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

Part II

Summary

Version: Completeness Check #1 (March 2012)

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 Belgium
b) Notification number Not available at the time of submission.
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][1].
d) Date of acknowledgement of notification Not available at the time of submission to EFSA.

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 cultivated, traded and used in the European Union in the same manner as commercial maize and by the same growers and operators currently involved in the production, storage, transport, processing and use of maize.	

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

3. Scope of the application

( )	GM plants for food use
( )	Food containing or consisting of GM plants
( )	Food produced from GM plants or containing ingredients produced from GM plants
( )	GM plants for feed use
( )	Feed containing or consisting of GM plants
( )	Feed produced from GM plants
( )	Import and processing (Part C of Directive 2001/18/EC)
(x)	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 (x)	No ( )
If *yes*, specify An application pursuant to Regulation (EC) No 1829/2003 on genetically modified food and feed (EFSA-GMO-NL-2007-37) was submitted in December 2006, which covers the use of MON 89034 for food, feed, import and processing as any other maize in the EU.	

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

Yes (x)	**No ( )**
If *yes*, specify MON 89034 has been notified to and evaluated by numerous international regulatory authorities. Approval for cultivation was granted in the US, Canada, Argentina, Brazil, Philippines and South Africa. Other countries around the world, such as Australia/New Zealand, Colombia, Honduras, Korea, Mexico, Japan, Russia, Singapore, Taiwan and the Philippines approved MON 89034 for import, food and feed uses. The status of other pending regulatory reviews, which are currently in progress in numerous countries around the world, depends 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 (*e.g.* European corn borer (ECB) and Mediterranean corn borer (MCB)). 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).

The use of MON 89034 enables the farmer to effectively control the targeted lepidopteran insect pests in maize, ensuring maximum realization of yield potential, while removing the environmental burden of the production, packaging and transport of insecticides, previously used to control those pests.

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

The range of uses of this maize will be identical to the full range of equivalent uses of conventional maize.

This application is for the authorization of MON 89034 production and cultivation in the EU. It complements the scope of Authorization for placing in the market of products containing, consisting of, or produced from genetically modified maize MON 89034 (Commission Decision 2009/813/EC).

c) Intended use of the product and types of users

MON 89034 will be cultivated, traded and used in the EU in the same manner as current commercial maize and by the same growers and operators currently involved in the production, storage, transport, processing and use of maize.

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

MON 89034 is substantially equivalent to other maize varieties except for its protection against 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. No specific instructions and recommendations for use, storage and handling are therefore warranted or required.

e) Any proposed packaging requirements

MON 89034 is substantially equivalent to conventional maize varieties (except for its protection from target lepidopteran insect). 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) A proposal for labelling in accordance with Articles 13 and 25 of Regulation (EC) 1829/2003. In the case of GMOs, food and/or feed containing, consisting of GMOs, a proposal for labelling has to be included complying with the requirements of Article 4, B(6) of Regulation (EC) 1830/2003 and Annex IV of Directive 2001/18/EC.

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

The applicant and his licensees will sell certified MON 89034 seed for planting in the EU. Seed vendors shall be required to label seed bags containing MON 89034 varieties with the words “genetically modified maize” or “contains genetically modified maize” as well as the product’s unique identifier MON-89Ø34-3.

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.

Growers and operators handling or using MON 89034 grain and derived foods and feeds in the EU 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, growers and 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 authorisation applied for. Any type of environment to which the product is unsuited

MON 89034 is suitable for cultivation in all maize production regions in the EU.

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

The scope of this application is for cultivation of varieties of MON 89034 in the EU.

MON 89034 is substantially equivalent to conventional maize except for the introduced lepidopteran-protection trait which is a trait of agronomic interest. Moreover, the information presented in this application established that MON 89034 is as safe and as nutritious as conventional maize and unlikely to pose any threat to the environment or to require special measures for its containment. Therefore, any measures for waste disposal and treatment of MON 89034 products are the same as those for conventional maize and no specific conditions are warranted or required for the placing on the market of MON 89034.

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 *mays*
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

<u>Out-crossing with cultivated *Zea* varieties</u>

In Europe, the potential for genetic transfer and exchange with other organisms is limited to other maize plants. Maize is wind pollinated, and the distance that viable pollen can travel depends on prevailing wind patterns, humidity, and temperature. All maize will interpollinate, except for certain popcorn varieties and hybrids that have one of the gametophyte factors (GaS, Ga, and ga allelic series) on chromosome 4. Maize pollen, therefore, moves freely within an area, lands on silks of the same variety or different varieties, germinate almost immediately after pollination, and within 24 hours completes fertilisation.

<u>Out-crossing with wild *Zea* species</u>

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 see 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, due to its relatively large mass and size (90 – 100 Om), the vast majority does not move more than a few meters from the crop in significant quantities. Most maize pollen falls within five meters of the field edge with smaller amounts of pollen deposited usually in a downwind direction.

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 EU 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**

Like other plants, cultivated maize is known to interact with other organisms in the environment including insects, birds and mammals. It is susceptible to a range of fungal diseases and nematode, insect and mite pests. Maize has a history of safe use for human food and animal feed and the toxic and allergenic risk posed from consumption of maize and derived products is likely to be very low.

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 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	Maize	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 Mediterranean corn borer (MCB, *Sesamia nonagrioides)* 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 (*e.g.* MCB and ECB). 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 the 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 organization 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.

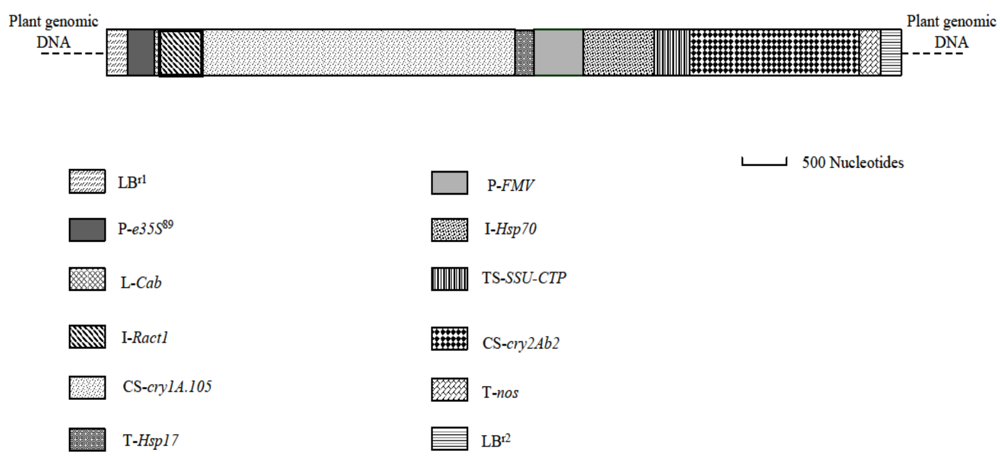
Genomic DNA from MON 89034 was analyzed by Southern blotting to confirm the presence and intactness of the elements comprising the *cry1A.105* and *cry2Ab2* expression cassettes and to determine the presence or absence of plasmid backbone sequences. The organisation of the elements within the insert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

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.

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 spread and replicates

MON 89034 and the conventional control maize were grown at five field sites in major maize-growing areas of the US and in 5 regions in Argentina during the 2004-2005 field season.

In addition, MON 89034 and the conventional control maize were grown at six field sites in geographical regions where maize is grown commercially in the EU (Germany and Spain) during the 2007 field season.

2004 US field season

MON 89034 and the conventional control maize were grown at five replicated field sites in major maize-growing areas of the US (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.

2007 EU field season

MON 89034 and the conventional control maize were grown at six replicated field sites across the EU (3 sites in Germany representing Northern Europe and 3 sites in Spain representing Southern Europe) during the 2004 field season.

b) the baseline used for consideration of natural variations

The two compositional studies compared MON 89034 (test) to the respective conventional 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[2] 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 studies were chosen on the basis of internationally accepted guidance provided by the OECD.

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 analyze other selected compounds in this maize.

[2] International Life Science Institute Crop Composition Database.

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 lepidopteran protection trait.

7.5 Product specification

MON 89034 will be used in the EU by growers and operators that have traditionally been involved in the production, commerce, processing and use of maize and maize-derived products in the EU.

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 US 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 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. In the EU, ECB and MCB are 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.

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

9.1 Persistence and invasiveness

Like for conventional maize, the likelihood of this maize to spread within cultivated fields or beyond the agricultural environment where it is grown is negligible, as maize is neither persistent nor invasive and these parameters are unaltered in MON 89034 when compared to conventional maize. In the unlikely event of the establishment of MON 89034 plants in the environment, the introduced traits would confer only a limited selective advantage (protection against ECB and MCB pests) of short duration, narrow spatial context and with negligible consequences for the environment. 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 inherited traits in MON 89034 would only confer a meaningful advantage under specific conditions, i.e. where target insect pest species (e.g. ECB and MCB) would be present in sufficiently high numbers and if no other more important factors limiting its survival in the environment were present. This introduced "advantage" is only relevant in agricultural habitats (i.e. in maize fields) and is short in duration. The risk of the insect pest protection trait in MON 89034 to be the cause of any adverse effects resulting from a competitive advantage or disadvantage is negligible, as maize is unlikely to establish outside cultivation under European conditions (see Section D.9.1). When viewed in the context of today's baseline agronomic practices for the production of maize, these advantages present negligible risk to the agricultural environment.

9.3 Potential for gene transfer

There is no potential for gene transfer from MON 89034 to wild plant species in the EU (as not present), while the likelihood for gene transfer to other maize crops depends mainly on wind, flowering synchrony and distance between the crops. In the event that an introduced gene outcrossed to other maize, its transfer would only confer a selective advantage under specific conditions (i.e. upon attack by the target insects), as discussed in Section 9.2. Therefore, gene transfer from MON 89034 to other maize crops is not considered to constitute an adverse environmental effect in itself and the environmental risk posed by this potential transfer to other maize crops, and hence by MON 89034, is negligible.

9.4 Interactions between the GM plant and target organisms

Target organisms for MON 89034 will be limited to those of the Cry1A.105 and Cry2Ab2 proteins, *i.e.* ECB and MCB in the EU.

Control of pest species is not considered adverse to the environment in an agro-ecosystem. The theoretical adverse effects of MON 89034 on non-target organisms (through indirect interactions) cannot be considered different from the effects produced by other insect pest control measures, such as insecticide applications in conventional maize. Therefore, MON 89034 poses no increased risk to these organisms, compared to conventional maize.

The only identified potential consequence from interactions between MON 89034 and its target insect pests, if it occurs, would be the development of resistance in the target pests to the insecticidal Cry proteins expressed in MON 89034. However, since an Insect Resistance Management (IRM) plan will be put in place in those countries where MON 89034 will be commercially planted, the risk for ECB and MCB resistance to the Cry1A.105 and Cry2Ab2 proteins to occur will be negligible.

9.5 Interactions of the GM plant with non-target organisms

As MON 89034 and conventional maize are not different with respect to their phenotypic, agronomic characteristics and ecological interactions (except for the lepidopteran-protection trait), it was concluded that the impact of MON 89034 on NTOs in the environment is not different from that of conventional maize. Furthermore, the potential exposure of NTOs to the inherited Cry1A.105 and Cry2Ab2 proteins presents no conceivable mechanism for causing adverse effects because of their specificity and properties.

A conclusion of negligible hazard for the Cry1A.105 and Cry2Ab2 proteins expressed in MON 89034 on NTOs is supported by the history of safe use of *Bt* microbial pesticides, the mode of action of Cry proteins and their spectrum of activity. The non-hazardous nature of the Cry1A.105 and Cry2Ab2 proteins to NTOs was further confirmed in first tier NTO studies of the environmental risk assessment. Therefore, the risk for any adverse effects to NTOs, through their ecological interactions with MON 89034 or through contact with the produced Cry1A.105 and Cry2Ab2 proteins, is negligible.

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

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 expresses the Cry1A.105 and Cry2Ab2 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 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

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 conventional maize (see Sections D.4, D.7.1 and D.7.4). The Cry1A.105 and Cry2Ab2 proteins are subjected to rapid degradation in soil.

9.9 Impacts of the specific cultivation, management and harvesting techniques

As MON 89034 is equivalent to conventional maize, except for the lepidopteran protection trait, all the agronomic practices currently used to grow maize in the EU remain applicable for growing MON 89034.

Pest control is an established baseline management technique in maize. The introduced ECB and MCB-protection trait in maize merely provide the farmer with an additional option or tool to control these pests. Therefore, cultivation of MON 89034 instead of conventional maize does not change any basic management technique in maize as such, but gives growers more flexibility to apply the existing tools for management, while creating at the same time new opportunities to grow maize in a more sustainable way (e.g. reduced insecticide applications or integrated pest management). It should be noted that the importance and aim of the basic management technique of removal of harmful insect pests from the MON 89034 field in order to achieve optimal yield of the crop, is neither new nor different compared to conventional maize.

In order to secure the valuable agronomic and other benefits of ECB and MCB-protected maize on a longer term, an IRM plan was developed, as described in Section D.9.4.

In conclusion, in comparison to any other maize, no typical characteristics of the genetically modified plant could be identified, which may cause adverse effects on the environment through a need to change management practices. Therefore, the environmental impact of farming practices to grow MON 89034 in the EU is considered no different from any other maize.

10. Potential interactions with the abiotic environment

Like other plants, cultivated maize is known to interact with the abiotic environment (soil, water and air), e.g. establishment of roots in the soil, nutrient and water uptake and gas exchange. Maize production in general is known to have indirect impacts on biophysical and biogeochemical processes in the soil through tillage, fertilizer application, and establishment of a monoculture in a defined area. All the agronomic practices currently used to grow maize in the EU remain applicable for growing MON 89034 and no specific techniques for cultivation, management and harvesting are required.

As MON 89034 was shown to be substantially equivalent to conventional maize (except for the introduced lepidopteran protection trait) with respect to its composition, phenotypic and agronomic characteristics, there is no evidence that this maize would be any different from conventional maize with regard to its baseline 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 and if the applicant has clearly shown that environmental exposure is absent or will be at levels or in a form that does not present a risk to other living organisms or the abiotic environment)

11.1 General (risk assessment, background information)

As the scope of this application under Regulation (EC) No 1829/2003 includes the use of MON 89034 for the cultivation of varieties in the EU, a monitoring plan in accordance with Annex VII of Directive 2001/18/EC was included, as required by Articles 5(5) and 17(5) of the said Regulation.

11.2 Interplay between environmental risk assessment and monitoring

An environmental risk assessment (ERA) for MON 89034 was conducted as required by Articles 5(5) and 17(5) of Regulation (EC) No 1829/2003. 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 proposed use of MON 89034 in the EU is consistently negligible.

The ERA describes, however, that specific strategies for risk management are required with regards to the interaction between the GM plant and target organisms. IRM measures will be put in place in MON 89034 cultivating countries to pro-actively avoid and in any case delay insect resistance development. Therefore, the applicant proposes to set up case-specific post marketing monitoring (CSM) actions, in the form of Insect Resistance Monitoring.

The monitoring will further concentrate on general surveillance (GS) to allow the identification of adverse effects of MON 89034 or its use on human health or the environment, which were not anticipated in the ERA.

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

The conclusions of the ERA (described in Section D.9) consistently show that the placing on the market of MON 89034 poses negligible risk to human and animal health and the environment. Specific strategies for risk management are however required with regard to the interactions between the GM plant and target organisms. IRM measures will be put in place in MON 89034 cultivating countries to proactively avoid and in any case delay insect resistance development. The party placing the GM plant on the market will therefore set up CSM actions in the form of insect resistance monitoring, as described in the IRM plan presented by the applicant.

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

Any potential adverse effects of MON 89034 on human health and the environment, which were not anticipated in the environmental risk assessment, are addressed by the GS plan. GS 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.

For GS, the party placing MON 89034 on the market will use several tools. The central tool is an annual farm questionnaire addressed to a subset of farmers cultivating MON 89034. Additionally, information from other sources (company stewardship programmes, scientific literature, official websites and existing observation networks) will be incorporated, where appropriate.

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 sciencebased 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.

11.5 Reporting the results of monitoring

Any recorded observations of adverse findings that are linked to the cultivation and/or use of this maize, which come to the attention of the party placing the GM plant on the market, will receive careful analysis in real time and remediating action, where applicable. Adverse reports will be discussed in the mandatory general surveillance report. The general surveillance reports will be sent to the European Commission, which will distribute to all Competent Authorities in the EU. General Surveillance reports will be prepared on an annual basis, except in case of adverse findings that need immediate risk mitigation, which will be reported as soon as possible.

Since monitoring of GM plants is a new topic and a creative process, the monitoring plan and especially the questionnaires can be improved based on experience from year to year.

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, Spain, Romania, Czech Rep., Slovakia since 2006.

b) Conclusions of post-release monitoring

The EU field trials with MON 89034 conducted to date aimed at collecting data for regulatory purposes. Post-release monitoring provided no significant evidence that MON 89034 is likely to pose any risk of adverse effects to human or animal health or to the environment.

c) Results of the release in respect to any risk to human health and the environment (submitted to the Competent Authority according to Article 10 of Directive 2001/18/EC)

Post-release monitoring provided no significant evidence that MON 89034 is likely to pose any risk of adverse effects to human or animal health or to the environment.

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

a) Release country

MON 89034 has been field tested in the US since 2004. It has also been tested in Argentina, Brazil, Canada, and South Africa.

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.

Brazil: CTNBio and Agriculture Ministry (MAPA)

South Africa: Department of Agriculture, Forestry and Fisheries

Philippines: Bureau of Plant Industry

c) Release site

US/Argentina/Brazil/Canada/South Africa/Philippines: major maize growing regions.

Japan: Ibaraki prefecture.

d) Aim of the release

US/Argentina/Canada/Brazil/South Africa/ Philippines: performance assessments (efficacy, yield, breeding, etc) and regulatory trials

Japan: stage III environmental assessment.

e) Duration of the release

Between 10-12 months

f) Aim of post-releases monitoring

US/Argentina/Brazil: assessment/removal of volunteers.

g) Duration of post-releases monitoring

Between 4-12 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 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[3] provides information related to the applications submitted under Regulation (EC) No 1829/2003 on genetically modified food and feed. MON 89034 was approved by Commission Decision 2009/813/EC of 30 October 2009[4]
b) Assessment Report of the Competent Authority (Directive 2001/18/EC) A notification for MON 89034 according to Part C of Directive 2001/18/EC has not been submitted by Monsanto.
c) EFSA opinion An EFSA opinion specifically for MON 89034, is not available at the time of submission of this application. However, favourable EFSA opinions have been issued for MON 89034[5].
d) Commission Register (Commission Decision 2004/204/EC) Once authorized, food and feed products will be entered in the Community Register of GM food and feed[6].
e) Molecular Register of the Community Reference Laboratory/Joint Research Centre Information on detection protocols can be found on the JRC website[7].

[3] http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?panel=GMO – Accessed December 7, 2010

[4] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:289:0021:0024:EN:PDF – Accessed December 7, 2010

[5] http://www.efsa.europa.eu/en/scdocs/doc/909.pdf – Accessed December 7, 2010

[6] http://ec.europa.eu/food/dyna/gm_register/index_en.cfm – Accessed December 7, 2010

[7] http://gmo-crl.jrc.ec.europa.eu/statusofdoss.htm – Accessed December 7, 2010

f) Biosafety Clearing-House (Council Decision 2002/628/EC) The publicly accessible portal site of the Biosafety Clearing-House (BCH) can be found online[8].
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[9] does provide a link to this summary of the application for MON 89034 under Regulation (EC) No 1829/2003.

[8] http://bch.biodiv.org – Accessed December 7, 2010

[9] http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?panel=GMO – Accessed December 7, 2010