Application for authorization to place on the market MON 87460 maize in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed

Part II

Summary

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 maize is MON 87460. Currently, no commercial name has been attributed to this product.

d) Date of acknowledgement of notification

Not available at the time of submission to EFSA.

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 87460 will be traded and used in the EU in the same manner as current commercial maize varieties and by the same operators currently involved in the trade and use of conventional maize.

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 87460 have been studied at multiple locations in North and South America that cover a range of environmental conditions. The data collected from these field releases have been used in the risk assessment presented in the MON 87460 application. A summary of the conclusions of the risk analysis that demonstrate the safety of MON 87460 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 U.S.A., Canada and Mexico but approvals from all agencies in these countries have not been obtained yet. The statuses of other 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 87460 was developed through *Agrobacterium*-mediated transformation of conventional maize variety embryos and expresses cold shock protein B (CspB) from *Bacillus subtilis* (Kingdom: Bacteria, Phylum: Firmicutes, Class: Bacilli) and NptII from Tn*5* of *Escherichia coli*. MON 87460 was developed to provide reduced yield loss under water-limited conditions compared to conventional maize.

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 import, processing and all uses of MON 87460 for food and feed. The range of uses of this maize for food and feed will be identical to the full range of equivalent uses of conventional maize.

c) Intended use of the product and types of users

MON 87460 will be traded and used in the EU in the same manner as current commercial maize varieties and by the same operators currently involved in the trade and use of conventional maize.

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 warranted or required for the placing on the market of MON 87460 for import, processing, and use as or in food and feed. MON 87460 is substantially equivalent to other maize varieties except for its reduced yield loss under water-limited conditions, which is a trait of agronomic interest. This maize was shown to be as safe and as nutritious as conventional maize. Therefore MON 87460 and derived products will be stored, packaged, transported, handled and used in the same manner as the commercial maize products.

e) Any proposed packaging requirements

MON 87460 is substantially equivalent to conventional maize varieties (except for its reduced yield loss under water-limited conditions). Therefore, MON 87460 and derived products will be used in the same manner as other maize and no specific packaging is foreseen. (For the labelling, *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, a labelling threshold of 0.9% is applied for the placing on the market of MON 87460 grain and derived products.

Operators shall be required to label products containing or consisting of MON 87460 with the words "genetically modified maize" or "contains genetically modified maize", and shall be required to declare the unique identifier MON-8746Ø-4 in the list of GMOs that have been used to constitute the mixture that contains or consists of this GMO.

Operators shall be required to label foods and feeds derived from MON 87460 with the words "produced from genetically modified maize". In the case of products for which no list of ingredients exists, operators shall 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 87460 grain and derived foods and feeds in the EU are 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 87460. 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)

MON-8746Ø-4

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 87460 is suitable for food and feed 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 and use MON 87460 as any other maize, not including the cultivation of varieties of MON 87460 in the EU, environmental release would be more likely to occur during import, storage and processing of MON 87460. However, modern methods of grain handling minimize losses of grain, so there is little chance of germination of spilt grain resulting in the development of mature plants of MON 87460 in the EU. Moreover, in the event of incidental spillage, the establishment of volunteer plants would be unlikely, since maize cannot survive without human assistance and is not capable of surviving as a weed. Although maize seed can over-winter in mild conditions and can germinate the following year, the appearance of maize in rotational fields is rare under European conditions. Maize volunteers, if they occurred, would be likely to be killed by frost or could be easily controlled by the use of selective herbicides. Moreover, the information presented in this application established that MON 87460 is unlikely to be different from other maize and, therefore, is unlikely to pose any threat to the environment or to require special measures for its containment.

No specific conditions are warranted or required for the placing on the market of MON 87460 for import, processing, or use for food and feed.

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

1. Complete name

a) Family name

Poaceae (formerly Gramineae)

b) Genus

Zea

c) Species

mays (2n=20)

d) Subspecies

N/A

e) Cultivar/breeding line

LH59

f) Common name

Maize; corn

2. a) Information concerning reproduction

(i) Mode(s) of reproduction

Maize (*Zea mays*) is an annual, wind-pollinated, monoecious species with separate staminate (tassels) and pistillate (silk) flowers, Self- and cross-pollination are generally possible, with frequencies of each normally determined by proximity and other physical influences on pollen transfer.

(ii) Specific factors affecting reproduction

Tasselling, silking, and pollination are the most critical stages of maize development and, consequently, grain yield may ultimately be greatly impacted by moisture and fertility stress.

(iii) Generation time

Maize is an annual crop with a cultural cycle ranging from as short as 60 to 70 days to as long as 43 to 48 weeks from seedling emergence to maturity.

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

Out-crossing with cultivated Zea *varieties*

The scope of the current application does not include cultivation of MON 87460 varieties in the EU. Outcrossing with cultivated *Zea* varieties is therefore not expected.

Out-crossing with wild Zea *species*

Closely related wild relatives of maize do not exist in Europe.

3. Survivability

a) Ability to form structures for survival or dormancy

Maize is an annual crop and seeds are the only survival structures. Natural regeneration from vegetative tissue is not known to occur.

b) Specific factors affecting survivability

Maize cannot survive without human assistance and is not capable of surviving as a weed due to past selection in its evolution. Volunteer maize is not found growing in fencerows, ditches or roadsides as a weed. Although maize seed from the previous crop year can over-winter in mild winter conditions and germinate the following year, it cannot persist as a weed. The appearance of "volunteer" maize in fields following a maize crop from the previous year is rare under European conditions. Maize volunteers are killed by frost or, in the unlikely event of their occurrence, are easily controlled by current agronomic practices including cultivation and the use of selective herbicides.

Maize grain survival is dependent upon temperature, moisture of seed, genotype, husk protection and stage of development. Freezing temperatures have an adverse effect on maize seed germination and have been identified as being a major risk in seed maize production. Temperatures above 45°C have also been reported as injurious to maize seed viability.

4. Dissemination

a) Ways and extent of dissemination

In general, dissemination of maize may occur by means of seed dispersal and pollen dispersal. Dispersal of the maize grain is highly restricted in domesticated maize due to the ear structure including husk enclosure. For maize pollen, the vast majority is deposited in the same field due to its large size (90 to 100 μm) with smaller amounts of pollen deposited usually in a downwind direction. However, the current application does not include cultivation of MON 87460 in the EU.

b) Specific factors affecting dissemination

Dispersal of maize seeds does not occur naturally because of the structure of the ears of maize. Dissemination of isolated seeds may result from mechanical harvesting and transport as well as insect or wind damage, but this form of dissemination is highly infrequent. Genetic material can be disseminated by pollen dispersal, which is influenced by wind and weather conditions. Maize pollen is the largest of any pollen normally disseminated by wind from a comparably low level of elevation. Dispersal of maize pollen is limited by its large size and rapid settling rate.

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

Because of its many divergent types, maize is grown over a wide range of climatic conditions. The bulk of the maize is produced between latitudes 30° and 55°, with relatively little grown at latitudes higher than 47° latitude anywhere in the world. The greatest maize production occurs where the warmest month isotherms range between 21 and 27°C and the freeze-free season lasts 120 to 180 days. A summer rainfall of 15 cm is

approximately the lower limit for maize production without irrigation with no upper limit of rainfall for growing maize, although excess rainfall will decrease yields.

There are no close wild relatives of maize 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

Maize is widely grown in the EU and represents a significant portion of global maize production. The most important areas of maize production in Europe include the Danube Basin, from southwest Germany to the Black Sea, along with southern France through the Po Valley of northern Italy.

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

There are no known toxic effects of the maize plant to humans, animals or livestock; it has a history of safe use for human food and animal feed. However, maize is known to interact with other organisms in the environment including insects, birds, and mammals. It is susceptible to a range of fungal diseases and nematode, insect and mite pests.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Description of the methods used for the genetic modification

A disarmed *Agrobacterium tumefaciens* plant transformation system including the double-border, binary vector PV-ZMAP595 was used to transform LH59 embryos producing MON 87460.

2. Nature and source of the vector used

The disarmed *Agrobacterium tumefaciens* transformation vector PV-ZMAP595 contains both left and right T-DNA border sequences facilitating the transformation. It was constructed using standard molecular biology techniques. The T-DNA region of this vector contains *cspB* and *nptII* expression cassettes and it is the portion that is integrated to maize genome during transformation event.

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

The genetic elements of PV-ZMAP595 intended for insertion into the maize genome comprised between the T-DNA borders are, from the right border region, promoter and leader from the rice actin gene (P-*Ract1*), a non-translated intron from the rice actin gene (I-*Ract1*), the *cspB* coding sequence (CS-*cspB*) and a polyadenylation sequence from the *transcript 7* gene (T-*tr7*). These elements together constitute the *cspB* expression cassette which is followed by the *nptII* expression cassette. The latter is flanked by two *loxP* sites and constitutes of a transcriptional promoter (P-*35S*), the *nptII* coding sequence (CS-*nptII*), and a polyadenylation sequence from the nopaline synthase gene (T-*nos*).

More detailed description of all elements is presented in Table 1.

Table 1. Summary of genetic elements intended for insertion

Genetic element[1]	Size (kb)	Function and source
		T-DNA
B-Right Border	0.36	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA
P-*Ract1*	0.92	Promoter and leader from the rice actin gene, *act1,* of *Oryza sativa*
I-*Ract1*	0.48	Intron from the rice actin gene, *act1,* of *Oryza sativa*
CS-*cspB*	0.20	Codon modified coding sequence of the *cspB* gene from *Bacillus subtilis* encoding CspB
T-*tr7*	0.50	3' non-translated sequence of *transcript* 7 gene from *Agrobacterium tumefaciens* that directs polyadenylation
loxP	0.03	Sequence from *Bacteriophage P1* for the recombination site recognized by Cre recombinase
P-*35S*	0.29	Promoter for the 35S RNA of the Cauliflower Mosaic Virus
CS-*nptII*	0.79	Coding sequence from Tn*5* in *E. coli* encoding neomycin and kanamycin resistance
T-*nos*	0.25	3' non-translated sequence of the nopaline synthase (*nos*) gene from *Agrobacterium tumefaciens* which terminates and directs polyadenylation
loxP	0.03	Sequence from *Bacteriophage P1* for the recombination site recognized by Cre recombinase
B-Left Border	0.44	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA

[1] CS – Coding Sequence; OR – Origin of Replication; B – Border; P – Promoter; I – Intron; T – 3' non-translated transcriptional termination sequence 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 87460 was developed to provide reduced yield loss under water-limited conditions compared to conventional maize. Efficacy in MON 87460 is derived by expression of the inserted *Bacillus subtilis* cold shock protein B (CspB). CspB is an extensively studied protein known to facilitate adaptation to environmental stresses in bacteria. CspB is known to bind and unfold secondary RNA structures that compromise the ability of the cell to translate those RNA molecules, thus helping to preserve normal cellular functions.

In addition to CspB, MON 87460 also expresses the NptII protein. Neomycin phosphotransferase II (NPTII; E.C. 2.7.1.95) inactivates aminoglycoside antibiotics such as neomycin and kanamycin but does not act on other aminoglycosides with clinical applications. The purpose of inserting the gene encoding the NptII protein into maize cells along with CspB was to have an effective method for selecting cells after transformation. In general, the efficiency of plant cells transformation is often low, ranging from 1×10^{-5} to 1×10^{-4} of cells treated. Therefore, the selectable marker, NptII, was used to facilitate the screening process, which was used to facilitate the selection process of transformed plant cells.

Under well-watered conditions, grain yield for MON 87460 is equivalent to conventional maize. Under water-limited conditions, grain yield loss is reduced compared to conventional maize. However, like conventional maize, MON 87460 is still subject to yield loss under water-limited conditions, particularly during flowering and grainfill periods when maize yield potential is most sensitive to stress, by disrupting kernel development. Under severe water deficit, maize grain yield for MON 87460, as well as conventional maize, can be reduced to zero.

2. Information on the sequences actually inserted or deleted

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

MON 87460 contains one copy of the insert at a single insertion site hosting both *cspB* and *nptII* intact expression cassettes. No additional elements from the transformation vector PV-ZMAP595, linked or unlinked to the *cspB* and *nptII* expression cassettes, were detected in the genome of MON 87460. Additionally, backbone sequence from the plasmid PV-ZMAP595 was not detected.

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

No deletion was intended in the development of MON 87460. However, following characterization of the insertion site the following deletions were observed:

- The DNA sequence containing the Left Border region was truncated in MON 87460 compared to the Left Border region on the PV-ZMAP595 plasmid: 194 bp were deleted;
- the B-Right border region is absent in the MON 87460 insert as well as a part of the P-*Ract1* element region of PV-ZMAP595;
- Comparing with the conventional maize genome sequence, a 22 bp DNA deletion occurred at the site of insertion in MON 87460.

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 87460 insert in the maize nuclear genome is best shown by the Chi square (χ^2) analysis of the segregation data. The results show that inheritance of the drought-tolerance trait in MON 87460 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 organization of the inserted genetic material at the insertion site

The insert in MON 87460 was characterized using Southern blot methods. Specifically, the insert number (number of insertions of the integrated DNA within the maize genome), the copy number (the number of copies of the integrated DNA within one insertion site), the integrity of the inserted *cspB* and *nptII* expression cassettes and the presence or absence of plasmid backbone sequence was assessed. DNA sequence analyses confirmed the sequence identity between the MON 87460 insert and the portion of the T-DNA from PV-ZMAP595 that was integrated into the maize genome. The results of PCR and sequence analyses further confirmed the organization of the genetic elements within the *cspB* and *nptII* expression cassettes of MON 87460, which was identical to that in plasmid PV-ZMAP595.

3. Information on the expression of the insert

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

The levels of the CspB and NptII proteins in various tissues of MON 87460 that are relevant to the risk assessment were assessed by a validated ELISA. Tissue samples for analysis were collected from six field sites in the U.S. (2006) and four field sites in Chile (2006-2007). The trial locations used represent the major maize-growing regions of the U.S. and Chile and provide a range of environmental conditions that would be encountered in the commercial production of maize. At each site, three replicated plots of MON 87460 and a conventional control hybrid were planted using a randomized complete block field design.

In tissues harvested throughout the growing season in the U.S., the mean CspB protein levels in MON 87460 across all sites ranged from 0.47 to 3.1 µg/g dw in leaf, 0.24 to 1.4 µg/g dw in root, and 0.67 to 2.8 µg/g

dw in whole plant. The range of NptII protein levels for MON 87460 in leaf, forage, and grain were 0.21 to 0.63, 0.017 to 0.053, and <LOQ µg/g fresh weight, respectively.

In tissues harvested throughout the growing season in Chile, mean CspB protein levels in MON 87460 across all sites ranged from 0.39 to 2.8 µg/g dw in leaves, 0.031 to 1.5 µg/g dw in roots, and 0.67 to 3.2 µg/g dw whole plants. The mean NptII protein levels across the sites were highest in young leaf (2.6 µg/g dw), followed by root (0.51 µg/g dw), and forage (0.17 µg/g dw). The levels of NptII protein in grain were below the NptII assay LOQ (0.0047 µg/g fw for grain).

b) Parts of the plant where the insert is expressed

The CspB and NptII proteins were found to be expressed in leaf, root, pollen, silk, forage, forage root, grain, stover and senescent root at appropriate times of plant development. Grain and forage are the most relevant tissues for the food and feed safety assessment of MON 87460, while leaf, root, pollen, silk and stover are relevant tissues in terms of environmental risk assessment.

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

a) Reproduction

According to the phenotypic and agronomic data collected from field trials conducted at locations representing the major maize growing regions in the U.S. and Chile, MON 87460 has not been altered in survival, multiplication or dissemination characteristics when compared to conventional maize under different irrigation practices. The introduced trait is an agronomic trait which has no influence on maize reproductive morphology and, hence, no changes in seed dissemination would be expected.

b) Dissemination

The introduced trait has no influence on maize reproductive morphology and, hence, no changes in seed dissemination are to be expected.

c) Survivability

Maize is known to be a weak competitor in the wild, which cannot survive outside cultivation without human intervention. Field observations have demonstrated that MON 87460 has not been altered in its survivability when compared to conventional maize.

d) Other differences

Comparative assessments in the field did not reveal any biologically significant differences between MON 87460 and conventional maize hybrids, except for the introduced trait that is of agronomic interest.

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

MON 87460 contains one insert with a single copy of the transformed DNA, which is stably integrated into the nuclear maize genome. The insert is inherited in a Mendelian fashion. This has been confirmed by Southern blot analyses.

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 inserted in MON 87460 has a genetic transfer function. Therefore, no changes are expected in the ability of this maize to transfer genetic material to bacteria.

b) Plant to plant gene transfer

Not applicable. The scope of the current application does not include the cultivation of MON 87460 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 87460 was compared with a conventional control maize with similar genetic background.

7.2 Production of material for comparative assessment

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

MON 87460 and a conventional control maize were grown at six field sites in major maize-growing areas of the U.S. during the 2006 field season, and at four field sites representative for the commercial maize production regions in Chile during the 2006-2007 growing season.

b) the baseline used for consideration of natural variations

The compositional study compared MON 87460 to the control. Reference hybrids were grown in the same field locations and under the same conditions as the test and control. Where statistical differences occurred, the measured analyte was compared to ILSI ranges for commercial varieties and ranges reported in literature.

7.3 Selection of material and compounds for analysis

The numerous compounds that were selected for analysis in the compositional study were chosen on the basis of internationally accepted guidance provided by the OECD (*See* consensus document for compositional analysis of maize), in addition to other selected compounds.

Based on the conclusion of compositional equivalence resulting from these extensive, compositional analyses conducted for MON 87460 compared to conventional maize hybrids, there is no indication to further analyze other selected compounds in this maize.

7.4 Agronomic traits

Field trials with MON 87460 were performed and the set of agronomic observations supports a conclusion that from an agronomic, phenotypic (morphological) and ecological interaction point of view, MON 87460 is equivalent to conventional maize, except for the introduced drought tolerance trait (see Section D.4).

7.5 Product specification

MON 87460 will be imported into the EU in mixed shipments of maize grain and products, produced in other world areas, for use by operators that have conventionally been involved in the commerce, processing and use of maize and maize derived products in the EU.

7.6 Effect of processing

Using both wet and dry milling processes, maize is converted into a diverse range of food and feed products and derivatives used as food and feed ingredients or additives. As MON 87460 is substantially equivalent and as safe and as nutritious as conventional maize, the use of MON 87460 for the production of foods and feeds is no different from that of conventional maize. Consequently, any effects of the production and processing of MON 87460 are not expected to be any different from the production and processing of the equivalent foods and feeds, originating from conventional maize.

7.7 Anticipated intake/extent of use

There are no anticipated changes in the intake and/or extent of use of maize or derived products for use as or in food or feed as a result of the addition of MON 87460 to the conventional maize supply. MON 87460 is expected to replace a portion of current maize hybrids such that its intake or use will represent some fraction of the total products derived from maize.

7.8 Toxicology

7.8.1 Safety assessment of newly expressed proteins

MON 87460 contains the *cspB* and *nptII* expression cassettes that produce the CspB and NPTII proteins, respectively. The assessment of human and animal safety of the CspB and NPTII proteins can be summarized as follows:

(i) The donor organisms, *Bacillus subtilis* and *E. coli* are safe.

The genes encoding the newly expressed proteins were isolated from donor organisms considered to be safe. *B. subtilis* has been extensively tested by numerous agencies and found to be non-pathogenic and safe for human consumption. *E. coli* is classified in all major national and international safety guidelines as a biologically safe organism for the propagation of a broad range of gene cloning and expression vectors and has been used as such for protein production in many commercial applications;

(ii) The CspB protein is similar to proteins with a history of safe useCspB has a long history of safe consumption in foods and feeds. Cold shock proteins have no known toxicity and are not associated with pathogenicity. Proteins homologous to CspB are present in many widely consumed foods. The NptII protein is ubiquitous in *E. coli* and, therefore, is normally present in the human gastrointestinal tract. Furthermore, NptII-containing crops, such as MON 863 maize and MON 863-containing stacks[1] have been commercialized on a broad acreage and safely consumed as foods and feeds since their initial introduction in 2003. The NptII protein produced in these maize crops is identical to NptII produced in MON 87460;

(iii) No structurally relevant similarity exisits between the CspB and NptII proteins and any known toxins or other biologically active proteins that would be harmful to human or animal health;

(iv) Acute oral toxicity studies conducted with the CspB and NpII proteins in mice reported no indications of toxicity in mice administered the CspB or NptII proteins by oral gavage;

(v) According to the dietary risk assessment, acceptable margins of exposure have been demonstrated for the CspB and NptII proteins compared to conservative estimates of human exposure. Therefore, there is a reasonable certainty that consumption of foods derived from MON 87460 will not adversely affect the health of adults or children in the EU.

All these data indicate that the CspB and NptII proteins are safe for human and animal consumption.

7.8.2 Testing of new constituents other than proteins

Maize has a long history of safe use and consumption around the world. As described in Section D.7.1, MON 87460 has been shown to be compositionally equivalent to traditional maize. Therefore, no testing of any constituent other than the introduced proteins is indicated.

[1] MON 863 × MON 810, MON 863 × NK603 and MON 863 × MON 810 × NK603

7.8.3 Information on natural food and feed constituents

Maize is known as a common source of food and feed with a centuries-long history of safe use and consumption around the world. No particular natural constituents of maize are considered to be of significant concern to require additional information or further risk assessment.

7.8.4 Testing of the whole GM food/feed

The compositional and nutritional equivalence of grain and forage from MON 87460 and conventional maize have been established by compositional analysis. Additionally, the wholesomeness of MON 87460 grain has been confirmed by repeat-dose animal feeding studies in rat and in broiler chickens using MON 87460 - containing diets.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein

The CspB and NptII proteins have been assessed for their potential allergenicity according to the recommendations of the Codex Alimentarius Commission. Both proteins are from non-allergenic sources, lack structural similarity to known allergens, are rapidly digested in simulated gastric and simulated intestinal fluids, and constitute a very small portion of the total protein present in the grain of MON 87460. Taken together these data lead to the conclusion that the CspB and NptII proteins are unlikely to have any allergenic potential, and MON 87460 is as safe as conventional maize regarding the risk for allergenicity.

7.9.2 Assessment of allergenicity of the whole GM plant or crop

Maize is not considered a common allergenic food. Food allergies to maize are of low frequency and mainly occur in populations of specific geographic areas. Rare cases of occupational allergy to maize dust have been reported.

As MON 87460 is substantially equivalent and as safe as conventional maize, there is no reason to expect that the use of MON 87460 will significantly increase the potential for allergenicity. Further, as the introduced proteins in MON 87460 do not have any allergenic potential, it was concluded that the use of MON 87460 for food or feed does not lead to an increased risk for allergenic reactions compared to the equivalent range of food and feed uses of conventional maize.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

As described in Section D.7.1, MON 87460 was shown to be compositionally equivalent to conventional maize. The introduced drought tolerance trait is of agronomic interest and is not intended to change any nutritional aspects of this maize. MON 87460 is not

expected to be more or less attractive for use as food (or feed), for processing or as a food (or feed) ingredient. Therefore, anticipated dietary intake of MON 87460 maize-derived foods is not expected to be altered in the EU upon commercialisation of MON 87460 and no nutritional imbalances are expected as a result.

7.10.2 Nutritional assessment of GM feed

MON 87460 was demonstrated to be compositionally equivalent to conventional maize. The safety assessment of MON 87460 showed that this drought tolerant maize does not pose any adverse effects for humans and animals. Animal feeding studies were nonetheless conducted with MON 87460 in broilers and established the nutritional equivalence and wholesomeness of this maize to conventional maize for use as feed.

7.11 Post-market monitoring of GM food/feed

The assessment of the human and animal safety of MON 87460 was conducted on the basis of its substantial equivalence to conventional maize (except for the introduced trait) and by extensive characterization of the newly expressed proteins.

There are no intrinsic hazards related to MON 87460 as no signs of adverse or unanticipated effects have been observed in a number of safety 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 87460 demonstrates that the risks of consumption of MON 87460 or its derived products are no different from the risks associated with the consumption of conventional maize and maize-derived products.

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

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

Not applicable. MON 87460 provides reduced yield loss under water-limited conditions and, as such, does not have any target organisms.

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

This application is limited to import for direct food or feed use or for processing. 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 maize.

9.1 Persistence and invasiveness

Like for conventional maize, the likelihood of MON 87460 spreading in the environment is negligible, as maize is neither persistent nor invasive and these parameters are unaltered in MON 87460 when compared to

conventional maize. Furthermore, MON 87460 has equivalent growth and development, morphology, yield, plant health and survival characteristics compared to conventional maize Hence, the risk of unintentional spreading of MON 87460 through increased weediness of this maize is negligible.

9.2 Selective advantage or disadvantage

Compared with conventional maize, the presence of the drought tolerance trait would only confer a selective advantage where water-limiting conditions are present at levels that would suppress yield, and if no other, more important factors limiting the survival of maize in the receiving environment would be present. In practice, however, this advantage would be of limited consequence because of the poor survival characteristics of maize under most European conditions and since the trait was shown not to provide a meaningful selective advantage or disadvantage that altered the survival of MON 87460 maize as volunteer plants or in areas that outside of agricultural production. In conclusion, the risk of the drought tolerance trait in MON 87460 to be the cause of any competitive advantage or disadvantage impacting the receiving environment is negligible.

9.3 Potential for gene transfer

MON 87460 is unchanged in its potential for gene transfer compared to conventional maize. There is no potential for gene transfer from MON 87460 to wild plant species in the EU and negligible likelihood for gene transfer to other maize crops, as this application is not for consent to cultivate MON 87460 varieties in the EU. The environmental risk of potential gene transfer is negligible.

9.4 Interactions between the GM plant and target organisms

MON 87460 provides reduced yield loss under water-limited conditions and, as such, has no target organisms with which to interact, either directly or indirectly.

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 87460 varieties in the EU, the likelihood for direct or indirect interactions of this maize with non-target organisms is considered to be negligible. In addition, the newly expressed protein presents a negligible hazard to non-target organisms, even if incidental spillage of MON 87460 grain during import, storage, transport or use would lead to the short survival of MON 87460 plants in the environment. As a consequence, there is negligible risk for harmful effects of MON 87460 on non-target organisms, either through direct or indirect interactions with this maize or through contact with the newly expressed protein.

Furthermore, no adverse effects were observed in field trials conducted in multiple years in the U.S. and Chile involving MON 87460 maize.

9.6 Effects on human health

The likelihood for any adverse effects, occurring in humans as a result of their contact with this maize, is no different from conventional maize. MON 87460 contains the CspB and NptII proteins, which have negligible potential to cause any toxic or allergenic effects in humans. Therefore, the risk of changes in the occupational health aspects of this maize is negligible.

9.7 Effects on animal health

The likelihood for any adverse effects, occuring in animals fed on MON 87460, is negligible. MON 87460 contains the CspB and NptII proteins, which have negligible potential to cause any toxic or allergenic effects in animals. Therefore, the risk of MON 87460 for the feed/food chain is also negligible.

9.8 Effects on biogeochemical processes

As for conventional maize, spillage of MON 87460 during transport or storage of grain could cause some kernels to fall to the ground. Although such kernels could eventually germinate if the local soil and environmental conditions are favorable, this maize 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 maize grain that is incidentally released.

There is no evidence that MON 87460 plants would be any different from conventional maize regarding their direct influence on biogeochemical processes or nutrient levels in the soil as MON 87460 is compositionally equivalent and has equivalent growth and development, morphology, yield, plant health and survival characteristics to conventional maize.

In conclusion, as for conventional maize, it is highly unlikely that there would be any significant immediate or delayed adverse effects from MON 87460 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 87460 in the EU and for the use of this maize as any other maize, excluding the cultivation of MON 87460 varieties in the EU.

10. Potential interactions with the abiotic environment

Since this application is for import of MON 87460 into the EU and use thereof as any other maize, excluding the cultivation of MON 87460 varieties, the interaction with the environment will be limited. Moreover, no deleterious impact of MON 87460 on the abiotic environment is expected to result from the import, processing or use of this product for food and feed in the EU.

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 87460 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 (EFSA, 2006).

11.2 Interplay between environmental risk assessment and monitoring

An environmental risk assessment (e.r.a.) was carried out for MON 87460 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 87460 in the e.r.a. (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 87460.

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

The scientific evaluation of the characteristics of MON 87460 in the e.r.a. 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. 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 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 e.r.a.

The authorisation holder is not involved in commodity trade with MON 87460. 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 87460. They are exposed to the imported viable MON 87460 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 87460 can be established. If the investigation establishes that MON 87460 was present when the adverse effect was identified, and confirms that MON 87460 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 an annual monitoring report containing information obtained from participating networks, and/or in case of an effect that was confirmed. 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 livestock health and/or the environment.

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

Southern blot or PCR techniques can be employed for the detection and identification of the inserted nucleotide sequences. ELISAs have been developed and can be used to detect the CspB and NPTII proteins in individual plants. A MON 87460-specific PCR assay allowing the identification and the quantification of MON 87460 has been provided to the Joint Research Center (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 87460 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 87460 has been field tested in the U.S.A. since 2003, as well as Chile and Argentina since 2006. It has also been tested in South Africa since 2007 and during 2007 in Canada.

b) Authority overseeing the release

U.S.A.: United States Department of Agriculture. Chile: Agricultural and Livestock Service (SAG). Argentina: Secretary of Agriculture (SAGPyA) – CONABIA. South Africa: Department of Agriculture. Canada: Canadian Food Inspection Agency.

c) Release site

U.S.A.: mainly in the states of the corn belt and western great plains as well as California, Hawaii and Puerto Rico. Chile: Metropolitana and Valparaiso. Argentina: Provincia de Buenos Aires. South Africa: Nothern Cape. Canada: Ontario and Quebec

d) Aim of the release

U.S.A./Chile/Argentina/South Africa: assess agronomic performance, efficacy, yield, breeding. Canada: agronomic evaluation.

e) Duration of the release

U.S.A./Chile/Argentina/South Africa: 12 months. Canada: 6 months.

f) Aim of post-releases monitoring

U.S.A./Argentina/Chile/South Africa/Canada: assessment/removal of volunteers.

g) Duration of post-releases monitoring

All countries: 12 months.

h) Conclusions of post-release monitoring

Volunteers have been eliminated when found during the post-release monitoring.

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

All countries: no evidence that MON 87460 is likely to cause any adverse effects to human or animal health and 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 87460.

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

A notification for MON 87460 according to Directive 2001/18/EC has not been submitted by Monsanto.

c) EFSA opinion

An EFSA opinion, specifically for MON 87460, was not available at the time of submission of this application.

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

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/.