Application for renewal of the authorisation for continued marketing of existing feed materials, feed additives and food additives produced from MON 863 × NK603 maize that were previously notified, according to Articles 8(1)(b) and 20(1)(b) of Regulation (EC) No 1829/2003 on genetically modified food and feed

Part II

Summary

April 2007

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 × NK603. In countries where MON 863 × NK603 varieties are being cultivated, packages of hybrid seed of this maize are marketed under the name of the hybrid variety, in association with the trademarks YieldGard® Rootworm and Roundup Ready®, indicating clearly to growers that the hybrid is protected from specific coleopteran insect pests and tolerant to Roundup® herbicide, containing the active ingredient glyphosate.

d) Date of acknowledgement of notification

Not available at the time of application

2. Applicant

a) Name of applicant

Monsanto Company, represented by Monsanto Europe S.A.

b) Address of applicant

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

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

c) Name and address of the person established in the Community who is responsible for the placing on the market, whether it be the manufacturer, the importer or the distributor, if different from the applicant (Commission Decision 2004/204/EC Art 3(a)(ii))

MON 863 × NK603 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, Roundup and Roundup Ready are registered trademarks of Monsanto Technology LLC

[1] Hereafter referred to as MON 863 × NK603

3. Scope of the application

( ) GM plants for food use
( ) Food containing or consisting of GM plants
(x) 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
( ) Import and processing (Part C of Directive 2001/18/EC)
( ) Seeds and plant propagating material for cultivation in Europe (Part C of Directive 2001/18/EC)

4. Is the product being simultaneously notified within the framework of another regulation (e.g. seed legislation)?

Yes ( )	**No (x)**
If *yes*, specify	

5. Has the GM plant been notified under Part B of Directive 2001/18/EC and/or Directive 90/220/EEC?

Yes ( )	**No (x)**
If *no*, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC *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 In November 2004, application EFSA-GMO-UK-2004-06 was submitted under Regulation (EC) No 1829/2003 for use of MON 863 × NK603 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 × NK603 is lawful in the U.S.A. and Canada, 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 × NK603 is produced by the combination of MON 863 and NK603 using traditional breeding methods. No additional genetic modification is involved in the production of MON 863 × NK603.

MON 863 × NK603 and the genetically modified parental lines, MON 863 and NK603, were developed by the Monsanto Company. Like MON 863, MON 863 × NK603 produces the MON 863 modified Cry3Bb1 protein[2], which confers protection against certain coleopteran insect pests (*Diabrotica* spp.). Like NK603, MON 863 × NK603 is tolerant to Roundup® herbicide (containing the active ingredient glyphosate).

The use of MON 863 × NK603 plants enables the farmer to effectively control certain coleopteran 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. The use of MON 863 × NK603 also enables farmers to use glyphosate for effective control of weeds during the growing season and to take advantage of the favourable environmental and safety characteristics of glyphosate herbicides.

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, feed additives and food additives produced from MON 863 × NK603 which are lawfully placed on the market in the E.U., as listed in the Community Register of GM Food and Feed[3]. The range of uses of these MON 863 × NK603 -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 × NK603-derived feed materials, feed additives and food additives, 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

[2] Hereafter referred to as the Cry3Bb1 protein

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

MON 863 × NK603 is substantially equivalent to conventional maize except for its introduced (*i.e.* inherited) traits: protection against the corn rootworm pest and tolerance to glyphosate, which are traits of agronomic interest. This maize was shown to be as safe and as nutritious as conventional maize. Therefore, MON 863 × NK603-derived feed materials, feed additives and food additives will be stored, packaged, transported, used, and handled in the same manner as products derived from current commercial maize. No specific conditions are warranted or required for the feed materials, feed additives and food additives produced from NK603 × MON 810.

e) Any proposed packaging requirements

MON 863 × NK603 is substantially equivalent to its parental lines MON 863 and NK603 and to conventional maize (except for its protection against targeted coleopteran insect pests and its tolerance to glyphosate). Therefore, MON 863 × NK603-derived feed materials, feed additives and food additives 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) A proposal for labelling in accordance with Articles 13 and 25 of Regulation (EC) 1829/2003. In the case of GMOs, food and/or feed containing, consisting of GMOs, a proposal for labelling has to be included complying with the requirements of Article 4, B(6) of Regulation (EC) 1830/2003 and Annex IV of Directive 2001/18/EC.

In accordance with Regulations (EC) N° 1829/2003 and 1830/2003, a labelling threshold of 0.9 % is applied for the placing on the market of MON 863 × NK603 grain and derived products.

Operators shall be required to label foods and feeds derived from MON 863 × NK603 with the words "produced from genetically modified maize". In the case of products for which no list of ingredients exists, operators shall ensure that an indication that the food or feed product is produced from GMOs is transmitted in writing to the operator receiving the product.

Operators handling or using MON 863 × NK603-derived foods and feeds in the E.U. 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 × NK603. Therefore, no further specific measures are to be taken by the notifier.

g) Unique identifier for the GM plant (Regulation (EC) 65/2004; does not apply to applications concerning only food and feed produced from GM plants, or containing

ingredients produced from GM plants)

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 × NK603 feed materials, feed additives and food additives 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, feed additives and food additives produced from MON 863 × NK603 is unlikely as the proposed uses of this maize are enclosed in the current food and feed uses of conventional maize.

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

No specific conditions are warranted or required for the continued marketing of MON 863 × NK603-derived feed materials, feed additives and food additives.

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

Not applicable

e) Cultivar/breeding line

MON 863 × NK603

f) Common name

Maize; Corn

2. a) Information concerning reproduction

(i) Mode(s) of reproduction

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

(ii) Specific factors affecting reproduction

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

(iii) Generation time

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

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

Out-crossing with cultivated *Zea* varieties

The scope of this renewal application does not include the environmental release of MON 863 × NK603. Outcrossing with cultivated *Zea* varieties is therefore not expected in the context of this application.

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 renewal application does not include the cultivation of MON 863 × NK603 varieties in the E.U. but only the continued use of existent food and feed products derived from MON 863 × NK603.

b) Specific factors affecting dissemination

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

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

Because of its many divergent types, maize is grown over a wide range of climatic conditions. The bulk of the maize is produced between latitudes 30° and 55°, with relatively little grown at latitudes higher than 47° latitude anywhere in the world. The greatest maize production occurs where the warmest month isotherms range between 21° and 27° C and the freeze-free season lasts 120 to 180 days. A summer rainfall of 15 cm is approximately the lower limit for maize production without irrigation with no upper limit of rainfall for growing maize, although excess rainfall will decrease yields.

There are no wild relatives of maize in Europe.

6. In the case of plant species not normally grown in the Member State(s), description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts

Maize is widely grown in the European Union. The most important areas of maize production in Europe include the Danube Basin, from southwest Germany to the Black Sea, along with southern France through the Po Valley of northern Italy.

7. Other potential interactions, relevant to the GM plant, of the plant with organisms in the ecosystem where it is usually grown, or used elsewhere, including information on toxic effects on humans, animals and other organisms

Maize is known to interact with other organisms in the environment including insects, birds, and mammals. It is susceptible to a range of fungal diseases and nematode, insect and mite pests. Maize has a history of safe use for human food and animal feed.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Description of the methods used for the genetic modification

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

2. Nature and source of the vector used

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

MON 863 was generated by the integration of sequences from the plasmid vector PV-ZMIR13, containing the *cry3Bb1* coding sequence of interest, which was derived from *Bacillus thuringiensis* subsp. *kumamotoensis*. MON 863 was produced using the particle acceleration method.

NK603 was produced by a particle acceleration transformation method using a gel-isolated *Mlu*I fragment of plasmid vector PV-ZMGT32, containing a 5 enolpyruvylshikimate-3-phosphate synthase (*epsps*) gene that was derived from the common soil bacterium *Agrobacterium* sp. strain CP4 (*cp4 epsps*).

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

MON 863 × NK603 results from a traditional cross of the parental lines MON 863 and NK603. By crossing MON 863 and NK603 inbreds, MON 863 × NK603 inherits the inserted DNA fragments from both its parental maize lines. 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[4]

Genetic element	Source	Size (kb)	Function
***MON 863 cry 3Bb1* gene cassette**			
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
Selectable marker elements			
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 confering a resistance towards a category of aminoglycosides comprising kanamycin, and neomycin
ble (truncated)	*Escherichia coli*	0.15	Non-functional. The bleomycin resistance gene *ble* has been subcloned together with the *nptII* ORF from which it shares the same prokaryotic operon
T-*nos*	*Agrobacterium tumefaciens*	0.26	Ends transcription and directs polyadenylation

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

Table 2. Genetic elements inherited from NK603			
Genetic element	**Source**	**Size (kb)**	**Function**
***cp4 epsps* gene cassette**			
P-*Ract1*/I-*Ract1*	*Oryza sativa*	1.4	Contains promoter, transcription start site and first intron.
TS-*CTP2*	*Arabidopsis thaliana*	0.2	Encodes chloroplast transit peptide, which directs the CP4 EPSPS protein to the chloroplast
CS-*cp4 epsps*	*Agrobacterium* sp. strain CP4	1.4	Encodes glyphosate-tolerant CP4 EPSPS protein
T-*nos*	*Agrobacterium tumefaciens*	0.3	Ends transcription and directs polyadenylation of the mRNA
***cp4 epsps l214p* gene cassette**			
P-*e35S*	Cauliflower mosaic virus	0.6	Promoter
I-*Hsp70*	*Zea mays L.*	0.8	Stabilizes the level of gene transcription.
TS-*CTP2*	*Arabidopsis thaliana*	0.2	Encodes chloroplast transit peptide, which directs the CP4 EPSPS protein to the chloroplast
CS-*cp4 epsps l214p*	*Agrobacterium* sp. strain CP4	1.4	Encodes glyphosate-tolerant CP4 EPSPS L214P protein[5]
T-*nos*	*Agrobacterium tumefaciens*	0.3	Ends transcription and directs polyadenylation of the mRNA

[5] The substitution of leucine by proline in the CP4 EPSPS, encoded by the second *cp4 epsps* gene in NK603 transformation event, is indicated by the suffix L214P.

D. INFORMATION RELATING TO THE GM PLANT

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

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

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

2. Information on the sequences actually inserted or deleted

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

As described in the respective applications for the single-trait parental maize lines, MON 863 and NK603 each contain a single DNA insert containing one copy of the introduced DNA fragment, and this at different loci in the maize genome.

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

As the parental maize lines used to produce MON 863 × NK603 are inbred lines that are homozygous in the MON 863 or NK603 insert, both of the inserts are inherited in MON 863 × NK603. The presence of these inserts in MON 863 × NK603 was confirmed through Southern blot analysis, and the size of the observed bands was as expected, indicating that the structure of the inserts has been conserved in MON 863 × NK603.

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

Not applicable

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

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

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

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

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

3. Information on the expression of the insert

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

Expression levels of the introduced proteins were measured in tissue samples collected from MON 863 × NK603, grown in Argentinean field trials conducted at multiple locations in the season 2002-2003. The levels of Cry3Bb1, NPTII and CP4 EPSPS proteins in grain and forage are presented below.

The mean levels of Cry3Bb1 and CP4 EPSPS in MON 863 × NK603 grain across all sites were 34 and 12 µg/g dry weight, respectively. The mean levels of Cry3Bb1 and CP4 EPSPS in MON 863 × NK603 forage across all sites were 40 and 110 µg/g dry weight, respectively. The values given for CP4 EPSPS represent the sum of both CP4 EPSPS and CP4 EPSPS L214P, as the ELISA analytical method recognizes both these proteins. All measured NPTII protein levels in grain were below the limit of detection (LOD < 0.21 µg/g fresh weight).

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

b) Parts of the plant where the insert is expressed

The levels of Cry3Bb1, CP4 EPSPS and NPTII were assessed in forage, grain, leaf, root and pollen, using validated enzyme-linked immunosorbent assays (ELISA). However, only results for forage and grain tissue samples are presented in this summary, because these are most relevant tissues for the evaluation of the food and feed safety of MON 863 × NK603.

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

a) Reproduction

Comparative assessments of the phenotypic and agronomic characteristics of MON 863 and NK603 and conventional maize have been conducted at multiple sites in the field. The experience gathered from these trials and from extensive commercial plantings demonstrates that, except for the respective protection against target coleopteran pests and tolerance to glyphosate, there are no biologically significant differences in the reproductive capability, dissemination or survivability of MON 863 or NK603 when compared to conventional maize.

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

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

Regardless, it should be noted that the scope of the current renewal application does not include the cultivation of MON 863 × NK603 varieties in the E.U. but only the renewal of the authorisation for the continued marketing of existing MON 863 × NK603-derived feed materials, feed additives and food additives, entered in the Community Register of GM Food and Feed, in the E.U.

b) Dissemination

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

c) Survivability

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

d) Other differences

Comparative assessments in the field did not reveal any biologically significant differences between MON 863 × NK603 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 × NK603 hybrid seed (F1) is produced by traditional breeding by crossing MON 863 and NK603 inbred lines (each homozygous for the respective insert). Thereby, each parental line passes on its inserted DNA sequence to the resulting MON 863 × NK603 plant.

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

Southern blot analysis of MON 863 × NK603 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 × NK603, no changes are to be expected in the ability of the GM plant to exchange genetic material with bacteria.

b) Plant to plant gene transfer

Since reproductive morphology in the single-trait parental lines and in MON 863 × NK603 are unchanged compared to conventional maize, pollen production and pollen viability are not expected to be affected by the genetic modification. Therefore, the outcrossing frequency to other maize varieties or to wild relatives (which are not present in the E.U.) is unlikely to be different for MON 863 × NK603 when compared to MON 863 and NK603 or to conventional maize varieties. However, it should be noted that the scope of the current renewal application does not include the cultivation of MON 863 × NK603 varieties in the E.U.

but only the renewal of the authorisation for continued marketing of existing MON 863 × NK603 -derived feed materials, feed additives and food additives, 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

Compositional analyses were performed on forage and grain samples from MON 863 × NK603, grown at four sites in Argentina during the 2002 – 2003 season. The study also included compositional analyses of forage and grain collected from a conventional control hybrid and thirteen conventional commercial reference hybrids, which were grown in replicated plots at the same field sites as MON 863 × NK603. Statistical analyses showed that 99.7% (309) of the 310 comparisons made between MON 863 × NK603 and the conventional control were either not statistically significantly different or they were with 95% confidence within the calculated 99% tolerance interval for the population of commercial reference hybrids.

The one remaining statistical difference was only noted at one site. In addition, this one value was within the range of values reported in previous compositional studies of maize. Therefore, this single statistical difference was not considered to be biologically meaningful.

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

7.2 Production of material for comparative assessment

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

The compositional analysis study of the forage and grain from MON 863 × NK603 was conducted at four replicated field sites in Argentina in 2002 – 2003. Two sites were located in the province of Buenos Aires and the other two sites in the province of Cordoba. At each field site, the seed starting materials were planted in a randomized complete block design with three replicates per block.

b) the baseline used for consideration of natural variations

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

7.3 Selection of material and compounds for analysis

Compositional analyses were conducted on grain and forage from MON 863 × NK603 hybrids and conventional counterpart.

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

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

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

7.4 Agronomic traits

The scope of this application is limited to the renewal of the authorisation for continued marketing of existing MON 863 × NK603-derived feed materials, feed additives and food additives in the E.U., but does not include the cultivation of MON 863 × NK603 varieties in the E.U. The observations from environmental releases provide evidence confirming the absence of significant unintended or unanticipated effects of the genetic modifications present in this maize.

The agronomic and phenotypic equivalence of MON 863 × NK603 to conventional maize was confirmed during the development and evaluation of MON 863 × NK603 in the field. Observations from field trials and commercial cultivation in North America show that, except for the introduced traits, MON 863 × NK603 is agronomically, phenotypically and morphologically equivalent to conventional maize (*see* Section D.4.a).

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

7.5 Product specification

MON 863 × NK603-derived feed materials, feed additives and food additives 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 × NK603 results from traditional breeding of MON 863

and MON 810, it contains both inserts in combination, which confer protection against coleopteran insect pests and tolerance to glyphosate. The presence of the coleopteran-protection and glyphosate-tolerance traits in MON 863 × NK603 -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 NK603. The unambiguous detection of MON 863 × NK603 in mixed consignments of maize product requires single samples to be subjected to detection methods for both MON 863 and NK603, and to test positive for both.

The event specific methods of detection of MON 863 and NK603 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 × NK603 on their website[6].

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 × NK603 is substantially equivalent and as safe and as nutritious as conventional maize, the use of MON 863 × NK603 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 × NK603 foods and feeds 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, feed additives and food additives produced from MON 863 × NK603 were first placed on the E.U. market in 2003. In July 2004 these products were notified to the European Commission, following Articles 8(1)(b) and 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 × NK603-derived feed materials, feed additives and food additives 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 × NK603-derived products.

7.8 Toxicology

7.8.1 Safety assessment of newly expressed proteins

MON 863 × NK603 is produced by traditional breeding of MON 863 and NK603 inbred lines. Both of the introduced traits from the parental lines are inherited in MON 863 × NK603. This results in the combined expression of the Cry3Bb1, NPTII and CP4 EPSPS proteins in the same plant. These introduced proteins are present at low levels and were demonstrated to be safe for animal and human health.

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

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

- History of safety for both proteins.
- Cry3Bb1 is selective for certain coleopteran insects, with no activity against other types of living organisms such as mammals, fish, birds or invertebrates.
- NPTII, which has no insecticidal activity, is ubiquitous in the environment and is found in microbes present in food and within the human digestive system.
- Rapid digestion of both proteins in *in vitro* simulated gastric fluids.
- No significant amino acid sequence similarity to known protein toxins (other than *B.t.* proteins for Cry3Bb1) and no immunologically relevant sequence similarity with known allergens.
- Lack of acute toxicity for both proteins as determined by a mouse acute gavage study at dose levels orders of magnitude higher than those encountered in human or animal diets.

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

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

7.8.2 Testing of new constituents other than proteins

Since maize is known as a common source of food and feed with a centuries-long history of safe use and consumption around the world, and as MON 863 × NK603 was shown to be compositionally 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 × NK603 and conventional maize have been established by compositional analysis. In addition, the wholesomeness and safety of MON 863 × NK603 has been confirmed in a 42-day 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 CP4 EPSPS proteins expressed in MON 863 × NK603 has previously been demonstrated for the single-trait parental lines. These proteins are present at very low levels in grain and were assessed for their potential allergenicity by a variety of tests, including a) whether the genes came from allergenic or non-allergenic sources, b) sequence similarity to known allergens, and c) pepsin stability of the protein in an *in vitro* digestion assay. In all cases, the proteins did not exhibit properties characteristic of allergens.

7.9.2 Assessment of allergenicity of the whole GM plant or crop

As the introduced proteins do not have allergenic potential, it was concluded that the use of MON 863 × NK603 for food or feed does not lead to an increased risk for allergenic reactions compared to the equivalent range of food and feed uses of traditional maize.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

MON 863 × NK603 inherits the inserts present in MON 863 and NK603. The introduced traits of coleopteran-protection and glyphosate-tolerance are of agronomic interest and do not change the nutritional aspects of this maize. Hence this maize is not expected to be more or less attractive for the production of feed materials, feed additives and food additives. Therefore, anticipated dietary intake of maize-derived products is not expected to be altered upon the renewal of the MON 863 × NK603 authorisation and no nutritional imbalances are expected as a result of the use of MON 863 × NK603-derived feed materials, feed additives and food additives.

7.10.2 Nutritional assessment of GM feed

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

When individual treatment comparisons were made, broilers in general performed and had similar carcass yields and meat composition when fed diets containing MON 863 × NK603, the conventional hybrid, and commercially available reference hybrids. The MON 863 × NK603 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 MON 863 × NK603, which showed that there were no biologically relevant differences in nutritional and compositional properties relative to control and reference maize. These data confirm the conclusion that MON 863 × NK603 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 × NK603 as no signs of adverse or unanticipated effects have been observed in a number of safety studies, including animal feeding studies using doses of administration that are orders of magnitude above expected consumption levels. The pre-market risk characterisation for food and feed use of MON 863 × NK603 demonstrates that the risks of consumption of this maize or its derived products are consistently negligible and no different from the risks associated with the consumption of 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 this maize for food and feed 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, feed additives and food additives produced from MON 863 × NK603 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 maize MON 863 and NK603, as well as the validation report for MON 863 × NK603, 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 × NK603 was grown commercially in U.S.A. Prior to commercialisation, this maize as well as its parental single-trait maize lines, MON 863 and NK603, were tested extensively at multiple locations in the field.

b) Authority overseeing the release

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

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 × NK603, indicating that post-release monitoring would not be necessary.

In addition, the commercialisation of MON 863 × NK603 is accompanied by stewardship programmes to ensure correct handling of this maize by downstream stakeholders (implementation of good agricultural practice for cultivation; ensure a channel of communication in the unlikely event that unanticipated adverse effects might occur).

No unanticipated effects have been observed during field testing or since commercialization of MON 863 × NK603.

g) Duration of post-releases monitoring

Please see question E.2.(f)

h) Conclusions of post-release monitoring

Please see question E.2.(f)

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

Field-testing and post-marketing experience provided no significant evidence that grain or derived products from MON 863 × NK603 are likely to cause adverse effects to human health, animal health or the environment.

3. Links (some of these links may be accessible only to the competent authorities of the Member States, to the Commission and to EFSA):

a) Status/process of approval

The EFSA website http://www.efsa.europa.eu/en/science/gmo/gm_ff_applications.html provides information related to the applications submitted under Regulation (EC) No 1829/2003 on genetically modified food and feed: EFSA-GMO-UK-2004-06.

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

A notification for MON 863 × NK603 according to Directive 2001/18/EC

has not been submitted by Monsanto.

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/703.html) on Application EFSA-GMO-UK-2004-06 for authorisation of MON 863 × NK603, 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)

EFSA provides a link to the publicly accessible summary of this renewal application, as well as to the summary of application EFSA-GMO-UK-2004-06 submited under Regulation (EC) No 1829/2003, at http://www.efsa.europa.eu/en/science/gmo/gm_ff_applications/more_info/703.html