analyses (see Section 2.3 c ii).

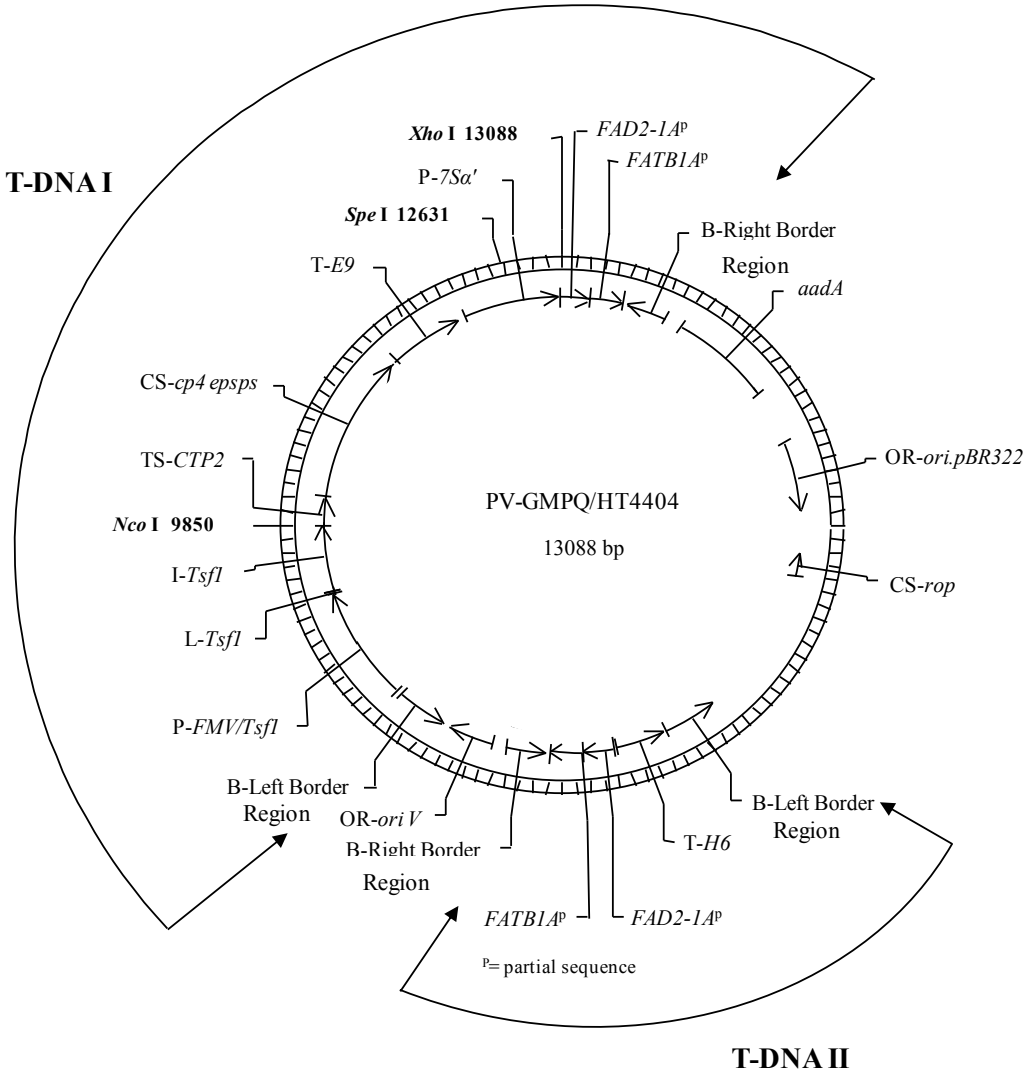


Figure 3. Circular Map of Plasmid PV-GMPQ/HT4404

Plasmid PV-GMPQ/HT4404 containing the T-DNAs used in *Agrobacterium*-mediated transformation to produce MON 87705. Approximate locations of the genetic elements (with approximate positions relative to the plasmid vector) are shown on the exterior of the map. PV-GMPQ/HT4404 contains two T-DNA regions designated T-DNA I and T-DNA II.

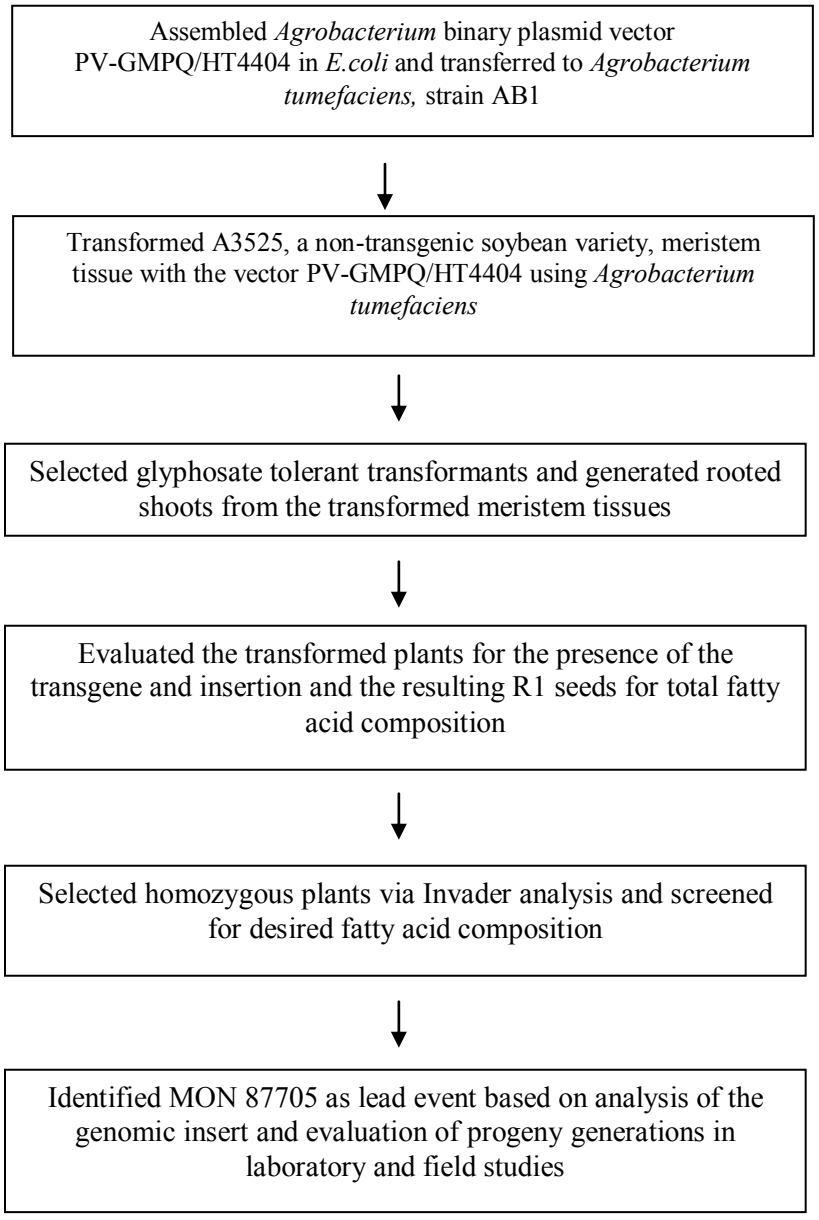


Figure 4. Schematic of the Development of MON 87705

Table 2. Summary of Genetic Elements in Plasmid Vector PV-GMPQ/HT4404

Genetic Element	Location in Plasmid	Function (Reference)
T-DNA I		
B[1]-Left Border Region	7657 – 8098	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983)
Intervening Sequence	8099 – 8134	Sequence used in DNA cloning
P[2]-*FMV/Tsf1*	8135 – 9174	Chimeric promoter consisting of enhancer sequences from the promoter of the Figwort Mosaic virus 35S RNA (Richins et al., 1987) combined with the promoter from the *Tsf1* gene of *Arabidopsis thaliana* that encodes elongation factor EF-1alpha (Axelos et al., 1989)
L[3]-*Tsf1*	9175 – 9220	5' untranslated leader (exon 1) from the *Tsf1* gene of *Arabidopsis thaliana* that encodes elongation factor EF-1 alpha (Axelos et al., 1989)
I[4]-*Tsf1*	9221 – 9842	Intron with flanking exon sequence from the *Tsf1* gene of *Arabidopsis thaliana* that encodes elongation factor EF-1alpha (Axelos et al., 1989)
Intervening Sequence	9843 – 9851	Sequence used in DNA cloning
TS[5]-*CTP2*	9852 – 10079	Targeting sequence from the *ShkG* gene encoding the transit peptide region of *Arabidopsis thaliana* EPSPS (Klee et al., 1987) that directs transport of the CP4 EPSPS protein to the chloroplast
CS[6]-*cp4 epsps*	10080 –11447	Codon modified coding sequence of the *aroA* gene from the *Agrobacterium sp.* strain CP4 encoding the CP4 EPSPS protein (Barry et al., 1997; Padgette et al., 1996a)
Intervening Sequence	11448 – 11505	Sequence used in DNA cloning
T[7]-*E9*	11506 – 12148	3′ untranslated region of the pea *RbcS2* gene which functions to direct polyadenylation of the mRNA (Coruzzi et al., 1984)
Intervening Sequence	12149 – 12236	Sequence used in DNA cloning
P-*7Sα′*	12237 – 13077	promoter and leader from the *Sphas1* gene of *Glycine max* encoding beta-conglycinin storage protein (alpha'-bcsp) (Doyle et al., 1986) that directs transcription in seed

[1] – Border; [2] – Promoter; [3]– Leader; [4] – Intron; [5] – Targeting Sequence; [6] – Coding Sequence;
[7] – 3' untranslated transcriptional termination sequence and polyadenylation signal sequences.

Table 2 (continued). Summary of Genetic Elements in Plasmid Vector PV-GMPQ/HT4404

Intervening Sequence	13078 – 11	Sequence used in DNA cloning
***FAD2-1A*^{p}**	12 – 277	Partial sequence from intron #1 of the *Glycine max FAD2-1A* gene that encodes the delta-12 desaturase (Fillatti et al., 2003) which forms part of the suppression cassette
***FATB1-A*^{p}**	278 – 578	Partial sequence from the 5' untranslated region and the plastid targeting sequence from *Glycine max FATB1-A* gene that encodes the palmitoyl acyl carrier protein thioesterase (Fillatti et al., 2003) which forms part of the suppression cassette
Intervening Sequence	579 – 616	Sequence used in DNA cloning
B-Right Border Region	617 – 973	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Zambryski et al., 1982)
Vector Backbone		
Intervening Sequence	974 – 1109	Sequence used in DNA cloning
aadA	1110 – 1998	Promoter, coding sequence, and 3' UTR for an aminoglycoside-modifying enzyme, 3"(9)-O-nucleotidyltransferase from the transposon *Tn7* (Fling et al., 1985) that confers spectinomycin and streptomycin resistance
Intervening Sequence	1999 – 2528	Sequence used in DNA cloning
OR^{8}-*ori-pBR322*	2529 – 3117	Origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1979)
Intervening Sequence	3118 – 3544	Sequence used in DNA cloning
CS-*rop*	3545 – 3736	Coding sequence for repressor of primer protein from the ColE1 plasmid for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989)
Intervening Sequence	3737 – 5127	Sequence used in DNA cloning
T-DNA II		
B-Left Border Region	5128 – 5569	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983)

^{P} – Partial sequence; ^{8} – Origin of Replication.

Table 2 (continued). Summary of Genetic Elements in Plasmid Vector PV-GMPQ/HT4404

Intervening Sequence	5570 – 5667	Sequence used in DNA cloning
T-*H6*	5668 – 6103	3' UTR sequence of the *H6* gene from *Gossypium barbadense* encoding a fiber protein involved in secondary cell wall assembly (John and Keller, 1995)
Intervening Sequence	6104 – 6115	Sequence used in DNA cloning
FAD2-1A^{p}	6116 – 6381	Partial sequence from intron #1 of the *Glycine max FAD2-1A* gene that encodes the delta-12 desaturase (Fillatti et al., 2003) which forms part of the suppression cassette
FATB1-A^{p}	6382 – 6682	Partial sequence from the 5' untranslated region and the plastid targeting sequence from *Glycine max FATB1-A* gene that encodes the palmitoyl acyl carrier protein thioesterase (Fillatti et al., 2003) which forms part of the suppression cassette
Intervening Sequence	6683 – 6693	Sequence used in DNA cloning
B-Right Border Region	6694 – 7024	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Zambryski et al., 1982)
Vector Backbone		
Intervening Sequence	7025 – 7173	Sequence used in DNA cloning
OR-*ori V*	7174 – 7570	Origin of replication from the broad host range plasmid RK2 for maintenance of plasmid in *Agrobacterium* (Stalker et al., 1981)
Intervening Sequence	7571 – 7656	Sequence used in DNA cloning

```
  1 MLHGASSRPA TARKSSGLSG TVRIPGDKSI SHRSFMFGGL ASGETRITGL LEGEDVINTG
 61 KAMQAMGARI RKEGDTWIID GVGNGGLLAP EAPLDFGNAA TGCRLTMGLV GVYDFDSTFI
121 GDASLTKRPM GRVLNPLREM GVQVKSEDGD RLPVTLRGPK TPTPITYRVP MASAQVKSAV
181 LLAGLNTPGI TTVIEPIMTR DHTEKMLQGF GANLTVETDA DGVRTIRLEG RGKLTGQVID
241 VPGDPSSTAF PLVAALLVPG SDVTILNVLM NPTRTGLILT LQEMGADIEV INPRLAGGED
301 VADLRVRSST LKGVTVPEDR APSMIDEYPI LAVAAAFAEG ATVMNGLEEL RVKESDRLSA
361 VANGLKLNGV DCDEGETSLV VRGRPDGKGL GNASGAAVAT HLDHRIAMSF LVMGLVSENP
421 VTVDDATMIA TSFPEFMDLM AGLGAKIELS DTKAA
```

Figure 5. Deduced Amino Acid Sequence of the Full- Length CP4 EPSPS Protein Produced in MON 87705

The amino acid sequence of the CP4 EPSPS protein was deduced from the full-length *cp4 epsps* coding sequence present in PV-GMPQ/HT4404.

c) Molecular Characterization

Southern blot analyses were used to determine the number of copies and the insertion sites of the integrated DNA as well as the presence or absence of plasmid backbone sequence. The Southern blot strategy was designed to ensure that all potential insertion segments would have been identified. The entire soybean genome was assayed with probes that spanned the complete transformation plasmid to detect the presence of the insertion as well as confirm the absence of any backbone sequence. This was accomplished by using probes designed to insure high sensitivity. This high level of sensitivity was demonstrated for each blot by detection of a positive control added at the equivalent of $1/10^{th}$ of a genome. Two restriction enzyme sets were specifically chosen to independently confirm the presence of the insert. This two enzyme design also minimizes the possibility of not detecting an insertion elsewhere in the genome which could be missed if that band comigrated with an expected band. Additionally, the restriction enzyme sets were chosen such that at least one enzyme from each set resides in the known 5′ or 3′ flanking sequence and that together the enzyme sets result in overlapping segments covering the entire insert. Therefore, at least one segment for each flank is of a predictable size and overlaps with another predictable size segment. This overlapping strategy confirms that the entire insert sequence is identified in a predictable hybridization pattern.

The results of these analyses for MON 87705 show that a single copy of the T-DNA inserted at a single locus of the genome. Generational stability analysis demonstrated that an expected Southern blot fingerprint of MON 87705 has been maintained through four generations of the breeding history, thereby confirming the stability of the insert. Results from segregation analyses show heritability and stability of the insert occurred 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.

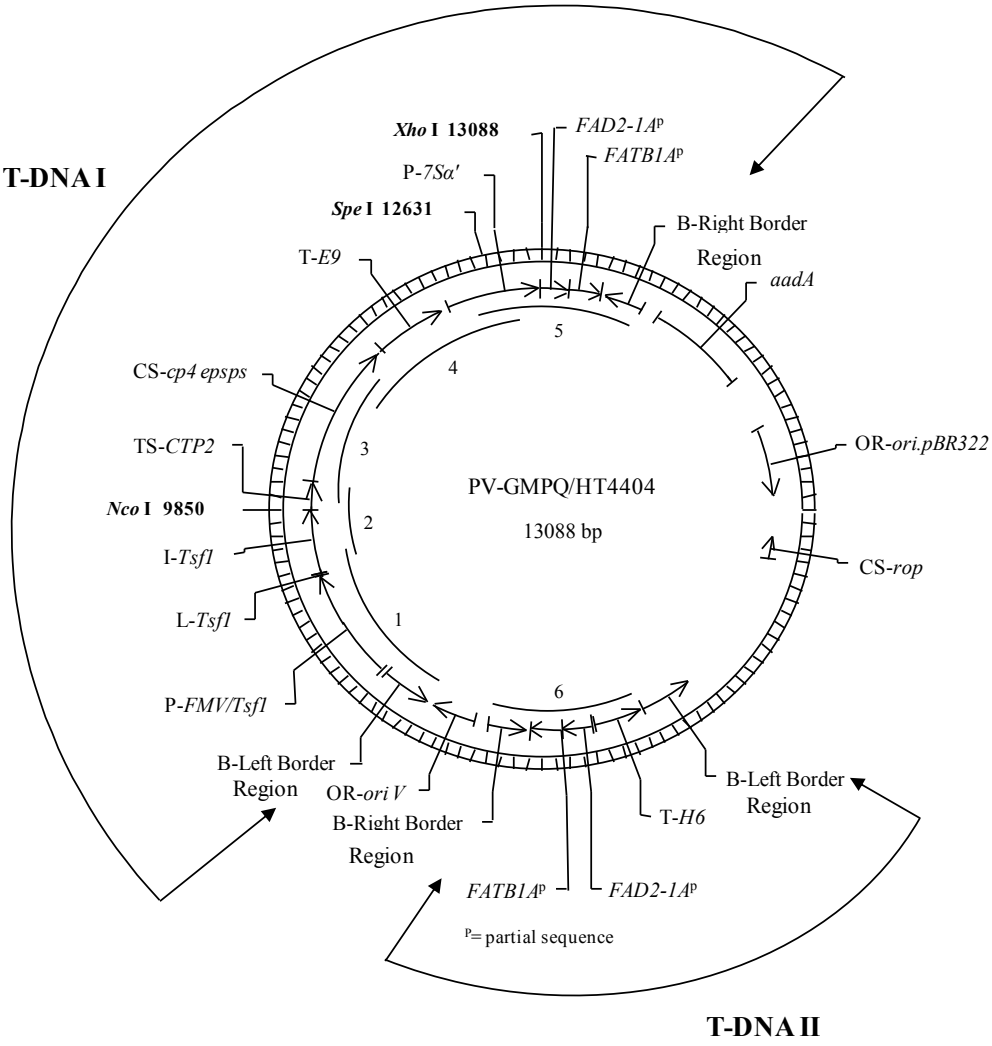
For each digest used to confirm copy number there were duplicated samples that consisted of equal amounts of digested DNA. One set of samples was run for a longer period of time (long run) than the second set (short run). The long run allows for greater resolution of large molecular weight DNA, whereas the short run allows the detection of small molecular weight

DNA. For estimating the sizes of bands present in the long run lanes of Southern blots, the molecular weight markers on the left of the figure were used. For estimating the sizes of bands present in the short run lanes, the molecular weight markers on the right of the figure were used.

The DNA sequencing analyses complement the Southern analyses. Southern analyses determined that MON 87705 contains T-DNA I and T-DNA II-derived sequences integrated at a single insertion site. Integration of inserted sequences into a single locus when using a two T-DNA system to create a biotechnology-derived soybean is not an unusual circumstance (Xing et al., 2000). Sequencing of the insert and the flanking genomic DNA confirmed the organization of the elements within the insert, determined the 5' and 3' insert-to-plant junctions, determined the complete DNA sequence of the insert and adjacent soybean genomic DNA, and confirmed that the genomic DNA sequences flanking the 5' and 3' ends of the insert in MON 87705 are native to the soybean genome. Each cassette is intact, and the sequence of the insert matches the corresponding sequence in PV-GMPQ/HT4404 as described in Table 2 and 4. In addition, genomic rearrangements at the insertion site were assessed by comparing the insert and flanking sequence to the insertion site in conventional soybean A3525 which has background genetics similar to MON 87705.

The stability of the DNA insert across multiple generations (R3-R6) also was demonstrated by Southern blot fingerprint analyses. Four generations of MON 87705 were digested with one of the enzyme sets used for the copy number analysis and were hybridized with probes that would detect restriction segments that encompass the entire insert (two hybridization bands). This fingerprint strategy consists of two border segments that assess not only the stability of the insert, but also the stability of genomic DNA directly adjacent to the insert.

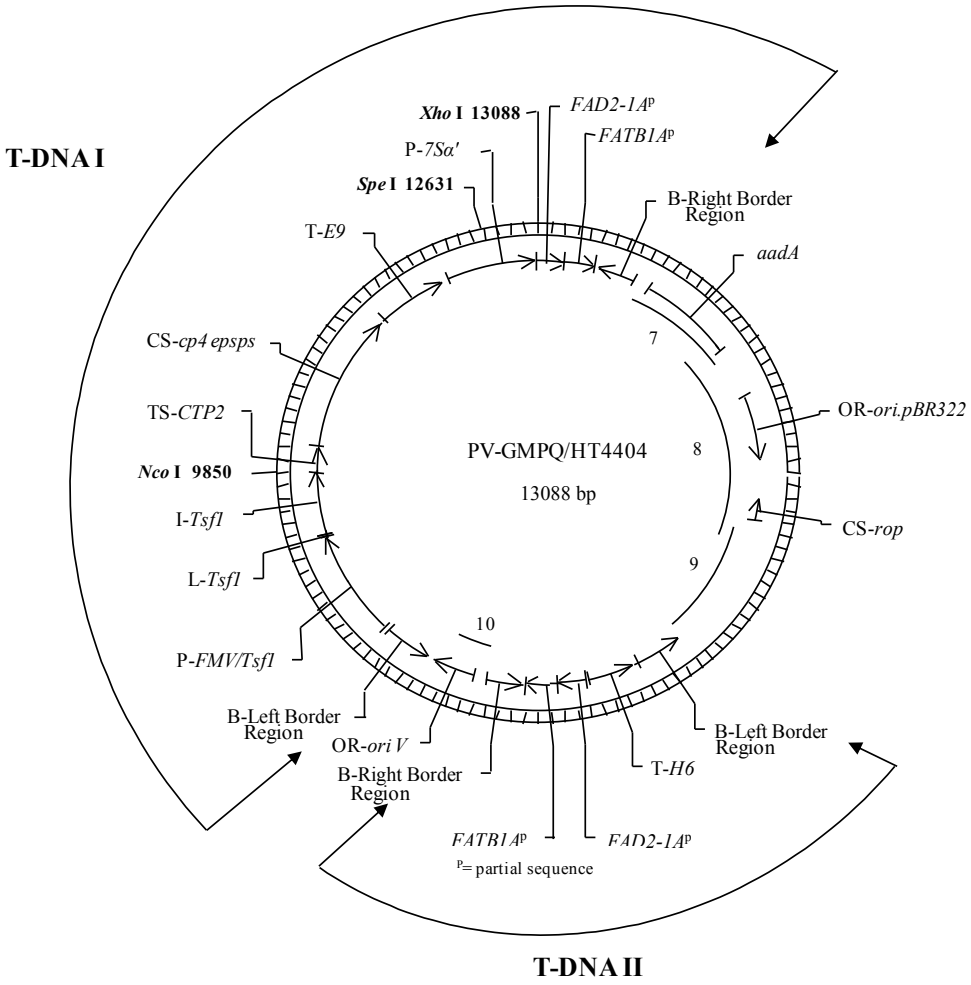
The Southern blot analysis confirmed the insert reported in Figure 8 represents the only detectable insert in MON 87705. The genetic elements integrated in MON 87705 are summarized in Table 4. Maps of plasmid vector PV-GMPQ/HT4404, used in the transformation to produce MON 87705 and annotated with the probes used in the Southern analysis are presented in Figure 6 and 7. Shown in Figure 8 is a linear map depicting restriction sites within the insert as well as within the known soybean genomic DNA immediately flanking the insert in MON 87705. Based on the linear map of the insert and the plasmid map, a table summarizing the expected DNA segments for Southern analyses is presented in Table 3. In some of the Southern blots, the migration of the genomic DNA is slightly different when compared to the migration of the molecular weight markers. These altered migrations are likely the result of different base pair composition and/or differences in salt concentration between the genomic DNA samples and the molecular weight marker (Sambrook, 1989). The generations used in these studies are depicted in the breeding history shown in Figure 16.



Probe	DNA Probe	Start Position	Stop Position	Total Length (~kb)
1	T-DNA I Probe 1	7657	9406	1.8
2	T-DNA I Probe 2	9270	10042	0.8
3	T-DNA I Probe 3	9943	11325	1.4
4	T-DNA I Probe 4	11151	160	2.1
5	T-DNA I Probe 5	13080	973	1.0
6	T-DNA II Probe 1	5570	6693	1.1

Figure 6. Circular Map of Plasmid PV-GMPQ/HT4404 Showing Probes 1-6

Plasmid PV-GMPQ/HT4404 containing the T-DNAs used in *Agrobacterium*-mediated transformation to produce MON 87705. Genetic elements (depicted in the exterior of the map) and restriction sites for enzymes used in Southern analyses (with positions relative to the size of the plasmid vector) are shown on the exterior of the map. The T-DNA I and T-DNA II probes used in the Southern analyses (labeled 1-6 within the interior of the map) are detailed in the table above.



Probe	DNA Probe	Start Position	Stop Position	Total Length (~kb)
7	Backbone Probe 1	974	2280	1.3
8	Backbone Probe 2	2140	4080	1.9
9	Backbone Probe 3	3631	5127	1.5
10	Backbone Probe 4	7025	7656	0.6

Figure 7. Circular Map of Plasmid PV-GMPQ/HT4404 Showing Probes 7-10

Plasmid PV-GMPQ/HT4404 containing the T-DNAs used in *Agrobacterium*-mediated transformation to produce MON 87705. Genetic elements (depicted in the exterior of the map) and restriction sites for enzymes used in Southern analyses (with positions relative to the size of the plasmid vector) are shown on the exterior of the map. The backbone probes used in the Southern analyses (labeled 7-10 within the interior of the map) are detailed in the table above.

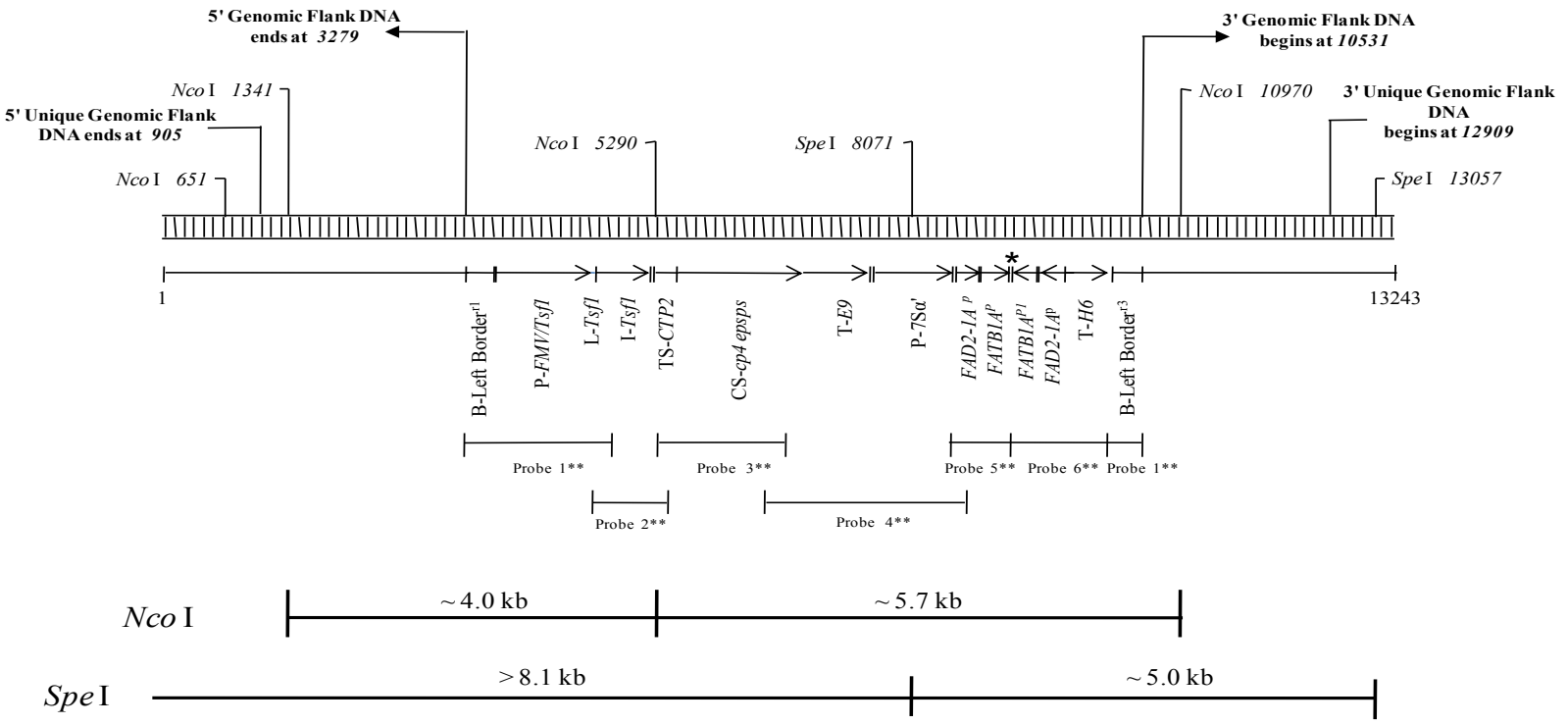


Figure 8. Schematic Representation of the Insert and Genomic Flanking Sequences in MON 87705

A linear map of the insert and genomic DNA flanking the insert in MON 87705 is shown. Identified on the map are genetic elements within the insert, as well as restriction sites with positions relative to the size of the linear map for enzymes used in the Southern analyses. Shown on the lower portion of the map are the expected sizes of the DNA segments after digestion with respective restriction enzymes. Arrowheads (→) indicate the end of the insert and the beginning of soybean genomic flanking sequence. The arrows (→) indicated the sequence direction of the elements in MON 87705. The * indicates partial sequences from the Left Border and Right Border sequences after integration into MON 87705 (Table 4). Base pairs 906-3279 in the 5' flanking genomic DNA, and 10535-12908 in the 3' flanking genomic DNA represent duplicated bases from the 3′ end of the flanking soybean genomic DNA. Therefore, bases 1-905 and 12909-13243 are indicated as "unique" genomic flank DNA. The * denotes sequences from Right Border and Left Border after integration into MON 87705 (Table 4). Base pairs 906-3279 in the 5' flanking genomic DNA, and 10535-12908 in the 3' flanking genomic DNA represent duplicated bases from the 3' end of the flanking soybean genomic DNA (Section 2.3c iii).

**These probes are not drawn to scale and are the estimated locations of the T-DNA I and T-DNA II probes in MON 87705. Refer to the Figure 6 for the description of the probes.

Table 3. Summary Chart of the Expected DNA Segments Based on Hybridizing Probes and Restriction Enzymes

Southern blot Figure	**9**	**10**	**11**	**12**	**13**	**17**
Probes Used in Analysis	1, 4, 6	2, 5	3	7, 9	8, 10	1, 6
Positive Hybridization Controls						
Xho I + *Nco* I Digested Plasmid	~3.2, ~9.9 kb	~3.2, ~9.9 kb	~3.2 kb	~9.9 kb	~9.9 kb	~9.9 kb
Probe Templates [1]	~1.8, ~2.1, and ~1.1 kb	~0.8 and ~1.0 kb	NA[2]	~1.3 and ~1.5 kb	~1.9 and ~0.6 kb	~1.8 and ~1.1 kb
MON 87705 DNA Digestion						
Nco I	~4.0 and ~5.7 kb	~4.0 and ~5.7 kb	~5.7 kb	No band	No band	~4.0 and ~5.7 kb
Spe I	> 8.1* and ~5.0kb	> 8.1* and ~5.0kb	> 8.1* kb	No band	No band	NA[3]

[1] Probe templates were added to predigested conventional soybean DNA when multiple probes are used in Southern blot analysis.
[2] 'NA (not assessed)' indicates that the plasmid template was the only positive control used, because the Southern blot was hybridized with one probe.
[3] 'NA (not assessed)'indicates that the particular restriction enzyme was not used in the analysis.
*Southern analysis indicates this segment to be ~ 11 kb.

Table 4. Summary of Genetic Elements in MON 87705

Genetic Element	Location in Sequence	Function (Reference)
Unique 5' flanking sequence of the insert	1 – 905	Soybean genomic DNA
Sequence flanking 5' end of the insert	906-3279	2374 bp of soybean genomic DNA duplicated from the 3' end of the flanking sequence of the insert
B[1]-Left Border	3280 – 3538	259 bp sequence from the B-Left Border region remaining after integration (Barker et al., 1983)
Intervening Sequence	3539 – 3574	Sequence used in DNA cloning
P[2]-*FMV/Tsf1*	3575 – 4614	Chimeric promoter consisting of enhancer sequences from the promoter of the Figwort Mosaic virus 35S RNA (Richins et al., 1987) combined with the promoter from the *Tsf1* gene of *Arabidopsis thaliana* that encodes elongation factor EF-1 alpha (Axelos et al., 1989)
L[3]-*Tsf1*	4615 – 4660	5' untranslated leader (exon 1) from the *Tsf1* gene of *Arabidopsis thaliana* that encodes elongation factor EF-1 alpha (Axelos et al., 1989)
I[4]-*Tsf1*	4661 – 5282	Intron with flanking exon sequence from the *Tsf1* gene of *Arabidopsis thaliana* that encodes elongation factor EF-1 alpha (Axelos et al., 1989)
Intervening Sequence	5283 – 5291	Sequence used in DNA cloning
TS[5]-*CTP2*	5292 – 5519	Targeting sequence from the *ShkG* gene encoding the transit peptide region of *Arabidopsis thaliana* EPSPS (Klee et al., 1987) that directs transport of the CP4 EPSPS protein to the chloroplast
CS[6]-*cp4 epsps*	5520 – 6887	Codon modified coding sequence of the *aroA* gene from the *Agrobacterium sp.* strain CP4 encoding the CP4 EPSPS protein (Barry et al., 1997; Padgette et al., 1996a)
Intervening Sequence	6888 – 6945	Sequence used in DNA cloning

[1]– Border; [2] – Promoter; [3]– Leader; [4] – Intron; [5] – Targeting Sequence; [6] – Coding Sequence;

Table 4 (continued). Summary of Genetic Elements in MON 87705

Genetic Element	Location in Sequence	Function (Reference)
T[7]-*E9*	6946 – 7588	3' untranslated region of the pea *RbcS2* gene which functions to direct polyadenylation of the mRNA (Coruzzi et al., 1984)
Intervening Sequence	7589 – 7676	Sequence used in DNA cloning
P-*7Sα'*	7677 – 8517	Non-coding promoter and leader from the *Sphas1* gene of *Glycine max* encoding beta-conglycinin storage protein (alpha'-bcsp) (Doyle et al., 1986) that directs transcription in seed
Intervening Sequence	8518 – 8539	Sequence used in DNA cloning
FAD2-1A[p]	8540 – 8805	Partial sequence from intron #1 of the *Glycine max FAD2-1A* gene that encodes the delta-12 desaturase (Fillatti et al., 2003) which forms part of the suppression cassette
FATB1-A[p]	8806 – 9106	Partial sequence from the 5' untranslated region and the plastid targeting sequence from *Glycine max FATB1-A* gene that encodes the palmitoyl acyl carrier protein thioesterase (Fillatti et al., 2003) which forms part of the suppression cassette
Intervening Sequence	9107 – 9114	Sequence used in DNA cloning
B-Right Border	9115 – 9134	20 bp sequence from the B-Right Border region remaining after integration (Zambryski et al., 1982)
B-Left Border	9135 – 9172	38 bp sequence from the B-Left Border region remaining after integration (Barker et al., 1983)
FATB1-A[p1]	9173 – 9443	Partial sequence from the 5' untranslated region and the plastid targeting sequence from *Glycine max FATB1-A* gene that encodes the palmitoyl acyl carrier protein thioesterase (Fillatti et al., 2003) which forms part of the suppression cassette
FAD2-1A[p]	9444 – 9709	Partial sequence from intron #1 of the *Glycine max FAD2-1A* gene that encodes the delta-12 desaturase (Fillatti et al., 2003) which forms part of the suppression cassette
Intervening Sequence	9710 – 9721	Sequence used in DNA cloning

[7] – 3' untranslated transcriptional termination sequence and polyadenylation signal sequences; ***P*** – Partial sequence; ***P1*** – Truncated partial sequence of *FATB1-A*.

Table 4 (continued). Summary of Genetic Elements in MON 87705

Genetic Element	Location in Sequence	Function (Reference)
T-*H6*	9722 – 10157	3' UTR sequence of the *H6* gene from *Gossypium barbadense* encoding a fiber protein involved in secondary cell wall assembly (John and Keller, 1995)
Intervening Sequence	10158 –10255	Sequence used in DNA cloning
B-Left Border	10256 - 10530	275 bp sequence from the B-Left Border region remaining after integration (Adang et al., 1985)
Sequence flanking 3' end of the insert	10531 – 12908	Soybean genomic DNA including the 2374 bases duplicated at the 5' end of the flanking sequence of the insert
Unique 3' flanking sequence of the insert	12909 – 13243	Soybean genomic DNA

i) Insert and Copy Number Determination

The copy number and insertion sites of T-DNA I and T-DNA II were assessed by digesting MON 87705 DNA with restriction enzymes *Nco* I or *Spe* I and hybridizing Southern blots with probes that span T-DNA I and T-DNA II (Figure 6 and 7). Each restriction digest is expected to produce a specific banding pattern on the Southern blots (Table 3). Since each detected segment contains flanking genomic DNA, any additional integration sites would produce a different banding pattern with additional bands.

The restriction enzyme *Nco* I cuts once in the MON 87705 insert and once in each of the known 5' and 3' flanking sequences of MON 87705. Therefore, if T-DNA I and T-DNA II sequences are present at a single integration site in MON 87705, the digestion with *Nco* I was expected to generate two border segments with expected sizes of ~4.0 kb and ~5.7 kb (Figure 8). The ~4.0 kb restriction segment contains genomic DNA flanking the 5' end of the insert, the Left Border, the *FMV/Tsf1* promoter, the *Tsf1* leader, and the *Tsf1* intron. The ~5.7 kb restriction segment contains the *CTP2* targeting sequence, *cp4 epsps* coding sequence, *E9* 3' untranslated sequence, *7Sα'* promoter, partial *FAD2-1A/FATB1-A* sense sequences, partial sequences of Right Border, Left Border, partial *FATB1-A/FAD2-1A* antisense sequences, *H6* 3' untranslated sequence, Left Border and genomic DNA flanking the 3' end of the insert.

The restriction enzyme *Spe* I cuts once in the MON 87705 insert and once in the known 3' flanking sequence of MON 87705. Therefore, if T-DNA I and T-DNA II sequences are present at a single integration site in MON 87705 digestion with *Spe* I is expected to release two border segments with expected sizes of ~5.0 kb and greater than 8.1 kb (Figure 8). Since the *Spe* I site in the soybean genome flanking the 5' end of the insert lies outside of the known sequence, it was not possible to predict a precise segment size. However, the segment size was determined by Southern blot analyses to be ~11 kb (Figure 9, 10, and 11). The ~11 kb DNA segment contains genomic DNA flanking the 5' end of the insert, Left Border, *FMV/Tsf1* promoter, *Tsf1* leader, *Tsf1* intron, *CTP2* targeting sequence, *cp4 epsps* coding sequence, *E9* 3' untranslated sequence, and a portion of *7Sα'* promoter. The ~5.0 kb restriction segment contains the remaining portion of the *7Sα'* promoter, partial *FAD2-1A/FATB1-A* sense sequences, partial sequences of Right Border and Left Border, partial *FATB1-A/FAD2-1A* antisense sequences, *H6* 3' untranslated sequence, Left Border and genomic DNA flanking the 3' end of the insert.

In the Southern blot analyses performed, each Southern blot contained a negative and several positive controls. Conventional soybean DNA digested with *Nco* I or *Spe* I was used as a negative control to determine if the probes hybridized to any endogenous soybean sequences. As a positive control on the Southern blots, digested plasmid and probe templates were used. Plasmid PV-GMPQ/HT4404 digested with a combination of *Xho* I and *Nco* I was mixed with predigested conventional soybean genomic DNA and loaded on the gel. For Southern blots hybridized with multiple probes, each probe template was mixed with predigested conventional soybean DNA. The positive hybridization control was spiked at 0.1 and 1 genome equivalent to demonstrate sufficient sensitivity of the Southern blot. Individual Southern blots were hybridized with the following probe sets: Probes 1, 4, and 6; Probes 2 and 5; Probe 3; Probes 7 and 9; and Probes 8 and 10 (refer to Figure 6 and 7 and Table 3). The results of these analyses are shown in Figure 9 through 13.

Probes 1, 4 and 6

Conventional soybean DNA digested with *Nco I* (Figure 9, lanes 1 and 8) or *Spe* I (Figure 9, lanes 3 and 10) and hybridized with the probes 1, 4, and 6 (Figure 6) produced several hybridization signals. These hybridization signals result from the probes (Probes 1, 4, and 6, Figure 6) hybridizing to endogenous sequences residing in the soybean genome and are not specific to the inserted DNA. These results were expected, because several genetic elements covered by probes 1, 4 and 6 are native to the soybean genome. These signals, as expected, were produced in both MON 87705 and conventional soybean lanes, and, therefore, the bands are considered to be endogenous background hybridization.

Probe template spikes (Probes 1, 4 and 6, Figure 6) generated from plasmid PV-GMPQ/HT4404 were mixed with the conventional soybean DNA predigested with *Spe* I and produced the expected bands at ~1.8, ~2.1, and ~1.1 kb, respectively, (Figure 9, lanes 5-6) in addition to the endogenous background hybridization observed in the conventional soybean DNA (Figure 9, lane 10). Plasmid PV-GMPQ/HT4404 digested with a combination of *Xho* I and *Nco* I and mixed with conventional soybean DNA predigested with *Spe* I (Figure 9, lane 7) produced the expected size bands of ~3.2 and ~9.9 kb (refer to Figure 6) in addition to the endogenous background hybridization observed in the conventional soybean DNA (Figure 9, lane 10). These results indicate that the probes are hybridizing to their target sequences.

MON 87705 DNA digested with *Nco I* (Figure 9, lanes 2 and 9) produced two unique bands of ~4.0 and ~5.7 kb in addition to the endogenous background hybridization observed in the conventional soybean DNA (Figure 9, lanes 1 and 8). The ~4.0 kb band is the expected size for the border segment containing the 5' end of the inserted DNA (T-DNA I) along with the adjacent genomic DNA flanking the 5' end of the insert (Figure 8). The ~5.7 kb band is the expected size for the border segment containing the 3' end of the inserted DNA (T-DNA I and II) along with the adjacent genomic DNA flanking the 3' end of the insert (Figure 8).

MON 87705 DNA digested with *Spe* I (Figure 9, lanes 4 and 11) produced two unique bands of ~5.0 and ~11 kb, in addition to the endogenous background hybridization observed in the conventional soybean DNA (Figure 9, lanes 3 and 10). The ~11 kb segment is consistent with the expected band being greater than 8.1 kb. This band in the short run appears slightly larger, at ~13 kb, than the corresponding band in the long run. This border segment contains the 5' end of the inserted DNA (T-DNA I) along with the adjacent genomic DNA flanking the 5' end of the insert (Figure 8). The ~5.0 kb band (Figure 9, lane 4) is consistent with the expected band of 5.0 kb; however, the migration of the segment is slightly higher at ~5.2 kb in the short run (Figure 9, lane 11) as indicated by the molecular weight marker. The ~5.0 kb band is the expected size for the border segment containing the 3' end of the inserted DNA (T-DNA I and II) along with the adjacent genomic DNA flanking the 3' end of the insert (Figure 8).

There were no additional bands detected using the probes 1, 4, and 6. Based on the results presented in Figure 9, it was concluded that T-DNA sequences covered by probes 1, 4, and 6 reside at a single integration locus in MON 87705.

Probes 2 and 5

Conventional soybean DNA digested with *Nco* I (Figure 10, lanes 1 and 8) or *Spe* I (Figure 10, lanes 3 and 10) and hybridized with probes 2 and 5 (Figure 6) produced several hybridization signals. These hybridization signals result from the probes (Probes 2 and 5, Figure 6) hybridizing to endogenous sequences residing in the soybean genome and are not specific to the inserted DNA. These results were expected, because several genetic elements covered by probes 2 and 5 are native to the soybean genome. These signals, as expected, were produced in both MON 87705 and conventional soybean lanes, and, therefore, the bands are considered to be endogenous background.

Probe template spikes (Probes 2 and 5, Figure 6) generated from plasmid PV-GMPQ/HT4404 mixed with the conventional soybean DNA predigested with *Spe* I produced the expected bands at ~0.8 and ~1.0 kb (Figure 10, lanes 5-6) in addition to the endogenous background hybridization observed in the conventional soybean DNA (Figure 10, lane 10). Plasmid PV-GMPQ/HT4404 digested with a combination of *Xho* I and *Nco* I and mixed with conventional soybean DNA predigested with *Spe* I (Figure 10, lane 7) produced the expected size bands of ~3.2 and ~9.9 kb (refer to Figure 8) in addition to the endogenous background hybridization observed in the conventional soybean DNA (Figure 10, lane 10). These results indicate that the probes are hybridizing to their target sequences.

MON 87705 DNA digested with *Nco* I (Figure 10, lanes 2 and 9) produced two unique bands of ~4.0 and ~5.7 kb (Figure 10, lanes 1 and 8) in addition to the endogenous background hybridization observed in the conventional soybean DNA. The ~4.0 kb band is the expected size for the border segment containing the 5' end of the inserted DNA (T-DNA I) along with the adjacent genomic DNA flanking the 5' end of the insert (Figure 8). The ~5.7 kb band is the expected size for the border segment containing the 3' end of the inserted DNA (T-DNA I and T-DNA II) along with the adjacent genomic DNA flanking the 3' end of the insert (Figure 8).

MON 87705 DNA digested with *Spe* I (Figure 10, lanes 4 and 11) produced two unique bands of ~5.0 and ~11 kb (Figure 10, lanes 3 and 10) in addition to the endogenous background hybridization observed in the conventional soybean DNA. The ~11 kb segment is consistent with the expected band being greater than 8.1 kb and with the ~11 kb segment seen with probes 1, 4, and 6 (Figure 9, lanes 4 and 11). The ~11 kb band is the expected size for the border segment containing the 5' end of the inserted DNA (T-DNA I) along with the adjacent genomic DNA flanking the 5' end of the insert (Figure 8). The ~5.0 kb border segment contains the 3' end of the inserted DNA (T-DNA I and T-DNA II) along with the adjacent genomic DNA flanking the 3' end of the insert (Figure 8). There were no additional bands detected using probes 2 and 5. Based on the results presented in Figure 10, it was concluded that sequence covered by probes 2 and 5 resides at a single integration locus in MON 87705.

Probe 3

Conventional soybean DNA digested with *Nco* I (Figure 11, lanes 1 and 7) or *Spe* I (Figure 11, lanes 3 and 9) and hybridized with probe 3 (Figure 6) showed no detectable hybridization bands, as expected for the negative control.

Plasmid PV-GMPQ/HT4404 digested with a combination of *Xho* I and *Nco* I and mixed with conventional soybean DNA predigested with *Spe* I (Figure 11, lanes 5-6) produced the expected size band of ~3.2 kb (refer to Table 3). This hybridization indicates that the probe is hybridizing to its target sequence.

MON 87705 DNA digested with *Nco* I (Figure 11, lanes 2 and 8) produced the expected band of ~5.7 kb. The ~5.7 kb band is the expected size for the border segment containing the 3' end of the inserted DNA (T-DNA I and T-DNA II) along with the adjacent genomic DNA flanking the 3' end of the insert (Figure 8).

MON 87705 DNA digested with *Spe* I (Figure 11, lanes 4 and 10) produced the expected band of ~11 kb. The ~11 kb band is consistent with the expected band being greater than 8.1 kb and with the ~11 kb segment seen in Figure 9 and 10 (lanes 4 and 11). The ~11 kb band represents the border segment containing the 5' end of the inserted DNA (T-DNA I) along with the adjacent genomic DNA flanking the 5' end of the insert (Figure 8).

There were no additional bands detected using probe 3. Based on the results presented in Figure 11, it was concluded that sequence covered by probe 3 resides at a single detectable integration locus in MON 87705.

Taken together, the data presented in Figure 9, 10, and 11 indicate that a single copy of the T-DNA I and T-DNA II sequences integrated into the soybean genome at a single detectable site in MON 87705.

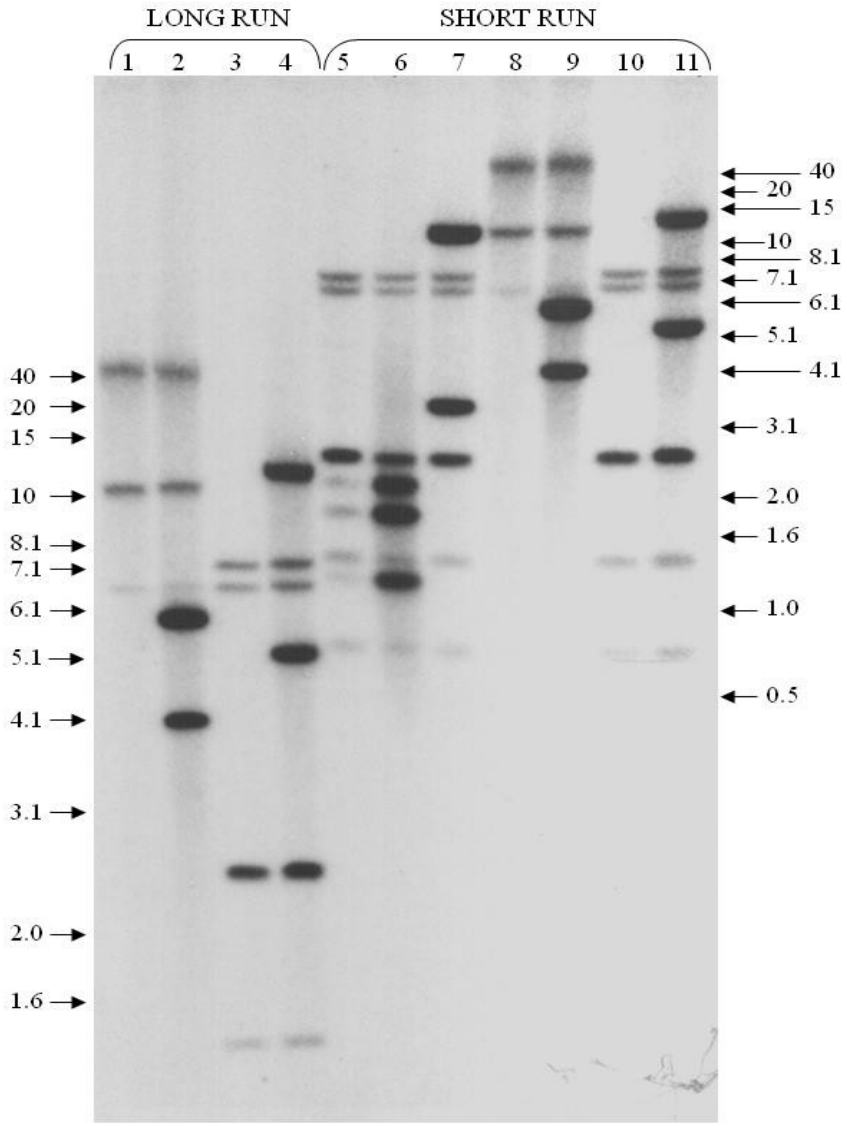


Figure 9. Southern Blot Analysis of MON 87705: Probes 1, 4 and 6

The blot was hybridized with ^{32}P-labeled probes that span a portion of T-DNA I and T-DNA II sequences (probes 1, 4, and 6, Figure 6). Each lane contains ~10 µg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane 1: Conventional soybean (*Nco* I)
2: MON 87705 (*Nco* I)
3: Conventional soybean (*Spe* I)
4: MON 87705 (*Spe* I)
5: Conventional soybean (*Spe* I) spiked with probe templates [~0.1 genomic equivalent]
6: Conventional soybean (*Spe* I) spiked with probe templates [~1 genomic equivalent]
7: Conventional soybean (*Spe* I) spiked with PV-GMPQ/HT4404 (*Xho* I/*Nco* I) [~1 genomic equivalent]
8: Conventional soybean (*Nco* I)
9: MON 87705 (*Nco* I)
10: Conventional soybean (*Spe* I)
11: MON 87705 (*Spe* I)

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

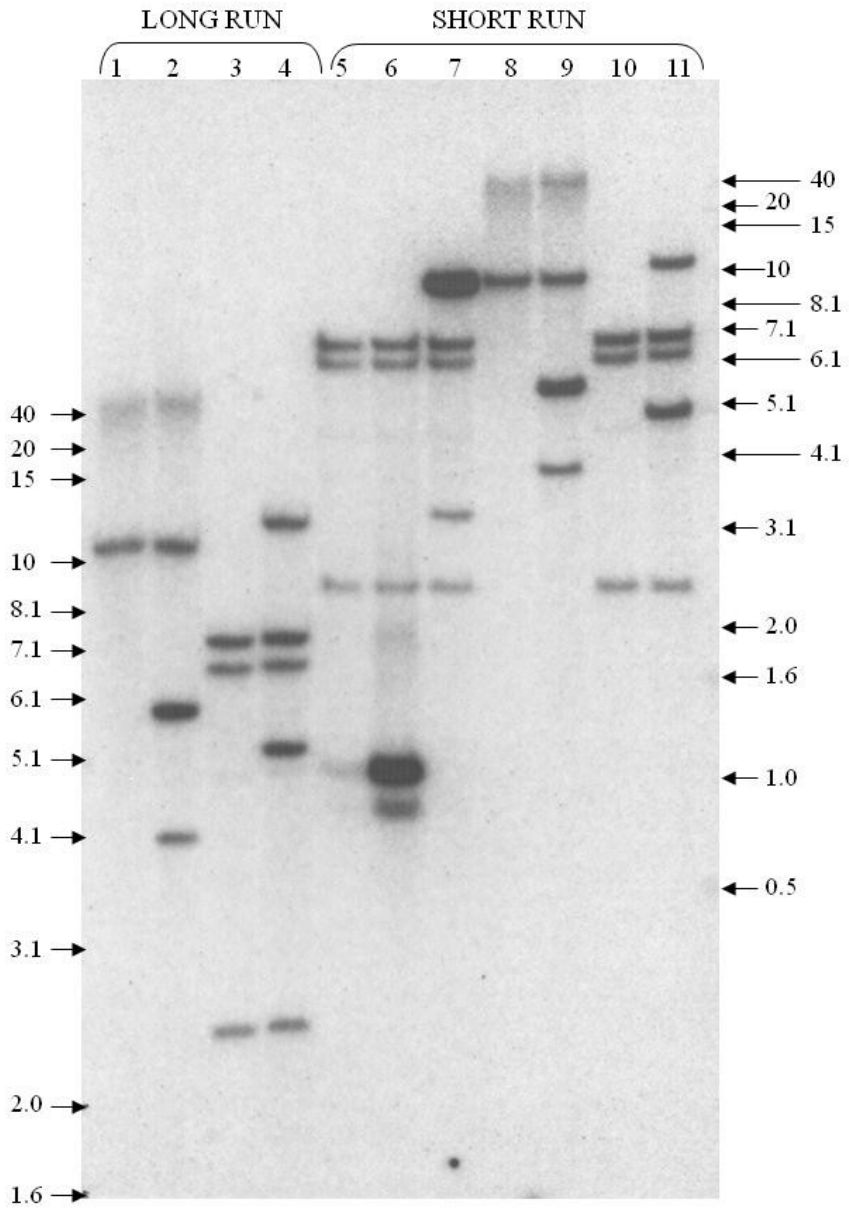


Figure 10. Southern Blot Analysis of MON 87705: Probes 2 and 5

The blot was hybridized with ^{32}P-labeled probes that span a portion of T-DNA I sequences (probes 2 and 5, Figure 6). Each lane contains ~10 µg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane 1: Conventional soybean (*Nco* I)
2: MON 87705 (*Nco* I)
3: Conventional soybean (*Spe* I)
4: MON 87705 (*Spe* I)
5: Conventional soybean (*Spe* I) spiked with probe templates [~0.1 genomic equivalent]
6: Conventional soybean (*Spe* I) spiked with probe templates [~1 genomic equivalent]
7: Conventional soybean (*Spe* I) spiked with PV-GMPQ/HT4404 (*Xho* I/*Nco* I) [~1 genomic equivalent]
8: Conventional soybean (*Nco* I)
9: MON 87705 (*Nco* I)
10: Conventional soybean (*Spe* I)
11: MON 87705 (*Spe* I)

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

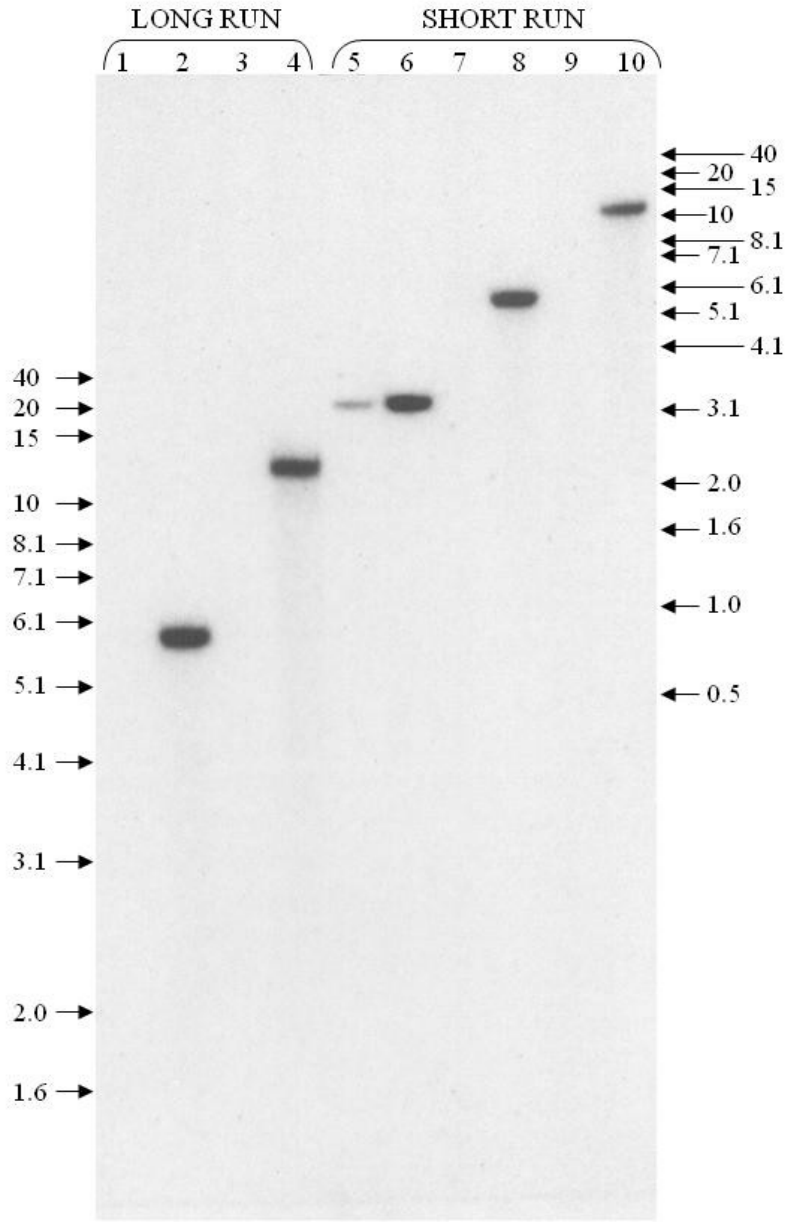


Figure 11. Southern Blot Analysis of MON 87705: Probe 3

The blot was hybridized with a ^{32}P-labeled probe that span a portion T-DNA I sequences (probe 3, Figure 6). Each lane contains ~10 µg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane 1: Conventional soybean (*Nco* I)
2: MON 87705 (*Nco* I)
3: Conventional soybean (*Spe* I)
4: MON 87705 (*Spe* I)
5: Conventional soybean (*Spe* I) spiked with PV-GMPQ/HT4404 (*Xho* I/*Nco* I) [~0.1 genomic equivalent]
6: Conventional soybean (*Spe* I) spiked with PV-GMPQ/HT4404 (*Xho* I/*Nco* I) [~1 genomic equivalent]
7: Conventional soybean (*Nco* I)
8: MON 87705 (*Nco* I)
9: Conventional soybean (*Spe* I)
10: MON 87705 (*Spe* I)

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

ii) Confirmation of the Absence of Plasmid PV-GMPQ/HT4404 Backbone

MON 87705 and conventional soybean genomic DNA were digested with the restriction enzymes *Nco* I or *Spe* I. Probe template spikes (probes 7-10, Figure 7) generated from plasmid PV-GMPQ/HT4404 were mixed with the predigested conventional soybean genomic DNA to serve as positive hybridization controls. Additionally, plasmid PV-GMPQ/HT4404 DNA previously digested with the combination of *Xho* I and *Nco* I was mixed with conventional soybean DNA digested with *Spe* I and loaded on the gel to serve as a positive hybridization control. The blots were hybridized with probes 7-10 (Figure 7) that covered the entire backbone sequence of PV-GMPQ/HT4404. If backbone sequences are present in MON 87705, then probing with backbone sequence should result in unique hybridizing bands. The results are shown in Figure 12 and 13.

MON 87705 DNA digested with either *Nco* I (Figure 12, lanes 2 and 9) or *Spe* I (Figure 12, lanes 4 and 11) showed no detectable hybridization signal, indicating that MON 87705 does not contain any detectable backbone sequence from the transformation vector PV-GMPQ/HT4404 that is covered by probes 7 and 9.

MON 87705 DNA digested with either *Nco* I (Figure 13, lanes 2 and 9) or *Spe* I (Figure 13, lanes 4 and 11) showed no detectable hybridization signal. These results in combination with Figure 12 indicate that MON 87705 does not contain any detectable backbone sequence from the transformation vector PV-GMPQ/HT4404.

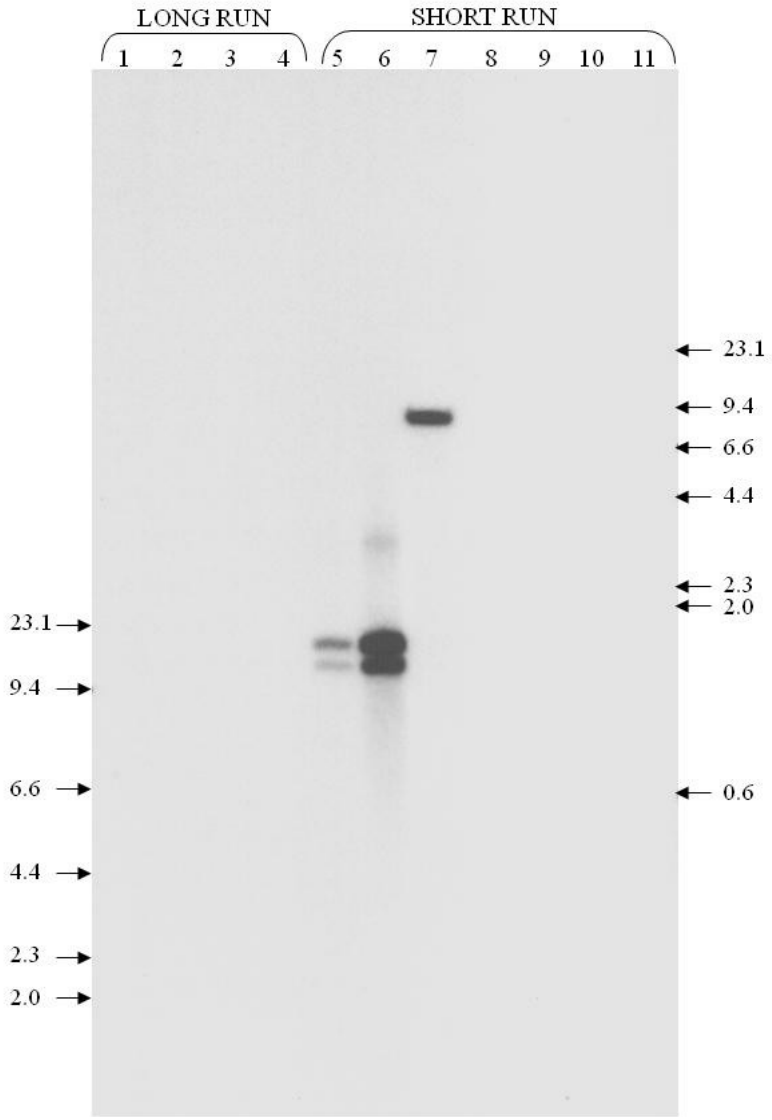


Figure 12. Southern Blot Analysis of MON 87705: PV-GMPQ/HT4404 Backbone Probes 7 and 9

The blot was hybridized with ^{32}P-labeled probes that span a portion of backbone sequences (probes 7 and 9, Figure 7) of plasmid PV-GMPQ/HT4404. Each lane contains ~10 µg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane 1: Conventional soybean (*Nco* I)
2: MON 87705 (*Nco* I)
3: Conventional soybean (*Spe* I)
4: MON 87705 (*Spe* I)
5: Conventional soybean (*Spe* I) spiked with probe templates [~0.1 genomic equivalent]
6: Conventional soybean (*Spe* I) spiked with probe templates [~1 genomic equivalent]
7: Conventional soybean (*Spe* I) spiked with PV-GMPQ/HT4404 (*Xho* I/*Nco* I) [~1 genomic equivalent]
8: Conventional soybean (*Nco* I)
9: MON 87705 (*Nco* I)
10: Conventional soybean (*Spe* I)
11: MON 87705 (*Spe* I)

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

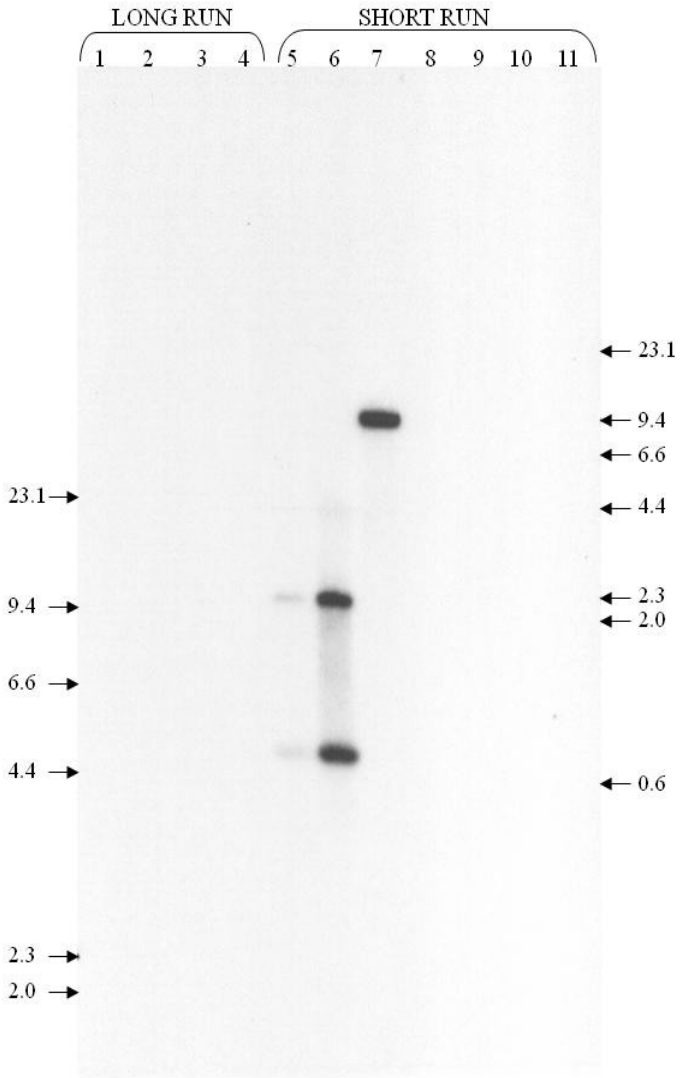


Figure 13. Southern Blot Analysis of MON 87705: PV-GMPQ/HT4404 Backbone Probes 8 and 10

The blot was hybridized with ^{32}P-labeled probes that span a portion of backbone sequences (probes 8 and 10, Figure 7) of plasmid PV-GMPQ/HT4404. Each lane contains ~10 µg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane 1: Conventional soybean (*Nco* I)
2: MON 87705 (*Nco* I)
3: Conventional soybean (*Spe* I)
4: MON 87705 (*Spe* I)
5: Conventional soybean (*Spe* I) spiked with probe templates [~0.1 genomic equivalent]
6: Conventional soybean (*Spe* I) spiked with probe templates [~1 genomic equivalent]
7: Conventional soybean (*Spe* I) spiked with PV-GMPQ/HT4404 (*Xho* I/*Nco* I) [~1 genomic equivalent]
8: Conventional soybean (*Nco* I)
9: MON 87705 (*Nco* I)
10: Conventional soybean (*Spe* I)
11: MON 87705 (*Spe* I)

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

iii) Organization and Sequence of the Insert in MON 87705

The organization of the elements within the MON 87705 insert was confirmed by DNA sequence analyses. The organization of the elements within the insert in MON 87705 was confirmed using PCR analysis to amplify six overlapping regions of DNA that span the entire length of the insert and the associated flanking genomic DNA. The amplified DNA fragments were subjected to DNA sequencing analyses. The insert in MON 87705 is 7251 base pairs and matches the sequence of PV-GMPQ/HT4404 as described in Table 2 and 4. The sequence analysis showed a 30 bp truncation at the 3' end of the *FATB1-A* antisense segment (Table 4) which did not have an impact on the ability of the suppression cassette to reduce levels of *FATB1-A* RNA and result in the intended fatty acid phenotype. The DNA sequence of the insert and the adjacent genomic regions is presented in Appendix 2 as confidential business information (CBI).

PCR and DNA sequence analyses were performed on genomic DNA extracted from MON 87705 and conventional soybean to demonstrate that the DNA sequences flanking the 5' and 3' ends of the insert in MON 87705 are native to the soybean genome and to examine the MON 87705 insertion site in conventional soybean (A3525). The results of the PCR analyses are shown in Figure 14. The control PCR containing no template DNA (Figure 14, lane 4) did not generate a PCR product, as expected. The reaction using the conventional soybean genomic DNA template (Figure 14, lane 2) generated a PCR product of ~3.7 kb band across the insertion site of MON 87705. As expected, a PCR product across the insert in MON 87705 (Figure 14, lane 3) was not generated in this analysis since the PCR conditions needed to generate a product of this size (13,349 bp) were not used.

The sequence comparison between the PCR product generated from the conventional soybean (A3525) and the sequence generated from the 5' and 3' flanking sequences of MON 87705 indicates there was a 36 bp deletion (bases 896-931) and a 2374 bp insertion just 5' to the MON 87705 insertion site. These 2374 bases are most likely from the 3' end of the flanking genomic DNA and were duplicated at the 5' end of the insertion site when T-DNA I and T-DNA II integrated into the genome, because of the following reasons: 1) there is a single nucleotide change detected in the duplicated 2374 base pairs at the 5' flanking sequence; and 2) there are 4 bases located at the 3' junction of the insert (10531-10534 bp, Appendix 2, CBI) that match conventional soybean (932-935 bp, Figure 14 of MSL 22384; see Figure 14), but these 4 bases are not present at the 5' end duplicated sequence. This analysis confirms that the genomic sequences flanking the insert in MON 87705 are native to the soybean genome and that a 36 base-pair deletion and a 2374 base pair duplication that contains a single base change occurred at the insertion site during integration of the T-DNA sequences. These molecular rearrangements presumably resulted from double-stranded break repair mechanisms in the plant during the *Agrobacterium*-mediated transformation process (Salomon and Puchta, 1998). Results confirm the presence and that the organization of the insert genetic elements in MON 87705 are as depicted in Table 4.

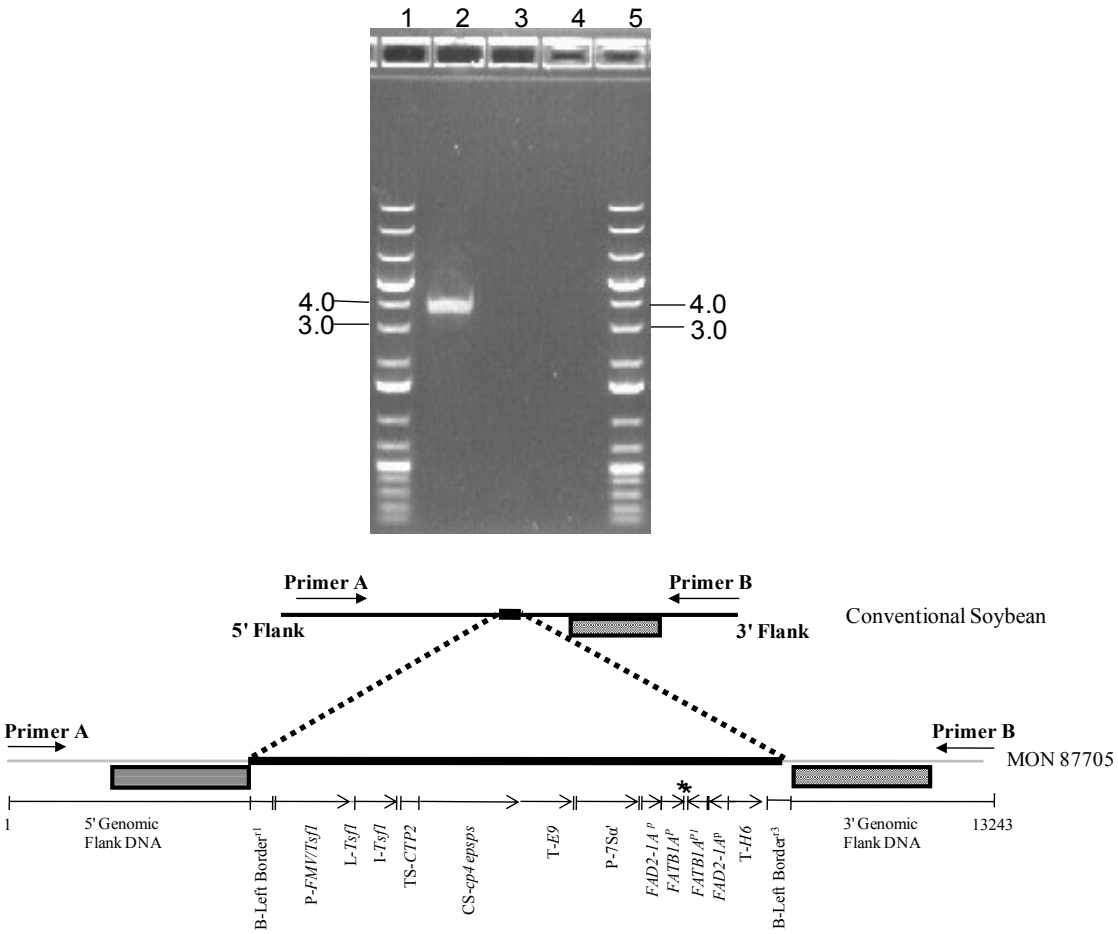


Figure 14. PCR Amplification of the MON 87705 Insertion Site in Conventional Soybean

The above figure is a depiction of the MON 87705 insertion site in conventional (upper panel) and MON 87705 soybean (lower panel). PCR amplification was performed using Primer A located in the 5' flanking sequence and Primer B located in the 3' flanking sequence of the insert in MON 87705 to verify that the flanking sequences are native to the soybean genome. Three to fifteen microliters of each of the PCR products were loaded on the gel. The box represents a 2374 bp direct repeat. The box represents that the 2374 bp repeat originated from the 3' flanking sequence and was duplicated at the 5' end of the insertion. Lane designations are as follows:

Lane 1: GeneRuler™ 1 kb Plus DNA Ladder
2: Conventional soybean
3: MON 87705
4: No template DNA control
5: GeneRuler™ 1 kb Plus DNA Ladder

⟶ Symbol denotes size of DNA, in base pairs, obtained from MW markers on gel.
* Symbol denotes sequences from Right Border and Left Border after integration into MON 87705

iv) Bioinformatics Assessment of Insert DNA Reading Frames

As part of a comprehensive safety assessment, bioinformatic analyses were performed to assess the potential of toxicity, allergenicity or biological activity of any putative peptides encoded by translation of reading frames 1 through 6 of the inserted DNA in MON 87705 (Figure 15). Translated sequences were compared to allergen (AD_2009), toxin (TOX_2009) and public domain (PRT_2009) sequence databases using the FASTA sequence comparison algorithm.

The FASTA sequence alignment tool was used to assess structural relatedness between the query sequences and any protein sequences in the AD_2009, TOX_2009, and PRT_2009 databases. Structural similarities shared between each putative polypeptide with each sequence in the database were examined. The extent of structural relatedness was evaluated by detailed visual inspection of the alignment, the calculated percent identity, and the *E*-score. Alignments having *E*-score less than 1×10^{-5} are deemed significant because they may reflect shared structure and function among sequences. In addition to structural similarity, each putative polypeptide was screened for short polypeptide matches using a pair-wise comparison algorithm. In these analyses, eight contiguous and identical amino acids were defined as immunologically relevant, where eight represents the typical minimum sequence length likely to represent an immunological epitope (Silvanovich et al., 2006) and evaluated against the AD_2009 database.

The results of the search comparisons showed that no relevant structural similarity to known allergens or toxins were observed for any of the putative polypeptides when compared to proteins in the allergen (AD_2009) or toxin (TOX_2009) databases. Furthermore, no short (eight amino acid) polypeptide matches were shared between any of the putative polypeptides and proteins in the allergen database.

When used as queries for a FASTA search of the PRT_2009 database, all frames yielded several alignments that displayed *E*-scores less than 1×10^{-5}. Two classes of alignments with frame 1 yielding *E*-scores of less than 1×10^{-5} were observed. One class, that yielded the lowest numerical *E*-score, was with an open reading frame (ORF) derived from the Figwort Mosaic Virus that aligned with sequence translated from the promoter for the CP4 EPSPS protein. The second class of alignments was with soybean acyl-acyl carrier protein (ACP) thioesterase. Neither of these classes of alignments is unexpected because the promoter for the CP4 EPSPS protein is partially derived from Figwort Mosaic Virus and soybean acyl-ACP thioesterase is the product of the *FATB* gene. Based on the insert, these two classes of alignments reflect expected conserved sequence, and do not indicate the potential for adverse biological activity.

Inspection of frames 2, 4, and 6 alignments revealed they were punctuated with numerous stop codons in the query sequence and required the insertion of numerous gaps, indicating a lack of true structural homology shared between the sequences. For those alignments with frame 3 yielding an *E*-score less than 1×10^{-5}, all were an identical 455 amino acid overlap displaying 100% identity with patent sequences described as being "5-enolpyruvylshikimate-3-phosphate synthase", CP4 EPSPS. While these alignments reflect conserved structure,

there is no indication that they reflect the potential for adverse biological activity as CP4 EPSPS protein is an intended translation product in MON 87705. For those alignments with frame 5 yielding an *E*-score less than 1×10^{-5}, all were to an identical 100 amino acid overlap displaying 82% identity with an acyl-ACP thioesterase or a presumptive acyl-ACP thioesterase. This alignment is not unexpected because the T-DNA contains segments of the *FATB1A* coding sequence on the forward and reverse complement strands. Based on the insert, these two classes of alignment reflect expected conserved sequence, and do not indicate the potential for adverse biological activity.

When combined, these data demonstrate the lack of relevant similarities between known allergens, toxins or other biologically active proteins for putative peptides derived from all six reading frames from the entire inserted DNA sequence of MON 87705. As mentioned, RNA-based suppression of *FATB* and *FAD2* soybean genes in MON 87705 is mediated by dsRNA molecules (Section 1). The assembled gene transcript has an inverted repeat that produces dsRNA that, via the RNAi pathway, suppresses endogenous *FATB* and *FAD2* genes, thereby producing the desired fatty acid phenotype of decreased saturate, increased oleic and decreased linoleic fatty acid composition in the oil. As a result, in the unlikely event that a translation product was derived from reading frames 1 to 6, which includes the suppression cassette, these putative polypeptides are not expected to possess functional cross-reactivity with known allergenic proteins or be toxic or display adverse biological activity.

v) Insert Junction Open Reading Frame Bioinformatics Analysis

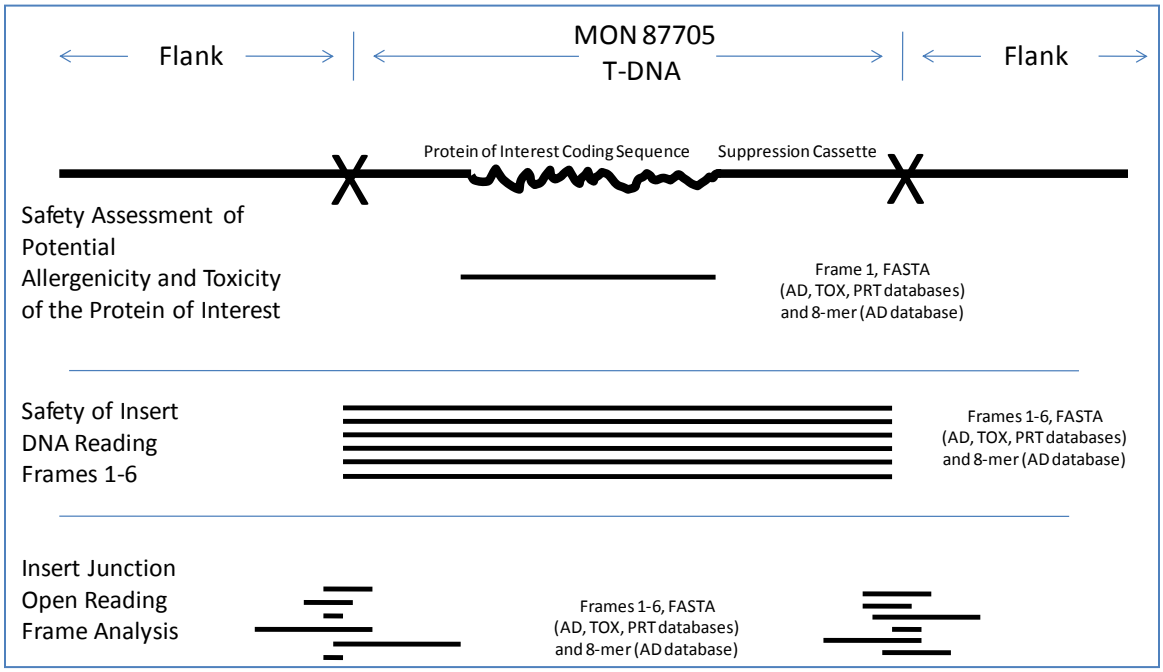
While there was no indication of additional polypeptides produced from the MON 87705 insert other than the CP4 EPSPS protein, analyses of putative polypeptides encoded by DNA spanning the 5′ and 3′ insert junctions of the MON 87705 inserted DNA were performed using a bioinformatic comparison strategy (Figure 15). The purpose of the assessment is to evaluate the potential for novel open reading frames (ORFs) that may have homology to known allergens and toxins. Sequences spanning the 5′ soybean genomic DNA-inserted DNA junction and the 3′ inserted DNA-soybean genomic DNA junction were translated from stop codon (TGA, TAG, TAA) to stop codon in all six reading frames. The resulting putative polypeptides from each reading frame, that were eight amino acids or greater in length, were compared to allergen (AD_2009), toxin (TOX_2009), and public domain (PRT_2009) database sequences using bioinformatic tools.

The FASTA sequence alignment tool was used to assess structural relatedness between the query sequences and protein sequences in the AD_2009, TOX_2009, and PRT_2009 databases. Structural similarities shared between each putative polypeptide with each sequence in the database were examined. The extent of structural relatedness was evaluated by detailed visual inspection of the alignment, the calculated percent identity, and the *E*-score. In addition to structural similarity, each putative polypeptide was screened for short polypeptide matches using a pair-wise comparison algorithm. In these analyses, eight contiguous and identical amino acids were defined as immunologically relevant, where eight

represents the typical minimum sequence length likely to represent an immunological epitope, and evaluated against the AD_2009 database.

No biologically relevant structural similarity to known allergens, toxins, or other biologically active proteins was observed for any of the putative polypeptides. Furthermore, no short (eight amino acid) polypeptide matches were shared between any of the putative polypeptides and proteins in the allergen database. As a result, in the unlikely event that a translation product was derived from DNA spanning the 5′ and 3′ insert junctions of MON 87705, these putative polypeptides are not expected to possess functional cross-reactivity with known allergenic proteins or be toxic or display adverse biological activity.

A conservative bioinformatic assessment of potential allergenicity, toxicity and adverse biological activity for putative polypeptides that span the 5' and 3' insert junctions or were derived from the entire insert was conducted for MON 87705. The data generated from these analyses confirm that even in the highly unlikely occurrence that a translation product was derived from frames 1 to 6 for the insert DNA, which includes the suppression cassette, or the insert junctions; they would not share a sufficient degree of sequence similarity with other proteins to indicate they would be potentially allergenic, toxic, or have other safety implications. Furthermore, no short (eight amino acid) polypeptide matches were shared between any of the putative polypeptides and proteins in the allergen database. Therefore, there is no evidence for concern regarding health implications of putative polypeptides for MON 87705.



AD= AD_2009; 8-mer = the eight amino acid sliding window search

Figure 15. Schematic Summary of MON 87705 Bioinformatic Analyses

d) Derivation of Line and Generational Stability

i) Southern Blot Analyses of MON 87705 Across Multiple Generations

In order to demonstrate the stability of the T-DNA I and T-DNA II insert in MON 87705 in multiple generations, Southern blot analyses were performed using DNA obtained from multiple generations of the MON 87705 breeding history. For reference, the breeding history of MON 87705 is presented in Figure 16. The specific generations tested are indicated in the legend of Figure 16. DNA samples from R_3, R_4, R_5, and R_6 generations of MON 87705 (refer to Figure 16) were digested with *Nco* I and were expected to release two border segments with the expected sizes of 4.0 and 5.7 kb (Figure 8). The detected hybridization bands in R_4, R_5, and R_6 generations are compared to the fully characterized MON 87705 R_3 generation to evaluate stability. Any instability associated with the insert would be detected as faint novel bands within the fingerprint on the Southern blot. The blot was hybridized simultaneously with two radiolabeled probes that cover both border segments generated by the digest (Probes 1 and 6, Figure 6). This blot has two of the same positive hybridization controls (Probes 1 and 6, Figure 6) as described in Section 2.3 c i. The result of this analysis is shown in Figure 17.

Conventional soybean DNA digested with *Nco* I and hybridized with probes 1 and 6 (Figure 17, lane 4) showed hybridization bands. These hybridization signals result from the probes hybridizing to endogenous targets residing in the soybean genome and are not specific to the inserted DNA.

Probe templates spikes (Probes 1 and 6, Figure 6) generated from plasmid PV-GMPQ/HT4404 and mixed with conventional soybean DNA predigested with *Nco* I (Figure 17, lanes 1 and 2) produced the expected size bands at ~1.8 and ~1.1 kb. The detection of the probe template positive hybridization controls demonstrates that both probes were hybridizing to the target DNA. Plasmid PV-GMPQ/HT4404 digested with a combination of *Xho* I and *Nco* I and mixed with conventional soybean DNA predigested with *Nco* I (Figure 17, lane 3) produced the expected size band at ~9.9 kb, which indicates that the probes are hybridizing to their corresponding sequence in the transformation vector. This expected band at ~9.9 kb migrated together with an endogenous hybridization signal observed in Figure 17, lane 4.

Digestion of MON 87705 from multiple generations (refer to Breeding History of MON 87705, Figure 16) with restriction enzyme *Nco* I produced two bands at ~4.0 and ~5.7 kb (Figure 17, lanes 5-8) in addition to the endogenous background hybridization observed in the conventional soybean DNA (Figure 17, lane 4). The ~4.0 kb band is the expected size for the border segment containing the 5' end of the inserted DNA (T-DNA I) along with the adjacent genomic DNA flanking the 5' end of the insert (Figure 8). The ~5.7 kb band is the expected size for the border segment containing the 3' end of the inserted DNA (T-DNA I and T-DNA II) along with the adjacent genomic DNA flanking the 3' end of the insert (Figure 8). However, the migration of this segment appears slightly lower than indicated by

the molecular weight marker most likely due to differences in salt concentrations between the samples and the marker. This restriction pattern is the same as the restriction pattern observed in the Southern blot analysis of the R3 generation shown in Figure 9 (lanes 2 and 9).

There were no additional unexpected bands detected, indicating that the single copy of T-DNA I and T-DNA II in MON 87705 is stably maintained in the selected generations.

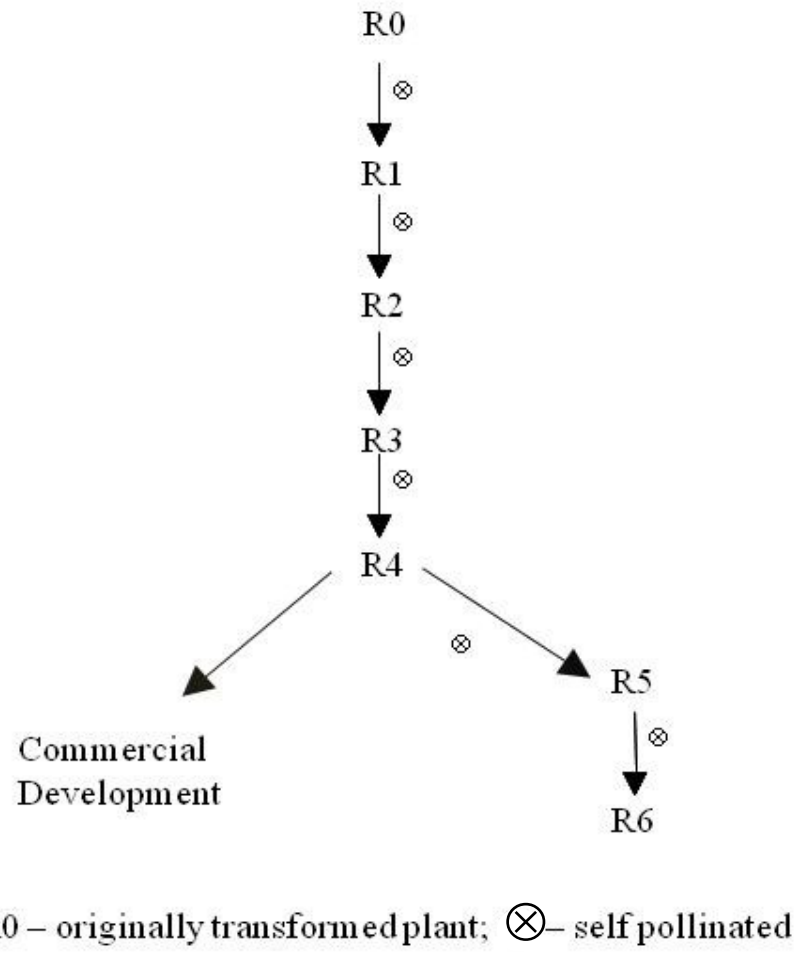


Figure 16. Breeding History of MON 87705

All generations were self pollinated (⊗). The R_3 generation was used for the molecular analyses of MON 87705 reported in Figure 9 through 13 and is referred to as MON 87705 in all Southern blot figures. The R_3, R_4, R_5, and R_6 generations were used for analyzing the stability of the insert and CP4 EPSPS protein expression in multiple generations. R_5 was used for seed and forage compositional analysis, and multiple tissue CP4 EPSPS protein expression ELISA analysis. R_6 was used for processed fraction compositional analysis.

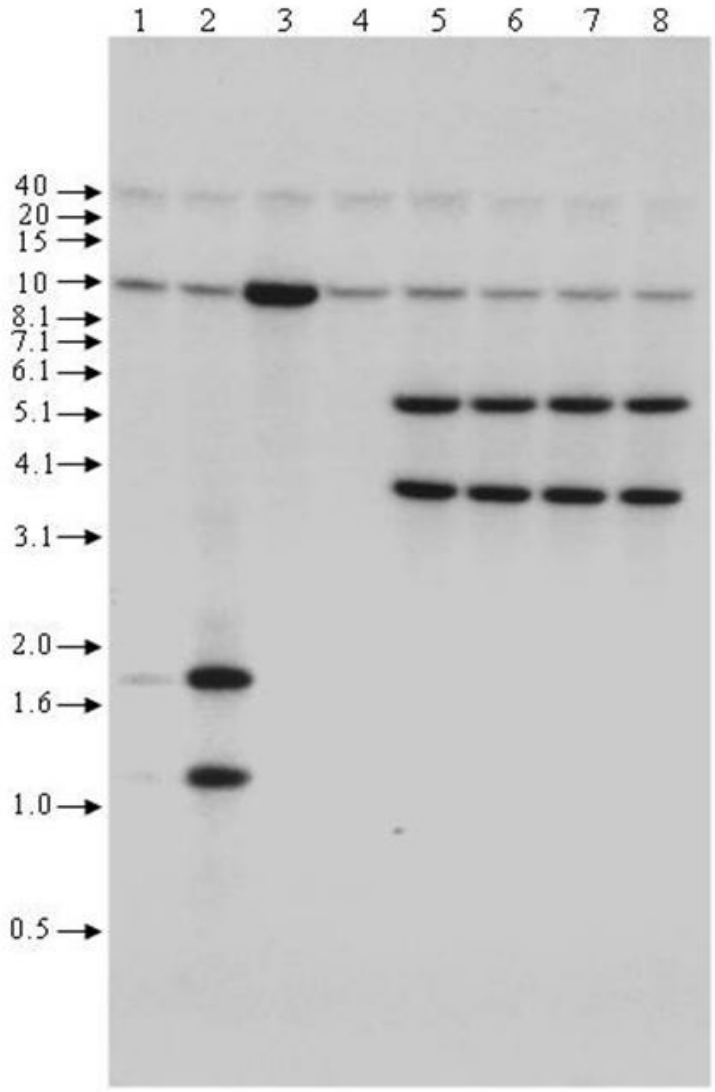


Figure 17. Generational Stability of MON 87705: Probes 1 and 6

The blot was hybridized with ^{32}P-labeled probes that spanned a portion of T-DNA I and T-DNA II sequences (probes 1 and 6, Figure 6). Each lane contains ~ 10 μg of digested genomic DNA isolated from leaf tissue. The breeding history of MON 87705 is illustrated in Figure 16. Lane designations are as follows:

Lane 1: Conventional soybean (*Nco* I) spiked with probe templates [~0.1 genomic equivalent]
2: Conventional soybean (*Nco* I) spiked with probe templates [~1 genomic equivalent]
3: Conventional soybean (*Nco* I) spiked with PV-GMPQ/HT4404 (*Xho* I/*Nco* I) [~1 genomic equivalent]
4: Conventional soybean (*Nco* I)
5: MON 87705 [R_3, (*Nco* I)]
6: MON 87705 [R_4, (*Nco* I)]
7: MON 87705 [R_5, (*Nco* I)]
8: MON 87705 [R_6, (*Nco* I)]

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

ii) Inheritance of the Genetic Insert in MON 87705

During development of MON 87705 segregation data were recorded to assess the heritability and stability of the coding sequences present in MON 87705. Chi-square analysis was performed over several generations to confirm the segregation and stability of the MON 87705 insertion. The Chi-square analysis is based on testing the observed segregation ratio to the expected segregation ratio according to Mendelian principles.

The MON 87705 breeding path from which segregation data were generated is described in Figure 18. The transformed R_0 plant was self-pollinated to produce R_1 seed. From the R_1 segregating population, an individual plant (#90, designated MON 87705) homozygous for a single copy of the *H6* 3'UTR was identified via Invader® (Third Wave Technologies, Inc.) and Southern blot analysis.

The selected R_1 MON 87705 plant was self-pollinated to give rise to a population of R_2 plants that were repeatedly self-pollinated through the R_4 generation. At each generation, the fixed homozygous plants were tested for the expected segregation pattern of 1:0 (positive: negative) for the *H6* 3'UTR using Invader analysis.

At the R_4 generation, homozygous MON 87705 plants were bred via traditional breeding with a soybean variety that did not contain the *H6* 3'UTR to produce F_1 hemizygous seed. The resulting F_1 plants were then self-pollinated to produce F_2 seed. The heritability and stability of the coding sequences present in MON 87705 were assessed from plants of the F_2, F_3, F_4, and F_5 generations. At each of these generations, the plants were tested for the presence of the *H6* 3'UTR by Invader analysis, and hemizygous positive plants were then selected and self-pollinated to produce seed of the next generation.

A Chi-square (χ^2) analysis was used to compare the observed segregation ratios to the expected ratios according to Mendelian principles. The Chi-square value was calculated as:

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

where o = observed frequency of the genotype and e = expected frequency of the genotype. The level of statistical significance was predetermined to be 5% ($p<0.05$).

The results of the χ^2 analysis of the segregating progeny of MON 87705 are presented in Table 5. The χ^2 values in the F_2 generation indicated no statistically significant difference between the observed and expected 1:2:1 (homozygous positive:hemizygous positive:homozygous negative) segregation ratio. The χ^2 value in the F_3 generation indicated a statistically significant difference between the observed and expected 1:2:1 segregation ratio. However, there were ten plants out of 91 total plants tested in the assay in which the zygosity could not be determined by Invader analysis. These missing data combined with a relatively small sample size (n=81) of tested plants may have skewed the segregation ratio. This caused the results of the analysis to be inconclusive and the data from the F_3 generation could not be used to accurately assess the heritability and stability of the coding sequences

present in MON 87705. Therefore, the F_4 and F_5 generations were tested using larger sample sizes to further assess the heritability and stability of the inserted coding sequences. The χ^2 values in the F_4 and F_5 generations indicated no statistically significant differences between the observed and expected 1:2:1 segregation ratios. Considering the data from three generations (F_2, F_4, and F_5), the results support the conclusion that the coding sequences present in MON 87705 reside at a single locus within the soybean genome and are inherited according to Mendelian inheritance principles. These results are also consistent with the molecular characterization data that indicate a single genomic insertion site in MON 87705 containing the genetic elements responsible for the improved fatty acid profile and glyphosate tolerance (Table 5).

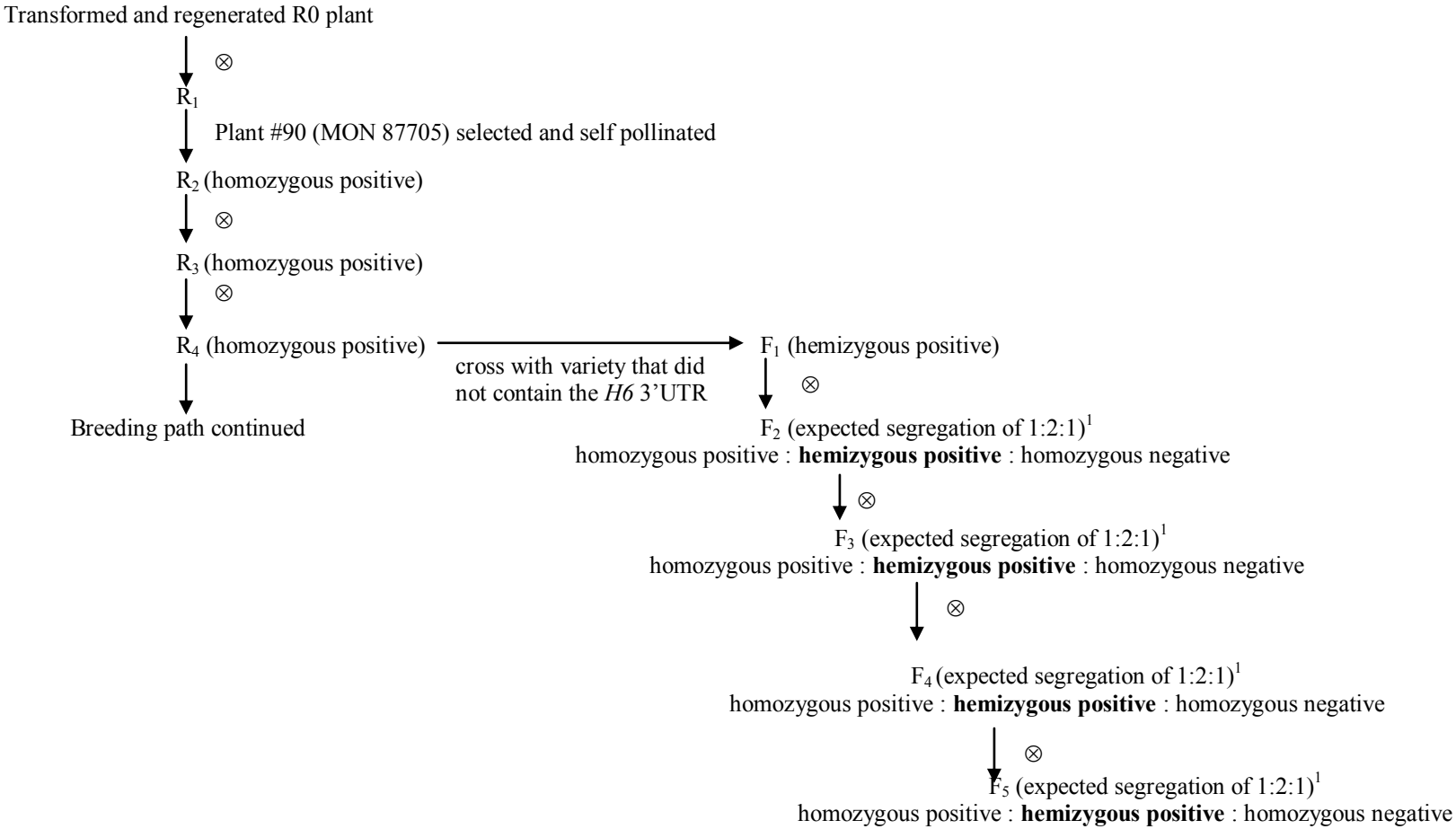


Figure 18. Breeding Path for Generating Segregation Data for MON 87705

[1] Chi-square analysis conducted on segregation data from the F_2, F_3, F_4 and F_5 generations.

Table 5. Segregation of the H6 3'UTR Gene During the Development of MON 87705

					1:2:1 Segregation				
Generation[1]	Total Plants Tested[2]	Observed # Plants Homozygous Positive	Observed # Plants Hemizygous Positive	Observed # Plants Homozygous Negative	Expected # Plants Homozygous Positive	Expected # Plants Hemizygous Positive	Expected # Plants Homozygous Negative	χ^2	Probability
F_2	4197	1009	2091	1097	1049.25	2098.5	1049.25	3.7	0.1538
F_3	81	30	35	16	20.25	40.5	20.25	6.3	0.0421
F_4	266	68	126	72	66.5	133	66.5	0.9	0.6514
F_5	175	44	88	43	43.75	87.5	43.75	0.0	0.9915

[1] F_2 progeny were from the cross of MON 87705 homozygous positive for the *H6* 3'UTR with a soybean variety that did not contain the *H6* 3'UTR. F_3, F_4, and F_5 progeny were from self-pollinated plants of the previous generation that were hemizygous positive for the *H6* 3'UTR.
[2] Plants were tested for the presence of the *H6* 3'UTR by Invader analysis. "Total plants" refers to the total number of plants in which zygosity could be determined using the assay.

2.4 Antibiotic Resistance Marker Genes

No genes that encode resistance to an antibiotic marker were inserted into the soybean genome during development of MON 87705. Molecular characterization data presented demonstrate the absence of the *aad*A antibiotic resistant marker in MON 87705.

a) Clinical / Veterinary Importance

Not applicable.

b) Viability

Not applicable.

c) Presence in Food

Not applicable.

2.5 Characterisation and Safety of MON 87705 Expression Products

As described in Section 2.3, the MON 87705 insert contains a *cp4 epsps* expression cassette and a *FAD2-1A/FATB1-A* suppression cassette. The *FAD2-1A/FATB1-A* suppression cassette encodes for dsRNA, and it is extremely unlikely to encode for a protein.

a) Safety of RNA-based Suppression Technology

Given the extensive history of safe consumption of nucleic acids (Burnside et al., 2008; Heisel et al., 2008; Ivashuta et al., 2009; Reddy et al., 2009; Zhou et al., 2009), the RNA-based suppression components of MON 87705 were excluded from traditional toxicity and allergenicity assessments utilized for introduced proteins. RNA-based suppression of *FATB* and *FAD2* soybean genes in MON 87705 is mediated by dsRNA molecules. The assembled gene transcript in MON 87705 has an inverted repeat that produces dsRNA that, via the RNAi pathway, suppresses endogenous *FATB* and *FAD2* genes, thereby producing the desired fatty acid phenotype of decreased saturates, increased oleic acid and decreased linoleic acid in the oil. Double stranded RNAs are commonly found in eukaryotes, including plants, for endogenous gene suppression and are composed of nucleic acids (Siomi and Siomi, 2009). Nucleic acids have a long history of safe consumption and are considered GRAS by the U.S. FDA (FDA, 1992). There is no evidence to suggest dietary consumption of RNA is associated with mammalian toxicity or allergenicity. Several biotechnology-derived plant products previously reviewed by the U.S. FDA and deregulated or approved by APHIS, as well as several international regulatory authorities, were developed using RNA-based suppression mechanisms, including virus-resistant papaya, high oleic soybean, virus resistant squash, FLAVR SAVR tomatoes, plum pox virus resistant plum trees, and high starch potato (ANZFA, 2000; CFIA, 2001; CFIA, 2009; EFSA, 2006; FDA, 1994; FDA, 1996; FDA, 1997a; FDA, 1997b; FDA, 2009a; FDA, 2009b; HC, 1999a; HC, 1999b; HC, 2002; MOE, 1999; USDA-APHIS, 1992; USDA-APHIS, 1996a; USDA-APHIS, 1996b; USDA-APHIS, 1997; USDA-APHIS, 2007).

Analysis of the MON 87705 DNA segments encoding dsRNA showed production of a protein from the dsRNA encoded by the insert in MON 87705 is highly unlikely. Based on this information, the inserted DNA and resulting dsRNA are safe and unlikely to produce a protein or polypeptide. Furthermore, even in the highly unlikely event a protein could be produced from the suppression cassette, bioinformatics analyses demonstrate the lack of relevant similarities between known allergens, toxins or other biologically active proteins for all putative peptides derived from all six reading frames from the entire inserted DNA sequence of MON 87705, including the sequences in the suppression cassette.

Based on the ubiquitous nature of the RNA-based suppression mechanism utilizing dsRNA, the history of safe consumption of RNA and the apparent lack of toxicity or allergenicity of dietary RNA, the RNA-based suppression technology used in MON 87705 poses no novel risks from a food or feed perspective. Thus, additional safety data on the expression product encoded by the *FATB1-A/ FAD2-1A* suppression cassette is not necessary.

b) Characterization of the CP4 EPSPS Protein from MON 87705

MON 87705 was developed to selectively down-regulate two key enzymes, FATB and FAD2, involved in the soybean seed fatty acid biosynthetic pathway. As a result, MON 87705 soybean oil contains lower levels of saturated fatty acids (6% vs. 15% of total fatty acids [FA]) and higher levels of monounsaturated 18:1 oleic acid (76% vs. 23% of total FA), with an associated decrease in polyunsaturated 18:2 linoleic acid levels (10% vs. 53% of total FA) relative to commodity soybean oil. MON 87705 also contains the 5-enolpyruvylshikimate-3-phosphate synthase gene derived from *Agrobacterium sp*. strain CP4 (*cp4 epsps*), which encodes the CP4 EPSPS protein.

i) CP4 EPSPS Identity and Equivalence

The safety assessment of crops derived through biotechnology includes characterization of the introduced protein produced from the inserted DNA, confirmation of its functional and physicochemical properties, and confirmation of the safety of the protein. The level of CP4 EPSPS protein produced in MON 87705 is too low to allow purification of sufficient quantities for use in subsequent safety assessment studies. Therefore, it is necessary to produce the protein in high-expressing recombinant host systems (such as bacteria) in order to obtain sufficient quantities of the CP4 EPSPS protein. CP4 EPSPS protein was produced in *E. coli*, and subsequently purified and characterized. A small quantity of the CP4 EPSPS protein was also purified from harvested MON 87705 seed. The equivalence of the physicochemical characteristics and functional activity between the MON 87705-produced and *E. coli*-produced CP4 EPSPS proteins was confirmed by a panel of analytical techniques, including: 1) N-terminal sequence analysis, 2) matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to generate a tryptic peptide map of MON 87705-produced CP4 EPSPS, 3) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to establish equivalence of the apparent molecular weight between MON 87705-produced CP4 EPSPS and the *E. coli*-produced CP4 EPSPS protein reference standard, 4) western blot analysis to establish immunoreactive equivalence between MON 87705-produced CP4 EPSPS and the *E. coli*-produced CP4 EPSPS protein reference standard using an anti-CP4 EPSPS antibody, 5) CP4 EPSPS enzymatic activity analysis to demonstrate functional equivalence between MON 87705-produced CP4 EPSPS and the *E. coli*-produced CP4 EPSPS protein reference standard, and 6) glycosylation analysis to establish equivalent glycosylation status between MON 87705-produced CP4 EPSPS and *E. coli*-produced CP4 EPSPS protein reference standard.

A comparison of the MON 87705-produced CP4 EPSPS to the *E. coli*-produced CP4 EPSPS reference protein standard confirmed the identity of the MON 87705-produced CP4 EPSPS protein and established the equivalence of the plant produced protein to the *E. coli*-produced CP4 EPSPS protein reference standard. The molecular weight of the MON 87705- and *E. coli*-produced CP4 EPSPS proteins was estimated by SDS-PAGE. The SDS-PAGE demonstrated that the proteins migrated to the same position on the gel, indicating that the CP4 EPSPS proteins from both sources are equivalent in their molecular weight. The electrophoretic mobility and immunoreactive properties of the MON 87705-produced CP4 EPSPS protein were shown to be equivalent to those of the *E. coli*-produced CP4 EPSPS protein reference standard by immunoblot. The N-terminus of the MON 87705-produced

CP4 EPSPS protein was consistent with the predicted amino acid sequence translated from the CP4 EPSPS coding sequence, and the MALDI-TOF mass spectrometry analysis yielded peptide masses consistent with the expected peptide masses from the translated CP4 EPSPS coding sequence. The MON 87705-produced CP4 EPSPS and the *E. coli*-produced CP4 EPSPS protein reference standard were also found to be equivalent based on the functional activities and the lack of glycosylation. Taken together, these data provide a detailed characterization of the CP4 EPSPS protein isolated from MON 87705 and established its equivalence to the *E. coli*-produced CP4 EPSPS protein reference standard. Furthermore, since CP4 EPSPS proteins isolated from other Roundup Ready crops (Roundup Ready soybean [40-3-2], Roundup Ready 2 Yield soybean [MON 89788], Roundup Ready Corn 2 [NK603], Roundup Ready canola [GT73], Roundup Ready sugar beet [H7-1], and Roundup Ready Flex cotton [MON 88913]) have previously established equivalence to the *E. coli*-produced protein standard, by inference, the MON 87705-derived CP4 EPSPS protein is expected to possess equivalent biochemical and physiological characteristics with the CP4 EPSPS proteins expressed in other Roundup Ready crops, all of which have previously been the subject of consultations with U.S. FDA.

N-terminal Sequence Analysis

N-terminal sequencing of the first 10 amino acids performed on MON 87705-produced CP4 EPSPS protein resulted in the sequence expected for the CP4 EPSPS protein (Figure 19) with the exception of the N-terminal methionine, which was not detected. This result is expected as removal of the N-terminal methionine, catalyzed by methionine aminopeptidase, is a common modification that occurs co-translationally before completion of the nascent protein chain and has no effect on protein structure or activity (Arfin and Bradshaw, 1988; Polevoda and Sherman, 2000; Schmidt et al., 1992). The N-terminal sequence information, therefore, confirms the identity of the CP4 EPSPS protein isolated from MON 87705 and the intactness of its N-terminus.

Amino acid residue # from the N-terminus	→	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Expected Sequence[1]	→	M	L	H	G	A	S	S	R	P	A	T
		|	|	|	|	|	|	|	|	|	|	|
Experimental Sequence[2]	→	-	L	H	G	A	S	S	R	P	A	T

[1] The expected amino acid sequence of the N-terminus of the CP4 EPSPS protein was deduced from the *cp4 epsps* gene present in MON 87705. The experimental sequence obtained from the MON 87705-produced CP4 EPSPS was compared to the expected sequence.
[2] The single letter IUPAC-IUB amino acid code is **M**, methionine; **L**, Leucine; **H,** histidine; **G**, glycine; **A**, alanine, **S**, serine; **R**, Arginine; **P**, proline; **T**, threonine; and (-) indicates the **M** residue was not observed.

Figure 19. N-Terminal Sequence of the MON 87705-Produced CP4 EPSPS

MALDI-TOF Mass Spectrum Analysis

The identity of the MON 87705-produced CP4 EPSPS protein was further confirmed by tryptic peptide mass mapping analysis using MALDI-TOF MS. In general, protein identification made by peptide mapping is considered to be reliable if the measured coverage of the sequence is 15% or higher with a minimum of five matched peptides (Jensen et al., 1997). Observed tryptic peptides were considered a match to the expected tryptic mass when differences in molecular weight of less than one Dalton (Da) were found between the observed and predicted fragment masses. Such matches were made without consideration for potential natural amino acid modifications such as glycosylation. The protein sample was digested with trypsin and the masses of the tryptic peptides were measured.

There were 30 unique peptide fragments identified that corresponded to the expected masses of the CP4 EPSPS trypsin-digested peptides (Table 9). The identified masses were used to assemble a coverage map indicating the matched peptide sequences for the entire CP4 EPSPS protein (Figure 20), resulting in approximately 80% (362 out of 455 amino acids) coverage of the total protein. This analysis confirmed the identity of the MON 87705-produced CP4 EPSPS protein.

CP4 EPSPS Molecular Weight Equivalence

The equivalence in apparent molecular weight of the purified MON 87705- and the *E. coli*-produced CP4 EPSPS proteins was demonstrated using SDS-PAGE (Figure 21). The MON 87705-produced CP4 EPSPS protein had an estimated molecular weight of 44.6 kDa (Table 6) and migrated to the same position on the SDS PAGE gel as the *E. coli*-produced protein standard analyzed concurrently (Figure 21). The apparent molecular weight of the *E. coli*-produced CP4 EPSPS reference protein in this gel is 43.8 kDa. The difference between the theoretical and the estimated molecular weights between the MON 87705-produced CP4 EPSPS protein and the apparent molecular weight of the *E. coli*-produced CP4 EPSPS

proteins in the gel is 1.8% (Table 6). Because the experimentally determined difference in apparent molecular weights met the preset acceptance criteria (+/-10% difference) the MON 87705- and *E. coli*-produced CP4 EPSPS proteins were determined to have equivalent apparent molecular weights.

Table 6. Molecular Weight Difference between the Full-Length MON 87705-Produced and *E.coli*-Produced CP4 EPSPS Proteins Based on SDS-PAGE

Molecular Weight of Full-Length MON 87705-Produced CP4 EPSPS Protein	**Molecular Weight of *E.coli*-Produced CP4 EPSPS Protein**	**Percent Difference from *E. coli*-Produced CP4 EPSPS Protein [1]**
44.6 kDa	43.8 kDa	1.8 %

[1] Percent difference was calculated as follows: $\frac{44.6 - 43.8}{44.6} \times 100\% = 1.8\%$

CP4 EPSPS Immunoreactivity Equivalence

A western blot analysis using goat anti-CP4 EPSPS serum was conducted to determine the relative immunoreactivity of the MON 87705-produced CP4 EPSPS protein and the *E. coli*-produced CP4 EPSPS reference standard. The results demonstrated that the anti-CP4-EPSPS antibody recognized the MON 87705-produced CP4 EPSPS protein that migrated to a similar position as the *E. coli*-produced CP4 EPSPS protein reference standard (Figure 22). Furthermore, the immunoreactive signal increased with increasing levels of CP4 EPSPS protein loaded. The observed immunoreactivities between the MON 87705- and *E. coli*-produced proteins were similar based on densitometric analysis of the western blot.

Densitometric analysis was conducted to compare the immunoreactivity of MON 87705- and *E. coli*-produced CP4 EPSPS proteins. The relative immunoreactivity of each protein with CP4 EPSPS-specific antibody was determined by averaging intensity values of six protein bands corresponding to the MON 87705-produced CP4 EPSPS and six bands corresponding to the *E. coli*-produced CP4 EPSPS (Table 7). The averaged band intensity of the signal from the MON 87705-produced CP4 EPSPS lanes was 10.5% more than the averaged band intensity of the signal from the *E. coli*-produced CP4 EPSPS lanes. The observed difference was within the preset acceptance criteria for immunoreactivity (±30% difference). Thus, the immunoblot analysis established identity of the MON 87705-produced CP4 EPSPS and demonstrated that the MON 87705- and *E. coli*-produced CP4 EPSPS have equivalent immunoreactivity with CP4 EPSPS-specific antibody.

Table 7. Comparison of Immunoreactive Signals Between Full Length MON 87705-Produced and *E. coli*-Produced CP4 EPSPS Proteins

Sample	*Gel lane*	*Amount (ng)*	*Density (OD x mm²)*	*Average Density*[1]	*Percent Difference*[2] *(%)*	*Average Difference*[3] *(%)*
E. coli CP4 EPSPS	2	1	0.994	0.957	9.8	10.5 ± 1.8
E. coli CP4 EPSPS	3	1	0.920			
Plant CP4 EPSPS	9	1	1.027	1.062		
Plant CP4 EPSPS	10	1	1.096			
E. coli CP4 EPSPS	4	2	1.904	2.163	13.0	
E. coli CP4 EPSPS	5	2	2.421			
Plant CP4 EPSPS	11	2	2.584	2.485		
Plant CP4 EPSPS	12	2	2.386			
E. coli CP4 EPSPS	6	3	3.296	3.766	8.7	
E. coli CP4 EPSPS	7	3	4.236			
Plant CP4 EPSPS	13	3	4.039	4.124		
Plant CP4 EPSPS	14	3	4.208			

The immunoreactivity of the MON 87705-produced CP4 EPSPS was compared to that of the *E. coli*-produced CP4 EPSPS reference standard. The density of each band was determined by image analysis of the quantitative immunoblot shown in Figure 22. Density values were compared across the same concentrations and an average difference was determined. The 30s exposure was used for this analysis.

[1]Average Density =∑[[Density]]/2

[2]$\text{Percent Difference (\%)} = \frac{|\text{Average Density plant} - \text{Average Density E.coli}|}{\text{Average Density plant}} \text{ X } 100\%$

[3]Average difference (%) =∑[[% difference]]/3. The standard deviation was calculated using Microsoft Office Excel 2007 (12.0.6324.5001) SP1 MSO (12.0.6320.5000).

CP4 EPSPS Functional Activity Equivalence

The functional activities of the *E. coli*- and MON 87705-produced CP4 EPSPS proteins were estimated using an assay that measures the EPSPS-catalyzed formation of inorganic phosphate (P_i) and 5-enolpyruvylshikimate-3-phosphate (EPSP) from shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP). In this assay, protein specific activity is expressed as units per milligram of protein (U/mg), where a unit is defined as one µmole of inorganic phosphate released from PEP per min at 25°C. The *E. coli*- and MON 87705-produced CP4 EPSPS proteins were considered functionally equivalent if the specific activity of one protein was within 50% of the other. The specific activity of the plant-produced CP4 EPSPS protein was determined using a phosphate release assay. This end-point type colorimetric assay measures the release of inorganic phosphate from one of the substrates, PEP, by the action of the CP4 EPSPS enzyme.

Results indicated that the specific activity was 4.10 U/mg protein for the MON 87705-produced CP4 EPSPS, and 4.38 U/mg protein for the *E. coli*-produced CP4 EPSPS reference

standard. The difference in specific activities was 6.4% (Table 8) which is within assay variability. These results demonstrate that the CP4 EPSPS proteins derived from MON 87705 and *E. coli* have equivalent functional activities.

Table 8. CP4 EPSPS Functional Assay

MON 87705-produced CP4 EPSPS[1] *(U/mg)*	*E. coli-produced CP4 EPSPS*[1] *(U/mg)*	*Difference (%, Plant vs E. coli)*[2]
4.10 ± 0.1	4.38 ± 0.33	6.4

[1] Value refers to mean and standard deviation calculated based on n= 6

[2] $\% \text{ Difference} = \frac{(\text{Activity } E.coli - \text{Activity MON 87705})}{\text{Activity } E.coli} \times 100\%$

CP4 EPSPS Glycosylation Equivalence

Some eukaryotic proteins are post-translationally modified by the addition of carbohydrate moieties (Rademacher et al., 1988). These carbohydrate moieties may be complex, branched polysaccharide structures, simple oligosaccharides or monosaccharides. In contrast, the non-virulent *E. coli* strains used for cloning and expression purposes lack the necessary biochemical synthetic capacity required for protein glycosylation. Therefore, determining whether the MON 87705-produced CP4 EPSPS is equivalent to the *E. coli*-produced CP4 EPSPS requires an investigation of its glycosylation status.

To assess whether potential post-translational glycosylation of the MON 87705-produced CP4 EPSPS protein occurred, the purified protein sample was subjected to glycosylation analysis. The *E. coli*-produced CP4 EPSPS reference standard represented a negative control. The positive controls were the transferrin and horseradish peroxidase (HRP) proteins which are known to have multiple covalently-linked carbohydrate modifications. The transferrin protein and HRP, as well as the purified CP4 EPSPS protein isolated from MON 87705 and *E. coli* were separated on SDS-PAGE, transferred to a PVDF membrane, and glycosylation analysis was performed to detect carbohydrate moieties on the proteins. The results of this analysis are shown in Figure 23. The positive controls, transferrin and HRP, were detected at the expected molecular weights of ~75 and ~50 kDa, respectively, in a concentration-dependent manner (Figure 23, Panel A, Lanes 4-5 and 2-3). A very faint signal, slightly above background, was observed for the MON 87705- and *E. coli*-produced CP4 EPSPS proteins (Figure 23, Panel A, Lanes 6-7 and 8-9). This low level signal could be due to low level oxidation of amino acid residues of the protein and/or nonspecific binding of florescent reagents. Further evidence that the signals observed for MON 87705- and *E. coli*-purified CP4 EPSPS proteins are non-specific are the following: 1) The faint signal was also associated with the *E. coli*-produced CP4 EPSPS and could therefore not be the product of a glycan moiety because *E. coli*-produced proteins are not typically glycosylated (Schmidt et al., 2003). 2) Mass spectrometry data demonstrated the absence of glycosylation of the MON 87705-produced CP4 EPSPS. Glycosylation would result in an increase in the protein

mass relative to the theoretically calculated mass. No increase in protein mass was observed for the MON 87705-produced CP4 EPSPS protein determined by MALDI-TOF mass spectrometry (47396 Da) as compared to its theoretical mass (47481.48 Da), (3) Four potential glycosylation sites can be identified in the amino acid sequence of the CP4 EPSPS protein: one O linked at T248 and three N-linked at N213, N271 and N392 (see Figure 20 for amino acid positions). The tryptic fragments containing these amino acids were identified for the MON 87705-produced CP4 EPSPS protein by MALDI-TOF mass spectrometry (see Table 9). All identified masses matched the expected non-modified peptide masses, indicating that no glycosylation had occurred.

To confirm that sufficient MON 87705- and *E. coli*-produced CP4 EPSPS proteins were present for carbohydrate detection and glycosylation analysis, the membrane was stained with Coomassie Blue R250 stain to detect proteins (Figure 23, Panel B). Both MON 87705- and *E. coli*-produced CP4 EPSPS were clearly detected on the membrane (Figure 23, Panel B, Lanes 6-9).

These results indicate that the MON 87705-produced CP4 EPSPS protein is not glycosylated and, thus, is equivalent to the *E. coli*-produced CP4 EPSPS reference standard.

CP4 EPSPS Protein Identity and Equivalence Conclusion

The identity and equivalence of the CP4 EPSPS protein in MON 87705 was established through several lines of evidence. The identity of MON 87705-produced CP4 EPSPS protein was confirmed by N-terminus analysis showing it was consistent with the predicted amino acid sequence for this region of the protein. In addition, the MALDI-TOF mass spectrometry analysis yielded peptide masses consistent with the expected peptide masses from the translated *cp4 epsps* coding sequence. The equivalence of the MON 87705-produced CP4 EPSPS protein to the *E. coli*-produced CP4 EPSPS protein was shown using established techniques. The molecular weight of the MON 87705- and *E. coli*-produced CP4 EPSPS proteins was estimated by SDS-PAGE. The SDS-PAGE demonstrated the proteins migrated at the same molecular weight, indicating that the CP4 EPSPS proteins from both sources are equivalent in their molecular weight. The electrophoretic mobility and immunoreactive properties of the MON 87705-produced CP4 EPSPS were shown to be equivalent to those of the *E. coli*-produced CP4 EPSPS. The MON 87705- and *E. coli*-produced CP4 EPSPS proteins also were found to be equivalent based on their functional activities and the lack of glycosylation.

Taken together, these data provide a detailed characterization of the CP4 EPSPS protein isolated from MON 87705 harvested seed and establish its equivalence to the *E. coli*-produced CP4 EPSPS protein.

001 MLHGASSRPA TARKSSGLSG TVRIPGDKSI SHRSFMFGGL ASGETRITGL

051 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 20. MALDI-TOF MS Coverage Map of the MON 87705-Produced CP4 EPSPS

The amino acid sequence of the CP4 EPSPS protein was deduced from the *cp4 epsps* gene present in MON 87705. Boxed regions correspond to tryptic peptides that were identified from the MON 87705-produced CP4 EPSPS protein sample using MALDI-TOF MS. In total, 79.6% (362 of 455 total amino acids) of the expected protein sequence was identified.

Table 9. Summary of the Tryptic Masses Identified for the MON 87705-Produced CP4 EPSPS Using MALDI-TOF Mass Spectrometry

Matrix						
α-Cyano	DHB	Sinapinic acid	Expected Mass[1]	Difference[2]	AA position[3]	Segment
	389.18		*389.25*	0.07	225-227	TIR
	416.23		416.30	0.07	70-72	IRK
	474.20		474.27	0.07	228-231	LEGR
	506.17		506.22	0.05	354-357	ESDR
599.31	599.27		599.33	0.02	29-33	SISHR
616.32	616.29	616.10	616.34	0.02	128-132	RPMGR
629.32	629.28		629.29	0.03	201-205	DHTEK
629.32	629.28		629.34	0.02	383-388	GRPDGK
711.45	711.42		711.45	0	133-138	VLNPLR
835.39	835.37	835.29	835.39	0	62-69	AMQAMGAR
863.46	863.44		863.46	0	15-23	SSGLSGTVR
872.45	872.43		872.45	0	313-320	GVTVPEDR
872.45	872.43		872.52	0.07	358-366	LSAVANGLK
948.52	948.50		948.52	0	161-168	TPTPITYR
991.56			991.55	0.01	14-23	KSSGLSGTVR
1115.58	1115.58		1115.57	0.01	295-305	LAGGEDVADLR
1357.73	1357.73		1357.71	0.02	146-157	SEDGDRLPVTLR
1359.67	1359.69	1359.54	1359.72	0.05	354-366	ESDRLSAVANGLK
1359.67	1359.69	1359.54	1359.64	0.03	34-46	SFMFGGLASGETR
	1558.90	1558.73	1558.83	0.07	47-61	ITGLLEGEDVINTGK
1646.86	1646.89		1646.84	0.02	389-405	GLGNASGAAVATHLDHR
1705.82	1705.88		1705.81	0.01	367-382	LNGVDCDEGETSLVVR
1994.03	1994.07	1993.82	1993.97	0.06	206-224	MLQGFGANLTVETDADGVR
2183.24	2183.30	2183.05	2183.17	0.07	275-294	TGLILTLQEMGADIEVINPR
2367.43	2367.50	2367.21	2367.33	0.10	178-200	SAVLLAGLNTPGITTVIEPIMTR
		2450.13	2450.23	0.10	24-46	IPGDKSISHRSFMFGGLASGETR
		2450.13	2450.22	0.09	105-127	LTMGLVGVYDFDSTFIGDASLTK
	3186.35	3186.30	3186.52	0.17	73-104	EGDTWIIDGVGNGGLLAPEAPLDFGNAATGCR
	3249.77	3249.46	3249.62	0.15	321-351	APSMIDEYPILAVAAAFAEGATVMNGLEELR
		4188.82	4188.26	0.56	234-274	LTGQVIDVPGDPSSTAFPLVAALLVPGSDVTILNVLMNPTR

[1]Only experimental masses that matched expected masses are listed in the table.
[2]The numbers represent the difference between the expected mass and the first column which has the corresponding numbers.
[3]AA position refers to amino acid position within the predicted CP4 EPSPS sequence as depicted in Figure 21.

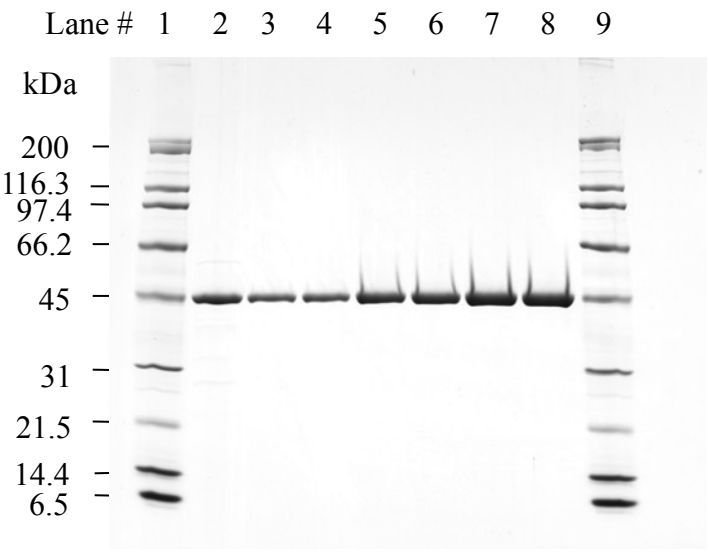


Figure 21. SDS-PAGE Molecular Weight and Purity Analysis of the MON 87705-Produced CP4 EPSPS Protein

Aliquots of the MON 87705- and the *E. coli*-produced CP4 EPSPS proteins were separated on a 4 to 20% Tris glycine polyacrylamide gradient gel and then stained with Brilliant Blue G-Colloidal stain. Approximate molecular weights (kDa) are shown on the left and correspond to the markers loaded in Lanes 1 and 9.

Lane	Sample	Amount loaded (µg)
1	Broad Range molecular weight markers	4.5
2	*E. coli* -produced CP4 EPSPS protein standard	1
3	MON 87705-produced CP4 EPSPS protein	1
4	MON 87705-produced CP4 EPSPS protein	1
5	MON 87705-produced CP4 EPSPS protein	2
6	MON 87705-produced CP4 EPSPS protein	2
7	MON 87705-produced CP4 EPSPS protein	3
8	MON 87705-produced CP4 EPSPS protein	3
9	Broad Range molecular weight markers	4.5

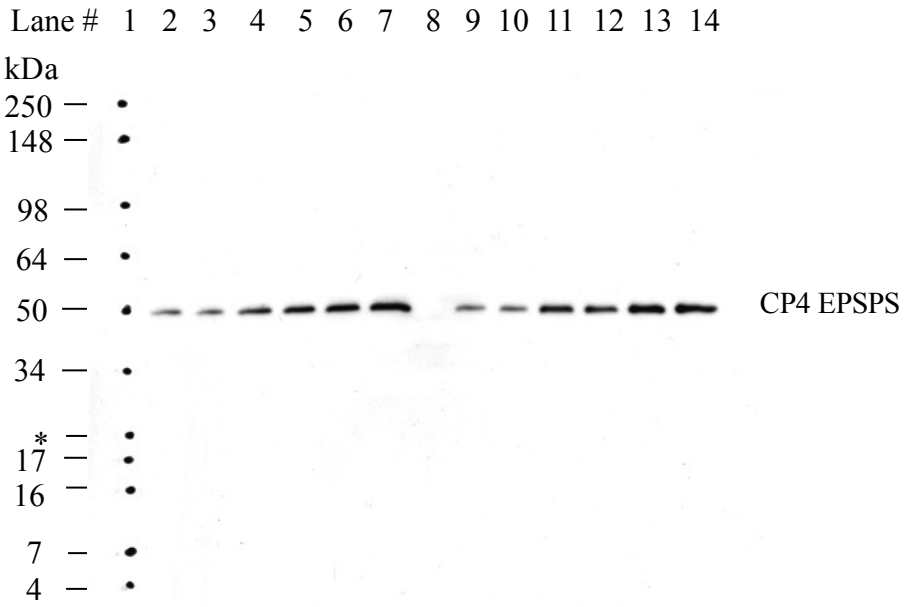


Figure 22. Western Blot Analysis of MON 87705- and *E.coli*-produced CP4 EPSPS Proteins

Aliquots of the purified, MON 87705- and *E. coli*-produced CP4 EPSPS proteins were separated by SDS-PAGE, and electrotransferred to a PVDF membrane. The membrane was probed with goat anti-CP4 EPSPS antibodies and developed using an ECL system (GE Healthcare). Approximate molecular weights (kDa) of markers loaded in Lane 1 are shown on the left side of the blot. The 30s exposure is shown. *: Non-assigned molecular weight in marker.

Lane	Sample	Amount Loaded (ng)
1	Blue Plus2 Pre-Stained molecular weight markers	-
2	*E. coli* -produced CP4 EPSPS reference standard	1
3	*E. coli* -produced CP4 EPSPS reference standard	1
4	*E. coli*- produced CP4 EPSPS reference standard	2
5	*E. coli* -produced CP4 EPSPS reference standard	2
6	*E. coli* -produced CP4 EPSPS reference standard	3
7	*E. coli* -produced CP4 EPSPS reference standard	3
8	Empty	
9	MON 87705-produced CP4 EPSPS protein	1
10	MON 87705-produced CP4 EPSPS protein	1
11	MON 87705-produced CP4 EPSPS protein	2
12	MON 87705-produced CP4 EPSPS protein	2
13	MON 87705-produced CP4 EPSPS protein	3
14	MON 87705-produced CP4 EPSPS protein	3

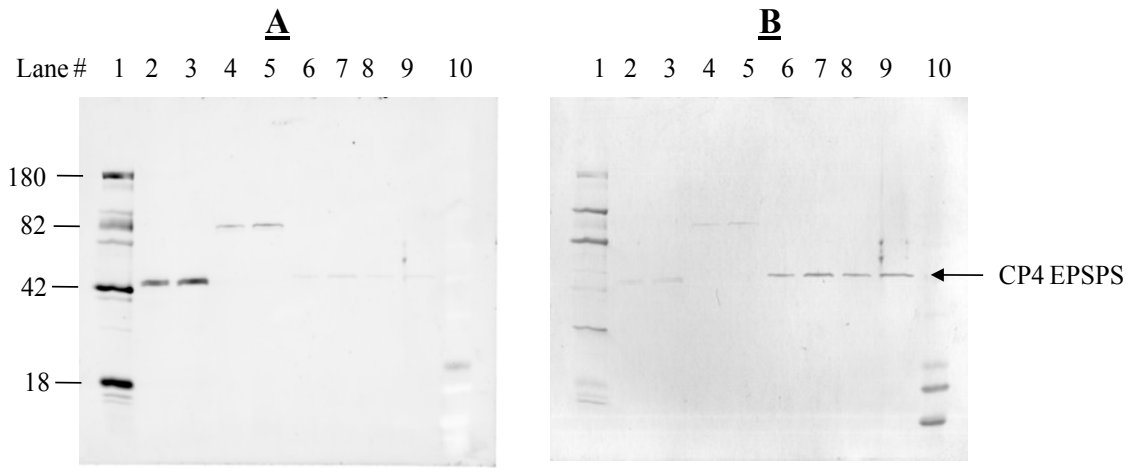


Figure 23. Glycosylation Analysis of the MON 87705-Produced CP4 EPSPS Protein

Aliquots of the MON 87705-produced CP4 EPSPS protein, *E. coli*-produced CP4 EPSPS reference standard (negative control), horseradish peroxidase (positive control) and transferrin (positive control) were separated by SDS-PAGE (4-20% gradient) and electrotransferred to a PVDF membrane. (**A**) Where present, periodate-oxidized protein-bound carbohydrate moieties reacted with Pro-Q Emerald 488 glycoprotein stain and emitted a fluorescent signal at 488 nm (Lanes 1-5). (**B**) The same blot was stained with Coomassie Blue R250. The signal was captured using a Bio-Rad GS-800 scanner. Approximate molecular weights (kDa) correspond to the CandyCane glycosylated markers loaded in Lane 1

Lane	Sample	Amount (ng)
1	CandyCane Glycoprotein molecular weight standards	—
2	Horseradish Peroxidase (positive control)	50
3	Horseradish Peroxidase (positive control)	100
4	Transferrin (positive control)	50
5	Transferrin (positive control)	100
6	MON 87705-produced CP4 EPSPS	50
7	MON 87705-produced CP4 EPSPS	100
8	*E. coli*-produced CP4 EPSPS (negative control)	50
9	*E. coli*-produced CP4 EPSPS (negative control)	100
10	Blue Plus2 Pre-Stained molecular weight markers	—

c) Expression Levels of CP4 EPSPS Protein in MON 87705

The levels of CP4 EPSPS protein in various tissues of MON 87705 that are relevant to the food/feed safety assessment were assessed by a validated enzyme-linked immunosorbent assay (ELISA). Tissues of MON 87705 and conventional control were collected during the 2007/2008 growing season from five field sites in Chile (City, Province): Quilapilum, Chacabuco; Melipilla, Melipilla; Calera de Tango, Maipo; Rancagua, Cachapoal; and San Fernando, Colchagua. These field sites were representative of soybean producing regions suitable for commercial production. At each site, three replicated plots of plants containing MON 87705, as well as the conventional soybean control A3525 with genetic background similar to MON 87705, were planted using a randomized complete block field design. Over-season leaf (OSL 1-4), root, forage, and mature seed tissues were collected from each replicated plot at all field sites. A description of tissues collected is provided below (Table 10).

Table 10. Tissues Collected for MON 87705

Tissue	Soybean development stage	Days after planting (DAP)
OSL-1	V3-V4	31-35
OSL-2	V6-V8	46-50
OSL-3	V10-V12	62-66
OSL-4	V14-V16	84-88
Forage	~R6	101-106
Root	~R6	101-106
Mature Seed	R8	154-159

The CP4 EPSPS protein levels were determined in all seven tissue types described above. The results obtained from ELISA analysis are summarized in Table 11. In summary, the 2007/2008 Chile expression study showed the CP4 EPSPS protein in MON 87705 was detected in all tissue types across all five sites with a range from 40 – 1000 µg/g dw. The levels of the CP4 EPSPS protein from the conventional control (A3525) were less than the assay limits of detection (LOD) or limit of quantitation LOQ in all tissue types. The mean CP4 EPSPS protein levels across the five sites were highest in leaf (ranging from 200 µg/g dw to 530 µg/g dw), followed by forage (120 µg/g dw), seed (110 µg/g dw) and root (77 µg/g dw).

Table 11. Summary of CP4 EPSPS Protein Levels in Leaf, Seed, Root, and Forage Tissues from MON 87705 Grown in 2007/2008 Chile Field Trials

Tissue Type[1]	CP4 EPSPS protein µg/g fw (SD)[2,4]	Range[3,4] (µg/g fw)	CP4 EPSPS protein µg/g dw (SD)[2,5]	Range (µg/g dw)[3,5]	LOQ/LOD (µg/g fw)[4]
OSL-1	36 (14)	16-65	200 (72)	84-340	0.57/0.26
OSL-2	110 (51)	60-230	530 (230)	290-1000	0.57/0.26
OSL-3	51 (21)	11-84	220 (94)	47-350	0.57/0.26
OSL-4	51 (21)	27-94	210 (92)	110-410	0.57/0.26
Forage	32 (5.3)	22-40	120 (24)	77-160	0.57/0.10
Root	24 (6.4)	14-34	77 (24)	41-120	0.57/0.11
Mature Seed	100 (39)	35-190	110 (44)	40-210	0.34/0.26

[1]The OSL-1, OSL-2, OSL-3, OSL-4 samples were collected approximately at V3 – V4, V6-8, V10-V12; and V14-V16 stages, respectively. The forage and root were collected approximately at R6 stage, and the mature seed was collected at R8 stage.
[2]The means and standard deviations were calculated for each tissue type across all sites (n=15 for all tissues, except OSL-2 where n=12 and OSL-3 where n=19).
[3]Minimum and maximum values were determined for each tissue type across all sites.
[4]Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fw) basis.
[5]Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a dry weight (dw) basis. The dry weight values were calculated by dividing the µg/g fw by the dry weight conversion factors obtained from moisture analysis data.

d) Non-expression

Not applicable

e) Assessment of the Potential for Toxicity of the CP4 EPSPS Protein

i) History of Safe Use

The 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) family of enzymes is ubiquitous to plants and microorganisms. EPSPS proteins have been isolated from both sources, and their properties have been extensively studied (Harrison et al., 1996; Haslam, 1993; Klee et al., 1987; Schonbrunn et al., 2001; Steinrücken and Amrhein, 1980). EPSPS exerts its function in the shikimate pathway that is integral to aromatic amino acid biosynthesis in plants and microorganisms (Levin and Sprinson, 1964; Steinrücken and Amrhein, 1980). The shikimate pathway and the EPSPS protein are absent in mammals, fish, birds, reptiles, and insects (Alibhai and Stallings, 2001). 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., 1996a), 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.

The *cp4 epsps* coding sequence encodes a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids (Padgette et al., 1996a). As stated, the CP4 EPSPS protein is similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate, the active ingredient in the Roundup family of agricultural herbicides, relative to endogenous plant EPSPS (Padgette et al., 1996a). 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 (Haslam, 1993; Steinrücken and Amrhein, 1980). In Roundup Ready plants, 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., 1996a). The CP4 EPSPS protein expressed in MON 87705 is identical to the CP4 EPSPS protein in other Roundup Ready crops, and the *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 (40-3-2), Roundup Ready 2 Yield soybean (MON 89788), Roundup Ready corn 2 (NK 603), Roundup Ready canola (GT73), Roundup Ready sugar beet (H7-1), and Roundup Ready Flex cotton (MON 88913). Furthermore, the U.S. 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).

ii) Structural Similarity of CP4 EPSPS to Known Toxins

The assessment of the potential for protein toxicity includes bioinformatic analysis of the amino acid sequence of the introduced protein. The goal of the bioinformatic analysis is to ensure that the introduced protein does not share homology to known toxins or anti-nutritional proteins associated with adverse health effects.

Potential structural similarities shared between the CP4 EPSPS protein and sequences in a protein database were evaluated using the FASTA sequence alignment tool (Figure 15). The FASTA program directly compares amino acid sequences (i.e., primary, linear protein structure) and the alignment data may be used to infer shared higher order structural similarities between two sequences (i.e., secondary and tertiary protein structures). Proteins that share a high degree of similarity throughout the entire sequence are often homologous. Homologous proteins usually have common secondary structures, common three-dimensional configuration, and, consequently, may share similar functions.

FASTA bioinformatic alignment searches using the CP4 EPSPS amino acid sequence were performed with the TOX_2009 database to identify possible homology with proteins that may be harmful to human and animal health. The TOX_2009 database is a subset of sequences derived from the GenBank protein database (PRT_2009), release 169.0 (December, 16, 2008). Sequences were selected using a keyword search and filtered to remove likely non-toxin proteins. The TOX_2009 database contains 7,651 sequences. Initially all header lines and the associated protein sequence in PROTEIN database were screened using all possible combinations of upper and lower case characters spelling the words “toxic” and “toxin.” The resulting 11,151 header lines and associated sequences then were filtered to exclude the following terms used in combination with “toxic” or ”toxin,” resulting in 7,651 sequences; these terms were “synthetic,” “anti,” “putative,” “like,” “insect,” “Cry,” “Thuringiensis”, and “toxin-reductase.”

An *E*-score acceptance criteria of $<1\times10^{-5}$ or less for any alignment was used to identify proteins from the TOX_2009 database with potential for significant shared structural similarity and function with CP4 EPSPS. The *E*-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 require an *E*-score of 1×10^{-5} or smaller to be considered to have sufficient sequence similarity to infer homology. The results of the search comparisons showed that no relevant alignments were observed against proteins in the TOX_2009 database. No FASTA alignment displayed an *E*-score of less than 1× 10-5.

The results of the bioinformatic analyses demonstrated that no structurally relevant similarity exists between the CP4 EPSPS protein and any known toxic or other biologically active proteins that would be harmful to human or animal health.

iii) Acute Oral Toxicity Study with the CP4 EPSPS Protein

Most known protein toxins act through acute mechanisms to exert toxicity (Hammond and Fuchs, 1998; Pariza and Johnson, 2001; Sjoblad et al., 1992). The primary exceptions to this

rule consist of certain antinutritional proteins such as lectins and protease inhibitors, which typically require a short-term (2-4 week) feeding study to manifest toxicity (Liener, 1994). The amino acid sequence of the CP4 EPSPS protein produced in MON 87705 is not similar to any of these antinutritional proteins or to any other known protein toxin. Therefore, an acute oral mouse toxicity study was considered sufficient to evaluate the toxicity of the CP4 EPSPS protein.

In this study, CP4 EPSPS protein was administered as a single dose by gavage to three groups of 10 male and 10 female CD-1 mice at dose levels up to 572 mg/kg (Harrison et al., 1996). The protein used in this study was produced by *E. coli* but shown to be physicochemically and functionally equivalent to the CP4 EPSPS protein produced in MON 87705. Additional groups of mice were administered comparable levels of either the buffer or bovine serum albumin (BSA) to serve as vehicle or protein controls. Following dosing, all mice were observed twice daily for mortality or signs of toxicity. Food consumption was measured daily. Body weights were measured prior to dosing and at study day 7. All animals were sacrificed on day 8 or 9 and subjected to a gross necropsy. There were no treatment-related effects on survival, clinical observations, body weight gain, food consumption or gross pathology. Therefore, the No Observable Adverse Effect Level (NOAEL) for CP4 EPSPS was considered to be 572 mg/kg, the highest dose tested.

iv) Dietary Safety Assessment of the CP4 EPSPS Protein

Estimated Human Exposure to the CP4 EPSPS Protein from MON 87705

Estimates of acute dietary exposure to the CP4 EPSPS protein from consumption of foods derived from MON 87705 were determined 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: seed, flour, milk, and oil. However, since soybean oil contains negligible amounts of protein (Martín-Hernández et al., 2008; Tattrie and Yaguchi, 1973), it would not be a significant source of dietary exposure to the CP4 EPSPS protein from MON 87705 and was thus excluded from this assessment. Estimated human exposure to CP4 EPSPS from MON 87705 in the U.S. was considered using a reasonable worst case scenario of the 95th percentile estimate of acute soybean consumption estimated on an "eater-only" basis.

MON 87705 is intended primarily for use as a broad acre field soybean and not for vegetable or garden soybean that are generally used to produce tofu, soybean sprouts, soymilk, edamame or other similar food items. Vegetable soybean generally has a different size, flavor, texture and other characteristics than field soybean, and is more easily cooked. After the extraction of the nutritionally-improved soybean oil from MON 87705, the remaining processed fractions derived from MON 87705 would likely be blended with those derived from other commercial soybean varieties before entering the human food supply. Estimating the percentage of consumed soybean products that would specifically be derived from MON 87705 is challenging. Therefore, for the purposes of this dietary risk assessment, the conservative assumption was made that 100% of all soybean products (excluding oil) consumed in the U.S. will be derived from MON 87705.

Because soybean is a blended commodity, the mean level of the CP4 EPSPS protein in each of the consumed food fractions (seed, flour, and milk) should be used when estimating total intake of this protein from consumption of MON 87705. However, specific values for each of these fractions are not available. Thus, the concentration of CP4 EPSPS protein in soybean seed and milk was assumed to be equal to the mean concentration in whole MON 87705 soybean seed grown in 2007/2008 field trials (100 µg/gram fresh weight). Protein content of soybean meal is concentrated approximately 1.35-fold relative to protein levels in soybean seed (Lundry et al., 2008). This value was derived from the ratio of total protein in processed meal to total protein in soybean seed. It was assumed that this same concentration occurs in soybean flour and, therefore, the concentration of CP4 EPSPS protein in soybean flour was assumed to be 135 µg/gram fresh weight, 1.35 times the mean concentration in whole MON 87705 soybean seed harvested in 2007/2008 field trials.

These assumptions for CP4 EPSPS levels in soybean fractions are very conservative, since they assume that there is no loss of CP4 EPSPS during storage, processing, and/or cooking. Soybean contains certain factors, such as trypsin inhibitors, which may act as antinutrients if the soybean is not properly heated during preparation (Rackis, 1974). Extensive heating processes are employed in extraction of soybean oil and in production of soybean meal and flour, soy protein concentrates, soy protein isolates, hydrolyzed vegetable protein, textured soy protein, soy milk, and tofu. Therefore, virtually all protein-containing soybean fractions are heated during processing prior to consumption by humans and most animals. Thus, the amounts of functionally active CP4 EPSPS protein present in consumed soybean products will be substantially lower than the levels assumed for this evaluation (EFSA, 2009).

Based on the above assumptions, the 95th percentile acute intake (eater-only) for CP4 EPSPS protein was estimated to be 0.013 mg/kg body weight/day for the overall U.S. population. The 95th percentile estimate of acute intake (eater-only) for non-nursing infants in the U.S., the most highly exposed sub-population, was 0.538 mg/kg body weight/day (Table 12).

Dietary Risk Assessment: Margins of Exposure for the CP4 EPSPS Protein Derived from MON 87705

A common approach used to assess potential health risks from chemicals or other potentially toxic products is to calculate a Margin of Exposure (MOE) between the lowest NOAEL from an appropriate animal toxicity study and an estimate of human exposure. No adverse health effects were observed when male or female mice were administered a dose of 572 mg/kg of CP4 EPSPS protein. Therefore, based on an apparent absence of hazard, a dietary risk assessment for this protein would normally not be considered necessary. Nevertheless, a dietary risk assessment for CP4 EPSPS was still conducted in order to provide further safety assurance.

Potential health risks from the acute dietary intake of CP4 EPSPS protein from consumption of food derived from MON 87705 were evaluated by calculating the MOE based on the acute mouse NOAEL for CP4 EPSPS and the 95th percentile "eater-only" estimates of acute dietary exposure from DEEM-FCID. The MOEs for acute dietary intake of CP4 EPSPS protein were estimated to be 43,600 and 1,100 for the general population and for non-nursing infants,

respectively (Table 12). These large MOEs indicate that there are no meaningful risks to human health from dietary exposure to CP4 EPSPS protein derived from MON 87705.

Table 12. Acute (95th Percentile, eater-only") Dietary Intake and Margins of Exposure for the CP4 EPSPS Protein from Consumption of MON 87705 Soybean Meal-Derived Food Products in the U.S.[1]

Population	CP4 EPSPS Intake [2] (mg/kg/day)	Margin of Exposure[3]
General Population	0.013	43,600
Non-nursing Infants	0.538	1,100

[1]Estimated using DEEM-FCID version 2.03, Exponent Inc., utilizing food consumption data from the 1994-1996 and 1998 USDA Continuing Surveys of Food Intakes by Individuals (CSFII). Includes soybean seed, flour, and milk. Assumes 100% of soybean products (excluding soybean oil) consumed in the U.S. are derived from MON 87705.
[2] Protein intake estimated with DEEM-FCID using an average expression level of 100 µg/gram fresh weight for CP4 EPSPS protein in whole seed. Assumes 1.35x concentration factor of protein in soybean flour relative to soybean seed (135 µg/gram; Lundry et al., 2008). Protein intake values were rounded to three significant figures.
[3]Calculated by dividing the NOAEL from the CP4 EPSPS acute mouse gavage study (572 mg/kg) by estimated dietary intake of CP4 EPSPS protein from MON 87705. MOEs were rounded to nearest hundred.

Estimated Animal Exposure to the CP4 EPSPS Protein from MON 87705

In the United States over 93% of the soybean grown is either crushed domestically or internationally with less than 7% of the soybean seed used as feed, seed or residual (USDA-ERS, 2007). During the crushing process soybean meal (SBM) is produced (approximately 0.74 kg of dehulled soybean meal from each kg of soybean). Of the soybean meal produced in the U.S., approximately 98% is consumed by the livestock industry (Soyatech, 2009). Poultry consumes 50%, swine 27%, cattle 17%, companion animals 3%, and the remainder by others (ASA, 2008). Full-fat soybean contains a trypsin inhibitor that affects protein digestion in monogastric animals and, thus, it must be heat-treated to deactivate the inhibitor before it can be fed to poultry and swine (Harris, 1990). Heat treatment also enhances the level of ruminal undegradeable protein that is beneficial to the ruminant so long as it is digestible in the lower gastro-intestinal (GI) tract. Typically, the feeding level of whole soybean to dairy cattle is 2.7 (2.3 -3.1) kg/cow/day (Harris, 1990; Hutjens, 1999) and is limited to a maximum of 20% of the swine diet due to its high oil content (Yacentiuk, 2008). Soybean forage can be fed to cattle and is limited to 50% of the total ration dry matter (Brown, 1999). Dehulled soybean meal consumption in different animal species is as follows:

1) 27.0 g dry matter/kg body wt/day for the four week old broiler (30.2% SBM in the diet, 161 g intake/day, diet 89% dry matter, 1.6 kg body wt)(Popescu and Criste, 2003);
2) 10.9 g dry matter/kg body wt/day for the young pig (24.3% SBM in the diet, 2.02 kg intake/day, diet 89% dry matter, 40 kg body wt)(Cromwell et al., 2002);
3) 3.8 g dry matter/kg body wt/day for the older pig (14% SBM in the diet, 3.04 kg of intake/day, diet 89% dry matter, 100 kg body wt)(Cromwell et al., 2002); and,
4) 7.8 g/kg body wt/day for the lactating dairy cow producing 37.4 kg of fat-corrected milk (18.6% SBM in the diet, 27.4 kg of dry matter intake/day, 655 kg body weight) (Bal et al., 2000). The soybean forage intake in lactating dairy cows may be up to 20.9 g/kg body wt/day (50% soybean forage in the diet dry matter [(Brown, 1999)]), 27.4 kg of dry matter intake/day, 655 kg body wt (Bal et al., 2000).

The exposure of poultry and livestock to MON 87705 primarily will be the result from feeding soybean meal with some animals being fed the heat treated full-fat soybean. For the following exposure calculations the intake of the soybean meal will be used since the diets contain a higher inclusion level of the protein from soybean meal than from the full-fat soybean. For example, if we assume that in the crushing process full-fat soybean yields 74% dehulled soybean meal then we can multiply the full-fat soybean inclusion levels mentioned above by 74%. The resulting dehulled soybean meal equivalent is equal to or less than the dehulled soybean meal inclusion levels used in this analysis. Soybean meal bought for animal feed would be expected to have gone through a series of commingling steps with non-MON 87705 soybean meal as it makes its way through commerce. MON 87705 soybean seed that is heat-treated and fed as full-fat soybean may not have been commingled with non-MON 87705 soybean; therefore, livestock could be exposed to the highest anticipated levels of CP4 EPSPS protein. For this assessment it will be assumed that MON 87705 soybean meal is the only source of soybean meal in the diet and has not been commingled with non-MON 87705 sources. The second assumption is that lactating dairy cows will be consuming both forage and soybean meal derived from MON 87705.

Animal Dietary Intake of CP4 EPSPS

Animals will be exposed to the CP4 EPSPS protein through dietary intake of feed derived from MON 87705 soybean seed and forage in the case of the lactating dairy cow. The quantity of soybean meal consumed on a daily basis by poultry and livestock, as well as the levels of CP4 EPSPS in MON 87705 soybean meal and forage, are necessary to derive an estimate of daily dietary intake (DDI). DDI is computed as follows:

DDI = [Daily soybean meal consumption (g) × CP4 EPSPS protein concentration (µg/g)] + [daily soybean forage consumption in the case of dairy cattle (g) × CP4 EPSPS protein concentration (µg/g)]

The intake calculations make the conservative assumption that there is no loss of the CP4 EPSPS protein during the processing of soybean into soybean meal. It also assumes that 100% of the soybean meal in animal feed is derived from MON 87705; however, in reality crushing plants that produce large quantities of soybean meal would be commingling soybean meal derived from MON 87705 with soybean meal from non-MON 87705 soybean.

The potential dietary intake of CP4 EPSPS from the consumption of MON 87705 soybean meal or forage can be estimated by multiplying the consumption of each commodity by the levels of the protein in that feedstuff.

For the purpose of this dietary intake calculation, which is to characterize a worst case scenario exposure of animals to CP4 EPSPS protein expressed in soybean meal derived from MON 87705 soybean seed and forage, allowing for some variability in expression levels, the mean and highest protein levels of the CP4 EPSPS protein reported for MON 87705 soybean seed and soybean forage were used. The mean and high end range values of the CP4 EPSPS protein levels in soybean seed and forage used in this assessment were from MON 87705 grown in Chile in 2007/2008.

The mean level of CP4 EPSPS protein in MON 87705 soybean seed is 110 µg/g dw with a maximum of 210 µg/g dw. Assuming a crushing yield of 74% (1 kg of soybean yield 0.74 kg of dehulled soybean meal), the calculated mean level of CP4 EPSPS protein in soybean meal derived from MON 87705 soybean seed is 148.65 µg/g dw (range 54.05 – 283.78 µg/g dw). The mean level of CP4 EPSPS protein in MON 87705 forage is 120 µg/g dw with a maximum of 160 µg/g dw.

The estimated mean and maximum daily intake of the CP4 EPSPS protein by poultry and livestock are shown in Table 13.

Table 13. Mean and Maximum Daily Intakes of the CP4 EPSPS Protein by Poultry and Livestock (g/kg body wt/day)

Species	Total Consumption of SBM (g DM/kg of body wt/day)	CP4 EPSPS Protein Intake (g/kg of body wt/day dw)	
		Mean	Highest Level
Chicken broiler[1]	27.0	0.004014	0.007662
Young pig[1]	10.9	0.001620	0.003093
Finishing pig[1]	3.8	0.000565	0.001078
Lactating dairy cow[2]	7.8 SBM + 20.9 forage	0.003667	0.005557

[1] Soybean meal consumed × concentration of CP4 EPSPS protein in the soybean meal.
[2] Soybean meal consumed × concentration of CP4 EPSPS protein in the soybean meal + forage consumed × concentration of CP4 EPSPS protein in the forage.

The broiler chicken, young pig, finishing pig, and lactating dairy would typically consume 18 g dietary protein/kg body wt (NRC, 1994), 14 g dietary protein/kg body wt (NRC, 1998), 4 g dietary protein/kg body wt (NRC, 1998), and 6 g dietary protein/kg bw (NRC, 2001), respectively. The highest percentage of CP4 EPSPS protein (g/kg bw) per total protein consumed was in the dairy cow due to the higher expression of CP4 EPSPS in the forage than in the seed, 0.0926% of the total dietary protein intake (0.005557 g CP4 EPSPS /kg body wt/day divided by 6 g dietary protein, which is the total dietary protein intake for the

dairy cow × 100). The percentages of the CP4 EPSPS protein consumed as part of the daily protein intake for the broiler and pig were less than 0.04256%.

Conclusion on Potential Toxicity of CP4 EPSPS Protein Produced in MON 87705
No adverse effects were noted in an acute mouse gavage study with the CP4 EPSPS protein expressed in MON 87705. In addition, large MOEs have been demonstrated for consumption of the CP4 EPSPS protein derived from MON 87705 for the general population and for non-nursing infants.

CP4 EPSPS has a long history of safe use and has been found to pose negligible risk to human and animal health upon consumption. Furthermore, it lacks structural similarity to known toxins or biologically active proteins known to have adverse effects on mammals. CP4 EPSPS is present at a very low level in the harvested seed of MON 87705 and, therefore, will constitute a very small portion of the total protein present in food and feed derived from MON 87705. CP4 EPSPS was readily digestible in simulated gastric and simulated intestinal fluids and showed no oral toxicity in mice. Based on the above information, the consumption of CP4 EPSPS protein from MON 87705 is safe for humans and animals.

f) Assessment of Potential Allergenicity of CP4 EPSPS Protein

According to guidelines adopted by the Codex Alimentarius Commission (Codex, 2003) for the assessment of potential allergenicity of introduced proteins, the allergenic potential of an introduced protein is assessed by comparing the biochemical characteristics of the introduced protein to characteristics of known allergens (Codex, 2003). A protein is not likely to be associated with allergenicity if: 1) the protein is from a nonallergenic source, 2) the protein represents only a very small portion of the total plant protein, 3) the protein does not share structural similarities to known allergens based on the amino acid sequence, and 4) the protein is rapidly digested in mammalian gastrointestinal systems. The CP4 EPSPS protein in MON 87705 has been assessed for its potential allergenicity according to these safety assessment guidelines.

Safety of the Donor Organism: *Agrobacterium* sp. Strain CP4
The donor organism, *Agrobacterium* sp. strain CP4, was isolated based on its tolerance to glyphosate brought about by the production of a naturally glyphosate-tolerant EPSPS protein (Padgette et al., 1996a). 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 (FAO/WHO, 2001), there is no known population of individuals sensitized to bacterial proteins.

The CP4 EPSPS Protein as a Proportion of Total Protein
The CP4 EPSPS protein was detected in all plant tissues assayed, at a number of time points during the growing season (Table 11). Among these tissues of MON 87705, harvested seed is the most relevant to the assessment of food allergenicity. The mean level of CP4 EPSPS in harvested seed is 110 µg/g dw. The mean % dry weight of total protein in harvested seed

from MON 87705 is 35.5% (or 353,000 µg/g). The percent of CP4 EPSPS in MON 87705 harvested seed is calculated as follows:

(110 µg/g ÷ 353,000 µg/g) × 100% ≈ 0.031% of total soybean protein

Therefore, the CP4 EPSPS protein represents a very small portion of the total protein in harvested seed of MON 87705.

Structural Similarity of CP4 EPSPS to Known Allergens

In 2003, the Codex Alimentarius Commission published guidelines for the evaluation of the potential allergenicity of introduced proteins (Codex, 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 recommends that a sliding window search with a scientifically justified peptide size could be used to identify immunologically relevant peptides in otherwise unrelated proteins. Therefore, the extent of sequence similarities between the CP4 EPSPS protein present in MON 87705 and known allergens, gliadins and glutenins was assessed using the FASTA sequence alignment tool and an eight-amino acid sliding window search (Codex, 2003; Thomas et al.,2005). 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 FASTA program directly compares amino acid sequences (i.e., primary, linear protein structure) and the alignment data may be used to infer shared higher order structural similarities between two sequences (i.e., secondary and tertiary protein structures). Proteins that share a high degree of similarity throughout the entire sequence are often homologous. Homologous proteins usually have common secondary structures, common three-dimensional configuration, and, consequently, may share similar functions. The allergen, gliadin, and glutenin sequence database (AD_2009) was obtained from Food Allergy Research and Resource Program Database (FARRP_2009) (http://www.allergenonline.com) and was used for the evaluation of sequence similarities shared between the CP4 EPSPS protein and all proteins. The AD_2009 database contains 1,386 sequences. 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. Results indicate the lack of sequence similarity between the CP4 EPSPS protein sequence and sequences in the allergen database. No alignment met nor exceeded the threshold of 35% identity over 80 amino acids recommended by Codex Alimentarius (2003) or had an *E*-score of 1×10^{-5} or smaller.

A second bioinformatic 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 (Hileman et al., 2002; Metcalfe et al., 1996). 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 useful to the allergy assessment process (Thomas et al.,2005). No eight contiguous amino acid identities were detected when the CP4 EPSPS protein sequence was compared to the AD_2009 sequence database.

Results indicate there were no biologically relevant sequence similarities to allergens or when the CP4 EPSPS protein sequence was used as a query for a FASTA search of the AD_2009 database. Furthermore, no short (eight amino acid) polypeptide matches were shared between the CP4 EPSPS protein sequence and proteins in the updated allergen database. These data indicate the lack of both structurally and immunologically relevant similarities between the CP4 EPSPS protein sequence and known allergens.

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). As with the previous assessment 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 protein was produced by *E. coli* but shown to be physicochemically and functionally equivalent to the CP4 EPSPS protein produced in MON 87705 (Section 2.5b i). The SDS-PAGE colloidal blue gel staining method demonstrated that greater than 98% of the CP4 EPSPS protein was digested within 15 seconds (Figure 24). Western blot analysis confirmed that greater than 95% of the *E. coli*-produced CP4 EPSPS protein was digested in SGF within 15 seconds (Figure 25). 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.

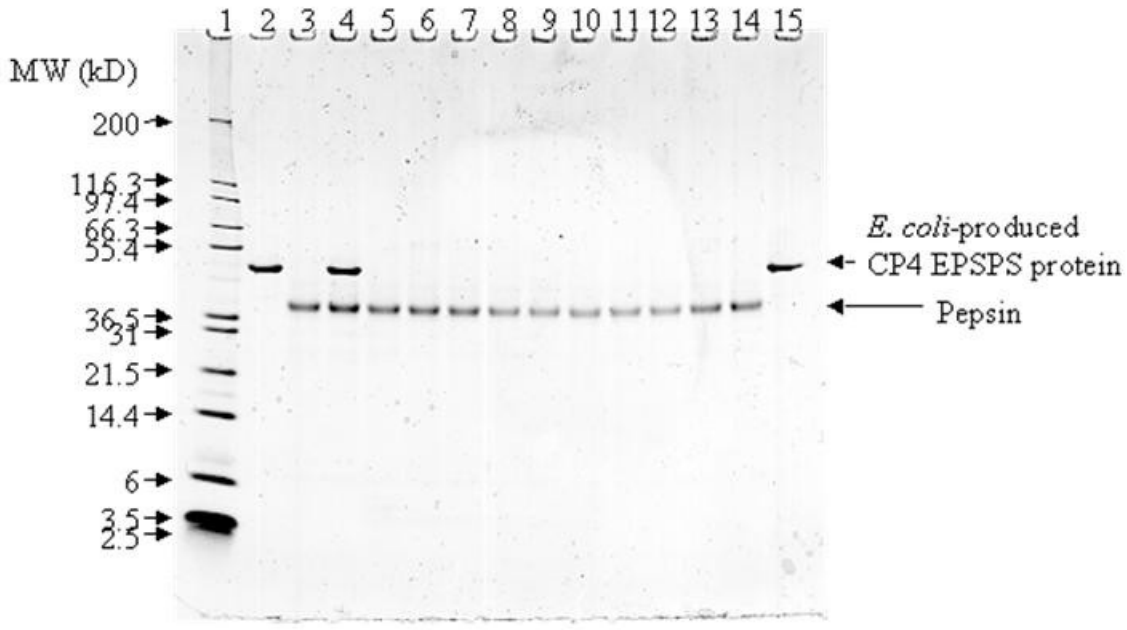


Figure 24. Colloidal Blue Stained SDS-PAGE Gel Showing the Digestion of Purified *E. coli*-produced CP4 EPSPS Protein in Simulated Gastric Fluid

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

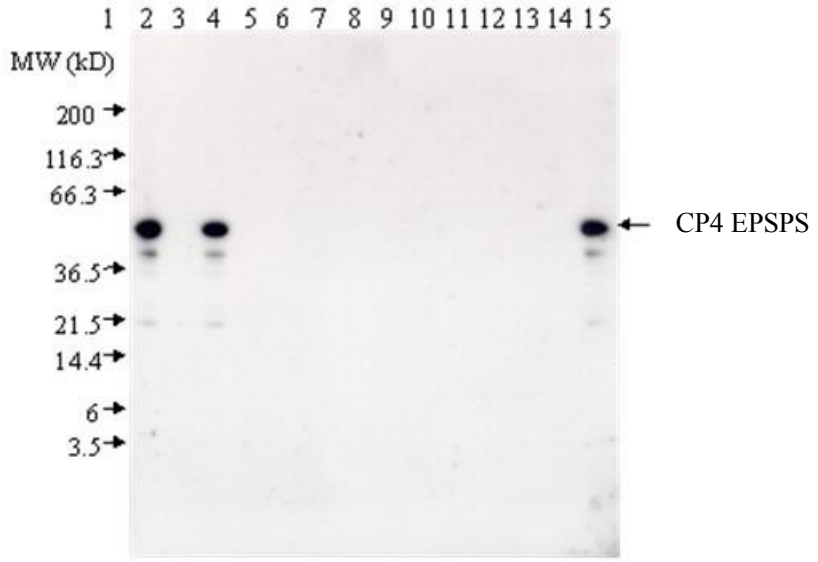


Figure 25. Western Blot Analysis of the Digestion of Purified *E.coli*-produced CP4 EPSPS Protein in Simulated Gastric Fluid

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

i) Assessment of the Endogenous Allergenicity of MON 87705

The purpose of this assessment was to quantitatively evaluate the soybean-specific IgE antibody in sera from clinically documented soybean allergic subjects. Soybean is one of eight allergenic foods that are responsible for approximately 90% of all food allergies (FAO, 1995). Soybean is less allergenic than other foods in this group and rarely responsible for severe, life-threatening reactions (Cordle, 2004). Allergy to soybean is more prevalent in children than adults and is considered a transient allergy of infancy/childhood (Sicherer et al., 2000). Since soybean is a known allergenic food crop, there is a need to ensure that the introduction of the genes did not cause an unintended change in the levels of endogenous allergenic proteins. This question can be addressed by comparing levels of soybean-specific IgE binding observed in the biotechnology-derived soybean to the set of binding values observed in reference soybean varieties that are already on the market. Determining the levels of direct IgE binding using an enzyme linked immunosorbent assay (ELISA) has been shown to be an appropriate method to perform such comparisons (Sten et al., 2004), especially when the assay is validated and calibrated prior to the production of the data (Ahlstedt, 2002; Holzhauser et al., 2008).

A quantitative evaluation of soybean-specific IgE provides an estimate of the endogenous allergens present in soybean seed. Protein extracts prepared from seed of MON 87705, a conventional soybean control (A3525), and 17 conventional soybean varieties (references) were evaluated. The reference soybean varieties were used to establish the range for soybean-specific IgE binding. The reference varieties are commercially available and included high protein, high oil, and food-grade (tofu) soybean that are already on the market and are being used for human consumption.

Sera from 13 clinically documented, soybean-allergic subjects and five non-allergic subjects were used to assess IgE binding to each soybean extract. Only soybean-allergic subjects with a documented case history of soybean allergy with anaphylaxis and a positive Double-Blind Placebo Controlled Food Challenge (DBPCFC) were included as soybean positive subjects.

Aqueous extracts were prepared from the ground soybean seed of MON 87705, the conventional soybean control, and reference varieties. These extracts were then analyzed for soybean-specific IgE antibody binding by a validated enzyme linked immunosorbent assay (ELISA). Each soybean extract was tested in triplicate. Soybean specific IgE binding was quantified by interpolation against a soybean-specific IgE standard curve and was expressed as ng of IgE/ml of serum. The standard curve was created by loading serial dilutions of human serum PEI 163 that contains a known amount of soybean-specific IgE into wells coated with internal reference soybean extract. Concentration of soybean-specific IgE in serum PEI 163 was 36 kU/l as measured by CAP-FEIA.

The IgE binding values obtained for the 17 reference soybean extracts were used to calculate a 99% tolerance interval for each subject's serum. The IgE binding values obtained for extracts prepared from MON 87705 and the conventional soybean control were compared to the tolerance interval derived for each serum. All of the IgE binding values for MON 87705 and the control were within the reference soybean tolerance limits for each subject's serum

(Figure 26). None of the soybean varieties showed IgE binding to sera from non-allergic subjects.

The results of this assessment demonstrate that soybean-specific IgE binding to endogenous allergens in MON 87705 and the conventional soybean control are comparable with the IgE binding to conventional soybean varieties currently on the market. Therefore, MON 87705 does not pose an increased endogenous soybean allergenic risk compared to conventional soybean.

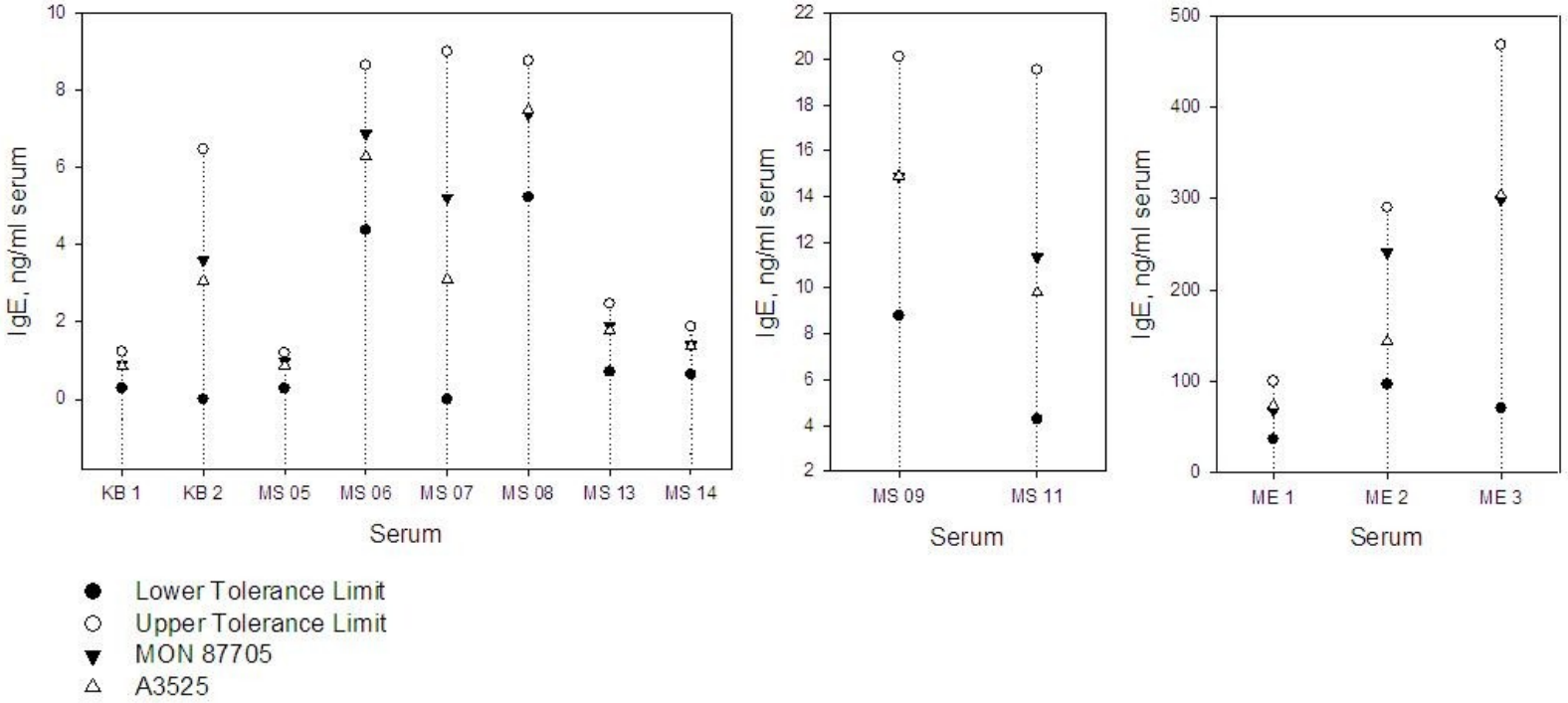


Figure 26. Serum IgE Binding Values from MON 87705, Conventional Control (A3525), and the Tolerance Limits for 17 Conventional References

The lower and upper tolerance limits for 99% tolerance intervals with 95% confidence for each serum are the result of a tolerance interval analysis for 17 conventional soybean varieties. Lower limits of the tolerance intervals that were calculated as less than zero were reported as zero in the analysis. Data are presented in three graphs due to the difference in the range of IgE concentration between individual sera. Abbreviations KB, MS and ME are subject designations

2.6 Characterisation of Other Novel Proteins

a) Identification

Not applicable

b) Toxicity

Not applicable

2.7 Comparison of the Composition and Nutritional Components of MON 87705 to Conventional Soybean

Compositional comparisons between biotechnology-derived and conventional crops represent an integral part of a nutritional and safety assessment. Compositional assessments are performed using the principles and analytes outlined in the OECD consensus documents for soybean composition (OECD, 2001). These principles are accepted globally and have been employed previously in assessments of soybean products derived through biotechnology.

Compositional equivalence between biotechnology-derived and conventional crops provides an "equal or increased assurance of the safety of foods derived from genetically modified plants" (OECD, 1998). The OECD consensus documents emphasize quantitative measurements of essential nutrients, and known antinutrients and toxicants. This is based on the premise that such comprehensive and detailed analyses will most effectively discern any compositional changes that imply potential safety and antinutritional concerns. Levels of the components in the seed and forage of the biotechnology-derived crop product are compared to: 1) corresponding levels in a non-modified comparator, typically the nontransgenic parental line grown under identical conditions, and 2) natural ranges generated from an in-study evaluation of commercial varieties or from data published in the scientific literature.

MON 87705 was developed to generate soybean oil with lower levels of saturated fatty acids (16:0 palmitic acid and 18:0 stearic acid) and higher levels of 18:1 oleic acid, with an associated decrease in 18:2 linoleic acid, through suppression of *FAD2* and *FATB* RNAs (Figure 1). MON 87705 contains the same major fatty acids that are found in conventional soybean, 16:0 palmitic, 18:0 stearic, 18:1 oleic, 18:2 linoleic and 18:3 linolenic acids in different proportions. MON 87705 has a fatty acid profile comparable to other widely consumed vegetable oils including olive oil and canola oil. MON 87705 also contains the *cp4 epsps* gene encoding the CP4 EPSPS protein that is expressed throughout the plant.

Compositional analyses were conducted to assess whether the nutrient and antinutrient levels in the seed and forage derived from MON 87705 are comparable to those in the conventional soybean control, A3525, which has background genetics similar to MON 87705, but lacks the introduced traits. A3525 was the soybean variety used as the recipient for the DNA insertion to create MON 87705. In addition, commercial conventional soybean varieties were included in the seed and forage composition analyses

to establish a range of natural variability for each analyte, defined by a 99% tolerance interval. Statistically significant differences were determined at the 5% level of significance ($p<0.05$) using established statistical methods. In addition, because MON 87705 has an intentionally improved soybean oil composition, compositional analyses of select processed fractions were included to assess whether MON 87705 soybean fractions are comparable to those derived from the conventional control in accordance with OECD guidance (OECD, 2001).

Seed and forage of MON 87705 and the conventional soybean control were harvested from soybean grown in three replicated plots, planted in a randomized complete block design, at each of five sites across Chile during the 2007-2008 growing season: Quilapilun, Chacabuco Province (QUI); Melipilla, Melipilla Province (MEL); Calera de Tango, Maipo Province (CdT); Rancagua, Cachapoal Province (RAN); and San Fernando, Colchagua Province (SFR). Samples from all three replicates of MON 87705 and the control were collected from all three plots and analyzed. Four different commercial reference soybean varieties also were grown at each site for a total of 20 varieties. Samples from the commercial reference varieties grown at each site were collected from all three plots. All replicates from 19 of 20 commercial conventional reference soybean varieties were analyzed; however, due to the slower maturity of one reference variety, all replicates of this variety at one site were damaged by an early frost and were excluded from the study. All MON 87705, control and reference soybean varieties were grown under normal agronomic field conditions for their respective geographic regions. Forage was collected at the R6 plant growth stage, and harvested soybean seed was collected at physiological maturity. The seed and forage collected from MON 87705, the conventional control, and the reference varieties were analyzed for nutrient composition.

In all, 67 analytical components were measured, 60 in seed and seven in forage. The analytes in forage included proximates (ash, fat, moisture, protein, and carbohydrates by calculation), acid detergent fiber (ADF), and neutral detergent fiber (NDF). Seed samples were analyzed for proximates, ADF, NDF, amino acids (18), fatty acids (26; C8-C24), trypsin inhibitors, phytic acid, lectin, isoflavones (daidzein, glycitein, and genistein), vitamin E, raffinose, and stachyose.

The composition data then were statistically compared to that of the conventional soybean control to evaluate compositional equivalence. Of the measured components, 17 fatty acids in seed had more than 50% of the observations below the assay limit of quantitation (LOQ) and could not be statistically analyzed. Thus, statistical analyses were conducted for 50 components (43 in seed and seven in forage). The data set was assessed using a mixed model of variance. Six sets of statistical analyses were conducted, five based on the data from each of the replicated field sites (individual-site) and the sixth analysis based on data from a combination of all five field sites (combined-site). Composition data were analyzed statistically for all components and their biological and nutritional significance were evaluated. If a significant difference ($p<0.05$) in an analyte was detected between MON 87705 and its conventional control in the combined-site comparison, an analysis was conducted to assess whether the difference was biologically meaningful from a food and feed safety or nutritional perspective. This analysis included reproducibility across individual sites, magnitude of differences, and comparisons of MON 87705 mean analyte values to the 99% tolerance interval for the population of commercial conventional soybean varieties (grown concurrently) and published values.

Table 14 shows a summary of the intended changes in the fatty acid levels for MON 87705 compared to conventional soybean control (A3525) in the combined-site analysis. A summary of the significant differences observed between MON 87705 and the control are presented in Table 15, and the combined-site composition data for MON 87705 and the control are presented in Table 17. The reported literature and the International Life Sciences Institute-Crop Composition Database (ILSI-CCD, 2006, ILSI-CCD at http://www.cropcomposition.org) ranges for the analytical components present in seed are provided in Table 18.

a) Overall Assessment of the Composition of Forage and Seed

Based on the comprehensive assessment procedures discussed above, MON 87705 is as safe and nutritious as conventional soybean. Combined-site analyses of both forage and seed samples showed no statistically significant differences ($p>0.05$) between MON 87705 and the control for 39 of 50 comparisons. Significant differences ($p<0.05$) between MON 87705 and the conventional soybean control were detected for 10 analytes in seed (arginine, cystine, fat, 16:0 palmitic acid, 18:0 stearic acid, 18:1 oleic acid, 18:2 linoleic acid, 18:3 linolenic acid, 20:0 arachidic acid, and 20:1 eicosenoic acid) and one analyte (ash) in forage.

The compositional analyses confirmed that MON 87705 had the intended changes in the levels of four major soybean oil fatty acids (16:0 palmitic, 18:0 stearic, 18:1 oleic and 18:2 linoleic). For the remaining seven comparisons where a significant difference ($p<0.05$) was detected, an analysis, including magnitude of the differences, reproducibility across individual sites, and comparisons of mean analyte values to the 99% tolerance interval and literature values, indicated they were not biologically meaningful from a food and feed safety and nutritional perspective. Further assessment of the statistically significant differences observed between MON 87705 and the conventional soybean control is provided in the following sections.

Results of the comparisons indicate that, except for the intended change in the levels of fatty acids, the composition of the seed and forage of MON 87705 is compositionally equivalent to that of the conventional soybean control A3525, in accordance with OECD guidelines. Moreover, a thorough analysis of MON 87705 soybean fatty acid composition demonstrated no fatty acids beyond those presently found in soybean were detected in MON 87705 seed above the limit of quantitation (LOQ) of the analytical method (LOQ = 0.02% of total fatty acids). Therefore, MON 87705 is regarded as being as safe and nutritious as conventional soybean for food and feed use.

b) Intended Changes to Fatty Acid Levels in MON 87705 Seed

As described previously, MON 87705 was developed to generate soybean oil with lower levels of saturated fatty acids (16:0 palmitic acid and 18:0 stearic acid) and higher levels of 18:1 oleic acid, with an associated decrease in 18:2 linoleic acid, through suppression of *FATB* and *FAD2* RNAs (Figure 1).

Table 14. Summary of Intended Changes in Fatty Acid Levels for MON 87705 vs. the Conventional Soybean Control (A3525) in the Combined-Site Analysis

Component (Units)	MON 87705 Mean [Range]	Control Mean [Range]	p-Value	Commercial [Tolerance Interval[1]]
(% Total FA)				
16:0 Palmitic	2.36 [2.25 - 2.44]	10.83 [10.51 – 11.08]	<0.001	[7.62, 12.55]
18:0 Stearic	3.31 [3.07 - 3.82]	4.50 [4.24 – 4.85]	<0.001	[2.87, 7.15]
18:1 Oleic	76.47 [73.13 - 79.17]	22.81 [21.41 – 25.08]	<0.001	[18.40, 30.22]
18:2 Linoleic	10.10 [7.85 - 12.42]	52.86 [51.68 – 53.89]	<0.001	[47.75, 56.46]

[1]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

Results described in Table 14 show the level of the saturated 16:0 palmitic acid decreased from 10.83% of total FA in the conventional control to 2.36% of total FA in MON 87705, and the level of the saturated 18:0 stearic acid decreased from 4.50% of total FA in the conventional control to 3.31% of total FA in MON 87705. Thus, total saturated fatty acids were decreased from approximately 15.3% of total FA in the conventional control to 5.7% of total FA in MON 87705. In addition, the mean level of 18:1 oleic acid increased from 22.81% of total FA in the conventional control to 76.47% of total FA in MON 87705. These changes were associated with a decrease in the mean level of 18:2 linoleic acid from 52.86% of total FA in the conventional control to 10.10% of total FA in MON 87705. As expected, all intended changes in fatty acid levels in the seed of MON 87705 were statistically significant in the combined-site analysis and consistently observed at all five individual sites. Thus, compositional analysis confirmed MON 87705 had the intended fatty acid profile.

c) Soybean Seed Fatty Acid Nutrient Levels

Of the 26 fatty acids analyzed in seed, 17 fatty acids had more than 50% of the observations below the assay limit of quantitation and, as a result, were excluded from the statistical analysis. Of the nine fatty acids that could be statistically analyzed, significant differences ($p<0.05$) were observed for seven fatty acids in the combined-site analysis. Four of these differences were due to the intended changes in the fatty acid levels, as described in Section 2.7b. The three remaining significant differences in the combined-site analysis were for 18:3 linolenic, 20:0 arachidic and 20:1 eicosenoic acids. The biological relevance of these differences was assessed based on the magnitude of the difference, reproducibility across sites, and comparison of mean analyte values to the 99% tolerance interval for the population of commercial conventional soybean varieties grown concurrently at the same field sites.

A statistical difference ($p<0.05$) between MON 87705 and the conventional control was observed in the levels of 18:3 linolenic acid in the combined-site analysis. The decrease in 18:3 linolenic acid is expected given that it is produced from 18:2 linoleic acid which was reduced by the suppression of the *FAD2* gene. Examination of the reproducibility within sites shows the levels of 18:3 linolenic acid were significantly lower in MON 87705 compared to the soybean control in four of five individual-site analyses, with the absolute magnitude of the differences being small (<1.5% of total FA; Table 15). In addition, all the mean levels of 18:3 linolenic acid in MON 87705 seed from the combined-site and individual-site analyses were well within the 99% tolerance interval for the population of conventional references and within the range of values found in the ILSI-CCD (Table 18), and therefore these differences are not considered biologically meaningful.

Combined-site statistical differences between MON 87705 and the conventional control also were observed in levels of two minor fatty acids, 20:0 arachidic acid, and 20:1 eicosenoic acid. The mean level of 20:0 arachidic acid in MON 87705 was significantly lower than in the conventional soybean control in the combined-site analysis. An examination of the reproducibility within sites showed that the levels of 20:0 arachidic acid were consistently lower in MON 87705 than in the soybean control in all five individual-site analyses. However, the absolute magnitude of the differences was small (<0.06% of total FA; Table 15), all combined-site and individual-site means were well within the 99% tolerance interval for the population of conventional references and within the range of values found in ILSI-CCD (Table 18), and therefore these differences are not considered biologically relevant compositional changes.

The mean level of 20:1 eicosenoic acid in MON 87705 was significantly higher than in the conventional soybean control in the combined-site analysis. An examination of the reproducibility within sites showed that the levels of 20:1 eicosenoic acid were consistently higher than in the soybean control in all five individual-site analyses. However, the absolute magnitude of these differences was small (<0.18% of total FA; Table 15). The combined-site mean for 20:1 eicosenoic (0.34% of total FA) was slightly (0.09% total FA) outside the upper end (0.25% of total FA) of the 99% tolerance interval but within the values reported in ILSI-CCD (Table 18). In addition, 20:1 eicosenoic acid has a history of safe consumption in other commonly consumed vegetable oils, such as canola (4.3% of total FA), corn (0.6% of total FA), mustard seed (13.0% of total FA), peanut (1.7% of total FA), high oleic safflower (0.5% of total FA, and high oleic sunflower (0.5% of total FA)(Codex, 2005). Therefore, the small change in the mean level of 20:1 eicosenoic acid in MON 87705 is not considered biologically meaningful for food and feed safety or nutrition.

These results lead to the conclusion that apart from the intended changes in the levels of four fatty acids (16:0 palmitic, 18:0 stearic, 18:1 oleic and 18:2 linoleic), the seed from MON 87705 is compositionally equivalent to conventional soybean with regard to the levels of other fatty acids. The differences observed for 18:3 linolenic is not unexpected, given the intended shift made in fatty acid metabolism (see Figure 1). The absolute magnitude of differences observed for 20:0 arachidic, and 20:1 eicosenoic fatty acids were small and were not biologically meaningful. Furthermore, the mean levels of these fatty acids were within the 99% tolerance interval and/or the ILSI-CCD and literature

values. Therefore, these differences are not considered biologically meaningful from a food and feed safety or nutritional perspective.

d) Soybean Seed Non-Fatty Acid Nutrient Levels

In addition to fatty acids (C8-C24), soybean seed also was analyzed for the following 26 nutrients: amino acids, trypsin inhibitors, phytic acid, lectins, isoflavones (daidzein, glycitein, and genistein), vitamin E, raffinose, and stachyose. No statistically significant differences ($p<0.05$) were observed for 23 nutrient analytes. Three analytes were statistically different ($p<0.05$) between MON 87705 and the conventional control in the combined-site analysis: total fat, arginine and cystine (Table 15). The biological relevance of these differences was assessed based on the magnitude of the difference, reproducibility across sites, and comparison of mean analyte values to the 99% tolerance interval for the population of commercial conventional soybean varieties grown concurrently at the same field sites. Mean analyte values were further compared to ILSI-CCD and literature ranges (Table 18).

The mean level of total fat was significantly lower ($p<0.05$) in MON 87705 than the conventional soybean control in the combined-site analysis; however, the absolute magnitude of the mean difference was small (1.04% dw; Table 15. There were no differences in total fat in any of the individual-site analyses. Furthermore, the mean level of total fat in MON 87705 was well within the 99% tolerance interval. Therefore, the difference in total fat in MON 87705 compared to the control is not considered biologically meaningful.

The mean level of cystine was significantly higher ($p<0.05$) in MON 87705 than the conventional soybean control in the combined-site analysis; however, the absolute magnitude of the mean difference was small (0.02% dw; Table 15). There were no differences in cystine levels in any of the individual-site analyses. Furthermore, the mean level of cystine in MON 87705 was within the 99% tolerance interval. Therefore, the difference in cystine in MON 87705 compared to the control is not considered biologically meaningful.

The mean level of arginine was significantly higher ($p<0.05$) in MON 87705 than the conventional soybean control in the combined-site analysis; however, the absolute magnitude of the mean difference was small (0.1% dw; Table 15). Examination of the reproducibility within sites shows that the mean level of arginine was significantly higher in only one of five individual-site analyses; however, the absolute magnitude of the mean difference was small (0.18% dw; Table 15)**.** These differences are not biologically meaningful, given the mean levels of arginine in MON 87705 in the combined-site and individual-site analyses were all well within the 99% tolerance interval.

These results lead to the conclusion that the seed from MON 87705 is compositionally equivalent to conventional soybean with regard to the levels of nutrients. The differences observed for nutrients were limited in number, not consistently observed across sites, and reflect the natural variation of conventional soybean. Furthermore, the mean levels of nutrient analytes were within the 99% tolerance interval for the population of

conventional references and within the range of values found in the published literature and/or the ILSI-CCD (Table 18). Therefore, these differences are not considered biologically meaningful from a food and feed safety or nutritional perspective.

e) Soybean Seed Naturally Occurring Anti-Nutrient Levels

Soybean seed contains several well-described antinutritional factors which include: trypsin inhibitors, phytic acid, lectins, isoflavones (daidzein, glycitein, and genistein), raffinose, and stachyose (OECD, 2001). Combined-site analysis of antinutrients showed no significant differences ($p>0.05$) between MON 87705 and the conventional soybean control (Table 15). Additional information is provided below to complete the discussion for the group of antinutrients.

Trypsin inhibitors are heat-labile antinutrients that interfere with the digestion of proteins and result in decreased animal growth (Liener, 1994). Lectins are also heat labile, and can inhibit growth and cause death in animals if raw soybean is consumed (Liener, 1994). Both trypsin inhibitors and lectins are inactivated during processing of soybean protein products or soybean meal and, when processed appropriately, the final edible soybean fractions should contain minimal levels of these antinutrients. No significant differences ($p>0.05$) were observed in trypsin inhibitor levels between MON 87705 and the conventional soybean control in the combined-site or individual-site analyses.

There are three principle isoflavones in soybean seed, namely daidzein, genistein, and glycitein. Although they have been reported to possess biochemical activities, including estrogenic and anti-estrogenic effects, it is not universally accepted that the isoflavones are antinutrients because they have also been reported to have beneficial antioxidant, anticarcinogenic and heart-healthy hypocholesterolemic effects (OECD, 2001). It is well documented that isoflavone levels in soybean seed are highly variable and are greatly influenced by many factors (Messina, 2001; Nelson and Renner, 2001; OECD, 2001). No significant differences ($p>0.05$) in isoflavone levels were observed between MON 87705 and the conventional soybean control for the combined-site or individual-site analyses.

Stachyose and raffinose are low molecular weight carbohydrates present in soybean seed that are considered to be antinutrients due to the gas production and resulting flatulence caused by consumption. No significant differences ($p>0.05$) in raffinose levels were observed between MON 87705 and the conventional soybean control in the combined-site or individual-site analyses. Stachyose levels showed no differences between MON 87705 and the conventional soybean control in the combined-site analysis, but were significantly different at one site (Table 15). This difference is not considered biologically relevant because it was observed only at one site and was not observed consistently across all sites.

Phytic acid present in soybean seed chelates mineral nutrients, including calcium, magnesium, potassium, iron and zinc, rendering them biologically unavailable to monogastric animals consuming the seed (Liener, 2000). Unlike trypsin inhibitors, phytic acid is not heat labile, and remains stable through most soybean processing steps. No significant differences ($p>0.05$) in phytic acid levels were observed between MON 87705 and the conventional soybean control for the combined-site or individual-site analyses.

Based on the data and information presented above, the seed from MON 87705 is compositionally equivalent to conventional soybean with regard to the levels of antinutrients.

f) Forage Proximate and Fiber Levels

Combined-site analysis of forage composition showed one significant difference ($p<0.05$) between MON 87705 and the conventional soybean control for ash (Table 15)**.** The mean level of ash was significantly higher ($p<0.05$) in MON 87705 than the conventional soybean control; however, the absolute magnitude of this difference was small (0.57% dw). There were no statistical differences in ash in any of the individual-site analyses. Furthermore, the mean level of ash in MON 87705 was well within the 99% tolerance interval. Therefore, the difference in ash in MON 87705 compared to the control is not considered biologically meaningful from a food and feed safety or nutritional perspective. These results demonstrate the forage from MON 87705 is compositionally equivalent to conventional soybean forage**.**

Compositional Equivalence of MON 87705 and Conventional Soybean Seed and Forage

Consistent with OECD guidelines for soybean composition (OECD, 2001), compositional analyses were conducted to assess whether levels of nutrients, antinutrients, and key secondary metabolites in seed and forage derived from MON 87705 are comparable to those in the conventional soybean control, A3525, which has background genetics similar to MON 87705 but lacks the introduced improved fatty acid profile and glyphosate tolerance traits. Intended changes in the levels of the seed fatty acids 16:0 palmitic, 18:0 stearic, 18:1 oleic, and 18:2 linoleic comprised four of the 11 significant differences in the combined-site analyses. For the remaining seven comparisons where a significant difference ($p<0.05$) was detected, an analysis, including magnitude of differences, reproducibility across individual sites, and comparisons of mean MON 87705 analyte values to the 99% tolerance interval and published values, indicates the differences are not materially different and/or not biologically meaningful from a food and feed safety or nutritional perspective. Therefore, the compositional and nutritional assessment of MON 87705 supports the conclusion that, except for intended changes in the levels of fatty acids in soybean seed, forage and seed produced from MON 87705 are compositionally equivalent to those of conventional soybean.

Table 15. Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of Control)	p-Value	MON 87705 [Range]	Commercial [Tolerance Interval[2]]
Statistical Differences Observed in Combined-Site Analysis						
Forage Proximate (% DW)						
Ash	8.75	8.18	6.99	0.020	[7.39 - 10.11]	[6.78, 9.91]
Seed Amino Acid (% DW)						
Arginine	2.78	2.68	3.74	0.048	[2.43 - 3.16]	[1.81, 3.62]
Cystine	0.61	0.59	3.66	0.043	[0.57 - 0.64]	[0.49, 0.69]
Seed Fatty Acid (% Total FA)						
16:0 Palmitic	2.36	10.83	-78.18	<0.001	[2.25 - 2.44]	[7.62, 12.55]
18:0 Stearic	3.31	4.50	-26.39	<0.001	[3.07 - 3.82]	[2.87, 7.15]
18:1 Oleic	76.47	22.81	235.20	<0.001	[73.13 - 79.17]	[18.40, 30.22]
18:2 Linoleic	10.10	52.86	-80.90	<0.001	[7.85 - 12.42]	[47.75, 56.46]

Table 15(continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of Control)	p-Value	MON 87705 [Range]	Commercial [Tolerance Interval[2]]
Statistical Differences Observed in Combined-Site Analysis						
Seed Fatty Acid (% Total FA)						
18:3 Linolenic	6.69	8.02	-16.59	<0.001	[5.55 - 7.81]	[4.97, 9.93]
20:0 Arachidic	0.30	0.34	-11.72	0.005	[0.28 - 0.36]	[0.22, 0.53]
20:1 Eicosenoic	0.34	0.19	79.85	<0.001	[0.27 - 0.40]	[0.13, 0.25]
Seed Proximate (% DW)						
Total Fat	18.29	19.33	-5.38	<0.001	[16.55 - 19.50]	[15.35, 25.95]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
16:0 Palmitic Site CdT	2.31	10.80	-78.62	<0.001	[2.29 - 2.32]	[7.62, 12.55]
16:0 Palmitic Site MEL	2.39	10.83	-77.92	<0.001	[2.35 - 2.42]	
16:0 Palmitic Site QUI	2.30	10.56	-78.24	0.005	[2.25 - 2.37]	

Table 15 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of Control)	p-Value	MON 87705 [Range]	Commercial [Tolerance Interval[2]]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
16:0 Palmitic Site RAN	2.40	10.96	-78.12	<0.001	[2.39 - 2.40]	[7.62, 12.55]
16:0 Palmitic Site SFR	2.42	11.00	-78.00	<0.001	[2.40 - 2.44]	
18:0 Stearic Site CdT	3.17	4.58	-30.88	<0.001	[3.09 - 3.23]	[2.87, 7.15]
18:0 Stearic Site MEL	3.33	4.39	-24.06	0.018	[3.20 - 3.47]	
18:0 Stearic Site QUI	3.51	4.82	-27.20	0.004	[3.15 - 3.82]	
18:0 Stearic Site RAN	3.34	4.50	-25.73	0.001	[3.28 - 3.41]	
18:0 Stearic Site SFR	3.22	4.31	-25.26	0.001	[3.07 - 3.41]	

Table 15 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of Control)	p-Value	MON 87705 [Range]	Commercial [Tolerance Interval[2]]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
18:1 Oleic Site CdT	76.44	23.02	232.08	<0.001	[76.35 - 76.60]	[18.40, 30.22]
18:1 Oleic Site MEL	76.10	22.31	241.09	<0.001	[75.68 - 76.33]	
18:1 Oleic Site QUI	78.61	24.95	215.05	0.003	[77.70 - 79.17]	
18:1 Oleic Site RAN	74.69	21.53	246.87	<0.001	[73.13 - 75.98]	
18:1 Oleic Site SFR	76.49	22.42	241.12	<0.001	[75.33 - 77.21]	
18:2 Linoleic Site CdT	10.09	52.43	-80.75	<0.001	[9.94 - 10.22]	[47.75, 56.46]
18:2 Linoleic Site MEL	10.50	53.48	-80.38	<0.001	[10.16 - 10.92]	

Table 15 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of Control)	p-Value	MON 87705 [Range]	Commercial [Tolerance Interval[2]]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
18:2 Linoleic Site QUI	8.75	51.70	-83.07	0.006	[7.85 - 10.02]	[47.75, 56.46]
18:2 Linoleic Site RAN	11.32	53.73	-78.92	<0.001	[10.37 - 12.42]	
18:2 Linoleic Site SFR	9.82	52.84	-81.42	<0.001	[9.33 - 10.55]	
18:3 Linolenic Site CdT	6.90	8.15	-15.32	0.001	[6.85 - 6.94]	[4.97, 9.93]
18:3 Linolenic Site MEL	6.58	8.00	-17.72	0.002	[6.53 - 6.65]	
18:3 Linolenic Site QUI	5.64	7.02	-19.69	0.029	[5.55 - 5.71]	
18:3 Linolenic Site SFR	6.98	8.49	-17.72	0.009	[6.79 - 7.26]	

Table 15 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of Control)	p-Value	MON 87705 [Range]	Commercial [Tolerance Interval[2]]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
20:0 Arachidic Site CdT	0.29	0.35	-18.10	0.016	[0.28 - 0.29]	[0.22, 0.53]
20:0 Arachidic Site MEL	0.30	0.34	-11.86	0.026	[0.29 - 0.30]	
20:0 Arachidic Site QUI	0.33	0.36	-8.84	0.041	[0.30 - 0.36]	
20:0 Arachidic Site RAN	0.28	0.33	-13.08	0.014	[0.28 - 0.29]	
20:0 Arachidic Site SFR	0.29	0.32	-8.18	0.006	[0.29 - 0.29]	
20:1 Eicosenoic Site CdT	0.36	0.21	76.81	<0.001	[0.36 - 0.38]	[0.13, 0.25]
20:1 Eicosenoic Site MEL	0.35	0.20	70.85	0.001	[0.34 - 0.36]	

Table 15 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of Control)	p-Value	MON 87705 [Range]	Commercial [Tolerance Interval[2]]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
20:1 Eicosenoic Site QUI	0.38	0.20	89.53	0.049	[0.37 - 0.40]	[0.13, 0.25]
20:1 Eicosenoic Site RAN	0.29	0.16	82.18	0.003	[0.27 - 0.31]	
20:1 Eicosenoic Site SFR	0.33	0.18	80.72	0.005	[0.32 - 0.35]	
Seed Fiber (% DW)						
Acid Detergent Fiber Site CdT	18.23	16.27	12.10	0.049	[17.57 - 18.58]	[12.71, 19.29]
Acid Detergent Fiber Site RAN	16.32	13.94	17.07	0.002	[15.71 - 16.78]	
Statistical Differences Observed in One Site						
Forage Proximate (% DW)						
Carbohydrates Site RAN	69.77	72.09	-3.22	0.027	[68.94 - 71.06]	[64.45, 80.50]
Total Fat Site MEL	5.79	6.76	-14.29	0.030	[5.37 - 6.57]	[0, 9.74]

Table 15 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of Control)	p-Value	MON 87705 [Range]	Commercial [Tolerance Interval[2]]
Statistical Differences Observed in One Site						
Seed Amino Acid (% DW)						
Alanine Site SFR	1.51	1.44	4.62	0.024	[1.49 - 1.54]	[1.25, 1.92]
Arginine Site SFR	2.52	2.34	7.56	0.047	[2.43 - 2.64]	[1.81, 3.62]
Aspartic Acid Site SFR	3.76	3.56	5.48	0.009	[3.67 - 3.88]	[3.02, 5.11]
Glutamic Acid Site SFR	5.90	5.53	6.62	0.008	[5.72 - 6.12]	[4.42, 8.48]
Histidine Site SFR	0.90	0.85	5.90	0.018	[0.88 - 0.94]	[0.74, 1.16]
Leucine Site SFR	2.54	2.41	5.12	0.014	[2.47 - 2.61]	[2.06, 3.41]
Lysine Site SFR	2.25	2.13	5.32	0.007	[2.19 - 2.30]	[1.87, 2.81]

Table 15 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of Control)	p-Value	MON 87705 [Range]	Commercial [Tolerance Interval[2]]
Statistical Differences Observed in One Site						
Seed Amino Acid (% DW)						
Phenylalanine Site SFR	1.68	1.60	4.83	0.019	[1.64 - 1.73]	[1.35, 2.31]
Proline Site SFR	1.62	1.55	5.02	0.021	[1.59 - 1.66]	[1.29, 2.21]
Tyrosine Site SFR	1.18	1.12	5.72	0.042	[1.17 - 1.20]	[0.99, 1.49]
Seed Fatty Acid (% Total FA)						
24:0 Lignoceric Site CdT	0.15	0.15	-3.24	0.008	[0.14 - 0.15]	[0.030, 0.26]
Seed Fiber (% DW)						
Neutral Detergent Fiber Site CdT	21.04	17.99	16.97	0.009	[20.47 - 22.18]	[12.07, 21.51]
Seed Proximate (% DW)						
Carbohydrates Site CdT	41.82	40.05	4.40	0.016	[41.62 - 42.00]	[30.78, 45.86]
Seed Vitamin (mg/100g DW)						
Vitamin E Site MEL	3.26	3.83	-15.05	0.005	[3.15 - 3.45]	[0, 7.36]

Table 15 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87705 vs. the Conventional Control (A3525) and Commercial Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of Control)	p-Value	MON 87705 [Range]	Commercial [Tolerance Interval[2]]
Statistical Differences Observed in One Site						
Seed Antinutrient (% DW)						
Stachyose Site CdT	3.76	3.10	21.27	0.046	[3.55 - 4.16]	[1.96, 4.41]

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

Table 16. Statistical Summary of Combined Site Soybean Forage Fiber and Proximate Content for MON 87705 vs Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fiber and Proximate (% DW)						
Acid Detergent Fiber	30.18 (2.20) [20.04 - 42.14]	29.17 (2.23) [19.21 - 40.67]	1.01 (1.64) [-10.04 - 12.39]	-2.38, 4.39	0.543	(23.18 - 42.11) [18.29, 41.02]
Ash	8.75 (0.22) [7.39 - 10.11]	8.18 (0.22) [7.21 - 9.32]	0.57 (0.23) [-0.68 - 1.79]	0.096, 1.05	0.020	(6.76 - 10.40) [6.78, 9.91]
Carbohydrates	72.30 (1.20) [68.94 - 78.93]	73.43 (1.21) [67.88 - 78.99]	-1.13 (0.59) [-4.74 - 5.27]	-2.36, 0.097	0.069	(63.74 - 80.60) [64.45, 80.50]
Moisture (% FW)	72.93 (1.16) [70.20 - 81.10]	72.22 (1.16) [69.40 - 77.50]	0.70 (0.48) [-1.50 - 7.20]	-0.64, 2.04	0.218	(65.80 - 82.00) [62.26, 83.45]
Neutral Detergent Fiber	35.79 (2.05) [26.12 - 46.30]	35.85 (2.06) [32.19 - 45.90]	-0.062 (2.14) [-7.01 - 8.75]	-6.00, 5.88	0.978	(24.70 - 46.55) [22.57, 46.52]
Protein	14.04 (1.13) [9.25 - 18.10]	13.34 (1.13) [9.71 - 17.85]	0.70 (0.55) [-5.20 - 3.75]	-0.43, 1.84	0.213	(9.51 - 19.93) [7.38, 21.27]

Table 16 (continued). Statistical Summary of Combined Site Soybean Forage Fiber and Proximate Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[2]]
Fiber and Proximate (% DW)						
Total Fat	4.91 (0.42) [2.29 - 7.01]	5.14 (0.42) [3.36 - 7.19]	-0.23 (0.38) [-2.23 - 2.80]	-1.01, 0.55	0.549	(1.19 - 8.22) [0, 9.74]

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

Table 17. Statistical Summary of Combined Site Soybean Seed Amino Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.[2]) [Range]	95% CI[3] (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Amino Acid (% DW)						
Alanine	1.59 (0.033) [1.49 - 1.70]	1.57 (0.033) [1.43 - 1.66]	0.025 (0.018) [-0.041 - 0.13]	-0.026, 0.075	0.243	(1.34 - 1.78) [1.25, 1.92]
Arginine	2.78 (0.11) [2.43 - 3.16]	2.68 (0.11) [2.31 - 2.99]	0.10 (0.036) [-0.062 - 0.39]	0.0012, 0.20	0.048	(2.15 - 3.23) [1.81, 3.62]
Aspartic Acid	4.08 (0.13) [3.67 - 4.49]	4.00 (0.13) [3.51 - 4.43]	0.075 (0.060) [-0.16 - 0.52]	-0.092, 0.24	0.279	(3.37 - 4.76) [3.02, 5.11]
Cystine	0.61 (0.0075) [0.57 - 0.64]	0.59 (0.0076) [0.55 - 0.63]	0.022 (0.0075) [-0.027 - 0.073]	0.0010, 0.042	0.043	(0.53 - 0.64) [0.49, 0.69]
Glutamic Acid	6.46 (0.24) [5.72 - 7.19]	6.32 (0.24) [5.42 - 7.09]	0.14 (0.12) [-0.32 - 1.02]	-0.19, 0.47	0.300	(5.14 - 7.73) [4.42, 8.48]
Glycine	1.59 (0.039) [1.47 - 1.74]	1.56 (0.039) [1.41 - 1.67]	0.028 (0.022) [-0.050 - 0.17]	-0.033, 0.089	0.265	(1.30 - 1.79) [1.19, 1.95]

Table 17 (continued). Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.[2]) [Range]	95% CI[3] (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Amino Acid (% DW)						
Histidine	0.96 (0.023) [0.88 - 1.04]	0.94 (0.023) [0.84 - 1.01]	0.027 (0.010) [-0.021 - 0.11]	-0.0029, 0.057	0.065	(0.79 - 1.07) [0.74, 1.16]
Isoleucine	1.71 (0.050) [1.56 - 1.88]	1.67 (0.051) [1.45 - 1.86]	0.039 (0.036) [-0.15 - 0.26]	-0.064, 0.14	0.344	(1.37 - 2.00) [1.23, 2.15]
Leucine	2.73 (0.083) [2.47 - 3.01]	2.69 (0.083) [2.37 - 2.94]	0.044 (0.040) [-0.13 - 0.32]	-0.065, 0.15	0.325	(2.26 - 3.14) [2.06, 3.41]
Lysine	2.39 (0.053) [2.19 - 2.55]	2.33 (0.053) [2.10 - 2.51]	0.057 (0.025) [-0.051 - 0.25]	-0.011, 0.13	0.080	(2.00 - 2.63) [1.87, 2.81]
Methionine	0.55 (0.0088) [0.51 - 0.58]	0.53 (0.0089) [0.49 - 0.57]	0.020 (0.011) [-0.035 - 0.056]	-0.010, 0.049	0.141	(0.46 - 0.59) [0.43, 0.63]
Phenylalanine	1.82 (0.056) [1.64 - 2.00]	1.80 (0.056) [1.58 - 1.99]	0.019 (0.027) [-0.16 - 0.22]	-0.056, 0.093	0.523	(1.50 - 2.11) [1.35, 2.31]

Table 17 (continued). Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.[2]) [Range]	95% CI[3] (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Amino Acid (% DW)						
Proline	1.77 (0.057) [1.59 - 1.95]	1.72 (0.057) [1.52 - 1.90]	0.054 (0.030) [-0.046 - 0.27]	-0.029, 0.14	0.145	(1.43 - 2.03) [1.29, 2.21]
Serine	1.75 (0.059) [1.49 - 1.98]	1.77 (0.059) [1.45 - 1.94]	-0.016 (0.035) [-0.22 - 0.24]	-0.087, 0.056	0.655	(1.55 - 2.05) [1.44, 2.15]
Threonine	1.33 (0.030) [1.20 - 1.45]	1.33 (0.030) [1.18 - 1.47]	-0.0031 (0.018) [-0.13 - 0.11]	-0.041, 0.035	0.867	(1.19 - 1.48) [1.12, 1.53]
Tryptophan	0.42 (0.011) [0.37 - 0.46]	0.41 (0.012) [0.35 - 0.44]	0.0016 (0.0069) [-0.028 - 0.046]	-0.017, 0.020	0.831	(0.33 - 0.48) [0.30, 0.50]
Tyrosine	1.25 (0.029) [1.17 - 1.33]	1.22 (0.029) [1.10 - 1.32]	0.026 (0.014) [-0.040 - 0.12]	-0.011, 0.064	0.124	(1.07 - 1.39) [0.99, 1.49]
Valine	1.83 (0.052) [1.69 - 2.02]	1.77 (0.053) [1.55 - 1.96]	0.051 (0.038) [-0.17 - 0.28]	-0.059, 0.16	0.260	(1.45 - 2.13) [1.31, 2.29]

Table 17 (continued). Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.[2]) [Range]	95% CI[3] (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Fatty Acid (% Total FA)						
16:0 Palmitic	2.36 (0.056) [2.25 - 2.44]	10.83 (0.056) [10.51 - 11.08]	-8.47 (0.055) [-8.68 - -8.13]	-8.62, -8.31	<0.001	(8.78 - 11.51) [7.62, 12.55]
18:0 Stearic	3.31 (0.067) [3.07 - 3.82]	4.50 (0.067) [4.24 - 4.85]	-1.19 (0.065) [-1.47 - -0.79]	-1.37, -1.01	<0.001	(3.82 - 7.21) [2.87, 7.15]
18:1 Oleic	76.47 (0.59) [73.13 - 79.17]	22.81 (0.59) [21.41 - 25.08]	53.65 (0.22) [51.71 - 55.05]	53.17, 54.13	<0.001	(20.77 - 27.19) [18.40, 30.22]
18:2 Linoleic	10.10 (0.39) [7.85 - 12.42]	52.86 (0.39) [51.68 - 53.89]	-42.77 (0.18) [-43.74 - -41.38]	-43.17, -42.37	<0.001	(48.62 - 54.74) [47.75, 56.46]
18:3 Linolenic	6.69 (0.28) [5.55 - 7.81]	8.02 (0.28) [6.86 - 8.60]	-1.33 (0.072) [-1.80 - -0.59]	-1.53, -1.13	<0.001	(5.89 - 9.11) [4.97, 9.93]
20:0 Arachidic	0.30 (0.0076) [0.28 - 0.36]	0.34 (0.0077) [0.31 - 0.36]	-0.039 (0.0071) [-0.082 - -0.022]	-0.059, -0.019	0.005	(0.28 - 0.54) [0.22, 0.53]

Table 17 (continued). Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

			Difference (MON 87705 minus Control)			
Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Mean (S.E.[2]) [Range]	95% CI[3] (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Fatty Acid (% Total FA)						
20:1 Eicosenoic	0.34 (0.013) [0.27 - 0.40]	0.19 (0.013) [0.15 - 0.21]	0.15 (0.0086) [0.12 - 0.20]	0.13, 0.18	<0.001	(0.15 - 0.22) [0.13, 0.25]
22:0 Behenic	0.29 (0.0037) [0.28 - 0.33]	0.30 (0.0038) [0.28 - 0.31]	-0.0052 (0.0051) [-0.029 - 0.020]	-0.017, 0.0070	0.346	(0.29 - 0.46) [0.22, 0.47]
24:0 Lignoceric	0.14 (0.017) [0.066 - 0.17]	0.13 (0.017) [0.067 - 0.16]	0.0046 (0.0046) [-0.019 - 0.027]	-0.0084, 0.018	0.372	(0.056 - 0.21) [0.030, 0.26]
Fiber (% DW)						
Acid Detergent Fiber	17.14 (0.54) [15.71 - 19.31]	16.14 (0.54) [13.36 - 18.02]	1.00 (0.74) [-1.84 - 4.03]	-1.06, 3.05	0.249	(12.46 - 21.25) [12.71, 19.29]
Neutral Detergent Fiber	18.44 (0.85) [13.41 - 22.18]	17.83 (0.86) [14.61 - 21.09]	0.60 (1.03) [-4.24 - 4.33]	-2.25, 3.46	0.590	(12.25 - 20.89) [12.07, 21.51]
Proximate (% DW)						
Ash	6.06 (0.13) [5.46 - 6.54]	6.13 (0.13) [5.48 - 6.55]	-0.072 (0.081) [-0.56 - 0.26]	-0.30, 0.15	0.421	(5.64 - 6.82) [5.26, 7.17]

Table 17(continued). Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.[2]) [Range]	95% CI[3] (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Proximate (% DW)						
Carbohydrates	40.35 (0.86) [36.69 - 43.52]	39.93 (0.86) [37.46 - 42.96]	0.42 (0.37) [-1.08 - 2.00]	-0.59, 1.43	0.317	(32.79 - 42.29) [30.78, 45.86]
Moisture (% FW)	10.76 (0.37) [8.96 - 12.30]	11.56 (0.37) [10.20 - 12.70]	-0.80 (0.44) [-3.40 - 0.90]	-2.02, 0.42	0.141	(6.89 - 12.50) [5.51, 13.37]
Protein	35.32 (0.99) [31.48 - 38.59]	34.66 (0.99) [30.71 - 37.40]	0.66 (0.36) [-0.94 - 3.08]	-0.33, 1.65	0.141	(29.51 - 40.25) [26.12, 43.51]
Total Fat	18.29 (0.39) [16.55 - 19.50]	19.33 (0.39) [17.63 - 20.32]	-1.04 (0.16) [-2.16 - -0.20]	-1.39, -0.69	<0.001	(16.91 - 23.48) [15.35, 25.95]
Vitamin (mg/100g DW)						
Vitamin E	2.83 (0.43) [1.23 - 4.36]	3.27 (0.43) [1.69 - 4.19]	-0.44 (0.17) [-1.40 - 0.76]	-0.92, 0.037	0.062	(1.09 - 5.10) [0, 7.36]
Antinutrient						
Lectin (H.U./mg DW)	2.21 (0.40) [0.72 - 3.77]	2.45 (0.41) [0.61 - 5.53]	-0.24 (0.57) [-4.80 - 3.04]	-1.57, 1.09	0.686	(0.65 - 8.10) [0, 6.44]

Table 17 (continued). Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.[2]) [Range]	95% CI[3] (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Antinutrient (% DW)						
Phytic Acid	1.82 (0.052) [1.52 - 2.13]	1.85 (0.053) [1.63 - 2.19]	-0.031 (0.041) [-0.27 - 0.18]	-0.12, 0.057	0.457	(1.42 - 2.27) [1.35, 2.35]
Raffinose	0.58 (0.029) [0.48 - 0.71]	0.58 (0.029) [0.50 - 0.70]	0.00036 (0.015) [-0.088 - 0.12]	-0.042, 0.043	0.981	(0.40 - 0.80) [0.27, 0.87]
Stachyose	3.87 (0.13) [3.39 - 4.48]	3.70 (0.13) [3.04 - 4.43]	0.17 (0.14) [-0.56 - 0.96]	-0.22, 0.56	0.290	(2.30 - 4.53) [1.96, 4.41]
Trypsin Inhibitor (TIU/mg DW)	38.14 (2.60) [26.73 - 52.01]	37.25 (2.61) [27.23 - 49.78]	0.89 (0.97) [-5.31 - 6.98]	-1.74, 3.51	0.408	(23.11 - 60.42) [8.75, 63.43]
Isoflavone (µg/g DW)						
Daidzein	1806.33 (229.35) [1145.72 - 2565.56]	1794.07 (229.50) [1092.43 - 2565.86]	12.26 (37.50) [-196.53 - 242.28]	-68.88, 93.40	0.748	(320.54 - 3061.22) [0, 3328.03]
Genistein	1160.30 (115.82) [809.79 - 1527.94]	1117.27 (115.95) [751.67 - 1466.21]	43.04 (24.92) [-104.61 - 208.66]	-10.74, 96.81	0.107	(433.41 - 2301.59) [0, 2727.33]

Table 17 (continued). Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate,Vitamin, Antinutrient and Isoflavone Content for MON 87705 vs. the Conventional Control (A3525)

Component (Units)[1]	MON 87705 Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (MON 87705 minus Control) Mean (S.E.[2]) [Range]	95% CI[3] (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Isoflavone (µg/g DW)						
Glycitein	132.85 (12.38) [49.11 - 196.59]	126.86 (12.53) [72.10 - 167.04]	5.98 (12.18) [-61.67 - 98.54]	-27.41, 39.38	0.648	(21.67 - 354.30) [0, 376.03]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; [2]S.E. = standard error; [3]CI = Confidence Interval.
[4]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

Table 18. Literature and ILSI Database Values for Components in Conventional Soybean Seed and Forage

Forage Tissue/Component[1]	Literature Range[2]	ILSI Range[3]
Proximate (% dw)		
Ash	5.36 – 8.91	6.72 – 10.78
Carbohydrates	62.25 – 72.28	59.8 – 74.7
Moisture (% fw)	68.50 – 78.40	73.5 – 81.6
Protein	16.48 – 24.29	14.38 – 24.71
Total Fat	2.65 – 9.87	1.302 – 5.132
Fiber (% dw)		
Acid Detergent Fiber (ADF)	23.86 – 50.69	not available
Neutral Detergent Fiber (NDF)	19.61 – 43.70	not available
Seed Tissue Component[1]	**Literature Range[2,4]**	**ILSI Range[3]**
Proximates (% dw)		
Ash	4.61 – 6.32	3.89 – 6.99
Carbohydrates	32.75 – 40.98	29.6 – 50.2
Moisture (% fw)	6.24 – 11.10	4.7 – 34.4
Protein	34.78 – 43.35	33.19 – 45.48
Total Fat	14.62 – 20.68	8.10 – 23.56
Fiber (% dw)		
Acid Detergent Fiber (ADF)	9.22 – 26.26	7.81 – 18.61
Neutral Detergent Fiber (NDF)	10.79 – 23.90	8.53 – 21.25
Amino Acids (% dw)		
Alanine	1.62 – 1.89	1.51-2.10
Arginine	2.57 – 3.27	2.29-3.40
Aspartic acid	4.16 – 5.02	3.81-5.12
Cystine/Cysteine	0.52 – 0.69	0.37-0.81
Glutamic acid	6.52 – 8.19	5.84-8.20
Glycine	1.59 – 1.90	1.46-2.00
Histidine	0.96 – 1.13	0.88-1.18
Isoleucine	1.59 – 2.00	1.54-2.08
Leucine	2.79 – 3.42	2.59-3.62
Lysine	2.36 – 2.77	2.29-2.84
Methionine	0.45 – 0.63	0.43-0.68
Phenylalanine	1.82 – 2.29	1.63-2.35
Proline	1.83 – 2.23	1.69-2.28
Serine	1.95 – 2.42	1.11-2.48
Threonine	1.44 – 1.71	1.14-1.86
Tryptophan	0.30 – 0.48	0.36-0.50
Tyrosine	1.27 – 1.53	1.02-1.61
Valine	1.68 – 2.09	1.60-2.20

Table 18. Literature and ILSI Database Values for Components in Conventional Soybean Seed and Forage (Continued).

Seed Tissue Component[1]	Literature Range[2,4]	ILSI Range[3]
Fatty Acids (% Total FA)		
8:0 Caprylic	not available	0.148 – 0.148
10:0 Capric	not available	not available
12:0 Lauric	not available	0.082 – 0.132
14:0 Myristic	not available	0.071 – 0.238
14:1 Myristoleic	not available	0.121 – 0.125
15:0 Pentadecanoic	not available	not available
15:1 Pentadecenoic	not available	not available
16:0 Palmitic	10.63-11.69	9.55 – 15.77
16:1 Palmitoleic	not available	0.086 – 0.194
17:0 Heptadecanoic	0.11-0.14	0.085 – 0.146
17:1 Heptadecenoic	not available	0.073 – 0.087
18:0 Stearic	3.85-4.55	2.70 – 5.88
18:1 Oleic	15.02-31.19	14.3 – 32.2
18:2 Linoleic	44.03-54.96	42.3 – 58.8
18:3 Gamma Linolenic	not available	not available
18:3 Linolenic	5.08-10.26	3.00 – 12.52
20:0 Arachidic	0.31-0.43	0.163 – 0.482
20:1 Eicosenoic	0.14-0.26	0.140 – 0.350
20:2 Eicosadienoic	not available	0.077 – 0.245
20:3 Eicosatrienoic	not available	not available
20:4 Arachidonic	not available	not available
22:0 Behenic	0.46-0.59	0.277 – 0.595
22:1 Erucic	not available	not available
24:0 Lignoceric	0.13-0.24	not available
Vitamins (mg/100g dw)		
Vitamin E	1.29 – 4.80	0.19-6.17
Antinutrients		
Lectin (H.U./mg fw)	0.45 – 9.95	0.09 – 8.46
Trypsin Inhibitor (TIU/mg dw)	20.79 – 59.03	19.59 – 118.68
Phytic Acid (% dw)	0.41 – 1.92	0.63 – 1.96
Isoflavones	**(µg/g dw)**	**(mg/kg dw)**
Daidzein	224.03 – 1485.52	60.0 – 2453.5
Genistein	338.24 – 1488.89	144.3 – 2837.2
Glycitein	52.72 – 298.57	15.3 – 310.4
Bio-Actives (% dw)		
Raffinose	0.26 – 0.84	0.21 – 0.66
Stachyose	1.53 – 2.98	1.21 – 3.50

[1]fw=fresh weight; dw=dry weight; H.U. = hemagglutinating unit; TIU = trypsin inhibitor unit.
[2]Lundry et al. (2008). [3]ILSI Crop Composition Database at: http://www.cropcomposition.org. [4]Padgette et al. (1996b).
Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw; g/100g dw x 10 = mg/g dw

g) Compositional Comparisons of Processed Fractions from Soybean Seed

An assessment of the composition of processed fractions was conducted in accordance with OECD guidance (OECD, 2001) because MON 87705 has an intentionally improved soybean oil composition. The processed fractions prepared from MON 87705 were compared to those of the conventional soybean control, A3525, which has background genetics similar to MON 87705, but lacks the intentionally modified fatty acid profile and glyphosate tolerance traits. Statistically significant differences were determined at the 5% level of significance ($p<0.05$) using established statistical methods. In addition, 12 commercial conventional soybean varieties were grown and harvested seed were used to produce several processed fractions. These fractions were analyzed 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.

To prepare soybean processed fractions, seed samples were collected from field trials conducted with MON 87705 and the conventional soybean control, A3525, at two field sites (Jefferson County, Iowa [IA] and Clinton County, Illinois [IL]) in the U.S. during the 2007 growing season. In addition, 12 commercial conventional soybean reference varieties were grown separately at three field sites in the U.S. to determine a 99% tolerance interval for each component analyzed. The seed samples were processed according to typical industry standards into toasted defatted soybean meal (TD soybean meal); refined, bleached, and deodorized soybean oil (RBD oil); protein isolate; and crude lecithin fractions. The processed fractions were analyzed according to the principles outlined in the OECD consensus document for soybean composition (OECD, 2001). Samples from all three replicates of MON 87705, the control, and the 12 references were analyzed from all plots. The TD soybean meal was analyzed for proximates (moisture, protein, fat, ash and carbohydrates by calculation), ADF, NDF, amino acids, trypsin inhibitors and phytic acid. The RBD oil was analyzed for fatty acids and vitamin E (α-tocopherol). The protein isolate fraction was analyzed for amino acids and moisture. The crude lecithin fraction was analyzed for phosphatides (α-phosphatidic acid, α-phosphatidylcholine, α-phosphatidylethanolamine, and α-phosphatidylinositiol.

The statistical analysis compared MON 87705 and the conventional control across the two sites (combined-site). A summary of the significant differences observed between the processed fractions prepared from the seed of MON 87705 and the conventional control is included in Table 19. Literature ranges are provided in Table 20 for soybean meal and Table 21 for soybean oil.

Results show that, except for the intended change in levels of fatty acids, minor differences in the levels of less abundant fatty acids in RBD oil and occurrence of low levels of minor fatty acids likely due to slight differences in oil refining process, the processed fractions produced from MON 87705 are compositionally equivalent to those of conventional soybean. Further details are provided below.

Composition of TD Soybean Meal

Comparison of the composition of TD soybean meal prepared from MON 87705 and the conventional soybean control showed no differences ($p>0.05$) for 21 of the 27 components analyzed. Significant differences ($p<0.05$) were observed for six components (Table 19): alanine, glycine, isoleucine, lysine, valine, and NDF. For the statistically significant amino acid differences, the absolute magnitude of the mean differences from the control were small (<0.1% dw) and the MON 87705 mean values fell within the 99% tolerance interval for the conventional soybean varieties and also within the range of published values for conventional soybean. For the statistically significant difference observed for NDF, the absolute magnitude of the mean difference from the control was small (1.7% dw) and the MON 87705 mean value was within the 99% tolerance interval for the conventional soybean varieties and also within the range of published values for conventional soybean (Table 20). Therefore, these differences were not considered biologically relevant from a food and feed safety or nutritional perspective.

As expected, the TD soybean meal from MON 87705 contained a small amount of total fat or oil (0.78% dw), which was also present at similar levels (0.86% dw) in the meal from conventional soybean. TD soybean meal is expected to contain oil, and accordingly, the National Oil Processors Association has established a minimum oil content (0.5% dw) for defatted soybean meal that meets quality standards and guidelines for soybean meal for domestic and international shipping (NOPA, 2006). Although not analyzed, the composition of the residual oil present in TD soybean meal is expected be consistent with the intended changes in the fatty acid levels observed in seed and in RBD oil (see discussion below). Based on these results, apart from the intended changes in the fatty acid levels in residual oil, the meal from MON 87705 soybean is considered compositionally equivalent to the meal from conventional soybean.

Composition of RBD Oil

Of the 38 fatty acids analyzed, 21 were excluded from statistical analysis since more than 50% of the observations were below the assay limit of quantitation. Of the 17 fatty acids that could be statistically analyzed, significant differences ($p<0.05$) between MON 87705 and control RBD oil were observed for 13 fatty acids (Table 19). Four of the 13 differences were expected as they were due to the intended changes in fatty acid levels. Thus, 16:0 palmitic acid levels were significantly lower in MON 87705 (2.49% of total FA) than the control (11.59% of total FA), 18:0 stearic acid levels were significantly lower in MON 87705 (3.22% of total FA) than the control (4.47% of total FA); 18:1 oleic acid levels were significantly higher in MON 87705 (71.51% of total FA) than the control (23.16% of total FA), and 18:2 linoleic acid levels were significantly lower in MON 87705 (14.41% of total FA) than the control (51.08% of total FA) (Table 19).

Of the remaining nine of 13 fatty acid differences, six differences were not detected in seed (14:0 myristic acid, 16:1 palmitoleic acid, 17:0 margaric (heptadecanoic) acid, 17:1 9c heptadecenoic acid, 18:2 other *trans* isomer fatty acids (excluding 9t,12t linolelaidic), and 18:2 6c,9c, octadecadienoic acid), and three differences were detected both in seed and oil (20:0 arachidic, 20:1 eicosenoic and 22:0 behenic acids).

For six of these nine fatty acid differences (14:0 myristic acid, 16:1 palmitoleic acid, 17:0

margaric [heptadecanoic] acid, 20:0 arachidic, 20:1 eicosenoic and 22:0 behenic acids), the absolute magnitude of the differences was small (<0.15% of total FA), and the MON 87705 mean values fell within the 99% tolerance intervals for the reference soybean varieties and/or within published ranges for conventional soybean oil (Table 21).

As discussed below, for the three remaining fatty acid differences (17:1 9c heptadecenoic acid, 18:2 other *trans* isomer fatty acids [excluding 9t,12t linolelaidic], and 18:2 6c,9c, octadecadienoic acid), no adverse food and feed safety or nutrition effects are expected for the levels detected.

A significant increase in the level of the minor fatty acid 17:1 9c heptadecenoic acid was observed in MON 87705 RBD oil (0.12% of total FA) compared to its level in control RBD oil (0.031% of total FA). The mean level of 17:1 9c heptadecenoic acid in MON 87705 RBD oil was higher than the level in the commercial reference varieties (<0.02% of total FA) and slightly outside reported literature values for soybean oil (ND-0.1% total FA; [Table 21]). However, 17:1 9c heptadecenoic acid is known to be present in other vegetable oils such as canola (0.3% of total FA), corn (0.1% of total FA), peanut (0.1% of total FA), high oleic safflower (0.1% of total FA), and high oleic sunflower (0.1% of total FA) (Codex, 2005). The presence of 17:1 9c heptadecenoic acid has also been documented in a variety of foods, with levels highest in meats and oils. As shown in the Table 22, comparable or higher intakes of 17:1 9c heptadecenoic acid can be achieved in a single serving of tofu, ground beef or soft-spread margarine compared to a serving of MON 87705 RBD oil. Therefore, there are no adverse food and feed safety or nutrition effects associated with the levels of 17:1 9c heptadecenoic acid observed in MON 87705 RBD oil.

The 18:2 other *trans* fatty acids and 18:2 6c,9c, octadecadienoic acid observed in both MON 87705 and control RBD oil were not observed in seed fatty acid analyses and are believed to arise from the minor differences in the oil refining process. The primary source of industrially produced *trans* fatty acids (TFAs) in the human diet is from hydrogenation of liquid and solid vegetable oils that results in food products that may contain in excess of 30% TFA (Chardigny et al., 1996; Ledoux et al., 2007). A secondary source of TFAs derives from ruminant-based meat and dairy products, due to naturally occurring bacterially produced TFAs accumulating in these animals (Chardigny et al., 1996; Ledoux et al., 2007).

The mean level of 18:2 other *trans* fatty acids in MON 87705 RBD oil was significantly lower than in control RBD oil, and both values were outside the range of the commercial reference varieties. This is likely due to minor differences in processing as the commercial reference soybean varieties were grown and processed separately from MON 87705 and the conventional control. However, the levels of 18:2 other *trans* fatty acids in MON 87705 RBD oil were within the range of total TFA content in samples of unhydrogenated commercial soybean oil (<3.5%)(Chardigny et al., 1996; Ledoux et al., 2007; Wolff, 1993). The contribution of MON 87705 RBD oil to overall dietary TFA intake will be minimal relative to commonly experienced dietary intakes, and, therefore, are not biologically meaningful from a food and feed safety or nutritional perspective.

The mean level of 18:2 6c,9c, octadecadienoic acid in MON 87705 RBD oil was significantly lower than in control RBD oil, and both values were outside the range of the

commercial references. This is likely due to minor differences in processing as the commercial reference soybean varieties were grown and processed separately from MON 87705 and the conventional control. It has been reported that although most fatty acid double bonds are in the *cis* configuration, some processes (such as heat treatment) may lead to the migration of double bonds from their naturally occurring positions in the carbon chain, leading to an increase in the levels of other cis isomers (Ledoux et al., 2007).

The RBD oil was also analyzed for vitamin E levels which were not significantly different ($p>0.05$) between MON 87705 and control RBD oil.

In summary, except for the intended changes in fatty acid levels, minor differences in the levels of less abundant fatty acids and occurrence of low levels of minor fatty acids due to spontaneous isomerisation during the oil refining process, the RBD oil from MON 87705 is considered compositionally equivalent to oil from conventional soybean.

Composition of Soybean Protein Isolate

There were no statistically significant differences ($p<0.05$) between MON 87705 and the conventional control for components measured in the protein isolate fraction. Based on these results, the protein isolate prepared from MON 87705 is considered compositionally equivalent to protein isolate from conventional soybean.

Composition of Crude Lecithin

There were no statistically significant differences ($p<0.05$) between MON 87705 and the conventional control for phosphatides of crude lecithin. Based on these results, the MON 87705 soybean lecithin is considered compositionally equivalent to conventional soybean lecithin.

Compositional Equivalence of MON 87705 and Conventional Soybean Processed Fractions

The processed fractions, TD soybean meal, RBD oil, protein isolate and crude lecithin, were analyzed according to the principles outlined in the OECD consensus document for soybean composition (OECD, 2001). There were no statistical differences ($p<0.05$) observed between MON 87705 and the conventional control (A3525) for the components measured in protein isolates or crude lecithin. Significant differences ($p<0.05$) were observed for six (alanine, glycine, isoleucine, lysine, valine, and NDF) of 27 components measured in TD soybean meal; however, the magnitude of the differences from the control was small and the MON 87705 mean values were within the 99% tolerance interval for the conventional reference soybean varieties and/or within the range of published values for conventional soybean meal, indicating that they were not biologically meaningful. The low levels of oil (0.78% dw, as total fat) present in TD soybean meal are expected to reflect the intended fatty acid changes observed in seed.

As expected, consistent with the intended changes in the fatty acid levels in seed, the intended changes in fatty acid levels were also observed in the RBD oil fraction from MON 87705. As observed in seed, levels of several less abundant fatty acids were

significantly different ($p<0.05$) between the RBD oil from MON 87705 and the control. Of the 17 fatty acids that could be statistically analyzed in RBD oil, significant differences ($p<0.05$) between MON 87705 and the control were observed for 13 fatty acids. Four of the 13 differences were expected as they were due to the intended changes in fatty acid levels (16:0 palmitic, 18:0 stearic, 18:1 oleic and 18:2 linoleic). For six of the nine remaining differences (14:0 myristic acid, 16:1 palmitoleic acid, 17:0 margaric [heptadecanoic] acid, 20:0 arachidic, 20:1 eicosenoic and 22:0 behenic acids), the magnitude of the differences was small (<0.15% total FA), and the MON 87705 mean values were within the 99% tolerance intervals for the reference varieties and/or within published ranges for conventional RBD soybean oil (Codex, 2005, Table 21).

The remaining three differences were for 17:1 9c heptadecenoic acid, 18:2 *trans* isomer fatty acids (excluding linolelaidic), and 18:2 6c,9c, octadecadienoic acid. A significant increase in the level of the minor fatty acid 17:1 9c heptadecenoic acid was observed in MON 87705 compared to control RBD oil. The mean level of 17:1 9c heptadecenoic acid in MON 87705 was outside the range of values obtained for the RBD oil from commercial reference soybean varieties. However, 17:1 9c heptadecenoic acid is present at similar or higher levels in a variety of oils (canola, corn, peanut, high oleic safflower, and high oleic sunflower) and foods (tofu, ground beef, and soft-spread margarine). Therefore, it is concluded that there are no adverse food and feed safety or nutrition effects associated with the levels of 17:1 9c heptadecenoic acid observed in MON 87705 soybean oil. The minor fatty acids, 18:2 other *trans* (excluding linolelaidic) and 18:2 6c,9c, octadecadienoic acid, are believed to arise from the minor differences in the oil refining process.

Therefore, this compositional assessment supports the conclusion that, except for the intended changes in fatty acid levels, minor differences in the levels of less abundant fatty acids and occurrence of low levels of minor fatty acids likely due to differences in the oil refining process, the processed fractions produced from MON 87705 are compositionally equivalent to those of conventional soybean.

h) Allergenic Proteins

Not applicable

Table 19. Summary of Differences (p<0.05) for the Comparison of Soybean Processed Fraction Component Levels for MON 87705 vs the Conventional Control (A3525) and Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of A3525)	p-Value	MON 87705 [Range]	Commercial Range[2] [99% Tolerance Interval[2,3]]
Meal Amino Acid (% DW)						
Alanine	2.26	2.33	-3.06	0.019	[2.22 - 2.29]	2.18 – 2.49 [1.87, 2.74]
Glycine	2.28	2.34	-2.44	0.023	[2.26 - 2.33]	2.19 – 2.46 [1.91, 2.68]
Isoleucine	2.45	2.53	-3.17	0.006	[2.40 - 2.51]	2.36 – 2.71 [2.03, 3.06]
Lysine	3.25	3.34	-2.49	0.030	[3.22 - 3.29]	3.07 – 3.48 [2.65, 3.85]
Valine	2.55	2.64	-3.47	0.003	[2.51 - 2.60]	2.48 – 2.91 [2.07, 3.26]
Meal Fiber (% DW)						
Neutral Detergent Fiber	8.55	6.81	25.47	0.016	[8.05 - 8.96]	6.20 – 10.58 [2.19, 13.59]
RBD Oil Fatty Acid (% Total FA)						
14:0 Myristic	0.031	0.090	-65.15	<0.001	[0.031 - 0.032]	0.066 – 0.11 [0.024, 0.14]
16:0 Palmitic	2.49	11.59	-78.50	<0.001	[2.36 - 2.69]	9.22 – 11.96 [7.75, 13.82]
16:1 Palmitoleic	0.13	0.11	22.10	0.012	[0.12 - 0.14]	0.072 – 0.11 [0.044, 0.14]
17:0 Margaric [Heptadecanoic]	0.036	0.10	-65.27	0.002	[0.031 - 0.048]	0.047 – 0.10 [0.0082, 0.16]
17:1 9c Heptadecenoic	0.12	0.031	279.62	0.006	[0.092 - 0.14]	0 < 0.02 not calculated
18:0 Stearic	3.22	4.47	-28.05	<0.001	[3.00 - 3.40]	3.58 – 5.00 [1.83, 6.48]

Table 19 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Processed Fraction Component Levels for MON 87705 vs. the Conventional Control (A3525) and Reference Varieties

Component (Units)[1]	MON 87705 Mean	Control Mean	Mean Difference (MON 87705 minus Control) Mean Difference (% of A3525)	p-Value	MON 87705 [Range]	Commercial Range[2] [99% Tolerance Interval[2]]
RBD Oil Fatty Acid (% Total FA)						
18:1 Oleic	71.51	23.16	208.71	<0.001	[69.30 - 73.01]	21.10 – 31.19 [11.72, 37.78]
18:2 6c,9c Octadecadienoic	0.20	0.65	-69.86	<0.001	[0.16 - 0.24]	0.031 – 0.074 [0, 0.13]
18:2 Linoleic	14.41	51.08	-71.78	<0.001	[12.25 - 17.39]	47.74 – 53.88 [42.34, 61.19]
18:2 Other Trans	0.18	0.63	-70.92	<0.001	[0.14 - 0.23]	0 < 0.02 Not calculated
20:0 Arachidic	0.29	0.36	-19.97	0.001	[0.27 - 0.31]	0.28 – 0.43 [0.13, 0.59]
20:1 11c Eicosenoic	0.33	0.19	73.17	<0.001	[0.29 - 0.37]	0.18 – 0.27 [0.066, 0.37]
22:0 Behenic	0.31	0.35	-9.74	0.001	[0.30 - 0.35]	0.30 – 0.50 [0.11, 0.70]

[1] DW = dry weight; FA = fatty acid.

[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

Table 20. Literature Ranges for Components in Soybean Meal

Tissue Component	Literature Range[1]
Proximates	**(% dw)**
Ash	5.2 – 9.1[a]
Carbohydrates	32.0 – 38.0[b]
Fat, total	0.5 -3.30[a]
Moisture (% fw)	5.58-11.7[a]
Protein	47.4 – 59.5[a]
Fiber	**(% dw)** [a]
Acid detergent fiber (ADF)	5.2 – 6.7
Neutral detergent fiber (NDF)	7.4 – 12.2
Amino Acids	**(%dw)**[a]
Alanine	2.18 – 2.59
Arginine	3.29 – 4.49
Aspartic acid	5.18 – 6.83
Cystine/Cysteine	0.6 – 0.92
Glutamic acid	8.05 – 11.21
Glycine	2.02 – 2.40
Histidine	1.32 – 1.63
Isoleucine	2.11 – 2.74
Leucine	3.62 – 4.72
Lysine	2.97 – 3.69
Methionine	0.5 – 0.9
Phenylalanine	2.39 – 3.19
Proline	2.32 – 3.05
Serine	1.97 – 3.3
Threonine	0.80 – 2.24
Tryptophan	0.60 – 2.08
Tyrosine	1.68 – 2.17
Valine	2.29 – 2.92
Anti-Nutrients	
Trypsin Inhibitors (TIU/mg dw)	3.8 – 17.9[a]
Phytic Acid (% dw)	1.3 – 4.1 [a]

[1]Literature range references: [a]Lundry et al. (2008). [b]Padgette et al. (1996b).

Table 21. Literature Ranges for Components in Soybean Oil

Tissue Component	Literature Range[1]
Fatty Acids (FA)	
14:0 Myristic	ND – 0.2[a]
16:0 Palmitic	7 - 12[b]
16:1 Palmitoleic	≤ 0.2[b]
17:0 Margaric [Heptadecanoic]	ND – 0.1[a]
17:1 9c Heptadecenoic	ND – 0.1[a]
18:0 Stearic	2 – 5[b]
18:1 Oleic	19 – 34[b]
18:2 Linoleic	48 – 60[b]
18:3 Linolenic	2 -10[b]
20:0 Arachidic	0.1 – 0.6[a]
20:1 Eicosenoic	ND – 0.5[a]
20:2 Eicosadienoic	ND – 0.1[a]
22:0 Behenic	ND – 0.7[a]
Vitamins	**mg/100g fw**
Vitamin E	0.9 – 35.2[a]

[1]Literature range references: [a]Codex, 2005 (% Total FA). [b]Lundry et al. (2008).
ND = not detected.

Table 22. Estimation of 17:1 9c Heptadecenoic Acid Intake in Selected Foods and MON 87705

Food	Level of 17:1 (g/100 g food)	RACC[1] (g)	Amount of 17:1 consumed per serving (mg/RACC)
Extra firm nigari tofu (16159)[2]	1.085	85	922.0
Ground beef, 80% lean (23572)[2]	0.135	85	115.0
Soft-spread margarine, 80% fat, canola based (04684)[2]	0.053	14	7.0
MON 87705	0.12	14	17.0

[1]Reference Amount Customarily Consumed, which is the U.S. serving size for labeling purposes (21 CFR 101.12).
[2]Number in parentheses refers to Nutrient Database Number used to identify a particular food within the USDA-ARS food composition database.

2.8 Nutritional Impact

MON 87705 soybean oil has an improved fatty acid profile compared to conventional soybean oil, therefore, the following sections describe the safety and nutritional assessment of the intended changes in MON 87705.

Safety and Nutritional Assessment of the Intended Changes in MON 87705
MON 87705 was developed to generate soybean oil with decreased levels of saturated fats (16:0 palmitic acid and 18:0 stearic acid) and increased levels of 18:1 oleic acid, with an associated decrease in 18:2 linoleic acid. Replacement of conventional soybean oil with MON 87705 soybean oil under the proposed food uses would result in a change in the fatty acid composition in the U.S. diet leading to higher oleic acid intake, and lower consumption of saturated fats (i.e., 16:0 palmitic and 18:0 stearic acid) with no impact on total fat intake. This assessment assumes all of the targeted oil components of the foods proposed for replacement that are consumed in the U.S. are replaced with MON 87705 soybean oil. Therefore, the results presented in this safety summary represent a theoretical maximal effect of MON 87705 soybean oil on fatty acid composition of the diet. The nutritional impact from the use of MON 87705 soybean oil in targeted foods under the intended conditions of use is estimated to result in changes in fatty acid consumption that are within current dietary guidelines for fatty acid intake (Lichtenstein et al., 2006; WHO, 2003).

Safety and Nutrition Assessment Conclusion
In conclusion, except for the intended changes in fatty acid profile, the compositional equivalence of MON 87705 seed, forage, and processed fractions to conventional soybean has been demonstrated in accordance with OECD guidelines. In addition, the nutritional impact from the use of MON 87705 soybean oil in targeted foods under the intended conditions of use is estimated to result in changes in fatty acid consumption that are within current dietary guidelines for fatty acid intake. Therefore, MON 87705 is regarded to be as safe and nutritious as conventional soybean for food and feed use.

a) Other Information Relevant to Nutritional Assessment of MON 87705

i) 90-day Study in the Rat

A 90-day toxicology assessment was conducted in rats to evaluate the potential health effects of processed soybean meal from MON 87705 as compared to processed meal from a conventional soybean variety, A3525 with similar background genetics to that of MON 87705, but does not express the biotechnology-derived traits. Additionally, diets were formulated using meal from three reference soybean varieties as groups representative of conventional soybean varieties.

Materials and Methods

Processed soybean meal (toasted and defatted) from MON 87705 was fed to rats for at least 90 days. Sprague-Dawley (Crl:CD®[SD]) rats, following acclimation, were randomly assigned to five groups consisting of 12 males and 12 females such that mean group body weights were ± 20% of the mean for each sex at the time of randomization (animal age six weeks). All diets were formulated to contain 30% (w/w) soybean meal. All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily and detailed physical examinations were performed weekly. Individual body weights and food consumption were recorded weekly. Clinical pathology evaluations (hematology, coagulation, serum chemistry and urinalysis) were performed on blood and urine samples collected from 10 randomly-selected, fasted animals by sex and group on the day of the scheduled necropsy (week 13). Complete necropsies were conducted on all animals, and selected organs were weighed at the scheduled necropsy. Approximately 23 tissues were examined microscopically from all animals fed the 30 % MON 87705 test diet and the 30% A3525 control diet.

Statistical analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 5% and 1%, comparing the MON 87705 treated group to the control group by sex. Body weight, cumulative body weight change, food consumption, clinical pathology and organ weight data were subjected to a parametric one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1980) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the MON 87705 treated group to the control group. Microscopic findings were compared using Fisher's exact test (Steel and Torrie, 1980).

Results

All animals survived to the scheduled necropsy. There were no MON 87705-related clinical observations. There were no statistical differences in body weights, cumulative body weight gains, or food consumption when test and control groups were compared. There were no test substance-related alterations in hematology, coagulation, serum chemistry or urinalysis parameters and no test substance-related effects on organ weights. Although there were a few statistical differences in these parameters, the differences were not considered MON 87705 related because the magnitude of changes was small, changes were not observed in both sexes, the values were typically within the range of those observed for control rats of the same age, sex and strain, and there were no apparent macroscopic or histological correlations to these findings. There were no MON 87705 related alterations in gross macroscopic or microscopic findings. Any alterations in macroscopic findings and microscopic findings were considered to be incidental, as there was no MON 87705 related alteration in the prevalence, severity or histologic character of any observed tissue alterations.

Conclusion

Administration of processed meal from MON 87705 for at least 90 consecutive days at a concentration of 30% (w/w) in the diet (equivalent to 22,095 mg/kg body weight/day for males and 25,433 mg/kg body weight/day for females) had no adverse effects on the growth

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or health of Sprague Dawley rats compared to the conventional soybean control. This 90-day rat assessment supports the conclusion that MON 87705 is as safe as conventional soybean from a food and feed perspective.

ii) 42-day Study in Broiler

A 42-day feeding assessment using Cobb × Cobb 500 broilers was conducted to compare the nutritional value of diets containing soybean meal produced from MON 87705 and a conventional soybean control, A3525, with similar background genetics to MON 87705. Six additional diets were included as references representative of the population of commercial soybean meal. The soybean meal component for the reference diets was provided by meal produced from processing of six conventional soybean varieties (Anand, Ozark, NK S38-T8, UA4805, NC+2A86, and NK25-J5).

Statistical Analyses

Statistical analyses were conducted on bird performance, carcass yield, and meat composition parameters. SAS®, version 9.2, was used to perform the analyses. Three types of statistical analyses were conducted based on analysis of variance (ANOVA) methods. The first analysis evaluated the effects of diet and gender. The main effects of diet and gender were evaluated at a 5% level of significance, whereas the diet × gender interaction was evaluated at a 15% level of significance. Diet means were averaged over gender unless the diet × gender interaction for a particular parameter was statistically significant. When a diet × gender interaction was detected ($p<0.15$), a second analysis was conducted for each gender at a 5% level of significance. A third analysis was performed to compare the response of broilers fed diets containing soybean meal produced from MON 87705 to that of the population of broilers fed the conventional soybean control and reference soybean meal containing diets.

Results

Broiler Mortality

Chick mortality by dietary treatment during the first seven days of the study, attributed predominantly to bacterial infection and dehydration, ranged from 0 to 2.5% (average of 1.1% across all dietary treatments). From Day 7 to Day 42 bird mortality averaged 1.3% and ranged from 0 to 2.0% across all dietary treatments. Mortality from Day 7 to Day 42 was 1.0% for birds receiving diets containing soybean meal produced from MON 87705. The surviving birds in all groups were in good health based on twice daily pen observations.

Performance and Carcass Measurements

Performance measurements, including Day 42 live bird weight (kg/bird), total feed intake (kg/bird), and unadjusted and adjusted feed to gain ratio (kg/kg), for broilers fed diets containing soybean meal produced from MON 87705 were not different ($p> 0.05$) than those of broilers fed diets containing conventional soybean control meal produced from conventional soybean with a genetic background comparable to MON 87705 (Table 23). Performance over the entire 42-day test period was also not different ($p>0.05$) for birds fed

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diets containing soybean meal produced from MON 87705 compared to the population of birds fed diets containing soybean meal produced from conventional soybean control or reference soybean varieties (Table 24). A diet × sex interaction was detected ($p<0.15$) for Day 42 bird weight (kg/bird) and bird weight gain (kg/bird) from Day 0 to Day 42; however, within sex analyses detected no treatment difference ($p>0.05$) in either parameter for male or female birds. Measures of bird performance were of similar magnitude for birds fed diets formulated to the same nutrient specifications with the soybean meal component of the diet provided by soybean meal produced from MON 87705, conventional soybean, or six conventional reference soybean varieties. No unexpected effects on broiler performance were observed when broilers were fed diets containing soybean meal produced from MON 87705 compared to diets containing soybean meal produced from conventional soybean control or reference soybean varieties.

Carcass yield measurements were not different ($p>0.05$) for broilers fed diets containing soybean meal produced from MON 87705 compared to those fed diets containing conventional soybean control soybean meal (Table 23). Similarly, carcass yield measurements were not different ($p>0.05$) for birds fed diets containing soybean meal produced from MON 87705 compared to the population of those fed diets containing conventional soybean control or reference soybean meal (Table 24). A diet × sex interaction was detected ($p<0.15$) for average pre-processing live weight (kg/bird), chilled carcass weight (kg/bird or % of live weight), fat pad weight (kg/bird), breast meat weight (kg/bird), thigh weight (kg/bird or % of chilled weight), and drum weight (kg/bird). Within sex analyses detected no difference ($p>0.05$) for any of these parameters between birds fed diets containing soybean meal produced from MON 87705 and the conventional soybean control for male or female birds, with exception of average breast weight for male birds (0.562 versus 0.592 kg/bird, respectively). No differences were detected ($p>0.05$) for any of the carcass yield measurements for which a diet × sex interaction was detected for either male or female birds fed diets containing soybean meal produced from MON 87705 versus the population of birds fed diets containing soybean meal produced from conventional soybean control or reference soybean varieties (Table 24). Average carcass measurements were of similar magnitude for birds fed diets formulated to the same nutrient specifications with the soybean meal component of the diet provided by soybean meal produced from MON 87705, conventional soybean control, or six conventional reference soybean varieties.

Meat composition (moisture, fat, and protein) was also not significantly different ($p>0.05$) for birds fed diets containing soybean meal produced from MON 87705 versus that of birds fed diets containing soybean meal produced from conventional soybean control or reference soybean based on individual diet comparisons or comparison to the population of control and reference soybean meal diets (Table 23 and 24). A diet × sex interaction was detected ($p< 0.15$) for breast meat moisture (%) and thigh meat moisture and protein (%); however, within sex comparison of these meat composition measurements detected no difference ($p>0.05$) among dietary treatments for any of the measurements for male or female birds.

Conclusions

In conclusion, there were no statistical differences in broiler performance, carcass yield, or meat composition between broilers fed diets containing soybean meal produced from

MON 87705 and those fed diets containing conventional soybean control soybean meal. The diets containing soybean meal produced from MON 87705 were as wholesome as the diets formulated with conventional soybean control or reference soybean meal regarding their ability to support the rapid growth of broiler chickens. These data support the conclusion that soybean meal produced from MON 87705 is as nutritious as conventional soybean meal.

Table 23. Performance, Carcass Yield, and Meat Quality of Broilers Fed Diets Containing MON 87705, Conventional Control, and Reference Soybean Meal (means[1] combined across males and females)

Treatment Number	3	8	4	7	6	5	1	2			
Soybean Meal ID	MON 87705	Control (A3525)	Anand	Ozark	NK S38-T8	UA4805	NC+ 2A86	NK 25-J5	SEM[2]	Treatment p-value[3]	LSD[4] 5.0%
Performance											
Average bird weight (g/bird), d 0	40.883	41.417	41.683	41.617	41.650	41.817	41.833	41.700	0.2811	0.3173	0.795
Average bird weight (kg/bird), d 42	2.607	2.622	2.640	2.560	2.591	2.625	2.614	2.678	0.0234	0.0506[8]	0.066
Average bird gain (kg/bird), d 0 to 42	2.566	2.580	2.598	2.519	2.549	2.583	2.572	2.636	0.0234	0.0507[8]	0.066
Feed intake (kg/bird), d 0 to 42	4.112^{bdc}	4.124^{bdc}	4.203^{ba}	4.091^{dc}	4.037^{d}	4.118^{bdc}	4.154^{bac}	4.234^{a}	0.0391	0.0225	0.111
Feed:gain (kg/kg), d 0 to 42	1.614	1.615	1.647	1.650	1.635	1.601	1.644	1.642	0.0199	0.5372	0.056
Adjusted feed:gain[5] (kg/kg), d 0 to 42	1.597^{dc}	1.592^{edc}	1.613^{ba}	1.622^{a}	1.577^{e}	1.589^{d}	1.608^{bac}	1.599^{bdc}	0.0055	<0.0001	0.016
Carcass Yield											
Processing live weight[6] (kg)	2.630	2.657	2.666	2.587	2.620	2.650	2.643	2.699	0.0244	0.0938[8]	0.069
Chilled carcass weight (kg)	1.909^{bac}	1.933^{ba}	1.931^{ba}	1.866^{c}	1.902^{bc}	1.924^{ba}	1.912^{bac}	1.960^{a}	0.0181	0.0387[8]	0.051
Chilled carcass weight (% of live wt.)	72.573^{a}	72.701^{a}	72.413^{ba}	72.091^{b}	72.593^{a}	72.582^{a}	72.349^{ba}	72.590^{a}	0.1288	0.0395[8]	0.364
Fat pad weight (kg)	0.040^{c}	0.040^{bc}	0.043^{a}	0.041^{bac}	0.041^{bac}	0.043^{a}	0.040^{bc}	0.042^{ba}	0.0009	0.0401[8]	0.003
Fat pad weight (% of live wt.)	1.519	1.522	1.620	1.577	1.564	1.631	1.530	1.573	0.0303	0.0718	0.086
Breast meat weight (kg)	0.549	0.562	0.558	0.541	0.539	0.551	0.546	0.565	0.0065	0.0520[8]	0.018
Breast meat weight (% of chilled wt.)	28.768	29.076	28.947	29.051	28.401	28.640	28.544	28.863	0.1687	0.0596	0.477
Thigh weight (kg)	0.331^{a}	0.334^{a}	0.333^{a}	0.320^{b}	0.332^{a}	0.335^{a}	0.331^{a}	0.339^{a}	0.0034	0.0307[8]	0.010
Thigh weight (% of chilled wt.)	17.295	17.280	17.233	17.147	17.453	17.408	17.309	17.273	0.0850	0.2818[8]	0.240
Drum weight (kg)	0.256^{a}	0.255^{a}	0.257^{a}	0.246^{b}	0.255^{a}	0.257^{a}	0.256^{a}	0.261^{a}	0.0026	0.0161[8]	0.007
Drum weight (% of chilled wt.)	13.390	13.181	13.310	13.179	13.393	13.348	13.375	13.320	0.0802	0.3286	0.227
Wing weight (kg)	0.200^{b}	0.200^{b}	0.201^{ba}	0.194^{c}	0.200^{ba}	0.201^{ba}	0.201^{ba}	0.206^{a}	0.0019	0.0228	0.006
Wing weight (% of chilled wt.)	10.465	10.341	10.426	10.418	10.536	10.456	10.498	10.501	0.0499	0.1917	0.141
Breast Meat Analysis[7]											
Moisture (%)	75.151	75.006	75.112	74.815	75.370	75.185	74.960	75.025	0.1567	0.3565[8]	0.443
Protein (%, as-is basis)	22.878	22.906	22.592	22.982	22.422	22.717	22.789	22.648	0.1519	0.1902	0.430
Fat (%, as-is basis)	1.130	1.031	1.159	1.178	1.094	0.993	1.125	1.131	0.0887	0.8241	0.251
Thigh Meat Analysis[7]											
Moisture (%)	77.179	76.990	77.294	77.036	77.268	77.193	77.276	77.338	0.1254	0.4428[8]	0.355
Protein (%, as-is basis)	20.248	20.340	20.338	20.261	20.141	20.295	19.851	19.744	0.2171	0.3636[8]	0.614
Fat (%, as-is basis)	1.528	1.726	1.880	1.954	1.796	1.681	1.608	1.733	0.1693	0.6961	0.479

[1] Each mean represents 10 observations (one per pen). [2] SEM = standard error of the mean for respective parameter. [3] p-value for test of dietary treatment effect, [a-d]Individual treatment means in the same row with the same superscript are not statistically different (p>0.05).

[4] LSD = least significant difference between two means (p<0.05). [5] Adjusted feed:gain is adjusted by adding the weight at removal of mortalities and culls to the weight of the live birds in a pen.

[6] Processing live weight = pre-processing weight on Day 43 (males) or Day 44 (females). [7] Mean values for skinless breast and thigh meat analyses and based on one bird per pen.

[8] A diet × sex interaction (p<0.15) was detected.

Table 24. Performance, Carcass Yield, and Meat Quality of Broilers Fed Diets Containing MON 87705 Soybean Meal Compared to Broilers Fed Diets Formulated with Conventional Control or Reference Soybean Meal (means[1] ± SEM[2] combined across males and females)

Parameter	Soybean Meal Diets		Treatment p-value[3]	LSD[4] 5%
	MON 87705	Control and References		
Performance				
Average bird weight (g/bird), d 0	40.883 ± 0.2661	41.674 ± 0.1006	0.0070	0.567
Average bird weight (kg/bird), d 42	2.607 ± 0.0369	2.618 ± 0.0139	0.7771[8]	0.097
Average bird gain (kg/bird), d 0 to 42	2.566 ± 0.0369	2.577 ± 0.0139	0.7916[8]	0.096
Feed intake (kg/bird), d 0 to 42	4.112 ± 0.0669	4.137 ± 0.0253	0.7389	0.175
Feed:gain (kg/kg), d 0 to 42	1.614 ± 0.0196	1.633 ± 0.0074	0.3560	0.042
Adjusted feed:gain[5] (kg/kg), d 0 to 42	1.597 ± 0.0154	1.600 ± 0.0058	0.8625	0.040
Carcass Yield				
Processing live wt[6] (kg/bird)	2.630 ± 0.0353	2.646 ± 0.0133	0.6848[8]	0.092
Chilled wt (kg/bird)	1.909 ± 0.0294	1.918 ± 0.0111	0.7804[8]	0.077
Chilled wt (% of live wt.)	72.573 ± 0.2115	72.474 ± 0.0800	0.6692[8]	0.493
Fat pad wt (kg/bird)	0.040 ± 0.0013	0.041 ± 0.0005	0.2216[8]	0.003
Fat pad wt (% of live wt.)	1.519 ± 0.0409	1.574 ± 0.0155	0.2531	0.107
Breast wt (kg/bird)	0.549 ± 0.0102	0.552 ± 0.0039	0.7775[8]	0.027
Breast wt (% of chilled wt)	28.768 ± 0.2627	28.789 ± 0.0993	0.9445	0.687
Thigh wt (kg/bird)	0.331 ± 0.0057	0.332 ± 0.0022	0.8058[8]	0.015
Thigh wt (% of chilled wt)	17.295 ± 0.1352	17.300 ± 0.0511	0.9707[8]	0.315
Drum wt (kg/bird)	0.256 ± 0.0046	0.255 ± 0.0017	0.9196[8]	0.012
Drum wt (% of chilled wt)	13.390 ± 0.0876	13.301 ± 0.0331	0.3756	0.229
Wing wt (kg/bird)	0.200 ± 0.0033	0.200 ± 0.0013	0.8562	0.009
Wing wt (% of chilled wt)	10.465 ± 0.0654	10.454 ± 0.0247	0.8778	0.171
Breast Meat Analysis[7]				
Moisture (%)	75.151 ± 0.1990	75.067 ± 0.0752	0.7020[8]	0.464
Protein (%, as-is basis)	22.878 ± 0.1909	22.722 ± 0.0721	0.4734	0.499
Fat (%, as-is basis)	1.130 ± 0.0880	1.102 ± 0.0332	0.7605	0.187
Thigh Meat Analysis[7]				
Moisture (%)	77.179 ± 0.1838	77.199 ± 0.0695	0.9194[8]	0.428
Protein (%, as-is basis)	20.248 ± 0.2769	20.139 ± 0.1047	0.7176[8]	0.645
Fat (%, as-is basis)	1.528 ± 0.1636	1.768 ± 0.0618	0.1734	0.349

[1] Each mean for MON 87705 represents 10 observations (one per pen) and the control and references represents 70 observations (one per pen).

[2] SEM = standard error of the mean for respective parameter.

[3] MON 87705 diet versus the population of the control and six reference diets.

[4] LSD = least significant difference between two means ($p<0.05$).

[5] feed:gain is adjusted by adding the weight at removal of mortalities and culls to the weight of the live birds in a pen.

[6] Processing live weight = pre-processing weight on Day 43 (males) or Day 44 (females).

[7] Mean values for skinless breast and thigh meat analyses based on one bird per pen.

[8] A diet × sex interaction ($p<0.15$) was detected.

2.9 Other Techincal Information

a) Detection Methodology

The partial sequence of the soybean FAD2-1A gene and the FATB1-A gene can be detected by isolating DNA from leaf tissue and digesting with appropriate restriction endonucleases. Southern Blot analysis following digestion of genomic DNA with the appropriate restriction endonucleases will produce banding patterns consistent with the presence of the insert in MON 87705.

b) Projected Market Penetration

Oil from MON 87705 is anticipated to have enhanced oxidative stability relative to conventional soybean oil due to its increase in monounsaturated fatty acid and decrease in PUFAs. Therefore, MON 87705 soybean oil provides more stable oil for use in food formulation.

The fatty acid profile of MON 87705 soybean oil is also well suited for industrial applications. Soybean oils have very good lubricating properties, and are highly biodegradable compared to mineral oils, but typically lack the stability needed to meet industrial requirements. Hydrogenation of soybean oil is not acceptable for most industrial uses because it leads to formation of saturated and *trans* fatty acids which can cause the oil to be solid at lower temperatures, resulting in excessive wear and tear of machinery (Kinney, 1998). The fatty acid profile of MON 87705 provides an industrial oil with improved stability that could serve as a lubricant without needing hydrogenation. In addition, soybean oil with elevated oleic acid is an attractive source for other industrial applications, such as a replacement for petrochemical-derived plasticizers (Kinney, 1998). The higher oleic acid and lower saturated fat levels of MON 87705 also make it much more suitable for use in biodiesel due to its greater stability, improved cold weather performance, and reduced nitrous oxide emissions (Bringe, 2005; Graef et al., 2009; Knothe, 2005).

As with all new biotechnology-derived traits, MON 87705 will be bred into soybean varieties with diverse genetic backgrounds. These varieties will include commercial varieties with low linolenic acid levels which can further enhance the oxidative stability of the soybean oil. In addition, MON 87705 will be combined using traditional breeding methods with other biotechnology-derived traits, including glyphosate tolerance, to deliver the best agronomic platform to growers.

To summarize, MON 87705 was developed to improve soybean oil's oxidative stability profile and lower the saturated fat content of the oil. Due to the compositional improvement in MON 87705 soybean oil, MON 87705 could expand the food market applicability of soybean oil, without contributing further to known dietary health risks or sacrificing food functionality. Similarly, MON 87705 soybean oil could also serve as an improved source for industrial and biofuel products.

There are no current plans to produce MON 87705 commercially in Australia or New Zealand. However, soybean grains and products containing processed soybean may potentially be imported into Australia and New Zealand from countries where MON 87705 is intended for commercialisation.

PART 3 REGULATORY / LEGISLATIVE IMPLICATIONS

3.1 Other Approvals

a) Relevant Overseas Approvals

Monsanto has submitted a food and feed safety and nutritional assessment summary for MON 87705 to the United States Food and Drug Administration (FDA) in October 2009 and has also requested a Determination of Nonregulated Status for MON 87705 including all progenies derived from crosses between MON 87705 and other soybean, from the United States Department of Agriculture-Animal and Plant Health and Inspection Service in July 2009.

Regulatory submissions have been or will be made to countries that import significant soybean or food and feed products derived from U.S. soybean and have functional regulatory review processes in place. These 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, the Korean Food and Drug Administration (KFDA) and the Rural Development Administration (RDA), Canadian Food Inspection Agency (CFIA) and Health Canada, and the European Food Safety Authority (EFSA). As appropriate, notifications will be made to countries that import significant quantities of U.S. soybean and soybean products and do not have a formal regulatory review process for biotechnology-derived crops.

b) Approval Refusal

No application has been rejected or withdrawn by any regulatory body.

3.2 Regulatory Impact Statement

As described previously, soybean is one of the most agriculturally important crops in the world. This application – if approved – will ensure imports of food derived from MON 87705 soybean comply with the *Australian New Zealand Food Standards Code*. This will ensure that there is no potential for trade disruption on regulatory grounds.

PART 4 STATUTORY DECLARATION – AUSTRALIA

I, Amanda Forster, declare that the information provided in this application fully sets out the matters required and that the same are true to the best of my knowledge and belief, and that no information has been withheld that might prejudice this application.

Signature: _____

Declared before me ……

This …….. day of …….. 2010.

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