PLANT PEST RISK ASSESSMENT FOR MON 87769 SOYBEAN

Monsanto Company (Monsanto) has petitioned APHIS for a determination that the genetically engineered (GE) stearidonic acid soybean (*Glycine max*) event MON 87769 **(**hereafter referred to as MON 87769**)** is unlikely to pose a plant pest risk (Monsanto 2009) and, therefore, is no longer a regulated article under the APHIS' 7 Code of Federal Regulations (CFR) part 340. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act of 2000[1]. This plant pest risk assessment was conducted to determine if MON 87769 is unlikely to pose a greater plant pest risk than the unmodified soybean line with which it was derived.

MON 87769 was produced by transformation of soybean tissue using *Agrobacterium tumefaciens,* a plant pest, and some of the sequences used in the transformation process are also from plant pests, including some either retained in MON 87769 (i.e., *tml* terminator sequence and left and right T-DNA border sequences from *A. tumefaciens*) or eliminated (35S RNA from figwort mosaic virus); therefore, the engineered soybean has been considered a regulated article under APHIS regulations at 7 CFR part 340.

Potential impacts to be addressed in this risk assessment are those that pertain to the use of MON 87769 and its progeny in the absence of confinement. APHIS uses data and information submitted by the applicant, in addition to current literature, to assess if MON 87769 is unlikely to pose a plant pest risk. If APHIS determines that a GE organism is unlikely to pose a plant pest risk, then APHIS has no regulatory authority over that organism.

Potential impacts in this Plant Pest Risk Assessment are those that pertain to plant pest risk characteristics. APHIS regulation 7 CFR 340.6(c) specifies the information needed for consideration in a petition for nonregulated status. APHIS will evaluate information submitted by the applicant related to plant pest risk characteristics, disease and pest susceptibilities, expression of the gene product, new enzymes, or changes to plant metabolism, weediness of the regulated article, any impacts on the weediness of any other plant with which it can interbreed, and transfer of genetic information to organisms with which it cannot interbreed.

An analysis of agricultural or cultivation practices and indirect plant pest effects on other agricultural products associated with MON 87769, and impacts of MON 87769 on the environment will be considered in the Environmental Assessment (EA) for MON 87769. Likewise, a thorough assessment of the effects of the determination on nontarget organisms, beneficial organisms, and threatened and endangered species will be considered in the EA.

[1] Plant Protection Act in 7 U.S.C. 7702 § 403(14) defines plant pest as: "Plant Pest - The term "plant pest" means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs."

Introduction

a. History of Development of MON 87769 Stearidonic Acid (SDA) Soybean

Soybean has been cultivated extensively and improved through conventional breeding programs following its introduction in the U.S. and subsequently has become a key source of nutrients for food and feed use in the U.S. (Hymowitz and Singh 1992). Monsanto developed SDA MON 87769 by using recombinant DNA technology to genetically engineer (GE) into a conventional soybean variety two foreign desaturase genes, *Primula juliae* Δ6 desaturase (*Pj.D6D)* and *Neurospora crassa* Δ15 desaturase (*Nc.Fad3),* whose combined action results in the production of two fatty acids, stearidonic acid (SDA) and γ- linolenic acid (GLA), that do not occur in conventional soybeans (Figure 1 below, adapted from Figure VII-1, p. 103, Monsanto 2010).

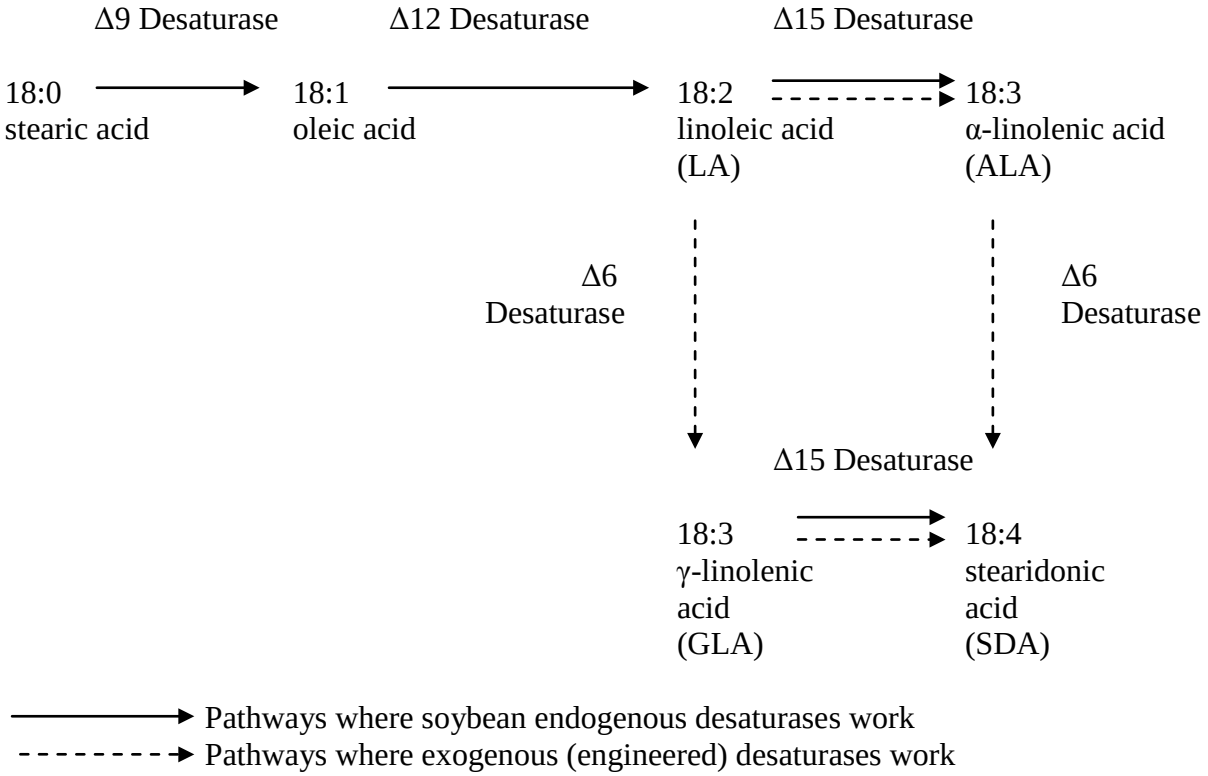

Figure 1. Fatty acid biosynthesis in plants and the introduced changes to changes to produce MON 87769 Soybean (adapted from Monsanto 2010, Figure VII-1)

MON 87769 seed oil consists of approximately 20-30 percent stearidonic acid (SDA), a sustainable alternate source of an omega-3 fatty acid to help meet the needed dietary intake of long chain omega-3 fatty acids (Harris et al. 2008). SDA is an omega-3 polyunsaturated fatty acid (PUFA) composed of an 18-carbon chain with four double bonds (18:4). Although SDA is found in fish, fish oils, green leafy vegetables, and some nuts and vegetable oils, it occurs relatively high levels in only a limited number of fish, and seaweed, and rarely in other plant species used as food sources (Figure 2 in Guil-Guerrero 2007; Whelan 2009). In mammals, SDA is a metabolic intermediate in the production of the longer carbon chain omega-3 PUFAs eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6) from alpha linolenic

acid (ALA; 18:3), a common dietary constituent of vegetable oils. In addition to SDA, MON 87769 seed oil contains approximately 7% γ- linolenic acid (GLA; 18:3), an omega-6 PUFA which is an *in vivo* metabolite in the conversion of linoleic acid (LA: 18:2) to arachidonic acid (20:4) in mammals (p. 4, Monsanto 2010). Although the therapeutic and health-promoting effects of consuming omega-3 long chain PUFAs from fish (SA, EPA, and DHA) are widely recognized, typical Western diets contain very little fish, and the same benefits may not be shared by their more commonly consumed precursor alpha linolenic acid (ALA). In contrast, comparative results suggest that SDA shares many of the biological effects of the omega 3 longer chain PUFAs and functions most similarly to dietary EPA, compared with ALA, when consumed in a typical Western diet (Whelan 2009). However, the lower number of double bonds in SDA compared to EPA and DHA makes it more stable to oxidation (less prone to fishy or rancid orders and taste) which is expected to expand its use as an alternative to fish oil in food products. While 6% of the world's fish oil is used for human consumption and an even amount for animal feed, 87 % is used in fish feed largely to support aquaculture (Whelan 2009). Oils enriched in SDA (such as those from *Echium*) or SDA itself have been shown to provide similar results as fish oil with respect to growth and feed efficiency in some fish species (e.g. Arctic char, cod, Atlantic salmon, and hybrid striped bass) (Bharadwaj et al 2010). According to Monsanto, MON 87769 can serve as an alternate source to help meet the needed dietary intake of long chain omega-3 fatty acids in food and animal feed (poultry and aquaculture).

b. Description of the Inserted Genetic Elements

MON 87769 was developed through *A. tumefaciens* mediated transformation of soybean line A3525, a nontransgenic, mid-maturity group III, conventional variety with very high yield potential (Steffen 2004). The disarmed *A. tumefaciens* used for the transformation harbors a plasmid vector PV-ZMPQ1972 that contains two separate T-DNAs (Figure IV-1, p. 45, Monsanto 2010). The first T-DNA, designated as T-DNA I, contains two expression cassettes: *Primula juliae* Δ6 desaturase (*Pj.D6D*) gene expression cassette and *Neurospora crassa* Δ15 desaturase 3 (*Nc.Fad3)* gene expression cassette. The second T-DNA region (T-DNA II) contains the *cp4 epsps* gene expression cassette that encodes the CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium sp.* strain CP4) that provides tolerance to the action of glyphosate, which is the active ingredient in Roundup® agricultural herbicides.

T-DNA I region incorporated into MON 87769 contained the following genetic elements (Table V-1, p. 50, Monsanto 2010):

- Border Sequence: Left and right T-DNA border sequences from *A. tumefaciens* to facilitate T-DNA transfer (Depicker et al. 1982; Barker et al. 1983).
- *7Sα* Promoter: Promoter and leader from the *Sphas1* gene of soybean encoding beta-conglycinin storage protein (Doyle et al. 1986) that directs mRNA transcription in seed.
- *Δ6D* gene: *Δ6d* gene coding sequence from *Primula juliae* (Ursin et al. 2008) that codes for Δ6 desaturase, which creates a double bond at the 6th position from the carboxyl end of a fatty acid.
- *tml* terminator: 3′ untranslated region of the *tml* gene from *A. tumefaciens* octopine-type Ti plasmid (Kemp et al. 2000).

- *7Sα* promoter: Promoter and leader from the *Sphas2* gene from soybean encoding the alpha subunit of beta-conglycinin storage protein (Wang and Dubois 2004) that directs mRNA transcription in seed.
- *Fad (fatty acid desaturase) 3* gene: *Fad3* gene coding sequence from *Neurospora crassa* (Ursin et al. 2006) that codes for Δ15 desaturase, which creates a double bond at the 15th position from the carboxyl end of a fatty acid.
- *E-9* terminator: 3′ untranslated region of the pea ribulose 1,5-bisphosphate carboxylase small subunit 2 (*rbcS2)* gene which functions to direct polyadenylation of the mRNA (Coruzzi et al. 1984).

In addition to the above-mentioned genetic elements, the insert also contained noncoding intervening sequences of 8-101 base-pair length that were used for DNA cloning.

T-DNA II region, ***which was bred out of the final MON 87669 event***, contained the following genetic sequences (Figure IV-1, pp. 45-47, Monsanto 2010):

- Border Sequence: Left and right T-DNA border sequences from *A. tumefaciens* to facilitate T-DNA transfer (Depicker et al. 1982; Barker et al. 1983).
- *FMV* promoter: Promoter for the 35S RNA from figwort mosaic virus (FMV) (Rogers, 2000) that directs transcription in plant cells.
- *ShkG* leader sequence: 5' untranslated leader sequence from the *Arabidopsis ShkG* gene encoding EPSPS (Klee et al. 1987) that helps regulate gene expression.
- *CTP2* coding sequence: Transit peptide region of *Arabidopsis thaliana* EPSPS (Klee et al. 1987) that directs transport of the CP4 EPSPS protein to the chloroplast.
- *cp4 epsps* coding sequence: 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. 1996).
- *E9 terminator:* 3' untranslated region of the pea *rbcS2* gene which functions to direct polyadenylation of the mRNA (Coruzzi et al. 1984).

Monsanto provided evidence demonstrating that,

- the *A. tumefaciens* strain ABI that was used to transform MON 87769 was made nonpathogenic by removing the pathogenic sequences encoding phytohormones that cause crown gall tumors from the Ti (tumor inducing) plasmid present in the *Agrobacterium* (Koncz and Schell 1986);
- the final product does not contain any of the backbone sequences outside of the T-DNA I borders from the transformation vector, PV-GMPQ1972 (Figures V-3, p. 53; V-5, p. 59, Monsanto 2010);
- the final product does not contain T-DNA II elements that contain the *cp4 epsps* gene (Figure V-6, p. 62, Monsanto 2010);
- the DNA inserted into the soybean genome is present at a single locus and contains one functional copy of *D6D* (Figure V-8, p. 69, Monsanto 2010) and *fad3* (Figure V-11, p. 72, Monsanto 2010) genes;
- the sequence and organization of inserted genes in MON 87769 are identical to their original sequences and arrangements in the donor plasmid PV-GMPQ1972 (based on the DNA sequence alignment between the MON 87769 insert sequence to the PV-

GMPQ1972 transformation vector sequence) (Figure V-3, p.53 and p. 81, Monsanto 2010);

- no novel open reading frames were created that spanned either the 5′ or 3′ junctions between the T-DNA and soybean genomic sequences;
- the stability of the introduced genes was demonstrated by the presence of introduced genes of T-DNA I via Southern blot fingerprint of MON 87769 (Figures V-14 and V-15, pp. 78-79, Monsanto 2010) from five segregating generations tested (Monsanto 2010, Figure V-13, p. 77) in the breeding history. The stability was further confirmed by the Mendelian inheritance of the T-DNA I in MON 87769 (Table V-3, p. 84, Monsanto 2010) in three segregating generations (Figure V-17, p. 83, Monsanto 2010).

Because the T-DNA II region, containing the herbicide resistant gene cp4 epsps and associated regulatory elements, has been removed from the final MON 877769 event seeking deregulation, those genetic sequences are excluded from the plant pest risk assessment discussion presented in the following paragraphs.

PLANT PEST RISK ASSESSMENT

Based on information on the biology of soybean (OECD 2000), data presented by Monsanto Company (Monsanto 2010), and scientific data relevant to the discussion of plant pest risk, APHIS makes the following conclusions for MON 87769:

Potential Impacts of Genetic Modifications on Disease and Pest Susceptibilities

USDA-APHIS assessed whether MON 87769 soybean is likely to have significantly increased disease and pest susceptibility. This assessment encompasses a thorough consideration of introduced traits, their impact on agronomic traits and plant composition, and interactions with pests and diseases.

Monsanto used well-established qualitative or quantitative techniques (Appendix I, pp. 344-346, Monsanto 2010) to measure field trials for damage due to diseases (Alternaria leaf spot, Anthracnose, Asian rust, Bacterial blight, Bacterial pustule, Brown spot, Brown stem rot, Cercospora leaf blight, Charcoal rot, Downy mildew, Frogeye leaf spot, *Fusarium*, *Phytophthora*, Powdery mildew, *Pythium*, *Rhizoctonia*, *Sclerotinia*, *Septoria*, Soybean mosaic virus, Soybean rust, Stem canker, Sudden death, White mold) and insect pests (Aphid, Bean leaf beetle, Blister beetle, Corn rootworm, Flea beetle, Grasshopper, Green cloverworm, Japanese beetle, Leafhopper, Leafroller, Mexican bean beetle, Spidermite, Seed corn maggot, Soybean stem borer, Stink bug, Tarnished plant bug, Thistle caterpillar, Thrips, Velvetbean caterpillar, White fly, Wireworm, and Woolybear caterpillar) for two years conducted across different agroecological conditions. Data were collected on pest and disease damage across 17 sites in 2006 and 4 sites in 2007, and 4 sites in 2006 and 2007 were quantitatively tested for abundance of arthropods. There was a slight difference in susceptibility to frog eye leaf spot at one site in 2006. There was a significant difference (increase) in the abundance of tarnished plant bug and two beneficial arthropods at one site (IL1) during one collection point and for leafhopper and stinkbug at one collection point at one location (MI). However, these differences were either within the range of the reference varieties and /or were not found to be significantly different in other locations. The data submitted by Monsanto indicated no meaningful differences between

MON 87769 soybean and the non-transgenic counterparts for diseases (Appendix Tables I-3 and I-4, pp. 349-350, Monsanto 2010), or insect pests (Appendix Tables I-5 to I-7, pp. 351-355; Table I-9, pp. 358-360, Monsanto 2010). Thus MON 87769 is expected to be susceptible to the same plant pathogens and insect pests as conventional soybean.
Monsanto's aforementioned data (expression of the gene products, nutritional and compositional analyses) indicate that MON 87769 is not biologically different from conventional soybean (with the exception of the Δ6D and Δ15D proteins) and modified fatty acid phenotypes did not alter the pest and disease incidences on Mon 87769; therefore, MON 87769 is no more susceptible to pests and diseases compared to conventional soybean cultivars.

Expression of the Gene Product, New Enzymes, or Changes to Plant Metabolism

The Δ6D protein expressed in MON 87769 is nearly identical to the native protein produced by *Primula juliae* containing 446 amino acids and three histidine motifs that are characteristic of integral membrane desaturases (Figure VI-1, pp. 87-89, Monsanto 2010; Ursin et al. 2008). Furthermore, the petitioner's analysis of the Δ6D deduced amino acid sequence has shown that, the MON 87769 Δ6D is similar to other Δ6 desaturases in amino acid composition and enzymatic structure (Nakamura and Nara 2004). Likewise, the Δ15D protein produced in MON 87769 is identical to the native protein produced by *Neurospora crassa*, with the exception of a single amino acid change from threonine to alanine at the first amino acid after the start codon. The petitioner deliberately introduced this change to facilitate the insertion of the gene into the plant transformation vector. The MON 87769-expressed Δ15D protein consists of 429 amino acids and three histidine motifs that are characteristic of integral membrane desaturases (Figure VI-3. p. 89, Monsanto 2010).

The functional activities of the Δ6D and Δ15D proteins were confirmed *in vivo* after expression from the yeast expression vector in *Saccharomyces cerevisiae* (see pp. 87-90, Monsanto 2010 for details). Monsanto collected tissue samples from leaf, forage, root, mature seed and immature seed to quantify Δ6D and Δ15D protein expression in MON 87769 (see Appendix D for the protein expression analysis, pp. 237-239, Monsanto 2010). Because the expression of the PjΔ6D and NcΔ15D proteins is driven by a 7Sα' and 7Sα seed specific promoter, respectively, those two proteins were not detected in leaf and root tissues of MON 87769, while both proteins were detected in immature seed and at much lower levels in mature seed and forage, which contains a small of amount of residual immature seeds (Tables VI-1, p. 95 and VI-2, p. 96, Monsanto 2010). The highest levels of PjΔ6D and NcΔ15D proteins measured in immature seeds of MON87769 were 210 and 330 μg/g of tissue on a dry weight (DW) basis, respectively, and in the mature seed they represent no more than 0.00043% and 0.00239% of the total protein. As expected, the Δ6D and Δ15D proteins were not detected in the conventional soybean control, A3525 (p. 94, Monsanto 2010).

USDA-APHIS assessed whether changes in plant metabolism or composition in MON 87769 is likely to alter plant pest risk. The assessment encompasses a consideration of the expressed protein or enzymes and their effect on plant metabolism and an evaluation of whether the nutrients and antinutrient levels in harvested seed and forage derived from MON 87769 are comparable to those in the conventional soybean control, A3525.

Detailed compositional and nutritional comparisons of MON 87769, the conventional soybean control A3525, and ten commercially available soybean varieties were conducted on samples collected from fives sites across the U.S. in 2006. The analysis included protein, fat, carbohydrates, fiber, ash, moisture, amino acids, fatty acids, vitamin E, and antinutrients and key secondary metabolites, consistent with OECD guidelines (OECD 2001). Appendix E (pp. 240-315, Monsanto 2010) describes the materials and methods used for the compositional analysis. In all, 75 different analytical components were measured, 68 in seed and seven in forage. Of the measured components, 26 in seed had more than 50% of the observations below the assay limit of quantitation (LOQ) and were excluded from the statistical analysis. The overall data set was examined for evidence of biologically relevant changes.

The compositional analyses confirmed that MON 87769 had the intended change in fatty acid composition, while the other components analyzed in MON 87769 were compositionally equivalent to conventional soybean. As intended, MON 87769 seed contained relatively high levels of SDA and GLA and low levels of two minor fatty acids, trans-SDA and trans-ALA that were not found in conventional soybean (Table VII-1, p. 101, Monsanto 2010). The two trans fatty acids are known to form during seed extraction and processing. The mean percent of total fatty acid and mean percent dry weight for each of these fatty acids combined across sites is summarized below for MON 87769.

Seed Fatty Acid (FA)	**% Total FA**	**Range**	**% Dry Weight**	**Range**
18:4 Stearidonic (SDA)	26.13	16.83 - 33.92	3.94	2.77 - 4.91
18:3 γ-Linolenic (GLA)	7.09	6.07 – 8.03	1.09	0.93 - 1.22
18:4 6c,9c,12c,15t (trans-SDA)	0.18	0.058 - 0.26	0.027	0.011 – 0.036
18:3 9c,12c,15t (trans-ALA)	0.44	0.38 – 0.48	0.068	0.055 – 0.081

Statistical analysis was conducted on the composition data for other components for evidence of biologically-relevant changes. 18:2 LA is the most abundant fatty acid in conventional soybean. Considering LA is the starting material from which SDA and GLA are produced, the LA levels were significantly different (lower) in MON 87769 (18.46 % of total FA) compared to conventional soybean A3525 (54.90 % of total FA). As anticipated, the LA values were also well outside the 99% tolerance interval for the population of conventional references as well as the range of values found in the published literature and the International Life Science Institute Crop Composition Database (http://www.cropcomposition.org/query/index.html; Ridley et al. 2004). In addition to LA, combined-site statistical differences were found in five other fatty acid levels (16:0 palmitic, 18:1 oleic, 18:3 linolenic, 20:0 arachidic, and 22:0 behenic acid). The difference in the level of each of these five fatty acids was relatively small in absolute magnitude (5% or less of total fatty acids) and/or their mean values and ranges in MON 87769 seed were within the 99% tolerance interval for the population of the conventional reference varieties (Table VII-2, pp. 106-112, and Table E-4 pp. 260-261, Monsanto 2010). Given the intended shift in the fatty acid metabolism toward an increase in SDA content in MON 87769, differences in fatty acid levels were expected.

Statistical comparisons between MON 87769 and the conventional control for the presence of other components showed that 22 non-fatty acid analytes in soybean seed were significantly different ($p<0.05$) in the combined-site analysis (Table VII-2, pp. 106-112 and Appendix Table

E-14, pp. 294-295, Monsanto 2010). For the 22 non-fatty acid analytes, most of the statistical differences between MON 87769 and conventional soybean control were seen in amino acids (17 out of the 22 significant differences) and the magnitude of these differences were small (10% or less). The mean and range values of these amino acids, as well as those of seed proteins, carbohydrates, and isoflavones which were also significantly different in the combined site analysis, were within the calculated 99% tolerance interval for the population of commercial conventional soybean varieties and also within the range of values found in the published literature and the International Life Science Institute Crop Composition Database (http://www.cropcomposition.org/query/index.html; Ridley et al. 2004). The three isoflavones, genistein, daidzein and glycitein, were the only antinutritional factors among those described as naturally occurring in soybean seed (which also include trypsin inhibitors, lectins, stachyose, raffinose, and phytic acid) that were found to be significantly different in the combined site analysis. These differences were not considered to be biologically meaningful from a food and feed safety or nutritional perspective. Combined-site analysis of forage showed no significant differences ($p>0.05$) between MON 87769 and the conventional soybean control.

In addition to the compositional analysis of seed and forage, four soybean processed fractions (oil, meal, lecithin, and protein isolate) were produced from MON 87769 seed and subjected to compositional analysis in accordance with OECD guidelines (OECD 2001). As expected, apart from the intended fatty acid change, the composition of the soybean processed fractions from MON 87769 is equivalent to the composition of soybean processed fractions from the conventional soybean control (Appendix Table E-15 to E-18, pp. 303-313, Monsanto 2010). Thus, the processed fractions from MON 87769 are concluded to be as safe and nutritious as the processed fractions prepared from conventional soybean. Collectively, the compositional data support the conclusion that MON 87769, with the exception of the expected changes in fatty acid composition, does not have biologically meaningful differences from conventional soybean from a food/feed safety and/or nutritional perspective.

Based on all the above noted considerations, APHIS concludes that MON 87769 poses no more of a plant pest risk from new gene products, changes to plant metabolism or composition than conventional soybean. The expression of the introduced Δ6 desaturase and Δ15 desaturase proteins and intended changes in fatty acid composition do not appear to affect pest or disease susceptibility.

Weediness of MON 87769

Soybean is a highly domesticated legume species, and cultivated varieties of soybean in the U.S. do not exhibit weedy characteristics, nor is soybean listed as a weed in any major weed references (Crockett 1977; Holm et al. 1979; Muenscher 1980). Likewise, soybean is not identified as a noxious weed in the Federal Noxious Weed List (7 CFR § 360; http://plants.usda.gov/java/noxious?rptType=Federal). Moreover, soybean does not possess any of the attributes commonly associated with weeds (Baker 1965), such as long persistence of seed in the soil, the ability to disperse, invade, and become a dominant species in new or diverse landscapes, or the ability to compete well with native vegetation.

Phenotypic and agronomic characteristics of MON 87769 were evaluated in a comparative manner to assess plant pest potential (OECD 1993). These assessments included 14 plant growth

and development characteristics, five seed germination parameters, two pollen characteristics, six plant-symbiont interaction characteristics, and observations for plant-insect and plant-disease interactions and plant responses to abiotic stressor (Table VIII-1, p. 116, Monsanto 2010). Monsanto presented the following results to show that Mon 87769 is phenotypically and agronomically similar to conventional control of reference varieties:

- Seed dormancy is one of the potential traits effecting volunteerism and weediness. A total of 25 comparisons were made between MON 87769 and the conventional control for seed germination characteristics (Tables VIII-1 and VIII-2, p. 121-122, Monsanto 2010). No statistically or biologically significant differences were detected between MON 87769 and the conventional control for percent normal germinated, abnormal germinated, viable hard, dead, or viable firm-swollen seed in the AOSA (Association of Official Seed Analysts) temperature regime (20/30 °C) or for percent germinated, viable hard, dead, or viable firm-swollen seed in the additional temperature regimes (10, 20, 30, 10/20, 10/30 °C; Table VIII-3. p. 122, Monsanto 2010). Furthermore, the germination of both MON 87769 (89.1%) and the conventional control (92.1%) at 20/30 ºC exceeded the accepted standard of 80% minimum germination for certified soybean seed recommended by the Official Seed Certifying Agencies of North America (AOSCA 2003). Thus, the germination values of MON 87769 were not different from the A3525 conventional control, an established commercial soybean variety, and were within the range of accepted germination values for certified soybean seed. Soybean lacks seed dormancy (Monsanto Field Test Reports) and a majority of viable seeds germinate under adequate temperature and moisture. Although soybean seeds can potentially grow as volunteer plants in a subsequent crop rotation, volunteer plants are most likely be killed by frost in the soybean growing regions during autumn or winter of the year they were produced. Even if soybean volunteers get established, there are effective weed management strategies to control such volunteers (OECD 2000; York et al. 2005).

- Fourteen plant growth, development, and yield characteristics (early stand count, seedling vigor, plant growth stage, days to 50% flowering, flower color, plant pubescence, plant height, lodging, pod shattering, final stand count, seed moisture, 100 seed weight, test weight, and yield.) were assessed under field conditions as part of the plant characterization assessment of MON 87769. Data were collected at 21 field locations over two years that provided a diverse range of environmental and agronomic conditions representative of commercial soybean production areas in the U.S. (Table VIII-4, p. 125, Monsanto 2010). The results of this assessment showed the introduced trait did not unexpectedly alter the phenotypic or agronomic characteristics of MON 87769 compared to conventional soybean and are indicative of no increased weediness of MON 87769 (Tables VIII-5 and VIII-6, pp. 126-127; Monsanto 2010; for a detailed field analysis see Appendix G, pp. 321-340, Monsanto 2010).

- Increased pollen viability and altered pollen morphology might be beneficial for plant species to acquire weedy characteristics such as higher seed production or increased pollen dispersal facilitating gene flow and invasiveness (Lu et al. 2008). Pollen was collected from MON 87769, the control (A3525), and four commercial reference soybean varieties grown under similar agronomic conditions. No statistically significant

differences were detected between MON 87769 and the control for percent viable pollen or pollen grain diameter (Table VIII-7, p. 130, Monsanto 2010). Furthermore, no visual differences in general pollen morphology were observed between MON 87769 and the control. These results demonstrate that the introduced genes and proteins produced from them did not alter the overall morphology or viability of MON 87769 pollen compared to the conventional soybean control. Since soybean is highly self-pollinating, this observation is consistent with the similar seed yield obtained from MON87769 and the control (A3525) soybean in field tests.

- A total of 703 comparative observations of plant response to abiotic stressors, disease damage, and arthropod damage were conducted in field trials at 21 sites over two consecutive years to assess environmental interactions of MON 87769 compared to the conventional control (see Appendix I, pp. 344-364, Monsanto 2010 for details). These data were used to assess the susceptibility and tolerance of MON 87769 to specific abiotic stressors, diseases, or arthropod pests compared to that of the conventional soybean control. No biologically meaningful differences were observed across sites over two U.S. soybean growing seasons between MON 87769 and the conventional control in their susceptibility or tolerance to specific abiotic stressors, diseases, or arthropod pests or in their interactions with specific pests.

Results of these evaluations indicate that there is no fundamental difference between MON 87769 and the conventional control for traits associated with weediness. Collectively, these findings support the conclusion that MON 87769 is no more likely to be a weed compared to conventional soybean.

Impacts on the Weediness of Any Other Plant with which MON 87769 can Interbreed

Gene flow is a natural biological process with significant evolutionary importance. A number of angiosperm taxa are believed to be derived from hybridization or introgression between closely related taxa (Grant 1981; Soltis and Soltis 1993; Rieseberg 1997; Hegde et al. 2006), and even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace 1987; Rieseberg and Wendel 1993; Peterson et al. 2002). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars. However, gene flow from crops to wild relatives is also thought of as having a potential to enhance the weediness of wild relatives, as observed in rice, sorghum, sunflower and few other crops (see Table 1 in Ellstrand et al. 1999).

Soybean is a predominantly a self-pollinated species (OECD 2000), yet a small amount of outcrossing does occur in this species (Table X-3, p. 178, Monsanto 2010). A majority of outcrossing occurs within a couple of meters from the source populations and falls to about 0.01% or less within 10 – 15 meters (see Table X-3, Monsanto 2010 for outcrossing investigations). Based upon these factors, it is unlikely that MON 87769 will naturally outcross or hybridize to a significant extent with other soybean varieties in agricultural settings.

In assessing the risk of gene introgression from MON 87769 into its sexually compatible relatives, APHIS considers two primary issues: 1) the potential for gene flow and introgression and, 2) the potential impact of introgression.

The genus *Glycine* is divided into two subgenera, *Glycine* and S*oja*. The subgenus *Soja* consists of three annual species: *G. soja* Sieb. and Zucc., the wild form of soybean; *G. gracilis* Skvortz., the weedy form of soybean; and *G. max*, the cultivated soybean. These species grow wild or semi-wild in Asia. Fertile hybrids between *G. max and G. soja* (Nakamaya and Yamaguchi 2002; Mizuguti et al. 2010), and between *G. max* and *G. gracilis* (Karasawa 1952) occur. *Glycine soja* and *G. gracilis* grow naturally only in Asia, not in the United States (see Table 9.1 in Lu 2005). The subgenus *Glycine* consists of twelve wild perennial species. These species grow wild in Australia, South Pacific Islands and Asia (Newell et al. 1978), and do not exist naturally in the U.S. Hybrids between perennial *Glycine* species are fertile.

Glycine max is the only *Glycine* species located in the United States, thus there are no other plant species with which *G. max* can interbreed. *Glycine max* has never been found in the wild (Hymowitz and Singh 1987) without human intervention. Therefore, it is highly unlikely that soybean plants in the United States will be found outside of an agricultural setting. It is also highly unlikely that gene flow and introgression will occur between MON 87769 and soybean plants in a natural environment. USDA has therefore determined that any adverse consequences of gene flow from MON 87769 to wild or weedy species in the United States are highly unlikely.

Effects of MON 87769 on Non-target Organisms

MON 87769 soybean is not engineered for pest resistance, thus there are no 'target' species, and thus no 'nontarget' species either. APHIS assessed whether exposure or consumption of MON 87769 soybeans would have an adverse affect on beneficial species or wildlife associated with soybeans. As discussed earlier, MON 87769 is similar in nutritional and compositional analysis to nonmodified control soybean variety A3525 except for the intended changes in the fatty acid composition associated with the production of SDA and GLA in the seed.

Monsanto also assessed the potential allergenicity and toxicity of introduced traits (Δ6D and Δ15D proteins obtained from *P. juliae* and *N. crassa*, respectively) according to the recommendations of the Codex Alimentarius Commission (Codex 2003). Donor organisms of the two genes are not known to be toxic, and one of the donor organisms, *N. crassa*, is found in the digestive tracts of vertebrate species, including humans. Monsanto's bioinformatic analyses of both proteins demonstrated that they do not share structurally or immunologically relevant amino acid sequence similarities with known allergens. Additionally, Monsanto conducted digestive fate experiments with the Δ6D and Δ15D proteins and found that the full-length proteins are rapidly digested in simulated gastric fluid (SGF), a characteristic shared among many proteins with a history of safe consumption. The transiently stable protein fragments in the SGF assay were quickly degraded during a short exposure to simulated intestinal fluid (SIF). Rapid digestion of the full-length proteins in SGF and SIF, together with rapid degradation of the transiently stable fragments from the SGF assay by SIF, indicates that it is highly unlikely that the Δ6D and Δ15D proteins and their fragments will reach absorptive cells of the intestinal mucosa. Finally, the PjΔ6D and NcΔ15D proteins are present at very low concentrations which

average no more than 0.00043% and 0.00239%, respectively, of the total protein in MON 87769 mature seed.

Monsanto also assessed both proteins for their potential toxicity. Structurally and functionally similar proteins (homologous desaturases) are present across life forms and also found in human and animal diets. The proteins lack structural similarity to known toxins or biologically active proteins known to have adverse effects to mammals. Both proteins occur at extremely low levels in the harvested seed, in other words, they make up a negligible portion of the total protein present in food and feed derived from MON 87769. Monsanto provided data showing that feeding high rates of the PjΔ6D and NcΔ15D proteins purified from MON87729 immature seed did not cause adverse effects on mice (p. 98, Monsanto 2010).

In addition, the changed fatty acid composition of mature MON 87769 soybean is not expected to adversely affect organisms that might feed on them. The environmental exposure and fate of fatty acids in MON87769 was analyzed in Section X.A.3.2 . and X.A.3.3. of the Monsanto 2010, (pp. 169-171). Monsanto provided the results of a published 90 day/one generation reproductive rat toxicity study in which a no observable adverse effect level (NOAEL) of 4 g of SDA soybean oil/kg/day (the maximum dose tested) was determined (Hammond et al. 2008) (see Appendix F for experimental details, pp. 316-320, Monsanto 2010). In practical terms, given that fatty acids constitute 18% of the dry weight of MON 87769 soybean seeds, and the 100 seed weight is 15.1 g (as reported in the Monsanto 2010,), this would constitute a consumption rate of approximately 66 seeds of MON 87769 soybean /day for a typical 500 g adult rat. The SDA and GLA are found in variety of sources (fish, plant seeds, algae), and in mammals these are intermediates in the *in vivo* metabolism of ALA to long chain omega-3 fatty acids and of LA to arachidonic acid, respectively.

Furthermore, Monsanto has submitted food and feed safety data to FDA as a voluntary consultation process. Based on the food and feed safety data, lack of toxicity and allergenicity of introduced gene products, APHIS concludes that feeding of MON 87769 plant or seed from mammals and other nontarget organisms is unlikely to cause any adverse impact on their survival and reproduction.

Beneficial arthropods, pollinators, symbiotic nitrogen fixing bacteria, and wildlife associated with soybeans are unlikely to be adversely affected by cultivation of MON 87769 soybean. No significant reduction in beneficial arthropods was detected in observations of field trials (see Section VIII.D.2.2, Monsanto 2010). Neither beneficial arthropods nor pollinators feed directly on soybean seed, and thus would not be expected to be affected. *Bradyrhizobium japonicum* are included in seed inoculums to improve nitrogen fixation in the field. The Monsanto included studies (summarized in Section VIII.D.4. on pp.130-133, Tables VIII-9 and VIII-10, and in Appendix K, Monsanto 2010) that demonstrate that MON 87769 soybean was not altered in its symbiotic relationship with *B. japonicum* compared to conventional soybean based on assessments of nodule number, shoot nitrogen, and biomass of nodules, shoot material, or root material.

Transfer of Genetic Information to Organisms with which MON 87769 cannot Interbreed

The horizontal gene transfer (HGT) between unrelated organisms is one of the most intensively studied fields since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Dröge et al. 1998). HGT has been implicated as a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria and the emergence of increased virulence in bacteria, eukaryotes, and viruses; and has contributed to major transitions in evolution. Gene exchange has been documented for nearly all types of genes and between unrelated organisms (Gogarten et al. 2002). For example, recently, Yoshida and colleagues (Yoshida et al. 2010) through a comparative genomics analysis implicated HGT for the presence of a similar genetic sequence between the parasitic plant purple witchweed (*Striga hermonthica*), which infests cereal fields (monocots), and sorghum (*Sorghum bicolor*).

APHIS examined the potential for the new genetic material inserted into MON 87769 to be horizontally transferred to other organisms without sexual reproduction and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants. The MON 87769 contains two noncoding sequences from bacteria. Horizontal gene transfer and expression of DNA from a plant species to other bacterial species is unlikely to occur based on the following observations.

Although there are many opportunities for plants to directly interact with fungi, bacteria, and parasitic plants (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores), so far there are no reports of significant horizontal gene transfer between sexually incompatible or evolutionarily distant organisms (as reviewed in Keese 2008). Accumulated evidence show that there are universal gene-transfer barriers, regardless of whether transfer occurs among closely or distantly related organisms (Kaneko et al. 2000; Koonin et al. 2001; Wood et al. 2001; Kaneko et al. 2002; Brown 2003; Sorek et al. 2007). Many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Kaneko et al. 2000; Kaneko et al. 2002; Wood et al. 2001). There is no evidence that these organisms contain genes derived from plants. In cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Koonin et al. 2001; Brown 2003), so also the case with the recent report about of HGT between sorghum and purple witchweed. According to authors (Yoshida et al. 2010), the incorporation of a specific genetic sequence occurred between sorghum and purple witchweed before speciation of purple witchweed (*S. hermonthica*) and related cowpea witchweed (*S. gesnerioides*), a parasitic plant of dicots, from their common ancestor. In other words, HGT is an extremely rare event, and a majority of those rare events occur over millions of years.

Transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression. Thus even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. FDA has evaluated horizontal gene transfer from the use of antibiotic resistance marker genes, and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is remote (FDA 1998: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Biotechnology/ucm096135.htm). Therefore APHIS concludes that horizontal gene transfer is

unlikely to occur from Mon 87769 to microorganisms and thus no significant plant pest risk is expected from horizontal gene transfer.

Conclusion

APHIS has reviewed the information submitted by the petitioner and conducted a plant pest risk assessment on MON 87769 soybean. Due to the lack of plant pest risk from the inserted genetic material, the lack of atypical responses to disease or plant pests in the field, the lack of weediness characteristics of MON 87769 soybean, the lack of deleterious effects on non-targets or beneficial organisms in the agro-ecosystem, and the lack of horizontal gene transfer, APHIS concludes that MON 87769 soybean is unlikely to pose a greater plant pest risk than the unmodified soybean line from which it was derived.

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