Assessment of Plant Pest Risk for MON 87705 Soybean

Monsanto Company (Monsanto) has petitioned APHIS for a determination that MON 87705 soybean is unlikely to pose a plant pest risk (Monsanto 2010) and, therefore, should no longer be a regulated article under 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 87705 is unlikely to pose a plant pest risk.

MON 87705 was produced by transformation of soybean tissue using *A. tumefaciens*. Because *A. tumefaciens* is a plant pest and some of the regulatory sequences (Figwort Mosaic Virus promoter) used to facilitate expression of these genes in soybean were derived from plant pests, this 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 87705 and its progeny in the absence of confinement. APHIS uses data and information submitted by the applicant, in addition to current literature, to determine if MON 87705 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 associated with MON 87705 and impacts on the environment will be considered in the Environmental Assessment (EA) for MON 87705 soybean. 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.

History of Development of MON 87705 soybean with an improved fatty acid profile

Soybean oil is relatively high in saturated fatty acids (15% of total fatty acids) compared to vegetable oil from other sources. Soybean oil is also relatively high in polyunsaturated fatty acids (23% of total fatty acids). These polyunsaturated fatty acids oxidize readily to

[1] Section 403 (14) of the Plant Protection Act (7USC Sec 7702(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."

produce off-flavors and reduce the performance of unprocessed soybean oil. The stability and flavor of soybean oil is improved by hydrogenation, which chemically reduces the double bonds. However, hydrogenation has the undesirable consequence of creating *trans*-fatty acids. In recent years, *trans*-fatty acids have come under considerable scrutiny by American Heart Association and others because of the negative effects by these substances on human health.

Monsanto has developed a transgenic soybean, event MON 87705 (hereafter referred to as MON 87705), that produces soybean seeds with decreased levels of saturated (palmitic and stearic) and polyunsaturated (linoleic) fatty acids, and increased levels of monounsaturated (oleic) fatty acid. MON 87705 contains DNA segments designed to suppress endogenous delta-12 desaturase (*FAD2*) and Acyl-ACP thioesterase (*FATB*) genes which encode for two enzymes in the soybean fatty acid biosynthetic pathway. MON 87705 also contains the 5-enolpyruvylshikimate-3-phosphate synthase (*cp4 epsps)* gene encoding the CP4 EPSPS protein. The *cp4 epsps* gene was used as a selectable marker to identify transgenic plants during the transformation process. The CP4 EPSPS protein confers tolerance to glyphosate and has been used in many Roundup Ready crops (e.g. canola, corn, cotton, soybean and sugar beet).

In plants, the microsomal delta-12 desaturase-catalyzed pathway is the primary route of production of polyunsaturated lipids. The *FAD2* gene is strongly expressed in developing seeds. The seed-specific expressed *FAD2* gene is likely to play a major role in controlling conversion of oleic acid (a monounsaturated fatty acid) to linoleic acid (a polyunsaturated fatty acid) within storage lipids during seed development (Heppard et al., 1996). The delta-12 desaturase (*FAD2*) gene in soybean is responsible for the conversion of oleic acid to linoleic acid. Suppression of the *FAD2* gene prevents linoleic acid from being synthesized and leads to the accumulation of oleic acid in the soybean seed.

The *FATB* gene is one of the key enzymes for oil biosynthesis in plants. The suppression of *FATB* results in a decrease in the transport of saturated fats out of the plastid, thus retaining the availability of those saturated fats for desaturation to oleic acid (Monsanto 2010, page 5, panel A and B). In MON 87705, the assembled gene transcripts have an inverted repeat that produces double stranded RNA that, via the RNA interference (RNAi) pathway, suppresses endogenous *FATB* and *FAD2* gene expression.

The increase in oleic acid and decrease in saturated fatty acids in MON 87705 is aimed at improving the stability of these soybean oils and promoting human heart health. The commercialization of MON 87705 could be beneficial for consumers. From a nutritional standpoint, the consumption of *trans* fatty acids results in considerable potential harm with no apparent known benefit to human health (Mozaffarian et al., 2006). Increased intake of oils high in monounsaturated fatty acids, such as oleic acid, has been recommended by American Heart Association because of their positive effect on total cholesterol levels when compared to equal intakes of hydrogenated oils (Lichtenstein et al., 2006). Likewise, increased intake of oils high in oleic acid is known to decrease LDL-cholesterol levels compared to an equal intake of oils high in saturated fats (Mensink et al., 1989) and known to increase HDL-cholesterol levels compared to an

equal intake of polyunsaturated oils (Mata et al., 1992). Moderate consumption of oil high in oleic acid has also been demonstrated to result in decreased systolic blood pressure (Bondia-Pons et al., 2007).

Description of the modification

MON 88705 was produced by *Agrobacterium*-mediated transformation of soybean with a binary vector that contains two T-DNAs (T-DNA I AND T-DNA II; T-DNA refers to the DNA that is transferred to the plant during transformation) as shown in petition (Monsanto 2010, figures IV-I and IV-II, p. 54-55 and Table V-2, p. 63-65).

(1) T-DNA I contains a *cp4 epsps* expression cassette and a partial suppression cassette containing sense segments of an FAD2-1A intron and FATB1-A 5´ untranslated region (UTR) (Monsanto 2010, Table IV-1, pp. 56-57).

The *cp4 epsps* expression cassette contains the following genetic elements:

- *FMV/TSF1* chimeric promoter: *FMV/TSF1* promoter consisting of enhancer sequences from the promoter of the Figwort Mosaic Virus (FMV) 35S RNA combined with the promoter from the *Tsf1* gene of *Arabidopsis thaliana* that encodes elongation factor EF-1 alpha (Axelos et al., 1989).
- *cp4 epsps* gene: the *cp4 epsps* gene is a gene fusion composed of the N-terminal chloroplast transit peptide (CTP2) sequence from *Arabidopsis thaliana, epsps* gene and the C-terminal synthetic 5-enolpyruvylshikimate-3-phosphate synthase gene (CP4) from an *Agrobacterium* species, strain CP4 and encoding CP4 EPSPS protein. The CP4 EPSPS protein confers tolerance to glyphosate (Padgette et al., 1996; Barry et al., 1997).
- E9 3´ UTR as transcriptional termination: 3´untranslated region of the *Pisum sativum* ribulose 1,5-bisphosphate carboxylase small subunit E9 (*RbcS2*) gene which functions to direct polyadenylation of the *cp4 epsps* mRNA (Coruzzi et al., 1984).

The partial suppression cassette contains the following genetic elements:

- *7Sα´* promoter: *7Sα´* seed specific promoter is from the *Sphas1* gene of *Glycine max* encoding beta-conglycinin storage protein (Doyle et al., 1986) that directs transcription in seed.
- The sense segment of the *FAD2-1A* intron: Partial sequence from the intron number 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* 5' UTR: Partial sequence from the 5' UTR 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.

(2) T-DNA II contains a partial suppression cassette that consists of antisense segments of *FAD2-1A* intron and *FATB1-A* 5'UTR.

- The antisense segment of *FAD2-1A* intron: is an intron from *FAD2-1A* gene that encodes the delta-12 desaturase.
- The antisense segment of *FATB1-A 5*' UTR: Partial sequence from 5' UTR and the plastid targeting sequence is from Glycine max *FATB1-A* gene that encodes the palmitoyl acyl carrier protein thioesterase.
- *H6* 3' UTR Terminator: *H6* 3'UTR from *Gossypium barbadense* is for transcriptional termination and polyadenylation of a fiber protein involved in secondary cell wall assembly.

In addition to the above-mentioned nucleotide sequences, T-DNA I and II also contains non-coding intervening sequences and elongation factors that facilitate DNA cloning and translational elongation, respectively. The assembled gene transcript has an inverted repeat that produces double stranded RNA (dsRNA) that, via the RNA interference (RNAi) pathway, suppresses endogenous FATB and FAD2 RNA.

Transformation system.
MON 87705 was developed using a disarmed *Agrobacterium* –mediated transformation system of soybean, which allows the generation of transformed plants without utilization of callus (Martinell et al., 2002). This technique using disarmed *Agrobacterium* followed by glyphosate selection has a long history of safe use.

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 87705 soybean:

Potential impacts of genetic modifications on disease and pest susceptibilities

USDA-APHIS assessed whether MON 87705 is likely to have significantly increased disease and pest susceptibility compared to its unmodified control. The assessment encompasses a consideration of introduced traits and interactions with pests and diseases.

The only introduced protein produced in MON 87705 is CP4 EPSPS. The 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) family of enzymes is ubiquitous in plants and microorganisms. The introduced *cp4 epsps* gene is from *Agrobacterium sp.* strain CP4. The CP4 EPSPS protein expressed in MON 87705 is similar and functionally identical to endogenous plant EPSPS enzymes and is identical to the CP4 EPSPSs in other Roundup Ready® crops including Roundup Ready® soybean (40-3-2 and MON 89788), Roundup Ready® canola, Roundup Ready® sugar beet and Roundup Ready flax and Roundup Ready® cotton. The first generation of Roundup Ready® soybean (40-3-2) (USDA-APHIS, 1993) was determined by APHIS to be no longer subject to the regulatory requirements of 7 CFR Part 340 or the plant pest provisions of the Plant Protection Act in 1995. The *cp4 epsps* gene has been assessed extensively in the last 15 years to confirm that it does not alter the desired agronomic characteristics of transgenic

crops, and that it does not present a plant pest risk. Therefore, incorporating *cp4 epsps* genes into MON 87705 is not likely to alter disease and pest susceptibility. The expression of the *epsps* gene is controlled by a chimeric promoter wherein a part of the sequence comes from Figwort Mosaic Virus. These plant virus-derived regulatory sequences are non-coding with known function and they are not known, in and of themselves, to cause plant disease.The introduced endogenous *FAD2* gene that suppresses lipid biosynthesis increases the amount of oleic acid in the seed, a constituent which is present in the parent cultivar. Such genes have been used in field trials previously and are not known to cause plant disease. There is no indication that inserting the *FAD2* gene will result in increased likelihood of introduction or dissemination of a plant pest. The higher concentration of oleic acid is not known to pose a plant pest risk in unconfined releases. APHIS has not identified any plant pest risk associated with the introduction of the high oleic acid trait in the DuPont product (USDA-APHIS, 1997) and Pioneer product (USDA-APHIS, 2006) that have been determined by APHIS to be no longer subject to the regulatory requirements of 7 CFR Part 340 or the plant pest provisions of the Plant Protection Act. The Plant Biosafety Office (PBO) of the Canadian Food Inspection Agency (CFIA, 2000), which also reviewed a dossier on this product, also concluded that this novel trait (high oleic acid) will not introduce any plant pest characteristics into MON 87705 soybean. The introduced endogenous *FATB* gene suppresses the acyl-acyl carrier protein (ACP) thioesterase and results in a decrease in the transport of saturated fats out of the plastid, thus retaining their availability for desaturation to 18:1 oleic acid. As a result, MON 87705 soybean oil is lower in saturated fats. The information collected from the field trials and literature, there is no indication that by suppressing *FATB* gene will result in altering plant pest risk.

Field evaluation of MON 87705 and conventional soybean varieties were carried out at 17 sites covering 10 States and assessed for differences in plant response to abiotic stress, disease damage, and arthropod damage. The petitioner rated soybeans for responses to abiotic stress, diseases, and arthropod pests that were present or typically present at each site. For the assessments of the plant-insect interactions, plant-disease interactions, and plant responses to abiotic stress, no differences were detected between MON 87705 and the controls for 574 of 579 observations at the 17 sites. There were statistical differences noted in the disease and arthropod damage in 5 of 579 observations. In three of the observations, MON 87705 has less damage from bacterial blight. In the other two observations, MON 87705 has less damage from aphids and leafhoppers (Monsanto 2010, p. 344-346). The 5 noted differences, however, were still within the reference response range of the control plants.

Based on the analysis of genetic modifications and their functions and field testing data submitted by petitioner, APHIS concludes that there are no significant differences between MON 87705 soybean and the non-transgenic counterparts relative to pest and disease susceptibility. Soybean (*Glycine max*) is not a plant pest in the United States (USDA-APHIS, 2000), and the introduced DNA in MON 87705 is unlikely to pose a plant pest risk.

Potential impacts from new gene products, changes to plant metabolism or composition

Assessment of Plant Pest Risk for MON 87705 Soybean

USDA-APHIS assessed whether changes in plant metabolism or composition in MON 87705 is likely to alter plant pest risk. The assessment encompasses a consideration of the inserted sequences, expressed protein and introduced traits.

There is no new protein expressed because of the insertion of the *FAD2 and FATB* gene fragments. The only novel protein expressed in MON 87705 is EPSPS (5-enolpyruvylshikimate-3phosphate synthase), which imparts glyphosate tolerance.

The EPSPS protein is found naturally in plants, fungi and bacteria, but is absent in mammals, fish, birds, reptiles, and insects (Alibhai et al., 2001). The EPSPS in MON 87705 is derived from an *Agrobacterium sp*. strain *CP4*. More than 200 *epsps* sequences are known (http://www.ncbi.nlm.nih.gov/nuccore). Even though there is significant diversity for amino acid sequences among EPSPS proteins, they all share a common structure and a conserved active site. The EPSPS protein in Mon 87705 is minimally modified compared to the endogenous form in soybean. The primary mode of action of glyphosate is the competitive inhibition of the plant enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) which catalyses the penultimate step in the shikimate pathway (Franz et al., 1997).

The scientific basis for glyphosate tolerance in all Monsanto Roundup Ready® GE crops (Monsanto 2010, page 4) is identical. These products have been grown widely across the U.S. since the 1990's and have a long history of safe use. Although EPSPS proteins are ubiquitous in nature, there is no evidence of unexpected plant pest risk from these proteins. Southern blots were used to determine the copy number of each of the genetic elements and to examine the integrity of each fragment inserted into the soybean genome. The petitioner has concluded that the CP4 EPSPS-expressing transgene is present in one copy, is integrated at a single locus, segregates as a single dominant Mendelian trait, and is stable within the soybean genome. The petitioner also notes that the trait is phenotypically stable over several generations.

The CP4 EPSPS transcript levels were measured in leaf, seed, root and forage tissues. The mean protein data for the measured plants are summarized in Table VI-2 (Monsanto 2010, p.89). The Margin of Exposure (MOE) was calculated based on the EPSPS protein expressed in different tissues. The Margin of Exposure (MOE) is the ratio between a defined point on the dose-response curve (reference point) for the adverse effect of the compound in an animal carcinogenicity study and the estimated human intake of the compound. The MOE is calculated for CP4 EPSPS protein produced by MON 87705 and results indicate that there is no meaningful risk to human health from dietary exposure. The conclusions of "no further questions" on nutritional and safety issues were reached by FDA for the CP4 EPSPS protein (FDA, 2000; FDA, 2004; FDA, 2005; FDA, 2006). Petitioner submitted a summary of its safety and nutritional assessment to FDA for MON 87705 soybean in 2009. A final FDA decision is pending.

The compositional assessment was conducted in accordance with the Organization of Economic Co-operation and Development (OECD) consensus document on

compositional considerations for new varieties of soybean (OECD, 2001). A total of 38 fatty acids were analyzed in MON 87705 and control lines. Twenty-one fatty acid concentrations were near or below the detection limits of the assay. Of the other acids that could be statistically analyzed, concentrations of 13 fatty acids showed statistically significant differences between MON 87705 and the controls. Four of the 13 differences were intended changes from control soybean lines: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1) and linoleic acid (18:2). Nine of the 13 fatty acids were significantly different from the control soybean lines: myristic acid, palmitoleic acid, margaric acid, heptadecenoic, octadecadienoic acid, arachidic acid, eicosenoic acid, behenic acid and 18:2 other trans isomer fatty acids (Monsanto 2010, pp.311-312, Table E15). However, the levels of these fatty acids were biologically not significant, as most of them are relatively minor fatty acids and are common at similar levels in vegetable oils (USDA-ARS, 2006). Therefore, APHIS concludes that MON 87705 is compositionally equivalent to conventional soybean with the exception of intended changes in fatty acid levels.

Compositional and nutritional data were collected on MON 87705 and comparisons were made to a conventional control line and a set of reference soybean varieties. Based on the results of comparative analysis APHIS concludes that MON 87705 is compositionally and nutritionally equivalent to conventional soybean varieties currently in commerce, except for the intended changes.

Based on all the considerations above, APHIS concludes that MON 87705 poses no more of a plant pest risk from new gene products, changes to plant metabolism or composition than conventional soybean.

Potential impacts from outcrossing of MON 87705 to wild relatives

Soybean is a self-pollinated species propagated by seed (OECD, 2000). In its papilionaceous flower, the anthers mature in the bud and directly pollinate the stigma of the same flower. As a result, soybeans exhibit a high percentage of self fertilization. Natural or artificial cross-pollination can only take place during the short time when the pollen is viable. The cross pollination rate (with and without pollinators) is less than 1.5% beyond one meter from the pollen source (Garber, et al., 1926; Carviness, 1966; Ahrent et al., 1994; Ray, et al., 2003; Yoshimura, et al., 2006). At greater distances from the pollen source, cross pollination rates decrease rapidly. Based upon these factors, it is unlikely that MON 87705 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 87705 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.

Cross pollination with wild species
The genus *Glycine* is divided into two subgenera, *Glycine* and S*oja*.

Assessment of Plant Pest Risk for MON 87705 Soybean

The subgenus *Soja* consists of three annual species: *G. soja* Sieb. and Zucc., the form of soybean, *G. gracilis* Skvortz., the weedy form of soybean and *G. max*, the cultivated soybean. They grow wild or semi-wild in Asia. Fertile hybrids between *G. max* and *G. soja* (Broich, 1978), and between *G. max* and *G. gracilis* (Karasawa, 1952) occur. *G. soja* and *G. gracilis* grow naturally only in Asia and Australia, not in the United States (Skvortzow, 1972).

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.

G. max is the only *Glycine* species located in the United States, thus there are no other plant species with which *G. max* can interbreed. *G. max* has never been found in the wild (Hymowitz et al., 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 87705 and soybean plants in a natural environment. USDA has therefore determined that any adverse consequences of gene flow from MON 87705 to wild or weedy species in the United States are highly unlikely.

Potential impacts based on the relative weediness of MON 87705.

APHIS assessed whether MON 87705 is any more likely to become a weed than the non-transgenic recipient soybean line, or other soybean currently cultivated. The assessment encompasses a thorough consideration of the basic biology of soybean and an evaluation of unique characteristics of MON 87705.

In the U.S., soybean is not listed as a weed in the major weed references (Crockett, 1977; Holm et al., 1979; Muenscher, 1980) nor is it listed as a noxious weed species by the U.S. Federal Government (USDA-APHIS, 2010a) and States (USDA-APHIS 2010b). Soybeans are not frost tolerant, do not survive freezing winter conditions (OECD, 2000), and do not reproduce vegetatively. After crop harvest, soybean may germinate as a volunteer weed in the succeeding crop (Padgette et al., 1996). If any seeds remain viable and germinate the following year, mechanical methods or herbicides can be used to control volunteers. Soybean is one of the largest acreage crops in the world. Soybean has never been reported as a serious weed. In the U.S., soybean is grown on over 77 million acres (USDA-NASS, 2009_a). Based on the familiarity of soybean as the parent plant, there has been no report of soybean escaping cultivation and becoming established as a weed in United States (Holm et al., 1979).

Weediness for the purposes of this part of the plant pest risk assessment is an attribute, which causes a crop to act as a weed due to the addition of genes (compared to the non-GE plant). If the fitness of MON 87705 improves in natural or agricultural ecosystems due to the inserted DNA, the potential for weediness could increase. The following analysis of the inserted DNA is intended to document that MON 87705 has a negligible likelihood of increased weediness.

Assessment of Plant Pest Risk for MON 87705 Soybean

MON 87705 differs from conventional soybeans only in the expression of the EPSPS protein and silencing of the endogenous *FAD2* and *FATB* genes. The RNA-based suppression of *FATB and FAD2* soybean genes are mediated by double stranded RNA (dsRNA) molecules and do not code for any protein. Double stranded RNAs are commonly used in plants for endogenous gene suppression and pose no novel risks from a plant pest perspective. The only introduced protein in MON 87705 is CP4 EPSPS. This protein has the same functional and enzymatic activity as the CP4 EPSPS in other Roundup Ready® crops. The large scale commercial cultivation of glyphosate tolerant soybean crop acreage has steadily increased from 1996, accounting for 91 % of soybean acreage in 2009 (USDA-NASS, 2009_b). No data of which APHIS is aware indicate that the presence of the *cp4 epsps* gene improves the ability of this soybean line to survive without human intervention, nor is there any foreseeable reason to conclude that this gene would facilitate MON 87705's survival in unmanaged areas.

In the 2007 growing season, Monsanto evaluated 14 phenotypic and agronomic characteristics in 17 field locations in the United States (Monsanto 2010, p. 124, Table VIII-3). Data such as emergence, seedling vigor, plant height, lodging, days to maturity, shattering, seed weight, yields, disease incidence and insect damage were used to assess whether there was an increase in weediness potential. Monsanto observed four statistically significant differences between MON 87705 and the control: (1) early stand count, (2) final stand count, (3) days to 50% flowering, and (4) seed weight. MON 87705 was lower than control for both early stand count and final stand count (Monsanto 2010, p. 349-350, Table G-3, Appendix G). MON 87705 flowered about one day later than the control and the weight of 100 seeds was lower for MON 87705 compared to the control (15.6g vs. 16.1g). APHIS is not aware of any information indicating that decreased stand counts, seed weight or differences in days to 50% flowering would contribute to an increased weediness potential of soybean.

Monsanto also did comparative assessments of seed dormancy and germination characteristics on MON 87705 and A3525 (a conventional variety), where A3525 served as a control because it has background genetics similar to MON 87705 but does not possess the transgenes. The data related to seed germination characteristics were used to assess whether there was an increase in weediness potential. Four statistically significant differences were detected between MON 87705 and the control: (1) MON 87705 had lower percent viable hard seed (0.0 vs. 0.3%) at 20°C, (2) MON 87705 had lower percent germinated seed at 30°C (92.8 vs. 95.5%), (3) MON 87705 had higher percent dead seed at 30°C (7.3 vs. 4.5%), and (4) MON 87705 had higher percent dead seed at 20/30ºC (2.7 vs. 1.3%). A decrease in hard seed, a lower percent germinated seed and higher percent dead seed would not contribute to increased weediness. Furthermore, all values were well within the recommended standards for certified soybean seed (AOSCA, 2009).

These data provide an evaluation of the phenotypic and agronomic characteristics of MON 87705 compared to the conventional A3525 control. Results from the phenotypic and agronomic assessments indicate that MON87705 does not possess characteristics that would confer a plant pest risk compared to conventional soybean. These data indicate

that the engineered plant is not different in any fitness characteristics from its parent that might cause MON 87705 to become weedy or invasive. Therefore, soybean is unlikely to become a weed through the introduction of the glyphosate tolerance trait.

A few earlier investigations (Kodama et al., 1994 and Kodama et al.,1995) indicated that the increases in levels of trienoic fatty acids such as hexadecatrienoic acid and linolenic acid could enhance cold tolerance in model plants such as *Arabidopsis* and tobacco. In MON 87705, the levels of linolenic acid are significantly decreased. The decreased levels of linolenic acid in MON 87705 would, therefore, not be expected to enhance cold tolerance. If the seed overwintering capacity improved, the potential for a successful weed could increase. There is no indication that MON 87705 possesses a selective advantage that would result in increased weediness potential. Therefore, the chances for MON 87705 to behave as a weed are negligible.

From these observations and data submitted in the petition, APHIS concludes that there are no weediness risk issues associated with MON 88705.

Potential impacts from transferring genetic information from MON 87705 to organisms with which it cannot interbreed.

Horizontal gene transfer and expression of DNA from a plant species to bacteria is unlikely to occur. First, many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Kaneko et al., 2000; Wood et al., 2001; Kaneko et al., 2002). There is no evidence that these organisms contain genes derived from plants. Second, 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). Third, 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. Fourth, the 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). Therefore APHIS concludes that horizontal gene transfer is unlikely to occur and thus poses no significant environmental or plant pest risk.

Conclusion

APHIS has reviewed the potential of MON 87705 to pose a plant pest risk and conducted an assessment based on the information provided by the petitioner on MON 87705. Due to lack of plant pest risk from the inserted genetic material, lack of atypical responses to disease or plant pests, lack of weediness characteristics, and lack of horizontal gene transfer, APHIS concludes that MON 87705 is unlikely to pose a plant pest risk.

References:

Ahrent, D.K., Caviness, C.E. (1994) Natural cross-pollination of twelve soybean cultivars in Arkansas. Crop Sci. 34:376-378.

Alibhai, M.F., and W.C. Stallings. (2001). Closing down on glyphosate inhibition - with a new structure for drug discovery. Proceedings of the National Academy of Sciences U.S.A. 98:2944-2946.

AOSCA (The Association of Official Seed Analysts). 2009. Operational Procedures, Crop Standards and Service Programs. Association of Official Seed Certifying Agencies, Meridian, Idaho.

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

Barry, G., Kishore, G., Padgette, S., Stallings, W. (1997) Glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthases. U.S.A. Patent 5, 633, 435.

Bondia-Pons, I., chroder, H., Covas, M., Castellote, A.I., Kaikkonen, J., Poulsen, H.E., gaddi, A.V., Machowetz, A., Kiesewetter, H., Lopez-Sabater, C. (2007) Moderate consumption of olive oil by healthy European men reduces systolic blood pressure in non-Mediterranean participants. The Journal of Nutrition (137):84-87.

Broich, S.L. (1978) The systematic relationships within the genus *Glycine* Willd. Subgenus *soja* (Moench) F.J. Hermann. M.S. thesis. Iowa State University, Ames.

Brown, J. R. (2003) Ancient horizontal gene transfer. Genetics. 4:121-132.

Caviness, C.E. (1966) Estimates of natural cross-pollination in Jackson soybeans in Arkansas. Crop Science 6:21.

CFIA (Canadian Food Inspection Agency) (2000) Decision Document 2001-34: Determination of the Safety of Optimum Quality Grains L.L.C.'s High Oleic Soybean (*Glycine max*) lines G94-1, G94-19, and G-168 Derived from Transformation Event Line 260-05 http://www.inspection.gc.ca/english/plaveg/bio/dd/dd0134e.shtml#a13. Accessed on 11/03/2008

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

Crockett, L. (1977) Wildly Successful Plants: North American Weeds. University of Hawaii Press, Honolulu, Hawaii. 609 pp.

Doyle, J., Schuler, M., Godette, W., Zenger, V., Beachy, R. (1986) The glycosylated seed storage proteins of Glycine max and Phaseolus vulgaris. Journal of Biological Chemistry 261:9228-9238

FDA (U.S. Food and Drug Administration) (1998) Guidance for industry: use of antibiotic resistance marker genes in transgenic plants, draft guidance. Sept. 4, 1998. http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Biotechnology/ucm096135.htm

FDA (U.S. Food and Drug Administration) (2000) Biotechnology Consultation Note to the File BNF No. 000071. http://www.fda.gov/Food/Biotechnology/Submissions/ucm155788.htm

FDA (U.S. Food and Drug Administration) (2004) Biotechnology Consultation Note to the File BNF No. 000090. http://www.fda.gov/Food/Biotechnology/Submissions/ucm155775.htm

FDA (U.S. Food and Drug Administration) (2005) Biotechnology Consultation Note to the File BNF No. 000098. http://www.fda.gov/Food/Biotechnology/Submissions/ucm155614.htm

FDA (U.S. Food and Drug Administration) (2006) Biotechnology Consultation Note to the File BNF No. 000104. http://www.fda.gov/Food/Biotechnology/Submissions/ucm155610.htm

Fillatti, J., Bringe, N., Dehesh, K. (2003) Nucleic acid constructs and methods for producing altered seed oil compositions. International Patent WO 2003/080802 A3.

Franz, J. E., Mao, M. K.; Sikorski, J. A. Glyphosate's Molecular Mode of Action. In *Glyphosate: A Unique Global Herbicide*. ACS Monograph 189; American Chemical Society: Washington, DC, 1997; pp 521-615.

Garber, R., Odland, T. (1926) Natural crossing in soybeans. Am Soc. Agron. J. 18:967-970

Heppard, E.P., Kineey, A.J., Stecca, K.L., Miao, G.-H. (1996) Developmental and growth temperature regulation of two different microsomal omega-6 desaturase genes in soybeans. Plant Physiology 110:311-319

Holm, L., Pancho, J. V., Herbarger, J. P., and Plucknett, D. L. (1979) A Geographical Atlas of World Weeds. John Wiley and Sons, New York. 391 pp.

Hymowitz, T., Singh, R.J. (1987) Taxonomy and Speciation in Soybean: Improvement, Production, and Uses-Second Edition. American Society of Agronomy.

Kaneko, T., Y. Nakamura, S. Sato, E. Asamizu, T. Kato, and S. Sasamoto. (2000) Complete genome structure of the nitrogen-fixing xymbiotic bacterium Mesorhizobium loti. DNA Research. 7:331-338.

Kaneko, T., Nakamura, Y., Sato , S., Minamisawa, K., Uchiumi, T., Sasamoto, S., Watanabe, A., Idesawa, K., Iriguchi, M., Kawashima, K., Kohara, M., Matsumoto, M., Shimpo, S., Tsuruoka, H., Wada, T., Yamada, M., Tabata, S. (2002) Complete genomic sequence of nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* USDA110. DNA Research 9(6):189-197

Karasawa, K. (1952) Crossing Experiments with *Glycine soja* and *G. gracilis.* Genetica 26:357-358.

Kodama, H., Hamada, T., Horiguchi, G., Nishimura, M., and Iba, K. (1994) Genetic enhancement of cold tolerance by expression of a gene for chloroplast ω-3 fatty acid desaturase in transgenic tobacco. Plant Physiology 105:601-605.

Kodama, H., Horiguchi, G., Nishiuchi, M., Nishimura, M., and Iba, K. (1995) Fatty acid desaturation during chilling acclimation is one of the factors involved in conferring low temperature tolerance to young tobacco leaves. Plant Pathology 107:1177-1185.

Koonin, E., Makarova, K., Aravind, L. (2001) Horizontal gene transfer in Prokaryotes: Quantification and Classification. Annu. Rev. Microbiol. 55:709-742

Lichtenstein, A., Mathan, N.R., Jalbert, S.M., Resteghini, N.A., Schaefer, E.j., Ausman, L.M. (2006) Novel soybean oils with different fatty acid profiles alter cardiovascular disease risk factors in moderately hyperlipidemic subjects. Am. J. Clin. Nutr. 84:497-504

Martinell, B., Julson, L., Emler, C., Yong, H., McCabe, D., Williams, E.,inventors. (2002) Soybean *Agrobacterium* transformation method.U.S. Patent Application No. 6384301.

Mata, P., Alvarez-Sala, L.A., Rubio, M.J., Oya, M.D. (1992) effects of long-term monounsaturated- vs polyunsaturated-enriched diets on lipoproteins in healthy men and women. Am J Clin Nutr 55:846-850

Mensink, R.P., Katan, M.B. (1989) Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high density lipoprotein

cholesterol in healthy women and men. New England Journal of Medicine Vol. 321 No 7.

Monsanto (2010) Petition for the Determination of Nonregulated Status for Improved Fatty Acid Profile MON 87705 Soybean. Submitted by G. Rogan. Monsanto Company, St. Louis, MO (See Table http://www.aphis.usda.gov/biotechnology/not_reg.html).

Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC. (2006) Trans fatty acids and cardiovascular disease. *N Engl J Med.* 13;354(15):1601-13.

Muenscher, W. C. (1980) Weeds. Second Edition. Cornell University Press, New York and London. 586 pp.

Newell, C.A., Hymowitz, T. (1978) A reappraisal of the subgenus. Am. J. Bot. 65(2):168-179

OECD (Organization of Economic Co-operation and Development). (2000) Consensus document on the biology of *Glycine max* (soybean). OECD, ENV/JM/MONO(2000)15.

OECD (Organization of Economic Co-operation and Development) (2001) Consensus Document on compositional considerations for new varieties of soybean: key food and feed nutrients and anti-nutrients. Organisation for Economic Co-operation and Development ENV/JM/MONO (2001)15.

Padgette, S.R., Re, D.B., Barry, G.F., Eichholtz, D.E., Delannay, X., Fuchs, R.L., Kishore, G.M., Fraley, R.T. (1996) New weed control opportunities: development of soybeans with a Roundup Ready gene. Pp53-84. In S.O. Duke (ed.). Herbicide Resistant Crops-Agricultural, Environmental, Economic, Regulatory, and Technical Aspects. CRC Press, Boca Raton, Florida.

Ray, J., Kilen, T., Abel, C., Paris, R. (2003) Soybean natural cross-pollination rates under field conditions. Environmental Biosafety Research 2(2003) 133-138.

Skvortzow, B.V. (1972). The soybean-wild and cultivated in Eastern Asia. Proc Manchurian Res. Soc Publ Ser A. Natural History Sect. No. 22:1-8

USDA-APHIS (1993) Monsanto Company Petition for a determination of nonregulated status for the glyphosate tolerant soybean (GTS) line 40-3-2: Environmental Assessment. http://www.aphis.usda.gov/brs/not_reg.html. Accessed 6/29/2010

USDA-APHIS (1997) DuPont Petition 97-008-01p for determination of nonregulated status for transgenic High Oleic Acid soybean sublines G94-1, G94-19, and G-168: Environmental Assessment and Finding of No Significant

Impact. http://www.aphis.usda.gov/brs/aphisdocs2/97_00801p_com.pdf. Accessed 6/29/2010

USDA-APHIS (2000) U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Regulated Plant Pest List. http://www.invasivespecies.org/RegulatedPestList.pdf. Accessed 10/14/2008

USDA-APHIS (2006) Pioneer Hi-Bred International Petitions 06-271-01p for determination of Nonregulated Status for transgenic herbicide tolerant 356043 soybean: Environmental Assessment. http://www.aphis.usda.gov/brs/not_reg.html. Accessed 11/10/2008

USDA-APHIS (2010 $_a$) Federal Noxious Weed List as of May 1, 2010 http://plants.usda.gov/java/noxiousDriver#federal Accessed on 6/29/2010

USDA-APHIS (2010 $_b$) States Noxious Weed Lists http://plants.usda.gov/java/noxiousDriver#state Accessed on 6/29/2010

USDA-ARS (2006) USDA food and nutrient database for dietary studies, 2.0 (2006) Beltsville, MD., ARS, Food Surveys Research Group (http://www.ars.usda.gov/ba/bhnrc/fsrg)

USDA-NASS (2009$_a$) Newsroom (Acrage) http://www.nass.usda.gov/Newsroom/2010/01_12_2010.asp. Accessed 6/9/2010

USDA-NASS (2009$_b$) Adoption of genetically engineered crops in the U.S. http://www.ers.usda.gov/Data/biotechcrops/. Accessed 6/9/2010

Wood, D. W., J. C. Setubal, R. Kaul, D. E. Monks, J. P. Kitajima, V. K. Okura, Y. Zhou, L. Chen, G. E. Wood, N. F. Almeida Jr., L. Woo, Y. Chen, I. T. Paulsen, J. A. Eisen, P. D. Karp, D. Bovee Sr., P. Chapman, J. Clendenning, G. Deatherage, W. Gillet, C. Grant, T. Kutyavin, R. Levy, M.-J. Li, E. McClelland, A. Palmieri, C. Raymond, G. Rouse, C. Saenphimmachak, Z. Wu, P. Romero, D. Gordon, S. Zhang, H. Yoo, Y. Tao, P. Biddle, M. Jung, W. Krespan, M. Perry, B. Gordon-Kamm, L. Liao, S. Kim, C. Hendrick, Z.-Y. Zhao, M. Dolan, f. Chumley, S. V. Tingey, J.-F. Tomb, M. P. Gordon, M. V. Olson, and E. W. Nester. (2001) The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. Science. 294:2317-2323.

Yoshimura, Y., Matsuo, K., Yahuda, K. (2006) Gene flow from GM glyphosate-tolerant to conventional soybeans under field conditions in Japan. Environmental Biosafety Research. 5:169-173.