

Determination of the Safety of Monsanto's  
Combined trait product corn: MON 810 x GA21  
for Direct Use Food, Feed, and Processing

---

**Food and Feed Safety:**

The product dossiers on Monsanto's Combined trait product corn: MON 810 x GA21 were reviewed for safety and nutritional differences compared with the conventional corn. The focus of the food/feed safety assessment is based on three major issues/concerns regarding stacked genes from different sources namely a) gene interaction; b) effect on metabolic pathways and c) differential gene expression due to stacking.

A biosafety notification for combined trait product corn: MON 810 x GA 21 and all progenies derived from crosses of the product with any conventionally-bred corn and corn containing approved-biotech events for direct use as food, feed or for processing were issued to Monsanto Philippines, Inc. on November 16, 2004. The notification is valid for five years and shall expire on November 15, 2009 subject to the terms and conditions set forth in DA Administrative order No. 8, Series of 2002, and Memorandum Circulars Nos. 6 and 8, Series of 2004. The said combined trait product was included in the Lists of Approval Registry (Delisting) being prepared by the Department of Agriculture-Bureau of Plant Industry.

*This approval is for use as Food, Feed and Processing only. This does not include cultivation of Combined trait product corn: MON810 x GA21 in the Philippines. Food and Feed use of Combined trait product corn: MON 810 x GA 21 its by-products is therefore authorized as of November 16, 2004. The biosafety notification (No. 04-001) stated that combined trait product corn: MON810 x GA21 is as safe for human food, livestock feed and for processing as its conventional counterparts".*

---

**I. Brief Identification of the Genetically Modified Organism (Living Modified Organism)**

**Designation:** Combined trait product corn: MON 810 x GA 21

**Applicant:** MONSANTO PHILIPPINES, INC.  
7<sup>th</sup> Floor, Ayala Life-FGU Center  
Alabang-Zapote Road cor Acacia Avenue  
Madrigal Business Park  
Alabang 1770, Muntinlupa City

**Plant Species:**

Name: corn (*Zea mays* L.)

Parent Material: Corn MON810 and Corn GA21

Center of Origin: Mexico, Central America and South America

Toxic Factors/Allergen(s): Trypsin inhibitor, phytic acid, and secondary metabolites such as raffinose, ferulic acid and p-coumaric acid are present in low amount 2-4 dihydroxy-7-methoxy-2H-1, 4 benzoxazin- 3(4H)-one (DIMBOA) a potential toxicant but declines rapidly as the plant grows

<b>Trait Description:</b>	Insect resistance and herbicide tolerance
<b>Trait Introduction Method:</b>	Conventionally breeding
<b>Donor Organisms:</b>	<i>Bacillus thuringiensis</i> var <i>kurstaki</i> , strain HD-1 ( <i>B.t.k</i> ), a widely distributed, non-pathogenic, spore-forming Gram positive bacteria, the source of the <i>cryIAb</i> gene which produces the Cry1Ab protein with insecticidal activity against Lepidopteran insects.  <i>Zea mays</i> L. (mEPSPS)
<b>Pathogenicity:</b>	<i>Bacillus thuringiensis</i> var. <i>kurstaki</i> which produces the delta-endotoxin insect control protein has been commercially prepared as spray application and in use over the 30 years in agriculture. The toxin produced by the gene <i>cryIAb</i> is known to be very specific to act on insect gut of lepidopteran insects. There are no receptors for the protein on the surface of mammalian intestinal cell hence; humans are not susceptible to these toxins. While target insects are susceptible to oral doses of <i>B.t.k.</i> protein, there was no evidence of any toxic effect observed on non-target laboratory mammals, fish or birds given the equivalent of up to 10 µg of protein per gram of body weight.  There is no evidence that the Cry1Ab protein can accumulate in the food chain. Most food processing requires the product to pass heat treatment beyond temperatures at which protein are denatured. Likewise, it has been known that Cry1Ab protein degrades rapidly in the soil and the degradation of the residual vegetative mass is not changed.  <i>Escherichia coli</i> is ubiquitous in the environment and in the digestive systems of vertebrates, including humans. The characteristics of this organism do not warrant additional analytical or toxicological testing.
<b>Proposed Use:</b>	For direct use as food, feed or for processing

## II. Background Information

To produce varieties of *Bt* lines stacked with the roundup Ready traits, process follows a typical backcrossing program. This takes from 5-6 generations to result in the progeny species containing the target gene in the background of the recurrent parent.

Monsanto Philippines, has filed an application with attached technical dossiers to the Bureau of Plant Industry on July 16, 2004 for a biosafety notification for direct use as food, feed and for processing under Administrative Order (AO) No. 8 Part 5 for Stacked trait product corn:MON 810 x GA 21 which has been genetically modified for insect resistance and herbicide tolerance.

A safety assessment of combined trait product corn: MON 810 x GA 21 was conducted by the Scientific and Technical Review Panel (STRP) as per Department of Agriculture Administrative

Order No. 8 Series of 2002. The focus of risk assessment is the gene interactions between the two transgenes.

Review of results of evaluation by the BPI Biotech Core Team in consultation with DA-Biotechnology Advisory Team (DA-BAT) completed the approval process.

### **III. Description of Novel (Introduced) Traits**

EPSPS is one of the most characterized enzymes of the aromatic amino acid pathway, which specifically catalyze the reversible transfer of the carboxyvinyl moiety of PEP to S3P, yield EPSP and inorganic phosphate. The plant and bacterial EPSPS are very similar and they are monomeric with molecular weights ranging from 44 to 48 kDa. Glyphosate, which is an amino acid analog, is a competitive inhibitor with respect to PEP and an uncompetitive inhibitor with respect to S3P.

Cry1Ab is only found in bacteria (under natural conditions) while EPSPS can be found in both bacteria and plants. Cry1Ab is not involved in any specific metabolic pathway simply because the entomopathogenic *Bacillus thuringiensis* uses this protein to infect and kill larvae. EPSPS, whether bacterial or plant origin, is involved in the biosynthetic pathway leading to formation of essential aromatic acids that is required for normal growth and development.

There is only a single copy of the Cry1Ab in MON 810 and two copies of mEPSPS in GA21. Furthermore, the gene expression levels for both proteins are very low (less than 0.1% of the total proteins) and the digestibility (metabolic fate) in simulated gastric and intestinal fluids is very fast.

A commercial inbred line with the inserted genes of MON 810 is developed by the conventional backcrossing of MON 810 and a non transgenic elite inbred line. The resulting commercial inbred line with the inserted genes of Mon 810 is then crossed with another inbred line, which contains the inserted genes of Corn GA21. The resulting seeds are stacked traits F1 hybrid variety.

#### **Safety of the Expressed Proteins**

Without stacking, both the insertion of Cry1Ab and mEPSPS in corn did not result in the occurrence of a new allergen or a new toxin. Both Cry1Ab and mEPSPS are non allergenic proteins because both these novel proteins could easily be digested by simulated gastric and intestinal fluids. Furthermore, using bioinformatics tools, both novel proteins do not share any significant homology or amino acid sequence similarity with known allergenic proteins. Acute oral toxicity studies using mice as experimental animals also show that there is a relatively high level of safety for these novel proteins. Ultimately, animal feeding studies using poultry and livestock animals showed no effect on the overall performance of these test animals.

The expression of the novel protein Cry1Ab in MON 810 is driven by a plant viral promoter called 35S CaMV, which is constitutive in nature. A constitutive promoter is one of that drives gene expression in all plant tissue parts at all stages of plant in all plant tissue parts at all stages of plant growth and development meaning that Cry1Ab protein will be present in the corn ears, the primary stalks and stems, leaves, roots and reproductive tissues (such as the pollen). However, the level of protein expression is very low (less than 30 micrograms per gram fresh weight of plant material which could not exceed 0.1% of the total protein).

The gene product of mEPSPS has a transit signal peptide that target the novel protein into the chloroplasts of leaf tissues because the effect of the herbicide glyphosate is primarily the biosynthesis of aromatic amino acids such as phenylalanine, tyrosine and tryptophan which occurs in plastid

organelles of chloroplasts. Therefore, mEPSPS can be found only in the leaves of corn plants. The expression of mEPSPS is also very low in the leaf tissues, which is less than 0.1% of the total protein

The mechanism and the target sites for Cry1Ab and mEPSPSs are entirely different than the target sites for Cry1Ab and mEPSPS are entirely different than the probability of protein-protein interaction that will lead to adverse health effects in non-target organisms such as animals and man is very low.

Cry1Ab specifically binds with receptor proteins found only in the plasma membrane of the intestinal midgut or lepidopteran insects such as the Asiatic or the European corn borer. Three dimensional structure of the Cry1Ab protein reveals three dominant domains involved in very specific protein-protein interaction with an insect receptor.

In the unstacked corn MON 810, no *NPTII* (kanamycin resistance gene) gene as proven by southern gel blot analyses and PCR.

Based on mode of action of mEPSPS and Cry1Ab proteins and location of these proteins' accumulation in the plant cells, the likelihood of interaction with one another is not considered to be significantly possible to affect stability and expression level of either one of the genes.

#### **IV. Nutritional Composition (Compositional Analysis)**

The World Health Organization (1995) stated that two plants that are substantially equivalent to conventional varieties are crosses by conventional breeding techniques; the combined trait product is expected to be substantially equivalent to the single event products.

#### **V. Anti-Nutritional Factors**

No known anti nutritional factors for individual events. Thus, MON 810 x GA 21 corn has no known anti nutritional factors.

#### **VI. Regulatory Decision**

After reviewing the scientific data and information relevant to the application of Monsanto Philippines Inc., it is concluded that combined trait product corn: MON 810 x GA 21 and all progenies derived from crosses of the product with any conventionally-bred corn, and corn containing approved-biotech events for direct use as food or feed or for processing is as safe and substantially equivalent to its unmodified counterpart. Monsanto's combined trait product corn MON 810 and GA 21 has no evidence of interaction leading to unintended effects and/or over expression/under expression of the two novel gene products and is therefore approved for direct use as food, or feed or for processing.