Application for authorization to place on the market MON 87769 × MON 89788 soybean in the European Union, according to Regulation (EC) No. 1829/2003 on genetically modified food and feed

Part III

Cartagena Protocol

Data protection.

This application contains scientific data and other information which are protected in accordance with Art. 31 of Regulation (EC) No. 1829/2003.

MON 87769 × MON 89788

A combined trait soybean product from Monsanto Company

Information on MON 87769 × MON 89788,
notified in accordance with Article 9(2) (Annex II) of Regulation (EC) No. 1946/2003 of 15 July 2003, concerning the conclusion, on behalf of the European Community, of the Cartagena Protocol on Biosafety

Monsanto Europe S.A.
Avenue de Tervuren 270-272
B-1150 Brussels
BELGIUM

Annex II of Regulation (EC) No. 1946/2003

INFORMATION REQUIRED CONCERNING LIVING MODIFIED ORGANISMS INTENDED FOR DIRECT USE AS FOOD OR FEED, OR FOR PROCESSING UNDER ARTICLE 9(2) of Annex II

This document contains the statutory information required by the European Union for the transboundary movement of a living genetically modified organism, as requested in Article 9 and Annex II of Regulation (EC) No. 1946/2003. Annex II details the information required to complete the procedure for living modified organisms intended for direct use as food or feed, or for processing (LMO-FFP). The subject LMO-FFP in this document, hereafter referred to as MON 87769 × MON 89788, is a herbicide-tolerant soybean product that produces stearidonic acid (SDA), an omega-3 fatty acid. MON 87769 × MON 89788 is a combined trait product developed through traditional breeding by Monsanto Company.

(a) The name and contact details of the applicant for a decision for domestic use

Monsanto Company, represented by Monsanto Europe S.A.

Monsanto Europe S.A.
Avenue de Tervuren 270-272
B-1150 Brussels
Belgium

Monsanto Company
800 N. Lindbergh Boulevard
St. Louis, Missouri 63167
U.S.A.

(b) The name and contact details of the authority responsible for the decision

The information on MON 87769 × MON 89788 included in this document will be notified by the EU Commission, DG SANCO to the Biosafety Clearing-House, and will be accessible to all Parties to the Cartagena Protocol on Biosafety.

Contact details:

European Commission, DG SANCO
Unit Biotechnology and Plant health
Rue Belliard 232 03/100
B-1049 Brussels
Belgium

(c) Name and identity of the living modified organism

The Monsanto development code for this product is MON 87769 × MON 89788. It produces SDA, and is tolerant to glyphosate, the active ingredient in Roundup® agricultural herbicides.

® Roundup is a registered trademark of Monsanto Technology LLC.

(d) Description of the gene[tic] modification, the technique used, and the resulting characteristics of the living modified organism

No novel method of genetic modification was utilised in the production of MON 87769 × MON 89788. Instead, traditional soybean breeding techniques were used to cross parental soybean plants of MON 87769 and MON 89788. MON 87769 × MON 89788 is an extension of the use of the parents, combining these traits in one soybean plant. MON 87769 × MON 89788 was developed using traditional breeding, however, genetic modification was used to develop the parents, MON 87769 and MON 89788.

MON 87769 was developed through *Agrobacterium*-mediated transformation of soybean meristem tissue using plasmid vector PV-GMPQ1972. *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-GMPQ1972 contains two separate T-DNAs (herein referred to as a 2T-DNA system). The first T-DNA, designated as T-DNA I, contains two expression cassettes: the *Pj.D6D* gene expression cassette and the *Nc.Fad3* gene expression cassette, which produce stearidonic acid (SDA), an omega-3 fatty acid. The second T-DNA region (T-DNA II) contains the *cp4 epsps* gene expression cassette that encodes the CP4 EPSPS protein (5-enolpyruvyl shikimate-3-phosphate synthase protein from *Agrobacterium sp.* Strain CP4), which provides tolerance to the action of glyphosate.

The use of the 2T-DNAs system is the basis for an effective approach to generate marker-free plants. It allows for insertion of the T-DNA with the trait of interest (*e.g.*, T-DNA I) and the T-DNA encoding the selectable marker (*e.g.*, *cp4 epsps*, T-DNA II) into two independent loci within the genome of the plant. Following selection of the transformants, the inserted T-DNA encoding the selectable marker (*e.g.*, T-DNA II) can be segregated from progeny through subsequent breeding and genetic selection, while the inserted T-DNA containing the trait of interest is maintained (*e.g.*, T-DNA I). This process is well documented (De Framond *et al.*, 1986; Depicker *et al.*, 1985; Komari *et al.*, 1996; Yoder and Goldsbrough, 1994) and was successfully used in transformation of barley (Matthews *et al.*, 2001), rice (Breitler *et al.*, 2004), maize (Miller *et al.*, 2002) and soybean (Xing *et al.*, 2000).

Molecular characterization of MON 87769 by Southern blot analyses demonstrated that the DNA inserted into the soybean genome is present at a single locus and contains functional copies of the PjΔ6D and NcΔ15D expression cassette. The *Pj.D6D* expression cassette consists of the *Pj.D6D* coding sequence under the regulation of the *7Sα'* promoter and the *tml* 3' non-translated region. The *Nc.Fad3* expression cassette consists of the *Nc.Fad3* coding sequence under the regulation of the *7Sα* promoter, and the *E9* 3' non-translated region.

The insert in MON 87769 is inherited in the expected Mendelian pattern as a single dominant gene and the stability of the insert has been demonstrated by molecular analysis.

The expression of the PjΔ6D and NcΔ15D genes result in the synthesis of stearidonic acid (SDA) and γ-linolenic acid (GLA) in the oil of MON 87769.

MON 89788 was developed using *Agrobacterium*-mediated transformation of soybean to introduce the *cp4 epsps* gene cassette into the soybean genome. MON 89788 produces the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) protein from *Agrobacterium tumefaciens*

sp. strain CP4 (CP4 EPSPS), which confers tolerance to glyphosate. Molecular characterization of MON 89788 by Southern blot analyses demonstrated that the DNA inserted into the soybean genome is present at a single locus and contains one functional copy of the *cp4 epsps* expression cassette. The *cp4 epsps* expression cassette contains: 1) the *cp4 epsps* coding sequence under the regulation of the *FMV/Tsf1* promoter, containing the *Arabidopsis thaliana Tsf1* gene promoter and enhancer sequences from the Figwort Mosaic Virus *35S* promoter which directs the constitutive expression of the CP4 EPSPS protein; 2) the nontranslated *Tsf1* leader sequence and the *Tsf1* intron to help regulate gene expression; 3) the *Arabidopsis thaliana epsps CTP2* chloroplast transit peptide to translocate the CP4 EPSPS protein to the chloroplasts, which is the site of aromatic amino acid biosynthesis; 4) the *cp4 epsps* coding sequence encoding the CP4 EPSPS protein; 5) the *E9* DNA sequence derived from *Pisum sativum*, containing the 3' nontranslated region of the pea ribulose-1,5-bisphosphate carboxylase small subunit (*RbcS2*) gene for transcriptional termination and polyadenylation of the *CTP2/cp4 epsps* mRNA.

The insert in MON 89788 is inherited in the expected Mendelian pattern as a single dominant gene and the stability of the insert has been demonstrated by molecular analysis and stability of the glyphosate-tolerance phenotype.

CP4 EPSPS provides tolerance to glyphosate (N-phosphonomethylglycine), the active ingredient in the nonselective, foliar-applied, broadspectrum, postemergent family of Roundup® agricultural herbicides.

The use of MON 87769 × MON 89788 will provide a sustainable alternate source of omega-3 fatty acid to help meet the needed dietary intake of long chain omega-3 fatty acids and will enable growers to utilize Roundup® agricultural herbicides for effective control of weeds. With the exception of the intended seed fatty acid compositional changes, particularly the presence of SDA and GLA, and the glyphosate-tolerance trait, MON 87769 × MON 89788 is substantially equivalent in composition and agronomics to conventional soybean.

(e) Any unique identification of the living modified organism

The OECD unique identifier for MON 87769 × MON 89788 is MON−87769−7 × MON−89788−1.

(f) Taxonomic status, common name, point of collection or acquisition, and characteristics of recipient organism or parental organisms related to biosafety

1. Taxonomic status

(i) Family name

Leguminosae

(ii) Genus

Glycine

(iii) Species

max, diploidized tetraploid (2n = 40)

(iv) Subspecies

N/A

2. Common Name

Soybean

3. Point of collection or acquisition

The original soybean varieties used in transformation to produce MON 87769 and MON 89788 were A3525 and A3244, respectively. A3525 and A3244 are commercial conventional varieties obtained from Asgrow, Monsanto Company's wholly-owned soybean seed company.

4. Characteristics of recipient organism or parental organism related to biosafety

In geographies around the world where soybean is grown, it has a long history of safe use as a common source of food and animal feed. The principle product of soybean production is the whole bean, commonly referred to as the seed, which is typically processed into oil and meal. The seed (an LMO) and resulting processed oil and meal (non-viable) are the articles of commerce in international trade. Soybean is produced in several world areas and is exported and imported as a viable grain without risk to the environment.

Domesticated soybean is not known to occur as a weed. It does not possess characteristics commonly associated with weeds and is not known to be invasive or a strong competitor outside of cultivation.

Soybean biology is extensively described in several publications, including an OECD consensus document (2000) and other publications (Carlson and Lersten, 1987; Hermann, 1962). In summary, soybean is a cultivated annual species of the legume family. Soybean grows as erect, bushy annual plants that are 0.3 to 1.2 meters high on hairy stems with trifoliate leaves. Soybean seed planted in a cultivated field will germinate when the soil temperatures reach 10ºC and will subsequently emerge 5-7 days afterward under favourable conditions (OECD, 2000). The plant is considered a quantitative short day plant that flowers more quickly under short day periods. The flowers are small in axillary racemes, usually white or purple. The male and female floral organs are enclosed within the corolla. The seeds are produced in pods, usually containing three spherical to oval seeds. The plants do not reproduce through vegetative means.

Soybean is largely autogamous, which greatly reduces the potential for cross pollination to related species. During anthesis, pollen viability outside of the soybean flower is limited (Fehr, 1987). Pollination typically takes place on the day the flower opens. At that stage, the anthers are closely grouped in a ring around the stigma, such that the plant's own pollen naturally comes in contact with the stigma during anthesis. Anthesis typically takes place in late morning with the pollen remaining viable for 2-4 hours, after which it germinates. Subsequently, natural cross-pollination is restricted to the short time that the pollen grains remain viable. When outcrossing does take place, it primarily occurs with surrounding plants and is usually less than one percent (Boerma and Moradshahi, 1975; Caviness, 1966). Insect activity can increase the outcrossing rate, with the honey bee as the primary insect vector, but soybean is not a preferred plant (Erickson, 1975; Erickson, 1984). In the event that outcrossing occurs, the only wild species that potentially can cross with cultivated soybean are members of the genus *Glycine*, which are native to Asian countries. No other genus is closely enough related to soybean to allow for the possibility of outcrossing (Hymowitz *et al.*, 1992).

Soybean reproduces solely by means of seed, which have no innate dormancy mechanism (TeKrony *et al.*, 1987). Furthermore, soybean is sensitive to cold (Raper and Kramer, 1987) and soybean plants do not normally overwinter. Due to the lack of dormancy, soybean seeds germinate quickly under adequate temperature and moisture, such that seed returned to the ground from mechanical harvesting or during transport will germinate, emerge and subsequently be killed by frost during the autumn or early winter months. If a volunteer MON 87769 × MON 89788 plant were to persist in an agricultural field, it could easily be controlled by currently available selective herbicides or by mechanical means.

(g) Centres of origin and centres of genetic diversity, if known, of the recipient organism and/or the parental organisms and a description of the habitats where the organisms may persist or proliferate

1. Centres of origin and genetic diversity

Soybean (*Glycine max*) is thought to have its origin in Northeast China, the Korean Pennisula and Southeast Russia (Hymowitz, 2004; Vavilov, 1992). Soybean is an annual plant that belongs to the subgenus *Soja*, which also contains the annual wild relative *G. soja* and a form know as *G. gracilis* (Hymowitz, 2004). *Glycine max* is thought to originate from *G. soja*, a wild species of soybean that grows in fields, hedgerows, roadsides and riverbanks in many Asian countries. The genetic relationship between *G. max* and *G. soja* is based on cytological, morphological and molecular evidence indicating that *G. soja* is the ancestor of *G. max*. *Glycine gracilis* is considered to be a weedy or semi-wild form of *G. max*, with some phenotypic characteristics intermediate to those of *G. max* and *G. soja*, such that *G. gracilis* may be an intermediate in the speciation of *G. max* or a hybrid between *G. soja* and *G. max*. *Glycine soja* and other annual and perennial wild soybean relatives are endemic in China, Korea, Japan, Taiwan and Russia (OECD, 2000), but are not known to naturally exist in North American or European countries.

2. Description of the habitats where the organism may persist or proliferate

Soybean is commonly considered one of the oldest cultivated crops in the world. It is grown as a commercial crop in over 35 countries (OECD, 2000). The major producers of soybean are the US, Brazil, Argentina and China.

Soybean cultivars are identified based on bands of adaptation that run east-west, determined by latitude and day length (OECD, 2000). They are classified into one of 13 maturity groups (000, 00, 0, I to X), which determines the area where it will be most productive (Palmer and Kilen, 1987). As soybean is a short day plant, time to maturity is strongly influenced by photoperiod. Cultivars of maturity group X are adapted to southern latitudes (*e.g.*, 0-10° N equatorial zones) where the photoperiod is short, whereas cultivars of maturity group 000 are adapted to the highest latitudes (>45° N) where the photoperiod is longest.

Based on experience from centuries of cultivation, domesticated soybean is known as a poor competitor outside of cultivation. Soybean does not persist or proliferate in most environments around the world without the aid of human intervention. Where *G. max* populations are established, it is accomplished via seed rather than vegetative material. Cultivated soybean is rarely found to 'volunteer', for example, in fence or hedgerows, ditches or roadsides (OECD, 2000).

Experience with soybean imported for processing into a food or feed, has demonstrated that stable populations do not establish, persist of proliferate as a result of incidental release during handling and transport.

(h) Taxonomic status, common name, point of collection or acquisition, and characteristics of the donor organism or organisms related to biosafety

1. Taxonomic status

MON 87769 × MON 89788 was produced through traditional breeding techniques using two genetically modified soybean varieties (*Glycine max*), MON 87769 and MON 89788. There is no evidence of any biosafety issues related to the use of either MON 87769 or MON 89788. There is no evidence of human or animal pathogenicity for any of the donor organisms of the DNA sequences that were used to produce MON 87769 and MON 89788.

The donor organisms for the *Pj.D6D* and *Nc.Fad3* coding sequences were *Primula juliae* and *Neurospora crassa*, respectively. *Primula* is a member of the large genus of plants commonly known as Primrose. They are familiar as a popular garden plant in colder climates. *Neurospora crassa* is a fungi that is ubiquitous in the environment, is not allergenic, and found in the digestive tracts of vertebrate species, including humans. In addition to *Pj.D6D* and *Nc.Fad3*, the regulatory genetic elements discussed in section (d), were obtained from the following sources: 7S α and 7S α' promoters from soybean, the *tml* nontranslated region from *Agrobacterium tumefaciens*, and the *E9* nontranslated region from pea (*Pisum sativum*).

The donor organism for the *cp4 epsps* coding sequence was *Agrobacterium* sp. strain CP4, a common soil-borne bacterium. In addition to *cp4 epsps*, the regulatory genetic elements discussed in section (d) were obtained from the following sources: *FMV*, Figwort Mosaic Virus; *Tsf1* and *CTP2*, *Arabidopsis thaliana*; *E9*, pea (*Pisum sativum*).

2. Common name

See section (h)1.

3. Point of collection or acquisition

All genetic elements were isolated or obtained from Monsanto research laboratories.

4. Characteristics of the donor organism(s) related to biosafety

There is no evidence of any human or animal pathogenicity for any of the donor organisms of the DNA sequences that were used to develop MON 87769 and MON 89788.

(i) Approved uses of the living modified organism

No uses for MON 87769 × MON 89788 have been approved to date. Regulatory submissions and reviews are currently in progress in selected countries around the world.

The scope of this application in the EU is considered to correspond to the following categories of use described in the revised *EFSA guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed*, Annex II: *Scope of the Application* (EFSA, 2006[1]).

1. Food

- 1.1 Genetically Modified (GM) plants for food use
- 1.2 Food containing or consisting of GM plants
- 1.3 Food produced from GM plants or containing ingredients produced from GM plants

2. Feed

- 2.1 GM plants for feed use
- 2.2 Feed containing or consisting of GM plants
- 2.3 Feed produced from GM plants

3. GM plants for environmental release

- 3.1 Import and processing

In accordance with the definitions of 'food' and 'feed' in Article 2(1), the scope of the application also includes all food additives and feed additives (approved according to Directives 89/107/EEC and 70/524/EEC, respectively).

The scope of this application does not include the cultivation of MON 87769 × MON 89788 varieties in the EU.

Once MON 87769 × MON 89788 is authorized in the EU, the approved uses of this soybean will be posted in the Community Register website[2].

The requested duration of the authorization is 10 years.

(j) A risk assessment report consistent with Annex III

Introduction

This section presents a risk assessment report consistent with Annex III of the Cartagena Protocol on Biosafety as required by Annex II.j. The information was collected following the general principles and methodology described in Annex III, which are, "to identify and evaluate the potential adverse effects of living modified organisms on the conservation and sustainable

[1] http://www.efsa.europa.eu/cs/BlobServer/Guidance_of_Panel/gmo_guidance_derived_feed_food.pdf?ssbinary=true – Accessed on 12 July 2010

[2] http://ec.europa.eu/food/dyna/gm_register/index_en.cfm – Accessed on 12 July 2010

use of biological diversity in the likely potential receiving environment, taking into account risks to human health". General principles outlined in Annex III paragraphs (3), (4), (5), and (6) were utilized in the risk assessment including: scientific soundness, transparency, consistency with international guidance and expert advice, and comparison of the non-modified recipient or parental organism within the likely receiving environment. The assessment was carried out considering the intended use and likely receiving environment. In addition, consideration was given to the potential risks to human health. The framework underlying the evaluation of MON 87769 × MON 89788 is consistent with guidance established by the OECD, United Nations World Health Organization (WHO), the FAO and Codex (Codex Alimentarius Commission, 2003; FAO, 1996; OECD, 2003; WHO, 1995), the US, Canada, Japan, the EU and other countries.

The conclusions of the risk assessment conducted herein demonstrate that MON 87769 × MON 89788 poses no increased risk to the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking into account risks to human health under the intended direct use as food or feed, or for processing compared to conventional soybean.

(a) Identification of any novel genotypic or phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health

MON 87769 × MON 89788, developed by traditional breeding of MON 87769 and MON 89788, expresses the PjΔ6D, NcΔ15D and CP4 EPSPS proteins. As a result, these plants produce SDA in the seeds and are tolerant to glyphosate herbicide.

The production of stearidonic acid (SDA), an omega-3 fatty acid, in seeds of MON 87769 × MON 89788 was achieved by the introduction of the *Pj.D6D* and *Nc.Fad3* genes encoding the production of the *Primula juliae* delta-6 desaturase (PjΔ6D) and *Neurospora crassa* delta-15 desaturase (NcΔ15D) proteins into the soybean genome. These two genes are controlled by promoters known to be spatially and temporally localised to the developing seed resulting in the production of SDA only in soybean seeds. The general mode of action of PjΔ6D and NcΔ15D are well understood. PjΔ6D catalyses the desaturation of α-linolenic acid (ALA, 18:3*n*-3) to form SDA (18:4*n*-3) in seeds of MON 87769 × MON 89788 and also converts linoleic acid (LA, 18:2*n*-6) to γ-linolenic acid (GLA, 18:3*n*-6). NcΔ15D catalyses the desaturation of LA to ALA and, therefore, reduces the substrate pool for GLA production and increases the substrate pool for SDA production.

Tolerance of MON 87769 × MON 89788 to glyphosate was achieved by the introduction of a DNA sequence encoding the glyphosate-tolerant EPSPS enzyme, derived from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). The EPSPS enzyme catalyses the penultimate step of the shikimic acid pathway for the biosynthesis of aromatic amino acids, which is present in all green plants. Inhibition of this enzyme by glyphosate leads to a reduction of aromatic amino acids, interfering with plant growth, and ultimately leading to plant death. Expression of the glyphosate-tolerant CP4 EPSPS enzyme in MON 87769 × MON 89788, however, ensures the continued function of the aromatic amino acid pathway in the presence of the herbicide, and enables farmers to use Roundup® herbicide for effective, in-season weed control in soybean. The amino acid sequence

of the CP4 EPSPS protein produced in MON 87769 × MON 89788 is identical to that of the mature CP4 EPSPS produced in a number of other Roundup® Ready crops, including Roundup® Ready soybean 40-3-2, Roundup® Ready canola and Roundup® Ready cotton.

MON 87769 × MON 89788 was characterised to assess what, if any, novel genotypic or phenotypic characteristics might have adverse effects on biological diversity as a result of importation for processing as a food or feed. The characterisation information included molecular, protein, compositional, and phenotypic analyses.

The results of the molecular characterisation indicated that MON 87769 × MON 89788 contains one copy of each DNA insert from MON 87769 and from MON 89788 at separate single integration loci and that no additional elements from either transformation vector were detected in the genome of MON 87769 × MON 89788.

As expected, expression of the PjΔ6D and NcΔ15D proteins in leaf and root tissue was below the limit of detection of 0.1 – 0.2 µg/g of tissue on a fresh weight (fwt) basis for PjΔ6D and 0.5 – 1.0 µg/g fwt for NcΔ15D. The mean PjΔ6D protein levels in immature seed, mature seed, and forage were 46, 3.4, and 10 µg/g of tissue on a dry weight (dwt) basis, respectively. The mean NcΔ15D protein levels in immature seed, mature seed, and forage were 120, 9.6, and 9.2 µg/g dwt, respectively. Both proteins were detected at low levels in forage because the samples contained small amounts of immature seed. The PjΔ6D and NcΔ15D proteins were not detected in the conventional control soybean variety, A3525. The mean protein levels of the CP4 EPSPS protein in MON 87769 × MON 89788 were 52 µg/g fwt in immature seed, 100 µg/g fwt in seed, 35 µg/g fwt in forage, 42 µg/g fwt in over season leaf, and 14 µg/g fwt in root.

Compositional analyses were conducted to assess whether the nutrient, anti-nutrient, and secondary metabolite levels in the seed and forage tissues derived from MON 87769 × MON 89788 are comparable to those in conventional soybean. The analysis established that forage and seed derived from MON 87769 × MON 89788 is compositionally equivalent to those of conventional soybean, with the exception of the intended production of SDA and GLA in seed, the expected changes in associated fatty acids and the tolerance to glyphosate As expected, composition analysis showed that the levels of SDA in MON 87769 × MON 89788 seed ranged from 19.32 to 25.14% of total fatty acids over two seasons, with a mean of 21.62%. Associated with the expected levels of SDA in MON 87769 × MON 89788 is the production of GLA from the Δ6 desaturation of linoleic acid (LA) by PjΔ6D. The GLA levels ranged from 5.79 to 7.05% of total fatty acids over two seasons, with a mean of 6.49%. The intended fatty acid composition change (*i.e.* production of SDA and GLA) decreased the levels of LA in MON 87769 × MON 89788 seed. Low levels of two other fatty acids, trans-SDA (mean = 0.14%, range = 0.063 - 0.18% of total fatty acids) and trans-ALA (mean = 0.20%, range = 0.15 - 0.26% of total fatty acids) also were observed in MON 87769 × MON 89788 seed over two seasons. The formation of trans-ALA and trans-SDA is due to the known spontaneous trans-isomerisation of unsaturated fatty acids, at rates that increase with increasing degree of unsaturation (Chardigny *et al.*, 1996). As SDA and ALA represent a significant proportion of total fatty acids in MON 87769 × MON 89788 (approximately 25 - 35% in total), trans-ALA and trans-SDA are expected to be detected in the fatty acid analysis of MON 87769 × MON 89788. Thus, results indicated that, with the exception of the intended seed fatty acid compositional changes and the production of the CP4 EPSPS protein,

MON 87769 × MON 89788 is compositionally and nutritionally equivalent to conventional soybean that is currently in commerce and has a history of safe human and animal consumption.

Field trials with MON 87769 × MON 89788 across a broad geographic range of environments have been conducted since 2007. These trials indicated that MON 87769 × MON 89788 demonstrated no biologically meaningful phenotypic differences from conventional soybean except for the introduced genes (*Pj.D6D, Nc.Fad3,* and *cp4-epsps*) and the resultant proteins (PjΔ6D, NcΔ15D, and CP4 EPSPS) with the intended fatty acid composition changes and the introduction of the glyphosate tolerant trait.

In conclusion, there are no meaningful differences in MON 87769 × MON 89788 except for the intended seed fatty acid compositional changes, particularly the presence of SDA and GLA, and the glyphosate tolerance trait. These traits are imparted by the production of the PjΔ6D, NcΔ15D, and CP4 EPSPS proteins encoded by the inserted DNA. Molecular analyses and comparative assessments of MON 87769 × MON 89788 with conventional soybean did not reveal any other novel characteristics or unintended adverse effects of the genetic modification, particularly changes to its persistence and invasiveness compared to conventional soybean.

The histories of safe use and data from multiple evaluations support the safety of the PjΔ6D and NcΔ15D proteins. Results from protein safety assessment indicated there is a reasonable certainty of no harm to mammals from exposure to the PjΔ6D and NcΔ15D proteins. The proteins have a well-defined mode of action that do not raise any safety concerns. Both proteins lack similarity to known allergens, toxins, or anti-nutritional proteins known to have adverse effects to mammals. Expression levels of both PjΔ6D and NcΔ15D proteins range from not detectable in vegetative tissues to very low in seed. Digestive fate experiments conducted with the PjΔ6D protein demonstrated that the full-length protein is 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 SGF were quickly degraded during a short exposure to simulated intestinal fluid (SIF). Rapid digestion of the full-length PjΔ6D protein in SGF and SIF, together with rapid degradation of the transiently stable fragment from the SGF assay by SIF, indicates that it is highly unlikely that the PjΔ6D protein and its fragment will reach absorptive cells of the intestinal mucosa. The NcΔ15D protein was also readily digested in SGF and SIF.

Additionally, the PjΔ6D and NcΔ15D proteins or their close structural and functional homologues have been present in foods and feeds for significant periods of time with no documented history of any adverse effects. Neither protein exhibits toxicity when administered orally to mammals even when doses are significantly greater than would be experienced under the most conservative dietary exposure scenarios. Ultimately, the safety assessment supports the conclusion that there are no meaningful risks of adverse effects to human or animal health from dietary exposure to either the PjΔ6D or NcΔ15D proteins present in MON 87769 × MON 89788.

The environmental and human safety of the CP4 EPSPS protein is well established. Numerous products containing CP4 EPSPS have been approved for intentional release or importation around the world including Argentina, Canada, the European Union (EU), Japan, and the US.

Products expressing CP4 EPSPS have been marketed since 1996 with no environmental or human health issues.

Results of a safety assessment of the intended fatty acid changes in MON 87769 × MON 89788 indicated there is a reasonable certainty of no harm to mammals from exposure to SDA or GLA in seed derived from MON 87769 × MON 89788. In general, polyunsaturated fatty acids (PUFA) are important compounds in most animals. They are oxidised in energy production, incorporated in phospholipids and are essential for cellular membrane formation and function, desaturated/elongated, and oxygenated into physiologically active eicosanoids (Macdonald and Sprecher, 1991; Watkins, 1991; Watkins, 1995).

SDA and GLA are *in vivo* intermediate PUFAs in the metabolism of ALA to long chain omega-3 fatty acids and LA to arachidonic acid, respectively, in most mammals, birds, and insects. SDA and GLA are present in many sources in the environment including plants, marine algae, and fish/fish oil without known adverse effects. The lack of toxicity of SDA and GLA in MON 87769 × MON 89788 seed is further demonstrated by the lack of test material-related adverse effects in animal feeding studies conducted using soybean oil and meal derived from MON 87769 × MON 89788. Ultimately, the safety assessment supports the conclusion that there are no meaningful risks of adverse effects to human or animal health from dietary exposure to either SDA or GLA present in MON 87769 × MON 89788.

Based on these properties, it is reasonable to conclude that the PjΔ6D, NcΔ15D, and CP4 EPSPS proteins, the presence of SDA and GLA, and the expected changes in associated fatty acids in the seed pose no meaningful potential to adversely affect biological diversity or human health in the EU from the intended use of soybean containing event MON 87769 × MON 89788 as a LMO imported for direct use in food, feed and processing, or due to the transboundary movement of this LMO.

Based on extensive characterization of MON 87769 × MON 89788 and its introduced traits, it is not likely that MON 87769 × MON 89788 will pose any meaningful adverse effects to human or animal health or to the biodiversity in any given environment. Nevertheless, in Sections j(b)-(f), a step-wise risk assessment is made to evaluate the potential of the introduced traits to cause adverse effects on the biodiversity in the receiving environment or potential toxic or allergenic effects to humans, resulting from transboundary movement of this living modified organism for direct use as food or feed, or for processing.

(b) Evaluation of the likelihood of [these] adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism

As noted above in Section j(a), no potentially adverse effects were detected based on extensive characterization of MON 87769 × MON 89788, which included molecular, protein, compositional analyses and phenotypic evaluation conducted in field trials over a wide range of environmental conditions. Testing of MON 87769 × MON 89788 showed no changes in its ability to persist in the environment without human intervention or to become invasive compared to the parents and conventional soybean.

The likelihood of adverse effects being realized is considered to be low for the following reasons. Firstly, MON 87769 × MON 89788 seed has a negligible hazard potential. Field trials and laboratory evaluations have demonstrated that the hazards associated with MON 87769 × MON 89788 are no greater than those associated with conventional soybean. Therefore, the hazard characterization is not expected to change as a result of importing soybean containing MON 87769 × MON 89788 for direct use in food, feed or processing.

Secondly, the containment systems used to transport and handle seed are characterized by highly reduced exposure to the biodiversity based on experience with conventional soybean. Seed imported for direct use as food or feed, or for processing is not intended for release into natural or agricultural environments. Rather, the harvested seed material (either biotechnology-derived or LMO or not) is held, transported and handled in a confined manner that restricts the potential for escape into the local environment. Because the seed will be confined to conditions that are fixed in location (seaports, grain elevators and processing facilities) and enclosed to minimize or prevent release (transport vehicles including trucks and railroad cars), they meet the conditions of Article 3(b) of the Cartagena Protocol on Biosafety. Such conditions significantly limit exposure to the environment. Although incidental release of spilled seed into the receiving environment occurs during export/import, handling, storage and processing of seed, modern methods of grain handling minimize such losses. Furthermore, the locations of any incidental release will be predictable, since they will be near the storage facilities and along transportation routes. Environmental conditions at these sites are unlikely to be conducive to germination, growth and reproduction of harvested soybean seed material that is incidentally released.

Compared to conventional soybean, MON 87769 × MON 89788 has not been altered with respect to its dispersal or survival characteristics as assessed by phenotypic characteristics including: early stand count, seedling vigor, plant growth stages, days to 50% flowering, flower color, plant pubescence, plant height, lodging, pod shattering, final stand count, grain moisture, 100 seed weight, test weight, yield, arthropod damage, disease damage and plant response to abiotic stressors. Field trial data for MON 87769 × MON 89788 have demonstrated that this soybean has not been altered in its phenotypic, agronomic, reproductive, survival and dissemination characteristics when compared to conventional soybean. Based on the overall characterization, the results support a conclusion that there are no changes in the plant phenotype of MON 87769 × MON 89788 that would be indicative of increased invasiveness or persistence relative to conventional soybean.

Importantly, there is no information to support a conclusion that MON 87769 × MON 89788 harvested seed material will establish, persist or disperse to a greater extent than conventional soybean. In cases where incidental release occurs and a MON 87769 × MON 89788 plant establishes, these plants will be easily controlled by currently available herbicides and by mechanical means. As such, MON 87769 × MON 89788 has no meaningful potential to disperse, persist without human intervention, or invade non-agricultural areas as a result of importation for direct use in food, feed or processing. Even if spillage of seed in the environment resulted in the short survival of MON 87769 × MON 89788 plants containing the inherited traits, their presence would pose negligible risk to non-target organisms dwelling in the vicinity of the incidental release.

Because of the lack of hazard potential and the factors that limit exposure to the environment, the likelihood of MON 87769 × MON 89788 to result in adverse environmental effects on the biodiversity and human health is comparable to the impact of conventional soybean imported for direct use in food, feed or processing.

(c) Evaluation of the consequences should [these] adverse effects be realized

As noted above in Section j(a), no potentially adverse effects were detected based on extensive characterization of MON 87769 × MON 89788, which included molecular, protein, compositional analyses and phenotypic evaluations conducted in field trials over a wide range of environmental conditions. Testing of MON 87769 × MON 89788 demonstrated no changes that would indicate an increased ability to persist in the environment without human intervention or to become invasive compared to conventional soybean. As such, the potential consequences to biodiversity resulting from importation of MON 87769 × MON 89788 for direct use in food, feed or processing are the same as with conventional soybean. Potential adverse consequences such as weediness, unfavorable impacts to non-target organisms and harmful effects on biodiversity and human health were considered in this assessment.

(d) Estimation of overall risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized

The overall estimated risk to biodiversity posed by the importation of soybean seed containing MON 87769 × MON 89788 for direct use in food, feed, or for processing is negligible. This conclusion is based on: a) the history of safe use of the host plant, soybean, b) the extensive characterization of the LMO MON 87769 × MON 89788 compared to conventionally bred soybean including, phenotypic, compositional, and nutritional equivalence (except for the intended seed fatty acid changes), c) the extensive characterization and history of safety of the expressed PjΔ6D, NcΔ15D, and CP4 EPSPS proteins, d) results of a safety assessment of the intended fatty acid changes in MON 87769 × MON 89788 seed, and e) the fact that, based on a combination of the history of experience with importing soybean and the characteristics of MON 87769 × MON 89788, there are no unique environmental conditions that would meaningfully change the environmental characteristics of MON 87769 × MON 89788 compared to conventional soybean.

(e) Recommendation whether the risks are acceptable or manageable; including where necessary, identification of strategies to manage these risks

Analysis of the characteristics of MON 87769 × MON 89788 did not reveal any potential for adverse effects to human health or the environment, as could be expected from the analysis of its parental varieties.

Therefore, there are no unacceptable environmental or human health risks associated with the importation of MON 87769 × MON 89788 for direct use as food or feed, or for processing. As the environmental and health risks of this soybean are consistently negligible and no different than conventional soybean, no specific strategies for risk management are required.

(f) Where there is uncertainty regarding the level of risk, it may be addressed by requesting further information on the specific issues of concern or by implementing appropriate risk management strategies and/or monitoring [of] the living modified organism in the receiving environment

The above risk assessment for MON 87769 × MON 89788 was undertaken in accordance with the general principles and methodology laid out in the Cartagena Protocol on Biosafety and in the context of the scope of transboundary movement for direct use as food or feed, or for processing.

Analysis of the characteristics of MON 87769 × MON 89788 and comparison to the long history of safety established for conventional soybean has shown that the risks for potential adverse effects on human health or the receiving environment are consistently negligible and no different from conventional soybean. Therefore, the overall risk posed by the living modified organism is negligible and no specific strategies for risk management are required.

Since the conclusions of this environmental risk assessment, including the positive conclusion on the safety of MON 87769 × MON 89788 to human health, have been derived from the results of scientific studies rather than major assumptions, no monitoring actions, typically aimed to address significant uncertainty regarding the levels of risk in this assessment, would be warranted or required.

(k) Suggested methods for the safe handling, storage, transport and use, including packaging, labeling, documentation, disposal and contingency procedures, where appropriate

In countries where the planting of MON 87769 × MON 89788 will be authorized, appropriate and comprehensive information will be provided on seed bags and in accompanying documents in order for purchasers to be fully informed about the use of this soybean.

Transboundary shipments of commodity soybean which "may contain" MON 87769 × MON 89788, will clearly be identified as such, and will bear an indication that the living modified organism is not intended for intentional introduction into the environment. A contact point for further information will be added to the documentation accompanying this living modified organism in accordance with Art.18(2)(a) of the Cartagena Protocol on Biosafety.

MON 87769 × MON 89788 is substantially equivalent to other soybean varieties except for the production of SDA, GLA, expected changes in associated seed fatty acids, and its tolerance to glyphosate. In order to derive commercial value from this product, the crop will be grown and processed in an identity-preserved manner (IDP). Therefore the seed and oil from MON 87769 × MON 89788 will be stored, packaged, transported, used, and handled in a manner consistent with IDP practices.

In the highly unlikely event of establishment of MON 87769 × MON 89788 in the environment, volunteer plants could be easily controlled by currently available herbicides or by mechanical means. Therefore no specific measures are recommended in case of unintended release (spillage or other means) of MON 87769 × MON 89788.

References

Bevan, M. (1984) Binary *Agrobacterium* vectors for plant transformation, *Nucleic Acids Res.*, **12**, 8711-8721.

Boerma, H. R. and Moradshahi, A. (1975) Pollen movement within and between rows to male-sterile soybeans, *Crop Sci.*, **15**, 858-861.

Breitler, J. C., Meynard, D., Van Boxtel, J., Royer, M., Bonnot, F., Cambillau, L. and Guiderdoni, E. (2004) A novel two T-DNA binary vector allows efficient generation of marker-free transgenic plants in three elite cultivars of rice (*Oryza sativa* L.), *Transgenic Res.*, **13**, 271-287.

Carlson, J. B. and Lersten, N. R. (1987) Reproductive morphology, *Agronomy*, **16**, 95-134.

Caviness, C. E. (1966) Estimates of natural cross-pollination in Jackson soybeans in Arkansas, *Crop Sci.*, **6**, 211-212.

Chardigny, J., Sebedio, J. and Berdeaux, O. (1996) *Trans* polyunsaturated fatty acids: occurrence and nutritional implications. In: *Advances in Applied Lipid Research*. JAI Press Inc, London, UK, Vol. 2, pp. 1-33.

Codex Alimentarius Commission. (2003) Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants, *Codex*, **CAC/GL 45-2003**, 1-13.

De Framond, A. J., Black, E. W., Chilton, W. S., Kayes, L. and Chilton, M. D. (1986) Two unlinked T-DNAs can transform the same tobacco plant cell and segregate in the F1 generation, *Mol. Gen. Genet.*, **202**, 125-131.

Depicker, A., Herman, L., Jacobs, A., SChell, J. and Van Montague, M. (1985) Frequencies of simultaneous transformation with different T-DNAs and their relevance to the *Agrobacterium*/plant cell interaction, *Mol. Gen. Genet.*, **201**, 477-484.

Erickson, E. H. (1975) Variability of floral characteristics influences honey bee visitation to soybean blossom, *Crop Sci.*, **15**, 767-771.

Erickson, E. H. (1984) Soybean pollination and honey production - a research progress report, *Am. Bee J.*, **124**, 775-779.

FAO. (1996) Biotechnology and Food Safety. Report of a Joint FAO/WHO consultation; FAO food and nutrition paper 61, *Food and Agriculture Organization of the United Nations.*, **61**, 1-31.

Fehr, W. R. (1987) Soybean. In: Fehr, W. R. (ed.) *Principles of Cultivar Development*, New York, Vol. 2, pp. 533-576.

Hermann, F. J. (1962) A revision of the genus *Glycine* and its immediate allies, *US dept. Agric. Techn. Bull.*, **1268**, 1-79.

Hymowitz, T., Palmer, R. G. and Singh, R. J. (1992) Cytogenetics of the genus *Glycine*. In: Tsuchiya, T., Gupta, P. K. (eds.), *Chromosome Engineering in Plants: Genetics, Breeding, Evolution, Part B.*, Amsterdam, Vol. 3, pp. 53-63.

Hymowitz, T. (2004) Speciation and cytogenetics. In: Boerma, H. R., Specht, J. E. (eds.), *Soybeans: improvement, production, and uses*. American Society of Agronomy, Crop Science Society of America & Soil Science Society of America, Madison, Wisconsin, U.S.A., pp. 97-136.

Komari, T., Hiei, Y., Saito, Y., Murai, N. and Kumashiro, T. (1996) Vectors carrying two separate T-DNAs for co-transformation of higher plants mediated by *Agrobacterium tumefaciens* and segregation of transformants free from selection markers, *The Plant Journal*, **10**, 165-174.

Macdonald, J. and Sprecher, H. (1991) Phospholipid fatty acid remodeling in mammalian cells, *Biochimica et Biophysica Acta*, **1084**, 105-121.

Matthews, P. R., Wang, M. B., Waterhouse, P. M., Thornton, S., Fieg, S. J., Glubler, F. and Jacobsen, J. V. (2001) Marker gene elimination from transgenic barley, using co-transformation with adjacent 'twin T-DNA's on a standard *Agrobacterium* transformation vector, *Mol. Breeding*, **7**, 195-202.

Miller, M., Tagliani, L., Wang, N., Berka, B., Bidney, D. and Zhao, Z. Y. (2002) High efficiency transgene segregation in co-transformed maize plants using an Agrobacterium tumefaciens 2 T-DNA binary system, *Transgenic Research*, **11**, 381-396.

OECD. (2000) Consensus document on the biology of *glycine max* (L.) merr. (soybean), *OECD*, **ENV/JM/MONO(2000)9**

OECD. (2003) Consensus Document on the Biology of Zea Mays Subsp. Mays (Maize). http://www.oecd.org/

Palmer, R. G. and Kilen, T. C. (1987) Qualitative genetics and cytogenetics. In: Wilcox, J. R. (ed.) *Soybeans: improvement, production, and uses*. American Society of Agronomy, Crop Science Society of America & Soil Science Society of America, Madison, Wisconsin, U.S.A., pp. 135-209.

Raper, C. D. and Kramer, P. J. (1987) Stress physiology. In: Wilcox, J. R. (ed.) *Soybean: improvement, production and uses*. American Society of Agronomy, Crop Science Society of America & Soil Science Society of America, Madison, Wisconsin, U.S.A., pp. 589-641.

TeKrony, D. M., Egli, D. B. and White, G. M. (1987) Seed production and technology. In: Wilcox, J. R. (ed.) *Soybeans: improvement, production, and uses*. American Society of Agronomy, Crop Science Society of America & Soil Science Society of America, Madison, Wisconsin, U.S.A., pp. 295-353.

Vavilov, N. I. (1992) Origin and geography of cultivated plants,

Watkins, B. (1991) Importance of essential fatty acids and their derivatives in poultry, *Journal of Nutrition*, **121**, 1475-1485.

Watkins, B. (1995) Biochemical and physiological aspects of polyunsaturates, *Poultry and Avian Biology Reviews*, **6**, 1-18.

WHO. (1995) Application of the principles of substantial equivalence to the safety evaluation of foods or food components from plants derived by modern biotechnology, *Report of a who workshop. WHO food safety unit WHO/FNU/FOS/95.1*, 1-78.

Xing, A., Zhangyuan, Z., Sato, S., Staswick, P. and Clemente, T. (2000) The use of the two T-DNA binary system to obtain maker free transgenic soybeans, *In Vitro Cell Dev. Biol.*, **36**, 456-463.

Yoder, J. I. and Goldsbrough, A. P. (1994) Transformation systems for generating marker-free transgenic plants, *Bio/technology*, **12**, 263-267.