Application for renewal of the authorisation for continued marketing of existing feed materials produced from MON 863 × MON 810 maize that were previously notified, according to Article 20(1)(b) of Regulation (EC) No 1829/2003 on genetically modified food and feed

Part II

Summary

April 2007

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

A. GENERAL INFORMATION

1. Details of application

a)	**Member State of application** Not applicable
b)	**Notification number** Not known 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. In countries where MON 863 × MON 810 varieties are 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** Not known 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 maize[1] will continue to be traded and used in the European Union in the same manner as current commercial maize and by the same operators currently involved in the trade and use of maize.

® YieldGard is a registered trademark of Monsanto Technology LLC.
[1] Hereafter referred to as MON 863 × MON 810

3. Scope of the application

( )	**GM plants for food use**
( )	**Food containing or consisting of GM plants**
( )	**Food produced from GM plants or containing ingredients produced from GM plants**
( )	**GM plants for feed use**
( )	**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)**

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. The application received a favourable opinion from the Rapporteur in April 2003, and from EFSA in June 2005, finally leading to approval for placing on the market in January 2006.

The notification according to Regulation (EC) No 258/97 concerning novel foods and novel foods ingredients, submitted in July 2002, was transformed in July 2004 into Application EFSA-GMO-DE-2004-03 under Regulation (EC) No 1829/2003 for use of MON 863 × MON 810 as food or feed. This application received a positive opinion from EFSA on 31 March 2006.

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

Yes (x **)**	**No ( )**

If *yes*, specify

Cultivation of MON 863 × MON 810 is lawful in the USA, Canada and the Philippines, while importation of derived foods and feeds is lawful in Australia, Japan, Korea, Mexico, New Zealand and Taiwan.

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 against 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 renewal application includes feed materials produced from MON 863 × MON 810 which are lawfully placed on the market in the E.U., as listed in the Community Register of GM Food and Feed[2]. The range of uses of these MON 863 × MON 810-derived products will be identical to the full range of equivalent uses of current commercial maize derived products.
c)	**Intended use of the product and types of users** MON 863 × MON 810-derived feed materials will continue to be traded and used in the European Union in the same manner as equivalent products from current commercial maize and by the same operators currently involved in the trade and use of 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 conventional maize 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 conventional maize. Therefore MON 863 × MON 810-derived feed materials will be stored, packaged, transported, handled and used in the same manner as feed materials derived from current commercial maize. No specific conditions are warranted or required for the feed materials produced from 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 conventional maize (except for its protection against targeted coleopteran and lepidopteran insect pests). Therefore, MON 863 × MON 810-derived feed materials will continue to be used in the same manner as other equivalent maize derived products and no specific packaging is required (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 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

[2] http://ec.europa.eu/food/dyna/gm_register/index_en.cfm

exists, operators shall ensure that an indication that the feed product is produced from GMOs is transmitted in writing to the operator receiving the product.

Operators handling or using MON 863 × MON 810-derived 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 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)

Not applicable

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 feed materials are suitable for 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 feed materials produced from MON 863 × MON 810 is unlikely, as this proposed feed use is enclosed in the current food and feed uses of conventional maize. MON 863 × MON 810 is substantially equivalent to other maize hybrids except for the introduced (*i.e.* inherited) traits: protection against coleopteran and lepidopteran insect pests, which are traits of agronomic interest. This maize has been shown to be as safe and as nutritious as conventional maize. Therefore, all measures for waste disposal and treatment of MON 863 × MON 810-derived products are the same as those for conventional maize. No specific conditions are warranted or required for the continued marketing of MON 863 × MON 810-derived feed materials in the E.U.

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

1. Complete name

a) Family name

Poaceae (formerly Gramineae)

b) Genus

Zea

c) Species

mays (2n=20)

d) Subspecies

N/A

e) Cultivar/breeding line

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 this renewal application does not include the environmental release of MON 863 × MON 810. Outcrossing with cultivated *Zea* varieties is therefore not expected in the context of this application.

Out-crossing with wild *Zea* species

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 renewal application does not include the deliberate release of MON 863 × MON 810 in the E.U but only the continued use of existing feed materials derived from MON 863 × MON 810.

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. 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. Instead, traditional breeding methods are used to cross inbreds of MON 863 and MON 810. MON 863 and MON 810 were modified by incorporation of a DNA fragment derived from plasmid vectors PV-ZMIR13 and PV-ZMBK07, respectively, into the maize genome using a particle acceleration method.

2. Nature and source of the vector used

While MON 863 × MON 810 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 also generated using the particle acceleration method, 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 hybrid varieties are produced by a single cross from MON 863 and MON 810 inbred lines (homozygous for the respective insert) by traditional breeding. By crossing MON 863 and MON 810, MON 863 × MON 810 inherits the inserted DNA fragments from both its parents 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. Genetic elements inherited from MON 863[3]

Genetic element	Source	Size (kb)	Function
***<u>MON 863 cry 3Bb1</u>* <u>gene cassette</u>**			
P-*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*
L-*CAB*	*Triticum aestivum*	0.06	Translation enhancement
I-R*act1*	*Oryza sativa*	0.49	Transcription enhancement
CS-*MON 863 cry3Bb1*	*Bacillus thuringiensis* subsp. *kumamotoensis*	1.96	Carries the insect protection trait
T-*Hsp 17 3'*	*Triticum aestivum*	0.23	Ends transcription and directs polyadenylation
<u>Selectable marker elements</u>			
P-*35S*	Cauliflower mosaic virus	0.32	Regulates expression in plant cells
CS-*nptII*	*Escherichia coli*	0.82	Allows the selection of the plant cells carrying the insect protection trait by conferring 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
T-*nos*	*Agrobacterium tumefaciens*	0.26	Ends transcription and directs polyadenylation

[3] The nomenclature of these genetic elements has been refined.

Table 2. Genetic elements inherited from MON 810			
Genetic element	**Source**	**Size (Kb)**	**Function**
P-*e35S*$^{MON\ 810}$	Cauliflower mosaic virus	0.3	Promoter
I-*Hsp70*	*Zea mays* L.	0.8	Stabilizes level of gene transcription.
CS-*cry1Ab*$^{MON\ 810}$	*Bacillus thuringiensis*	2.5	Encodes a variant of Cry1Ab1 protein, which targets specific lepidopteran insect pests.

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 is produced by traditional methods of maize breeding, starting from MON 863 and MON 810 single-trait lines. MON 863 × MON 810, therefore, expresses:

1. the modified Cry3Bb1 protein, derived from *Bacillus thuringiensis* subsp. *kumamotoensis*, which provides protection from certain coleopteran pests (*Diabrotica* spp.),
2. the NPTII protein which provides an effective method for selecting cells that contain the insecticidal gene and can be used in bacterial selection during construction of the plasmid. The *nptII* gene is inserted into maize cells along with the *MON 863 cry3Bb1*.

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).

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 to produce MON 863 × MON 810 are inbred lines that are homozygous in the MON 863 or MON 810, respectively, both of the inserted fragments are inherited by MON 863 × MON 810, *i.e.* one fragment for the coleopteran-protection and one for the lepidopteran-protection traits. The presence of these inserts in MON 863 × MON 810 was confirmed through Southern blot analysis, showing that the integrity of the inserts has been conserved in the combined-trait product.

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

MON 863 × MON 810 contains both of the parental inserts on separate chromosomes in the nuclear genome, as they were present in parental MON 863 and MON 810. The presence of these inserts in the combined-trait product was confirmed through Southern blot analysis.

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

As MON 863 × MON 810 is the product of traditional breeding of MON 863 and MON 810 and no additional genetic modification methods have been applied, and as the inherited DNA fragments have negligible potential to interact with one another, it is highly likely that the insert sequences of MON 863 and MON 810 are conserved in MON 863 × MON 810. 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, NPTII and Cry1Ab 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. The levels of these proteins in grain and forage are summarized below:

The mean levels of Cry3Bb1 and Cry1Ab in MON 863 × MON 810 grain across all sites were 61.1 and 0.84 µg/g dry weight, respectively. The mean levels of Cry3Bb1 and Cry1Ab in MON 863 × MON 810 forage across all sites were 23.6 and 7.9 µg/g dry weight, respectively. NPTII protein levels were below the limit of detection (LOD < 0.076 µg/g fresh weight) in grain and equal to 0.19 µg/g fresh weight in forage.

Overall, the ranges across four sites for the Cry3Bb1, NPTII and Cry1Ab protein levels in MON 863 × MON 810 were comparable to the corresponding ranges in either MON 863 or MON 810. Enzyme-linked immunosorbent assay (ELISA) methods were developed and validated for each protein.

b) Parts of the plant where the insert is expressed

Cry3Bb1, NPTII and Cry1Ab protein levels were estimated in forage and grain, which are the most relevant tissues in terms of feed safety evaluation. Additionally, these proteins were also measured in young leaf, pollen, young root and mature root for the Cry3Bb1 protein, young leaf and pollen for the Cry1Ab protein and young leaf for the NPTII protein.

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

a) Reproduction

Agronomic data collected from trials performed with MON 863 × 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 conventional maize. The introduced traits for insect-protection have no influence on maize reproductive morphology and hence no changes in seed dissemination would be expected.

Regardless, it should be noted that the scope of the current renewal application does not include the cultivation of MON 863 × MON 810 varieties in the E.U. but only the renewal of the authorisation for the continued marketing of existing MON 863 × MON 810-derived feed materials, entered in the Community Register of GM Food and Feed, in the E.U.

b) Dissemination

The introduced coleopteran and lepidopteran-protection 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 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 conventional maize.

d) Other differences

Comparative assessments of the phenotypic and agronomic characteristics in the field did not reveal any biologically significant differences between MON 863 × MON 810 and conventional 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 hybrid seed (F1) is produced by traditional breeding starting from MON 863 and MON 810 inbred lines (made homozygous for MON 863 or MON 810, respectively). Thereby, each parental line passes on its inserted DNA sequence to the resulting MON 863 × MON 810 plant.

The single-trait products, 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. In the progeny of MON 863 and MON 810, 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.

Southern blot analysis of MON 863 × MON 810 confirmed that the inserts from MON 863 and MON 810 are stably inherited in the combined-trait progeny, which could be expected on the basis of the characterization of the single-trait products.

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

a) Plant to bacteria gene transfer

In comparison with the possible transfer of genetic material between bacteria and conventional maize, and based on the nature of the DNA elements used in the inserts that have been inherited by MON 863 × MON 810, 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 products and the MON 863 × MON 810 combined-trait product are unchanged compared to conventional maize, pollen production and pollen viability are not expected to be affected by the genetic modifications. Therefore, the outcrossing frequency to other maize or to wild relatives (which are not present in the E.U.) is unlikely to be different for MON 863 × MON 810 when compared to conventional maize. However, it should be noted that the scope of the current renewal application does not include the cultivation of MON 863 × MON 810 varieties in the E.U. but only the renewal of the authorisation for continued marketing of existing MON 863 × MON 810-derived feed materials, entered in the Community Register of GM Food and Feed, in the E.U.

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 × MON 810 was compared with control lines that had not been genetically modified and with commercial hybrids.

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. The four replicated trials provided a variety of environmental conditions representative of regions in Argentina where maize is grown commercially. At each site, the MON 863 × 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[8] statistical comparisons were made between the test (MON 863 × MON 810) and the conventional 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.

Comparisons with baseline data from numerous other field trials and from the peer-reviewed literature were also 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.

Grain samples were analysed to measure proximate (protein, fat, ash, carbohydrate, moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), amino acids, fatty acids, vitamin B1, vitamin B2, vitamin E, minerals (calcium, copper, iron, magnesium, manganese,

[8] 5 sets of comparisons : data from each of the 4 trials and data from a combination of all four trials for 58 components

phosphorus, potassium, sodium and zinc), folic acid, phytic acid, trypsin inhibitor, ferulic acid, inositol, raffinose, 2-furaldehyde (furfural) and p-coumaric acid content; forage samples were analysed for proximate, ADF and NDF.

Based on the positive results of these extensive, compositional analyses conducted for MON 863 × MON 810 compared to conventional 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.

7.4 Agronomic traits

The scope of this application is limited to the renewal of the authorisation for continued marketing of existing MON 863 × MON 810-derived feed materials in the E.U., but does not include the cultivation of MON 863 × MON 810 varieties in the E.U. The observations from agronomic and phenotypic assessments provide additional evidence confirming the absence of unintended or unanticipated adverse effects of the genetic modifications present in this maize.

Synergistic or antagonistic effects are not expected as a result of combining the genetic modifications of the parental lines which could alter the agronomic characteristics of MON 863 × MON 810. Furthermore, field trials with MON 863 × 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-derived feed materials are currently imported into the EU in mixed shipments of maize products, produced in other world areas. These products are handled by operators that have traditionally been involved in the commerce, processing and use of maize and maize derived products in the European Union.

As MON 863 × MON 810 results from traditional breeding of MON 863 and MON 810, it contains both inserts in combination, which confer protection against coleopteran and lepidopteran insect pests. The presence of the coleopteran and lepidopteran-protection traits in MON 863 × MON 810-derived products can be detectable using either the insert-specific PCR method for detecting the introduced DNA present in MON 863, or the equivalent method for MON 810. The unambiguous detection of MON 863 × MON 810 in mixed consignments of maize product requires single samples to be subjected to detection methods for both MON 863 and MON 810, and to test positive for both.

The event specific methods of detection of MON 863 and MON 810 were validated by the Community Reference Laboratory (CRL), in collaboration with the European Network of GMO Laboratories (ENGL), and published together with the validation report for MON 863 × MON 810 on their website[9].

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 conventional maize, the use of MON 863 × MON 810 for the production of foods and feeds is no different from that of conventional maize. Consequently, any effects of the production and processing of MON 863 × MON 180 are not expected to be any different from the production and processing of the equivalent foods and feeds, originating from conventional maize.

7.7 *Anticipated intake/extent of use*

Feed materials produced from MON 863 × MON 810 were first placed on the E.U. market in 2003. In July 2004 these products were notified to the European Commission, following Article 20(1)(b) of Regulation (EC) No 1829/2003, in order to allow for their continued marketing in the E.U. given that they had been lawfully placed on the market before Regulation (EC) No 1829/2003 came into force, on 18 April 2004.

MON 863 × MON 810-derived feed materials replace a portion of current commercial maize products. Anticipated dietary intake and/or extent of use of current commercial maize products is not expected to be altered upon renewal of the authorisation of existing MON 863 × MON 810-derived feed materials.

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 and animals 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.

[9] http://gmo-crl.jrc.it/statusofdoss.htm

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 conventional maize, testing of any constituent other than the introduced proteins is not 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 conventional 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 conventional maize.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

MON 863 × MON 810 is produced from MON 863 and MON 810 using traditional breeding methods, and thereby inherits both inserts from the single-trait parental lines. The introduced traits for coleopteran and lepidopteran-protection are of agronomic interest, 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 the production of feed, materials. Therefore, anticipated dietary intake of maize-derived products is not expected to be altered upon the renewal of the MON 863 × MON 810 authorisation, and no nutritional imbalances are expected as a result of the use of MON 863 × MON 810-derived feed materials.

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 conventional 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 conventional 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 conventional hybrid, and commercially available reference maize hybrids. The MON 863 × MON 810 diet was as wholesome as its corresponding conventional 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 conventional maize.

7.11 Post-market monitoring of GM food/feed

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 conventional maize and maize-derived products.

As a consequence, and as previously stipulated in the Community Register of GM food and feed, no specific risk management measures are indicated, and post-market monitoring of the use of feed materials produced from this maize is not appropriate.

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

Not applicable as this renewal application under Regulation (EC) No 1829/2003 only includes feed materials produced from MON 863 × MON 810 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

Not applicable as neither the GMO, nor the food and feed containing or consisting of the GMO, are within the scope of this renewal application under Regulation (EC) No 1829/2003.

10. Potential interactions with the abiotic environment

Not applicable, as neither the GMO, nor the food and feed containing or consisting of the GMO, are within the scope of this renewal application under Regulation (EC) No 1829/2003.

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

Not applicable, as neither the GMO, nor the food and feed containing or consisting of the GMO, are within the scope of this renewal application under Regulation (EC) No 1829/2003.

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

The validated event-specific methods for detection of MON 863 and MON 810, as well as the validation report for NK603 × MON 810, prepared by the Community Reference Laboratory (CRL) in collaboration with the European Network of GMO Laboratories (ENGL), are published on the CRL website since 14 March 2006.

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 was grown commercially in U.S.A. Prior to commercialisation, this maize as well as its parental single-trait maize lines, MON 863 and MON 810, have been extensively tested in the field in several countries around the world.
b)	**Authority overseeing the release** U.S.A.: United States Department of Agriculture (USDA) and Environmental Protection Agency (EPA). It should be noted that only in a few countries around the world, stacked products require separate approvals by regulatory agencies. In most countries, including the U.S.A., stacked products are not regulated, provided that each of the single-trait parental lines is already approved.
c)	**Release site** Please see question E.2.(a)
d)	**Aim of the release** Commercial release for all uses as conventional maize.
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, indicating that post-release monitoring would not be necessary. In addition, the commercialisation of MON 863 × MON 810 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.
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 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 JRC websites http://gmoinfo.jrc.it and http://gmo-crl.jrc.it/statusofdoss.htm and the EFSA website http://www.efsa.europa.eu/en/science/gmo/gm_ff_applications.html, provide publicly accessible links to up-to-date databases on the regulatory progress of notifications under Directive 2001/18/EC and applications under Regulation (EC) No 1829/2003, including the Monsanto dossiers for MON 863 × MON 810: C/DE/02/9 and EFSA-GMO-DE-2004-03.
b)	**Assessment Report of the Competent Authority (Directive 2001/18/EC)** The JRC website http://gmoinfo.jrc.it/gmc_browse.aspx?DossClass=3 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** On 31 March 2006, the EFSA issued its overall opinion (http://www.efsa.europa.eu/en/science/gmo/gm_ff_applications/more_info/505.html) on Application EFSA-GMO-DE-2004-03 for authorisation of NK603 × MON 810, according to Regulation (EC) No 1829/2003 on genetically modified food and feed, concluding on its safety for humans, animals and the environment.
d)	**Commission Register (Commission Decision 2004/204/EC)** http://ec.europa.eu/food/dyna/gm_register/index_en.cfm
e)	**Molecular Register of the Community Reference Laboratory/Joint Research Centre** Information on detection protocols is posted at http://gmo-crl.jrc.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.aspx?DossClass=3 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: C/DE/02/9.

Further, EFSA provides a link to the publicly accessible summary of this renewal application, as well as to the summary of application EFSA-GMO-DE-2004-03 under Regulation (EC) No 1829/2003 at http://www.efsa.europa.eu/en/science/gmo/gm_ff_applications/more_info/505.html.