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

Part II

Summary

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. In countries where MON 89034 is being cultivated, packages of this maize are marketed under the name of the hybrid variety, in association with the trademark YieldGard VT PRO™[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 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™ 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 U.S.A., Canada, and Colombia, but approvals from all agencies in these countries have not been obtained yet. The status of other 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

MON 89034 has been developed to produce the Cry1A.105 and the Cry2Ab2 proteins that confer protection against certain lepidopteran pests. MON 89034 was produced by *Agrobacterium*-mediated transformation of maize cells with plasmid vector PV-ZMIR245.

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 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 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 for import, processing, and use as or in food and feed. MON 89034 is substantially equivalent to other maize varieties except for its protection from target lepidopteran pests, which is a trait of agronomic interest. This maize was shown to be as safe and as nutritious as conventional maize. Therefore MON 89034 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 is substantially equivalent to conventional maize varieties (except for its protection from targeted lepidopteran insect pests). Therefore, MON 89034 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 grain and derived products.

Operators shall be required to label products containing or consisting of MON 89034 with the words "genetically modified maize" or "contains genetically modified maize", and shall be required to declare the unique identifier MON-89Ø34-3 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 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 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. 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

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 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 MON 89034 as any other maize, not including the cultivation of varieties of MON 89034 in the E.U., environmental release would be more likely to occur during import, storage and processing of MON 89034. 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 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 likely to 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 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 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

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 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 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 was produced by *Agrobacterium*-mediated transformation of immature embryos of maize tissue

2. Nature and source of the vector used

The plasmid vector PV-ZMIR245, used for the transformation of maize cells to produce MON 89034, contains 2 T-DNA. 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.

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

The T-DNA I region containing the *cry1A.105* and *cry2Ab2* gene expression cassettes is the portion of the plasmid PV-ZMIR245 intended for insertion.

The expression cassette for the coding sequence of the Cry1A.105 protein consists of the promoter (P-e35S) and leader for the cauliflower mosaic virus (CaMV) 35S RNA containing a duplicated enhancer region. It contains the 5' untranslated leader of the wheat chlorophyll a/b/ binding protein (L-*Cab*), the intron from the rice actin gene (*I-Ract1*), the *cry1A.105* coding sequence that was optimized for expression in monocots, and the 3' nontranslated region of the coding sequence for wheat heat shock protein 17.3 (T-*Hsp17*), which terminates transcription and provides the signal for mRNA polyadenylation.

The *cry2Ab2* gene expression cassette that produces the Cry2Ab2 protein consists of the 35S promoter from figwort mosaic virus (P-*FMV*), the first intron from the maize heat shock protein 70 gene (I-*Hsp* 70). It contains a *cry2Ab2* coding sequence with a modified codon usage (CS-*cry2Ab2*) fused to a chloroplast transit peptide region of maize ribulose 1,5-biphosphate carboxylase small subunit including the first intron (TS-*SSU-CTP*). The 3' nontranslated region of the nopaline synthase (T-*nos*) coding region from *Agrobacterium tumefaciens* T-DNA terminates transcription and directs polyadenylation.

Detailed description of all elements is presented in Table 1.

Table 1. Size and intended function of each constituent fragment of the region intended for insertion in MON 89034

Sequence	Size (Kb)	Source	Function
		T-DNA I	
B-Right Border	0.36	*Agrobacterium tumefaciens*	Border
P-*e35S*	0.62	Cauliflower mosaic virus	Promotor
L-*Cab*	0.06	Wheat	Leader
I-*Ract1*	0.48	Rice	Intron
CS-*cry1A.105*	3.53	*Bacillus thuringiensis*	Coding sequence
T-*Hsp17*	0.21	Wheat	Transcript termination sequence
P-*FMV*	0.56	Figwort mosaic virus	Promotor
I-*Hsp70*	0.80	Wheat	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	0.44	*Agrobacterium tumefaciens*	Border

D. INFORMATION RELATING TO THE GM PLANT

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

MON 89034 produces the Cry1A.105 and Cry2Ab2 insecticidal proteins and is protected from feeding damage caused by the European corn borer (ECB, *Ostrinia nubilalis*) and other lepidopteran insect pests.

Cry1A.105 is a modified *Bt* Cry1A protein while Cry2Ab2 is a *Bt* subsp. *kurstaki* protein. The combination of the Cry1A.105 and Cry2Ab2 insecticidal proteins in a single plant provides better insect control and offers an additional insect resistance management (IRM) tool.

The Cry1A.105 protein provides increased activity against fall armyworm (FAW, *Spodoptera* sp.) and black cutworm (BCW, *Agrotis ipsilon*) compared to Cry1Ab. The Cry2Ab2 protein provides improved control over Cry1Ab products from damage caused by corn earworm. This wider spectrum of activity also will potentially contribute to the further reduction of mycotoxins in grain that result from fungal invasion after insect feeding damage. MON 89034 provides an effective insect resistance management tool by producing two insecticidal proteins, Cry1A.105 and Cry2Ab2. Each of these proteins effectively controls primary lepidopteran insect pests, therefore reducing chances of developing insect resistance. Taken together, adoption of MON 89034 is likely to enhance the economic benefits to farmers and improve the quality of grain and the safety of derived food and feed products. In addition, MON 89034 was developed to allow the efficient introgression of two insect protection traits into improved maize germplasm, which will reduce the time and costs for new improved variety introductions into the marketplace. MON 89034 was developed using a single transformation vector containing both the *cry1A.105* and *cry2Ab2* genes. This approach, known as vector stacking, increases the efficiency of breeding multiple traits into new maize hybrids, thereby providing growers an earlier access to improved germplasm containing these traits rather than through conventional inbred stacking.

2. Information on the sequences actually inserted or deleted

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

MON 89034 contains a single DNA insert containing a single copy of the introduced DNA fragment, and this at a single locus in the maize genome.

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

Not applicable.

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

The Chi square analysis of the segregation pattern, according to Mendelian genetics, was consistent with a single site of insertion into the maize nuclear DNA.

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

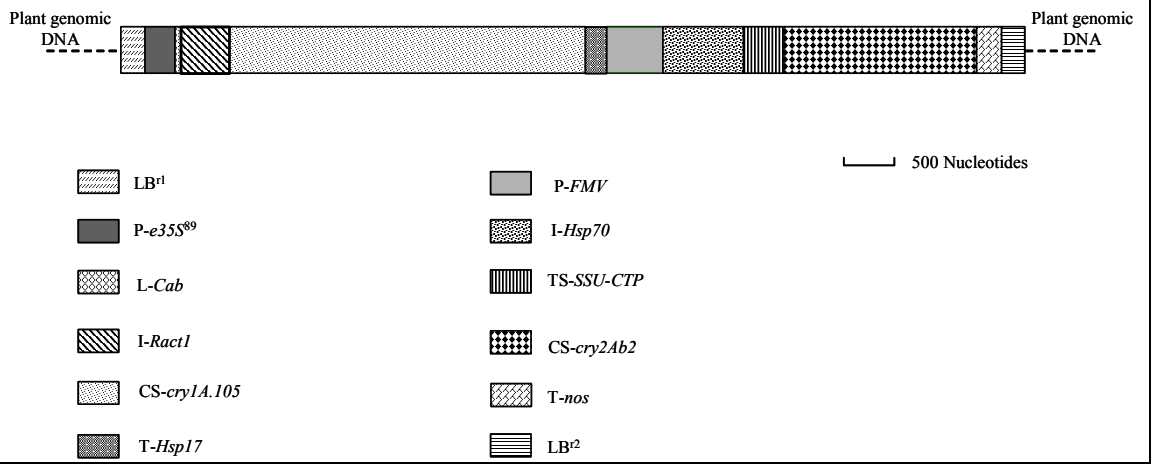
MON 89034 was developed through *Agrobacterium*-mediated transformation of maize to produce the *Bt* insecticidal proteins Cry1A.105 and Cry2Ab2 using the binary plasmid vector, PV-ZMIR245 that contains 2 T-DNAs. T-DNA I includes the *cry1A.105* and the *cry2Ab2* expression cassettes, while T-DNA II includes the *nptII* expression cassette.

Genomic DNA from MON 89034 was analyzed by Southern blotting to confirm the presence and intactness of the elements comprising the *cry1A.105* and *cry2Ab2* expression cassettes and to determine the presence or absence of plasmid backbone sequences. The organisation of the elements within the insert in MON 89034 was further confirmed using PCR analysis and sequencing of the insert.

It has been demonstrated that MON 89034 contains one copy of T-DNA I that resides at a single locus of integration on ~13 kb *Nde* I restriction fragment. No additional elements from the transformation vector PV-ZMIR245 were detected in the genome of MON 89034. Additionally, MON 89034 does not contain any detectable plasmid backbone sequence.

A schematic representation of the MON 89034 insert is given in Figure 1.

Figure 1. Schematic representation of the MON 89034 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 and Cry2Ab2 proteins in various tissues of MON 89034 were estimated using enzyme-linked immunosorbent assay (ELISA). To produce the tissues for analysis, MON 89034 and conventional maize were planted at five field locations during the 2005 growing season. The sites were located in the major maize-growing region of the U. S.A. A randomized complete block design with three replications was used at all sites. The tissues were collected over the season from the V2 to the R6 vegetative growth stages: overseason leaf samples (OSL 1-4), overseason whole plant (OSWP 1-4), and overseason root (OSR 1-4).

In tissues harvested throughout the growing season, mean Cry1A.105 protein levels across all sites ranged from 72-520 µg/g dwt in leaf, 42-380 µg/g dwt in the whole plant, and 11-79 µg/g dwt in root tissues. Mean Cry2Ab2 protein levels across all sites ranged from 130-180 µg/g dwt in leaf, 38-130 µg/g dwt in the whole plant, and 21-58 µg/g dwt in root. In general, levels of the Cry1A.105 and Cry2Ab2 proteins declined over the growing season.

b) Parts of the plant where the insert is expressed

Young leaf, pollen, silk, forage, forage root, grain, stover and senescent root were collected at appropriate times of plant development. In addition, grain being the most relevant tissue to food and feed safety a second year study was performed in Argentina during the 2004 season.

The first year, the mean Cry1A.105 levels across all sites were 520 µg/g dwt in young leaf, 12 µg/g dwt in pollen, 26 µg/g dwt in silk, 42 µg/g dwt in forage, 12 µg/g dwt in forage root, 5.9 µg/g dwt in grain, 50 µg/g dwt in stover, and 11 µg/g dwt in senescent root.

The mean Cry2Ab2 protein levels across all sites were 180 µg/g dwt in young leaf, 0.64 µg/g dwt in pollen, 71 µg/g dwt in silk, 38 µg/g dwt in forage, 21 µg/g dwt in forage root, 1.3 µg/g dwt in grain, 62 µg/g dwt in stover, and 26 µg/g dwt in senescent root.

The second year confirmed the data obtained for the expression in grain for both Cry1A.105 and Cry2Ab2.

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

a) Reproduction

Agronomic data collected from trials performed with MON 89034 have demonstrated that MON 89034 has not been altered in survival, multiplication or dissemination characteristics when compared to conventional maize varieties. The introduced trait for insect-protection has no influence on maize reproductive morphology and hence no changes in seed dissemination would be expected.

b) Dissemination

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

c) Survivability

Maize is known to be a weak competitor in the wild, which cannot survive outside cultivation without the aid of human intervention. Field observations have demonstrated that MON 89034 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 and conventional maize hybrids, except for the introduced trait that is of agronomic interest.

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

MON 89034 contains one insert with a single copy of the transformed DNA, which is stably integrated into the nuclear maize genome. The insert is inherited in a Mendelian fashion. This has been confirmed by Southern blot analyses.

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

a) Plant to bacteria gene transfer

None of the genetic elements inserted in MON 89034 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 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 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

2004 U.S. field season
MON 89034 and the conventional control maize were grown at five replicated field sites in major maize-growing areas of the U.S.A. (Iowa, Illinois, Ohio and Nebraska) during the 2004 field season.

2004-2005 Argentinean field season
MON 89034 and the conventional control maize were grown at five replicated field sites across Argentina during the 2004-2005 field season.

b) the baseline used for consideration of natural variations

2004 U.S. field season
For the compositional study, altogether a total of 366[6] statistical comparisons were made between the test hybrid (MON 89034) and the non-transgenic control. The 47 test values observed to be statistically different ($p<0.05$) between MON 89034 and the conventional control were within the 99% tolerance interval, except for calcium in grain (which showed a difference for only one of the six comparisons). All test values for maize grain calcium were within ILSI ranges or literature ranges. Based on these data it is unlikely that these differences are biologically meaningful.

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[6] Six sets (five based on the date from each of the replicated field sites and the sixth based on data from the combination of all five field sites) of comparisons x 52 components for grain and x nine components for forage. In all 77 different analytical components (nine in forage and 68 in grain) were analysed. Of these evaluated components, 16 had more than 50% of the observations below the limit of quantitation of the assay and, as a result, were excluded from the statistical analysis.

[7] Six sets (five based on the date from each of the replicated field sites and the sixth based on data from the combination of all five field sites) of comparisons x 52 components for grain and x nine components for forage. In all 77 different analytical components (nine in forage and 68 in grain) were analysed. Of these evaluated components, 16 had more than 50% of the observations below the limit of quantitation of the assay and, as a result, were excluded from the statistical analysis.

2004-2005 Argentinean field season
For the compositional study, altogether a total of 366[7] statistical comparisons were made between the test hybrid (MON 89034) and the non-transgenic control. The 29 test values observed to be statistically different ($p<0.05$) between MON 89034 and the conventional control were within the 99% tolerance interval as well as within ILSI ranges. Based on these data it is unlikely that these differences are biologically meaningful.

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 compared to conventional maize hybrids, there is no indication to further analyse other selected compounds in this maize.

7.4 Agronomic traits

Field trials with MON 89034 were performed and the set of agronomic observations supports a conclusion that from an agronomic and phenotypic (morphological) point of view, MON 89034 is equivalent to conventional maize, except for the introduced insect-protection trait.

7.5 Product specification

MON 89034 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 is substantially equivalent and as safe and as nutritious as conventional maize, the use of MON 89034 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 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 or in food or feed as a result of the addition of MON 89034 to the conventional maize supply. MON 89034 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

The Cry1A.105 and Cry2Ab2 proteins have been assessed for their potential toxicity according to the recommendations of Codex. The proteins are functionally and structurally similar to Cry proteins with a demonstrated history of safe use, lack structural similarity to known toxins or biologically active proteins known to have adverse effects on mammals, do not show acute oral toxicity in mice, and constitute a very small portion of the total protein present in feed and food derived from MON 89034.

The Cry1A.105 and Cry2Ab2 proteins are from *Bacillus thuringiensis,* an organism that has been used commercially in the U.S.A. since 1958 to produce microbial-derived products with insecticidal activity and whose safety has been demonstrated by over 45 years of use. Bioinformatics analyses demonstrated that the proteins do not share structural or sequence similarities to known toxins or biologically active proteins that are known to cause adverse health effects in humans or animals. Results from acute oral toxicity studies with mice demonstrated that the Cry1A.105 and Cry2Ab2 proteins are not acutely toxic and do not cause any adverse effects even at maximum attainable dose levels. Finally, the Cry1A.105 and Cry2Ab2 proteins represent no more than 0.005% and 0.001% of the total protein in the grain of MON 89034, respectively. Taken together these data lead to the conclusion that the Cry1A.105 and Cry2Ab2 proteins are unlikely to have any toxic effect on on animals or humans.

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 was shown to be substantially equivalent to conventional 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 MON 89034 and conventional maize have been established by compositional analysis. Additionally, the wholesomeness of MON 89034 grain has been confirmed by a repeat-dose animal feeding study in broiler chickens using MON 89034-containing diets.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein

Cry1A.105 and Cry2Ab2 proteins were assessed for their potential allergenicity by a variety of tests, including a) whether the genes came from allergenic or non-allergenic sources, b) sequence similarity to known allergens, and c) pepsin stability of the protein in an *in vitro* digestion assay. In all cases, the proteins did not exhibit properties characteristic of allergens.

7.9.2 Assessment of allergenicity of the whole GM plant or crop

As the introduced proteins do not have any allergenic potential, it was concluded that the use of MON 89034 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 trait in MON 89034 is of agronomic interest, and is not intended to change any nutritional aspects of this maize. In addition to the extensive compositional analyses which demonstrated the substantial equivalence of MON 89034 to conventional maize (except for the introduced trait), a confirmatory feed performance study was conducted in rapidly growing broiler chickens. Broilers were fed diets containing grain from MON 89034, 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 for use as food, and demonstrates the absence of any pleiotropic or unanticipated effects from the introduced trait.

In conclusion, MON 89034 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 introduced trait in MON 89034 is of agronomic interest, and is not intended to change any nutritional aspects of this maize. In addition to the extensive compositional analyses which demonstrated the substantial equivalence of MON 89034 to conventional maize (except for the introduced trait), a confirmatory feed performance study was conducted in rapidly growing broiler chickens. Broilers were fed diets containing grain from MON 89034, 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 for use as feed, and demonstrates the absence of any pleiotropic or unanticipated effects from the introduced trait.

In conclusion, MON 89034 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 was conducted on the basis of its substantial equivalence to conventional maize (except for the introduced trait) and by extensive characterisation of the introduced trait, which is of agronomic interest, resulting in the expression of the Cry1A.105 and Cry2Ab2 proteins.

There are no intrinsic hazards related to MON 89034 as no signs of adverse or unanticipated effects have been observed in a number of safety studies, including animal feeding studies using doses of administration that are orders of magnitude above expected consumption levels. The pre-market risk characterisation for food and feed use of MON 89034 demonstrates that the risks of consumption of MON 89034 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 provide protection from feeding damage caused by a wide spectrum of lepidopteran insect pests. Those lepidopteran insects may be considered the target organisms which interact with MON 89034.

The general mechanism of insecticidal activity of the Cry proteins is well understood. A generalized mode of action of Cry1A.105, and Cry2Ab2 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 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 varieties in the E.U. is not within the scope of this application. The likelihood that the import and use of MON 89034 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 spreading in the environment is negligible, as maize is neither persistent nor invasive and these parameters are unaltered in MON 89034 when compared to conventional maize. Hence the risk of establishment and spreading of MON 89034 in the environment is negligible.

9.2 Selective advantage or disadvantage

Compared with conventional maize, the presence of the lepidopteran-protection trait would only confer a selective advantage where target lepidopteran pest species would be present at sufficiently high numbers to limit reproductive success, and if no other, more important factors limiting the survival of maize in the receiving environment would be present. The potential for the lepidopteran-protection trait in MON 89034 to cause a selective advantage of maize outside an agro-ecosystem is exceedingly 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 is unchanged in its potential for gene transfer compared to conventional maize. There is no potential for gene transfer from MON 89034 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 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 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 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 introduced Cry1A.105 and Cry2Ab2 proteins present a negligible hazard to non-target organisms, even if incidental spillage of MON 89034 grains during import, storage, transport or use would lead to the short survival of MON 89034 plants in the environment. As a consequence, there is negligible risk for harmful effects of MON 89034 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 U.S.A.

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. MON 89034 contains the Cry1A.105 and Cry2Ab2 proteins, which have negligible potential to cause any toxic or allergenic effects in man. 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 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 for the feed/food chain is also negligible.

9.8 Effects on biogeochemical processes

In the event of an incidental release of MON 89034 in the environment, the risk for direct or indirect, immediate or delayed adverse effects on biogeochemical processes can be considered as negligible. There is no evidence that MON 89034 plants would be any different from conventional maize regarding their direct influence on biogeochemical processes or nutrient levels in the soil, as MON 89034 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 and Cry2Ab2 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 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 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 Cry1A.105 and Cry2Ab2 are introduced proteins in maize, they already have a safe history of use and have no known negative interactions with the abiotic environment. The Cry1A.105 and Cry2Ab2 proteins are subjected to rapid degradation in soil (Cry1A.105 - DT_{50}: 7 days or less, DT_{90}: 19 days or less; Cry2Ab2 - DT_{50}: 6 days or less, DT_{90}: 14 days or less) and is therefore not expected to negatively affect soil or water.

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

1. *Case-specific monitoring*

   An environmental risk assessment (e.r.a.) of MON 89034 was undertaken in the context of the scope of the application, that is, for import, processing and food and feed use of MON 89034, but not including the cultivation of MON 89034 varieties in the E.U. Analysis of the characteristics of MON 89034 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 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.

2. *General surveillance*

   Any potential adverse effects of MON 89034 on human health and the environment, which were not anticipated in the e.r.a., 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 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 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.

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

MON 89034 will be detectable using the insert-specific PCR method for detecting the introduced DNA present in MON 89034. The proteins present in MON 89034 may also be detected by an appropriate ELISA method.

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, Germany and Spain in 2006.

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 has been field tested in the U.S.A. and Argentina since 2002. It has also been tested in Canada (2005) and Japan (2006).

b)	**Authority overseeing the release** U.S.A.: United States Department of Agriculture and Environmental Protection Agency. Japan: Ministry of Agriculture Fisheries and Forestry. Argentina: Secretary of Agriculture (SAGPyA) – CONABIA. Canada: Canadian Food Inspection Agency.
c)	**Release site** U.S.A.: mainly in the states of the corn belt and in Hawaii and Puerto Rico. Japan: Ibaraki prefecture. Argentina: Buenos Aires, Cordoba, Santa Fe. Jujy y Chaco. Canada: Ontario (Ridgetown (2), Branchton, Springford)
d)	**Aim of the release** U.S.A./Argentina: assess the performances: agronomic performance, efficacy, yield, breeding, Japan: stage III environmental assessment. Canada: agronomic evaluation.
e)	**Duration of the release** U.S.A./Argentina: 12 months. Japan/Canada: 6 months.
f)	**Aim of post-releases monitoring** U.S.A./Argentina/Canada: assessment/removal of volunteers.
g)	**Duration of post-releases monitoring** U.S.A./Argentina/Canada: 12 months.
h)	**Conclusions of post-release monitoring** Volunteers have been eliminated when found during the post-release monitoring
i)	**Results of the release in respect to any risk to human health and the environment** All countries: no evidence that MON 89034 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[8] 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 according to Directive 2001/18/EC has not been submitted by Monsanto.
c)	**EFSA opinion** An EFSA opinion, specifically for MON 89034, was not available at the time of submission of this application.
d)	**Commission Register (Commission Decision 2004/204/EC)** The authorized food and feed are entered in the Community Register of GM food and feed[9].
e)	**Molecular Register of the Community Reference Laboratory/Joint Research Centre** Information on detection protocols can be found on the JRC website[10].
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. The EFSA website[11] does provide a link to this summary of the application for MON 89034 under Regulation (EC) No 1829/2003.

8 http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html
9 http://europa.eu.int/comm/food/dyna/gm_register/index_en.cfm
10 http://gmo-crl.jrc.it/statusofdoss.htm
11 http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html