Application for renewal of the authorisation for continued marketing of existing feed materials, feed additives and food additives produced from NK603 × MON 810 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: NK603 × MON 810. In countries where NK603 × MON 810 varieties are being cultivated, packages of hybrid seed of this maize are marketed under the name of the hybrid variety, in association with the trademarks Roundup Ready® Corn 2 with YieldGard® Corn Borer, indicating clearly to growers that the hybrid is tolerant to Roundup® herbicide and protected from specific lepidopteran insect pests.

d) Date of acknowledgement of notification

Not known at the time of application

2. Applicant

a) Name of applicant

Monsanto Company, represented by Monsanto Europe S.A.

b) Address of applicant

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

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

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

NK603 × MON 810 maize[1] will continue to be traded and used in the European Union in the same manner as current commercial maize and by the same operators currently involved in the trade and use of maize.

® Roundup, Roundup Ready and YieldGard are registered trademarks of Monsanto Technology LLC.

[1] Hereafter referred to as NK603 × MON 810

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 *ye*s, specify	

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

Yes (x)	**No ( )**
If *n*o, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC	

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 *ye*s, specify	

A notification pursuant to Directive 2001/18/EC (C/GB/02/M3/3) for import and processing of NK603 × MON 810 in the E.U., excluding the cultivation of varieties, was submitted in April 2002. The notification received a favourable opinion from the UK Lead Member State in March 2004, progressed through the Member States consultation and finally received a favourable opinion from the EFSA GMO Panel on 13 October 2005.

In addition, an application under Regulation (EC) No 1829/2003 (EFSA-GMO-UK-2004-01) for use of NK603 × MON 810 as food or feed, was submitted in June 2004. This application received a positive opinion from EFSA on 31 March 2006.

In January 2004, a notification according to Directive 2001/18/EC (C/ES/04/01) for import of NK603 × MON 810 in the E.U. and use thereof as any other maize for import, processing and food and feed, including the cultivation of varieties, was submitted. Progress of this application depends on the advancement of Application EFSA-GMO-NL-2005-26 under Regulation (EC) No 1829/2003, for cultivation of NK603 × MON 810 in the E.U., which was submitted in October 2005.

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

Yes (x)	**No ( )**

If *yes*, specify

Cultivation of NK603 × MON 810 is lawful in the U.S.A., Canada, Honduras, the Philippines and South Africa, while importation of derived foods and feeds is lawful in Australia, Japan, Korea, New Zealand, Mexico and Taiwan.

8. General description of the product

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

NK603 × MON 810 consists of maize produced from NK603 and MON 810, using traditional methods. Although genetic modification was used in the development of NK603 and MON 810, no additional genetic modifications were involved for the production of NK603 × MON 810.

Like the single-trait product NK603, NK603 × MON 810 expresses CP4 EPSPS proteins derived from *Agrobacterium* sp. strain CP4, which confers tolerance to glyphosate containing herbicides, such as Roundup®. Like the second parental single-trait product MON 810, NK603 × MON 810 also expresses the Cry1Ab protein, derived from *Bacillus thuringiensis* subsp. *kurstaki*, which confers protection against predation by certain lepidopteran insect pests, including the European Corn Borer (*Ostrinia nubilalis*) and pink borers (*Sesamia* spp).

The use of NK603 × MON 810 plants enables the farmer 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. The use of NK603 × MON 810 also enables farmers to effectively control certain lepidopteran insect

pests in maize, ensuring maximum realization of yield potential, while removing the environmental burden of the production, packaging and transport of insecticides, previously used to control *Ostrinia nubilalis* and *Sesamia* spp.

b) Types of products planned to be placed on the market according to the authorisation applied for

The scope of the current renewal application includes feed materials, feed additives and food additives produced from NK603 × MON 810 which are lawfully placed on the market in the E.U., as listed in the Community Register of GM Food and Feed[2]. The range of uses of these NK603 × MON 810-derived products will be identical to the full range of equivalent uses of current commercial maize derived products.

c) Intended use of the product and types of users

NK603 × MON 810-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

NK603 × MON 810 is substantially equivalent to conventional maize except for its introduced (*i.e.* inherited) traits: tolerance to glyphosate and protection against certain lepidopteran insect pests, which are traits of agronomic interest. This maize was shown to be as safe and as nutritious as conventional maize. Therefore, NK603 × MON 810-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 NK603 × MON 810.

e) Any proposed packaging requirements

NK603 × MON 810 is substantially equivalent to conventional maize (except for the introduced glyphosate-tolerance and lepidopteran-protection traits). Therefore, NK603 × MON 810-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)

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

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 NK603 × MON 810 grain and derived products.

Operators shall be required to label foods and feeds derived from NK603 × MON 810 with the words "produced from genetically modified maize". In the case of products for which no list of ingredients exists, operators shall ensure that an indication that the food or feed product is produced from GMOs is transmitted in writing to the operator receiving the product.

Operators handling or using NK603 × MON 810-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 NK603 × MON 810. Therefore, no further specific measures are to be taken by the notifier.

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

Not applicable

h) If applicable, geographical areas within the EU to which the product is intended to be confined under the terms of the authorisation applied for. Any type of environment to which the product is unsuited

NK603 × MON 810 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 NK603 × MON 810 is unlikely, as the proposed uses for this maize are included in the current food and feed uses of conventional maize. NK603 × MON 810 hybrids are substantially equivalent to other maize hybrids except for the introduced (*i.e.* inherited) traits: tolerance to glyphosate and protection against certain lepidopteran insect pests, 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 NK603 × MON 810-derived products are the same as those for conventional maize. No specific conditions are warranted or required for the continued marketing of NK603 × MON 810-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** NK603 × MON 810
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 NK603 × MON 810. Outcrossing with

cultivated *Zea* varieties is therefore not expected in the context of this application.

Out-crossing with wild *Zea* species

Wild relatives of maize do not exist in Europe.

3. Survivability

a) Ability to form structures for survival or dormancy

Maize is an annual crop and seeds are the only survival structures. Natural regeneration from vegetative tissue is not known to occur.

b) Specific factors affecting survivability

Maize cannot survive without human assistance and is not capable of surviving as a weed due to past selection in its evolution. Volunteer maize is not found growing in fencerows, ditches or roadsides as a weed. Although maize seed from the previous crop year can over-winter in mild winter conditions and germinate the following year, it cannot persist as a weed. The appearance of "volunteer" maize in fields following a maize crop from the previous year is rare under European conditions. Maize volunteers are killed by frost or, in the unlikely event of their occurrence, are easily controlled by current agronomic practices including cultivation and the use of selective herbicides.

Maize grain survival is dependent upon temperature, moisture of seed, genotype, husk protection and stage of development. Freezing temperatures have an adverse effect on maize seed germination and have been identified as being a major risk in seed maize production. Temperatures above 45 °C have also been reported as injurious to maize seed viability.

4. Dissemination

a) Ways and extent of dissemination

In general, dissemination of maize may occur by means of seed dispersal and pollen dispersal. Dispersal of the maize grain is highly restricted in domesticated maize due to the ear structure including husk enclosure. For maize pollen, the vast majority is deposited in the same field due to its large size (90 to 100 µm) with smaller amounts of pollen deposited usually in a downwind direction. However, the current renewal application does not include the deliberate release of NK603 × MON 810 in the E.U but only the continued use of existing food and feed products derived from NK603 × MON 810.

b) Specific factors affecting dissemination

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

level of elevation. Dispersal of maize pollen is limited by its large size and rapid settling rate.

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

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

There are no 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 used in the production of NK603 × MON 810. Instead, NK603 × MON 810 is produced from a traditional breeding cross between NK603 and MON 810. Genetic modification (particle acceleration transformation method) was used in the development of the single-trait, parental NK603 and MON 810 lines.

2. Nature and source of the vector used

While NK603 × MON 810 results from traditional breeding, genetic modification was used in the development of the single-trait parental maize lines. 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*). MON 810 was also generated using the particle acceleration method, by the integration of sequences from the plasmid vector PV-ZMBK07, containing the *cry1Ab* coding sequence of interest, which was derived from *Bacillus thuringiensis* subsp. *kurstaki*.

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

NK603 × MON 810 is produced from NK603 and MON 810 single-trait lines by traditional breeding. Typically, NK603 × MON 810 hybrid varieties are produced by a single cross from NK603 and MON 810 inbred lines (homozygous for the respective insert). Less frequently, NK603 × MON 810 hybrid varieties can also be produced from a single cross of a traditionally-bred NK603 × MON 810 inbred line with a conventional maize inbred.

NK603 × MON 810 inherits the NK603 and MON 810 inserts from its parental lines. The individual components of the inserts and the function of these inherited DNA sequences are given in Tables 1 and 2.

Table 1. Genetic elements inherited from NK603

Genetic Element	Source	Size (kb)	Function
***cp4 epsps* gene cassette**			
P-*Ract1* / I-R*act1*	*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	Transcript termination sequence: 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-CTP*2*	*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[3]
T-*nos*	*Agrobacterium tumefaciens*	0.3	Ends transcription and directs polyadenylation of the mRNA.

Table 2. Genetic elements inherited from MON 810

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

[3] The substitution of leucine by proline in the CP4 EPSPS encoded by the second *cp4 epsps* gene in the 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

NK603 × MON 810 is produced by traditional methods of maize breeding, starting from NK603 and MON 810 single-trait lines. NK603 × MON 810 therefore expresses the CP4 EPSPS protein, which provides tolerance to glyphosate and the Cry1Ab protein, which confers protection against certain lepidopteran insect pests.

- As NK603, NK603 × MON 810 expresses the CP4 EPSPS protein, which imparts tolerance to glyphosate (N-phosphonomethyl-glycine), the active ingredient in the non-selective, foliar-applied, broad-spectrum, post-emergent herbicide Roundup.

- As MON 810, NK603 × MON 810 expresses the Cry1Ab protein derived from *Bacillus thuringiensis* subsp. *kurstaki*, which provides protection from certain lepidopteran insect pests, including European corn borer (*Ostrinia nubilalis*) and pink borers (*Sesamia* spp).

2. Information on the sequences actually inserted or deleted

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

As described in the respective applications for the single-trait parental maize lines, NK603 and MON 810 each contain a single DNA insert containing a single copy of the introduced DNA fragment, and this at different loci in the maize genome.

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

As the parental maize lines used to produce NK603 × MON 810 are inbred lines that are homozygous for the NK603 or MON 810 inserts, respectively, both of the inserted fragments are inherited by NK603 × MON 810, *i.e.* one fragment conferring the glyphosate-tolerance and one for the lepidopteran-protection trait. The presence of both inserts in NK603 × MON 810 was confirmed through Southern blot analysis, showing that the integrity of the inserts has been conserved in the combined-trait product.

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

Not applicable

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

NK603 × MON 810 contains both of the parental inserts on separate chromosomes in the nuclear genome, as they were present in parental NK603 and MON 810. The presence of these inserts in the combined-trait product was confirmed through Southern blot analysis.

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

As NK603 × MON 810 is the product of traditional breeding of NK603 and MON 810 and no additional genetic modification methods have been applied, and as the inherited DNA fragments have negligible potential to interact with one another, it is highly likely that NK603 × MON 810 contains each of the inserts as they were present in the parental NK603 and MON 810 lines. Therefore, the molecular characteristics of the introduced DNA sequences, known for the single-trait NK603 and MON 810 products, also apply to NK603 × MON 810, including the structural organization and integrity of the inserts, as well as the characteristics of the sites of insertion and the flanking sequences, immediately adjacent to the introduced sequences.

3. Information on the expression of the insert

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

Expression levels of the introduced proteins were measured in tissues collected from NK603 × MON 810 grown in the field. The levels of CP4 EPSPS and Cry1Ab proteins were measured in forage and grain samples of NK603 × MON 810 and are summarized below.

The mean levels of CP4 EPSPS across all sites were 36.3 µg/g fw in forage samples of NK603 × MON 810 and 12.7 µg/g fw in the grain. The mean level of the Cry1Ab protein across all sites was 6.06 µg/g fw in the forage and 0.73 µg/g fw in grain samples. Overall, the ranges across all sites for the CP4 EPSPS and Cry1Ab proteins levels in NK603 × MON 810 were comparable to the corresponding ranges in either NK603 or MON 810.

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 expressed in NK603 × MON 810 and NK603.

b) Parts of the plant where the insert is expressed

CP4 EPSPS and Cry1Ab proteins were estimated in forage and grain, which are the most relevant tissues in terms of food and feed safety evaluation.

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

a) Reproduction

Comparative assessments of the characteristics of the single-trait products versus conventional maize have been conducted at multiple sites in the field. The experience gathered from these trials and from extensive commercial planting of NK603 and MON 810 varieties demonstrates that, except for the introduced tolerance to glyphosate or the protection against certain lepidopteran insect pests, respectively, there are no biologically significant differences in the reproductive capability, dissemination or survivability of NK603 and MON 810 when compared to conventional maize.

Confirmatory observational data from comparative assessments in the field using NK603 × MON 810 and experience from commercial plantings in the U.S.A. confirm that no biologically relevant differences exist in maize that contains the NK603 and MON 810 inserts in combination. Compared to conventional maize, NK603 × MON 810 has not been significantly changed with respect to its dispersal or survival characteristics as assessed by a number of phenotypic (developmental, morphological, and agronomic) characteristics. NK603 × MON 810 is also not different from conventional maize in terms of potential invasiveness into natural environments and persistence in the environment. No significant differences in incidence of stressor symptoms were observed, other than the introduced protection of NK603 × MON 810 against certain target lepidopteran insect pests. Importantly, there is no information to indicate that there is a potential for NK603 × MON 810 to establish, persist and disperse to a greater extent than conventional maize.

Regardless, it should be noted that the scope of the current renewal application does not include the cultivation of NK603 × MON 810 varieties in the E.U. but only the renewal of the authorisation for the continued marketing of existing NK603 × MON 810-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 herbicide-tolerance and lepidopteran-protection have no influence on maize reproductive morphology and hence no changes in seed dissemination are to be expected.

c) Survivability

Maize is known to be a weak competitor in the wild, which cannot survive in Europe outside cultivation without human intervention. Field observations have demonstrated that NK603 × MON 810 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 NK603 × MON 810 and conventional maize, except for the two

introduced traits.

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

NK603 × MON 810 hybrid seed (F_1) is produced by traditional breeding, starting from NK603 and MON 810 inbred lines (made homozygous for either the NK603 or the MON 810 insert, respectively). Thereby, each parental line passes on its inserted DNA sequence to the resulting NK603 × MON 810 plant.

The single-trait products, NK603 and MON 810, each contain one insert with a single copy of the respective transformed DNA, which is stably integrated into the nuclear maize genome. In the progeny of NK603 and MON 810, each introduced 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 NK603 × MON 810 confirmed that the inserts from NK603 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 NK603 × MON 810, no changes are to be expected in the ability of the GM plant to exchange genetic material with bacteria.

b) Plant to plant gene transfer

Since reproductive morphology in the single-trait products and the NK603 × MON 810 combined-trait product are unchanged compared to conventional maize, pollen production and pollen viability are not expected to be affected by the genetic modifications. Therefore, the outcrossing frequency to other maize or to wild relatives (which are not present in the E.U.) is unlikely to be different for NK603 × MON 810 when compared to conventional maize. However, it should be noted that the scope of the current renewal application does not include the cultivation of NK603 × MON 810 varieties in the E.U. but only the renewal of the authorisation for continued marketing of existing NK603 × MON 810-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

NK603 and MON 810 were previously shown to be substantially equivalent to conventional maize. Confirmatory compositional analyses were performed on forage and grain samples from the combined-trait product, NK603 × MON 810, grown under representative field conditions in the E.U. in 2000. The study also included compositional analyses of forage and grain collected from a near-isogenic, conventional control hybrid with similar background genetics as the test product. The analytical results have shown that NK603 × MON 810 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 NK603 × MON 810 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 NK603 and MON 810 to conventional maize, the field trials confirmed that NK603 × MON 810 is compositionally equivalent to conventional maize, with the exception of the introduced (*i.e.* inherited) glyphosate-tolerance and lepidopteran-protection traits.

7.2 Production of material for comparative assessment

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

A comparative assessment of the composition of NK603 × MON 810 and conventional maize was conducted in France in 2000. The NK603 × MON 810 test hybrid, the near-isogenic control and five commercial reference products were grown at three sites located within important maize producing regions, representing a diversity of environmental conditions representative for regions in Europe where maize is grown commercially. The plants were grown under agricultural practices that are typical of maize production. At each location, the test product, the conventional control, and the five commercial reference hybrids were planted in single plots randomly assigned within each of four replication blocks. Within each replicate, products were blocked according to their glyphosate-tolerance.

b) the baseline used for consideration of natural variations

Compositional analyses were made for forage and grain samples from NK603 × MON 810. The study also included analyses of

forage and grain collected from a conventional control hybrid and five different conventional commercial maize hybrids, grown in replicated plots at the same field sites as NK603 × MON 810. Additional data from six commercial reference hybrids grown in the E.U. during the 1999 growing season were included for the construction of a statistically valid 99% tolerance interval for component concentrations in the population of commercial hybrids. 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

As described in Section D.7.1, compositional analyses were conducted on grain and forage from NK603 × MON 810 and conventional counterpart.

Grain samples were analysed for proximates (protein, fat, ash, moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), amino acids, fatty acids, vitamins (B1, B2, E), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), folic acid, phytic acid and trypsin inhibitor content. The amounts of the secondary metabolites, raffinose, inositol, 2-furaldehyde (furfural), ferulic acid and p-coumaric acid, were also determined in grain. In forage, the proximate, ADF and NDF contents were determined. Carbohydrate values in forage and grain were estimated by calculation. The selected compounds in the compositional study were chosen on the basis of internationally accepted guidance provided by the OECD.

Based on the positive results of the compositional analyses conducted for NK603 × MON 810 and its parental single-trait lines, NK603 and MON 810, 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 NK603 × MON 810-derived feed materials, feed additives and food additives in the E.U., but does not include the cultivation of NK603 × MON 810 varieties in the E.U. The observations from agronomic and phenotypic assessments provide additional evidence confirming the absence of unintended or unanticipated adverse effects of the genetic modifications present in this maize.

The phenotypic and agronomic characteristics of NK603 and MON 810 and the efficacy of the newly introduced agronomic traits have already been studied in the context of the single-trait products. Supplementary field trials in Iowa (IA), Missouri (MO), Nebraska (NE) and Ohio (OH) in the U.S.A. showed no biologically significant differences between NK603 × MON 810 and conventional maize in agronomic and phenotypic characteristics (such as growth and developmental parameters, plant morphology, vigour, and yield characteristics), except for the tolerance of NK603 × MON 810 plants to glyphosate and their protection from certain lepidopteran insect pests. No statistically

significant differences were detected for NK603 × MON 810 compared to the near-isogenic control for any of the measured phenotypic characteristics pooled across sites. In addition, no ecological observational differences between the test and control substances for insect, disease, or abiotic stressor incidences supports a conclusion of no increased weed potential of NK603 × MON 810 and no unintentionally altered plant interactions with non-target organisms. These results also infer that NK603 × MON 810 has equivalent reproductive, dissemination and survival characteristics to conventional maize, resulting in a similar lack of persistence and invasiveness as known for conventional maize.

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 NK603 × MON 810 is substantially equivalent to conventional maize except for the introduced traits of glyphosate-tolerance and lepidopteran-protection.

7.5 Product specification

NK603 × MON 810-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 NK603 × MON 810 results from traditional breeding of NK603 and MON 810, it contains both inserts in combination, which confer tolerance to glyphosate and protection against lepidopteran insect pests. The presence of the glyphosate-tolerance and lepidopteran-protection traits in NK603 × MON 810-derived products can be detectable using either the insert-specific PCR method for detecting the introduced DNA present in NK603, or the equivalent method for MON 810. The unambiguous detection of NK603 × MON 810 in mixed consignments of maize product requires single samples to be subjected to detection methods for both NK603 and MON 810, and to test positive for both.

The event specific methods of detection of NK603 and MON 810 were validated by the Community Reference Laboratory (CRL), in collaboration with the European Network of GMO Laboratories (ENGL), and published together with the validation report for NK603 × MON 810 on their website[4].

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 NK603 × MON 810 is substantially equivalent and as safe and as nutritious as conventional maize, the use of NK603 × MON 810 for the production of foods and feeds is not different from that of conventional maize. Consequently, any effects of the production and processing of NK603 × MON 180 foods

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

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 NK603 × MON 810 were first placed on the E.U. market in autumn 2002. 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.

NK603 × MON 810-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 NK603 × MON 810-derived products.

7.8 Toxicology

7.8.1 Safety assessment of newly expressed proteins

NK603 × MON 810 results from traditional breeding of two genetically modified parental lines, *i.e.* one containing the NK603 insert and one containing the MON 810 insert. Both of the introduced traits are inherited by the NK603 × MON 810 combined-trait progeny. This results in the combined expression of the CP4 EPSPS proteins and the Cry1Ab protein in the same plant. These introduced proteins are present at low levels in the plant and have previously been demonstrated as safe for animal and human health, as part of the safety evaluation of the single-trait products, NK603 and MON 810.

The CP4 EPSPS and Cry1Ab proteins have negligible potential to cause adverse effects to animal or human health. The CP4 EPSPS proteins belong to the safe class of EPSPS enzymes, that are commonly found in a wide variety of food sources and which have a long history of safe use. In addition, there is a history of safe use of CP4 EPSPS-expressing crops, such as glyphosate-tolerant soybean, glyphosate-tolerant canola and glyphosate-tolerant maize (NK603). Cry1Ab has a highly specific, insecticidal mode of action in the gut of target insects that is based on binding to specific receptors for *Bt* proteins. The long history of safe use of this protein in microbial *Bt* products and its history of safe use in previously approved GM products, such as products derived from MON 810, further support its safety to humans and animals.

In addition to their long history of safe use, the acute toxicity of each protein was directly assessed in an acute oral gavage study. There were no indications of acute toxicity for either of these proteins when administered by gavage to laboratory mice at doses which are orders of magnitude higher than expected consumption levels from food or feed products containing or consisting of NK603 × MON 810. This lack of toxicity was expected based on the absence of a toxic mechanism in animals, the history of exposure, and the rapid degradation of each protein in simulated human gastric fluids. In addition, CP4 EPSPS and Cry1Ab are not homologous to any known allergens or protein toxins

(except for the expected homology of Cry1Ab to other *Bt* proteins). Compared to other proteins, CP4 EPSPS and Cry1Ab are present at very low levels in NK603 × MON 810, and their expression levels are consistent with those observed for the respective single-trait products. As the potential for CP4 EPSPS and Cry1Ab proteins to interact is negligible, the conclusions of the safety assessments for the individual proteins are unaffected when the proteins are expressed in combination in NK603 × MON 810.

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 NK603 × MON 810 was shown to be substantially equivalent to conventional maize, testing of any constituents 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 NK603 × MON 810 and conventional maize have been established by compositional analysis. In addition, the wholesomeness and safety of NK603 × MON 810 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 CP4 EPSPS and Cry1Ab proteins expressed in NK603 × MON 810 has previously been demonstrated for the single-trait products. CP4 EPSPS and Cry1Ab are present at very low levels in maize grain. These proteins were assessed for their potential allergenicity by a variety of tests, including a) whether the genes came from allergenic or non-allergenic sources, b) sequence similarity to known allergens, and c) pepsin stability of the protein in an *in vitro* digestion assay. In all cases, the proteins did not exhibit properties characteristic of allergens.

7.9.2 Assessment of allergenicity of the whole GM plant or crop

As the introduced proteins do not have any allergenic potential, it was concluded that the use of NK603 × MON 810-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

NK603 × MON 810 is produced from NK603 and MON 810 using traditional breeding methods, and thereby inherits both inserts from the single-trait parental lines. The introduced traits of glyphosate-tolerance and lepidopteran-protection 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 NK603 × MON 810 authorisation, and no nutritional imbalances are expected as a result of the use of NK603 × MON 810-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 NK603 × MON 810 and conventional control grain, as well as commercial reference 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 NK603 × MON 810-containing diet and the conventional control diet. In addition, when individual treatment comparisons were made, broilers in general performed and had similar carcass yields and meat composition when fed diets containing NK603 × MON 810, the conventional hybrid, and commercially available reference maize hybrids. The NK603 × MON 810-diet was as wholesome as its corresponding conventional control diet and commercially available reference diets regarding its ability to support the rapid growth of broiler chickens. This conclusion was consistent with the evaluation of the composition of NK603 × MON 810, which showed that there were no biologically relevant differences in nutritional and compositional properties relative to control and reference maize hybrids. These data confirm the conclusion that NK603 × MON 810 and its derived products are as safe and nutritious as conventional maize.

7.11 Post-market monitoring of GM food/feed

There are no intrinsic hazards related to NK603 × MON 810 as no signs of adverse or unanticipated effects have been observed in a number of safety studies, including animal feeding studies using doses of administration that are orders of magnitude above expected consumption levels. The pre-market risk characterisation for food and feed use of NK603 × MON 810 demonstrates that the risks of consumption of NK603 × MON 810 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 this renewal application under Regulation (EC) No 1829/2003 only includes feed materials, feed additives and food additives produced from NK603 × MON 810 and does not include deliberate release of grains into the environment.

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

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

10. Potential interactions with the abiotic environment

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

11. Environmental monitoring plan (not if application concerns only food and feed produced from GM plants, or containing ingredients produced from GM plants 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 maize NK603 and MON 810, as well as the validation report for NK603 × MON 810, prepared by the Community Reference Laboratory (CRL) in collaboration with the European Network of GMO Laboratories (ENGL), are published on the CRL website since 16 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

B/FR/00/02/06; B/DE/04/163; B/ES/04/18; B/ES/04/20; B/FR/04/02/01; B/ES/06/07; B/ES/06/09; B/FR/06/01/04.

b) Conclusions of post-release monitoring

The E.U. field trials with NK603 × MON 810 which were conducted to date, relate to the assessment of agronomic performance, morphological characteristics, yield potential, residues determination, protein expression and compositional analysis. Trials were conducted in France, Germany and Spain, principal growing areas in the European Union. Post-release surveillance provided no significant evidence that this maize would likely cause any adverse effects to human or animal health or to the environment.

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)

Post-release surveillance from environments inside and outside the E.U. provided no significant evidence that NK603 × MON 810 would pose any risk of adverse effects to human or animal health or to the environment.

2. History of previous releases of the GM plant carried out outside the Community by the same notifier

a) Release country

NK603 × MON 810 was grown commercially in U.S.A., Canada and the Philippines.

Prior to commercialisation of NK603 × MON 810 varieties, this maize as well as the single-trait products, NK603 and MON 810, have been extensively tested 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)

Canada: Canadian Food Inspection Agency (CFIA) and Health Canada.

The Philippines: Department of Agriculture.

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. and Canada, stacked products are not regulated provided that each of the single-trait parental lines is

already approved.

c) Release site

Please *see* Section E.2.(a).

d) Aim of the release

Commercial release for all uses as conventional maize.

e) Duration of the release

Please see Section 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 NK603 × MON 810, indicating that a requirement for post-release monitoring would not be appropriate.

In addition, NK603 × MON 810 is commercialized alongside stewardship programmes such as insect resistance management programmes, involving downstream stakeholders in the use of this maize, in order to ensure the implementation of good agricultural practice in its cultivation and to ensure a channel of communication in the unlikely event that unanticipated adverse effects might occur.

However, no such unanticipated effects have been observed since the large-scale commercialization of NK603 × MON 810 in North America, nor during the field-testing programmes inside and outside the E.U.

g) Duration of post-releases monitoring

Please see Section E.2.(f)

h) Conclusions of post-release monitoring

Please see Section 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 NK603 × MON 810 are likely to cause any adverse effects to human or animal health, or to the environment.

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

a) Status/process of approval

The JRC websites http://gmoinfo.jrc.it/ and http://gmo-crl.jrc.it/statusofdoss.htm and the EFSA website http://www.efsa.europa.eu/en/science/gmo/gm_ff_applications.html provide publicly accessible links to up-to-date databases on the regulatory progress of notifications under Directive 2001/18/EC and

applications under Regulation (EC) No 1829/2003, including the Monsanto dossiers for NK603 × MON 810: C/GB/02/M3/3, C/ES/04/01, EFSA-GMO-UK-2004-01 and EFSA-GMO-NL-2005-26.

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

The JRC website http://gmoinfo.jrc.it/gmc_browse.aspx?DossClass=0 provides a link to the publicly accessible Initial Assessment Report from the UK Lead Member State for Monsanto notification C/GB/02/M3/3 on NK603 × MON 810.

c) EFSA opinion

On 31 March 2006, the EFSA issued its overall opinion (http://www.efsa.europa.eu/en/science/gmo/gm_ff_applications/more_info/486.html) on Application EFSA-GMO-UK-2004-01 for authorisation of NK603 × MON 810, according to Regulation (EC) No 1829/2003 on genetically modified food and feed, concluding on its safety for humans, animals and the environment.

d) Commission Register (Commission Decision 2004/204/EC)

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

e) Molecular Register of the Community Reference Laboratory/Joint Research Centre

Information on detection protocols is posted at http://gmo-crl.jrc.it/

f) Biosafety Clearing-House (Council Decision 2002/628/EC)

The publicly accessible portal site of the Biosafety Clearing-House (BCH) can be found at http://bch.biodiv.org/

g) Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC)

The JRC website http://gmoinfo.jrc.it/gmc_browse.aspx?DossClass=0 provides a link to the publicly accessible SNIF summary of notifications under Directive 2001/18/EC, including the pending Monsanto notifications for NK603 × MON 810: C/GB/02/M3/3 and C/ES/04/01.

Further, EFSA provides a link to the publicly accessible summary of this renewal application, as well as to the summaries of applications EFSA-GMO-UK-2004-01 and EFSA-GMO-NL-2005-26 under Regulation (EC) No 1829/2003, at http://www.efsa.europa.eu/en/science/gmo/gm_ff_applications/more_info/486.html.