# Application for authorisation of MON 88017 × MON 810 maize in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed

**Part III**

Cartagena Protocol

**MON 88017 × MON 810**
**A combined trait maize product from Bayer CropScience LP**

**Information on MON 88017 × MON 810, notified in accordance with Article 9(2) (Annex II) of Regulation (EC) No 1946/2003 of 15 July 2003 concerning the conclusion, on behalf of the European Community, of the Cartagena Protocol on Biosafety**

**Annex II of Regulation (EC) No 1946/2003**

**INFORMATION REQUIRED CONCERNING LIVING MODIFIED ORGANISMS INTENDED FOR DIRECT USE AS FOOD OR FEED, OR FOR PROCESSING UNDER ARTICLE 9(2) OF ANNEX II**

This document contains the statutory information required by the European Union (EU) for the transboundary movement of a living genetically modified organism, as requested in Article 9 and Annex II of Regulation (EC) No 1946/2003. Annex II details the information required to complete the procedure for living modified organisms intended for direct use as food or feed, or for processing (LMO-FFP). The subject LMO-FFP in this document is an herbicide-tolerant and insect-protected maize from Bayer CropScience LP, hereafter referred to as MON 88017 × MON 810.

**(a) The name and contact details of the applicant for a decision for domestic use**

Bayer Agriculture BV *on behalf of* Bayer CropScience LP.


| | |
|---|---|
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**(b) The name and contact details of the authority responsible for the decision**

The information on MON 88017 × MON 810 included in this document will be notified by the EU Commission, DG SANTE to the Biosafety Clearing-House, and will be accessible to all Parties to the Cartagena Protocol on Biosafety.

*Contact details:*

European Commission, DG SANTE
Unit Biotechnology and Plant health
Rue Belliard 232 03/100
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**(c) Name and identity of the living modified organism**

The development code for this product is MON 88017 × MON 810. It is tolerant to the action of Roundup® agricultural herbicides and is protected from feeding damage caused by specific coleopteran and lepidopteran insect pests.

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**(d) Description of the gene[tic] modification, the technique used, and the resulting characteristics of the living modified organism**

No novel method of genetic modification was utilised in the production of MON 88017 × MON 810. Instead, traditional maize breeding techniques were used to cross parental inbred maize plants of MON 88017 and MON 810. MON 88017 × MON 810 is an extension of the use of the single-trait parents, combining both traits in one maize plant.

Even though MON 88017 × MON 810 results from traditional breeding, genetic modification was used to develop the single-trait parents, MON 88017 and MON 810.

MON 88017, produced by *Agrobacterium*-mediated transformation of maize cells, contains a single, stably integrated DNA insert that is inherited in a Mendelian fashion. The inserted DNA contains two expression cassettes, one that codes for CP4 EPSPS (5-enolpyruvyl-3-phosphoshikimic acid synthase) and one that codes for Cry3Bb1. The *cp4 epsps* cassette consists of the ract1 promoter, the ract1 intron enhancing the transcription, the chloroplast transit peptide 2 targeting sequence, the *cp4 epsps* coding sequence, the *NOS 3*' which ends transcription and directs polyadenylation. The *MON 88017 cry3Bb1* cassette consists of the e35 S promoter, the wt CAB enhancing the translation, the ract1 intron enhancing the transcription, the *MON 88017 cry3Bb1* coding sequence, and the tahsp17 3' which ends transcription and directs polyadenylation.

MON 810, produced by transformation using particle acceleration, contains a single, stably integrated DNA insert that is inherited in a Mendelian fashion. The inserted DNA contains the *cry1Ab* cassette consisting of the truncated e35S promoter, the maize hsp70 intron and the truncated *cry1Ab* coding sequence.

CP4 EPSPS provides tolerance to glyphosate (N-phosphonomethylglycine), the active ingredient in the nonselective, foliar-applied, broadspectrum, postemergent family of Roundup® agricultural herbicides. Cry3Bb1 provides protection from specific coleopteran insect pests, including *Diabrotica* spp. Cry1Ab provides protection from specific lepidopteran insect pests, including the European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp. The use of MON 88017 × MON 810 will enable the grower to utilise Roundup agricultural herbicides for effective control of weeds and to control specific coleopteran and lepidopteran insect pests. With the exception of these introduced traits, MON 88017 × MON 810 is substantially equivalent in composition and agronomics to conventional maize.

**(e) Any unique identification of the living modified organism**

MON-88Ø17-3 × MON- ØØ81Ø-6

**(f) Taxonomic status, common name, point of collection or acquisition, and characteristics of recipient organism or parental organisms related to biosafety**

**1. Taxonomic status**

***(i) Family name***

Poaceae (formerly Gramineae)

***(ii) Genus***

*Zea*

***(iii) Species***

*mays* (2n = 20)

***(iv)* *Subspecies***

*mays*

**2. Common Name**

*Zea mays* is known by many common names, including “corn” and “maize” in many English-speaking countries.

**3. Point of collection or acquisition**

The original transformations that produced MON 88017 × MON 810 used privately owned maize germplasm.

**4. Characteristics of recipient organism or parental organism related to biosafety**

Maize (*Zea mays)* is one of the most frequently cultivated crops in the world, together with rice (*Oryza sativa* L.) and wheat (*Triticum* sp.)[1]. It has a long history of safe use as a raw material for processed products, and direct uses as a human food or animal feed. Today, maize is produced on every continent except Antarctica, and is exported and imported as viable grain for use as foods or feeds, or directly in processing, without risk to the environment.

According to OECD (2003), “Maize has lost the ability to survive in the wild due to its long process of domestication, and needs human intervention to disseminate its seed.” In addition, “Maize is incapable of sustained reproduction outside of domestic cultivation” and “maize plants are non-invasive in natural habitats.” Despite maize frequently appearing as a volunteer maize plant in a subsequent rotation, it has no inherent ability to persist or propagate. In all regions of the world, volunteer plants are managed with herbicides, tillage, or manual removal of plants. Thus, maize is not considered a pest anywhere in the world. When maize plants occur outside of cultivation, they have no impact on the conservation of biological diversity.

Gene flow from maize occurs through dispersal of seed and pollen-mediated exchange of genes to sexually compatible plants. Because maize has no biological mechanism to scatter seed, low-level, incidental dispersal of viable grain occurs only as a result of human-based activities such as transport and harvesting operations. As noted by OECD, the few plants that might result from incidental release will not persist or meaningfully reproduce without human intervention. In most world areas, gene flow via pollen is only possible to other maize plants. The exceptions are Mexico and Guatemala, where wild relatives occur (*see* section (g)). Maize is a sexually reproductive, wind-pollinated, monoecious species with separate staminate (tassels) and pistillate (silk) flowers. These characteristics encourage natural cross-pollination between maize plants. The distance that viable pollen can travel depends on prevailing wind patterns, humidity, and temperature. Generally, the pollen dissemination period lasts three to seven days. Because maize is not competitive and incidental release of maize during importation occurs at very low levels, pollen-mediated gene flow between local maize and rare volunteers would have no effect on the conservation and sustainable use of biological diversity.

[1] FAOSTAT - http://faostat.fao.org/site/339/default.aspx – Accessed on 18 March 2019.

**(g) Centres of origin and centres of genetic diversity, if known, of the recipient organism and/or the parental organisms and a description of the habitats where the organisms may persist or proliferate**

**1. Centres of origin and genetic diversity**

Maize originated in the highlands of Mexico 7 000 to 10 000 years ago. By the time Columbus discovered the Western Hemisphere, maize was being grown by the indigenous people from Chile to southern Canada. Columbus noted the presence of maize on the north coast of Cuba in November 1492, and introduced maize to Europe upon his return to Spain (Goodman, 1988). Within two generations after the introduction of maize to Europe, maize became distributed throughout those regions of the world where it could be cultivated.

Maize is a member of the genus *Zea*, which is broken into 2 sections: Zea and Luxuriantes. The section Zea includes one species (*mays*), which includes three subspecies: ssp. *mays*, ssp. *mexicana* (formerly *Euchlaena mexicana)*, and ssp. *parviglumis*. The former subspecies is known as maize (or corn) while the latter comprise a portion of the complex known as teosinte. Furthermore, ssp. *mexicana* and ssp. *parviglumis* are further separated into several races (OECD, 2003). Section Luxuriantes encompasses 3 species: an annual *Z. luxurians*, and perennials *Z. diploperennis* and *Z. perennis.*

**2. Description of the habitats where the organism may persist or proliferate**

As noted by the OECD (2003), maize does not invade natural habitats, persist, or disperse anywhere in the world without human intervention. Early domestication and diversification through selection occurred in Mesoamerica. Maize is grown across a wide range of ecological conditions including soil types, altitude and rainfall. The bulk of the maize is produced between latitudes 30° and 55°, with relatively little grown at latitudes higher than 47° anywhere in the world. The greatest maize production occurs where the warmest month isotherms range between 21 and 27° C and the frost-free season lasts 120 to 180 days. A summer rainfall of 15 cm is approximately the lower limit for maize production without irrigation.

Experience with maize imported for direct use as a food or feed, or for processing, has demonstrated that stable populations do not establish, persist or proliferate as a result of incidental release during handling and transport.

**(h) Taxonomic status, common name, point of collection or acquisition, and characteristics of the donor organism or organisms related to biosafety**

**1. Taxonomic status**

MON 88017 × MON 810 was produced by traditional breeding of two genetically modified maize lines (*Zea mays* L.), MON 88017 and MON 810. There is no evidence of any biosafety issues related to the use of either MON 88017 or MON 810. There is no evidence of human or animal pathogenicity for any of the donor organisms of the DNA sequences that were used to produce MON 88017 and MON 810.

<u>MON 88017</u>

The donor organism for the *epsps* and *cry3Bb1* coding regions were *Agrobacterium* sp. strain CP4 and *Bacillus thuringiensis* subsp. *kumamotoensis*, respectively. The regulatory genetic element discussed in section (d) were obtained from the following sources: ract1, rice (*Oryza sativa*); CTP2, *Arabidopsis thaliana*; NOS, *Agrobacterium tumefaciens*; wt CAB leader, wheat (*Triticum sativum*); tahsp17 3’, wheat.

MON 810

The donor organisms for the cry1Ab coding region was *Bacillus thuringiensis* subsp. *kurstaki*. The regulatory genetics elements, e35S and the heat shock protein hsp70, were obtained from the cauliflower mosaic virus and maize (*Zea mays* L**.**), respectively.

**2. Common name**

*See* section (h)1.

**3. Point of collection or acquisition**

All genetic elements were isolated or obtained in the U.S.A.

**4. Characteristics of the donor organism(s) related to biosafety**

There is no evidence of any human or animal pathogenicity for any of the donor organisms of the DNA sequences that were used to develop MON 88017 × MON 810.

MON 88017

CP4 EPSPS

The *epsps* coding region (hereafter referred to as *cp4 epsps*) was derived from the common soil-borne bacterium *Agrobacterium* sp. strain CP4. A 3' nontranslated region of the nopaline synthase gene (*NOS 3'*) from *A. tumefaciens* was used to end transcription and direct polyadenylation of the mRNA (Fraley *et al.*, 1983). There is no human or animal pathogenicity known for *Agrobacterium* species. The *cp4 epsps* and *NOS* 3' sequences are not determinants of *Agrobacterium* plant pathogenesis. CP4 EPSPS belongs to a class of EPSPS enzymes that are ubiquitous in nature (Schulz *et al.*, 1985). Furthermore, an extensive characterisation and safety evaluation has confirmed the human, animal and environmental safety of the introduced CP4 EPSPS protein (see Section (j)).

The *cp4 epsps* coding sequence is fused to a chloroplast transit peptide sequence (designated CTP2), which is derived from the *CTP* sequence from *Arabidopsis thaliana* (Klee *et al.*, 1987). The CTP directs the CP4 EPSPS enzymes to the chloroplast, the location of EPSPS in plants and the site of aromatic amino acid biosynthesis (Kishore and Shah, 1988). CTPs are typically cleaved from the "mature" protein following delivery to the plastid (Della-Cioppa *et al.*, 1986). The *A. thaliana CTP* sequences and polypeptides are not known to impact biosafety.

The *cp4 epsps* coding sequence is regulated by the rice actin promoter (P-ract1) and the rice actin (ract1) intron (McElroy *et al.*, 1990). Both the promoter and intron were derived from *Oryza sativa* L. (rice), which has a long history of safe use.

Cry3Bb1

The *cry3Bb1* coding region was derived from the soil bacterium *Bacillus thuringiensis* subsp. *kumamotoensis*. The Cry3Bb1 protein has a history of safe consumption by humans and animals and is present in microbial pesticides. Such microbial products are used as environmentally acceptable insecticides because they are specific for the target insect pest and are typically harmless to plants and other nontarget organisms. Furthermore, an extensive characterisation and safety evaluation has confirmed the human, animal and environmental safety of the introduced Cry3Bb1 protein.

The e35S promoter derived from the cauliflower mosaic virus was used to produce a high level of protein expression (Lam and Chua, 1990; Odell *et al.*, 1985). The promoter sequence is followed by a CAB leader sequence from wheat chlorophyll a/b binding protein, which facilitates mRNA translation (Lamppa *et al.*, 1985), and the first intron of the rice actin 1 sequence, which enhances DNA transcription (McElroy *et al.*, 1990). The final element in the *cry3Bb1* cassette is a polyadenylation sequence from the wheat heat shock protein 17.3 (McElwain and Spiker, 1989). There is no scientific evidence for the cauliflower mosaic virus to lead to adverse environmental effects or health effects in animals or humans. Wheat and rice have a long history of safe use.

MON 810

Cry1Ab

The *cry1Ab* coding sequence was derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* and encodes the insecticidally active Cry1Ab protein. The Cry1Ab protein has a long history of safe use in microbial formulations for nearly 40 years. Cry1Ab binds to specific receptors in the midgut of sensitive insects, but does not affect organisms that lack those receptors. Therefore, the Cry1Ab protein has selective toxicity to specific lepidopteran insects but is harmless to humans, fish, and wildlife, as well as beneficial insects that can help control other pests.

The *cry1Ab* gene is regulated by the e35S promoter from CaMV and the hsp70 intron (Rochester *et al.*, 1986) from maize. Maize has a long history of safe use, and there is no scientific evidence for the enhanced CaMV 35S promoter to lead to adverse environmental effects or health effects in animals or humans.

In conclusion, all components of the inherited DNA in MON 88017 × MON 810 are well understood and there is no evidence of any impact on biosafety for any of the DNA sequences or donor organisms that have been used.

**(i) Approved uses of the living modified organism**

The scope of the current global regulatory reviews for this LMO-FFP depends on the country and its local regulatory framework.

**(j) A risk assessment report consistent with Annex III**

Introduction

This section presents a risk assessment report consistent with Annex III of the Cartagena Protocol on Biosafety as required by Annex II.(j). The information was collected following the general principles and methodology described in Annex III., which are, "to identify and evaluate the potential adverse effects of living modified organisms on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking into account risks to human health." General principles outlined in Annex III paragraphs (3), (4), (5), and (6) were used in the risk assessment including: scientific soundness, transparency, consistency with international guidance and expert advice, and comparison with the non-modified recipient or parental organism within the likely receiving environment. The assessment was carried out considering the intended use and likely receiving environment. In addition, consideration was given to the potential risks to human health. The framework underlying the evaluation of MON 88017 × MON 810 is consistent with guidance established by the Organisation for Economic Co-operation and Development (OECD), United Nations World Health Organisation (WHO), the Food and Agriculture Organisation (FAO) and Codex (OECD, 1993; WHO/FAO, 1996; CODEX, 2003), the U.S.A., Canada, Japan, the EU and other countries.

The risk assessment conducted herein considers the risks to human health under the intended use of MON 88017 × MON 810 for direct use as food, feed, or for processing. It is concluded that compared to conventional maize, no increased risk is posed to the conservation and sustainable use of biological diversity in the likely potential receiving environment.

*(a) Identification of any novel genotypic or phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health*

MON 88017 × MON 810, developed by traditional breeding of MON 88017 and MON 810, expresses the CP4 EPSPS, Cry3Bb1 and Cry1Ab proteins. These plants are tolerant to Roundup agricultural herbicides and are protected from feeding damage by corn rootworm larvae and lepidopteran insect pests.

CP4 EPSPS

The EPSPS enzyme, which is present in all green plants, catalyses the penultimate step of the shikimic acid pathway for the biosynthesis of aromatic amino acids. Glyphosate binds to the endogenous plant EPSPS enzyme and blocks the biosynthesis of EPSPS, thereby depriving plants of essential amino acids (Steinrücken and Amrhein, 1980; Haslam, 1993). This leads to reduced plant growth and ultimately plant death. MON 88017 expresses the CP4 EPSPS protein, which is structurally similar and functionally identical to endogenous plant EPSPS enzymes but has a much reduced affinity for glyphosate relative to endogenous plant EPSPS (Padgette *et al.*, 1996). Expression of this glyphosate-tolerant CP4 EPSPS enzyme in MON 88017 ensures the continued function of the aromatic amino acid pathway in the presence of glyphosate and enables farmers to use Roundup agricultural herbicides for effective, in-season weed control.

The environmental and human safety of the CP4 EPSPS protein is well established. Numerous products containing CP4 EPSPS have been approved for intentional release or importation in numerous countries around the world including Argentina, Canada, the European Union, Japan, and the U.S.A. Products expressing CP4 EPSPS have been marketed since 1996 with no environmental or human health issues.

In addition, the CP4 EPSPS protein was evaluated to assess any potential impact on biological diversity. The CP4 EPSPS protein is nontoxic to animals including mammals, birds, fish, and arthropods. EPSPSs proteins are ubiquitous in plants and microorganisms (Levin and Sprinson, 1964). Crops producing the CP4 EPSPS protein have been grown extensively around the world and thus have a history of safe use. Due to a lack of toxicity, ubiquity in nature including foods and feeds, lack of homology to known toxins and allergens, and history of growing Roundup Ready crops on millions of acres crops, it is reasonable to conclude that CP4 EPSPS poses no meaningful potential to adversely affect biological diversity or human health due its importation for direct use as food or feed, or for processing.

Cry3Bb1

The Cry3Bb1 protein, derived from *Bacillus thuringiensis* subsp. *kumamotoensis*, provides protection from certain coleopteran insect pests, including members of the corn rootworm complex (*Diabrotica* spp.) comprised primarily of the Western, Northern and Southern corn rootworms (*Dibrotica virgifera virgifera* LeConte, *Diabrotica barberi*, *Diabrotica undecimpunctata howardi* Barber). The mode of action of a model Cry3 protein, Cry3A, was studied by Slaney *et al.* (1992) and does not differ significantly from that of the more familiar Cry1 mode of action (Gill *et al.*, 1992; English and Slatin, 1992; Yamamoto and Powell, 1993; Knowles, 1994; Dean *et al.*, 1996). Like Cry1 proteins, Cry3 proteins must be ingested by the susceptible insect to produce an insecticidal effect. Following ingestion, Cry3 proteins are solubilised and are relatively proteolytically stable (Slaney *et al.*, 1992) but, unlike Cry1A proteins, Cry3 proteins do not have a large C-terminal domain that is processed to the active core protein (Donovan *et al.*, 1988). The active form of the Cry3 protein must traverse the insect midgut peritrophic membrane and selectively bind to specific receptors to produce insecticidal activity (Slaney *et al.*, 1992; Donovan *et al.*, 1988). Cation-selective pores formed by the Cry3Bb protein disrupt cell homeostasis, ultimately leading to the death of the insect (Von Tersch *et al.*, 1994).

The European Food Safety Authority has issued a positive safety opinion for genetically modified maize, MON 863 (EFSA, 2004), which produces a Cry3Bb1 protein derived from *Bacillus thuringiensis* subsp. *kumamotoensis*. The Cry3Bb1 proteins produced in MON 88017 and MON 863 share an amino acid sequence identity of 99.8%, differing by only one of 653 amino acids. The physicochemical characteristics and functional activity of the Cry3Bb1 protein produced in MON 88017 are equivalent to those of the Cry3Bb1 protein produced in MON 863.

In addition, the Cry3Bb1 protein was evaluated to assess any potential impact on biological diversity. The Cry3Bb1 protein is nontoxic to animals including mammals, birds, fish and non-coleopteran arthropods. The selectivity of Cry3 proteins against specific coleopteran insects is well established (Frankenhuyzen, 1993; Shelton *et al.*, 2002). Tests with earthworms, *Collembola*, honey bees (larvae and adults), green lacewings, parasitic wasp, two lady bird beetle species, monarch butterfly larvae, and daphnia show no evidence of toxicity.

The genotypic and phenotypic characteristics specific to MON 88017 have been studied extensively to assess any potential adverse impacts on biological diversity in the European Union as a result of importation for the direct use as food or feed or for processing. MON 88017 was developed as a commercial product based upon agronomic parameters including tolerance to Roundup agricultural herbicides and protection against corn rootworm larvae. Trials conducted in the U.S. and Canada over several years across a broad geographic range of environments have shown that MON 88017 is equivalent to conventional maize in its nutrient composition (protein, fat, carbohydrates, moisture, amino acids, fatty acids, and minerals) and agronomic performance, except for tolerance to glyphosate and protection against feeding damage caused by corn rootworm larvae. This demonstrates that MON 88017 and its progeny are no different than conventional maize with the exception of the introduced traits, conferred by the production of CP4 EPSPS and the Cry3Bb1 protein. Finally, molecular characterisation and expression analysis confirm that MON 88017 contains one copy of the inserted DNA, which is stably integrated into the genome and functioning as expected.

Extensive composition and phenotypic analysis of MON 88017 demonstrate that the genetic modification did not result in any unintended effects, including persistence or invasiveness, compared to conventional maize. As such, no novel phenotypic characteristics with potentially adverse effects on biological diversity or human health in the European Union were detected or are considered likely from the intended use of maize containing MON 88017 as a LMO-FFP imported for direct use in food, feed or for processing.

Both the CP4 EPSPS and Cry3Bb1 proteins expressed in MON 88017 lack toxicity except for the intended toxic effect of the Cry3Bb1 protein to specific coleopteran pests. They also lack homology with known toxins (other than from *Bacillus thuringiensis*) and allergens. Based on these properties and from the experience with commercial production of maize containing CP4 EPSPS and Cry3Bb1, it is reasonable to conclude that these proteins pose no meaningful potential to adversely affect biological diversity or human health in the European Union from the intended use of MON 88017 as a LMO imported for direct use in food, feed or for processing.

Cry1Ab

MON 810, available commercially as YieldGard® Corn Borer corn, produces the Cry1Ab protein derived from *B. thuringiensis* subsp. *kurstaki*, which provides protection from certain lepidopteran insect pests, including the European corn borer (*Ostrinia nubilalis*) and pink borers (*Sesamia* spp.).

The environmental and human safety of the Cry1Ab protein is well established. MON 810 has been approved for intentional release and for importation in a number of countries around the world including Argentina, Canada, the European Union, Japan and the United States.

The mode of action of *B. thuringiensis* delta-endotoxins such as the Cry1Ab protein has been well studied and reviewed (Gill *et al.*, 1992; English and Slatin, 1992; Yamamoto and Powell, 1993; Knowles, 1994; Dean *et al.*, 1996). Cry1Ab is insecticidal only to certain lepidopteran insects (Schnepf, 1998) and must be ingested by the susceptible insect to produce an insecticidal effect (Huber and Lüthy, 1981). Following ingestion, the Cry1Ab protein is solubilised and proteolytically processed to the active core. The active form of the Cry1Ab protein traverses the insect midgut peritrophic membrane and selectively binds to specific receptors (Hofmann *et al.*, 1988a and 1988b). Cation-selective pores form and disrupt midgut ion flow, ultimately leading to the death of the insect.

MON 810 was extensively characterised to assess whether it possesses any novel genotypic or phenotypic characteristics that might affect biological diversity. The characterisation information includes molecular characterisation, compositional analyses, expression analysis to determine the levels of Cry1Ab in MON 810, and extensive phenotypic analysis. In field trials, MON 810 was selected based upon agronomic parameters including protection against damage by the European corn borer. These trials, established originally in 1993 in the U.S., and conducted over three years across a broad geographic range of environments, have shown no significant phenotypic differences between MON 810 and conventional maize, except for protection against feeding by ECB and other lepidopteran pests. This demonstrates that MON 810 and its progeny are no different than conventional maize with the exception of the introduced trait. The compositional and nutritional equivalence of MON 810 to conventional maize was confirmed by analyses of key nutrients including protein, fat, carbohydrates, moisture, amino acids, fatty acids, and minerals (Sanders *et al.*, 1998). Finally, the results of molecular characterisation and expression analysis confirmed that MON 810 contains one copy of the inserted DNA, which is stably integrated into the genome and functions as expected.

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The conclusion of this extensive characterisation is that the novel genotypic characteristics in this maize are limited to the intentionally introduced DNA and the presence of the Cry1Ab protein. The result is that MON 810 is protected against feeding by ECB and other lepidopteran pests, a trait which is of agronomic interest. In addition, extensive comparative assessment consisting of compositional and phenotypic analysis of MON 810 and conventional maize did not reveal any other novel characteristics or unintended adverse effects of the genetic modification. As such, no novel phenotypic characteristics with potential effects on biological diversity or human health were detected or are considered likely from the intended importation of MON 810 for direct use as food or feed, or for processing.

In addition, information was available to evaluate the potential for the Cry1Ab protein produced to impact biological diversity. The Cry1Ab protein is nontoxic to animals including mammals, birds, fish and non-lepidopteran arthropods. The selectivity of Cry1 proteins toward specific lepidopteran insects is well established (Frankenhuyzen, 1993; Shelton *et al.*, 2002). This specificity was confirmed in a series of laboratory studies that exposed a wide variety of nontarget organisms to high doses of Cry1Ab, either as a purified protein or in plant tissues. Tests with earthworms, *Collembola*, honey bees (larvae and adults), green lacewings, a parasitic wasp, a lady bird beetle and *Daphnia* showed no evidence of toxicity. Numerous field tests conducted around the world have also confirmed the lack of nontarget impacts associated with MON 810. Due to the lack of toxicity, the lack of homology with known toxins (other than ones from *B. thuringiensis*) and allergens, and the extensive experience based on the production of millions of acres of MON 810, it is reasonable to conclude that this protein poses no meaningful potential to affect biological diversity or human health from the intended importation MON 810 for direct use as food or feed or for processing.

Finally, the assessment took into account the extensive history of safe use of MON 810 around the world. There is no indication that the use of MON 810 in agricultural production has resulted in any effect on biological diversity or human health. As such, we have concluded that there are no novel genotypic or phenotypic characteristics of MON 810 that pose a meaningful potential to affect biological diversity or human health due to its importation for direct use as food or feed, or for processing.

MON 88017 × MON 810

Any “novel characteristics” in MON 88017 × MON 810 are limited to the intended traits of herbicide tolerance and insect protection present in the respective parental products. The molecular analysis of the single-trait parents and of MON 88017 × MON 810, as well as comparative assessments with conventional maize, did not reveal any other novel characteristics or unintended effects of the combined presence of these traits.

Given the history of safety of the CP4 EPSPS, Cry3Bb1, and Cry1Ab proteins, MON 88017 × MON 810 and its expressed traits are not expected to have any effect on human or animal health or to the biodiversity in any given environment. Furthermore, traditional breeding of maize to combine various traits has historically been safe. There is no reason to believe that the breeding process itself would create novel characteristics not inherited from the parents. Nevertheless, in sections j(b)-(f) below, a step-wise risk assessment is made to evaluate the potential of the introduced traits to affect biodiversity in the receiving environment, resulting from importation for direct use as feed or food, or for processing.

We have concluded that there are no novel genotypic or phenotypic characteristics of MON 88017 × MON 810 that are likely to adversely affect biological diversity or human health in the European Union due to the transboundary movement of this LMO for direct use as food, feed, or for processing.

*(b) Evaluation of the likelihood of [these] adverse effects being realised, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism*

As noted above in j(a), no potentially adverse effects were detected based on extensive characterisation of MON 88017 and MON 810, which included molecular analysis, expression analysis, compositional analyses and phenotypic evaluation conducted in field trials over a wide range of environmental conditions. Furthermore, no potential to adversely impact nontarget organisms was detected.

Grain from MON 88017 × MON 810 has a negligible hazard potential and the likelihood of adverse effects being realised is considered to be exceedingly low for the following reasons.

First, field trials and laboratory evaluations have demonstrated that there is no hazard associated with MON 88017 or MON 810 that is greater than that present with conventional maize. Based on the available information and experience with conventional maize, the hazard characterisation is not expected to change as a result of importing grain from MON 88017 × MON 810 for direct use as feed or food, or for processing.

Second, the containment systems used to transport and handle grain are characterised by highly reduced exposure based on experience with conventional maize. Grain imported for direct use as feed or food, or for processing is not intended for release into natural or agricultural environments. Rather, the grain is normally held, transported and handled in a confined manner that restricts the potential for escape into the local environment. Because the grain will be confined to conditions that are fixed in location (seaports, grain elevators and processing facilities) and enclosed to minimize or prevent release (transport vehicles including trucks and railroad cars), they meet the conditions of Article 3(b) of the Cartagena Protocol on Biosafety. Such conditions significantly limit exposure to the environment. Although incidental release of spilled grain into the receiving environment occurs during export/import, handling, storage and processing of bulk mixtures of grain, modern methods of grain handling minimize such losses. Furthermore, the locations of any incidental release will be predictable, since they will be near the storage facilities and along transportation routes. Environmental conditions at these sites are unlikely to be conducive to germination, growth and reproduction of maize grain that is incidentally released.

Compared to conventional maize, MON 88017 × MON 810 has not been changed with respect to its dispersal or survival characteristics as assessed by phenotypic characteristics including: dormancy, seedling vigor, early and final stand count, days to 50% pollen shed or silking, stay green, ear height, plant height, dropped ears, stalk or root lodging, yield, and plant-insect, plant-disease or plant-abiotic stressor interactions. MON 88017 × MON 810 is also unchanged compared to conventional maize in terms of invasiveness of natural environments and persistence in the environment as assessed by the phenotypic characteristics described above. Importantly, there is no information to indicate that there is a reasonable potential for grain from MON 88017 × MON 810 to establish, persist or disperse to a greater extent than conventional maize. In cases where incidental release were to occur and MON 88017 × MON 810 were to establish, these plants would be easily controlled by currently available selective herbicides and by mechanical means. As such, MON 88017 × MON 810 has no meaningful potential to disperse, persist without human intervention, or invade non-agricultural areas as a result of importation for direct use as feed or food, or for processing.

Because of the lack of hazard potential and the factors that limit exposure to the environment, the likelihood of MON 88017 × MON 810 resulting in adverse environmental effects on the biodiversity and human health is comparable to the impact of conventional maize grain imported for direct use as feed or food, or for processing.

*(c) Evaluation of the consequences should [these] adverse effects be realised*

As noted above in j(a), no potentially adverse effects were detected based on extensive characterisation of MON 88017 × MON 810, which included molecular analysis, expression analysis, compositional analyses and phenotypic evaluation conducted in field trials over a wide range of environmental conditions. Furthermore no potential to adversely impact non-target organisms was detected. As such, the potential consequences to biodiversity resulting from importation of MON 88017 × MON 810 for direct use as feed or food, or for processing are the same as conventional maize.

*(d) Estimation of overall risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realised*

The overall estimated risk to biodiversity posed by the importation of grain from MON 88017 × MON 810 for direct use as feed or food, or for processing is negligible. This conclusion is based on the a) the history of safe use of the host plant, maize, b) the extensive characterisation of MON 88017 × MON 810 compared to conventionally bred maize including compositional, nutritional and phenotypic equivalence, c) the extensive characterisation and history of safety of the expressed protein, and d) the fact that, based on a combination of the history of experience with importing maize and the characteristics of MON 88017 × MON 810, there are no unique environmental conditions that would meaningfully change the environmental characteristics of MON 88017 × MON 810 compared to conventional maize.

*(e) Recommendation whether the risks are acceptable or manageable; including where necessary, identification of strategies to manage these risks*

Analysis of the characteristics of MON 88017 × MON 810 did not reveal any potential for adverse effects to human health or the environment, as could be expected from the analysis of its parental lines.

Therefore, there are no unacceptable environmental, animal or human health risks associated with importation of MON 88017 × MON 810 for direct use as feed or food, or for processing. As the environmental and health risks of this maize are consistently negligible and not different from conventional maize, no specific strategies for risk management are required.

*(f) Suggested methods for the safe handling, storage, transport and use, including packaging, labelling, documentation, disposal and contingency procedures, where appropriate*

In countries where the planting of MON 88017 × MON 810 will be authorised, appropriate and comprehensive information will be provided on seed bags and in accompanying documents in order for purchasers to be fully informed about the use of this maize.

Transboundary shipments of commodity maize which may contain MON 88017 × MON 810 will be identified clearly and will bear an indication that the LMO is not intended for intentional introduction into the environment. A contact point for further information will be provided on the documentation accompanying the grain in accordance with Art.18(2)(a) of the Cartagena Protocol on Biosafety.

MON 88017 × MON 810 is substantially equivalent to other maize. Therefore the grain, forage and derived products of MON 88017 × MON 810 can be stored, packaged, transported, used, and handled in the same manner as conventional maize. The measures for waste disposal and treatment of MON 88017 × MON 810 are the same as those for conventional maize.

In the highly unlikely event that MON 88017 × MON 810 establishes in the environment, volunteer plants could be easily controlled by currently available selective herbicides or by mechanical means. Therefore no specific measures are recommended in case of unintended release such as spillage.

**REFERENCES**


CODEX. 2003. Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. CAC/GL 45-2003

Dean, D.H., F. Rajamohan, M.K. Lee, S.J. Wu, X.J. Chen, E. Alcantara and S.R. Hussain. 1996. Probing the mechanism of action of Bacillus thuringiensis insecticidal proteins by site-directed mutagenesis – a minireview. Gene. 179:111-117.

Della-Cioppa, G., S.C. Bauer, B.K. Klein, D.M. Shah, R.T. Fraley, and G.M. Kishore. 1986. Translocation of the precursor of 5-enolpyruvyl-shikimate-3-phosphate synthase into chloroplasts of higher plants in vitro. Proc. Natl. Acad. Sci. USA 83:6873-6877.

Donovan, W. P., C. C. Dankocsik, M. P. Gilbert, W. C. Gawron-Burke, R. R. Groat, and B. C. Carton. 1988. Amino acid sequence and entomocidal activity of the P2 crystal protein. An insect toxin from Bacillus thuringiensis var. kurstaki. J. Biol. Chem. 263:561-567.

EFSA. 2004. Opinion of the Scientific Panel on genetically modified organisms on a request from the Commission related to the safety of foods and food ingredients derived from insect-protected genetically modified maize MON 863 × MON 810, for which a request for placing on the market was submitted under Article 4 of the Novel Food Regulation (EC) No 258/97 by Monsanto (Question No EFSA-Q-2003-121).

English, L. and S.L. Slatin. 1992. Mode of action of delta-endotoxins from Bacillus thuringiensis: a comparison with other bacterial toxins. Insect Biochem. Molec. Biol. 22: 1-7.

Fraley, R.T., S.G. Rogers, R.B. Horsch, P.R. Sanders, J.S. Flick, S.P. Adams, M.L. Bittner, L.A. Brand, C.L. Fink, J.S. Fry, G.R. Galluppi, G.R., S.B. Goldberg, N.L. Hoffmann, and S.C. Woo. 1983. Expression of bacterial genes in plant cells. Proc. Natl Acad Sci USA. 80:4803-4807.

Frankenhuyzen, K.V. 1993. The challenge of Bacillus thuringiensis. In Bacillus thuringiensis, an Environmental Biopesticide: Theory and Practice. Entwistle, P.F.; Core, J.S.; Bailey, M.J.; and Higgs, D. editors. John Wiley and Sons. pps. 1-35.

Gill, S.S., E.A. Cowles, and P.V. Pietrantonio, 1992. The mode of action of Bacillus thuringiensis endotoxins. Ann. Rev. Entomol. 37:615-636.

Goodman MM, 1988. The history and evolution of maize. CRC Crital Rev. Plant Sciences, 7, 197-220.

Haslam, E., 1993. Shikimic Acid: Metabolism and Metabolites. John Wiley and Sons, Chichester, England.

Hofmann, C., Luthy, P., Hutter, R. and Pliska, V. 1988a. Binding of the delta endotoxin from Bacillus thuringiensis to brush- border membrane vesicles of the cabbage butterfly (Pieris brassicae). Eur. J. Biochem. 173:85-91.

Hofmann, C., Vanderbruggen, H., Hofte, H., Van Rie, J., Jansens, S. and Van Mellaert, H. 1988b. Specificity of Bacillus thuringiensis delta-endotoxins is correlated with the presence of high-affinity binding sites in the brush border membrane of target insect midguts. Proc. Natl. Acad. Sci. USA. 85:7844-7848.

Huber, H.E. and Lüthy, P. 1981. Bacillus thuringiensis delta-endotoxin: composition and activation. In Pathogenesis of Invertebrate Microbial Diseases. E.W. Davidson, ed. pp. 209-234. Allanheld, Osmun Publishers, New Jersey, U.S.A.

Kishore, G.M. and D.M. Shah. 1988. Amino acid biosynthesis inhibitors as herbicides. Ann. Rev. Biochem. 57:627-663.

Klee, H.J., Y.M. Muskopf, and C.S. Gasser, C.S. 1987. Cloning of an Arabidopsis gene encoding 5-enolpyruvylshikimate-3-phosphate synthase: sequence analysis and manipulation to obtain glyphosate-tolerant plants. Mol. Gen. Genet. 210:437–442.

Knowles, B.H. 1994. Mechanism of action of Bacillus thuringiensis insecticidal Delta-endotoxins. Adv. Insect Physiol. 24:275-308.

Lam, E. and N.H. Chua. 1990. GT-1 binding site confers light responsive expression in transgenic tobacco. Science. 248:417-474.

Lamppa, G.K., G. Morelli, and N.H. Chua. 1985. Structure and developmental regulation of a wheat gene encoding the major chlorophyll a/b-binding polypeptide. Mol. Cell. Biol. 5:1370-1378.

Levin, J.G. and D.B. Sprinson. 1964. The enzymatic formation and isolation of 5-enolpyruvylshikimate-3-phosphate synthase. J. Biol. Chem. 239:1142-1150.

McElroy, D., W. Zhang, J. Cao, and R. Wu. 1990. Isolation of an efficient actin promoter for use in rice transformation. Plant Cell. 2:163-171.

McElwain, E.F. and S. Spiker. 1989. A wheat cDNA clone which is homologous to the 17 kd heat-shock protein gene family of soybean. Nucleic Acids Res. 17:1764.

Odell, J.T., F. Nagy, and N.H. Chua. 1985. Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. Nature. 313:810-812.

OECD. 1993. Food safety and biotechnology: concepts and principles. Safety evaluations of food derived by modern biotechnology: concepts and principles: OECD publications.

OECD. 2003. Consensus Document on the Biology of Zea mays subsp. mays (Maize), OECD publications.

Padgette, S.R., D.B. Re, G.F. Barry, D.E. Eichholtz, X. Delannay, R.L. Fuchs, G.M. Kishore and R.T. Fraley. 1996. New Weed Control Opportunities: Development of soybeans with a Roundup Ready gene. Pp 53-84. *In* Herbicide-resistant crops, agricultural, environmental, economic, regulatory and technical impact, S.O. Duke (ed.). CRC Press, Boca Raton, Florida.

Rochester,D.E., J.A. Wirier, and D.M. Shah. 1986. The Structure and Expression of Maize Genes Encoding the Major Heat Shock Protein, hsp70. EMBO Journal. 5:451-458.

Sanders, P., T. Lee, M. Groth, J. Astwood, and R. Fuchs. 1998. Safety Assessment of Insect-Protected Corn. In: Biotechnology and Safety Assessment, 2nd edition, pp. 241-256. J.A. Thomas, ed. Taylor and Francis.

Schnepf, E., N. Crickmore, J. Van Rie, D. Lereclus, J. Baum, J. Feitelson, D.R. Zeigler and D.H. Dean. 1998. Bacillus thuringiensis and its pesticidal crystal proteins. Microbiol. Mol. Biol. Rev. 62:775-806.

Schulz, A., A. Krüper, N. Amrhein. 1985. Differential sensitivity of bacterial 5-enolpyruvylshikimate-3-phosphate synthases to the herbicide glyphosate. FEMS Microbiological Letters 28:297-301.

Shelton, A.M., J-Z. Zhou, and R.T. Roush. 2002. Economic, Ecological, Food Safety and Social Consequences of the Deployment of Bt Transgenic Plants. Annu. Rev. Entomol. 47:845-881.

Slaney, A.C., H.L. Robbins, and L. English. 1992. Mode of action of Bacillus thuringiensis toxin CryIIIA: an analysis of toxicity in Leptinotarsa decemlineata (Say) and Diabrotica undecimpunctata Howardi Barber. Insect Biochem. Molec. Biol. 22:9-18.

Steinrücken, H.C. and N. Amrhein. 1980. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic acid-3-phosphate synthase. Biochem. Biophys. Res. Comm. 94:1207-1212.

Von Tersch, M.A., S.L. Slatin, C.A. Kulesza, and L,H, English. 1994. Membrane-permeabilizing activities of Bacillus thuringiensis coleopteran-active toxin CryIIIB2 and CryIIIB2 domain I peptide. Appl. Environ. Microbiol. 60:3711-3717.

WHO/FAO. 1996. Biotechnology and food safety. http://www.fao.org

Wilkes, H.G. 1972. Maize and its wild relatives. Science. 177:1071-1077.

Yamamoto, T. and G.K. Powell. 1993. Structure and function of the insecticidal protein produced by Bacillus thuringiensis. Rec. Adv. Mol. Biochem. Res. Proteins, Proc. IUBMB Symp. Protein Struct. Funct., 137-144.