Application for authorisation 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 II

Summary

Data protection.

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

A. GENERAL INFORMATION

1. Details of application

a) Member State of application

The Netherlands.

b) Notification number

Not available at the time of submission.

c) Name of the product (commercial and other names)

The Monsanto development code for this genetically modified soybean is MON 87769 × MON 89788. Currently, no commercial name has been attributed to this product.

d) Date of acknowledgement of notification

Not available at the time of submission.

2. Applicant

a) Name of applicant

Monsanto Company, represented by Monsanto Europe S.A.

b) Address of applicant

Monsanto Europe S.A.	Monsanto Company
Avenue de Tervuren 270-272	800 N. Lindbergh Boulevard
B-1150 Brussels	St. Louis, Missouri 63167
BELGIUM	U.S.A.

c) Name and address of the person established in the Community who is responsible for the placing on the market, whether it be the manufacturer, the importer or the distributor, if different from the applicant (Commission Decision 2004/204/EC Art 3(a)(ii))

MON 87769 × MON 89788 will be produced in other world areas and will be imported and used in the European Union by operators that have traditionally been involved in the commerce, transport, processing and use of soybean and soybean-derived products in the EU.

3. Scope of the application

(X) GM plants for food use

(X) Food containing or consisting of GM plants

(X) Food produced from GM plants or containing ingredients produced from GM plants

(X) GM plants for feed use

(X) Feed containing or consisting of GM plants

(X) Feed produced from GM plants

(X) Import and processing (Part C of Directive 2001/18/EC)

( ) Seeds and plant propagating material for cultivation in Europe (Part C of Directive 2001/18/EC)

4. Is the product being simultaneously notified within the framework of another regulation (e.g. Seed legislation)?

Yes ( )	**No (X)**
If *yes*, specify	

5. Has the GM plant been notified under Part B of Directive 2001/18/EC and/or Directive 90/220/EEC?

Yes ( )	**No (X)**
If *no*, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC The protein expression, the composition, the safety, the agronomic and the phenotypic characteristics of MON 87769 × MON 89788 have been studied at multiple locations in North and/or South America that cover a range of environmental conditions. The risk assessment presented in the MON 87769 × MON 89788 application includes data collected from these field trials. A summary of the conclusions of the risk analysis that demonstrate the safety of MON 87769 × MON 89788 to humans, animals and to the environment, have been presented in the respective sections throughout this summary.	

6. Has the GM plant or derived products been previously notified for marketing in the Community under Part C of Directive 2001/18/EC or Regulation (EC) 258/97?

Yes ( )	No (X)
If *yes*, specify	

7. Has the product been notified in a third country either previously or simultaneously?

Yes (X)	No ( )

If *yes*, specify

Regulatory submissions were made to the US regulatory agencies (FDA and USDA) and to countries that import significant soybean or food and feed products derived from US soybean and have functional regulatory review processes in place. These included submissions to a number of additional governmental regulatory agencies including, but not limited to Ministry of Health, Labor and Welfare (MHLW) and Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan, Canadian Food Inspection Agency (CFIA) and Health Canada, and Mexico's Intersectoral Commission for Biosafety of Genetically Modified Organisms (CIBIOGEM). Submission will soon be made to the Ministry of Agriculture (MOA) of China. As appropriate, notifications will be made to countries that import significant quantities of US soybean and soybean products and do not have a formal regulatory review process for biotechnology-derived crops. The reviews in all the above mentioned countries are in progress.

In addition, a Generally Recognised As Safe (GRAS) notice was filed with the US FDA on soybean oil from MON 87769 (hereafter referred to as SDA soybean oil) containing 20 to 30% SDA (GRAS Notice No. GRN 000283[1]). A scientific panel has confirmed the safety of SDA soybean oil on 4 September 2009[2].

[1] GRAS notices filed in 2009: http://www.accessdata.fda.gov/scripts/fcn/gras_notices/grn000283.pdf - Accessed July 20, 2010

[2] FDA: http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm185688.htm - Accessed July 20, 2010

8. General description of the product

a) Name of the recipient or parental plant and the intended function of the genetic modification

MON 87769 × MON 89788 was obtained by traditional breeding of two parental lines, one derived from MON 87769 and the other one derived from MON 89788.

MON 87769 soybean was developed to produce stearidonic acid (SDA) through *Agrobacterium*-mediated transformation of soybean meristem tissues using the binary transformation plasmid PV-GMPQ1972. Two desaturase genes were introduced, *Primula juliae* Δ6 desaturase (*Pj.D6D*) and *Neurospora crassa* Δ15 desaturase (*Nc.Fad3*). These proteins desaturate certain endogenous fatty acids resulting in the production of SDA at approximately 20-30% of total fatty acids. SDA is an omega-3 fatty acid which is a normal metabolic precursor to the long chain, poly-unsaturated omega-3 fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in humans and animals. The cardiovascular benefits of long-chain PUFAs are well established.

MON 89788 was developed through *Agrobacterium*-mediated transformation of soybean tissues using the double-border, binary transformation plasmid PV-GMGOX20. The vector PV-GMGOX20 carries a glyphosate[3] tolerant 5-enolpyruvyl shikimate-3-phosphate synthase (*epsps*) gene derived from *Agrobacterium* sp. strain CP4 (*cp4 epsps*). MON 89788 produces the CP4 EPSPS protein conferring glyphosate tolerance to the plant.

b) Types of products planned to be placed on the market according to the authorisation applied for

The scope of the current application is for authorisation of MON 87769 × MON 89788 in the EU for all uses according to Art 3(1) and 15(1) of Regulation (EC) No. 1829/2003, with the exception of cultivation. The range of uses of this soybean will be identical to the full range of equivalent uses of conventional soybean.

c) Intended use of the product and types of users

MON 87769 × MON 89788 is being developed as a sustainable means to produce oil enriched in an omega-3 fatty acid that will be utilised as a component of human foods. The refined oil produced from MON 87769 × MON 89788 contains approximately 20 to 30% SDA (weight % of total fatty acids) and it can be used for the production of margarine, mayonnaise, shortenings, salad dressings and ready-to-eat foods. The use of SDA soybean oil in selected food categories could provide a wide range of dietary alternatives for increasing human omega-3 fatty acid intake. Given the targeted commercial applications, MON 87769 × MON 89788 is intended

[3] N-phosphonometyl glycine is the active ingredient in the non-selective, foliar applied, broad spectrum, post-emergent Roundup® herbicides. Roundup is a registered trademark of Monsanto Technology LLC.

for use as a small area crop planned initially for production in North America. In order to derive commercial value from this product, the MON 87769 × MON 89788 soybean crop will be grown in an identity preserved manner. MON 87769 × MON 89788 will be processed in dedicated oil processing facilities that will also be operated in an identity preserved manner and SDA soybean oil will be sold to food processors for food formulation. The oil will be used in food applications where omega-3 products are currently being used. The co-product, soybean meal and other soybean derivatives will be used in a manner similar to conventional soybean meal and derivatives.

d) Specific instructions and/or recommendations for use, storage and handling, including mandatory restrictions proposed as a condition of the authorisation applied for

No specific conditions or instructions are required for the placing on the market of MON 87769 × MON 89788 in the context of the scope of the current application, which does not include cultivation. MON 87769 × MON 89788 is substantially equivalent to conventional soybean, except for its SDA production trait, the associated changes in the levels of fatty acids and its glyphosate tolerance trait. This soybean was shown to be as safe as conventional soybean. Therefore, MON 87769 × MON 89788 and its derived products will be stored, packaged, transported, used and handled in the same manner as current commercial soybean with the exception of those practices needed to retain the commercial value of the MON 87769 oil and to ensure its functional suitability for incorporation into food and feed products.

e) Any proposed packaging requirements

MON 87769 × MON 89788 is substantially equivalent to conventional soybean, except for its SDA production trait, the associated changes in the levels of fatty acids and its glyphosate tolerance trait. Therefore, MON 87769 × MON 89788 and derived products will be used in the same manner as other soybean and no specific packaging is required (for labelling, please *see* question A.8.f).

f) A proposal for labelling in accordance with Articles 13 and 25 of Regulation (EC) 1829/2003. In the case of GMOs, food and/or feed containing, consisting of GMOs, a proposal for labelling has to be included complying with the requirements of Article 4, B(6) of Regulation (EC) 1830/2003 and Annex IV of Directive 2001/18/EC.

In accordance with Regulations (EC) No. 1829/2003 and 1830/2003, the current labelling threshold of 0.9% is applied for the marketing of MON 87769 × MON 89788 and derived products.

As MON 87769 × MON 89788 differs from conventional soybean in terms of fatty acid composition and nutritional value, labelling in accordance with Article 13(2)(a) and Article 25(2) is proposed. Monsanto proposes that operators shall be required to label products containing or consisting of MON 87769 × MON 89788 with the

words “genetically modified soybean containing SDA omega-3 oil” or “contains genetically modified soybean containing SDA omega-3 oil” and shall declare the unique identifier MON-87769-7 × MON-89788-1 in the list of GMOs that have been used to constitute a mixture that contains or consists of this GMO.

Operators shall be required to label foods and feeds derived from MON 87769 × MON 89788 with the words “produced from genetically modified soybean containing SDA omega-3 oil”, and products containing or consisting of oil produced from MON 87769 × MON 89788 with the words “oil produced from genetically modified soybean containing SDA omega-3”.

Monsanto proposes that products containing or consisting of derivatives (other than the oil) from MON 87769 × MON 89788 are labelled with the words “produced from genetically modified soybean”.

Operators handling or using MON 87769 × MON 89788 and derived foods and feeds in the EU shall be required to be aware of the legal obligations regarding traceability and labelling of these products. Given that explicit requirements for the traceability and labelling of GMOs and derived foods and feeds are laid down in Regulations (EC) No. 1829/2003 and 1830/2003, and that authorized foods and feeds shall be entered in the Community Register, operators in the food/feed chain will be fully aware of the traceability and labelling requirements for MON 87769 × MON 89788. Therefore, no further specific measures are to be taken by the applicant.

g) Unique identifier for the GM plant (Regulation (EC) 65/2004; does not apply to applications concerning only food and feed produced from GM plants, or containing ingredients produced from GM plants)

The unique identifier for this genetically modified soybean is MON-87769-7 × MON-89788-1

h) If applicable, geographical areas within the EU to which the product is intended to be confined under the terms of the authorisation applied for. Any type of environment to which the product is unsuited

MON 87769 × MON 89788 is suitable for use throughout the EU.

9. Measures suggested by the applicant to take in case of unintended release or misuse as well as measures for disposal and treatment

In the context of the scope of the current application, which does not include cultivation of MON 87769 × MON 89788 in the EU, environmental release would more likely occur during import, storage and processing. As described in Section A.8.c, to retain commercial value of the product, MON 87769 × MON 89788 soybean will be processed in dedicated facilities and therefore it is not expected that significant quantities of MON 87769 × MON 89788 soybean will commingle with general soybean supply in the EU.

Modern methods of soybean handling minimize losses of seed, so there is little chance of germination of spilt soybeans resulting in the development of mature MON 87769 × MON 89788 plants in the EU. Moreover, in the case of incidental spillage, the establishment of volunteer plants would be unlikely, since soybean cannot survive without human assistance and is not capable of surviving as a weed due to selection over centuries of cultivation. Soybean is not documented as a source of volunteer plants in rotational crops, which results from the combination of absence of seed dormancy, poor seed survivability in soils, frost sensitivity of soybean seedlings and soil preparations prior to the planting of a subsequent crop (which includes destruction of any existing vegetation and soil cultivation). MON 87769 × MON 89788 is shown to be substantially equivalent to conventional soybean, except for the inherited SDA production trait, the associated changes in the levels of fatty acids and the inherited glyphosate tolerance trait. Therefore, MON 87769 × MON 89788 is unlikely to pose any threat to the EU environment or to require special measures for its containment. Furthermore, soybean volunteers can be easily controlled using currently available selective herbicides or by mechanical means. Therefore, no specific conditions are warranted or required for the placing on the market of MON 87769 × MON 89788 in the context of the scope of the current application.

B. INFORMATION RELATING TO (A) THE RECIPIENT OR (B) (WHERE APPROPRIATE) PARENTAL PLANTS

1. Complete name

a) Family name

Leguminosae

b) Genus

Glycine

c) Species

max

d) Subspecies

Not applicable

e) Cultivar/breeding line

A3525

f) Common name

Soybean

2. a) Information concerning reproduction

(i) Mode(s) of reproduction

Soybean is a diploidized tetraploid (2n = 40) and is a self-pollinated species, propagated by seed.

Pollination typically takes place on the day the flower opens. Anthesis normally occurs in late morning (usually between 10.00 and 11.00 am, depending on the environmental conditions). The pollen usually remains viable for 2-4 hours, and no viable pollen can be detected by late afternoon. Natural or artificial cross-pollination can only take place during the short time of the day that the pollen is viable.

(ii) Specific factors affecting reproduction

Soybean is a quantitative short day plant and hence flowers more quickly under short days. As a result, photoperiodism and temperature response are important in determining areas of cultivar adaptation.

During the reproductive stages of development, soybean plants are particularly sensitive to hydric and thermal (low temperature) stress which can cause significant flower abortion and yield loss. Soybeans do not yield well on acid soils and the addition of limestone may be required.

(iii) Generation time

Soybean is an annual crop which is planted from April to May in the northern hemisphere, and from November to February in the southern hemisphere including second cropping. Soybean seed germinates when the soil temperature reaches 10°C and emerges in a 5-7 day period under favourable conditions

Soybeans grow most rapidly when air temperatures are between 25°C and 35°C. The life cycle of soybean is approximately 100 to 160 days, depending on the variety and the region in which it is cultivated.

2. b) Sexual compatibility with other cultivated or wild plant species

Outcrossing with cultivated soybean species

Although soybean is a self-pollinated species, natural cross-pollination can occur, at very low rate. Cross-pollination frequencies may vary due to growing season and genotype, and most outcrossing occurs with immediately surrounding plants. Insect activity increases the outcrossing rate, but soybeans generally are not the preferred plant for pollinators.

It has to be noted, however, that the scope of the current application does not include the cultivation of MON 87769 × MON 89788 varieties in the EU. Therefore, any outcrossing between MON 87769 × MON 89788 and cultivated *Glycine* varieties is highly unlikely.

Outcrossing with wild soybean species

From a taxonomic standpoint, both the wild annual species of subgenus *Soja* and the wild perennial species of subgenus *Glycine* are candidates for gene exchange with the cultivated soybean. No other genus is closely enough related to soybean to allow for the possibility of outcrossing.

There are no known reports of successful natural hybridization between cultivated soybean and wild perennial species of subgenus *Glycine*. Moreover, there are no wild relatives of subgenus *Glycine* in Europe.

The wild annual species *G. soja*, can hybridize naturally with the cultivated soybean, *G. max*, since they are both members of the subgenus *Soja*. Therefore,

gene transfer between cultivated soybean and wild species of subgenus *Soja* may occur, but not in Europe, where the wild relatives of subgenus *Soja* are not present.

3. Survivability

a) Ability to form structures for survival or dormancy

Cultivated soybean plants are annuals and they reproduce solely by means of seeds. Mature soybean seeds have no innate dormancy, are sensitive to cold and are not likely to survive from one growing season to the next if left in the field over winter. Commercial soybean seeds are selected for lack of dormancy, enabling them to germinate quickly under adequate temperature and moisture which could potentially allow them to grow as volunteers in a field. However, volunteers likely would be killed by frost during autumn or winter of the year they were produced. If they did establish, volunteers would not compete well with the succeeding crop, and could be controlled readily either mechanically or chemically.

b) Specific factors affecting survivability

See Section B.3.a.

4. Dissemination

a) Ways and extent of dissemination

In theory, soybean dissemination may occur by means of seed dispersal or pollen dispersal. Soybean pods and seed do not have dispersal mechanisms that facilitate seed or pod movement over long distances. Furthermore, neither the soybean seedpod, nor the seed have morphological characteristics that would facilitate animal transportation. Primary movement of soybean seed is facilitated by human activities during planting, harvesting and transport of seed; however, few seeds are typically lost due to the relatively large seed size.

Soybean pollen may also be considered as a vehicle for dissemination, but the pollen viability outside of the soybean flower is limited by the fact that soybean is a predominately a self-pollinated species. The major barrier that prevents dissemination of soybean pollen and therefore cross-pollination, is the enclosure of both the stigma and anthers within the flower, even during maturation of the pollen. As a consequence, the potential for the pollen to become disseminated is reduced and the chance for self-pollination greatly increases. However, natural cross-pollination may occur to a certain extent as discussed in B.2.a.

b) Specific factors affecting dissemination

See Section B.4.b.

5. Geographical distribution and cultivation of the plant, including the distribution in Europe of the compatible species

Soybean was domesticated in the eastern half of northern China around the 11th century B.D. or earlier and its cultivation subsequently extended throughout south-east Asia. From the first century A.D. to approximately the 15th to 16th centuries, soybeans were introduced into several countries, with land races eventually developing in Japan, Indonesia, Philippines, Vietnam, Thailand, Malaysia, Myanmar, Nepal and northern India. Soybean cultivation was probably introduced in Europe starting in the late 16th and throughout the 17th century and in the US in the 18th century. Today, soybean is the most prevalently grown oilseed in over 35 countries worldwide. The major producers of soybean are the US, Brazil, Argentina, and China. The largest soybean producers in the European Union are Italy and Romania, followed by France and Hungary.

There are no compatible species for cultivated soybean in Europe.

6. In the case of plant species not normally grown in the Member State(s), description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts

Not applicable, as soybean is grown in Europe.

7. Other potential interactions, relevant to the GM plant, of the plant with organisms in the ecosystem where it is usually grown, or used elsewhere, including information on toxic effects on humans, animals and other organisms

Soybean is known to interact with other organisms in the agricultural environment. Soybean is sensitive to a number of economically important diseases and insect predators and is susceptible to competition from surrounding weeds. In addition, soybean is involved in the fixation of atmospheric nitrogen into organic nitrogen through a symbiotic association with the bacterium *Bradyrhizobium japonicum*.

Soybean seed is known to contain a number of natural anti-nutritional components, which are completely or partially inactivated during processing. Trypsin (proteinase) inhibitors are known to have anti-nutritive properties in animals fed unprocessed soybeans. Other anti-nutrients include lectins, stachyose, raffinose and phytic acid. Some of these anti-nutrients relate to their impact on human nutrition, while others relate to animal nutrition in general including livestock.

Soybean is one of the eight food groups that are known to elicit food allergic responses in humans. It contains several endogenous proteins that have been shown to elicit an allergenic response when ingested. Relatively few of the specific soybean proteins involved in allergenic reactions in soybean have been uniquely identified or characterised. Allergic responses to soybean are experienced by a very small percentage of the human population, but are considered clinically important. However, allergic reactions to soybean proteins are mostly manifested in atopic symptoms (*e.g.* dermatitis), they are rarely life-threatening and can be outgrown by the age of three. Moreover, individuals seem to become tolerant to soybean products within 3-5 years after the initial diagnosis.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Description of the methods used for the genetic modification

MON 87769 × MON 89788 is produced by crossing soybean plants of MON 87769 and MON 89788 using traditional breeding methods. Both parental lines, MON 87769 and MON 89788, were developed through *Agrobacterium*-mediated transformation of soybean tissues.

2. Nature and source of the vector used

MON 87769 × MON 89788 results from traditional breeding of MON 87769 and MON 89788 and no vector has been used to produce this combined trait soybean.

However, genetic modification has been used in the development of the parental soybean lines by *Agrobacterium*-mediated transformation using vectors PV-GMPQ1972 (in the case of MON 87769) and PV-GMGOX20 (in the case of MON 89788).

Vector PV-GMPQ1972 contains two T-DNAs (therein referred to as 2T-DNAs 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. The second T-DNA, designated as T-DNA II, contains the *cp4 epsps* expression cassette. Utilizing a vector with two T-DNAs is the basis for an effective approach to generate marker-free plants. It allows for the T-DNA with the traits of interest (T-DNA I) and the T-DNA encoding the selectable marker (T-DNA II) to be inserted into two independent loci within the genome of the plant. Following selection of the transformants, the inserted T-DNA encoding the selectable marker can be segregated from progeny through subsequent traditional breeding and genetic selection processes, while the inserted T-DNA containing the trait(s) of interest is maintained. The result is a marker-free, biotechnology-derived soybean that contains two desaturase genes, *Pj.D6D* and *Nc.Fad3,* resulting in expression of the PjΔ6D and NcΔ15D desaturases and subsequently, the production of SDA in soybean seed.

Vector PV-GMGOX20 is a binary vector containing a T-DNA which hosts the *cp4 epsps* expression cassette.

3. Source of donor DNA, size and intended function of each constituent fragment of the region intended for insertion

MON 87769 × MON 89788 results from traditional breeding of MON 87769 and MON 89788 and therefore, MON 87769 × MON 89788 inherits the inserted DNA fragments from both parental soybean lines.

The genetic elements of PV-GMPQ1972 inserted into MON 87769 comprised between the T-DNA I borders are: the P-*7Sα'* seed-specific promoter and leader sequence from the *Sphas1* gene encoding the alpha prime subunit of the beta-conglycinin storage protein of *Glycine max*, the *Pj.D6D* gene from *Primulae juliae,* and the 3' non-translated region of the *tml* gene from the octopine-type Ti plasmid of *Agrobacterium tumefaciens.* These elements together comprise the *Pj.D6D* gene expression cassette. The *Nc.Fad3* gene expression cassette is comprised of the seed-specific promoter and leader sequence from the *Sphas2* gene (*7Sα'*), the *Nc.Fad3* gene isolated from *Neurospora crassa*, and the 3' nontranslated region of the pea (*Pisum sativum*) ribulose-1,5-bisphosphate carboxylase small subunit (*rbcS2)* gene transcript termination sequence (*E9*).

The genetic elements of PV-GMGOX20 inserted into MON 89788 comprised between the T-DNA borders are: the *cp4 epsps* coding sequence (*cp4 epsps*) from *Bacillus thuringiensis*, the *FMV/Tsf1* promoter (*FMV/Tsf1*) from the Figwort Mosaic Virus and *Arabidopsis thaliana*, the non-translated *Tsf1* leader sequence (*Tsf1*) from *Arabidopsis thaliana*, and the *Tsf1* intron (*Tsf1*) from *Arabidopsis thaliana,* the *CTP2* chloroplast transit peptide (*CTP2*) from *Arabidopsis thaliana* and the *E9* transit termination sequence (*E9'*) from *Glycine max*.

The individual components and the function of the DNA sequences in MON 87769 and MON 89788 are given in Tables 1 and 2.

Table 1. Summary of inserted genetic elements inherited from MON 87769

Genetic element	Size (kb)	Source	Function
B – Right Border	0.34	*Agrobacterium tumefaciens*	Portion of the right border region used for transfer of the T-DNA remaining after integration
P – 7S α'	0.84	*Glycine max*	Seed specific promoter
CS – Pj.Δ6D	1.34	*Primula juliae*	Coding sequence for the fatty acid delta-6 desaturase
T – tml	0.94	*Agrobacterium tumefaciens*	Transcript termination sequence
P – 7S α	1.68	*Glycine max*	Seed specific promoter
CS – Nc.Fad3	1.29	*Neurospora crassa*	Coding sequence for the fatty acid delta-15 desaturase
T – E9	0.64	*Pisum sativum*	Transcript termination sequence
B – Left Border	0.44	*Agrobacterium tumefaciens*	Portion of the left border region used for transfer of the T-DNA remaining after integration

B – Border; P – Promoter; CS – Coding sequence; T – 3' nontranslated transcriptional termination and polyadenylation signal sequences.

Table 2. Summary of inserted genetic elements inherited from MON 89788

Genetic element	Size (kb)	Source	Function
B – Right Border	0.041	*Agrobacterium tumefaciens*	Portion of the right border region used for transfer of the T-DNA remaining after integration
P – *FMV/Tsf1*	1.04	Figwort Mosaic Virus and *Arabidopsis thaliana*	Promotor
L – *Tsf1*	0.05	*Arabidopsis thaliana*	Leader
I – *Tsf1*	0.62	*Arabidopsis thaliana*	Intron
TS – *CTP2*	0.23	*Arabidopsis thaliana*	Targeting sequence
CS – *cp4 epsps*	1.37	*Bacillus thuringiensis*	Coding sequence for the CP4 EPSPS
T – *E9'*	0.64	*Glycine max*	Transcript termination sequence
B – Left Border	0.15	*Agrobacterium tumefaciens*	Portion of the left border region used for transfer of the T-DNA remaining after integration

B – Border; P – Promoter; L – Leader; I – Intron; TS – Targeting sequence; CS – Coding sequence; T – 3' nontranslated transcriptional termination and polyadenylation signal sequences.

D. INFORMATION RELATING TO THE GM PLANT

1. Description of the trait(s) and characteristics which have been introduced or modified

MON 87769 × MON 89788 is produced by crossing MON 87769 and MON 89788 using traditional breeding methods and expresses:

- Two desaturase genes, *Primulae juliae* Δ6 desaturase (*Pj.D6D*) and *Neurospora crassa* Δ15 desaturase (*Nc.Fad3*) that result in the seed-specific production of the PjΔ6D and NcΔ15D proteins. Soybean plants lack the Δ6 desaturase gene, which is a minimal requirement for the production of 18:4 stearidonic acid (SDA). However, Δ6 desaturase also converts 18:2 linoleic acid (LA) to 18:3 gamma linoleic acid (GLA). The addition of a Δ15 desaturase with temporal expression similar to the Δ6 desaturase increases the flux of 18:3 alpha linolenic acid (ALA) to SDA and lowers the substrate pool for GLA production. To produce SDA in soybean, the conventional soybean variety A3525 was transformed with vector PV-GMPQ1972 that contained the *Pj.D6D* and *Nc.Fad3* genes driven by promoters that are known to be spatially and temporally active only in the developing soybean seed. Recommendations to increase consumption of long chain omega-3 polyunsaturated fatty acids have been made by a number of world-wide government and public health agencies and scientific organisations. Although the benefits of omega-3 fatty acid consumption are widely recognised, typical Western diets contain very little fish, and the dietary intake of omega-3 fatty acids is generally quite low relative to recommended intake. An alternative approach to increase omega-3 fatty acid intake is to provide a wider range of foods that are enriched in omega-3 fatty acids so that people can choose foods that suit their usual dietary habits. The oil derived from MON 87769 (SDA soybean oil) contains increased levels of SDA (approximately 20-30%) and GLA (~7%) and can serve as an alternate sustainable source of omega-3 fatty acid and help meet the need for increased dietary intake of long chain omega-3 fatty acids. In mammals, SDA is a metabolic intermediate in the production of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from ALA, a common dietary constituent. SDA is an eighteen carbon fatty acid with four double bonds (18:4). Since SDA has fewer double bonds than either EPA (20:5) or DHA (22:6), SDA soybean oil is more stable to oxidation (*i.e.* less prone to fishy or rancid odours and taste) than fish oils, thereby expanding the potential formulation options for food companies.
- The CP4 EPSPS protein which imparts tolerance to glyphosate herbicide. Glyphosate has excellent weed control capabilities and well-known, favourable environmental and safety characteristics. However, the sensitivity of crop plants to glyphosate has prevented the in-season use of this herbicide over-the-top of crops. The extension of its use to allow in-season application in major crops such as soybean provides a novel weed control option for farmers.

The use of MON 87769 × MON 89788 would provide a sustainable source of omega-3 fatty acid and substantial benefits to growers by offering an easier weed management, increasing subsequently yield.

2. Information on the sequences actually inserted or deleted

a) The copy number of all detectable inserts, both complete and partial

The genome of MON 87769 × MON 89788 contains two different inserts, one derived from MON 87769 and one derived from MON 89788. The results of Southern blot analyses on MON 87769 and MON 89788 indicate that each of these parental lines contain a single copy of the T-DNA of interest at a single insertion site. The presence of these two inserts in the combined trait soybean MON 87769 × MON 89788 was confirmed through Southern blot analysis.

b) In case of deletion(s), size and function of the deleted region(s)

MON 87769 × MON 89788 is produced by traditional breeding crossing MON 87769 and MON 89788. Since the inserts present in MON 87769 × MON 89788 correspond to those of the parental lines, the characteristics of the insertions and the 5' and 3' flanking sequences should be conserved in this combined-trait product. No deletion was intended in the development of the single parental lines. However, the analysis of the molecular structure at the insertion site of MON 87769 identified a 9 bp deletion and the analysis of the genomic organization of the MON 89788 insertion site identified a 40 bp deletion. The bioinformatic analyses of the DNA sequences flanking the insertion sites of MON 87769 and MON 89788 revealed that there is no known function associated with these deleted regions and therefore it is not expected they could affect the safety of these two products, neither of the combined product MON 87769 × MON 89788.

c) Chromosomal location(s) of insert(s) (nucleus, chloroplasts, mitochondria, or maintained in a non-integrated form), and methods for its determination

The respective analyses of the segregation results for MON 87769 and MON 89788 are consistent with single active sites of integration of the inserts into the nuclear genomic DNA. Southern blot analyses further demonstrate the stability of the inserted sequences of MON 87769 and MON 89788, respectively, and their progeny. The traditionally bred MON 87769 × MON 89788 contains both of the parental inserts and Southern analyses confirm that no detectable rearrangements of these inserts occurred.

d) The organisation of the inserted genetic material at the insertion site

Since the inserts present in MON 87769 × MON 89788 correspond to those of the parental lines, the characteristics of the insertions and the 5' and 3' flanking sequences should be conserved in this combined trait product.

3. Information on the expression of the insert

a) Information on developmental expression of the insert during the life cycle of the plant

MON 87769 × MON 89788 produces three functional proteins:

-PjΔ6D and NcΔ15D which are driven under a seed-specific promoter. These proteins desaturate certain endogenous fatty acids resulting in the production of SDA at approximately 20-30% of total fatty acids.

-CP4 EPSPS provides tolerance to glyphosate herbicide.

The levels of the PjΔ6D, NcΔ15D and CP4 EPSPS proteins in various tissues of MON 87769 × MON 89788 collected from a field trial conducted in the US during 2007 were assessed by validated immunoblot assay or enzyme-linked immunosorbent assay (ELISA). Over-season leaf, root, forage and harvested seed tissues were collected from each replicated plot at five field sites.

The expression of the PjΔ6D protein is driven by a 7Sα' seed specific promoter, and as expected, in MON 87769 × MON 89788, PjΔ6D protein was undetectable across all field sites in OSL-1, OSL-2, OSL-3, OSL-4, and root tissues as determined by the lack of measurable specific protein in the immunoblot assay. PjΔ6D protein was detected in immature seed, mature seed, and at low levels in forage tissues of MON 87769 × MON 89788 because forage usually contains small quantities of immature seed. The mean PjΔ6D protein levels in immature seed, mature seed and forage were 46, 3.4 and 10 µg/g dwt, respectively. The PjΔ6D protein levels in MON 87769 × MON 89788 tissues are comparable to the levels determined in MON 87769 tissues collected from the same trial.

The expression of the NcΔ15D protein is driven by a 7Sα seed specific promoter, and as expected, in MON 87769 × MON 89788, NcΔ15D protein was undetectable across all field sites in OSL-1, OSL-2, OSL-3, OSL-4, and root tissues as determined by the lack of measurable specific protein in the immunoblot assay. NcΔ15D protein was detected in immature seed, mature seed, and at low levels in forage tissues of MON 87769 × MON 89788 because forage usually contains small quantities of immature seed. The mean NcΔ15D protein levels in MON 87769 × MON 89788 across all five sites in immature seed, mature seed and forage were 120, 9.6 and 9.2 µg/g dwt, respectively. The NcΔ15D protein levels in MON 87769 × MON 89788 tissues are comparable to the levels determined in MON 87769 tissues collected from the same trial.

The mean CP4 EPSPS protein levels in MON 87769 × MON 89788 in immature seed, mature seed, OSL, root and forage were 190, 120, 210 – 250, 47 and 150 µg/g

dwt, respectively. Immature seed samples of MON 89788 were not collected and therefore not analysed. The CP4 EPSPS protein levels in MON 87769 × MON 89788 tissues are comparable to the levels determined in the same tissues from the MON 89788 tissues collected from the same trial.

b) Parts of the plant where the insert is expressed

The expression of the PjΔ6D and NcΔ15D proteins is driven by 7Sα' and 7Sα seed specific promoters, respectively. As expected in MON 87769 × MON 89788, both PjΔ6D and NcΔ15D proteins were undetectable across all field sites in OSL-1, OSL-2, OSL-3, OSL-4, and root tissues. Both proteins were detected in immature seed, mature seed, and at low levels in forage tissues (because forage usually contains small quantities of immature seed). The expression of the CP4 EPSPS protein occurs throughout the plant at appropriate times of plant development, as described in A.3.a.

4. Information on how the GM plant differs from the recipient plant in

a) Reproduction

Agronomic data collected from five sites at field trials conducted in major Argentinean soybean growing regions. In each of these assessments MON 87769 × MON 89788 was compared to an appropriate conventional soybean (control) which has a genetic background similar to MON 87769 × MON 89788 but does not possess the *Pj.D6D, Nc.Fad3* or *cp4 epsps* expression cassettes. In addition, multiple commercial soybean varieties (reference) were employed to provide a range of baseline values that are common to the existing commercial soybean varieties for each measured phenotypic, agronomic, and ecological interaction characteristic.

Results of this field study showed that there are no unexpected changes in the phenotype or ecological interactions indicative of increased pest or weed potential of MON 87769 × MON 89788 compared to the conventional soybean control. These results concur with those obtained previously for the single traits MON 87769 and MON 89788.

On the basis of the studies described above, it is possible to conclude that no differences in the mode or rate of reproduction, dissemination, survivability or other agronomic, phenotypic or ecological characteristics are expected in MON 87769 × MON 89788 and that MON 87769 × MON 89788 is equivalent to conventional soybean in its phenotypic and agronomic behaviour, except for the SDA production trait and the associated changes in the levels of fatty acids and glyphosate tolerance trait.

b) Dissemination

See Section D.4.a.

c) Survivability

See Section D.4.a.

d) Other differences

See Section D.4.a.

5. Genetic stability of the insert and phenotypic stability of the GM plant

MON 87769 × MON 89788 is produced by crossing MON 87769 and MON 89788 parental lines by traditional breeding. Thereby, each parental line passes on its inserted DNA sequence to the resulting MON 87769 × MON 89788 combined trait seed.

The respective analyses of the segregation results for MON 87769 and MON 89788 are consistent with single active sites of integration of the inserts into the nuclear genomic DNA. Southern blot analyses further demonstrate the stability of the inserted sequences of MON 87769 and MON 89788, respectively, and their progeny.

6. Any change to the ability of the GM plant to transfer genetic material to other organisms

a) Plant to bacteria gene transfer

None of the genetic elements in MON 87769 and MON 89788 has a genetic transfer function. Therefore, no changes are expected in the ability of these soybeans or MON 87769 × MON 89788 to transfer genetic material to bacteria

b) Plant to plant gene transfer

Based on the observation that reproductive morphology in MON 87769, MON 89788 and MON 87769 × MON 89788 is unchanged compared to conventional soybean, the out-crossing frequency to other soybean varieties or to wild relatives (which are not present in the EU) would be unlikely to be different for MON 87769 × MON 89788 when compared to MON 87769 and MON 89788, or when compared to other conventional soybean varieties.

Moreover, the scope of the current application does not include the cultivation of MON 87769 × MON 89788 varieties in the EU.

7. Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed

7.1 Comparative assessment

Choice of the comparator

MON 87769 × MON 89788 was compared to a conventional soybean variety with background genetics similar to MON 87769 × MON 89788, as well as with other commercially available soybean varieties.

7.2 Production of material for comparative assessment

a) number of locations, growing seasons, geographical spread and replicates

Compositional analyses were conducted on MON 87769 × MON 89788 and conventional control soybean seed and forage grown at five field sites in major soybean-growing areas of US during the 2007 field season. Three commercially available soybean varieties were grown also at each of the same field sites to provide reference substances representative for their respective growing regions. At each field site, the test, control and reference seed were planted in a randomized complete block design with three replicates per block. All the plants were grown under normal agronomic field conditions for their respective geographic regions. The test MON 87769 × MON 89788 was treated with glyphosate herbicide.

Compositional analysis confirmed that MON 87769 × MON 89788 harvested seed had the expected change in fatty acid composition, while the other components analysed in MON 87769 × MON 89788 seed were compositionally equivalent to conventional soybean seed. MON 87769 × MON 89788 seed had expected levels of SDA and GLA and small changes in two minor fatty acids, trans-SDA and trans-ALA. As anticipated, LA values were also significantly different. Forage analysis showed no meaningful differences between MON 87769 × MON 89788 and conventional control or values found in the ILSI Crop Compositional Database.

b) the baseline used for consideration of natural variations

Levels of the components in seed and forage of MON 87769 × MON 89788 were compared to the corresponding levels in the control conventional comparator, which has similar genetic background to MON 87769 × MON 89788. Reference varieties were grown in the same field locations and under the same conditions as the test and control to provide data for the development of a 99% tolerance interval for each analyte analyzed. Where statistical differences occurred, the measured analyte was compared to a confidence interval developed from these references. Finally differences were also compared to ILSI ranges and ranges reported in literature.

7.3 Selection of material and compounds for analysis

The key nutrients and other nutritionally important components that were selected for analysis in the compositional studies were chosen on the basis of internationally accepted guidance provided by the OECD on compositional considerations for new varieties of soybean.

7.4 Agronomic traits

Field trials with MON 87769 × MON 89788 were conducted and the set of agronomic observations supports the conclusion that from an agronomic and phenotypic (morphological) point of view, MON 87769 × MON 89788 is equivalent to traditional soybean, except for the introduction of genes (*Pj.D6D, Nc.Fad3* and *cp4 epsps*), the production of the proteins from the introduced genes (PjΔ6D, NcΔ15D and CP4 EPSPS), the expected fatty acid changes and the tolerance to glyphosate (*see* Section D.4.).

7.5 Product specification

MON 87769 × MON 89788 inherited a Δ6 desaturase gene and a Δ15 desaturase gene from MON 87769. The inheritance of these two genes results in the seed-specific production of PjΔ6D and NcΔ15D proteins. These proteins desaturate certain endogenous fatty acids resulting in the production of SDA at approximately 20-30% of total fatty acids. MON 87769 × MON 89788 inherited a *cp4 epsps* gene from MON 89788. The inheritance of this gene results in the production of the CP4 EPSPS protein conferring glyphosate tolerance to the plant.

MON 87769 × MON 89788 is developed by traditional crossing of MON 87769 and MON 89788 and it contains both inserts in combination. Therefore, MON 87769 × MON 89788 is detectable using either the event-specific PCR method for detecting the introduced DNA present in MON 87769 or the equivalent method for MON 89788. As for all plants in which one or more events are combined by traditional breeding, the unambiguous detection of MON 87769 × MON 89788 in mixed consignments of seed will require single seeds to be subjected to detection methods for both MON 87769 and MON 89788, and to test positive for both.

7.6 Effect of processing

Although MON 87769 × MON 87769 soybeans will be processed in dedicated oil processing facilities, operated in an identity preserved manner to retain the commercial value of the product, processing is not expected to be any different from that of conventional soybeans.

7.7 Anticipated intake/extent of use

The main commercial product from MON 87769 × MON 87769 is the oil which may be used as a replacement for fish oil or other omega-3 fatty acid rich products. It is expected that initially, MON 87769 × MON 87769 will be grown and processed in an identity preserved manner in the northern US soybean growing region. Because of the identity preserved status of this product, it would not be expected that MON 87769 × MON 87769 soybean will enter the current soybean or soybean oil supply to the EU in significant quantities, however may potentially enter the EU in a variety of food products. Products that might be derived from MON 87769 × MON 87769 soybean meal and entering the human food supply would likely be blended with other commercial soybean meal or meal-derived products. The effect of the introduction of SDA soybean oil is discussed further in Section 7.10.

7.8 Toxicology

7.8.1 Safety assessment of newly expressed proteins

MON 87769 × MON 89788 is produced by traditional breeding of MON 87769 and MON 89788. Both of the introduced traits from the parental lines are inherited by the MON 87769 × MON 89788 progeny. This results in the combined expression of the PjΔ6D, NcΔ15D and CP4 EPSPS proteins in the same plant.

The conclusion of safety to humans of the PjΔ6D, NcΔ15D and CP4 EPSPS proteins was based upon the following considerations:

- Those proteins have a demonstrated history of safe use;
- They have no structural similarity to known toxins or other biologically active proteins that could cause adverse effects in humans or animals;
- They do not exert any acute toxicity to mammals.
- They have large margins of exposure (MOE)

In addition, their low concentration in tissues that are consumed and their rapid digestibility in simulated digestive fluids provide additional assurance for their safety.

It is therefore possible to conclude that the PjΔ6D, NcΔ15D and CP4 EPSPS proteins are safe and pose no concerns for humans, animals and the environment.

7.8.2 Testing of new constituents other than proteins

Soybean has a long history of safe use and consumption around the world. As described in Section D.7.1., compositional analysis confirmed that MON 87769 × MON 89788 has the expected change in fatty acid composition, while the other components analysed in MON 87769 × MON 89788 were compositionally equivalent to conventional soybean. As expected, MON 87769 × MON 89788 seed had expected levels of SDA and GLA and small changes in two minor fatty acids, trans-SDA and trans-ALA. The safety of these fatty acids is discussed in Section D.7.8.3. There are no other new constituents present in MON 87769 × MON 89788 and therefore, no further testing is required.

7.8.3 Information on natural food and feed constituents

MON 87769 × MON 89788 contains SDA, GLA, trans-ALA and trans-SDA, slightly increased levels of ALA and reduced concentrations of LA. Since SDA, GLA, and ALA are produced at the expense of LA, the level of LA is lower in MON 87769 × MON 89788 compared to conventional soybean. The saturated and unsaturated fatty acids in SDA soybean oil are typical of those consumed from other food sources, and indeed occur at low levels in fish oils and other foods. Therefore, SDA soybean oil and the fatty acids present in the oil are expected to be absorbed, distributed, and metabolised in the same general manner as fatty acids from other sources.

Soybean is known to contain a number of natural anti-nutritional components, such as trypsin inhibitors, lectins, isoflavones (daidzein, genistein and glycitein), stachyose, raffinose and phytic acid, which are inactivated when the beans are toasted or heated during processing. Nonetheless, these antinutrients were evaluated in MON 87769 × MON 89788 compositional analyses and their levels were demonstrated to be comparable in MON 87769 × MON 89788 and in conventional soybean.

Therefore, except for the fatty acid changes and the presence of the introduced proteins, PjΔ6D and NcΔ15D, there have been no biologically-relevant changes to the composition (including nutrients and anti-nutrients) of food or feed derived from MON 87769 × MON 89788 compared to other conventional soybean varieties.

7.8.4 Testing of the whole GM food/feed

The safety assessment demonstrates that MON 87769 × MON 89788 is as safe as conventional soybean for food and feed use through:

• The compositional equivalence of MON 87769 × MON 89788 harvested seed (except for the expected fatty acid changes) and forage to harvested seed and forage from conventional soybean;

• The safety of the SDA soybean oil and expected change in fatty acids levels;

• The history of safe use of the introduced proteins;

• The familiarity of the host organism from which the genes are derived.

The compositional equivalence of MON 87769 × MON 89788 seed and forage to that of conventional soybean has been established by compositional analysis. Additionally, the wholesomeness of MON 87769 × MON 89788 seed has been confirmed by a repeat-dose animal feeding study in broiler chickens fed diets containing soybean meal produced from MON 87769 × MON 89788. These studies confirm the absence of any toxic effects associated to the inherited proteins and the absence of any unanticipated or pleiotropic effects linked to the genetic modification.

Taken altogether, there was no evidence of any adverse effects on human or animal health.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein

The assessment of the allergenic potential of the PjΔ6D, NcΔ15D and the CP4 EPSPS proteins compares the biochemical characteristics of these proteins to characteristics of known allergens, according to the recommendations of Codex Alimentarius Commission.

It is unlikely that the PjΔ6D, NcΔ15D and the CP4 EPSPS proteins will cause allergenic concerns due to the following considerations:

- They were obtained from non-allergenic sources (*Primula juliae* for PjΔ6D, *Neurospora crassa* for NcΔ15D and *Agrobacterium* for CP4 EPSPS);
- They lack structural similarity to known allergens, as demonstrated by bioinformatics analyses;
- They are rapidly digested in simulated digestive fluid;
- They constitute a very small portion of the total protein present in the seed of MON 87769 × MON 89788.

Taken together, it can be concluded that the allergenic potential of the PjΔ6D, NcΔ15D and CP4 EPSPS proteins is negligible and therefore, these proteins do not pose a significant allergenic risk.

7.9.2 Assessment of allergenicity of the whole GM plant or crop

MON 87769 × MON 89788 is produced by traditional breeding of MON 87769 and MON 89788. Both of the introduced traits from the parental lines are inherited by the MON 87769 × MON 89788 progeny. The assessment of a potential allergenicity of each of the parental lines against a conventional soybean has been previously performed. Results of these assessments support the conclusion that both MON 87769 and MON 89788 are comparable to conventional soybean in terms of allergenicity potential.

As the PjΔ6D, NcΔ15D and CP4 EPSPS proteins expressed in MON 87769 × MON 89788 are not allergenic and as there are no new genetic modifications in MON 87769 × MON 89788, there are no reasons to believe that the expression of these proteins in MON 87769 × MON 89788 would alter its endogenous allergen content compared to commercial soybean.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

Detailed compositional and nutritional comparisons of MON 87769 × MON 89788, a conventional soybean control and commercially available reference soybean varieties confirmed that MON 87769 × MON 89788 had the expected change in fatty acid composition, while the other components analysed were compositionally equivalent to conventional soybean.

In addition to the extensive compositional analyses which demonstrated the substantial equivalence of MON 87769 × MON 89788 to conventional soybean (except for the inherited traits), a confirmatory feed performance study was conducted in rapidly growing broiler chickens which were fed MON 87769 × MON 89788 derived soybean meal.

Finally, an assessment of the anticipated intake of SDA from the proposed uses of SDA soybean oil in selected food categories and the impact on the intakes of individual fatty acids was conducted. This analysis demonstrated that fatty acid consumption would not appreciably change compared to dietary recommendations with the introduction of MON 87769 × MON 89788, except for an increase in the intake of omega-3 fatty acids, SDA and ALA, and would have minimal effects on the intake of fatty acids present in the diet.

Therefore, no nutritional imbalances are expected as a result of the use of MON 87769 × MON 89788 for food or feed or processing.

7.10.2 Nutritional assessment of GM feed

The dietary safety of the respective proteins within the soybean matrix was further confirmed by an animal feeding study in broiler chickens using diets containing soybean meal produced from MON 87769 × MON 89788. This study confirms the absence of any toxic effects associated to the introduced proteins and the absence of any unanticipated or pleiotropic effects linked to the genetic modification. There was no evidence of any adverse effects on human or animal health.

7.11 Post-market monitoring of GM food/feed

There are no intrinsic hazards related to MON 87769 × MON 89788 as no signs of adverse or unanticipated effects have been observed in a number of safety assessment studies, including an animal feeding study using doses of administration that are orders of magnitude above expected consumption levels.

The pre-market risk characterization for food and feed use of MON 87769 × MON 89788 demonstrates that the risks of consumption of MON 87769 × MON 89788 or its derived products are consistently negligible and no different from the risks associated with the consumption of conventional soybean. Furthermore, the scientific evidence presented does not indicate any potential for adverse effects in humans following the consumption of SDA soybean oil under the conditions of intended use in foods. As a consequence, specific risk management measures for MON 87769 or SDA soybean oil are not considered necessary.

8. Mechanism of interaction between the GM plant and target organisms (if applicable)

MON 87769 × MON 89788 contains SDA, an omega-3 fatty acid, and tolerance to glyphosate herbicide. Therefore MON 87769 × MON 89788 is not pesticidal to any target organism.

9. Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification

As the scope of this application under Regulation (EC) No. 1829/2003 includes the import and use of the viable GMO, an environmental risk assessment in accordance with the principles of Annex II to Directive 2001/18/EC is included in this section.

9.1 Persistence and invasiveness

Based on centuries of experience with conventional, domesticated soybean in Europe, there is no potential for soybean to be invasive of natural habitats or persist in the environment without human intervention.

MON 87769 × MON 89788 is substantially equivalent to conventional soybean, except for the inherited SDA production trait, the associated changes in the levels of fatty acids and the inherited glyphosate tolerance trait. Field trial data demonstrated that this soybean has not been altered in its phenotypic, agronomic, reproductive, survival and dissemination characteristics when compared to conventional soybean. The scope of the current application does not include cultivation of MON 87769 × MON 89788 in the EU. As such, exposure to the environment will be rare. In the event MON 87769 × MON 89788 seed was spilt in the environment, its introduced trait would have negligible consequences for the environment.

Therefore, the risk to the environment from MON 87769 × MON 89788 through increased persistence and invasiveness of this soybean is negligible.

9.2 Selective advantage or disadvantage

It was demonstrated previously that the inherited genetic sequences in MON 87769 × MON 89788 did not lead to any biologically meaningful alterations of other phenotypic characteristics, such as plant growth and development, morphology, or agronomic performance, when compared to conventional soybean. Therefore, it was concluded that MON 87769 × MON 89788 is not substantially different from conventional soybean, with the exception of the inherited traits. Compared with conventional soybean, the presence of the inherited traits would only confer a selective advantage to MON 87769 × MON 89788, where its control was attempted using glyphosate alone, and if no other more important factors limiting the survival of soybean in the receiving environment were present. In practice, however, this advantage would be of short duration and of limited consequence because of the poor survival characteristics of soybean under most European conditions

Therefore, the likelihood is negligible for the inherited traits in MON 87769 × MON 89788 to confer any meaningful competitive advantage or disadvantage of relevance to the environment.

9.3 Potential for gene transfer

There is no potential for gene transfer from MON 87769 × MON 89788 to wild plant species in the EU since soybean is not sexually compatible with any indigenous or introduced wild plant species present in European countries. Furthermore, there is negligible likelihood for gene transfer from MON 87769 × MON 89788 to other soybean crops since this application is not for consent to cultivate MON 87769 × MON 89788 varieties in the EU but limited to import of MON 87769 × MON 89788 seed into the EU and use of it thereof as any

other soybean commodity seed.

In the case that an introduced gene outcrossed to other soybean, its transfer would only confer a selective advantage under specific conditions (*i.e.* applications of glyphosate-containing herbicide), as discussed in Section 9.2.

In the highly unlikely event that the introduced genes would outcross to another soybean plant, its transfer would, in any event, have negligible consequences for the environment. The environmental risk posed by this transfer, and hence by the intended import and use of MON 87769 × MON 89788 for food, feed and processing is negligible.

9.4 Interactions between the GM plant and target organisms

MON 87769 × MON 89788 is not pesticidal and thus does not have any target organisms.

9.5 Interactions of the GM plant with non-target organisms

The only meaningful differences between MON 87769 × MON 89788 and conventional soybean are the expected seed fatty acid compositional changes, particularly the presence of SDA and GLA, conferred by the introduced PjΔ6D and NcΔ15D proteins. Thus, the baseline interaction of MON 87769 × MON 89788 with other organisms in the environment is considered no different from traditional soybean, except for the additional direct exposure to the PjΔ6D and NcΔ15D proteins, SDA, and GLA that are produced in MON 87769 × MON 89788 of soybean pests and animals that feed on soybean seeds. Through trophic interactions and decomposition processes, additional organisms such as predators and prey of the soybean pests could be exposed to some very low levels of the PjΔ6D and NcΔ15D proteins, SDA, and GLA. Potential exposure of non-target organisms in the receiving environment to the PjΔ6D and NcΔ15D proteins, SDA, and GLA, produced in MON 87769 is a characteristic of the GMHP that may, theoretically, cause an adverse environmental effect. 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. The fatty acids present in MON 87769 × MON 89788 exist in many sources in the environment without known adverse effects. There are many plants producing SDA in the environment, such as plants of the *Echium* family, black currant *etc*. GLA is present in oats, barley, and human breast milk while small concentrations are found in meats, fish and a variety of foods.

Given the scope of the current application, which does not include the cultivation of MON 87769 × MON 89788 varieties in the EU, the likelihood for direct or indirect interactions of this soybean with non-target organisms is considered to be negligible.

In addition, even if incidental spillage of MON 87769 × MON 89788 seed during import, storage, transport or use would lead to the short survival of

MON 87769 × MON 89788 plants, the newly produced proteins in MON 87769 × MON 89788, PjΔ6D, NcΔ15D and CP4 EPSPS present a negligible hazard to NTOs. As a consequence, there is negligible risk for harmful effects of MON 87769 × MON 89788 on non-target organisms, either through direct or indirect interactions with this soybean or through contact with the newly expressed protein.

Furthermore, no adverse effects were observed in field trials conducted since 2007 across a broad geographic range of environments involving MON 87769 × MON 89788.

9.6 Effects on human health

MON 87769 × MON 89788 was shown to be compositionally equivalent to conventional soybean, except for expected seed fatty acid compositional changes, particularly the presence of SDA conferred by the introduced PjΔ6D and NcΔ15D proteins, and tolerance to glyphosate conferred by the CP4 EPSPS protein. There are no substantial differences from conventional soybean with respect to safety characteristics and agronomic and phenotypic characteristics. The likelihood for any adverse effects occurring in humans as a result of their contact with MON 87769 × MON 89788 is no different from that of conventional soybean, as MON 87769 × MON 89788 contains the PjΔ6D, NcΔ15D and CP4 EPSPS proteins, which have negligible potential to cause any toxic or allergenic effects in humans.

Furthermore, the potential for the PjΔ6D, NcΔ15D and CP4 EPSPS proteins to interact when expressed in combination in MON 87769 × MON 89788 is negligible.

Therefore, the risk of changes in the occupational health aspects of this soybean is negligible.

9.7 Effects on animal health

The likelihood for any adverse effects occurring in animals fed on MON 87769 × MON 89788 is negligible. MON 87769 × MON 89788 contains the PjΔ6D, NcΔ15D and CP4 EPSPS proteins which have negligible potential to cause any toxic or allergenic effects in animals.

MON 87769 × MON 89788 is substantially equivalent to conventional soybean as well as to soybean varieties in commerce, except for the inherited SDA production trait, the associated changes in the levels of fatty acids and the inherited glyphosate-tolerance trait imparted by the CP4 EPSPS proteins. As previously discussed, the PjΔ6D, NcΔ15D and CP4 EPSPS proteins safety has been extensively investigated when assessed individually and in combination in MON 87769 × MON 89788.

In conclusion, the MON 87769 × MON 89788 combined trait product is expected to pose no meaningful health risks to farm animals that would consume it. Therefore, the risk of MON 87769 × MON 89788 for the feed/food chain is also negligible.

9.8 Effects on biogeochemical processes

The scope of the current application does not include cultivation of MON 87769 × MON 89788 in the EU. As such, exposure to the environment will be rare, occurring only through incidental release during shipment and handling. As for conventional soybean, spillage of MON 87769 × MON 89788 during transport or storage of seed could cause some seed to fall to the ground. Although such seed could eventually germinate if the local soil and environmental conditions are favourable, this soybean is a poor competitor and cannot persist as a weed. Environmental conditions at the sites of handling are, however, unlikely to be conducive to germination, growth and reproduction of soybean seed that is incidentally released.

Soybean production in general is known to have indirect impacts on biogeochemical processes through tillage, fertilizer application, and establishment of a monoculture in a defined area. As MON 87769 × MON 89788 was shown to be compositionally equivalent to conventional soybean with no biologically meaningful differences in agronomic and phenotypic characteristics, except for the inherited traits and the expected change in fatty acids levels, there is no evidence that this soybean would be any different from conventional soybean regarding its influence on biogeochemical processes and nutrient levels in the soil. Furthermore, any indirect interactions of the GMO with other organisms in the vicinity of an incidental release of the grain are not likely to cause hazardous effects on the biogeochemical processes in the soil. As previously discussed, CP4 EPSPS is widely present in the environment.

In conclusion, as for conventional soybean, it is highly unlikely that there would be any significant immediate or delayed adverse effects from MON 87769 × MON 89788 on the biogeochemical processes in the soil.

9.9 Impacts of the specific cultivation, management and harvesting techniques

Not applicable. The scope of the current application does not include cultivation of MON 87769 × MON 89788 in the EU.

10. Potential interactions with the abiotic environment

The scope of the current application does not include cultivation of MON 87769 × MON 89788 in the EU. As such, exposure to the environment will be rare.

Therefore, no negative impact of MON 87769 × MON 89788 on the abiotic environment is expected to result from the import of MON 87769 × MON 89788 seed into the EU and use thereof as any other soybean commodity seed.

11. Environmental monitoring plan (not if application concerns only food and feed produced from GM plants, or containing ingredients produced from GM plants and if the applicant has clearly shown that environmental exposure is absent or will be at levels or in a form that does not present a risk to other living organisms or the abiotic environment)

11.1 General (risk assessment, background information)

As required by Article 5(5)(b) and 17(5)(b) of Regulation (EC) No. 1829/2003, a environmental monitoring plan in accordance to Annex VII of Directive 2001/18/EC is included.

11.2 Interplay between environmental risk assessment and monitoring

An environmental risk assessment (ERA) of MON 87769 × MON 89788 was undertaken in the context of the scope of the application, that is, for import, processing, food and feed use of MON 87769 × MON 89788, but not including the cultivation of MON 87769 × MON 89788 varieties in the EU. Analysis of the characteristics of MON 87769 × MON 89788 has shown that the risk for potential adverse effects on human health and the receiving environment, resulting from the proposed use of MON 87769 × MON 89788 in the EU is consistently negligible. Therefore, the overall environmental risk posed by this genetically modified higher plant is negligible, and no specific strategies for risk management and no case-specific post-market monitoring actions are considered required.

11.3 Case-specific GM plant monitoring (approach, strategy, method and analysis)

As the overall environmental risk posed by this genetically modified higher plant is negligible, and as the conclusions of the environmental risk assessment are derived from the results of scientific studies, rather than major assumptions, no case-specific postmarket monitoring actions, typically aimed at testing assumptions made in this assessment, would be warranted or required.

11.4 General surveillance of the impact of the GM plant (approach, strategy, method and analysis)

Any potential adverse effects of MON 87769 × MON 89788 on human health and the environment, which were not anticipated in the ERA, can be addressed under the general surveillance. General surveillance is largely based on routine observation and implies the collection, scientific evaluation and reporting of reliable scientific evidence, in order to be able to identify whether unanticipated, direct or indirect, immediate or delayed adverse effects have been caused by the placing on the market of a genetically modified (GM) crop in its receiving environment.

In order to allow detection of the broadest possible scope of unanticipated adverse effects, general surveillance is performed by either selected, existing networks, or by specific company stewardship programmes, or by a combination of both. The consent holder will ensure that appropriate technical information on MON 87769 × MON 89788 and relevant legislation will be available for the relevant networks, in addition to further relevant information from a number of sources, including industry and government websites, official registers and government publications.

Following the approval of this soybean in the EU, the consent holder will approach key stakeholders and key networks of stakeholders of the product (including international grain traders, soybean processors and users of soybean seed for animal feed) and inform them that the product has been authorised. The consent holder will request key stakeholders and networks for their participation in the general surveillance of the placing on the market of this soybean, in accordance with the provisions of Directive 2001/18/EC and the consent. Key stakeholders and networks will be requested to be aware of their use of this soybean and to inform the consent holder in case of potential occurrence of any unanticipated adverse effects to health or the environment, which they might attribute to the import or use of this product. Appropriate technical information on MON 87769 × MON 89788 will be provided to them. Where there is scientifically valid evidence of a potential adverse effect (whether direct or indirect), linked to the genetic modification, then further evaluation of the consequence of that effect should be science-based and compared with available baseline information. Relevant baseline information will reflect prevalent use practices and the associated impact of these practices on the environment. Where scientific evaluation of the observation confirms the possibility of an unanticipated adverse effect, this would be investigated further to establish a correlation, if present, between the use of MON 87769 × MON 89788 and the observed effect. The evaluation should consider the consequence of the observed effect and remedial action, if necessary, should be proportionate to the significance of the observed effect.

11.5 Reporting the results of monitoring

The authorisation holder will submit a monitoring report annually, containing information obtained from participating networks, and/or in case of a confirmed adverse effect. If information that confirms an adverse effect which alters the existing risk assessment becomes available, Monsanto will submit a report, consisting of a scientific evaluation of the potential adverse effect and a conclusion on the safety of the product. The report will also include, where appropriate, the measures that were taken to ensure the safety of human or animal health and/or the environment.

12. Detection and event-specific identification techniques for the GM plant

As MON 87769 × MON 89788 is the result of a traditional cross of MON 87769 and MON 89788, it contains both inserts in combination. Therefore, MON 87769 × MON 89788 is detectable using either the event-specific PCR method for detecting the introduced DNA present in MON 87769 or the equivalent method for MON 89788. However, as for all plants in which one or more events are combined by traditional breeding, the unambiguous detection of MON 87769 × MON 89788 in mixed consignments of seed will require single soybean seeds to be subjected to detection methods for both MON 87769 and MON 89788, and to test positive for both.

A MON 87769-specific PCR-based assay allowing the identification and quantification of MON 87769 has been provided to the Joint Research Centre (JRC), acting as the European Union Reference Laboratory (EURL). The detection method for MON 89788 has already been validated by the EURL.

E. INFORMATION RELATING TO PREVIOUS RELEASES OF THE GM PLANT AND/OR DERIVED PRODUCTS

1. History of previous releases of the GM plant notified under Part B of the Directive 2001/18/EC and under Part B of Directive 90/220/EEC by the same notifier

a) Notification number

There is no history of release of MON 87769 × MON 89788 in the EU.

b) Conclusions of post-release monitoring

Not applicable.

c) Results of the release in respect to any risk to human health and the environment (submitted to the Competent Authority according to Article 10 of Directive 2001/18/EC)

Not applicable.

2. History of previous releases of the GM plant carried out outside the Community by the same notifier

a) Release country

MON 87769 x MON 89788 has been field tested in the US since 2007, Argentina from 2007-2008, Chile since 2007, Canada since 2008 and Puerto Rico in 2007.

b) Authority overseeing the release

US and Puerto Rico: United States Department of Agriculture (USDA)

Argentina: Secretary of Agriculture, livestock, fishery and feed (SAGPyA) – National Advisory Commission on Agricultural Biotechnology (CONABIA)

Chile: Agriculture and Livestock Service (SAG)

Canada: Canadian Food Inspection Agency (CFIA)

c) Release site

US: In major soybean growing states (Arkansas, Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, Pennsylvania, South Dakota and Wisconsin).

Argentina: Cordoba, Santa Fe, Buenos Aires

Chile: Rancagua

Puerto Rico: Isabela

Canada: Ontario, Quebec

d) Aim of the release

US/Argentina/Chile/Puerto Rico/Canada: regulatory trials, efficacy, yield, breeding, product development.

e) Duration of the release

US/Argentina/Chile/Puerto Rico/Canada: One growing season

f) Aim of post-releases monitoring

US/Argentina/Chile/Puerto Rico/Canada: Assessment of volunteers

g) Duration of post-releases monitoring

US/Argentina/Chile/Puerto Rico/Canada: 12 months.

h) Conclusions of post-release monitoring

US/Argentina/Chile/Puerto Rico/Canada: In general, no volunteers have been observed since soybean is an annual crop. If volunteers occur, the practice is to eliminate them manually or chemically to prevent occurrence in subsequent crops.

i) Results of the release in respect to any risk to human health and the environment

Field-testing provided no evidence that MON 87769 × MON 89788 or derived products would be the cause of any adverse effects to human health or to the environment.

3. Links (some of these links may be accessible only to the competent authorities of the Member States, to the Commission and to EFSA):

a) Status/process of approval

The EURL websites http://gmoinfo.jrc.ec.europa.eu/gmp_browse.aspx and http://gmo-crl.jrc.ec.europa.eu/statusofdoss.htm and the EFSA website http://www.efsa.europa.eu/ provide publicly accessible links to up-to-date databases on the regulatory progress of notifications under Directive 2001/18/EC and applications under Regulation (EC) No. 1829/2003, including the Monsanto dossier for MON 87769 × MON 89788.

b) Assessment Report of the Competent Authority (Directive 2001/18/EC)

A notification for MON 87769 × MON 89788 according to Directive 2001/18/EC has not been submitted by Monsanto Company.

c) EFSA opinion

No EFSA opinion is available at the time of submission of this application.

d) Commission Register (Commission Decision 2004/204/EC)

The Commission Register can be seen in the at http://ec.europa.eu/food/dyna/gm_register/index_en.cfm.

e) Molecular Register of the Community Reference Laboratory/Joint Research Centre

Information on detection protocols is posted at http://gmo-crl.jrc.ec.europa.eu/default.htm

f) Biosafety Clearing-House (Council Decision 2002/628/EC)

The publicly accessible portal site of the Biosafety Clearing-House (BCH) can be found at http://bch.cbd.int/ /

g) Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC)

EFSA provides a link to the publicly accessible summary of this application under Regulation (EC) No. 1829/2003 at http://www.efsa.europa.eu/.