Application for authorization of MON 863 × MON 810 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

Germany

b) Notification number

EFSA-GMO-DE-2004-03

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

The Monsanto development code for this genetically modified maize is: MON 863 × MON 810. In countries where MON 863 × MON 810 is being cultivated, packages of hybrid seed of this maize are marketed under the name of the hybrid variety, in association with the trademark YieldGard® Plus, indicating clearly to growers that the hybrid is protected from specific coleopteran and lepidopteran insect pests.

d) Date of acknowledgement of notification

July 02, 2004 (Date of reception EFSA)

2. Applicant

a) Name of applicant

Monsanto Company, represented by Monsanto Europe S.A.

b) Address of applicant

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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 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 (x) GM plants for feed use (x) Feed containing or consisting of GM plants (x) Feed produced from GM plants or containing ingredients produced from GM plants ( ) Import and processing (Part C of Directive 2001/18/EC) ( ) Seeds and plant propagating material fro cultivation in Europe (Part C of Directive 2001/18/EC) A separate application requesting authorization to import MON 863 x MON 810 grains into the E.U. is pending under Directive 2001/18/EC (C/DE/02/9).

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 *See* following sections	

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 (x)	**No ( )**
If *yes*, specify An application pursuant to Directive 2001/18/EC (C/DE/02/9) for import of MON 863 × MON 810 in the E.U. and use thereof as any other maize, excluding the cultivation of varieties, was submitted in July 2002, has received a positive Rapporteur opinion in April 2003 and has been reviewed by the other E.U. Member States and by the European Food Safety Authority (EFSA).	

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

Yes (x)	No ( )

If *yes*, specify

In more than a third country outside the E.U., such as the U.S.A. and Canada, MON 863, MON 810 and MON 863 × MON 810 is authorised for all uses, corresponding to the full range of uses of traditional maize. However, the scope of the approvals already granted for these genetically modified organisms and the status of 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 863 × MON 810 consists of hybrid maize varieties, produced using traditional methods of maize breeding by crossing parental inbred lines of MON 863 and MON 810. Although genetic modification was used in the development of MON 863 and MON 810, no additional genetic modifications were involved for the production of MON 863 × MON 810.

Like parental MON 863, MON 863 × MON 810 expresses i) a Cry3Bb1 protein variant from *Bacillus thuringiensis* subsp. *kumamotoensis*, which confers protection to certain coleopteran pests (*Diabrotica* spp.) and ii) the NPTII protein (neomycin phosphotransferase II) which provides resistance towards kanamycin for maize plant cell selection purposes. Like its second parental MON 810, MON 863 × MON 810 also expresses the Cry1Ab protein, derived from *Bacillus thuringiensis* subsp. *kurstaki*, which confers protection from predation by certain lepidopteran insect pests, including the European Corn Borer (*Ostrinia nubilalis*) and pink borers (*Sesamia* spp).

The use of MON 863 × MON 810 enables the farmer to effectively control the targeted coleopteran and lepidopteran insect pests in maize, ensuring maximum realization of yield potential, while removing the environmental burden of the production, packaging and transport of insecticides, previously used to control *Diabrotica* spp., *Ostrinia nubilalis* and *Sesamia* spp.

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 all uses of MON 863 × MON 810 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 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

MON 863 × MON 810 is substantially equivalent to other maize varieties except for its introduced (*i.e.* inherited) traits, namely protection from target coleopteran and lepidopteran pests, 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 and derived products will be stored, packaged, transported, handled and used in the same manner as the commercial maize products. No specific conditions are warranted or required for the food and feed use of MON 863 × MON 810.

e) Any proposed packaging requirements

MON 863 × MON 810 is substantially equivalent to its parental maize lines MON 863 and MON 810, and to traditional maize varieties (except for its protection from targeted coleopteran and lepidopteran insect pests). Therefore, MON 863 × MON 810 and derived products will be used in the same manner as other maize and no specific packaging is foreseen. (For the labelling, *See* question 8.(f)).

f) Any proposed labelling requirements in addition to those required by Community law (Annex IV of Directive 2001/18/EC; Regulation 1829/2003 art. 13 and 25)

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 grain and derived products.

Operators shall be required to label products containing or consisting of MON 863 × MON 810 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 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 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 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 863 × MON 810. 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 863 × MON 810 is uniquely identified using this combination of the unique identifiers for MON 863 (MON-ØØ863-5), and MON 810 (MON-ØØ81Ø-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 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

Misuse of MON 863 × MON 810 is unlikely, as the proposed food and feed uses for this maize include all the current food and feed uses of traditional maize. MON 863 × MON 810 is substantially equivalent to other maize hybrids except for the introduced traits, which are traits of agronomic interest. This maize has been shown to be as safe and as nutritious as traditional maize. Therefore, any measures for waste disposal and treatment of MON 863 × MON 810 products are the same as those for traditional maize. No specific conditions are warranted or required for the placing on the market of MON 863 × MON 810 for food and feed.

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

1. Complete name

a)	**Family name** Gramineae
b)	**Genus** *Zea*
c)	**Species** *mays* (2n=20)
d)	**Subspecies** N/A
e)	**Cultivar/breeding line** MON 863 × MON 810
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 the environmental release of MON 863 × MON 810.

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 the environmental release of MON 863 × MON 810 in the European Union.

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 European Union 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

No novel method of genetic modification is utilized in the production of MON 863 × MON 810. Instead, traditional maize breeding methods are used to cross inbreds of MON 863 and MON 810. While MON 863 × MON 810 results from traditional breeding, MON 863 and MON 810 were modified by incorporation of a DNA fragment derived, respectively, from plasmid vectors PV-ZMIR13 and PV-ZMBK07 into the maize genome using a particle acceleration method.

2. Nature and source of the vector used

MON 863 x MON 810 has been obtained by traditional breeding of MON 810 and MON 863 and no vector has been used to produce this maize hybrid.

The vector used to amplify the DNA fragment which was introduced in MON 863 is composed of a pUC plasmid replication origin associated with a selectable marker, *nptII*. The functions carried by the vector are required to allow its maintenance and amplification in *E. coli* bacterial cells. Like the original pUC vectors, this vector does not contain transfer origins, *i.e.*, sequences allowing transfer from bacteria to bacteria.

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*.

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

MON 863 × MON 810 results from a single traditional cross of the inbred parental lines MON 863 and MON 810, which are made homozygous for their respective inserted sequences.

By crossing MON 863 and MON 810, MON 863 × MON 810 inherits the inserted DNA fragments from both its parental maize lines as they were present in the parental line.

The individual components and the function of these inherited DNA sequences are given in Tables 1 and 2.

Table 1. Components of the inserted DNA fragment inherited from MON 863

Sequence	Size (Kb)	Source	Function
MON 863 cry3Bb1 gene cassette			
4AS1	0.22	Cauliflower mosaic virus	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	0.06	Wheat (*Triticum aestivum*)	Translation enhancement
ract1 intron	0.49	Rice (*Oryza sativa*)	Transcription enhancement
MON 863 cry3Bb1	1.96	*Bacillus thuringiensis* subsp. *kumamotoensis*	Carries the insect protection train
tahsp 17 3'	0.23	Wheat (*Triticum aestivum*)	Ends transcription and directs polyadenylation
Selectable marker elements			
35 S	0.32	Cauliflower mosaic virus	Regulates expression in plant cells
nptII	0.82	*Escherichia coli*	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)	0.15	*Escherichia coli*	Non-functional The bleomycin resistance gene *ble* has been subcloned together with the *nptII* coding sequence from which it shares the same prokaryotic operon
NOS 3'	0.26	*Agrobacterium tumefaciens*	Ends transcription and directs polyadenylation

Table 2. Components of the inserted DNA fragment inherited from MON 810

Sequence	Size (Kb)	Source	Function
e35S	0.32	Cauliflower mosaic virus	DNA sequences derived from cauliflower mosaic virus (CaMV) containing a portion of the CaMV promoter with the duplicated enhancer region and 5' untranslated region.
zmhsp70	0.81	Maize (*Zea mays* L.)	DNA sequences derived from corn containing the intron sequence from the maize *hsp* 70 gene (heat-shock protein) present to stabilize the level of gene transcription.
cry1Ab	2.45	*Bacillus thuringiensis* subsp. *kurstaki*	DNA sequence containing synthetic linker and a portion of the synthetic coding sequence for a variant of Cry1Ab1 protein from *Bacillus thuringiensis* subsp. *kurstaki*.

D. INFORMATION RELATING TO THE GM PLANT

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

MON 863 x MON 810 consists of varieties developed using traditional methods of maize breeding, which express:

1. the modified Cry3Bb1 protein, derived from *Bacillus thuringiensis* subsp. *kumamotoensis*, which provides protection from certain Coleopteran pests (*Diabrotica* spp.),
2. the Cry1Ab protein, derived from *Bacillus thuringiensis* subsp. *kurstaki*, which provides protection from certain Lepidopteran insect pests (including *Ostrinia nubilalis* (European corn borer) and *Sesamia* spp).

MON 863 that expresses the modified Cry3Bb1 protein from *B.t.* offers an entirely new means to control corn rootworm (CRW) that is safe for humans and the environment. As the leading pest of U.S. maize, based on insecticide use, there is an ongoing need for efficacious CRW control measures. CRW-protected maize will offer growers a unique new management tool for CRW, which will reduce or eliminate the risks associated with chemical transportation, storage, application, disposal, and stewardship. Agro-ecosystems will benefit from the specificity of the product to CRW and the lack of harmful effects on beneficial insects or wildlife. CRW-protected maize is fully compatible with current management protocols for CRW, including integrated pest management (IPM).

The *B.t.* protein, MON 863 Cry3Bb1 has shown high levels of activity against the CRW complex. MON 863 demontrates levels of control that are superior to those of commercial soil-applied insecticides.

As its second parental line MON 810, MON 863 × MON 810 expresses the Cry1Ab protein derived from *Bacillus thuringiensis* subsp. *kurstaki*, which provides protection from certain lepidopteran insect pests, including European corn borer (*Ostrinia nubilalis*) and pink borers (*Sesamia* spp).

2. Information on the sequences actually inserted or deleted

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

As described in the respective application dossiers for the single-trait parental maize lines, MON 863 and MON 810 each contains a single DNA insert containing a single copy of the introduced DNA fragment, and this at different loci in the maize genome.

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

As the parental maize lines used in the traditional cross to produce MON 863 × MON 810 are inbred lines that are homozygous in the MON 863 or MON 810, both of the inserted fragments are inherited by the MON 863 × MON 810. The presence of these inserts in the hybrid was confirmed through Southern blot analysis.

Therefore, MON 863 × MON 810 contains both of the parental inserts, as they were present in the parental MON 863 and MON 810.

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

The MON 863 and MON 810 inserts are non-allelic sequences that have been stably integrated in the nuclear maize genome. The respective analyses of the segregation results for MON 863 and MON 810 are consistent with single active sites of integration of the inserts into the genomic DNA. Southern blot analyses confirmed that the size and structure of each insert are identical to those of their respective parents and therefore that their molecular structure has been preserved during the breeding process.

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

Based on the molecular characterisation of the single-trait lines, MON 863 and MON 810, we consider it highly likely that the insert sequences of MON 863 and MON 810 are conserved in MON 863 x MON 810, since there is no scientific basis to support the fact that these sequences would be intrinsically more unstable when combined together by traditional breeding ("stacked"). Therefore, the molecular characteristics of the respective introduced DNA sequences, known for the single-trait MON 863 and MON 810, also apply to MON 863 × MON 810, including the structural organisation and integrity of the inserts, as well as the characteristics of the sites of insertion and the flanking sequences, immediately adjacent to the introduced sequences.

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 Cry3Bb1, Cry1Ab and NPTII proteins were measured in various tissues collected from MON 863 × MON 810 plants produced in multi-site field trials in Argentina during the 1999-2000 growing season.

Cry3Bb1 and Cry1Ab protein levels were estimated in forage and grain because these tissues are most relevant to food and animal feed product safety. Since protein levels are relevant to the insect control performance of the maize plants, and are also necessary to assess exposure of non-target species where the maize is planted, protein levels were also measured in additional maize tissues. Levels of Cry3Bb1 protein were measured in young leaf, root and pollen, while levels of Cry1Ab protein were measured in leaf and pollen. Levels of the NPTII protein were measured in leaf, forage and grain.

Enzyme-linked immunosorbent assay (ELISA) methods were developed and validated for each protein. All protein values are reported as micrograms (µg) of the specific protein per gram (g) of tissue on a fresh weight (fw) basis.

Overall, the ranges across four sites for the Cry3Bb1, Cry1Ab and NPTII protein levels in MON 863 × MON 810 were comparable to the corresponding ranges in either MON 863 or MON 810. However, the average Cry3Bb1 and Cry1Ab protein levels in most tissues types of MON 863 × MON 810 were slightly higher than the corresponding levels in the single-trait hybrids. Similar Cry3Bb1 protein levels were estimated in the over-season root for MON 863 x MON 810 and MON 863. NPTII protein levels were similar in all tissue types for MON 863 × MON 810 and MON 863.

b) Parts of the plant where the insert is expressed

Levels of proteins are summarized in Tables 3 (Cry3Bb1), 4 (Cry1Ab) and 5 (NPTII). The Cry3Bb1 and NPTII protein levels in MON 863 x MON 810 were compared to MON 863, whereas, the Cry1Ab protein levels in MON 863 x MON 810 were compared to MON 810.

Cry3Bb1

Average levels of the Cry3Bb1 protein in tissues of MON 863 x MON 810 were estimated to be 1-2 fold higher than in MON 863. However, the highest levels of Cry3Bb1 were significantly lower in young leaf, and approximately the same in pollen and grain, as the levels obtained in 1999 U.S. trials. Limits of detection (LODs) were previously established for the Cry3Bb1 protein ELISA assay: 0.087 µg/g for leaf, 0.22 µg/g for forage, 0.096 µg/g for grain, 0.70 µg/g for pollen and 0.76 µg/g for root.

Table 3. Cry3Bb1 protein in MON 863 x MON 810 and MON 863

Tissue Type	Collection time (Days Post-Planting)	Average Cry3Bb1 protein levels (µg/g fw) (Range)	
		MON 863 x MON 810	MON 863
Young Leaf	18	46.7 (35.6 - 53.2)	30.0 (21.3 - 47.2)
Forage	90	23.6 (6.7 - 39.7)	12.8 (<0.22 - 28.8)
Grain	117	61.1 (38.5 - 83.1)	43.7 (<0.096 - 84.1)
Pollen	60	79.6 (65.1 - 96.5)	60.4 (29.7 - 90.7)
Mature root	90	19.7 (6.0 - 41.7)	16.2 (<0.76 - 49.8)
Over-season Root	46	22.0 (N/A)	20.0 (N/A)

Cry1Ab

Levels of the Cry1Ab protein in MON 863 x MON 810 were estimated to be slightly higher than in MON 810, but within the range of natural variability. LODs were previously established for the Cry1Ab protein ELISA assay: 0.12 µg/g for leaf, 0.26 µg/g for forage, 0.13 µg/g for grain, and 0.08 µg/g for pollen.

Table 4. Cry1Ab protein levels in MON 863 x MON 810 and MON 810

Tissue Type	Collection time (Days Post-Planting)	Average Cry3Bb1 protein levels (µg/g fw) (Range)	
		MON 863 x MON 810	MON 863
Young Leaf	18	17.9 (14.1-27.5)	13.0 (9.8-15.4)
Forage	90	7.9 (3.9-11.9)	5.6 (3.0-8.2)
Grain	117	0.84 (0.63-1.2)	0.46 (0.24-0.77)
Pollen	60	<0.08 (<0.08)	<0.08 (<0.08-0.18)

NPTII

Levels of the NPTII protein in MON 863 x MON 810 were similar to those measured in MON 863. LODs were previously established for the NPTII protein ELISA assay: 0.093 µg/g for leaf, 0.075 µg/g for forage and 0.076 µg/g for grain.

Table 5. NPTII protein levels in MON 863 x MON 810 and MON 863

Tissue Type	Collection time (Days Post-Planting)	Average Cry3Bb1 protein levels (µg/g fw) (Range)	
		MON 863 × MON 810	MON 863
Young Leaf	18	1.6 (0.53-2.3)	1.06 (0.58-1.6)
Forage	90	0.19 (0.13-0.27)	0.17 (<0.075-0.33)
Grain	117	<0.076 (<0.076)	<0.076 (<0.076)

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

a) Reproduction

Agronomic data collected from trials performed with MON 863 x MON 810 have demonstrated that MON 863 × MON 810 has not been altered in survival, multiplication or dissemination characteristics when compared to its parental maize lines (MON 863 and MON 810) or compared to traditional maize varieties. The introduced traits for insect-protection have no influence on maize reproductive morphology and hence no changes in seed dissemination would be expected.

b) Dissemination

The introduced traits have 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 the aid of human intervention. Field observations have demonstrated that MON 863 × MON 810 has not been altered in its survivability when compared to its parental maize lines (MON 863 and MON 810) or compared to traditional maize.

d) Other differences

Comparative assessments in the field did not reveal any biologically significant differences between MON 863 × MON 810 and traditional maize hybrids, 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 hybrid seed (F1) is produced by a single cross of the MON 863 and MON 810 parental inbred lines (made homozygous for MON 863 or MON 810, respectively) by traditional breeding. Thereby, each parental line passes on its inserted DNA sequence to the resulting MON 863 × MON 810 F1 hybrid seed, which is sown by the grower.

The single-trait modified maize lines MON 863 and MON 810 each contain one insert with a single copy of the respective transformed DNA, 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.

The harvested (F2) grain of MON 863 × MON 810 is marketed by the grower for food, feed or industrial use and is not used for further breeding. Therefore, since MON 863 × MON 810 hybrid maize seed exists only for a single generation, there is no opportunity for its 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

None of the genetic elements in MON 863 and MON 810 has a genetic transfer function. Therefore, no changes are expected in the ability of these maize lines or MON 863 x MON 810 to transfer genetic material to bacteria.

b) Plant to plant gene transfer

Not applicable. This application under Regulation (EC) No 1829/2003 includes food and feed of MON 863 x MON 810 for uses equivalent to any other maize. Plant to plant gene transfer is assessed in the import application under Directive 2001/18/EC for this maize.

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 863 x MON 810 was compared with control lines that had not been genetically modified and with commercial hybrids. This MON 863 x MON 810 F_1 was used for the studies and its self-pollination produced the respective F_2 seed generations, which was the grain material tested.

7.2 Production of material for comparative assessment

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

Materials for the compositional analysis were produced in 1999/2000 replicated field trials at four sites in Argentina (study plan 99-04-39-01). The four replicated trials, Fontezuela 1, Fontezuela 2, Salto and Rojas provided a variety of environmental conditions representative of regions in Argentina where maize is grown commercially. At each site, the MON 863 x MON 810 test, the control, the single trait references and four commercial maize hybrids were planted in two-row plots in four replicates, in a randomized complete block design, with the exception of MON 810 which was planted only in a single row due to shortage of seeds.

b) the baseline used for consideration of natural variations

For the compositional study, altogether a total of 290[5] statistical comparisons were made between the test (MON 863 x MON 810) and the non-transgenic control. For all 71 significant differences ($p<0.05$), the range of the values for the test were within the 99% tolerance interval or, in cases where a 99% tolerance interval was not available, the ranges of values for the commercial hybrids.

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

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 positive results of these extensive, compositional analyses conducted for MON 863 × MON 810 compared to traditional maize hybrids (*See* Section D.7.1), as well as the results from similar analyses previously conducted for the single-trait maize lines containing either MON 863 or MON 810, there is no indication to further analyse other selected compounds in this maize.

[5] 5 sets of comparisons : data from each of the 4 trials and data from a combination of all four trials for 58 components

7.4 Agronomic traits

We do not anticipate synergistic or antagonistic effects as a result of combining the genetic modifications of the parental lines which could alter the agronomic characteristics of MON 863 x MON 810. Furthermore, field trials with MON 863 x MON 810 were performed and the set of agronomic observations supports a conclusion that combining MON 863 and MON 810 through traditional breeding does not cause any unexpected and adverse performance impacts for this hybrid.

7.5 Product specification

MON 863 × MON 810 will be imported into the E.U. in mixed shipments of maize grain and 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 E.U.

MON 863 × MON 810 actually comprises all traditionally bred hybrid maize varieties produced by the combination of genetically modified maize inbreds derived from MON 863 and MON 810.

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 is substantially equivalent and as safe and as nutritious as traditional maize, the use of MON 863 × MON 810 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 180 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 use as or in food or feed as a result of the addition of MON 863 × MON 810 to the traditional maize supply. MON 863 × MON 810 are expected to replace a portion of current maize hybrids such that their intake or use will represent some fraction of the total products derived from maize.

7.8 Toxicology

7.8.1 Safety evaluation of newly expressed proteins

MON 863 × MON 810 is produced by a single traditional cross of two genetically modified parental inbred maize lines, i.e. one derived from MON 863 and one derived from MON 810. Both of the introduced traits in the single-trait, parental lines are inherited by the MON 863 × MON 810 progeny. This results in the combined expression of the Cry3Bb1, NPTII and the Cry1Ab proteins in the same plant, MON 863 × MON 810. These introduced proteins are present at low levels in the plant and have previously been demonstrated as safe for animal and human health.

The conclusion of safety to humans of the Cry3Bb1, NPTII and Cry1Ab proteins was based upon the following considerations:

(1) no amino acid sequence similarity to known toxins, other than *B.t.* proteins in the case of Cry3Bb1 and Cry1Ab, and no immunologically relevant sequence similarity with known allergens, (2) rapid degradation under conditions which simulate mammalian digestive systems, (3) no indications of acute toxicity in mice administered Cry3Bb1, NPTII or Cry1Ab protein by oral gavage, (4) very low dietary exposure, and (5) a history of safe use.

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 was shown to be substantially 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 compositional and nutritional equivalence of grain and forage from MON 863 × MON 810 and traditional maize have been established by compositional analysis. Additionally, the wholesomeness of MON 863 × MON 810 grain has been confirmed in a highly sensitive feeding study using broiler chickens.

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 and Cry1Ab proteins expressed in MON 863 × MON 810 has previously been demonstrated for the single-trait parental lines containing either MON 863 or MON 810.

These proteins 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 any allergenic potential, it was concluded that the use of MON 863 × MON 810 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 hybrids are F1 maize hybrids, that have inherited the genetic modification MON 863 and MON 810 from their single-trait, genetically modified parental lines. The introduced traits for insect protection are of agronomic interest (input traits), and are not intended to change any nutritional aspects of this maize. Hence this maize 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 maize-derived foods and feeds is not expected to be altered upon commercialisation of MON 863 × MON 810, and no nutritional imbalances are expected as a result of the use of MON 863 × MON 810.

7.10.2 Nutritional assessment of GM feed

A confirmatory feeding study in broiler chickens was conducted to compare the nutritional value of the stacked MON 863 × MON 810 grain and non-transgenic control grain as well as additional commercial maize hybrids, and to provide confirmation of the safety of this hybrid 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 diet and the non-transgenic control diet. In addition, 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, the non-transgenic hybrid, and commercially available reference maize hybrids. The MON 863 × MON 810 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 the MON 863 × MON 810, which showed that there were no biologically relevant differences in nutritional and compositional properties relative to control and reference maize hybrids. These data confirm and support the conclusion that the MON 863 × MON 810 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 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, which are of agronomic interest, resulting in the expression of the Cry3Bb1, NPTII and Cry1Ab proteins.

There are no intrinsic hazards related to MON 863 × MON 810 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 demonstrates that the risks of consumption of MON 863 × MON 810 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 are not indicated, and post-market monitoring of the use of this maize for food, feed or processing is neither warranted, nor appropriate.

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

Not applicable as this application under Regulation (EC) No 1829/2003 includes food and feed of MON 863 x MON 810 for uses equivalent to any other maize and does not include deliberate release of grains into the environment.

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

9.1 Persistence and invasiveness

Not applicable as this application under Regulation (EC) No 1829/2003 includes food and feed of MON 863 x MON 810 for uses equivalent to any other maize and does not include deliberate release of grains into the environment.

The environmental aspects associated with the import of MON 863 x MON 810 are covered in the application under Directive 2001/18/EC.

9.2 Selective advantage or disadvantage

Please see question **D.9.1**

9.3 Potential for gene transfer

Please see question **D.9.1**

9.4 Interactions between the GM plant and target organisms

Please see question **D.9.1**

9.5 Interactions of the GM plant with non-target organisms

Please see question **D.9.1**

9.6 Effects on human health

Please see question **D.9.1**

9.7 Effects on animal health

Please see question **D.9.1**

9.8 Effects on biogeochemical processes

Please see question **D.9.1**

9.9 Impacts of the specific cultivation, management and harvesting techniques

Please see question **D.9.1**

10. Potential interactions with the abiotic environment

Not applicable as this application under Regulation (EC) No 1829/2003 includes food and feed of MON 863 x MON 810 for uses equivalent to any other maize and does not include deliberate release of grains into the environment.

The interactions with the abiotic environment associated with the import of MON 863 x MON 810 are covered in the application under Directive 2001/18/EC.

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

11.1 General (risk assessment, background information)

The environmental release of MON 863 × MON 810 in the E.U. is not within the scope of this application under Regulation (EC) No 1829/2003. The scope of the current application only includes the use of this maize for food and feed.

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

Please see question **D.11.1**

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

Please see question **D.11.1**

11.4 Reporting the results of monitoring

Please see question **D.11.1**

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

As MON 863 × MON 810 is the result of a traditional cross of MON 863 and MON 810, they contain both transformation events in combination. Therefore, MON 863 × MON 810 is detectable using either the event-specific PCR method for detecting the introduced DNA present in MON 863 or the equivalent method for MON 810. However, as for all plants in which one or more events are combined by traditional breeding, the unambiguous detection of MON 863 × MON 810 in mixed consignments of grain will require single grains to be subjected to detection methods for both MON 863 and MON 810, and to test positive for both.

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** MON 863 × MON 810 has been field tested in Argentina and in the USA since 1999 and 2000, respectively. In addition, the two parental single-trait maize varieties, MON 863 and MON 810, have been extensively tested in the field prior to their approval and subsequent commercialisation in several countries around the world.
b)	**Authority overseeing the release** Argentina: Secretary of Agriculture (SAGPyA) - CONABIA USA: United States Department of Agriculture and Environmental Protection Agency
c)	**Release site** Argentina: Bragado, Salto, Rojas USA: Mainly in the states of the corn belt and in Hawaii and Porto Rico
d)	**Aim of the release** Argentina/USA: Assess the performances: efficacy, yield, breeding, …

e)	**Duration of the release** Argentina/USA: 12 months
f)	**Aim of post-releases monitoring** Argentina/USA: Assess for volunteers
g)	**Duration of post-releases monitoring** Argentina/USA: 12 months
h)	**Conclusions of post-release monitoring** Argentina: nothing to report USA: volunteers have been eliminated to prevent persistence in the environment
i)	**Results of the release in respect to any risk to human health and the environment** Argentina/USA: No evidence that MON 863 × MON 810 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 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)** The JRC website http://gmoinfo.jrc.it/gmc_browse.asp provides a link to the publicly accessible Initial Assessment Report from the German Lead Member State for Monsanto notification C/DE/02/9 on MON 863 × MON 810.
c)	**EFSA opinion** A favourable EFSA opinion, specifically for MON 863 × MON 810, was not available at the time of submission of this application. A favourable EFSA opinion has been issued, however, for the MON 863 single-trait product, which were posted at http://www.efsa.eu.int/science/gmo/gmo_opinions/catindex_en.html.

d)	**Commission Register (Commission Decision 2004/204/EC)** The authorised food and feed are entered in the Community Register of GM food and feed: 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 is likely to 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)** The JRC website http://gmoinfo.jrc.it/gmc_browse.asp provides a link to the publicly accessible SNIF summary of notifications under Directive 2001/18/EC, including the Monsanto notification for MON 863 × MON 810.