Application for authorization of NK603 × MON 810 maize in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed

ANNEX IV

Summary of application for NK603 × MON 810 maize for food and feed use, according to Annex IV of the *EFSA Draft Guidance Document for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed* of 7 April 2004

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A. GENERAL INFORMATION

1. Details of application

a) Member State of application

United Kingdom

b) Notification number

Not available 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® and YieldGard®, 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 available at the time of application

2. Applicant

a) Name of applicant

Monsanto Company, represented by Monsanto Europe S.A.

b) Address of applicant

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

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

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

NK603 × MON 810 maize will be traded and used in the European

Union in the same manner as current commercial maize varieties and by the same operators currently involved in the trade and use of traditional maize.

3. Scope of the application

( ) Cultivation (Part C of Directive 2001/18/EC)
( ) Import and processing (Part C of Directive 2001/18/EC)
(x) Use as food/food ingredient (Regulation 1829/2003)
(x) Use as feed/feed material (Regulation 1829/2003)

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 (x)	**No** ( )
If *no*, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC NK603 × MON 810 hybrids have been field-tested in the E.U. from 2000 to 2003 (*see* B/FR/00.02.06).	

6. Has the GM plant or derived products been previously notified for marketing in the Community under Part C of Directive 2001/18/EC or Regulation (EC) 258/97?

Yes (x)	**No** ( )
If *yes*, specify An application pursuant to Directive 2001/18/EC (C/GB/02/M3/3) for import of NK603 × MON 810 maize in the E.U. and use thereof as any other maize, excluding the cultivation of varieties, was submitted in April 2001, has received a favourable UK Rapporteur opinion in March 2004 and is currently under review by the E.U. Member States. In addition, an application pursuant to Directive 2001/18/EC (C/ES/04/01) for import of NK603 × MON 810 maize in the E.U. and use thereof as any other maize, including the cultivation of varieties, has been submitted in January 2004 and is currently under review by the Rapporteur Member State Spain.	

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

Yes (x)	**No** ( )

If *yes*, specify

In more than one third country outside the E.U., such as the U.S.A. and Canada, NK603 maize, MON 810 maize and NK603 × MON 810 maize are authorised for all uses, corresponding to the full range of uses of traditional maize. However, the scope of the approvals already granted for these genetically modified organisms and the status of pending regulatory reviews, which are currently in progress in numerous countries around the world, typically depend on the country and its local regulatory framework.

8. General description of the product

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

NK603 × MON 810 maize consists of hybrid maize varieties produced using traditional methods of maize breeding by crossing parental inbred lines of NK603 and MON 810 maize. Although genetic modification was used in the development of NK603 and MON 810 maize, no additional genetic modifications were involved for the production of NK603 × MON 810 hybrids.

Like parental NK603 maize, NK603 × MON 810 maize expresses CP4 EPSPS proteins derived from *Agrobacterium* sp. strain CP4, which confer tolerance to Roundup® agricultural herbicide (containing glyphosate). Like its second parental maize line derived from event MON 810, NK603 × MON 810 maize also expresses the Cry1A(b) protein, derived from *Bacillus thuringiensis* subsp. *kurstaki*, which confers protection from predation by certain Lepidopteran insect pests, including the European Corn Borer (*Ostrinia nubilalis*) and pink borers (*Sesamia* spp).

The use of NK603 × MON 810 maize plants enables the farmer to use Roundup herbicide for effective control of weeds during the growing season and to take advantage of the favourable environmental and safety characteristics of Roundup herbicide. The use of NK603 × MON 810 maize also enables the farmer to effectively control the targeted 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 application is for all uses of NK603 × MON 810 maize for food and feed. The range of uses of this maize for food and feed will be identical to the full range of equivalent uses of traditional maize.

c) Intended use of the product and types of users

NK603 × MON 810 maize will be traded and used in the European Union in the same manner as current commercial maize varieties and by the same operators currently involved in the trade and use of traditional maize.

d) Specific instructions and/or recommendations for use, storage and handling, including mandatory restrictions proposed as a condition of the authorisation applied for

NK603 × MON 810 maize is substantially equivalent to other maize varieties except for its introduced (*i.e.* inherited) traits: tolerance to Roundup herbicide and protection from target Lepidopterans, which are traits of agronomic interest. This maize was shown to be as safe and as nutritious as traditional maize. Therefore NK603 × MON 810 maize and derived products will be stored, packaged, transported, handled and used in the same manner as the commercial maize products. No specific conditions are warranted or required for the food and feed use of NK603 × MON 810 maize.

e) Any proposed packaging requirements

NK603 × MON 810 maize is substantially equivalent to its parental maize lines NK603 and MON 810 maize, and to traditional maize varieties (except for its tolerance to Roundup herbicide and its protection from targeted Lepidopteran insect pests). Therefore, NK603 × MON 810 maize and derived products will be used in the same manner as other maize and no specific packaging is required. (For the labelling, see question 8.(f)).

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

Not applicable.

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-ØØ6Ø3-6 × MON-ØØ81Ø-6

NK603 × MON 810 maize is uniquely identified using this combination of the unique identifiers for event NK603 (MON-ØØ6Ø3-6), and event MON 810 (MON-ØØ81Ø-6).

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

NK603 × MON 810 maize is suitable for food and feed use throughout the E.U.

9. Measures suggested by the applicant to take in case of unintended release or misuse as well as measures for disposal and treatment

Misuse of NK603 × MON 810 maize is unlikely, as the proposed food and feed uses for this maize include all the current food and feed uses of traditional maize. NK603 × MON 810 hybrids are substantially equivalent to other

maize hybrids except for the introduced (*i.e.* inherited) traits: tolerance to Roundup herbicide and protection from target Lepidopterans, which are traits of agronomic interest. This maize is shown to be as safe and as nutritious as traditional maize. Therefore, any measures for waste disposal and treatment of NK603 × MON 810 maize products are the same as those for traditional maize. No specific conditions are warranted or required for the placing on the market of NK603 × MON 810 maize for food and feed.

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 the current application does not include the environmental release of NK603 × MON 810 maize. The information required in this Section can be found in the relevant application for environmental release of this maize according to Directive 2001/18/EC.

Out-crossing with wild *Zea* species

Closely related wild relatives of maize do not exist in Europe.

3. Survivability

a) Ability to form structures for survival or dormancy

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

b) Specific factors affecting survivability

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

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

4. Dissemination

a) Ways and extent of dissemination

In general, dissemination of maize may occur by means of seed dispersal and pollen dispersal. Dispersal of the maize grain is highly restricted in domesticated maize due to the ear structure including husk enclosure. For maize pollen, the vast majority is deposited in the same field due to its large size (90 to 100 µm) with smaller amounts of pollen deposited usually in a downwind direction. However, the current application does not include the environmental release of NK603 × MON 810 maize in the European Union.

b) Specific factors affecting dissemination

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

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

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

There are no wild relatives of maize in Europe.

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

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

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

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

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Description of the methods used for the genetic modification

No novel method of genetic modification is utilized in the production of NK603 × MON 810 maize hybrid varieties. Instead, traditional maize breeding methods are used to cross inbreds of NK603 maize and MON 810

maize. While NK603 × MON 810 hybrid maize results from traditional breeding, genetic modification was used in the development of the single-trait, parental NK603 and MON 810 maize. NK603 and MON 810 maize were produced using the particle acceleration transformation method.

2. Nature and source of the vector used

While NK603 × MON 810 hybrid maize results from traditional breeding, genetic modification was used in the development of the single-trait parental maize lines. NK603 maize 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 maize was generated by the integration of sequences from the plasmid vector PV-ZMBK07, containing the *cry1A(b)* coding sequence of interest, which was derived from *Bacillus thuringiensis* subsp. *kurstaki*. MON 810 maize was produced using the particle acceleration method.

3. Size, source (name) of donor organism(s) and intended function of each constituent fragment of the region intended for insertion

NK603 × MON 810 maize results from a single traditional cross of the inbred parental lines NK603 maize and MON 810 maize, which are made homozygous in their respective inserted sequences.

By crossing NK603 and MON 810 inbreds, NK603 × MON 810 hybrid maize inherits the inserted DNA fragments from both its parental maize lines.

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

Table 1. Components of the NK603 insert

Genetic Element	Source	Size (kb)	Function
First *cp4 epsps* gene cassette			
P-ract1/ ract1 intron	*Oryza sativa*	1.4	Contains promoter, transcription start site and first intron.
ctp 2	*Arabidopsis thaliana*	0.2	Encodes chloroplast transit peptide, which directs the CP4 EPSPS protein to the chloroplast
cp4 epsps	*Agrobacterium* sp. strain CP4	1.4	Encodes glyphosate-tolerant CP4 EPSPS protein
NOS 3'	*Agrobacterium tumefaciens*	0.3	Ends transcription and directs polyadenylation of the mRNA.
Second *cp4 epsps* gene cassette			
e35S	Cauliflower mosaic virus	0.6	Promoter
Zmhsp70	*Zea mays L.*	0.8	Stabilizes the level of gene transcription.
ctp 2	*Arabidopsis thaliana*	0.2	Encodes chloroplast transit peptide, which directs the CP4 EPSPS protein to the chloroplast
cp4 epsps l214p	*Agrobacterium* sp. strain CP4	1.4	Encodes glyphosate-tolerant CP4 EPSPS L214P protein[1]
NOS 3'	*Agrobacterium tumefaciens*	0.3	Ends transcription and directs polyadenylation of the mRNA.

Table 2. Components of the MON 810 insert

Genetic Element	Source	Size (kb)	Function
e35S	Cauliflower mosaic virus	0.6	Promoter
Zmhsp70	*Zea mays* L.	0.8	Stabilizes level of gene transcription.
Cry1A(b)	*Bacillus thuringiensis*	3.5	Encodes Cry1A(b) protein, which targets specific lepidopteran insect pests

[1] 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 maize consists of hybrid varieties, produced by traditional methods of maize breeding, that express the CP4 EPSPS protein, which is tolerant to Roundup herbicide (containing glyphosate) and the Cry1A(b) protein, which confers protection from certain Lepidopteran insect pests.

As NK603 maize, 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. Roundup has excellent weed control capabilities and well-known, favourable environmental and safety characteristics. However, the sensitivity of crop plants to Roundup herbicide has prevented the in-season use of this herbicide in the crop. The extension of the use of Roundup agricultural herbicide to allow in-season application in major crops such as maize provides a novel weed control option for farmers. The use of Roundup in maize is significant as it enables the farmer to take advantage of the herbicide's favourable environmental properties.

As its second parental line containing event MON 810, NK603 × MON 810 maize expresses the Cry1A(b) 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 maize 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 maize, each fragment is inherited as a single gene in a Mendelian fashion.

As the parental maize lines used in the traditional cross to produce NK603 × MON 810 maize are inbred lines that are homozygous in the NK603 or MON 810 event, both of the inserted fragments are inherited by the NK603 × MON 810 hybrid, *i.e.* one fragment conferring the Roundup tolerance and one for the insect-protection trait. The presence of these events in the hybrid was confirmed through Southern blot analysis, suggesting that the integrity of the inserts has been conserved in the hybrid..

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 maize contains both of the parental inserts on separate chromosomes in the nuclear genome. The presence of these events in the hybrid was confirmed through Southern blot analysis.

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

As NK603 × MON 810 maize is the product of a single traditional cross of NK603 and MON 810 inbreds and no additional genetic modification methods have been applied, and as the inherited modification events have negligible potential to interact with one another, it is highly likely that the NK603 × MON 810 hybrid contains each of the inserts as they were present in the parental NK603 and MON 810 maize lines. Therefore, the molecular characteristics of the introduced DNA sequences, known for the single-trait NK603 and MON 810 maize lines, most likely also apply to NK603 × MON 810 maize, 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 maize grown in the field. The levels of CP4 EPSPS and Cry1A(b) proteins were measured in forage and grain samples of NK603 × MON 810 maize and are summarized below.

The mean levels of CP4 EPSPS across all sites was 36.3 µg/g fw in forage samples of NK603 × MON 810 maize and 12.7 µg/g fw in the grain. The mean level of the Cry1A(b) protein across all sites was 6.06 µg/g fw in the forage and 0.73 µg/g fw in grain samples.

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

The expression of the CP4 EPSPS and Cry1A(b) proteins occurs throughout the whole plant since the rice actin and CaMV *e35S* promoters have been shown to drive constitutive expression of the encoded protein in genetically modified maize.

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

a) Reproduction

NK603 × MON 810 maize has been field tested in the E.U. since 2000.

Agronomic data collected from these trials have demonstrated that NK603 × MON 810 maize has not been altered in survival, multiplication or dissemination characteristics when compared to its parental maize lines (NK603 and MON 810) or compared to traditional maize varieties. The introduced traits for herbicide tolerance and insect-protection have no influence on maize reproductive morphology and hence no changes in seed dissemination are to be expected.

b) Dissemination

The introduced herbicide tolerance and insect-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 outside cultivation without the aid of human intervention. Field observations have demonstrated that NK603 × MON 810 maize has not been altered in its survivability when compared to its parental maize lines (NK603 and MON 810) or compared to traditional maize.

d) Other differences

Comparative assessments in the field did not reveal any biologically significant differences between NK603 × MON 810 hybrids and traditional maize hybrids, except for the introduced traits that are of agronomic interest.

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

NK603 × MON 810 hybrid seed (F1) is produced by a single cross of the NK603 and MON 810 parental inbred lines (made homozygous for event NK603 or MON 810, respectively) by traditional breeding. Thereby, each parental line passes on its inserted DNA sequence to the resulting NK603 × MON 810 F1 hybrid seed, which is sown by the grower.

The single-trait modified maize lines 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. Each trait is inherited as a single dominant gene in a Mendelian fashion. This has been confirmed by Southern blot analyses and by studies of the inheritance pattern of these traits in maize.

The harvested (F2) grain of NK603 × MON 810 maize is marketed by the grower for food, feed or industrial use and is not used for further breeding. Therefore, since NK603 × MON 810 hybrid maize seed exists only for a single generation, there is no opportunity for its stability to be compromised.

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

a) Plant to bacteria gene transfer

In comparison with the possible transfer of genetic material between

bacteria and traditional maize, and based on the nature of the DNA elements used in the modification events that have been inherited by NK603 × MON 810 maize, 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

Based on the observation that the reproductive morphology of NK603 × MON 810 maize is unchanged compared to traditional maize, we infer that pollen production and pollen viability are unchanged by the genetic modification. Therefore, in a cultivation scenario, the out-crossing frequency between NK603 × MON 810 maize and other maize would not to be any different from traditional maize varieties. However, the scope of the current application does not include the cultivation of NK603 × MON 810 maize varieties in the E.U.

7. Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed

7.1 Comparative assessment

Choice of the comparator

Compositional analyses were performed on forage and grain samples from NK603 × MON 810 maize, grown under field conditions in the E.U. in 2000.

The study also included compositional analyses of forage and grain collected from a non-transgenic control hybrid and five different non-transgenic commercial maize hybrids, which were grown in replicated plots at the same field sites as NK603 × MON 810 maize.

The analytical results have shown that the composition of NK603 × MON 810 hybrid maize is substantially equivalent to that of the non-transgenic control hybrid and falls within the wide compositional range that is known for traditional maize.

In conclusion, NK603 × MON 810 maize may be considered compositionally equivalent to traditional maize.

7.2 Field trials

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

In addition to extensive field observations in the U.S.A., field trials were conducted at multiple locations in the E.U. since 2000 (*see* approval for deliberate release under Directive 90/220/EEC, superseded by Directive 2001/18/EC, B/FR/00.02.06). These trials included the field portion of a protein expression study and compositional analysis study. Samples of forage and grain from three replicated sites grown with NK603 × MON 810 test hybrid were analyzed and compared to a control hybrid and commercial comparators. Furthermore, the agronomic performance of NK603 × MON 810 maize was studied, and comparative assessments of the morphology, growth and development of NK603 × MON 810 versus traditional maize were conducted at six locations. The field trials confirmed that this maize is

substantially equivalent to traditional maize, with the exception of the introduced (*i.e.* inherited) Roundup tolerance and insect protection traits.

b) the baseline used for consideration of natural variations

Compositional analyses were made for forage and grain samples from NK603 × MON 810 maize. The study also included analyses of forage and grain collected from a non-transgenic control hybrid and five different non-transgenic commercial maize hybrids, grown in replicated plots at the same field sites as NK603 × MON 810 maize. 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 compounds for analysis

As described in Section C.7.1, compositional analyses were conducted on grain and forage from NK603 × MON 810 hybrids and non-transgenic counterpart.

Grain was analyzed for its proximate content (protein, fat, ash, moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), amino acids, fatty acids, vitamin B1, vitamin B2, vitamin 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. In addition, the carbohydrate content of forage and grain was determined by calculation.

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

Based on the long history of safe use of the host plant, maize, as well as the positive results of the compositional analyses conducted for NK603 × MON 810 maize and its parental single-trait lines, containing either event NK603 or MON 810, there is no indication of a need to further analyse other selected compounds in this maize.

7.4 Agronomic traits

This application under Regulation (EC) N° 1829/2003 does not include the environmental release of NK603 × MON 810 maize in the European Union. However, the observations from environmental releases provide additional evidence confirming the absence of any significant unintended or unanticipated effects of the genetic modifications present in this maize. Field data and commercial experience from North America provided a weight of evidence showing that, in comparison with traditional maize hybrids, NK603 × MON 810 hybrids have:

- equivalent growth, developmental and morphological characteristics,
- equivalent plant health, vigour and pest susceptibility (except for predation by specific Lepidopteran insect pests and secondary

diseases),

- equivalent agronomic performance, including yield potential.

These results also infer that NK603 × MON 810 maize has equivalent biological fitness, dissemination and survival characteristics as any other maize.

It is concluded that from an agronomic and phenotypic (morphological) point of view, NK603 × MON 810 maize is equivalent to traditional maize, except for the introduced traits conferring tolerance to Roundup agricultural herbicide and protection from certain Lepidopteran insect pests.

7.5 Product specification

NK603 × MON 810 maize actually comprises all traditionally bred hybrid maize varieties produced by the combination of genetically modified maize inbreds derived from maize transformation events NK603 and MON 810.

7.6 Effect of the production and 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 maize is substantially equivalent and as safe and as nutritious as traditional maize, the use of NK603 × MON 810 maize for the production of foods and feeds is no different from that of traditional maize. Consequently, any effects of the production and processing of NK603 × MON 180 maize are not expected to be any different from the production and processing of the equivalent foods and feeds, originating from traditional maize.

7.7 Anticipated intake/extent of use

There are no anticipated changes in the intake and/or extent of use of maize or derived products for use as or in food or feed as a result of the addition of NK603 × MON 810 maize varieties to the traditional maize supply. NK603 × MON 810 maize hybrids are expected to replace a portion of current maize hybrids such that their intake or use will represent some fraction of the total products derived from maize.

7.8 Toxicology

7.8.1 Safety evaluation of newly expressed proteins

NK603 × MON 810 hybrid maize is produced by a single traditional cross of two genetically modified parental inbred maize lines, i.e. one derived from event NK603 and one derived from event MON 810. Both of the introduced traits in the single-trait, parental lines are inherited by the NK603 × MON 810 hybrid progeny. This results in the combined expression of the CP4 EPSPS proteins and the Cry1A(b) protein in the same plant, NK603 × MON 810 maize. 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 parental lines containing either event NK603 or event MON 810

The CP4 EPSPS and Cry1A(b) 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 an extensive history of safe use of CP4 EPSPS-expressing crops, such as Roundup Ready soybean, Roundup Ready canola and NK603 Roundup Ready maize. Cry1A(b) 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 maize 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 maize. 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 Cry1A(b) are not homologous to any known allergens or protein toxins (except for the expected homology of Cry1A(b) to other *Bt* proteins). Compared to other proteins CP4 EPSPS and Cry1A(b) are present at very low levels in NK603 × MON 810 maize. These expression levels were virtually identical to those observed for the respective single-trait parental maize line. As the potential for CP4 EPSPS and Cry1A(b) 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 maize.

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

7.8.3 Information on natural food and feed constituents

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

7.8.4 Testing of the whole GM food/feed

The compositional and nutritional equivalence of grain and forage from NK603 × MON 810 maize and traditional maize have been established by compositional analysis. In addition, the wholesomeness and safety of NK603 × MON 810 maize 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 Cry1A(b) proteins expressed in NK603 × MON 810 maize has previously been demonstrated for the single-trait parental lines containing either event NK603 or event MON 810. CP4 EPSPS and Cry1A(b) 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 maize for food or feed does not lead to an increased risk for allergenic reactions compared to the equivalent range of food and feed uses of traditional maize.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

NK603 × MON 810 hybrids are maize hybrids, that have inherited the genetic modification events NK603 and MON 810 from their single-trait, genetically modified parental lines. The introduced traits of Roundup tolerance and insect-resistance are of agronomic interest (input traits), and do not change the nutritional aspects of this maize. Hence this maize is not expected to be more or less attractive for use as food (or feed), for processing, or as a food (or feed) ingredient. Therefore, anticipated dietary intake of maize-derived foods and feeds is not expected to be altered upon commercialisation of NK603 × MON 810 maize, and no nutritional imbalances are expected as a result of the use of NK603 × MON 810 maize.

7.10.2 Nutritional assessment of GM feed

A confirmatory feeding study in broiler chickens was conducted to compare the nutritional value of the stacked NK603 × MON 810 maize grain and non-transgenic control grain as well as additional commercial maize hybrids, and to provide additional confirmation of the safety of this hybrid maize. The results of this study show that there were no biologically relevant differences in the parameters tested between broilers fed the NK603 × MON 810 diet and the non-transgenic control diet. In addition, when individual treatment comparisons were made, broilers in general performed and had similar carcass yields and meat composition when fed diets containing NK603 × MON 810 maize, the non-transgenic hybrid, and commercially available reference maize hybrids. The NK603 × MON 810 maize diet was as wholesome as its corresponding non-transgenic control diet and commercially available reference diets regarding its ability to support the rapid growth of broiler chickens. This conclusion was consistent with the evaluation of the composition of the NK603 × MON 810 maize, 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 the NK603 × MON 810 maize hybrid is as safe and nutritious as traditional maize.

7.11 Post-market monitoring of GM food/feed

In summary, the assessment of the human and animal safety of NK603 × MON 810 maize was conducted on the basis of its substantial equivalence to traditional maize (except for the introduced traits) and by extensive characterisation of the two introduced traits, which are of agronomic interest, resulting in the expression of the CP4 EPSPS and Cry1A(b) proteins.

There are no intrinsic hazards related to NK603 × MON 810 maize 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 maize demonstrates that the risks of consumption of NK603 × MON 810 maize or its derived products are consistently negligible and no different from the risks associated with the consumption of traditional maize and maize-derived products.

As a consequence, no specific risk management measures are indicated, and post-market monitoring of the use of this maize for food or feed is neither warranted, nor appropriate.

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

The parental maize line NK603 is herbicide-tolerant and does not have any target organisms. The spectrum of target organisms of NK603 × MON 810 maize is therefore identical to the target organisms of the second parental line, *i.e.* MON 810 maize, which has already been approved for cultivation and use under Directive 90/220/EEC since 1998.

Any interactions of NK603 × MON 810 maize with target organisms are limited to those countries where the cultivation of this maize has been authorized. However, the environmental release of NK603 × MON 810 maize in the E.U. is not within the scope of this application for food and feed use. The information required in this Section can be found in the relevant application for environmental release of this maize according to Directive 2001/18/EC.

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

9.1 Persistence and invasiveness

Based on our familiarity with maize breeding practices and the testing and selection of new hybrids, combined with the demonstrated efficacy of NK603 × MON 810 maize in the field, there is no reason to believe that its interaction with non-target organisms is meaningfully altered when compared to the traditional maize. Furthermore, there are no scientific data to indicate that either of the introduced traits in NK603 and MON 810 maize will alter the interaction of the other with non-

target organisms when combined in NK603 × MON 810 maize.

However, any interactions of this maize with non-target organisms are limited to those countries where the environmental release of this maize has been authorized. As mentioned previously, the environmental release of NK603 × MON 810 maize in the E.U. is not within the scope of this application under Regulation (EC) No 1829/2003. The information required in this Section can be found in the relevant application for environmental release of this maize according to Directive 2001/18/EC.

9.2 Selective advantage or disadvantage

Please see question **D.9.1**

9.3 Potential for gene transfer

Please see question **D.9.1**

9.4 Interactions between the GM plant and target organisms

Please see question **D.9.1**

9.5 Interactions of the GM plant with non-target organisms

Please see question **D.9.1**

9.6 Effects on human health

Please see question **D.9.1**

9.7 Effects on animal health

Please see question **D.9.1**

9.8 Effects on biogeochemical processes

Please see question **D.9.1**

9.9 Impacts of the specific cultivation, management and harvesting techniques

Please see question **D.9.1**

10. Potential interactions with the abiotic environment

NK603 × MON 810 maize is substantially equivalent to traditional maize, with the exception of the two introduced (*i.e.* inheritied) traits of agronomic interest, which are imparted by the expression of the CP4 EPSPS and Cry1A(b) proteins. CP4 EPSPS and Cry1A(b) have a safe history of use and have no known negative interactions with the abiotic environment.

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

11.1 General (risk assessment, background information)

The environmental release of NK603 × MON 810 maize in the E.U. is not within the scope of this application under Regulation (EC) No 1829/2003. The information required in this Section can be found in the relevant application for environmental release of this maize according to Directive 2001/18/EC.

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

Please see question **D.11.1**

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

Please see question **D.11.1**

11.4 Reporting the results of monitoring

Please see question **D.11.1**

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

As NK603 × MON 810 hybrids are the result of a traditional cross of NK603 and MON 810 maize inbreds, they contain both transformation events in combination. Therefore, NK603 × MON 810 maize is detectable using either the event-specific PCR method for detecting the introduced DNA present in NK603 maize or the equivalent method for MON 810 maize. However, as for all plants in which one or more events are combined by traditional breeding, the unambiguous detection of NK603 × MON 810 maize in mixed consignments of grain will require single grains to be subjected to detection methods for both NK603 and MON 810 maize, and to test positive for both.

E. INFORMATION RELATING TO PREVIOUS RELEASES OF THE GM PLANT AND/OR DERIVED PRODUCTS

1. History of previous releases of the GM plant notified under Part B of the Directive 2001/18/EC and under Part B of Directive 90/220/EEC by the same notifier

a) Notification number

B/FR/00.02.06

b) Conclusions of post-release monitoring

The conclusions of the E.U. field trials with NK603 × MON 810 maize which were conducted to date, relate to the assessment of agronomic performance, morphological equivalence, yield potential, residues determination, protein expression and compositional analysis. Trials

were conducted in France, a principal growing area 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 general surveillance from environments inside and outside the E.U. provided no significant evidence that NK603 × MON 810 maize 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

Monsanto have commercialised NK603 × MON 810 maize in the U.S.A. and Canada since 2002.

Prior to commercialisation, this maize as well as its parental single-trait maize lines, NK603 and MON 810 maize, 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)

c) Release site

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

d) Aim of the release

Since 2002, NK603 × MON 810 maize has been commercially grown in North America.

e) Duration of the release

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

f) Aim of post-releases monitoring

Extensive pre-market risk assessment did not provide evidence of adverse effects potentially associated with the cultivation, handling or use of NK603 × MON 810 maize, indicating that a requirement for post-release monitoring would not be appropriate.

In addition, NK603 × MON 810 maize is commercialized alongside stewardship 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

commercialization of NK603 × MON 810 maize in North America, nor during the field-testing programmes inside and outside the E.U.

g) Duration of post-releases monitoring

Please see question **E.2.(f)**

h) Conclusions of post-release monitoring

Please see question **E.2.(f)**

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

Field-testing and post-marketing experience provided no significant evidence that grain or derived products from NK603 × MON 810 maize 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/gmc_browse.asp 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 and Regulation (EC) No 1829/2003, including the Monsanto notifications for NK603 × MON 810 maize (C/GB/02/M3/3 and C/ES/04/01).

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

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

c) EFSA opinion

No EFSA opinion was available at the time of submission of this application.

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

The exact link to the publicly accessible part of the Commission Register was yet to be released at the time of this application.

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

Information on detection protocols will likely be posted at http://gmo-crl.jrc.it/

f)	**Biosafety Clearing-House (Council Decision 2002/628/EC)** The publicly accessible portal site of the Biosafety Clearing-House (BCH) can be found at http://bch.biodiv.org/
g)	**Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC)** The JRC website http://gmoinfo.jrc.it/gmc_browse.asp provides a link to the publicly accessible SNIF summary of notifications under Directive 2001/18/EC, including the Monsanto notifications for NK603 × MON 810 maize (C/GB/02/M3/3 and C/ES/04/01).