Application for renewal of the authorisation for continued marketing of existing feed materials, feed additives and food additives produced from MON 863 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. In countries where MON 863 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, indicating clearly to growers that the hybrid is protected from specific coleopteran 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 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.

[1] Hereafter referred to as MON 863

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 **Approved for commercial release**	

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 July 2002, Monsanto submitted an application for import and use of MON 863 as any other maize (excluding cultivation) under Directive 90/220/EEC to Germany, the country acting as *Rapporteur* for this file. The application was then amended to fulfill the new 2001/18/EC requirements. Germany issued an Initial Assessment Report, which was forwarded to the EU Member States for further review in April 2003. The European Food Safety Authority (EFSA) evaluated the application and adopted a positive scientific opinion on 02 April 2004. After consideration by a Regulatory Committee, composed of Member States experts, and the Council of Environment Ministers, MON 863 was approved for import, feed and processing by the European Commission on the 8th of August 2005 (Commission Decision 2005/608/EC).

In July 2002, Monsanto also submitted an application under the novel food and novel food ingredients Regulation (EC) N° 258/97 to Germany as lead Member State in July 2002. Germany issued an Initial Assessment Report requesting a scientific opinion on the *nptII* antibiotic resistance marker gene, which was forwarded to the EU Member States for review in June 2003. The EFSA evaluated the complete dossier and adopted a scientific opinion on 02 April 2004, concluding that "the placing on the market of MON 863 is unlikely to have an adverse effect on human and animal health or the environment in the context of its proposed use." (http://www.efsa.eu.int/science/gmo/gmo_opinions/383_en.html). On that same date, EFSA issued an opinion with regard to the *nptII* gene, stating that "(…) there is no rationale for inhibiting or restricting its use, either for field experimentation or for the purpose of placing on the market." (http://www.efsa.eu.int/science/gmo/gmo_opinions/384_en.html). After consideration by a Regulatory Committee, composed of Member States experts, and the Council of Agriculture Ministers, MON 863 was approved by the European Commission on the 13th of January 2006 (Commission Decision 2006/68/EC).

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

Yes (x)	No ( )

If *yes*, specify

MON 863 has been approved in the United States, Canada, Japan, Korea, Taiwan, the Philippines, Australia/New Zealand, China, Russia, Singapore, Mexico and the EU.

The scope of the approvals granted for these genetically modified organisms and the status of pending regulatory reviews typically depend on the country and its local regulatory framework.

8. General description of the product

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

MON 863 has been developed to produce the Cry3Bb1 protein that confers protection against certain coleopteran pests. MON 863 was produced with particle bombardment technology using the plasmid vector PV-ZMIR13.

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 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-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-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

MON 863 is substantially equivalent to conventional maize except for its introduced trait: protection against certain coleopteran insect pests, which is a trait of agronomic interest. This maize was shown to be as safe and as nutritious as conventional maize. Therefore, MON 8963-derived feed materials, feed additives and food additives will be stored, packaged, transported, handled and used 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 MON 863.

e) Any proposed packaging requirements

MON 863 is substantially equivalent to conventional maize (except for the introduced coleopteran-protection trait). Therefore, MON 863-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)).

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

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 grain and derived products.

Operators shall be required to label foods and feeds derived from MON 863 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-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 labeling requirements for MON 863 Therefore, no further specific measures are to be taken by the notifier.

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

MON-ØØ863-5

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 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 is unlikely, as the proposed uses for this maize are included in the current food and feed uses of conventional maize. MON 863 hybrids are substantially equivalent to other maize hybrids except for the introduced trait: protection against certain coleopteran insect pests, which is a trait 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-derived products are the same as those for conventional maize. No specific conditions are warranted or required for the continued marketing of MON 863-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

N/A

e) Cultivar/breeding line

MON 863

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 consent for the environmental release of MON 863 according to Directive 2001/18/EC, Part C. 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 in the E.U but only the continued use of existing food and feed products derived from MON 863.

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

A DNA sequence encoding: i) a Cry3Bb1 protein variant, MON 863 Cry3Bb1 protein, which confers protection to certain coleopteran insects and ii) the NPTII protein (neomycin phosphotransferase II) which provides resistance towards kanamycin for maize plant cell selection purposes, was inserted into maize cells. The insertion of this DNA fragment was performed by using the particle bombardment technology.

2. Nature and source of the vector used

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

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

The transformation fragment PV-ZMIR13L contained the two genes to be introduced in the plant cells, *i.e.*, the chimearic *cry3Bb1* gene (encoding the agronomic trait) and the *nptII* gene (selectable marker) designed to be expressed in the maize cells. The expression cassettes corresponding to these two genes consist of respectively: i) a *cry3Bb1* open reading frame (ORF) regulated by the 4-AS1 plant promoter and the wtCAB leader, rice actin intron and tahsp17 3' polyadenylation sequence and ii) the *nptII* ORF regulated by the 35S promoter and the NOS 3' polyadenylation sequence. A description of each of these elements is given in Table 1.

Table 1. Elements of the transformation vector PV-ZMIR13

Size (Kb)	Genetic element	Origin	Function
Vector			
0.65	Ori-pUC	pUC	plasmid replication
0.95	*nptII*	Tn5, *E.coli*	resistance to a category of aminoglycosides comprising kanamycin, and neomycin
Transformation fragment			
MON 863 cry3Bb1 gene cassette			
0.22	4AS1	Promoter 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	Associated with high level of protein expression in roots
0.06	wtCAB	5' untranslated leader of the wheat chlorophyll a/b-binding protein	Translation enhancement
0.49	*ract1* intron	Intron from the rice actin 1 gene	Transcription enhancement
1.96	MON 863 *cry3Bb1*	Open reading frame (ORF) encoding a synthetic variant of Cry3Bb1 protein of *Bacillus thuringiensis* subsp. *kumamotoensis*	Carries the insect protection trait
0.23	tahsp 17 3'	3' non-translated region of the gene encoding the wheat heat shock protein 17.3	Ends transcription and directs polyadenylation
Selectable marker elements			
0.32	35S	35S cauliflower mosaic virus promoter	Regulate expression in plant cells
0.82	*nptII*	ORF encoding the neomycin phosphotransferase II enzyme (NPTII). Derived from the *E.coli* transposon Tn5.	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
0.15	*ble* (truncated)	ORF located downstream to the *nptII* gene derived from the *E. coli* transposon Tn5. In *E. coli* it codes for a truncated non-functional protein (the full length protein confers resistance to bleomycin). It is not expressed in plants	Non-functional *ble* has been subcloned together with the *nptII* ORF from which it shares the same prokaryotic operon
0.26	NOS 3'	3' non-translated region of the nopaline synthase gene of *Agrobacterium tumefaciens* T-DNA	Ends transcription and directs polyadenylation

D. INFORMATION RELATING TO THE GM PLANT

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

MON 863 maize plants are protected from damage due to feeding by the larvae of a major corn pest, corn rootworm (*Diabrotica virgifera*, CRW). The tissues of MON 863 maize contain a modified *cry3Bb1* gene, derived from *Bacillus thuringiensis*, which encodes a modified *B.t.* Cry3Bb1 protein for CRW control. The specific CRW control protein which is detailed in this application is MON 863 Cry3Bb1, which differs only by slight modifications from the *Bacillus thuringiensis* Cry3Bb1 protein. The MON 863 Cry3Bb1 protein is almost 99% amino acid sequence identical to *Bacillus thuringiensis* Cry3Bb1 and falls well within the >95% amino acid sequence identity range defined for Cry proteins in the Cry3Bb class. This protein has almost 99% identity in amino acid sequence with the Cry3Bb1 protein contained in the registered product Raven® (Ecogen, Inc.).

Corn rootworm-protected maize provides an alternative to soil insecticides that are currently used for control of this coleopteran pest. CRW-protected maize offers growers a unique new management tool for CRW, which reduces or eliminates the risks associated with chemical transportation, storage, application, disposal, and stewardship. Agro-ecosystems benefit from the specificity of the product to CRW and the lack of harmful effects on beneficial insects or wildlife. CRW-protected maize is fully compatible with current management protocols for CRW, including integrated pest management (IPM).

2. Information on the sequences actually inserted or deleted

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

MON 863 contains a single DNA insert containing a single copy of the introduced DNA fragment, and this at a single locus in the maize genome.

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

One single complete copy of the transformation fragment has been inserted at one site of the plant nuclear genome. This copy contains two ORFs, *MON 863 cry3Bb1* and *nptII*, which are expressed. Segregation of the traits occurs according to Mendelian genetics.

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

MON 863 maize was produced by particle acceleration technology using a *Mlu*I DNA restriction fragment from plasmid PV-ZMIR13 containing

the *nptII* and *MON 863 cry3Bb1* cassettes. The MON 863 event contains one DNA insert located on a 5.0 Kb *Nde*I fragment. This insert contains one copy of the fragment used in transformation. No additional elements from the DNA fragment used in transformation, linked or unlinked to intact cassettes, were detected in the genome. PCR and DNA sequencing were used to verify the 5' and 3' junction sequences of the insert with the plant genome, as well as the intactness of the 5' and 3' ends of the insert. Homologies with mitochondrial DNA were seen on the 5' end and homologies with plant genomic DNA on the 3' end. Approximately 10 bp from the 3' end of PV-ZMIR13L, including the *Hind*III restriction site, are missing; however, the tahsp17 3' polyadeylation sequence is intact. Additionally, event MON 863 does not contain any detectable plasmid backbone sequence including *ori*-pUC or the *nptII* coding region regulated by a bacterial promoter. These data support the conclusion that only the two expected full length proteins, MON 863 Cry3Bb1 and NPTII, should be encoded by the insert in event MON 863. In addition, the genetic stability of the inserted DNA was demonstrated by Southern blot analysis on genomic DNA from nine consecutive generations

3. Information on the expression of the insert

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

Levels of MON 863 Cry3Bb1 and NPTII proteins were determined in tissues collected from MON 863 maize plants grown under field conditions at multiple sites.

Tissue samples were collected from plants grown in four U.S. field trials during the 1999 growing season. Three additional sites in Argentina were used for harvesting of pollen. Collectively these sites provided a variety of environmental conditions representative of regions where corn rootworm-protected maize would be grown as commercial products. MON 863 and MON 846 maize were planted in four plots at each location.

Mean levels of MON 863 Cry3Bb1 protein declined during the growing season in leaf tissue, whole plant and root tissue of MON 863 maize. Mean levels in root tissue ranged from a high of 58 µg/g in young plants to a low of 24 µg/g in senescent plants. MON 863 Cry3Bb1 protein levels in root tissue were sufficient to confer protection from CRW feeding damage during the critical early periods of plant development. MON 863 Cry3Bb1 levels in pollen and silk were 62 and 10 µg/g, respectively.

b) Parts of the plant where the insert is expressed

Young leaf, grain, root, forage and pollen were collected at appropriate times of plant development.

Mean levels of MON 863 Cry3Bb1 protein were 81 µg/g in young leaf, 70 µg/g in grain, 41 µg/g in root, 39 µg/g in forage and 62 µg/g in pollen.

NPTII protein levels in all tissues tested ranged from non-detectable (<0.076 µg/g) to 1.4 µg/g. A control molecular analysis of DNA from MON 863 maize leaf and grain tissues confirmed the preservation of

event identity across all sites in the U.S.

Molecular analysis also confirmed the expected absence of *MON 863 cry3Bb1* and *nptII* coding sequences in the control plants.

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

a) Reproduction

Agronomic data collected from trials performed with MON 863 have demonstrated that MON 863 has not been altered in survival, multiplication or dissemination characteristics when compared to conventional maize varieties. The introduced trait for insect-protection has no influence on maize reproductive morphology and hence no changes in seed dissemination would be expected.

b) Dissemination

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

c) Survivability

Maize is known to be a weak competitor in the wild, which cannot survive in Europe outside cultivation without the aid of human intervention. Field observations have demonstrated that MON 863 has not been altered in its survivability when compared to conventional maize.

d) Other differences

Comparative assessments of the phenotypic and agronomic characteristics did not reveal biologically significant differences between MON 863 and conventional maize, except for the introduced trait.

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

MON 863 contains one insert with a single copy of the transformed DNA, which is stably integrated into the nuclear maize genome. The insert is inherited in a Mendelian fashion. This has been confirmed by Southern blot analyses.

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

a) Plant to bacteria gene transfer

None of the genetic elements introduced in MON 863 maize has a genetic transfer function. Therefore, no changes are expected in the ability of this maize to transfer genetic material to bacteria.

b) Plant to plant gene transfer

Since reproductive morphology in MON 863 is 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 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 varieties in the E.U. but only the renewal of the authorisation for continued marketing of existing MON 863-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

MON 863 was previously shown to be substantially equivalent to conventional maize. Confirmatory compositional analyses were performed on forage and grain samples from MON 863 maize grown under representative field conditions in the E.U. in 1999/2000. The study also included compositional analyses of forage and grain collected from a near-isogenic, non-transgenic control hybrid with similar background genetics as the test product. The analytical results have shown that MON 863 is compositionally equivalent to the near-isogenic comparator used in the study. Some statistically significant differences between the test and control product were observed for some of the components, but further investigation showed that they likely occurred by chance and none of them were considered to be of biological significance. The observed differences were generally small and were not consistent across trial sites. Moreover, the component concentrations in MON 863 were consistent with baseline concentrations from commercial reference hybrids, and they fell within the wide compositional variability of component concentrations known for conventional maize.

In conclusion, as expected based on the substantial equivalence of MON 863 to conventional maize, the field trials confirmed that MON 863 is compositionally equivalent to conventional maize, with the exception of the introduced coleopteran-protection trait.

7.2 Production of material for comparative assessment

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

<u>1999 U.S. field season</u>

MON 863 and the conventional control maize were grown at four replicated field sites in major maize-growing areas of the U.S.A. (Iowa (2 sites), Illinois and Nebraska) during the 1999 field season.

Four commercial reference hybrids were also grown at two sites and five commercial reference hybrids at the remaining two sites to provide a total of 18 different reference hybrids. At each field site, the test, control and reference seed were planted in a randomized complete block design with four replicates per block except the reference lines that only had two replicates.

1999 / 2000 Argentinean field season
MON 863 and the conventional control maize were grown at four replicated field sites in three areas of Argentina (Fontezuela (2 sites), Salto and Rojas) during the 1999 / 2000 field season. Four commercial reference hybrids were grown also at each of the same field sites to provide a total of 16 different reference substances. At each field site, the test, control and reference seed were planted in a randomized complete block design with four replicates per block.

b) the baseline used for consideration of natural variations

These studies compared MON 863 to the control. Reference hybrids were grown in the same field locations and under the same conditions as the test and control. Where statistical differences occurred, the measured analyte was compared to a confidence interval developed from the reference hybrids. Differences were also compared to ILSI ranges and ranges reported in literature.

7.3 Selection of material and compounds for analysis

As described in Section D.7.1, compositional analyses were conducted on grain and forage from MON 863 and non-transgenic counterparts.

Forage samples were analyzed for proximates (ash, carbohydrates, total fat moisture and protein), acid detergent fibre (ADF) and neutral detergent fibre (NDF). Compositional analyses of the grain samples included amino acids, fatty acids (C8-C22), fibre (ADF, NDF), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc), proximates (ash, carbohydrates, total fat moisture and protein), vitamin E and anti-nutrients (phytic acid and trypsin inhibitor). In all, 51 different analytical components (nine in forage and 68 in grain) were analyzed.

The results of this extensive compositional analysis conducted for MON 863, compared to conventional maize hybrids, do not indicate a need for further analysis of 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-derived feed materials, feed additives and food additives in the E.U., but does not include the cultivation of MON 863 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.

Field trials with MON 863 were performed and the set of agronomic observations supports a conclusion that from an agronomic and

phenotypic (morphological) point of view, MON 863 is equivalent to conventional maize, except for the introduced insect-protection trait.

The lack of differences in phenotypic and agronomic characteristics is consistent with the results of the compositional analyses (Section D.7.1.) Taken together, the agronomic, phenotypic and compositional analyses support the conclusion that MON 863 is substantially equivalent to conventional maize except for the introduced traits of glyphosate-tolerance and insect pest protection.

7.5 Product specification

MON 863 will be imported into the E.U. in mixed shipments of maize grain and products, produced in other world areas, for use by operators that have conventionally been involved in the commerce, processing and use of maize and maize derived products in the E.U.

The event specific methods of detection of MON 863 was 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 on their website[3].

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 is substantially equivalent and as safe and as nutritious as conventional maize, the use of MON863 for the production of foods and feeds is not different from that of conventional maize. Consequently, any effects of the production and processing of MON 863 foods and feeds are not expected to be 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 were first placed on the E.U. market in autumn 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-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-derived products.

7.8 Toxicology

7.8.1 Safety assessment of newly expressed proteins

The assessment of a potential for toxicity of the Cry3Bb1 and NPTII proteins is based on the established premise that a protein is not likely to

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

have a toxic effect if: 1) the proteins have a demonstrated history of safe use; 2) the proteins have no structural similarity to known toxins or other biologically active proteins that could cause adverse effects in humans or animals; and 3) the proteins do not exert any acute toxic effects to mammals.

In addition, the low concentration of introduced proteins in tissues that are consumed and the rapid digestibility in simulated digestive fluids provide additional assurance for their safety.

Based on the multiple studies performed to assess the human and animal safety of MON 863 Cry3Bb1 and NPTII, these proteins are unlikely to cause any adverse effects. In addition, compositional analysis has demonstrated that MON 863 maize is compositionally and nutritionally equivalent to other maize varieties. These results are also correlated by the agronomical equivalence between MON 863 and conventional maize.

MON 863 maize can therefore be considered as safe as any other maize variety in commerce today.

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 was shown to be substantially equivalent to conventional maize, no testing of any constituents other than the introduced proteins is indicated.

7.8.3 Information on natural food and feed constituents

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

7.8.4 Testing of the whole GM food/feed

The compositional and nutritional equivalence of grain and forage from MON 863 and conventional maize have been established by compositional analysis. In addition, the wholesomeness and safety of MON 863 has been confirmed in a 42-day feeding study using broiler chickens and a 90-day study using rats. Published literature and information presented at scientific meetings also indicates no effects of MON 863 on lactating cows, beef cattle or swine when introduced into their diets as any other maize.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein

Cry3Bb1 and NPTII 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-derived feed materials, feed additives and food additives, 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

The introduced traits in MON 863 maize are of agronomic interest and are not intended to change any nutritional aspects of this maize. The presence of these traits is not expected to alter patterns or volumes of maize consumption.

In addition to the extensive compositional analyses which demonstrated the substantial equivalence of MON 863 maize to conventional maize (except for the introduced traits), a confirmatory feed performance study was conducted in rapidly growing broiler chickens. This study confirmed the nutritional equivalence of MON 863 maize for use as food or feed and demonstrated the absence of any pleiotropic or unanticipated effects from the two introduced traits.

In conclusion, MON 863 maize is nutritionally equivalent to non-transgenic control maize, as well as to conventional maize varieties in commerce. Hence this maize is not expected to be more or less attractive for use as food (or feed), for processing or as a food (or feed) ingredient. Therefore, anticipated dietary intake of maize-derived foods and feeds is not expected to be altered upon commercialisation of MON 863 maize and no nutritional imbalances are expected as a result of the use of MON 863 maize for food or feed or processing..

7.10.2 Nutritional assessment of GM feed

The introduced trait in MON 863 is of agronomic interest, and is not intended to change any nutritional aspects of this maize. In addition to the extensive compositional analyses which demonstrated the substantial equivalence of MON 863 to conventional maize (except for the introduced trait), a confirmatory feed performance study was conducted in rapidly growing broiler chickens. Broilers were fed diets containing grain from MON 863, and their performance was compared to control groups fed diets containing a non-transgenic control hybrid or commercially available reference hybrids. This study confirms the nutritional equivalence of MON 863 for use as feed, and demonstrates the absence of any pleiotropic or unanticipated effects from the introduced trait.
In conclusion, MON 863 is nutritionally equivalent to non-transgenic control maize, as well as to maize varieties in commerce..

7.11 Post-market monitoring of GM food/feed

There are no intrinsic hazards related to MON 863 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 demonstrates that the risks of consumption of MON 863 and its derived products are consistently negligible and not 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, feed additives and food additives produced from this maize is not appropriate.

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

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.

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 and if the applicant has clearly shown that environmental exposure is absent or will be at levels or in a form that does not present a risk to other living organisms or the abiotic environment)

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 maize, as well as the validation report, prepared by the Community Reference Laboratory (CRL) in collaboration with the European Network of GMO Laboratories (ENGL), are published on the CRL website (http://gmo-crl.jrc.it/statusofdoss.htm)

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

No release has been notified under Part B of the Directive 90/220/EEC and under Part B of Directive 2001/18/EC.

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

Since 1998, MON 863 has been field tested in several countries, including USA, Argentina, Japan, Canada and Chile. First commercialisation of MON 863 varieties took place in the U.S.A. in 2003.

b) Authority overseeing the release

USA : United States Department of Agriculture and Environmental Protection Agency

Argentina : Secretary of Agriculture (SAGPyA) - CONABIA

Japan : Ministry of Agriculture, Forestry and Fisheries (MAFF)

Canada : Canadian Food Inspection Agency

Chile : Ministry of Agriculture and Cattle (SAG : Servicio Agrícola y Ganadero) and CALT: Comité Asesor para la Liberación de Transgénicos

c) Release site

USA :Mainly in the states of the corn belt and in Hawaii and Porto Rico

Argentina : Bragado, Salto, Rojas

Japan : Monsanto Japan Kawachi Research Farm in Ibaraki prefecture

Canada : Various sites in Southern Ontario/Quebec

Chile : Different site from the seeds Companies fields along Chile

d) Aim of the release

USA : Assess the performances : efficacy, yield, breeding, ...

Argentina : Compare different hybrid phenotypes and yielding performance

Japan : Requirement for import approval

Canada : Assess agronomic performance

Chile : Requirement for import and re-export approval

e) Duration of the release

USA : Field releases - one year

Argentina : One year

Japan : May 2000 - March 2001

Canada : Typical growing season (May-October 00/01/02)

Chile : One year

f) Aim of post-releases monitoring

USA : Destroy volunteers to prevent persistence in the environment of the regulated material

Argentina : Assess for volunteers

Japan : Not required

Canada : Removal of volunteers

Chile : Destroy volunteers to prevent persistence in the environment

g) Duration of post-releases monitoring

USA: One year

Argentina : One year

Japan : Not applicable

Canada : One year

Chile : One year

h) Conclusions of post-release monitoring

USA : Volunteers have been eliminated to prevent persistence in the environment

Argentina : Nothing to report

Japan : Not applicable

Canada : No conclusion required. Monitoring ensured containment of the novel genetic material

Chile : Nothing to report

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

USDA : The reports of these field releases do not indicate any adverse effects on the environment and do not address human health.

Argentina : No risk to human health and environment.

Japan : MAFF granted the environmental safety approval for the grain import on May 8, 2001 and also granted the feed safety approval on February 28 in 2002. Ministry of Health, Labor and Welfare (MHLW) granted the food safety approval on February 21 in 2002.

Canada : Nothing special to report

Chile : Nothing special to report

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/gmc_browse.aspx?DossClass=3 and http://gmo-crl.jrc.it/statusofdoss.htm provide publicly accessible links to up-to-date databases on the regulatory progress of notifications under Directive 2001/18/EC, including the Monsanto dossier for MON 863.

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 Germany, the Lead Member State for Monsanto notification on MON 863 (Application C/DE/02/9).

c) EFSA opinion

On 02 April 2004, the EFSA issued its opinion (http://www.efsa.europa.eu/etc/medialib/efsa/science/gmo/gmo_opinions/383.Par.0001/File.dat/opinion_gmo_07_en1.pdf) on the applications for authorisation of MON 863 according to Directive 2001/18 and Regulation (EC) No. 258/97, 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.