Application for authorization of MON 863 × MON 810 ×NK603 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

Belgium

b) Notification number

Not available at the time of application

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

The Monsanto development code for this genetically modified maize is **MON 863 × MON 810 × NK603**. In countries where MON 863 × MON 810 × NK603 varieties are being cultivated, packages of hybrid seed of this maize are marketed under the name of the hybrid variety, in association with the trademarks YieldGard®[1] Plus and Roundup Ready®[1] corn 2, indicating clearly to growers that the hybrid is protected from certain coleopteran and lepidopteran insect pests and tolerant to Roundup®[1] herbicide, containing the active ingredient glyphosate.

d) Date of acknowledgement of notification

Not available at the time of application

2. Applicant

a) Name of applicant

Monsanto Company, represented by Monsanto Europe S.A.

b) Address of applicant

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

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

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 863 × MON 810 × NK603 maize[2] will be traded and used in the European Union in the same manner as current commercial maize varieties and by the same operators currently involved in the trade and use of traditional 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

[1] Roundup®, YieldGard® and Roundup Ready® are registered trademarks of Monsanto Technology LLC
[2] Hereafter referred to as MON 863 × MON 810 × NK603

(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, composition, safety, and agronomic and phenotypic characteristics of MON 863 × MON 810 × NK603 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 assessments presented in this application. A summary of the conclusions of the risk analyses that demonstrate the safety of MON 863 × MON 810 × NK603 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 Since 2003, maize products containing the MON 863 insert have been deregulated in the U.S.A. for all uses, corresponding to the full range of uses of traditional maize. MON 863 × MON 810 × NK603 was first authorized for intentional release into the environment in the U.S.A. and Canada in 2004, and has been evaluated for food and feed safety in Korea in 2004.	

8. General description of the product

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

MON 863 × MON 810 × NK603 is produced by the combination of genetically modified YieldGard® Rootworm maize (MON 863), YieldGard® Corn Borer maize (MON 810) and Roundup Ready® maize (NK603)[3] using traditional breeding methods. No additional genetic modification is involved in the production of MON 863 × MON 810 × NK603.

MON 863 × MON 810 × NK603 and the genetically modified parental lines, MON 863, MON 810 and NK603, were developed by the Monsanto Company. MON 863 produces the MON 863 modified Cry3Bb1 protein[4], which confers protection against certain coleopteran insect pests (*Diabrotica* spp.). MON 810 contains the Cry1Ab protein protecting the crop against certain lepidopteran insect pests, including the European Corn Borer (*Ostrinia nubilalis*) and pink borers (*Sesamia* spp.). NK603 is tolerant to Roundup® herbicide (containing the active ingredient glyphosate).

Each of the introduced traits from the parental maize lines are inherited in MON 863 × MON 810 × NK603.

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 863 × MON 810 × NK603 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 traditional maize.

c) Intended use of the product and types of users

MON 863 × MON 810 × NK603 will be traded and used in the European Union in the same manner as current commercial maize varieties and by the same operators currently involved in the trade and use of traditional 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 considered necessary for the placing on the market of MON 863 × MON 810 × NK603 for import, processing, and use as or in food and feed. As demonstrated in this application, MON 863 × MON 810 × NK603 is equivalent to traditional maize except for its protection against the corn rootworm pest, certain lepidopteran insect pests, and its tolerance to glyphosate, which are traits of agronomic interest. This maize was shown to be as safe and as nutritious as traditional maize. Therefore, MON 863 × MON 810 × NK603 and derived products from MON 863 × MON 810 × NK603 will be stored, packaged, transported, used, and handled in the same manner as for current commercial maize varieties, and the measures for waste disposal and treatment of MON 863 × MON 810 × NK603 products are the same as those for traditional maize.

e) Any proposed packaging requirements

MON 863 × MON 810 × NK603 is equivalent to its parental lines MON 863,

[3] Hereafter referred to as MON 863, MON 810 and NK603, respectively
[4] Hereafter referred to as the Cry3Bb1 protein

MON 810 and NK603 and to traditional maize (except for its protection against targeted coleopteran and lepidopteran insect pests and its tolerance to glyphosate). Therefore, MON 863 × MON 810 × NK603 and derived products will be used in the same manner as other maize and no specific packaging is required (for the labelling, *see* question 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) N° 1829/2003 and 1830/2003, a labelling threshold of 0.9 % is applied for the placing on the market of MON 863 × MON 810 × NK603 grain and derived products.

Operators shall be required to label products containing or consisting of MON 863 × MON 810 × NK603 with the words "genetically modified maize" or "contains genetically modified maize", and shall be required to declare the unique identifier MON-ØØ863-5 × MON-ØØ81Ø-6 × MON-ØØ6Ø3-6 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 863 × MON 810 × NK603 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 863 × MON 810 × NK603 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 labeling requirements for MON 863 × MON 810 × NK603. Therefore, no further specific measures are to be taken by the notifier.

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-ØØ863-5 × MON-ØØ81Ø-6 × MON-ØØ6Ø3-6.

MON 863 × MON 810 × NK603 is uniquely identified using this combination of the unique identifiers for MON 863 (MON-ØØ863-5), MON 810 (MON-ØØ81Ø-6) and NK603 (MON-ØØ6Ø3-6).

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 863 × MON 810 × NK603 is suitable for food and feed use throughout the E.U.

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 863 × MON 810 × NK603 as any other maize, not including the cultivation of varieties of MON 863 × MON 810 × NK603 in the E.U., environmental release would be more likely to occur during import, storage and processing of MON 863 × MON 810 × NK603. 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 863 × MON 810 × NK603 in the E.U. 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 863 × MON 810 × NK603 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.

Misuse of MON 863 × MON 810 × NK603 is unlikely since the proposed uses for this maize include all the current uses of traditional maize, except for the cultivation of varieties in the E.U. Misuse of imported grain as seed for planting is thought to be an unlikely source of unintended release, since imports of MON 863 × MON 810 × NK603 grain will be of the F2 generation. As with all F1 hybrid maize seed, MON 863 × MON 810 × NK603 F1 hybrid varieties do not "breed true". Consequently, the harvested F2 grain does not produce a high yield of quality grain if replanted as seed, a fact that is widely known among growers. Furthermore, growers are aware that all maize hybrid varieties are highly specific to the climatic zones for which they are developed and may fail to thrive outside that zone, further diminishing the possibility of misuse of MON 863 × MON 810 × NK603 grain as seed. Finally, only certified hybrid seed can be legally marketed for planting in the E.U.

MON 863 × MON 810 × NK603 is equivalent to other maize, except for the introduced traits (protection against target coleopteran and lepidopteran insect pests and tolerance to glyphosate), which are traits of agronomic interest. This maize is shown to be as safe and as nutritious as traditional maize. Therefore, any measures for waste disposal and treatment of MON 863 × MON 810 × NK603 products are the same as those for traditional maize.

No specific conditions are required for the placing on the market of MON 863 × MON 810 × NK603 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

mays

e) Cultivar/breeding line

MON 863 × MON 810 × NK603

f) Common name

Maize; Corn

2. a) Information concerning reproduction

(i) Mode(s) of reproduction

Maize (*Zea mays* L.) 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

<u>Out-crossing with cultivated *Zea* varieties</u>

The scope of the current application does not include cultivation of MON 863 × MON 810 × NK603 varieties in the E.U. Outcrossing with cultivated *Zea* varieties is therefore not expected.

<u>Out-crossing with wild *Zea* species</u>

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 the cultivation of MON 863 × MON 810 × NK603 varieties in the E.U.

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 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 European Union. 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**

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. Maize has a history of safe use for human food and animal feed.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Description of the methods used for the genetic modification

No novel method of genetic modification is utilized in the production of MON 863 × MON 810 × NK603. Instead, traditional maize breeding methods are used to cross inbreds of MON 863, MON 810 and NK603. Whereas MON 863 × MON 810 × NK603 results from traditional breeding, genetic modification was used in the development of the single-trait parents. MON 863, MON 810 and NK603 were produced using the particle acceleration method.

2. Nature and source of the vector used

Whereas MON 863 × MON 810 × NK603 results from traditional breeding, genetic modification was used in the development of the single-trait parental maize lines.

- MON 863 was generated by the integration of sequences from the plasmid vector PV-ZMIR13, containing the *cry3Bb1* coding sequence of interest, which was derived from *Bacillus thuringiensis* subsp. *kumamotoensis*. MON 863 was produced using the particle acceleration method.
- MON 810 was generated by the integration of sequences from the plasmid vector PV-ZMBK07, containing the *cry1Ab* coding sequence of interest, which was derived from *Bacillus thuringiensis* subsp. *kurstaki*. MON 810 maize was produced using the particle acceleration method.
- NK603 was produced by a particle acceleration transformation method using a gel-isolated *Mlu*I fragment of plasmid vector PV-ZMGT32, containing a 5 enolpyruvylshikimate-3-phosphate synthase (*epsps*) gene that was derived from the common soil bacterium *Agrobacterium* sp. strain CP4 (*cp4 epsps*).

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

MON 863 × MON 810 × NK603 results from a traditional cross of the parental lines MON 863, MON 810 and NK603, and inherits the inserted DNA fragments from each its parental maize lines. The individual components and the function of these inherited DNA sequences are given in Tables 1 to 3.

Table 1.	**Components of the insert in MON 863**		
Genetic element	**Source**	**Size (kb)**	**Function**
***MON 863 Cry 3Bb1* gene cassette**			
4AS1	Cauliflower mosaic virus	0.22	Promoter associated with high level of expression in roots containing 4 tandem copies of the activating sequence 1 (AS1) which is a 21 bp sequence derived from the cauliflower mosaic virus 35s promoter (35S) fused to an additional portion of the 35S
wt CAB	*Triticum aestivum*	0.06	Translation enhancement
ract1 intron	*Oryza sativa*	0.49	Transcription enhancement
MON 863 cry3Bb1	*Bacillus thuringiensis* subsp. *kumamotoensis*	1.96	Carries the insect protection trait
tahsp 17 3'	*Triticum aestivum*	0.23	Ends transcription and directs polyadenylation
Selectable marker *(nptII)*			
35S	Cauliflower mosaic virus	0.32	Regulates expression in plant cells
nptII	*Escherichia coli*	0.82	Allows the selection of the plant cells carrying the insect protection trait by confering a resistance towards a category of aminoglycosides comprising kanamycin, and neomycin
ble (truncated)	*Escherichia coli*	0.15	Non-functional The bleomycin resistance gene *ble* has been subcloned together with the *nptII* ORF from which it shares the same prokaryotic operon
NOS 3'	*Agrobacterium tumefaciens*	0.26	Ends transcription and directs polyadenylation

Table 2.	**Components of the insert in MON 810**		
Genetic element	**Source**	**Size (kb)**	**Function**
e35S	Cauliflower mosaic virus	0.32	Promoter
Zmhsp70	*Zea mays* L.	0.8	Stabilizes level of gene transcription.
cry1Ab	*Bacillus thuringiensis*	3.5	Encodes Cry1Ab protein, which targets specific lepidopteran insect pests

Table 3.	**Components of the insert in NK603**		
Genetic element	**Source**	**Size (kb)**	**Function**
First *cp4 epsps* gene cassette			
P-ract1/ ract1 intron	*Oryza sativa*	1.4	Contains promoter, transcription start site and first intron.
ctp 2	*Arabidopsis thaliana*	0.2	Encodes chloroplast transit peptide, which directs the CP4 EPSPS protein to the chloroplast
cp4 epsps	*Agrobacterium* sp. strain CP4	1.4	Encodes glyphosate-tolerant CP4 EPSPS protein
NOS 3'	*Agrobacterium tumefaciens*	0.3	Ends transcription and directs polyadenylation of the Mrna
Second *cp4 epsps* gene cassette			
e35S	Cauliflower mosaic virus	0.6	Promoter
Zmhsp70	*Zea mays L.*	0.8	Stabilizes the level of gene transcription.

ctp 2	*Arabidopsis thaliana*	0.2	Encodes chloroplast transit peptide, which directs the CP4 EPSPS protein to the chloroplast
cp4 epsps l214p	*Agrobacterium* sp. strain CP4	1.4	Encodes glyphosate-tolerant CP4 EPSPS L214P protein, a variant of CP4 EPSPS, containing a proline residue instead of leucine at position 214
NOS 3'	*Agrobacterium tumefaciens*	0.3	Ends transcription and directs polyadenylation of the mRNA

D. INFORMATION RELATING TO THE GM PLANT

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

MON 863 × MON 810 × NK603 is produced by crossing the parental single-trait maize lines MON 863, MON 810 and NK603 by means of traditional breeding methods. MON 863 × MON 810 × NK603, therefore, expresses:

- The Cry3Bb1 protein from MON 863, which confers protection against certain coleopteran insect pests (*Diabrotica* spp.)
- The NPTII protein from MON 863. The *nptII* gene is inserted into maize cells along with the *MON 863 cry3Bb1* gene to have an effective method for selecting cells that contain the insecticidal gene and can be used in bacterial selection during construction of the plasmid.
- The Cry1Ab protein from MON 810, which confers protection against certain lepidopteran insect pests, including the European Corn Borer (*Ostrinia nubilalis*) and pink borers (*Sesamia* spp).
- The CP4 EPSPS proteins from NK603, which imparts tolerance to glyphosate (N-phosphonomethyl-glycine), the active ingredient of the non-selective broad-spectrum herbicide Roundup.

2. Information on the sequences actually inserted or deleted

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

MON 863, MON 810 and NK603 each contain a single DNA insert containing one copy of the introduced DNA fragment, and this at different loci in the maize genome.

In the progeny of MON 863, MON 810 and NK603, each fragment is inherited as a single gene in a Mendelian fashion. Therefore, each of the inserts are inherited in MON 863 × MON 810 × NK603.

The presence of these inserts in MON 863 × MON 810 × NK603 was confirmed through Southern blot analysis, and the size of the observed bands was as expected, indicating that the structure of the inserts has been conserved in MON 863 × MON 810 × NK603.

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

Not applicable

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

Traditionally bred (F1) MON 863 × MON 810 × NK603 contains each of the parental inserts on separate chromosomes in the nuclear genome, as they were present in parental MON 863, MON 810 and NK603. The presence of the inserts from MON 863, MON 810 and NK603 in MON 863 × MON 810 × NK603 was confirmed by Southern blot analysis.

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

As MON 863 × MON 810 × NK603 is the product of a traditional cross of MON 863, MON 810 and NK603 and no additional genetic modification methods have been applied, and as the inserts inherited in MON 863 × MON 810 × NK603 have negligible potential to interact with one another, it is highly likely that MON 863 × MON 810 × NK603 contains each of the inserts as they were present in MON 863, MON 810 and NK603, respectively.

Therefore, the respective molecular characteristics known for MON 863, MON 810 and NK603 have likely been conserved in MON 863 × MON 810 × NK603, including the structural organization and the integrity of the inserts, as well as the characteristics of the sites of insertion and the flanking sequences of the inserts.

3. Information on the expression of the insert

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

Expression levels of the introduced proteins were measured in tissue samples collected from MON 863 × MON 810 × NK603, grown in Argentinean field trials conducted at multiple locations in the season 2002-2003. The levels of Cry3Bb1, NPTII, Cry1Ab and CP4 EPSPS proteins in grain and forage are presented below, because these tissues are most relevant for the evaluation of the food and feed safety of MON 863 × MON 810 × NK603.

The mean Cry3Bb1 protein level across all sites was 37 µg/g dry weight (dw) in grain and 45 µg/g dw in forage samples from MON 863 × MON 810 × NK603. The mean Cry1Ab protein level in grain samples was 0.65 µg/g dw and 12 µg/g dw in the forage. The mean CP4 EPSPS protein level was 11 µg/g dw in the grain and 95 µg/g dw in forage samples. The values given for CP4 EPSPS represent the sum of both CP4 EPSPS and CP4 EPSPS L214P, as the ELISA analytical method recognizes both these proteins. The expression levels of NPTII protein in MON 863 × MON 810 × NK603 grain were below the ELISA assay limit of detection of 0.21 µg/g fw for this protein in grain. Therefore the NPTII protein levels could not be converted to a mean value on dry weight basis.

The observed expression levels in MON 863 × MON 810 × NK603 were consistent with the levels observed in the corresponding single-trait controls included in this study and with levels that have been previously reported for the single-trait products from trials conducted during other production years.

b) Parts of the plant where the insert is expressed

The expression of the Cry3Bb1, Cry1Ab and CP4 EPSPS proteins occurs throughout the whole plant. The NPTII protein was not detected in grain samples.

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

a) Reproduction

Comparative assessments of the phenotypic and agronomic characteristics of MON 863, MON 810 and NK603 and traditional maize have previously demonstrated that, except for the respective protection against target coleopteran or lepidopteran insect pests or the tolerance to Roundup herbicide (glyphosate), there are no biologically significant differences in the reproductive capability, dissemination or survivability of MON 863, MON 810 or NK603 when compared to traditional maize.

Observations from field trials with MON 863 × MON 810 × NK603 and first commercial plantings in the U.S.A. in 2004 confirm that no biologically relevant differences exist in varieties that contain the introduced agronomic traits in combination. Compared to traditional maize, MON 863 × MON 810 × NK603 has not been significantly changed with respect to its dispersal or survival characteristics as assessed by phenotypic characteristics including dormancy, growth habit, ear drop, and morphological and developmental characteristics including seedling vigour, early and final stand count, date of 50% silk and pollen shed, ear height, stalk or root lodging, stay green and yield. MON 863 × MON 810 × NK603 grain is also unchanged compared to traditional maize in terms of invasiveness of natural environments and persistence in the environment as assessed by the phenotypic characteristics described above. Importantly, there is no information to indicate that there is a potential for MON 863 × MON 810 × NK603 to establish, persist and disperse to a greater extent than traditional maize. In cases where incidental release occurs and a MON 863 × MON 810 × NK603 plant would establish, these plants will be easily controlled by currently available selective herbicides (except glyphosate) and by mechanical means. As such, MON 863 × MON 810 × NK603 has no meaningful potential to disperse, persist without human intervention, or invade non-agricultural areas as a result of importation for direct use in food, feed or processing.

It conclusion, MON 863 × MON 810 × NK603 does not differ from traditional maize with regard to reproduction, dissemination, survivability or other agronomic and phenotypic traits.

b) Dissemination

The introduced insect-protection traits and the herbicide tolerance trait have no influence on maize reproductive morphology and hence no changes in seed dissemination are to be expected (*see* Section D.4.a).

c) Survivability

Maize is known to be a weak competitor in the wild, which cannot survive outside cultivation without the aid of human intervention. Field observations have demonstrated that MON 863 × MON 810 × NK603 has not been altered in its survivability when compared to single-trait parental maize or compared to traditional maize (*see* Section D.4.a).

d) Other differences

Comparative assessments in the field did not reveal any biologically significant differences between MON 863 × MON 810 × NK603 and traditional maize, except for the introduced traits that are of agronomic interest.

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

MON 863 × MON 810 × NK603 hybrid seed (F1) is produced by traditional breeding by crossing MON 863, MON 810 and NK603 lines. Thereby, each parental line passes on its inserted DNA sequence to the resulting MON 863 × MON 810 × NK603 F1 hybrid seed, which is planted by the grower.

The single-trait modified maize lines MON 863, MON 810 and NK603 each contain one insert with a single copy of the respective DNA insert, which is stably integrated into the nuclear maize genome. Each trait is inherited as a single dominant gene in a Mendelian fashion. This has been confirmed by Southern blot analyses and by studies of the inheritance pattern of these traits in maize.

The harvested (F2) grain of MON 863 × MON 810 × NK603 is marketed by the grower for food, feed or industrial use and is not used for further breeding. Therefore, there is negligible opportunity for its genetic or phenotypic stability to be compromised.

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

a) Plant to bacteria gene transfer

No elements known to be involved in DNA mobility are contained in either of the inserted DNA fragments. Therefore, in comparison to traditional maize, no changes are to be expected in the ability of the GM plant to exchange genetic material with bacteria.

b) Plant to plant gene transfer

Since reproductive morphology in the single-trait parental lines and in MON 863 × MON 810 × NK603 are unchanged compared to traditional maize, pollen production and pollen viability are not expected to be affected by the genetic modification. Therefore, the outcrossing frequency to other maize varieties or to wild relatives (which are not present in the E.U.) is unlikely to be different for MON 863 × MON 810 × NK603 when compared to traditional maize. As cultivation of MON 863 × MON 810 × NK603 varieties is not in scope of the current application, the probability of plant to plant gene transfer is considered to be negligible.

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

Compositional analyses were performed on forage and grain samples from MON 863 × MON 810 × NK603, grown at four sites in Argentina during the 2002-2003 season. The study also included compositional analyses of forage and grain collected from a non-transgenic control hybrid and thirteen non-transgenic commercial reference hybrids, which were grown in replicated plots at the same field sites as MON 863 × MON 810 × NK603. Statistical analyses showed that 99.7% (309) of the 310 comparisons made between MON 863 × MON 810 × NK603 and the traditional control were either not statistically significantly different or they were with 95% confidence within the calculated 99% tolerance interval for the population of commercial reference hybrids. The one remaining statistically significant difference (folic acid) was noted at only one site and did not show a consistent trend across sites. Moreover, the values for folic acid were in overall agreement with the range reported for this component in the literature on the composition of maize. Therefore, this single statistical difference was not considered to be biologically meaningful.

Based on these data, it is concluded that MON 863 × MON 810 × NK603 is compositionally equivalent to traditional maize.

7.2 Production of material for comparative assessment

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

The compositional analysis study of the forage and grain from MON 863 × MON 810 × NK603 was conducted at four replicated field sites in Argentina in 2002 – 2003. The sites were Pergamino (Buenos Aires), Tacuari-Salto (Buenos Aires), Manfredi (Cordoba) and Marcos Juarez (Cordoba). At each field site, the seed starting materials were planted in a randomized complete block design with three replicates per block.

b) the baseline used for consideration of natural variations

Compositional analyses were made for forage and grain samples from MON 863 × MON 810 × NK603. The study also included analyses of forage and grain collected from a non-transgenic control hybrid and 13 different non-transgenic commercial maize hybrids, grown in replicated plots at the same field sites as MON 863 × MON 810 × NK603. Finally, also comparisons with baseline data from numerous other field trials and from the peer-reviewed literature were made. The literature on the composition of maize reveals a wide compositional variability across maize hybrids.

7.3 Selection of material and compounds for analysis

Compositional analyses were conducted on grain and forage from MON 863 × MON 810 × NK603 hybrids and non-transgenic counterpart.

Grain samples were analyzed for proximates (protein, fat, ash and moisture), acid detergent fiber (ADF), neutral detergent fibre (NDF), total dietary fibre (TDF), amino acids, fatty acids, minerals (calcium, copper, iron, magnesium, mangabese, phosphorus, potassium, sodium and zinc), vitamins (B_1, B_2, B_6, E, niacin, and folic acid), anti-nutrients (phytic acid and raffinose) and secondary metabolites (furfural, ferulic acid and p-coumaric acid). Forage samples were analyzed for proximates (protein, fat, ash, and moisture), ADF, NDF and minerals (calcium, phosphorus). Carbohydrate values in forage and grain were estimated by calculation.

The numerous compounds that were selected for analysis in the compositional study were chosen on the basis of internationally accepted guidance provided by OECD.

Based on the long history of safe use of the host plant, maize, as well as the positive results of the compositional analyses conducted for MON 863 × MON 810 × NK603 and its parental single-trait lines, there is no indication of a need to further analyse other selected compounds in this maize.

7.4 Agronomic traits

This application does not include the cultivation of MON 863 × MON 810 × NK603 varieties in the E.U. However, the observations from environmental releases provide evidence confirming the absence of significant unintended or unanticipated effects of the genetic modifications present in this maize. The agronomic and phenotypic equivalence of MON 863 × MON 810 × NK603 to traditional maize was confirmed during the development and evaluation of MON 863 × MON 810 × NK603 in the field. Observations from field trials and commercial cultivation in North America show that, except for the introduced traits, MON 863 × MON 810 × NK603 is agronomically, phenotypically and morphologically equivalent to traditional maize (*see* Section D.4.a).

From an agronomic, phenotypic and morphological point of view, MON 863 × MON 810 × NK603 is equivalent to traditional maize, except for the three intentionally introduced traits of agronomic interest: protection from certain coleopteran and lepidopteran insect pests and tolerance to glyphosate herbicide.

7.5 Product specification

MON 863 × MON 810 × NK603 will be imported into the European Union in mixed shipments of maize products, produced in other world areas, for use by operators that have traditionally been involved in the commerce, processing and use of maize and maize derived products in the European Union.

MON 863 × MON 810 × NK603 comprises all traditionally bred maize, produced by the combination of MON 863, MON 810 and NK603.

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 863 × MON 810 × NK603 is substantially equivalent and as safe and as nutritious as traditional maize, the use of MON 863 × MON 810 × NK603 for the production of foods and feeds is no different from that of traditional maize. Consequently, any effects of the production and processing of MON 863 × MON 810 × NK603 are not expected to be any different from the production and processing of the equivalent foods and feeds, originating from traditional 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 food and feed use as a result of the addition of MON 863 × MON 810 × NK603 to the traditional maize supply. MON 863 × MON 810 × NK603 and derived products are expected to replace a portion of current maize products such that their 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 863 × MON 810 × NK603 is produced by traditional breeding of MON 863, MON 810 and NK603 lines. Each of the introduced traits from the parental lines are inherited in MON 863 × MON 810 × NK603, which results in the combined expression of the Cry3Bb1, NPTII, Cry1Ab and CP4 EPSPS proteins in the same plant. These introduced proteins are present at low levels and were demonstrated to be safe for animal and human health.

The conclusion that the Cry3Bb1 and NPTII proteins are safe to humans was based upon the following findings:

- History of safety for both proteins.
- Cry3Bb1 is selective for certain coleopteran insects, with no activity against other types of living organisms such as mammals, fish, birds or invertebrates.
- NPTII, which has no insecticidal activity, is ubiquitous in the environment and is found in microbes present in food and within the human digestive system.
- Rapid digestion of both proteins in *in vitro* simulated gastric fluids.
- No significant amino acid sequence similarity to known protein toxins (other than *B.t.* proteins for Cry3Bb1) and no immunologically relevant sequence similarity with known allergens.

- Lack of acute toxicity for both proteins as determined by a mouse acute gavage study at dose levels orders of magnitude higher than those encountered in human or animal diets.

Similarly, the conclusion that the Cry1Ab protein is safe to humans was based upon the following findings:

- There is a long history of safe use for the Cry1Ab protein,
- Cry1Ab was demonstrated to be highly selective for certain Lepidopteran insects, with no activity against non-organisms such as mammals, fish, birds or invertebrates.
- Cry1Ab does not show significant amino acid sequence similarity to known protein toxins, other than *B.t.* proteins, and no immunologically relevant sequence similarity with known allergens.
- The Cry1Ab protein is rapidly degraded, and its insecticidal activity lost, under conditions which simulate mammalian digestion.
- There were no indications of acute toxicity in mice administered Cry1Ab protein by oral gavage at dose-levels that were orders of magnitude higher than the levels man or animals would encounter in their diet.

Finally, for the CP4 EPSPS proteins, the conclusion of safety to humans was based upon the following weight of evidence:

- History of safe use of the CP4 EPSPS protein for human and animal consumption.
- Extensive characterization of the CP4 EPSPS protein and its comparability to EPSPS enzymes commonly found in a wide variety of food sources, which also have histories of safe use.
- Rapid digestion of CP4 EPSPS proteins in *in vitro* simulated gastric fluids.
- Absence of significant homology with known protein toxins and allergens.
- Lack of acute toxicity of CP4 EPSPS proteins as determined by a mouse gavage study. No substance-related toxicity was observed in the test animals when administered purified CP4 EPSPS at dose levels orders of magnitude higher than the levels encountered in the human or animal diet.

7.8.2 Testing of new constituents other than proteins

Since maize is known as a common source of food and feed with a centuries-long history of safe use and consumption around the world, and as MON 863 × MON 810 × NK603 was shown to be compositionally equivalent to traditional maize, no testing of any constituent other than the introduced proteins is indicated.

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 conclusions of the safety assessments for the individual proteins are not changed when combined in MON 863 × MON 810 × NK603, since a) the proteins are unlikely to interact or have synergistic effects; b) the CP4 EPSPS enzymes and Cry proteins have very different and well-documented modes of action; c) the CP4 EPSPS and Cry proteins are localized to different subcellular compartments; d) the introduced proteins are produced in very low quantities in MON 863 × MON 810 × NK603; and e) were shown to be safe in their individual safety assessments. Furthermore, a confirmatory animal feeding experiment was conducted using MON 863 × MON 810 × NK603 fed to broiler chickens, which are known to be a sensitive animal model. As expected, this study did not indicate any nutritional effects or safety concerns for MON 863 × MON 810 × NK603 (*see* Section D.7.10.2). Finally, the Cry3Bb1, NPTII, Cry1Ab and CP4 EPSPS proteins have a history of safe use, *i.e.* through consumption of MON 863, MON 810 and NK603, individually and in grain mixtures, but also from their combined use in MON 863 × NK603, MON 863 × MON 810, NK603 × MON 810 and MON 863 × MON 810 × NK603 cultivated outside the E.U. These products and derivatives thereof have been handled and consumed without any adverse health effects.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein

Absence of any allergenic potential associated with the introduced Cry3Bb1, NPTII, Cry1Ab and CP4 EPSPS proteins expressed in MON 863 × MON 810 × NK603 has previously been demonstrated for the single-trait parental lines. These proteins are present t very low levels in grain and were assessed for their potential allergenicity by a variety of tests, including a) whether the genes came from allergenic or non-allergenic sources, b) sequence similarity to known allergens, and c) pepsin stability of the protein in an *in vitro* digestion assay. In all cases, the proteins did not exhibit properties characteristic of allergens.

7.9.2 Assessment of allergenicity of the whole GM plant or crop

As the introduced proteins do not have allergenic potential, it was concluded that the use of MON 863 × MON 810 × NK603 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 traditional maize.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

MON 863 × MON 810 × NK603 inherits the inserts present in MON 863, MON 810 and NK603. The introduced insect-resistance traits and glyphosate-tolerance are of agronomic interest and do not change the nutritional aspects of this maize. The presence of these traits is not expected to alter patterns or volumes of maize consumption. Compared to other maize, no differences in intake or extent of use are therefore expected for MON 863 × MON 810 × NK603.

MON 863 × MON 810 × NK603 and derived products are expected to replace a portion of the currently available maize products, such that their intake and use will represent some fraction of the total intake or use of maize products.

7.10.2 Nutritional assessment of GM feed

A confirmatory feeding study in broiler chickens was conducted to compare the nutritional value of MON 863 × MON 810 × NK603 and non-transgenic control as well as additional commercial maize hybrids, and to provide additional confirmation of the safety of this maize. The results of this study show that there were no biologically relevant differences in the parameters tested between broilers fed the MON 863 × MON 810 × NK603 diet and the non-transgenic control diet.

When individual treatment comparisons were made, broilers in general performed and had similar carcass yields and meat composition when fed diets containing MON 863 × MON 810 × NK603, the non-transgenic hybrid, and commercially available reference hybrids. The MON 863 × MON 810 × NK603 diet was as wholesome as its corresponding non-transgenic control diet and commercially available reference diets regarding its ability to support the rapid growth of broiler chickens.

This conclusion was consistent with the evaluation of the composition of MON 863 × MON 810 × NK603, which showed that there were no biologically relevant differences in nutritional and compositional properties relative to control and reference maize. These data confirm the conclusion that MON 863 × MON 810 × NK603 is as safe and nutritious as traditional maize.

7.11 Post-market monitoring of GM food/feed

The assessment of the human and animal safety of MON 863 × MON 810 × NK603 was conducted on the basis of its substantial equivalence to traditional maize (except for the introduced traits) and by extensive characterisation of the introduced traits, resulting in the expression of the Cry3Bb1, NPTII, Cry1Ab and CP4 EPSPS proteins.

There are no intrinsic hazards related to MON 863 × MON 810 × NK603 as no signs of adverse or unanticipated effects have been observed in a number of safety studies, including animal feeding studies using doses of administration that are orders of magnitude above expected consumption levels. The pre-market risk characterisation for food and feed use of MON 863 × MON 810 × NK603 demonstrates that the risks of consumption of this maize or its derived products are consistently negligible and no different from the risks associated with the consumption of traditional maize and maize-derived products.

As a consequence, specific risk management measures or 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)

The Cry3Bb1 protein present in MON 863 and MON 863 × MON 810 × NK603 confers protection against certain economically damaging coleopteran insect pests, in particular the larvae of *Diabrotica* spp. (corn rootworm). The Cry1Ab protein present in MON 810 and MON 863 × MON 810 × NK603 confers protection against certain lepidopteran insect pests, including the European Corn Borer (*Ostrinia nubilalis*) and pink borers (*Sesamia* spp.).These species may be considered the target organisms which interact with MON 863 × MON 810 × NK603. The Cry3Bb1 and Cry1Ab proteins are known to provide protection in the field to their specific target organisms and not to have synergistic effects.

The Cry3Bb1 and Cry1Ab proteins must be ingested by the relevant, susceptible insect to produce their insecticidal effect. Following ingestion, Cry1Ab and Cry3Bb1 proteins are solubilized and are relatively proteolytically stable, but unlike Cry1A proteins, Cry3 proteins do not have a large C-terminal domain that is processed to the active core protein. The active form of the respective Cry protein must traverse the insect midgut peritrophic membrane and selectively bind to specific receptors to exert its insecticidal activity. Cation-selective pores are formed by the Cry protein, disrupting cell homeostasis and ultimately leading to the death of the specific pest organism.

Any significant interactions of MON 863 × MON 810 × NK603 with its target pest organisms are, however, limited to those countries where the cultivation of this maize has been authorized. The cultivation of MON 863 × MON 810 × NK603 varieties in the E.U. is not within the scope of this application. The likelihood that the import and use of MON 863 × MON 810 × NK603 for food, feed or processing will result in plants of this maize being present in the environment is negligible.

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

9.1 Persistence and invasiveness

Like for traditional maize, the likelihood of MON 863 × MON 810 × NK603 spreading in the environment is negligible, as maize is neither persistent nor invasive and these parameters are unaltered in MON 863 × MON 810 × NK603 when compared to traditional maize. Hence the risk of establishment and spreading of MON 863 × MON 810 × NK603 in the environment is negligible.

9.2 Selective advantage or disadvantage

Compared with traditional maize, the presence of the introduced traits in a MON 863 × MON 810 × NK603 volunteer would only confer a meaningful advantage where target coleopteran or lepidopteran pest species would be present in high numbers or where plants would be treated with glyphosate herbicide, and if no other more important factors limiting its establishment in the environment would be present. The risk of the protective trait against target coleopteran and lepidopteran pests or the introduced glyphosate-tolerance in MON 863 × MON 810 × NK603 to be the cause of any competitive advantage or disadvantage impacting the environment is negligible, as maize is unlikely to establish outside cultivation under European conditions (*see* Section D.9.1).

9.3 Potential for gene transfer

There is no potential for gene transfer from MON 863 × MON 810 × NK603 to wild plant species in the E.U. and negligible likelihood for gene transfer to other maize crops, as this application is not for consent to cultivate MON 863 × MON 810 × NK603 varieties in the E.U. The environmental risk of potential gene transfer is negligible.

9.4 Interactions between the GM plant and target organisms

Since the likelihood is negligible that the import, processing and food and feed use of MON 863 × MON 810 × NK603 will result in plants of this maize establishing in the environment, any significant interactions with target organisms as described in Section D.8 are unlikely. The insecticidal traits in MON 863 × MON 810 × NK603 are identical to those in its MON 863 and MON 810 parental lines, respectively. Extensive testing of MON 863 and MON 810 in the laboratory and in the field did not indicate any direct or indirect adverse environmental effects on non-target organisms. Therefore, even if incidentally spilt kernels were to germinate and result in the short survival of some MON 863 × MON 810 × NK603 plants in the environment, or in the unlikely case of misuse of imported grain for planting in the E.U., the plants of this maize would have no potential to cause harm on non-target organisms through their interaction with target organisms.

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 863 × MON 810 × NK603 varieties in the E.U., the likelihood for direct or indirect interactions of this maize with non-target organisms is considered to be negligible.

In addition, the introduced Cry3Bb1, NPTII, Cry1Ab and CP4 EPSPS proteins present a negligible hazard to non-target organisms, even if incidental spillage of MON 863 × MON 810 × NK603 grains during import, storage, transport or use would lead to the short survival of MON 863 × MON 810 × NK603 plants in the environment. Numerous studies have established that Cry3Bb1 exhibits specific toxicity towards Coleoptera and Cry1Ab to specific Lepidoptera, but not to other insect orders or other non-target organisms. Based on the ubiquitous occurrence of natural EPSPSs in the environment and the history of safe use of CP4 EPSPS-expressing crops such as Roundup Ready soybean, it is highly unlikely that the introduced CP4 EPSPS enzymes in MON 863 × MON 810 × NK603 would possess biological activity towards any non-target organisms.

As a consequence, there is negligible risk for harmful effects of MON 863 × MON 810 × NK603 on non-target organisms, either through direct or indirect interactions with this maize or through contact with the newly expressed proteins.

Furthermore, no evidence of any adverse effects was found since the commercial introduction of MON 863, MON 810, NK603 and MON 863 × MON 810 × NK603 in North America. No evidence has been brought forward by the many farmers and operators handling these products of any harmful or undesirable effects associated with this maize or with the introduced proteins.

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 traditional maize. MON 863 × MON 810 × NK603 contains the Cry3Bb1, NPTII, Cry1Ab and CP4 EPSPS proteins, which have negligible potential to cause any toxic or allergenic effects in man. Therefore, the risk of changes in the occupational health aspects of this maize is negligible.

9.7 Effects on animal health

The likelihood of potential adverse effects in animals fed on MON 863 × MON 810 × NK603 and in humans, consuming those animals, is negligible (*see* Sections D.7.8, D.7.9, D.7.10). Therefore, the risk of MON 863 × MON 810 × NK603 for the feed/food chain is also negligible.

9.8 Effects on biogeochemical processes

In the event of an incidental release of MON 863 × MON 810 × NK603 in the environment, the risk for direct or indirect, immediate or delayed adverse effects on biogeochemical processes can be considered as negligible. There is no evidence that MON 863 × MON 810 × NK603 plants would be any different from traditional maize regarding their direct influence on biogeochemical processes or nutrient levels in the soil, as MON 863 × MON 810 × NK603 is compositionally equivalent and has equivalent growth and development, morphology, yield, plant health and survival characteristics to non-transgenic maize (*see* Sections D.4, D.7.1 and D.7.4). Furthermore, any indirect interactions of the GMO and target or non-target 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. The Cry3Bbl and Cry1Ab proteins are subjected to rapid degradation in soil. The NPTII protein is widely present in the environment and the CP4 EPSPS proteins belong to the safe class of EPSP synthases that are ubiquitous in the environment.

9.9 Impacts of the specific cultivation, management and harvesting techniques

Not applicable. This application is for consent to import MON 863 × MON 810 × NK603 in the E.U. and for the use of this maize as any other maize, excluding the cultivation of varieties in the E.U.

10. Potential interactions with the abiotic environment

No adverse impact of MON 863 × MON 810 × NK603 on the abiotic environment is expected to result from the import, processing or use of this product for food and feed in the E.U. Although Cry3Bb1, NPTII, Cry1Ab and CP4 EPSPS are introduced proteins in maize, they already have a safe history of use and have no known negative interactions with the abiotic environment. The CP4 EPSPS proteins in MON 863 × MON 810 × NK603 are innocuous and belong to a large class of EPSPS proteins that are ubiquitous in nature. The Cry3Bbl and Cry1Ab proteins are subjected to rapid degradation in soil and are therefore not expected to negatively affect soil or water. The NPTII protein is already present in the environment.

11. Environmental monitoring plan (not if application concerns only food and feed produced from GM plants, or containing ingredients produced from GM plants)

1. *Case-specific monitoring*

An environmental risk assessment (e.r.a.) of MON 863 × MON 810 × NK603 was undertaken in the context of the scope of the application, that is, for import, processing and food and feed use of MON 863 × MON 810 × NK603, but not including the cultivation of MON 863 × MON 810 × NK603 varieties in the E.U. Analysis of the characteristics of MON 863 × MON 810 × NK603 has shown that the risk for potential adverse effects on human health and the receiving environment, resulting from the import and use of MON 863 × MON 810 × NK603 in the E.U. is consistently negligible. Therefore, the overall environmental risk posed by this genetically modified higher plant is negligible, and no specific strategies for risk management and no case-specific post-marketing monitoring actions are considered required.

2. *General surveillance*

Any potential adverse effects of MON 863 × MON 810 × NK603 on human health and the environment, which were not anticipated in the e.r.a., can be addressed under general surveillance in accordance with Directive 2001/18/EC. General surveillance is largely based on routine observation and implies the collection, scientific evaluation and reporting of reliable scientific evidence, in order to be able to identify whether unanticipated, direct or indirect, immediate or delayed adverse effects have been caused by the placing on the market of a genetically modified (GM) crop in its receiving environment.

In order to allow detection of the broadest possible scope of unanticipated adverse effects, general surveillance is performed by either selected, existing networks, or by specific company stewardship programmes, or by a combination of both. The notifier will ensure that appropriate technical information on MON 863 × MON 810 × NK603 and relevant legislation will be available for the relevant networks, in addition to further relevant information from a number of sources, including industry and government websites, official registers and government publications.

Where there is scientifically valid evidence of a potential adverse effect (whether direct or indirect), linked to the genetic modification, then further evaluation of the consequence of that effect should be science-based and compared with available baseline information. Relevant baseline information will reflect prevalent use practices and the associated impact of these practices on the environment. Where scientific evaluation of the observation confirms the possibility of an unanticipated adverse effect, this would be investigated further to establish a correlation, if present, between the use of MON 863 × MON 810 × NK603 and the observed effect. The evaluation should consider the consequence of the observed effect and remedial action, if necessary, should be proportionate to the significance of the observed effect.

Monsanto will submit a General Surveillance 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

As MON 863 × MON 810 × NK603 results from a traditional cross of the single-trait MON 863, MON 810 and NK603 lines, it contains the inserts from MON 863, MON 810 and NK603 in combination. Therefore, MON 863 × MON 810 × NK603 is detectable using either the insert-specific PCR method for detecting the introduced DNA present in MON 863 or one of the equivalent methods for MON 810 or NK603. However, as for all plants in which inserts are combined by traditional breeding, the presence of MON 863 × MON 810 × NK603 maize may only be confirmed where single grains are subjected to the detection methods for MON 863, MON 810 and NK603 and test positive for all three.

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

Not applicable

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

Monsanto commercialised MON 863 × MON 810 × NK603 in the U.S.A. in 2004. Prior to commercialisation, this maize as well as its parental single-trait maize lines, MON 863, MON 810 and NK603, were tested extensively at multiple locations in the field.

b) Authority overseeing the release

U.S.A.: United States Department of Agriculture (USDA) and Environmental Protection Agency (EPA)

c) Release site

	Please see question **E.2.(a)**
d)	**Aim of the release** Since 2004, MON 863 × MON 810 × NK603 is grown commercially in North America.
e)	**Duration of the release** Please see question **E.2.(a)**
f)	**Aim of post-releases monitoring** Extensive pre-market risk assessment did not provide evidence of adverse effects potentially associated with the cultivation, handling or use of MON 863 × MON 810 × NK603, indicating that post-release monitoring would not be necessary. In addition, the commercialisation of MON 863 × MON 810 × NK603 is accompanied by stewardship programmes to ensure correct handling of this maize by downstream stakeholders (implementation of good agricultural practice for cultivation; ensure a channel of communication in the unlikely event that unanticipated adverse effects might occur). No unanticipated effects have been observed during field testing or since commercialization of MON 863 × MON 810 × NK603.
g)	**Duration of post-releases monitoring** Please see question **E.2.(f)**
h)	**Conclusions of post-release monitoring** Please see question **E.2.(f)**
i)	**Results of the release in respect to any risk to human health and the environment** Field-testing and post-marketing experience provided no significant evidence that grain or derived products from MON 863 × MON 810 × NK603 are likely to cause adverse effects to human health, animal health or 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 EFSA website http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html provides information related to the applications submitted under Regulation (EC) No 1829/2003 on genetically modified food and feed.
b)	**Assessment Report of the Competent Authority (Directive 2001/18/EC)** A notification for MON 863 × MON 810 × NK603 according to Directive 2001/18/EC has not been submitted by Monsanto.
c)	**EFSA opinion** An EFSA opinion, specifically for MON 863 × MON 810 × NK603, was not available at the time of submission of this application.

d)	**Commission Register (Commission Decision 2004/204/EC)** http://europa.eu.int/comm/food/food/biotechnology/authorisation/commun_register_en.htm	
e)	**Molecular Register of the Community Reference Laboratory/Joint Research Centre** Information on detection protocols will likely be posted at http://gmo-crl.jrc.it/	
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.biodiv.org/	
g)	**Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC)** A notification and SNIF according to Directives 2001/18/EC and 2002/812/EC, respectively, have not been submitted for MON 863 × MON 810 × NK603. The EFSA website http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html does provide a link to this summary of the application for MON 863 × MON 810 × NK603 under Regulation (EC) No 1829/2003.	