Application for authorization of MON 87701 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 87701. 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 87701 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 87701 have been studied at multiple locations in North and South America that cover a range of environmental conditions. The risk assessment presented in the MON 87701 application includes data collected from these field trials. A summary of the conclusions of the risk analysis that demonstrate the safety of MON 87701 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

Applications for the full range of uses have been made in US, Brazil, Japan and Canada. Applications for the full range of uses have been made in US, Brazil, Japan and Canada. Regulatory submissions have been made to countries that import significant quantities of soybean from US and South America and that have regulatory approval processes in place. These include submissions to a number of countries and regulatory authorities, including Australia/New Zealand, The Philippines, Thailand, Mexico, Taiwan and Korea and will include a submission to China. The status of the pending regulatory reviews, which are currently in progress in numerous countries around the world, typically depend on the country and its local regulatory framework.

8. General description of the product

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

MON 87701 was developed through *Agrobacterium*-mediated transformation of soybean meristem tissues using the binary transformation plasmid PV-GMIR9. The vector PV-GMIR9 contains two T-DNAs delineated by left and right border sequences which facilitate transformation. MON 87701 produces the Cry1Ac insecticidal crystal (Cry) protein (δ-endotoxin) derived from *Bacillus thuringiensis* (Bt) subsp. *kurstaki.* The Cry1Ac protein provides protection from feeding damage caused by targeted lepidopteran pests, such as primary target pests velvetbean caterpillar (*Anticarcia gemmatalis*), soybean looper (*Pseudoplusia includens*), soybean anxil borer (*Epinotia aporema*), and sunflower looper (*Rachiplusia nu).*

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 authorization of MON 87701 in the EU for import, processing, and all uses as any other soybean, according to Articles 5 and 17 of Regulation (EC) No 1829/2003 on genetically modified food and feed. The range of uses of this soybean will be identical to the full range of equivalent uses of conventional soybean. The scope of this application does not include the cultivation of MON 87701 in the EU.

c) Intended use of the product and types of users

MON 87701 will be used and traded in the EU in the same manner as

current commercial soybean and by the same operators currently involved in the trade and use of soybean.

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

MON 87701 is substantially equivalent to conventional soybean, except for its lepidopteran-protection trait, which is a trait of agronomic interest. This soybean was shown to be as safe and nutritious as conventional soybean. Therefore, MON 87701 and its derived products will be stored, packaged, transported, used and handled in the same manner as current commercial soybean. No specific conditions or instructions are required for the import of MON 87701.

e) Any proposed packaging requirements

MON 87701 is substantially equivalent to conventional soybean, except for its lepidopteran-protection trait. Therefore, MON 87701 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% will continue to be applied for the marketing of MON 87701 and derived products.

Operators shall be required to label products containing or consisting of MON 87701 with the words "genetically modified soybean" or "contains genetically modified soybean" and shall continue to declare the unique identifier MON-877Ø1-2 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 87701 with the words "produced from genetically modified soybean". In the case of products for which no list of ingredients exists, operators shall continue to ensure that an indication that the food or feed product is produced from GMOs is transmitted in writing to the operator receiving the product.

Operators handling or using MON 87701 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 87701. 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-877Ø1-2.

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 87701 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

Because this application is for consent to import, process and use MON 87701 as any other soybean, not including the cultivation of varieties of MON 87701 in the EU, environmental release would more likely occur during import, storage and processing of MON 87701. However, 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 87701 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 87701 is shown to be substantially equivalent to conventional soybean, except for the inserted protection against lepidopteran pests and, therefore, 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 import of MON 87701.

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 MON 87701
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. The pollen usually remains viable for 2-4 hours, after which it germinates. Natural or artificial cross-pollination can only take place during the short time of the day that the pollen is viable. As a result, cross-pollination is usually less than one percent.
(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 87701 varieties in the EU. Therefore, any outcrossing between MON 87701 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 87701 was developed through *Agrobacterium*-mediated transformation of soybean meristem tissues using the binary transformation plasmid PV GMIR9.

2. Nature and source of the vector used

The vector used to generate MON 87701 by *Agrobacterium*-mediated transformation is PV-GMIR9. The vector PV-GMIR9 contains two T-DNAs (therein referred to as 2T-DNAs system) delineated by left and right border sequences which facilitate transformation. The first T-DNA, designated as T-DNA I, contains the *cry1Ac* 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, insect-protected soybean containing only the *cry1Ac* expression cassette.

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

The genetic elements of PV-GMIR9 inserted into MON 87701 comprised between the T-DNA I borders are: the RbcS4 promoter (P-*RbcS4*) and leader (L-*RbcS4*), the CTP1 targeting sequence (TS-*CTP1*), the cry1Ac coding sequence (CS-*cry1Ac*) and the 7S α' 3' transcript termination sequence (T-*7S α'*). These elements together constitute the *cry1Ac* expression cassette.

The individual components and the function of the DNA sequences in MON 87701 are given in Table 1.

Table 1. Summary of genetic elements intended for insertion in MON 87701

Genetic element	Size (kb)	Source	Function
B-Right Border [r1]	0.05	*Agrobacterium tumefaciens*	Portion of the right border region remaining after integration
P-*RbcS4*	1.72	*Arabidopsis thaliana*	Promotor
TS-*CTP1*	0.26	*Arabidopsis thaliana*	Targeting sequence
CS-Cry1Ac	3.54	*Bacillus thuringiensis*	Coding sequence
T-*7S α'*	0.44	*Glycine max*	Transcript termination sequence
B-Left Border [r1]	0.26	*Agrobacterium tumefaciens*	Portion of the left border region remaining after integration

D. INFORMATION RELATING TO THE GM PLANT

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

MON 87701 produces the Cry1Ac insecticidal crystal (Cry) protein (δ-endotoxin) derived from *Bacillus thuringiensis* (Bt) subsp. *kurstaki*. The Cry1Ac protein provides protection from feeding damage caused by targeted lepidopteran pests in the soybean production system, such as velvetbean caterpillar (*Anticarcia gemmatalis*), soybean looper (*Pseudoplusia includens*), soybean anxil borer (*Epinotia aporema*) and sunflower looper (*Rachiplusia nu*).

The use of MON 87701 would provide substantial benefits to growers by offering an easier insect management, increasing health of the plant and, subsequently yield, while at the same time reducing the risk from insecticide use to humans and the environment.

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 87701 contains one insert. The results of Southern blot analyses on MON 87701indicate that a single copy of T-DNA of interest is present at a single insertion site.

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

No deletion was intended in the development of MON 87701. However, the analysis of the molecular structure at the insertion site identified a 32 bp deletion. The BLAST analyses of the DNA sequences flanking the insertion site revealed that there is no known function associated with the deleted region and therefore it is not expected it could affect the safety of MON 87701.

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

The presence of the MON 87701 insert in the soybean nuclear genome is best shown by the Chi square (χ^2) analysis of the segregation data. The results show that inheritance of the lepidopteran-protection trait in MON 87701 follows Mendelian principles. This indicates that the single insert is stably integrated in the nuclear genome and is neither located in the mitochondria nor in the chloroplasts.

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

The insert in MON 87701 was characterised using Southern blot methods. Specifically, the insert number (number of insertions of the

integrated DNA within the soybean genome), the copy number (the number of copies of the integrated DNA within one insertion site), the integrity of the inserted *Cry1Ac* expression cassette and the presence or absence of plasmid backbone sequence and T-DNA II was assessed. DNA sequence analyses confirmed the sequence identity between the MON 87701 insert and the portion of the T-DNA from PV-GMIR9 that was integrated into the soybean genome. The results of PCR and sequence analyses further confirmed the organisation of the genetic elements within the *Cry1Ac* expression cassettes of MON 87701, which were identical to that in plasmid PV-GMIR9.

3. Information on the expression of the insert

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

MON 87701 produces the Cry1Ac protein, providing protection against target lepidopteran pests.

The levels of the Cry1Ac protein in various tissues of MON 87701 collected from field trials conducted in the US during 2007 and in Argentina during 2007-2008 were assessed by validated enzyme-linked immunosorbent assay (ELISA). Over-season leaf, root, forage and harvested seed tissues were collected from each replicated plot at five field sites.

In tissues harvested in the 2007 US trials, the mean Cry1Ac protein levels in MON 87701 across sites were highest in leaf (OSL-4, 340 µg/g dw), followed by forage (29 µg/g dw) and mature seed (4.7 µg/g dw). If present in root, Cry1Ac levels are less than the ELISA assay LOD of 0.347 µg/g fw. In tissues harvested throughout the growing season, mean Cry1Ac protein levels in MON 87701 across all sites ranged from 220 – 340 µg/g dw.

In tissues harvested in the 2007-2008 Argentina trials, the mean Cry1Ac protein levels in MON 87701 across sites were highest in leaf (OSL-1, 450 µg/g dw), followed by forage (70 µg/g dw) and mature seed (5.1 µg/g dw). If present in root, Cry1Ac levels are less than the ELISA assay LOD of 0.347 µg/g fw. In tissues harvested throughout the growing season, mean Cry1Ac protein levels in MON 87701 across all sites ranged from 140 - 450 µg/g dw.

Overall, comparison between the 2007 US and 2007-2008 Argentina field trials indicates that the ranges of the Cry1Ac protein levels were comparable.

b) Parts of the plant where the insert is expressed

The expression of the Cry1Ac protein occurs throughout the plant at appropriate times of plant development, as described in Section D.3(a). The Cry1Ac protein was, however, not detected in root tissues analysed. In terms of food and feed safety assessment of MON 87701, seed and forage are the most relevant tissues.

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

a) Reproduction

Agronomic data was collected at field trials conducted in major US and Argentinean soybean growing regions (16 and 8 locations respectively). In each of these assessments MON 87701 was compared to an appropriate conventional soybean (control) which has a genetic background similar to MON 87701 but does not possess the *cry1Ac* expression cassette. 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 these field studies showed that there are no unexpected changes in the phenotype or ecological interactions indicative of increased pest or weed potential of MON 87701 compared to the conventional soybean control.

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 87701 and that MON 87701 is equivalent to conventional soybean in its phenotypic and agronomic behaviour, except for the lepidopteran-protection 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

The analyses of the segregation results for MON 87701 are consistent with a single active site of integration of the insert into the nuclear genomic DNA. Southern blot analyses further demonstrate the stability of the inserted sequence of MON 87701 and its 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 87701 has a genetic transfer function. Therefore, no changes are expected in the ability of these soybeans or MON 87701 to transfer genetic material to bacteria

b) Plant to plant gene transfer

Based on the observation that reproductive morphology in MON 87701 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 87701 when compared to other conventional soybean varieties.

However, the scope of the current application does not include the cultivation of MON 87701 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 87701 was compared to a conventional soybean variety with similar background genetics, 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 87701 and conventional control soybean seed and forage grown at five field sites in major soybean-growing areas of the US during 2007 and Argentina during the 2007-2008 field season. Four commercially available soybean varieties were grown also at each of the same field sites to provide a total of 20 different 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.

MON 87701 was found to be compositionally equivalent to conventional soybeans and thus as safe as conventional soybeans for uses in food and feed applications.

b) the baseline used for consideration of natural variations

Levels of the components in seed and forage of MON 87701 were compared to the corresponding levels in the control conventional comparator, which has similar genetic background to MON 87701. 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 87701 were conducted and the set of agronomic observations supports the conclusion that from an agronomic and phenotypic (morphological) point of view, MON 87701 is equivalent to traditional soybean, except for the inserted lepidopteran-protection trait (*see* Section D.4.).

7.5 Product specification

MON 87701 was created by introducing a c*ry1Ac* gene from *Bacilius thuringiensis,* into conventional soybean. The introduction of this gene results in the production of the Cry1Ac protein. This protein provides a lepidopteran-protection trait to MON 87701.

The presence of the *cry1Ac* gene and/or the Cry1Ac protein in soybean or in soybean derived products can be identified by employing different techniques. Southern blot or PCR techniques can identify the inserted nucleotide sequence, while the Cry1Ac protein can be detected in immature and mature seed from MON 87701, by optimised tissue extraction, standardised electrophoretic, blotting and immunodetection methodologies.

7.6 Effect of processing

MON 87701 has been shown to be substantially equivalent to conventional soybean, except for the inserted Cry1Ac protein. Therefore, it is highly likely that MON 87701 and its derived food and feed products are not different from the equivalent foods and feeds originating from conventional soybean.

7.7 Anticipated intake/extent of use

There are no anticipated changes in the intake and/or extent of use of soybean or derived products for use as or in food or feed as a result of the addition of MON 87701 to the soybean supply.

MON 87701 is not expected to affect current usage patterns of soybean, but to replace a portion of the commodity seed from current soybean varieties such that their intake or use will represent some fraction of the total products derived from soybean.

7.8 Toxicology

7.8.1 Safety assessment of newly expressed proteins

The conclusion of safety to humans of the Cry1Ac protein was based upon the following considerations:

- The protein has a demonstrated history of safe use;

- The protein has no structural similarity to known toxins or other biologically active proteins that could cause adverse effects in humans or animals;
- The protein does not exert any acute toxicity to mammals.

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

It is therefore possible to conclude that the Cry1Ac protein is 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. Compositional analysis confirmed that MON 87701 is compositionally equivalent to conventional soybean. Therefore, no testing of any constituent other than the introduced protein is required.

7.8.3 Information on natural food and feed constituents

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 87701 compositional analyses and their levels were demonstrated to be comparable in MON 87701 and in conventional soybean.

7.8.4 Testing of the whole GM food/feed

The compositional equivalence of MON 87701 seed and forage to that of conventional soybean has been established by compositional analysis. Additionally, the dietary safety of MON 87701 was further confirmed by repeat-dose animal feeding studies in broiler chickens and rats fed diets containing soybean meal produced from MON 87701. These studies confirm the absence of any toxic effects associated to the inserted protein 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.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein

The assessment of the allergenic potential of the Cry1Ac protein compares the biochemical characteristics of this protein to characteristics of known allergens, according to the recommendations of Codex Alimentarius Commission.

It is unlikely that the Cry1Ac protein will cause allergenic concerns due to the following considerations:

- It was obtained from non-allergenic sources (*Bacillus thuringiensis*)
- It lacks structural similarity to known allergens, as demonstrated by bioinformatics analyses
- It is rapidly digested in simulated gastric fluid
- It constitutes a very small portion of the total protein present in the seed of MON 87701.

Taken together, it can be concluded that the allergenic potential of the Cry1Ac protein is negligible and therefore, this protein does not pose a significant allergenic risk.

7.9.2 Assessment of allergenicity of the whole GM plant or crop

To assess whether MON 87701 has altered endogenous allergenic potential compared to traditional soybean, the potential allergenicity of MON 87701 against a conventional soybean variety was performed. Results of this assessment support the conclusion that MON 87701 is comparable to conventional soybean in terms of allergenicity potential. Thus, it is concluded that MON 87701 has no greater allergenic potential than soybean varieties that are currently on the market.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

The inserted lepidopteran-protection trait in MON 87701 is of agronomic interest, and is not intended to change any nutritional aspects of this soybean. The presence of this trait is not expected to alter patterns or volumes of soybean consumption.

In addition, the dietary safety of MON 87701 was further confirmed by repeat-dose animal feeding studies in the rat and broiler chickens. These studies found no adverse effects related to the consumption of diets containing MON 87701.

Therefore, no nutritional imbalances are expected as a result of the use of MON 87701 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 87701. 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. In the following paragraphs, this study is discussed in further detail.

7.11 Post-market monitoring of GM food/feed

There are no intrinsic hazards related to MON 87701 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 87701 demonstrates that the risks of consumption of MON 87701 or its derived products are consistently negligible and no different from the risks associated with the consumption of conventional soybean.

As a consequence, specific risk management measures are not indicated, and post-market monitoring of the use of soybean for food and feed is not considered appropriate.

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

MON 87701 carries a protection against certain lepidopteran insect pests trait.

A generalized mode of action of the Cry proteins includes the following steps: ingestion of the protoxin crystal by the insect, solubilization of the crystal in the insect midgut, proteolytic processing of the released Cry protein by digestive enzymes to produce an active toxin termed delta-endotoxin, binding of the endotoxin to receptors on the surface of midgut epithelial cells of target organisms, formation of membrane ion channels or pores, and consequent disruption of cellular homeostasis. Electrolyte imbalance and pH changes render the gut paralyzed, which causes the insect to stop eating and die.

However, any significant interactions of MON 87701 with its target pest organisms are limited to those countries where the cultivation of this soybean will be authorized. The cultivation of MON 87701 varieties in the EU is not within the scope of this application. The scope of the current application only includes the import, the processing and the use of this soybean in the EU as any other soybean, including food and feed use. In the context of the current application, the likelihood is negligible that the import of MON 87701 will result in plants of this soybean being present in the environment, and the potential for interactions of MON 87701 and its target organisms is, therefore, considered to be minimal.

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

This application under Regulation (EC) No 1829/2003 is for the authorisation of MON 87701 for import, processing and all uses as any other soybean, including food and feed use but excluding the cultivation of MON 87701 in the EU.

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 87701 is substantially equivalent to conventional soybean, except for the introduced lepidopteran-protection 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. In the event MON 87701 seed was spilt in the environment, its introduced trait would have negligible consequences for the environment.

Therefore, the risk to the environment from MON 87701 through increased persistence and invasiveness of this soybean is negligible.

9.2 Selective advantage or disadvantage

MON 87701 is substantially equivalent to conventional soybean, except for the inserted lepidopteran-protection trait.

Compared with conventional soybean, the presence of the lepidopteran-protection trait would only confer a selective advantage to MON 87701 where target lepidopteran pest species would be present at sufficiently high numbers to limit reproductive success, 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 inserted trait in MON 87701 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 87701 to wild plant species in the EU (as not present) while there is negligible likelihood for gene transfer from MON 87701 to other soybean crops since this application is not for consent to cultivate MON 87701 varieties in the EU.

In the case that an introduced gene outcrossed to other soybean, its transfer would only confer a selective advantage under specific conditions (*i.e.* upon attack by the target insects), as discussed in Section 9.2.

Therefore, gene transfer from MON 87701 to other soybean crops is not considered to constitute an adverse environmental effect in itself and the environmental risk posed by this potential transfer to other soybean crops, and hence by MON 87701, is negligible.

9.4 Interactions between the GM plant and target organisms

The (intended) insecticidal action of the Cry1Ac protein targets certain

lepidopteran pests. However, this application is limited to import of MON 87701 seed into the EU and use thereof as any other soybean commodity seed. As such, exposure to the environment will be rare, occurring only through incidental release during shipment and handling. The conditions where incidental release will occur are not conducive to establishment of soybean.

9.5 Interactions of the GM plant with non-target organisms

Given the scope of the current application, which does not include the cultivation of MON 87701 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 87701 seed during import, storage, transport or use would lead to the short survival of MON 87701 plants, the newly produced protein in MON 87701, Cry1Ac presents a negligible hazard to NTOs. As a consequence, there is negligible risk for harmful effects of MON 87701 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 87701.

9.6 Effects on human health

The likelihood for any adverse effects occurring in humans as a result of their contact with MON 87701 is no different from that of conventional soybean, as MON 87701 contains the Cry1Ac protein, which has negligible potential to cause any toxic or allergenic effects in humans.

MON 87701 is substantially equivalent to conventional soybean, except for the inserted lepidopteran-protection trait. This trait of agronomical interest is imparted by the production of the Cry1Ac protein, for which safety has been extensively investigated.

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 87701 is negligible. MON 87701 contains the Cry1Ac protein which has negligible potential to cause any toxic or allergenic effects in animals.

MON 87701 is substantially equivalent to conventional soybean as well as to soybean varieties in commerce, except for the inserted lepidopteran-protection trait imparted by the Cry1Ac protein. As previously discussed, the Cry1Ac protein has a history of safe use and its safety has been extensively investigated.

In conclusion, MON 87701 is expected to pose no meaningful health risks to farm animals that would consume it. Therefore, the risk of

MON 87701 for the feed/food chain is also negligible.

9.8 Effects on biogeochemical processes

This application is limited to import of MON 87701 seed into the EU and use thereof as any other soybean commodity seed. 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 87701 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 87701 was shown to be compositionally equivalent to conventional soybean with no biologically meaningful differences in agronomic and phenotypic characteristics, except for the inserted lepidopteran-protection trait, 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.

In conclusion, as for conventional soybean, it is highly unlikely that there would be any significant immediate or delayed adverse effects from MON 87701 on the biogeochemical processes in the soil.

9.9 Impacts of the specific cultivation, management and harvesting techniques

Not applicable. This application is for consent to import MON 87701 in the EU and for the use of this soybean as any other soybean, excluding the cultivation of MON 87701 varieties in the EU.

10. Potential interactions with the abiotic environment

MON 87701 carries a lepidopteran-protection trait of agronomic interest. As MON 87701 was shown to be substantially equivalent to conventional soybean (with the exception of the inserted lepidopteran-protection trait, imparted by the expression of the Cry1Ac protein), with respect to its composition, phenotypic and agronomic characteristics, there is no evidence that this soybean would be any different from conventional soybean with regard to its baseline interactions with the abiotic environment.

Although the Cry1Ac is an introduced protein in soybean, it already has a safe history of use and has no known negative interactions with the abiotic environment. The insecticidal protein Cry1Ac is subjected to

rapid degradation in soil and is therefore not expected to negatively affect soil or water.

Therefore, no negative impact of MON 87701 on the abiotic environment is expected to result from the import of MON 87701 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 the proposed monitoring plan for MON 87701 has been developed according to the principles and objectives outlined in Annex VII of Directive 2001/18/EC and Decision 2002/811/EC establishing guidance notes supplementing Annex VII to Directive 2001/18/EC. The structure of the monitoring plan also takes into account the guidance on presentation of applications provided in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed[1].

11.2 Interplay between environmental risk assessment and monitoring

An environmental risk assessment (ERA) was carried out for MON 87701 according to the principles laid down in Annex II to Directive 2001/18/EC and Decision 2002/623/EC establishing guidance notes supplementing Annex II to Directive 2001/18/EC. The scientific evaluation of the characteristics of MON 87701 in the ERA (Section D.9) has shown that the risk for potential adverse effects on human and animal health or the environment is negligible in the context of the intended uses of MON 87701.

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

The scientific evaluation of the characteristics of MON 87701 in the ERA has shown that the risk for potential adverse effects on human and animal health or the environment is negligible in the context of the intended uses of this soybean. It is therefore considered that there is no need for case-specific monitoring.

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

In accordance with Council Decision 2002/811/EC, general surveillance is not based on a particular hypothesis and it should be used to identify the

[1] http://www.efsa.europa.eu/cs/BlobServer/Guidance_of_Panel/gmo_guidance_derived_feed_food.pdf?ssbinary=true

occurrence of unanticipated adverse effects of the viable GMO or its use for human and animal health or the environment that were not predicted in the ERA.

The authorisation holder is not involved in commodity trade with MON 87701. The monitoring methodology hence needs to be predominantly based on collaboration with third parties, such as operators involved in the import, handling and processing of viable MON 87701. They are exposed to the imported viable MON 87701 and therefore are the best placed to observe and report any unanticipated adverse effects in the framework of their routine surveillance of the commodities they handle and use.

The general surveillance information reported to and collected by the authorisation holder from the European trade associations or other sources will be analysed for its relevance. Where information indicates the possibility of an unanticipated adverse effect, the authorisation holder will immediately investigate to determine and confirm whether a significant correlation between the effect and MON 87701 can be established. If the investigation establishes that MON 87701 was present when the adverse effect was identified and confirms that MON 87701 is the cause of the adverse effect, the authorisation holder will immediately inform the European Commission, as described in Section D.11.5.

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

MON 87701 is detectable using the event-specific PCR method for detecting the introduced DNA.

A MON 87701-specific PCR-based assay allowing the identification and quantification of MON 87701 has been provided to the Joint Research Centre (JRC), acting as the Community Reference Laboratory (CRL).

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 87701 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 87701 has been field tested in the US since 20012007 growing season, in Argentina in the 2002/2003, 2004/2005, 2005/2006, 2006-2007 and 2007-2008 growing seasons and in Brazil in the 2007-2008 and 2008-2009 growing seasons.

b) Authority overseeing the release

US: United States Department of Agriculture (USDA).

US EPA: United States Environmental Protection Agency

Argentina: Secretary of Agriculture, livestock, fishery and feed (SAGPyA) – National advisory commission on agricultural biotechnology (CONABIA).

Brazil: National technical committee of biosafety (CTNBio)

c) Release site

US: In the major soybean growing states (Alabama, Arkansas, Georgia, Illinois, Indiana, Kansas, Louisiana, Mississippi, North & South Carolina Texas and Virginia) and Puerto Rico.

Argentina: Buenos Aires, Cordoba, Santa Fe.

Brazil: Minas Gerais, Mato Grosso, Rio Grande do Sul, Paraná.

d) Aim of the release

US/Argentina/Brazil: Regulatory trials, efficacy, yield, breeding, product development.

e) Duration of the release

US/Argentina/Brazil: One growing season

f) Aim of post-releases monitoring

US/Argentina/Brazil: Assessment of volunteers

g) Duration of post-releases monitoring

US: Two to 12 months, depending on the field conditions

Argentina: Generally, 12 months

Brazil: Four to six months depending on the field conditions

h) Conclusions of post-release monitoring

US/Argentina/Brazil: 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 87701 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 JRC 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 87701.

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

A notification for MON 87701 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/.