Application for authorization of MON 88017 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** Czech Republic
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 88017. In countries where MON 88017 is being cultivated, packages of this maize are marketed under the name of the hybrid variety, in association with the trademark YieldGard® Rootworm/Roundup Ready®[1], indicating clearly to growers that the hybrid is protected from specific coleopteran insect pests and tolerant to glyphosate[2].
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

[1] YieldGard® Rootworm and Roundup Ready® are registered trademarks of Monsanto Technology LLC.

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

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 88017 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.

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 In more than a third country outside the E.U., applications for the full range of uses have been made in U.S.A. and Canada, 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 88017 has been developed to produce the MON 88017 Cry3Bb1[3] and the CP4 EPSPS proteins that confer protection against certain coleopteran pests (*Diabrotica* spp.) and tolerance to glyphosate, respectively. MON 88017 was produced by *Agrobacterium*-mediated transformation of maize cells with plasmid vector PV-ZMIR39.

The use of MON 88017 enables the farmer to effectively control the targeted coleopteran 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 *Diabrotica* spp. In addition, growers will have the ability to apply glyphosate over the top of maize for broad-spectrum weed control.

[3] Cry3Bb1 protein expressed in MON 88017

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

The scope of the current application is for import, processing and all uses of MON 88017 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 88017 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 authorisation applied for

No specific conditions or instructions are warranted or required for the placing on the market of MON 88017 for import, processing, and use as or in food and feed. MON 88017 is substantially equivalent to other maize varieties except for its protection from target coleopteran pests and its tolerance to glyphosate, which are traits of agronomic interest. This maize was shown to be as safe and as nutritious as conventional maize. Therefore MON 88017 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 88017 is substantially equivalent to conventional maize varieties (except for its protection from targeted coleopteran insect pests and its tolerance to glyphosate). Therefore, MON 88017 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 88017 grain and derived products.

Operators shall be required to label products containing or consisting of MON 88017 with the words “genetically modified maize” or “contains genetically modified maize”, and shall be required to declare the unique identifier MON-88Ø17-3 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 88017 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 88017 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 88017. Therefore, no further specific measures are to be taken by the notifier.

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

MON-88Ø17-3

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 88017 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 88017 as any other maize, not including the cultivation of varieties of MON 88017 in the E.U., environmental release would be more likely to occur during import, storage and processing of MON 88017. 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 88017 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 88017 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 88017 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** *mays*
e)	**Cultivar/breeding line** A x Hi-II
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 88017 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 88017 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 88017 was produced by *Agrobacterium*-mediated transformation of immature embryos of A x Hi-II maize tissue

2. Nature and source of the vector used

The plasmid vector PV-ZMIR39 was used for the transformation of maize cells to produce MON 88017. It was constructed using standard molecular biology techniques. It is a disarmed, binary *Agrobacterium tumefaciens* transformation vector that contains both left and right transfer-DNA (T-DNA) border sequences to facilitate transformation. The T-DNA region contains the *cp4 epsps* and *MON 88017 cry3Bb1* gene expression cassettes, and is the portion of plasmid PV-ZMIR39 that is integrated into the maize genome during the transformation process.

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

Starting from the left border, the region intended for insertion contains: 1) the *cp4 epsps* coding sequence joined to a chloroplast transit peptide (CTP2) sequence, regulated by the 5' noncoding end of the rice actin 1 sequence containing the promoter (P-ract1) and first intron (ract1 intron), and the nopaline synthase 3' polyadenylation sequence (NOS 3'); and 2) the *MON 88017 cry3Bb1* coding sequence regulated by the enhanced 35S plant promoter (P-e35S), a 5' untranslated leader of the wheat chlorophyll a/b/-binding protein (wt CAB leader), the ract1 intron, and the 3' nontranslated region of the coding sequence for wheat heat shock protein 17.3 (tahsp17 3'), which ends transcription and provides the signal for mRNA polyadenylation.

The specific genetic elements and origins of the various components of the T-DNA are provided in Table 1.

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

Sequence	Size (Kb)	Source	Function
LB (left border)	0.02	Octopine Ti plasmid, pTi15955	Left border sequence essential for transfer of T-DNA from the octopine Ti plasmid, pTi15955
***cp4 epsps* gene cassette**			
P-ract1	0.93	Rice actin gene	Promoter
ract1 intron	0.46	Rice actin gene	Intron
CTP2	0.23	*Arabidopsis thaliana*	DNA sequence coding for the N-teminal chloroplast transit peptide
cp4 epsps	1.37	*Agrobacterium* sp. Strain CP4	DNA sequence coding for the native CP4 EPSPS protein
NOS 3'	0.26	*Agrobacterium tumefaciens*	3' nontranslated region of the nopaline synthase (NOS) gene which terminates transcription and directs polyadenylation
***MON 88017 cry3Bb1* gene cassette**			
P-e35S	0.61	Cauliflower mosaic virus	Promoter with the duplicated enhancer region
wt CAB leader	0.07	Wheat	5' untranslated leader of the wheat chlorophyll a/b-binding protein
ract1 intron	0.46	Rice actin gene	Intron
MON 88017 cry3Bb1	1.96	*Bacillus thuringiensis* subsp. k*umamotoensis*	DNA sequence coding for a synthetic variant of Cry3Bb1 protein
tahsp17 3'	0.23	Wheat heat shock protein	3' nontranslated region of the DNA sequence coding for wheat 17.3 kDa heat-shock protein, which ends transcription and directs polyadenylation

D. INFORMATION RELATING TO THE GM PLANT

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

MON 88017 expresses:

1. the modified Cry3Bb1 protein, derived from *Bacillus thuringiensis* subsp. *kumamotoensis*, which provides protection from certain Coleopteran pests (*Diabrotica* spp.),
2. the CP4 EPSPS protein, derived from *Agrobacterium* sp. strain CP4 which provides tolerance to glyphosate.

By using MON 88017, growers will have the ability to control corn rootworm larvae, a major insect pest of maize in the U.S.A. and to apply glyphosate herbicides over the top of maize for broad-spectrum weed control with a minimal risk of crop injury.

2. Information on the sequences actually inserted or deleted

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

MON 88017 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

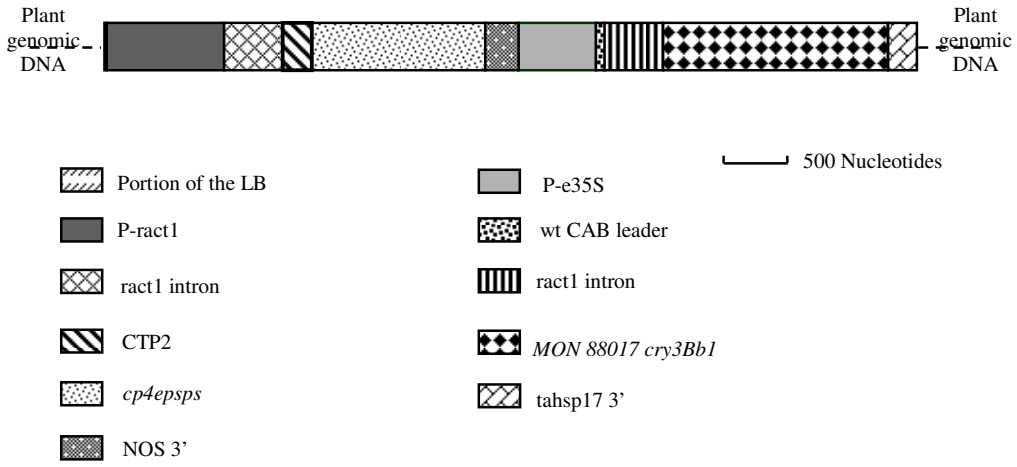
Genomic DNA from MON 88017 was analyzed by Southern blotting to determine the intactness of both expression cassettes, and the presence or absence of plasmid backbone sequences. The organisation of the elements within the insert in MON 88017 was further confirmed using PCR analysis and sequencing of the insert.

MON 88017 was produced by *Agrobacterium* sp. mediated transformation of an inbred maize line with the T-DNA from the plasmid vector PV-ZMIR39 that contains the *cp4 epsps* and *MON 88017 cry3Bb1* expression cassettes. MON 88017 contains one

copy of the T-DNA at a single integration locus on an approximately 13 kb *Sca* I restriction fragment. No additional elements from the transformation vector PV-ZMIR39, linked or unlinked to intact cassettes, were detected in the genome of MON 88017. Additionally, MON 88017 does not contain any detectable plasmid backbone sequence. Finally, PCR and DNA sequence analyses allowed to determine the 5' and 3' insert-to-plant junctions and confirmed that the organisation of the elements within the insert correspond to the "intended for insertion" one (*see* Table 1). These data support the conclusion that only the two expected full-length proteins, MON 88017 Cry3Bb1 and CP4 EPSPS, are encoded by the DNA insert present in MON 88017.

A schematic representation of the MON 88017 insert is given in Figure 1.

Figure 1. Schematic representation of the MON 88017 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 MON 88017 Cry3Bb1 and CP4 EPSPS proteins in various tissues of MON 88017 were estimated using enzyme-linked immunosorbent assay (ELISA). To produce the tissues for analysis, MON 88017 and conventional maize were planted at three field locations during the 2002 growing season. The sites were located in the major maize-growing region of the U. S. A randomized complete block design with three replications was used at all sites. The following tissues were collected over the season from the V2 to the R1 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 MON 88017 Cry3Bb1 protein levels across all sites ranged from 260-570 µg/g dw in leaf, 220-500 µg/g dw in the whole plant, and 100-370 µg/g dw in root tissues. Mean CP4 EPSPS protein levels across all sites ranged from 150-220 µg/g dw in leaf and 70-150 µg/g dw in root. In general, levels of the MON 88017 Cry3Bb1 and CP4 EPSPS proteins declined over the growing season.

b) Parts of the plant where the insert is expressed

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 2003-2004 season.

The first year, the mean MON 88017 Cry3Bb1 levels across all sites were 570 µg/g dw in young leaf, 25 µg/g dw in pollen, 380 µg/g dw in silk, 95 µg/g dw in forage, 130 µg/g dw in forage root, 15 µg/g dw in grain, and 88 µg/g dw in stover.

The mean CP4 EPSPS protein levels across all sites were 220 µg/g dw in young leaf, 390 µg/g dw in pollen, 57 µg/g dw in forage, 70 µg/g dw in forage root, and 5.8 µg/g dw in grain. CP4 EPSPS levels were not assessed in whole plant, silk and stover.

The second year confirmed the data obtained for the expression in grain for both MON 88017 Cry3Bb1 and CP4 EPSPS.

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

a) Reproduction

Agronomic data collected from trials performed with MON 88017 have demonstrated that MON 88017 has not been altered in survival, multiplication or dissemination characteristics when compared to conventional maize varieties. The introduced traits for insect-protection and tolerance to glyphosate have no influence on maize reproductive morphology and hence no changes in seed dissemination would be expected.

b) Dissemination

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

c) Survivability

Maize is known to be a weak competitor in the wild, which cannot survive outside cultivation without the aid of human intervention. Field observations have demonstrated that MON 88017 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 88017 and conventional maize hybrids, except for the introduced traits that are of agronomic interest.

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

MON 88017 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 88017 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 88017 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 88017 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

2002 U.S. field season
MON 88017 and the conventional control maize were grown at three replicated field sites in major maize-growing areas of the U.S.A. (Iowa, Illinois and Nebraska) during the 2002 field season.

2003-2004 Argentinean field season
MON 88017 and the conventional control maize were grown at four replicated field sites across Argentina during the 2003-2004 field season.

b) the baseline used for consideration of natural variations

2002 U.S. field season
For the compositional study, altogether a total of 248[8] statistical comparisons were made between the test hybrid (MON 88017) and the non-transgenic control. For all 16 significant differences ($p<0.05$), the range of the values for the test were within the 99% tolerance interval. Therefore it is unlikely that these differences are biologically meaningful.

2003-2004 Argentinean field season
For the compositional study, altogether a total of 296[9] statistical comparisons were made between the test hybrid (MON 88017) and the non-transgenic control. The 39 test values observed to be statistically different ($p<0.05$) between MON 88017 and the conventional control were within the 99% tolerance interval, except for isoleucine (which showed a difference for only one of the five comparisons). All test values for isoleucine were within literature and historical ranges for maize grain. For 18:2 linoleic acid and 18:1 oleic acid, which were consistently different between the test and control for all five comparisons (each individual site and the combination of all sites), the magnitude of the differences was small (<20%). In addition, all test values for these analytes were with the 99% tolerance interval as well as within literature

[8] Four sets of comparisons: data from each of the three trials and data from a combination of all three trials for 62 components (77 different analytical components (nine in forage and 68 in grain) were analysed – 15 compositional analytes with >50% of observations below the limit of quantitation of the assay were excluded from statistical analysis)

[9] Five sets (four based on the date from each of the replicated field sites and the fifth based on data from the combination of all four field sites) of comparisons x 52 components for grain and four sets of comparisons x nine components for forage, test and control substances from one of the four sites were not analyzed (The test forage samples were not received at Monsanto Company in St. Louis). 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.

and historical ranges for maize grain. Based on these data it is unlikely that these differences are biologically meaningful.

Also comparisons with baseline data from numerous other field trials and from the peer-reviewed literature were made. The literature on the composition of maize reveals a wide compositional variability across maize hybrids.

7.3 Selection of material and compounds for analysis

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 88017 compared to conventional maize hybrids (*see* Section D.7.1), there is no indication to further analyse other selected compounds in this maize.

7.4 Agronomic traits

Field trials with MON 88017 were performed and the set of agronomic observations supports a conclusion that from an agronomic and phenotypic (morphological) point of view, MON 88017 is equivalent to conventional maize, except for the introduced insect-protection and glyphosate tolerance traits.

7.5 Product specification

MON 88017 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 88017 is substantially equivalent and as safe and as nutritious as conventional maize, the use of MON 88017 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 88017 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 88017 to the conventional maize supply. MON 88017 is expected to replace a portion of current maize hybrids such that its intake or use will represent some fraction of the total products derived from maize.

7.8 Toxicology

7.8.1 Safety evaluation of newly expressed proteins

MON 88017 expresses the MON 88017 Cry3Bb1 and CP4 EPSPS proteins in the same plant. An assessment of their human and animal safety was conducted based upon the extensive characterization of these two proteins.

An assessment of safety of the MON 88017 Cry3Bb1 leads to the following conclusions, which are similar to the conclusions reached for the MON 863 Cry3Bb1[10] protein that was considered as safe by EFSA:

a) A history of safe use of Cry3Bb1 protein can be established, based on similarity to the wild type Cry3Bb1 protein present in Raven bioinsecticide and to the Cry3Bb1 protein produced in MON 863. MON 863, since its commercial introduction in the U.S.A. since 2003, has been grown on more than 550 000 ha.
b) The Cry3Bb1 protein purified from *E. coli* was found to be physicochemically and functionally equivalent to the protein produced in MON 88017.
c) Large margins of exposure for the MON 88017 Cry3Bb1 protein indicate that there is virtually no risk to human and animal health associated with dietary exposure to food and feed products derived from MON 88017.
d) Digestibility studies demonstrate that the MON 88017 Cry3Bb1 protein is rapidly degraded in simulated digestive fluids and would be unlikely to elicit potential toxic effects.
e) No biologically relevant structural similarities were observed between the MON 88017 Cry3Bb1 protein and pharmacologically active proteins that are known to cause adverse health effects in humans or animals.
f) Results from the acute oral toxicity study demonstrate that the MON 88017 Cry3Bb1 protein is not toxic and does not cause any adverse effects.

The analysis of the CP4 EPSPS protein leads to the following conclusions, which are similar to the conclusions reached for the CP4 EPSPS protein produced in a number of

[10] Cry3Bb1 protein expressed in MON 863

glyphosate tolerant crops, such as NK603 that was considered as safe by EFSA:

a) The donor organism, *Agrobacterium* sp. strain CP4, is not known for human or animal pathogenicity and is not commonly allergenic. Additionally, *Agrobacterium* sp. strain CP4 and the CP4 EPSPS protein it produces have been reviewed previously as a part of the safety assessment for other glyphosate tolerant crops.
b) A history of the safe use of CP4 EPSPS protein has been demonstrated, based on the similarity of the CP4 EPSPS protein in MON 88017 to EPSPSs naturally present in food crops (*e.g.*, soyabean and maize) and in microbial food sources such as Baker's yeast (*Saccharomyces cerevisiae*), and to the CP4 EPSPS protein produced in a number of other glyphosate tolerant crops that have already completed the regulatory process, including soyabean, NK603 maize, cotton and canola. CP4 EPSPS-expressing crops have been cultivated since 1996 on an aggregate area of more than 100 million hectares worldwide.
c) The CP4 EPSPS protein purified from *E. coli* was found to be physicochemically and functionally equivalent to the protein produced in MON 88017.
d) Large margins of exposure for CP4 EPSPS protein indicate that there is virtually no risk to human and animal health associated with dietary exposure to food and feed products derived from MON 88017.
e) Digestibility studies demonstrate that the CP4 EPSPS protein is rapidly degraded in simulated digestive fluids and would be unlikely to elicit potential toxic effects.
f) No biologically relevant structural similarities were observed between the CP4 EPSPS protein and pharmacologically active proteins that are known to cause adverse health effects in humans or animals.
g) Results from the acute oral toxicity study demonstrate that the CP4 EPSPS protein is not toxic and does not cause any adverse effects.

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 88017 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 88017 and conventional maize have been established by compositional analysis. Additionally, the wholesomeness of MON 88017 grain has been confirmed by repeat-dose animal feeding studies in rat and in broiler chickens using MON 88017-containing diets.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein

Cry3Bb1 and CP4 EPSPS have already been evaluated for allergenicity in the context of MON 863 and NK603 maize applications (2001/18/EC Directive and Regulation (EC) N° 258/97) that received EFSA positive scientific opinions.

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

7.9.2 Assessment of allergenicity of the whole GM plant or crop

As the introduced proteins do not have any allergenic potential, it was concluded that the use of MON 88017 for food or feed does not lead to an increased risk for allergenic reactions compared to the equivalent range of food and feed uses of conventional maize.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

The introduced traits in MON 88017 are of agronomic interest, and are not intended to change any nutritional aspects of this maize. Hence this maize is not expected to be more or less attractive for use as food (or feed), for processing, or as a food (or feed) ingredient. Therefore, anticipated dietary intake of maize-derived foods and feeds is not expected to be altered upon commercialisation of MON 88017, and no nutritional imbalances are expected as a result of the use of MON 88017.

7.10.2 Nutritional assessment of GM feed

Rats and broiler chicken feeding studies were conducted to compare the nutritional value of MON 88017 grain and non-transgenic control grain as well as commercial maize hybrids, and to provide confirmation of the safety of this maize. The results of these studies show that there were no biologically relevant differences in the parameters tested between rat/broilers fed the MON 88017 diet and the non-transgenic control diet. The MON 88017 diet was as wholesome as its corresponding non-transgenic control diet and commercially available reference diets regarding its ability to support the growth of rats/broiler chickens. This conclusion was consistent with the evaluation of the composition of the MON 88017, which showed that there were no biologically relevant differences in nutritional and compositional properties relative to control and reference maize hybrids. These data confirm and support the conclusion that the MON 88017 is as safe and nutritious as conventional maize.

7.11 Post-market monitoring of GM food/feed

The assessment of the human and animal safety of MON 88017 was conducted on the basis of its substantial equivalence to conventional maize (except for the introduced traits) and by extensive characterisation of the introduced traits, which are of agronomic interest, resulting in the expression of the MON 88017 Cry3Bb1 and CP4 EPSPS proteins.

There are no intrinsic hazards related to MON 88017 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 88017 demonstrates that the risks of consumption of MON 88017 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 MON 88017 Cry3Bb1 protein present in MON 88017 confers protection against certain economically damaging coleopteran insect pests, in particular the larvae of *Diabrotica* spp (corn rootworm). This species may be considered the target organism which interacts with MON 88017.

The Cry3Bb1 protein must be ingested by a susceptible insect to produce an insecticidal effect. Following ingestion, Cry3 proteins are solubilized and are relatively proteolytically stable, but Cry3 proteins do not have a large C-terminal domain that is processed to the active core protein. The active form of the Cry3 protein must traverse the insect midgut peritrophic membrane and selectively bind to specific receptors to exert its insecticidal activity. In susceptible organisms, cation-selective pores are formed by the Cry3Bb1 protein disrupting cell homeostasis and ultimately leading to the death of the insect.

Any significant interactions of MON 88017 with its target pest organisms are, however, limited to those countries where the cultivation of this maize has been authorized. The cultivation of MON 88017 varieties in the E.U. is not within the scope of this application. The likelihood that the import and use of MON 88017 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

9.1 Persistence and invasiveness

Like for conventional maize, the likelihood of MON 88017 spreading in the environment is negligible, as maize is neither persistent nor invasive and these parameters are unaltered in MON 88017 when compared to conventional maize. Hence the risk of establishment and spreading of MON 88017 in the environment is negligible.

9.2 Selective advantage or disadvantage

Compared with conventional maize, the presence of the introduced traits in a MON 88017 volunteer would only confer a meaningful advantage where target coleopteran pest species would be present in high numbers or where plants would be treated with glyphosate herbicide, and if no other more important factors limiting its establishment in the environment would be present. The risk of the coleopteran pest protection and the glyphosate-tolerance traits in MON 88017 to be the cause of any competitive advantage or disadvantage impacting the environment is negligible, as maize is unlikely to establish outside cultivation under European conditions (*see* Section D.9.1).

9.3 Potential for gene transfer

There is no potential for gene transfer from MON 88017 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 88017 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 88017 will result in plants of this maize being present in the environment and since MON 88017 plants pose negligible hazard to non-target organisms through their interaction with target organisms, the environmental risk of MON 88017 is considered negligible. Extensive testing of MON 88017 in the laboratory and in the field did not indicate any direct or indirect adverse environmental effects on non-target organisms. Therefore, even if incidentally spilt kernels were to germinate and result in the short survival of some MON 88017 plants in the environment, or in the unlikely case of misuse of imported grain for planting in the E.U., the plants of this maize would have no potential to cause harm on non-target organisms through their interaction with corn rootworm larvae.

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 88017 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 MON 88017 Cry3Bb1 and CP4 EPSPS proteins present a negligible hazard to non-target organisms, even if incidental spillage of MON 88017 grains during import, storage, transport or use would lead to the short survival of MON 88017 plants in the environment. Numerous studies have established that Cry3Bb1 exhibits specific toxicity towards Coleoptera, but not to other families of beetles, other insect orders or other non-target organisms. Based on the ubiquitous occurrence of natural EPSPSs in the environment and the history of safe use of CP4 EPSPS-expressing crops such as Roundup Ready soyabean, it is highly unlikely that the introduced CP4 EPSPS in MON 88017 would possess biological activity towards any non-target organisms.

As a consequence, there is negligible risk for harmful effects of MON 88017 on non-target organisms, either through direct or indirect interactions with this maize or through contact with the newly expressed proteins.

Furthermore, no evidence of any adverse effects was found since the commercial introduction of MON 863, NK603 and MON 863 × NK603, expressing similar proteins, in North America. No evidence has been brought forward by the many farmers and operators handling these products of any harmful or undesirable effects associated with this maize or with the introduced proteins.

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 88017 contains the MON 88017 Cry3Bb1 and CP4 EPSPS 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 88017 and in humans, consuming those animals, is negligible (*see* Sections D.7.8, D.7.9, D.7.10). Therefore, the risk of MON 88017 for the feed/food chain is also negligible.

9.8 Effects on biogeochemical processes

In the event of an incidental release of MON 88017 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 88017 plants would be any different from conventional maize regarding their direct influence on biogeochemical processes or nutrient levels in the soil, as MON 88017 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 MON 88017 Cry3Bbl protein is subjected to rapid degradation in soil and the CP4 EPSPS proteins belong to the safe class of EPSP synthases that are ubiquitous in the environment.

9.9 Impacts of the specific cultivation, management and harvesting techniques

Not applicable. This application is for consent to import MON 88017 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 88017 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 MON 88017 Cry3Bb1 and CP4 EPSPS are introduced proteins in maize, they already have a safe history of use and have no known negative interactions with the abiotic environment. The MON 88017 Cry3Bbl protein is subjected to rapid degradation in soil (DT50: 0.7 – 1.8 days, DT90: 3.5 – 7.8 days) and is therefore not expected to negatively affect soil or water. The CP4 EPSPS protein in MON 88017 is innocuous and belongs to a large class of EPSPS proteins that are ubiquitous in nature.

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 88017 was undertaken in the context of the scope of the application, that is, for import, processing and food and feed use of MON 88017, but not including the cultivation of MON 88017 varieties in the E.U. Analysis of the characteristics of MON 88017 has shown that the risk for potential adverse effects on human health and the receiving environment, resulting from the import and use of MON 88017 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 88017 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 notifier will ensure that appropriate technical information on MON 88017 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 88017 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 88017 will be detectable using the insert-specific PCR method for detecting the introduced DNA present in MON 88017. The proteins present in MON 88017 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

B/FR/05/04.01

b) Conclusions of post-release monitoring

No field trials performed in 2005.

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)

No field trials performed in 2005.

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

a) Release country

MON 88017 has been field tested in the U.S.A. since 2000. It has also been tested in: Japan in 2002, Argentina in 2003-2004, and Canada in 2003.

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: Kawasaki prefecture. Argentina: Buenos Aires, Cordoba, Santa Fe. Canada: Ontario (Vienna, Branchton, Exter, Ingersoll, Ayr), Quebec (St. Huques).

d)	**Aim of the release** U.S.A./Argentina: assess the performances: 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: assess for volunteers.
g)	**Duration of post-releases monitoring** U.S.A./Argentina: 12 months.
h)	**Conclusions of post-release monitoring** U.S.A.: volunteers have been eliminated to prevent persistence in the environment. Argentina: nothing to report.
i)	**Results of the release in respect to any risk to human health and the environment** All countries: no evidence that MON 88017 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[11] 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 88017 according to Directive 2001/18/EC has not been submitted by Monsanto.

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

c) EFSA opinion

An EFSA opinion, specifically for MON 88017, was not available at the time of submission of this application. Favourable EFSA opinions have been issued, however, for the MON 863 and NK603, expressing similar proteins and were posted on the EFSA website[12].

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

The authorised food and feed are entered in the Community Register of GM food and feed[13].

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

Information on detection protocols can be found on the JRC website[14].

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 88017. The EFSA website[15] does provide a link to this summary of the application for MON 88017 under Regulation (EC) No 1829/2003.

[12] http://www.efsa.eu.int/science/gmo/gmo_opinions/catindex_en.html
[13] http://europa.eu.int/comm/food/dyna/gm_register/index_en.cfm
[14] http://gmo-crl.jrc.it/statusofdoss.htm
[15] http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html