Application for authorization of MON 89034 × NK603 maize in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed

Part II
Summary

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

A. GENERAL INFORMATION

1. Details of application

a)	**Member State of application** The Netherlands
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: MON 89034 × NK603. In countries where MON 89034 × NK603 is being cultivated, packages of this maize are marketed under the name of the hybrid variety, in association with the trademark YieldGard VT PRO/RR2™[1].
d)	**Date of acknowledgement of notification** Not available at the time of application.

2. Applicant

a)	**Name of applicant** Monsanto Company, represented by Monsanto Europe S.A.
b)	**Address of applicant** Monsanto Europe S.A. Avenue de Tervuren 270-272 B-1150 Brussels BELGIUM Monsanto Company 800 N. Lindbergh Boulevard St. Louis, Missouri 63167 U.S.A
c)	**Name and address of the person established in the Community who is responsible for the placing on the market, whether it be the manufacturer, the importer or the distributor, if different from the applicant (Commission Decision 2004/204/EC Art 3(a)(ii))** MON 89034 × NK603 will be traded and used in the E.U. in the same manner as current commercial maize varieties and by the same operators currently involved in the trade and use of conventional maize.

[1] YieldGard VT PRO/RR2™ is a trademark of Monsanto Technology LLC.

3. Scope of the application

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

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

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

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

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

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

Yes (x)	No ( )
If *yes*, specify Applications for the full range of uses have been made in Japan but approvals from the agencies in this country have not been obtained yet.	

8. General description of the product

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

MON 89034 × NK603 was obtained by traditional breeding of two inbred lines, one derived from MON 89034 and the other one derived from NK603. MON 89034 × NK603, as well as the genetically modified parental lines containing either the MON 89034 or NK603 insert, have been developed by Monsanto Company.

MON 89034 has been developed to produce the Cry1A.105 and the Cry2Ab2 proteins that confer protection against certain lepidopteran pests. The Cry1A.105 protein provides increased activity against fall armyworm (FAW, *Spodoptera* sp.) and black cutworm (BCW, *Agrotis ipsilon*) compared to Cry1Ab, produced in current product MON 810 (*Ostrinia nubilalis* and *Sesamia* spp.). The Cry2Ab2 protein provides improved control over Cry1Ab products from damage caused by corn earworm (CEW, *Helicoverpa zea*). MON 89034 was produced by *Agrobacterium*-mediated transformation of maize cells with plasmid vector PV-ZMIR245, that contains two separate T-DNAs (2 T-DNA system plasmid vector).

NK603 has been developed to produce the CP4 EPSPS and CP4 EPSPS L214P[2] proteins that confer tolerance to glyphosate[3]-containing herbicides. NK603 was generated by particle acceleration technology using the DNA fragment PV-ZMGT32L containing a 5-enolpyruvylshikimate-3-phosphate synthase gene (*epsps*) from *Agrobacterium* sp. strain CP4 (*cp4 epsps*).

As MON 89034 × NK603 inherits the introduced traits from its parental inbreds, it is protected from the targeted lepidopteran insect pests as well as tolerant to glyphosate.

The use of MON 89034 × NK603 enables the farmer to effectively control the targeted lepidopteran insect pests in maize, ensuring maximum realization of yield potential, while removing the environmental burden

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

[3] Active ingredient of Monsanto's Roundup family of agricultural herbicides. Roundup® is a registered trademark of Monsanto Technology LLC.

of the production, packaging and transport of insecticides, previously used to control those pests. In addition, growers will have the ability to apply glyphosate over the top of maize for broad-spectrum weed control.

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

The scope of the current application is for import, processing and all uses of MON 89034 × NK603 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 conventional maize.

c) Intended use of the product and types of users

MON 89034 × NK603 will be traded and used in the E.U. in the same manner as current commercial maize varieties and by the same operators currently involved in the trade and use of conventional maize.

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

No specific conditions or instructions are warranted or required for the placing on the market of MON 89034 × NK603 for import, processing, and use as such or in food and feed. MON 89034 × NK603 is substantially equivalent to other maize varieties except for its protection against target lepidopteran pests and its tolerance to glyphosate, which are traits of agronomic interest. This maize was shown to be as safe and as nutritious as conventional maize. Therefore MON 89034 × NK603 and derived products will be stored, packaged, transported, handled and used in the same manner as the commercial maize products.

e) Any proposed packaging requirements

MON 89034 × NK603 is substantially equivalent to conventional maize varieties (except for its protection against target lepidopteran insect pests and its tolerance to glyphosate). Therefore, MON 89034 × NK603 and derived products will be used in the same manner as other maize and no specific packaging is foreseen. (For the labelling, *see* question A.8.(f)).

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

In accordance with Regulations (EC) No 1829/2003 and 1830/2003, a labelling threshold of 0.9 % is applied for the placing on the market of MON 89034 × NK603 grain and derived products.

Operators shall be required to label products containing or consisting of MON 89034 × NK603 with the words "genetically modified maize" or "contains genetically modified maize", and shall be required to declare the unique identifier MON-89Ø34-3 × MON-ØØ6Ø3-6 in the list of GMOs that have been used to constitute the mixture that contains or consists of this GMO.

Operators shall be required to label foods and feeds derived from MON 89034 × NK603 with the words "produced from genetically modified maize". In the case of products for which no list of ingredients exists, operators shall ensure that an indication that the food or feed product is produced from GMOs is transmitted in writing to the operator receiving the product.

Operators handling or using MON 89034 × NK603 grain and derived foods and feeds in the E.U. are required to be aware of the legal obligations regarding traceability and labelling of these products. Given that explicit requirements for the traceability and labelling of GMOs and derived foods and feeds are laid down in Regulations (EC) No 1829/2003 and 1830/2003, and that authorized foods and feeds shall be entered in the Community Register, operators in the food/feed chain will be fully aware of the traceability and labelling requirements for MON 89034 × NK603. Therefore, no further specific measures are to be taken by the applicant.

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-89Ø34-3 × MON-ØØ6Ø3-6

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

MON 89034 × NK603 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

Because this application is for consent to import and use of MON 89034 × NK603 as any other maize, not including the cultivation of varieties of MON 89034 × NK603 in the E.U., environmental release would be more likely to occur during import, storage and processing of MON 89034 × NK603. However, modern methods of grain handling minimize losses of grain, so there is little chance of germination of spilt grain resulting in the development of mature plants of MON 89034 × NK603 in the E.U. Moreover, in the event of incidental spillage, the establishment of volunteer plants would be unlikely, since maize cannot survive without human assistance and is not capable of surviving as a weed. Although maize seed can over-winter in mild conditions and can germinate the following year, the appearance of maize in rotational fields is rare under European conditions. Maize volunteers, if they occurred, would be killed by frost or could be easily controlled by the use of selective herbicides. Moreover, the information presented in this application established that MON 89034 × NK603 is unlikely to be different from other maize and, therefore, is unlikely to pose any threat to the environment or to require special measures for its containment.

No specific conditions are warranted or required for the placing on the market of MON 89034 × NK603 for import, processing, or use 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** MON 89034 × NK603

f) Common name

Maize; Corn

2. a) Information concerning reproduction

(i) Mode(s) of reproduction

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

(ii) Specific factors affecting reproduction

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

(iii) Generation time

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

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

Out-crossing with cultivated *Zea* varieties

The scope of the current application does not include cultivation of MON 89034 × NK603 varieties in the E.U. Outcrossing with cultivated *Zea* varieties is therefore not expected.

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 MON 89034 × NK603 in the E.U.

b) Specific factors affecting dissemination

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

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

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

There are no close wild relatives of maize in Europe.

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

Maize is widely grown in the E.U. and represents a significant portion of global maize production. 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

There are no known toxic effects of the maize plant to humans, animals or livestock; it has a history of safe use for human food and animal feed. However, 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.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Description of the methods used for the genetic modification

MON 89034 × NK603 was produced by crossing inbred plants of MON 89034 and NK603, using traditional breeding methods.

The inbred parental plants were both produced by genetic modification, *Agrobacterium*-mediated transformation of immature embryos of maize tissue for MON 89034 and particle acceleration technology for NK603.

2. Nature and source of the vector used

MON 89034 × NK603 has been obtained by traditional breeding of MON 89034 and NK603 and no vector has been used to produce this maize hybrid.

The plasmid vector PV-ZMIR245, used for the transformation of maize cells to produce MON 89034, contains two T-DNAs. T-DNA I includes the *cry1A.105* and the *cry2Ab2* expression cassettes, while T-DNA II includes the *nptII* expression cassette.

Plasmid vector PV-ZMIR245 was constructed using standard molecular biology techniques. It is a binary *Agrobacterium tumefaciens* transformation vector that contains sequences that are necessary for transfer of T-DNA into the plant cell. These sequences are contained in the Right and Left Border regions which flank both T-DNA I and T-DNA II allowing for independent integration of each T-DNA into the plant genome during transformation. The T-DNA I region containing the *cry1A.105* and *cry2Ab2* gene expression cassettes is the portion of plasmid PV-ZMIR245 maintained in MON 89034.

PV-ZMGT32L, the agarose gel-isolated *MluI* restriction fragment of plasmid vector PV-ZMGT32, was utilized for transformation of NK603. It contains two adjacent plant gene expression cassettes, each containing a single copy of the 5 enolpyruvylshikimate-3-phosphate synthase (*epsps*) gene, derived from the common soil bacterium *Agrobacterium* sp. strain CP4 (*cp4 epsps*), which were integrated into the maize genome during the transformation process.

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

MON 89034 × NK603 results from a traditional cross of the inbred parental lines MON 89034 and NK603, which are made homozygous for their respective inserted sequences.

By crossing MON 89034 and NK603, MON 89034 × NK603 inherits the inserted DNA fragments from both its parental maize lines.

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

Table 1. Components of the inserted DNA fragment inherited from MON 89034

Sequence	Size (Kb)	Source	Function
		T-DNA I	
B-Left Border r1	0.24	*Agrobacterium tumefaciens*	Border
P-*e35S*^{89}	0.30	Cauliflower mosaic virus	Promotor
L-*Cab*	0.06	Wheat	Leader
I-*Ract1*	0.48	Rice actin gene	Intron
CS-*cry1A.105*	3.53	*Bacillus thuringiensis*	Coding sequence
T-*Hsp17*	0.21	Wheat heat shock protein	Transcript termination sequence
P-*FMV*	0.56	Figwort mosaic virus	Promoter
I-*Hsp70*	0.80	Maize heat shock protein	Intron
TS-*SSU-CTP*	0.40	Maize	Targeting sequence
CS-*cry2Ab2*	1.91	*Bacillus thuringiensis*	Coding sequence
T-*nos*	0.25	*Agrobacterium tumefaciens*	Transcript termination sequence
B-Left Border r2	0.23	*Agrobacterium tumefaciens*	Border

Table 1. Components of the inserted DNA fragment inherited from NK603

Sequence	Size (Kb)	Source	Function
		***cp4 epsps* gene cassette**	
P-*Ract1*/ I-*Ract1*	1.40	Rice actin gene	Promoter and Intron
TS-*CTP2*	0.20	*Arabidopsis thaliana*	Targeting sequence
CS-*cp4 epsps*	1.40	*Agrobacterium tumefaciens* strain CP4	Coding sequence
T-*nos*	0.30	*Agrobacterium tumefaciens*	Transcript termination sequence
		***cp4 epspsl214p* gene cassette**	
P-*e35S*	0.60	Cauliflower mosaic virus	Promoter
I-*Hsp70*	0.80	Maize	Intron
TS-*CTP2*	0.20	*Arabidopsis thaliana*	Targeting sequence
CS-*cp4 epsps l214p*	1.40	*Agrobacterium tumefaciens* strain CP4	Coding sequence
T-*nos*	0.30	*Agrobacterium tumefaciens*	Transcript termination sequence

D. INFORMATION RELATING TO THE GM PLANT

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

MON 89034 × NK603 consists of varieties developed using traditional methods of maize breeding, which express:

1. the Cry1A.105 and the Cry2Ab2 insecticidal proteins which provide protection from feeding damage caused by the European corn borer (ECB, *Ostrinia nubilalis*) and other lepidopteran insect pests,
2. the CP4 EPSPS protein, derived from *Agrobacterium* sp. strain CP4 which provides tolerance to glyphosate.

Commercialization of MON 89034 × NK603 will therefore provide substantial benefits to growers by reducing the risk from insecticide and herbicide use to humans and the environment and by limiting yield losses from insects feeding damage while at the same time limiting weed pressure.

2. Information on the sequences actually inserted or deleted

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

MON 89034 and NK603 each contains a single DNA insert with a single copy of the introduced DNA fragment, and this at different loci in the maize genome. In their progeny, each fragment is inherited as a single gene in a Mendelian fashion.

As the parental maize lines used in the traditional cross to produce MON 89034 × NK603 are inbred lines that are homozygous in the MON 89034 and NK603 introduced traits, both of the inserted fragments are inherited by the MON 89034 × NK603. The presence of these inserts in the hybrid was confirmed through Southern blot analysis.

Therefore, MON 89034 × NK603 contains both of the parental inserts, as they were present in the parental MON 89034 and NK603.

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

Not applicable.

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

The traditionally bred F_1 MON 89034 × NK603 contains the parental inserts on separate chromosomes in the nuclear genome, as they were present in the parental lines MON 89034 and NK603, respectively. The presence of the inserts from MON 89034 and NK603 in MON 89034 × NK603 was confirmed by Southern blot analyses.

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

The molecular comparison of MON 89034 × NK603 to the parental lines, MON 89034 and NK603, indicates that the inserts are preserved in MON 89034 × NK603. Note that there is no scientific basis to support the fact that those inserts would be intrinsically more unstable when combined together by traditional breeding. The molecular characteristics of the respective introduced DNA sequences, present in MON 89034 and NK603, also apply to MON 89034 × NK603, including the structural organisation and integrity of the inserts, as well as the characteristics of the sites of insertion and the flanking sequences, immediately adjacent to the introduced sequences.

A schematic representation of MON 89034 and NK603 inserts is given in Figures 1 and 2.

Figure 1. Schematic representation of the MON 89034 insert

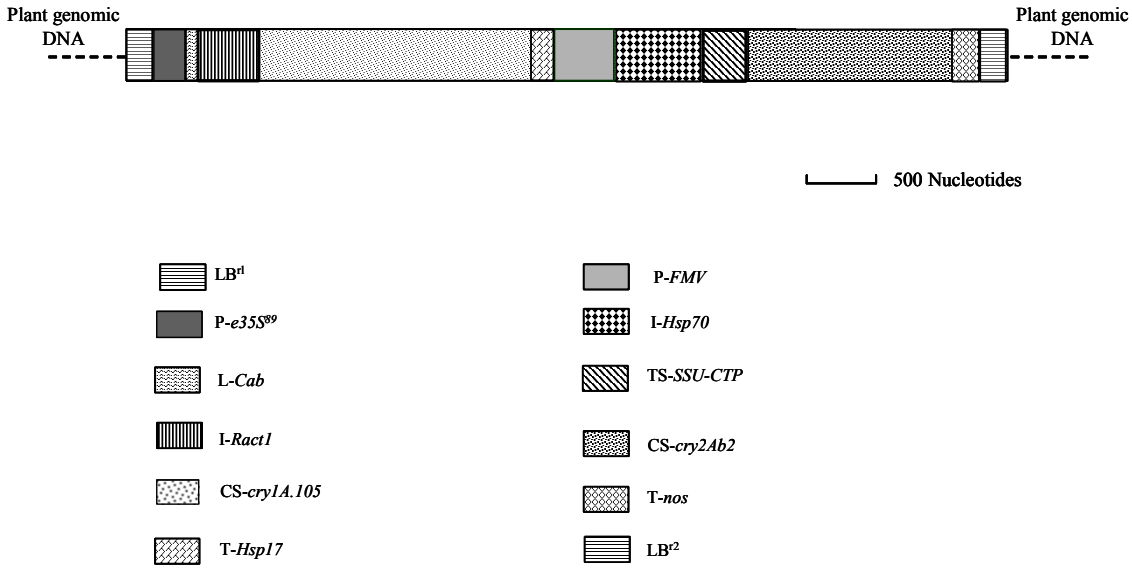
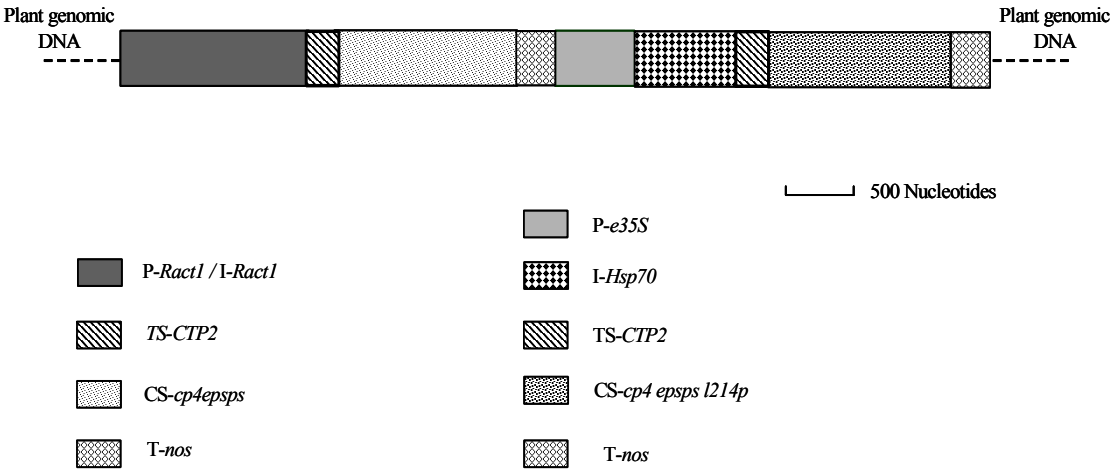


Figure 2. Schematic representation of the NK603 insert



3. Information on the expression of the insert

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

The levels of the Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins in various tissues of MON 89034 × NK603 were assessed by validated enzyme-linked immunosorbent assay (ELISA). Tissue samples for analysis were collected from five field trials conducted in Argentina during 2004. The trials were located in the provinces of Buenos Aires, Córdoba and Santa Fe, which represent the major maize growing region of Argentina and provide a variety of environmental conditions. At each site, three replicated plots of MON 89034 × NK603, MON 89034 and NK603, as well as the conventional control, were planted using a randomized complete block field design.

Expression levels of the introduced proteins were estimated in various tissues, grain being the most relevant tissues to food and feed safety. Since protein levels are relevant to the insect control performance of the maize plants, and are also necessary to assess exposure of non-target species where the maize is planted, protein levels were also measured in additional maize tissues, such as young leaf, young root, whole plant, forage, forage root and pollen.

b) Parts of the plant where the insert is expressed

Young leaf, young root, whole plant, forage, forage root, pollen and grain of MON 89034 × NK603 were collected at appropriate times of plant development. The Cry1A.105 and Cry2Ab2 protein levels in MON 89034 × NK603 were compared to those in MON 89034, whereas, the CP4 EPSPS protein levels in MON 89034 × NK603 were compared to those in NK603.

Overall, the ranges across all sites for the Cry1A.105, Cry2Ab2 and CP4 EPSPS protein levels in MON 89034 × NK603 were comparable to the corresponding ranges in either MON 89034 or NK603.

Cry1A.105 protein levels

The mean Cry1A.105 protein levels in MON 89034 × NK603 across all sites were 220 µg/g dwt in OSL-1, 66 µg/g dwt in OSR-1, 83 µg/g dwt in OSWP-3, 30 µg/g dwt in forage, 24 µg/g dwt in forage-root, 9.6 µg/G dwt in pollen and 3.1 µg/g dwt in grain.

Cry2Ab2 protein levels

The mean Cry2Ab2 protein levels in MON 89034 × NK603 across all sites were 140 µg/g dwt in OSL-1, 37 µg/g dwt in OSR-1, 72 µg/g dwt in OSWP-3, 33 µg/g dwt in forage, 27 µg/g dwt in forage-root, 0.66 µg/g dwt in pollen and 1.2 µg/g dwt in grain.

CP4 EPSPS protein levels

The mean CP4 EPSPS protein levels in MON 89034 × NK603 across all sites were 240 µg/g dwt in OSL-1, 78 µg/g dwt in OSR-1, 210 µg/g dwt in OSWP-3, 74 µg/g dwt in forage, 48 µg/g dwt in forage root, 390 µg/g dwt in pollen and 8.1 µg/g dwt in grain.

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

a) Reproduction

Agronomic data collected from trials performed with MON 89034 × NK603 have demonstrated that MON 89034 × NK603 has not been altered in survival, multiplication or dissemination characteristics when compared to conventional maize varieties. The inherited lepidopteran protection and glyphosate tolerance traits have no influence on maize reproductive morphology and hence no changes in seed dissemination would be expected.

b) Dissemination

The inherited traits have no influence on maize reproductive morphology and hence no changes in seed dissemination are to be expected.

c) Survivability

Maize is known to be a weak competitor in the wild, which cannot survive outside cultivation without the aid of human intervention. Field observations have demonstrated that MON 89034 × NK603 has not been altered in its survivability when compared to conventional maize.

d) Other differences

Comparative assessments in the field did not reveal any biologically significant differences between MON 89034 × NK603 and conventional 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

MON 89034 × NK603 hybrid seed (F_1) is produced by crossing MON 89034 and NK603 parental inbred lines (homozygous) by traditional breeding. Thereby, each parental line passes on its inserted DNA sequence to the resulting MON 89034 × NK603 F_1 hybrid seed, which is sown by the grower.

The parental lines MON 89034 and NK603 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.

The harvested (F_2) grain of MON 89034 × NK603 is marketed by the grower for food, feed or industrial use and is not used for further breeding. Therefore, since MON 89034 × NK603 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

None of the genetic elements inserted in MON 89034 × NK603 has a genetic transfer function. Therefore, no changes are expected in the ability of these maize lines to transfer genetic material to bacteria.

b) Plant to plant gene transfer

Not applicable. The scope of the current application does not include the cultivation of MON 89034 × NK603 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

MON 89034 × NK603 was compared with a conventional control maize with similar genetic background, as well as with other commercially available maize hybrids.

7.2 Production of material for comparative assessment

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

MON 89034 × NK603 and the conventional control maize were grown at five field sites in major maize-growing areas of Argentina (provinces of Buenos Aires, Córdoba and Santa Fe) during the 2004-2005 field season.

b) the baseline used for consideration of natural variations

The composition study compared MON 89034 × NK603 to the control. Reference hybrids were grown in the same field locations and under the same conditions as the test and control. Where statistical differences occurred, the measured analyte was compared to a confidence interval developed from the reference hybrids. Differences were also compared to ILSI ranges and ranges reported in literature.

7.3 Selection of material and compounds for analysis

The numerous compounds that were selected for analysis in the compositional study were chosen on the basis of internationally accepted guidance provided by the OECD (*see* consensus document for compositional analysis of maize), in addition to other selected compounds.

Based on the positive results of these extensive, compositional analyses conducted for MON 89034 × NK603 compared to conventional maize hybrids, there is no indication to further analyze other selected compounds in this maize.

7.4 Agronomic traits

Field trials with MON 89034 × NK603 were performed and the set of agronomic observations supports a conclusion that from an agronomic and phenotypic (morphological) point of view, MON 89034 × NK603 is equivalent to conventional maize, except for the inherited lepidopteran protection and glyphosate tolerance traits.

7.5 Product specification

MON 89034 × NK603 will be imported into the E.U. in mixed shipments of maize grain and products, produced in other world areas, for use by operators that have conventionally been involved in the commerce, processing and use of maize and maize derived products in the E.U.

7.6 Effect of processing

Using both wet and dry milling processes, maize is converted into a diverse range of food and feed products and derivatives used as food and feed ingredients or additives. As MON 89034 × NK603 is substantially equivalent and as safe and as nutritious as conventional maize, the use of MON 89034 × NK603 for the production of foods and

feeds is no different from that of conventional maize. Consequently, any effects of the production and processing of MON 89034 × NK603 are not expected to be any different from the production and processing of the equivalent foods and feeds, originating from conventional maize.

7.7 Anticipated intake/extent of use

There are no anticipated changes in the intake and/or extent of use of maize or derived products for use as such or in food or feed as a result of the addition of MON 89034 × NK603 to the conventional maize supply. MON 89034 × NK603 is expected to replace a portion of current maize hybrids such that its 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

MON 89034 × NK603 is produced by traditional breeding of MON 89034 with NK603. Both of the introduced traits from the parental lines are inherited by the MON 89034 × NK603 progeny. This resulted in the combined expression of the Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins in the same plant.

The conclusion of safety to humans of those proteins was based upon the following considerations:

- Those proteins have a demonstrated history of safe use;
- They have no structural similarity to known toxins or other biologically active proteins that could cause adverse effects in humans or animals;
- They do not exert any acute toxicity to mammals.

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

It is therefore highly unlikely that Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins would cause any toxic effects on human or animal health.

7.8.2 Testing of new constituents other than proteins

Since maize is known as a common source of food and feed with a centuries-long history of safe use and consumption around the world and as MON 89034 × NK603 was shown to be substantially equivalent to conventional maize, no testing of any constituent other than the inherited 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 from MON 89034 × NK603 and conventional maize have been established by compositional analysis. Additionally, the wholesomeness of MON 89034 × NK603 grain has been confirmed by a feed performance study conducted in rapidly growing broiler chickens fed MON 89034 × NK603-containing diets.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein

The Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins have been assessed for their potential allergenicity according to the recommendations of Codex Alimentarius Commission. The proteins are from non-allergenic sources, lack structural similarity to known allergens, are rapidly digested in simulated gastric fluid and constitute a very small portion of the total protein present in the grain of MON 89034 × NK603. Taken together, these data lead to the conclusion that the Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins are unlikely to have any allergenic potential, and MON 89034 × NK603 is as safe as conventional maize regarding the risk for allergenicity.

7.9.2 Assessment of allergenicity of the whole GM plant or crop

As the introduced proteins do not have any allergenic potential, it was concluded that the use of MON 89034 × NK603 for food or feed does not lead to an increased risk for allergenic reactions compared to the equivalent range of food and feed uses of conventional maize.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

The introduced traits in MON 89034 × NK603 are of agronomic interest, and are not intended to change any nutritional aspects of this maize. In addition to the extensive compositional analyses which demonstrated the substantial equivalence of MON 89034 × NK603 to conventional maize (except for the introduced traits), a confirmatory feed performance study was conducted in rapidly growing broiler chickens. Broilers were fed diets containing grain from MON 89034 × NK603, and their performance was compared to control groups fed diets containing a non-transgenic control hybrid or

commercially available reference hybrids. This study confirms the nutritional equivalence of MON 89034 × NK603 for use as food, and demonstrates the absence of any pleiotropic or unanticipated effects from the introduced trait.

In conclusion, MON 89034 × NK603 is nutritionally equivalent to non transgenic control maize, as well as to maize varieties in commerce.

7.10.2 Nutritional assessment of GM feed

The inherited traits in MON 89034 × NK603 are of agronomic interest, and are not intended to change any nutritional aspects of this maize. In addition to the extensive compositional analyses which demonstrated the substantial equivalence of MON 89034 × NK603 to conventional maize (except for the inherited traits), a confirmatory feed performance study was conducted in rapidly growing broiler chickens. Broilers were fed diets containing grain from MON 89034 × NK603, and their performance was compared to control groups fed diets containing a non-transgenic control hybrid or commercially available reference hybrids. This study confirms the nutritional equivalence of MON 89034 × NK603 for use as feed, and demonstrates the absence of any pleiotropic or unanticipated effects from the introduced trait.

In conclusion, MON 89034 × NK603 is nutritionally equivalent to non transgenic control maize, as well as to maize varieties in commerce.

7.11 Post-market monitoring of GM food/feed

The assessment of the human and animal safety of MON 89034 × NK603 was conducted on the basis of its substantial equivalence to conventional maize (except for the introduced traits) and by extensive characterisation of the introduced traits, which are of agronomic interest, resulting in the expression of the Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins.

There are no intrinsic hazards related to MON 89034 × NK603 as no signs of adverse or unanticipated effects have been observed in a number of safety studies, including an animal feeding study using doses of administration that are orders of magnitude above expected consumption levels. The pre-market risk characterisation for food and feed use of MON 89034 × NK603 demonstrates that the risks associated to the consumption of MON 89034 × NK603 or its derived products are consistently negligible and no different from the risks associated with the consumption of conventional maize and maize-derived products.

As a consequence, specific risk management measures are not indicated, and post-market monitoring of the use of this maize for food, feed or processing is neither warranted, nor appropriate.

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

The Cry1A.105 and Cry2Ab2 proteins produced in MON 89034 × NK603 provide protection against lepidopteran pests. Those lepidopteran insects may be considered the target organisms which interact with MON 89034 × NK603 plant, as the CP4 EPSPS protein (also produced by MON 89034 × NK603) does not have target organisms.

A generalized mode of action of Cry proteins includes the following steps: ingestion of the protoxin crystal by the insect, solubilization of the crystal in the insect midgut, proteolytic processing of the released Cry protein by digestive enzymes to produce an active toxin termed delta-endotoxin, binding of the endotoxin to receptors on the surface of midgut epithelial cells of target organisms, formation of membrane ion channels or pores, and consequent disruption of cellular homeostasis. Electrolyte imbalance and pH changes render the gut paralyzed, which causes the insect to stop eating and die.

Any significant interactions of MON 89034 × NK603 with its target pest organisms are, however, limited to those countries where the cultivation of this maize has been authorized. The cultivation of MON 89034 × NK603 varieties in the E.U. is not within the scope of this application. The likelihood that the import and use of MON 89034 × NK603 for food, feed or processing will result in plants of this maize being present in the environment is negligible.

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

This application is limited to import for direct food or feed use or for processing. As such, exposure to the environment will be rare, occurring only through incidental release during shipment and handling. The conditions where incidental release will occur are not conducive to establishment of maize.

9.1 Persistence and invasiveness

Like for conventional maize, the likelihood of MON 89034 × NK603 spreading in the environment is negligible, as maize is neither persistent nor invasive and these parameters are unaltered in MON 89034 × NK603 when compared to conventional maize. Hence, the risk of establishment and spreading of MON 89034 × NK603 in the environment is negligible.

9.2 Selective advantage or disadvantage

The introduced lepidopteran protection and glyphosate tolerance traits confer a selective advantage only under specific conditions (*i.e.* upon attack by the target insects or following treatment with glyphosate), which are short in duration. The advantage is of purely agronomic interest and presents negligible risk to the non-agricultural environments, because of the poor survival characteristics of maize under most European conditions.

The potential for the lepidopteran protection and glyphosate tolerance traits in MON 89034 × NK603 to cause a selective advantage of maize outside an agro-ecosystem is low. Therefore, the risk of adversely impacting the receiving environment is negligible under the intended use for food, feed or processing.

9.3 Potential for gene transfer

MON 89034 × NK603 is unchanged in its potential for gene transfer compared to conventional maize. There is no potential for gene transfer from MON 89034 × NK603 to wild plant species in the E.U. and negligible likelihood for gene transfer to other maize crops, as this application is not for consent to cultivate MON 89034 × NK603 varieties in the E.U. The environmental risk of potential gene transfer is negligible.

9.4 Interactions between the GM plant and target organisms

Since the likelihood is negligible that the import, processing and food and feed use of MON 89034 × NK603 will result in plants of this maize being present in the environment at meaningful levels, it is not expected that the target organisms will be exposed to Cry1A.105 and Cry2Ab2 proteins.

9.5 Interactions of the GM plant with non-target organisms

Given the scope of the current application, which does not include the cultivation of MON 89034 × NK603 varieties in the E.U., the likelihood for direct or indirect interactions of this maize with non-target organisms is considered to be negligible. In addition, the newly expressed proteins present a negligible hazard to non-target organisms, even if incidental spillage of MON 89034 × NK603 grain during import, storage, transport or use would lead to the short survival of MON 89034 × NK603 plants in the environment. As a consequence, there is negligible risk for harmful effects of MON 89034 × NK603 on non-target organisms, either through direct or indirect interactions with this maize or through contact with the newly expressed proteins.

Furthermore, no adverse effects were brought forward by the people handling these products during the extensive field trials conducted in the Argentina.

9.6 Effects on human health

The likelihood for any adverse effects occurring in humans as a result of their contact with this maize is no different from conventional maize, as MON 89034 × NK603 contains the Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins, which have negligible potential to cause any toxic or allergenic effects in humans. Therefore, the risk of changes in the occupational health aspects of this maize is negligible.

9.7 Effects on animal health

The likelihood of potential adverse effects in animals fed on MON 89034 × NK603 and in humans, consuming those animals, is negligible (*see* Sections D.7.8, D.7.9, D.7.10). Therefore, the risk of MON 89034 × NK603 for the feed/food chain is also negligible.

9.8 Effects on biogeochemical processes

In the event of an incidental release of MON 89034 × NK603 in the environment, the risk for direct or indirect, immediate or delayed adverse effects on biogeochemical processes can be considered negligible.

There is no evidence that MON 89034 × NK603 plants would be any different from conventional maize regarding their direct influence on biogeochemical processes or nutrient levels in the soil, as MON 89034 × NK603 is compositionally equivalent and has equivalent growth and development, morphology, yield, plant health and survival characteristics to non-transgenic maize (*see* Sections D.4, D.7.1 and D.7.4). Furthermore, any indirect interactions of the GMO and target or non-target organisms in the vicinity of an incidental release of the grain are not likely to cause hazardous effects on the biogeochemical processes in the soil. The Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins are subjected to rapid degradation in soil.

9.9 Impacts of the specific cultivation, management and harvesting techniques

Not applicable. This application is for consent to import MON 89034 × NK603 in the E.U. and for the use of this maize as any other maize, excluding the cultivation of varieties in the E.U.

10. Potential interactions with the abiotic environment

No adverse impact of MON 89034 × NK603 on the abiotic environment is expected to result from the import, processing or use of this product for food and feed in the E.U. Although the Cry1A.105, Cry2Ab2 and CP4 EPSPS are introduced proteins in maize, they already have a safe history of use and have no known negative interactions with the abiotic environment. The insecticidal proteins Cry1A.105 and Cry2Ab2 are subjected to rapid degradation in soil and are therefore not expected to negatively affect soil or water. The CP4 EPSPS protein is innocuous and belongs to a large class of EPSPS enzymes that are ubiquitous in nature. The family of EPSPS proteins has 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)

As required by Article 5(5)(b) of Regulation (EC) No 1829/2003, a general surveillance plan in accordance to Annex VII of Directive 2001/18/EC is included.

11.2 Interplay between environmental risk assessment and monitoring

An environmental risk assessment (ERA) of MON 89034 × NK603 was undertaken in the context of the scope of the application, that is, for import, processing and food and feed use of MON 89034 × NK603, but not including the cultivation of MON 89034 × NK603 varieties in the E.U. Analysis of the characteristics of MON 89034 × NK603 has shown that the risk for potential adverse effects on human health and the receiving environment, resulting from the import and use of MON 89034 × NK603 in the E.U. is consistently negligible. Therefore, the overall environmental risk posed by this genetically modified higher plant is negligible, and no specific strategies for risk management and no case-specific post-marketing monitoring actions are considered required.

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

As the overall environmental risk posed by this genetically modified higher plant is negligible, and as the conclusions of this environmental risk assessment are derived from the results of scientific studies, rather than major assumptions, no case-specific post-market monitoring actions, typically aimed at testing assumptions made in this assessment, would be warranted or required.

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

Any potential adverse effects of MON 89034 × NK603 on human health and the environment, which were not anticipated in the ERA, can be addressed under general surveillance in accordance with Directive 2001/18/EC. General surveillance is largely based on routine observation and implies the collection, scientific evaluation and reporting of reliable scientific evidence, in order to be able to identify whether unanticipated, direct or indirect, immediate or delayed adverse effects have been caused by the placing on the market of a genetically modified (GM) crop in its receiving environment.

In order to allow detection of the broadest possible scope of unanticipated adverse effects, general surveillance is performed by either selected, existing networks, or by specific company stewardship programmes, or by a combination of both. The applicant will ensure that appropriate technical

information on MON 89034 × NK603 and relevant legislation will be available for the relevant networks, in addition to further relevant information from a number of sources, including industry and government websites, official registers and government publications.

Where there is scientifically valid evidence of a potential adverse effect (whether direct or indirect), linked to the genetic modification, then further evaluation of the consequence of that effect should be science-based and compared with available baseline information. Relevant baseline information will reflect prevalent use practices and the associated impact of these practices on the environment. Where scientific evaluation of the observation confirms the possibility of an unanticipated adverse effect, this would be investigated further to establish a correlation, if present, between the use of MON 89034 × NK603 and the observed effect. The evaluation should consider the consequence of the observed effect and remedial action, if necessary, should be proportionate to the significance of the observed effect. Monsanto will submit a General Surveillance Report containing information obtained from participating networks, and/or in case of an effect that was confirmed. If information that confirms an adverse effect which alters the existing risk assessment becomes available, Monsanto will submit a Report, consisting of a scientific evaluation of the potential adverse effect and a conclusion on the safety of the product. The report will also include, where appropriate, the measures that were taken to ensure the safety of human or livestock health and/or the environment

11.5 Reporting the results of the monitoring

Monsanto will submit a General Surveillance Report containing information obtained from participating networks, and/or in case of an effect that was confirmed. If information that confirms an adverse effect which alters the existing risk assessment becomes available, Monsanto will submit a Report, consisting of a scientific evaluation of the potential adverse effect and a conclusion on the safety of the product. The report will also include, where appropriate, the measures that were taken to ensure the safety of human or livestock health and/or the environment.

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

As MON 89034 × NK603 is the result of a traditional cross of MON 89034 and NK603, it contains both inserts in combination. Therefore, MON 89034 × NK603 is detectable using either the event-specific PCR method for detecting the introduced DNA present in MON 89034 or the equivalent method for NK603. However, as for all plants in which one or more events are combined by traditional breeding, the unambiguous detection of MON 89034 × NK603 in mixed consignments of grain will require single grains to be subjected to detection methods for both MON 89034 and NK603, 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** Submissions made in France (B/FR/06/12/08), Spain (B/ES/07/02) and Germany in 2006[8].
b)	**Conclusions of post-release monitoring** Field trials to be performed in 2007.
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)** N/A

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

a)	**Release country** MON 89034 × NK603 has been tested in Argentina in 2004-2005 and 2005-2006.
b)	**Authority overseeing the release** Secretary of Agriculture (SAGPyA) – CONABIA.
c)	**Release site** Argentina 2004: Provinces of Buenos Aires, Cordoba and Santa Fe. Argentina 2005: Provinces of Buenos Aires, Cordoba, Santa Fe, Chaco and Jujuy.
d)	**Aim of the release** Assess the performances: efficacy, yield, breeding.
e)	**Duration of the release** 12 months

[8] http://gmoinfo.jrc.it/gmp_browse.aspx

f)	**Aim of post-releases monitoring** Assessment/removal of volunteers.
g)	**Duration of post-releases monitoring** 12 months
h)	**Conclusions of post-release monitoring** Volunteers have been eliminated to prevent persistence in the environment.
i)	**Results of the release in respect to any risk to human health and the environment** No evidence that MON 89034 × NK603 is likely to cause any adverse effects to human or animal health and the environment.

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

a)	**Status/process of approval** The EFSA website[9] provides information related to the applications submitted under Regulation (EC) No 1829/2003 on genetically modified food and feed.
b)	**Assessment Report of the Competent Authority (Directive 2001/18/EC)** A notification for MON 89034 × NK603 according to Directive 2001/18/EC has not been submitted by Monsanto.
c)	**EFSA opinion** An EFSA opinion, specifically for MON 89034 × NK603, was not available at the time of submission of this application.
d)	**Commission Register (Commission Decision 2004/204/EC)** Once authorized, food and feed products will enter in the Community Register of GM food and feed[10].

9 http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html
10 http://europa.eu.int/comm/food/dyna/gm_register/index_en.cfm

e)	**Molecular Register of the Community Reference Laboratory/Joint Research Centre** Information on detection protocols can be found on the JRC website[11].
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)** A notification and SNIF according to Directives 2001/18/EC and 2002/812/EC, respectively, have not been submitted for MON 89034 × NK603. The EFSA website[12] does provide a link to this summary of the application for MON 89034 × NK603 under Regulation (EC) No 1829/2003.

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

[12] http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html