

Determination of the Safety of Monsanto's
Combined trait product corn: MON 810 x NK 603 x MON 863
For Direct use Food, Feed, and Processing

Food and Feed Safety:

The product dossiers on Monsanto's combined trait product corn: MON 810 x NK 603 x MON 863 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 NK 603 x MON 863 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 was issued to Monsanto Philippines Inc. on February 7, 2005. The notification is valid for five years and shall expire on February 6, 2012 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: MON 810 x NK 603 x MON 863 in the Philippines. Food and Feed use of combined trait product corn: MON 810 x NK 603 x MON 863 its by-products is therefore authorized as of February 7, 2005. The biosafety notification (No. 05-007) stated that combined trait product corn: MON 810 x NK 603 x MON 863 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: MON810 x NK603 x MON 863
Applicant:	MONSANTO PHILIPPINES, INC. 7 th Floor, Ayala Life-FGU Center Alabang-Zapote Road cor Acacia Avenue Madrigal Business Park Alabang 1770, Muntinlupa City
Plant Species:	
Name:	Corn (<i>Zea mays</i> L.)
Parent Material:	Inbred corn lines (and/or isolines) developed and produced by Monsanto
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

Trait Description: Insect resistance and herbicide resistance

Trait Introduction Method: Conventionally breeding

Donor Organisms: *Bacillus thuringiensis* subs *kumamotoensis*, is a non-pathogenic spore-forming Gram positive bacterium that is found naturally in soil. It is a source of the *cry3Bb1* gene which produces a Cry3Bb1 protein with insecticidal activity against the coleopteran pest, corn rootworm.

Bacillus thuringiensis var *kurstaki*, strain HD-1 (*B.t.k*), a widely distributed, non-pathogenic, spore-forming Gram positive bacteria, the source of the *cry1Ab* gene which produces the Cry1Ab protein with insecticidal activity against Lepidopteran insects.

Agrobacterium sp. Strain CP4, which encodes only for the naturally occurring, glyphosate-tolerant EPSPS protein.

Pathogenicity: *Bacillus thuringiensis* subsp. *kumamotoensis*, the donor for the *cry3Bb1* coding sequence, is a non-pathogenic spore-forming gram positive bacterium that is found naturally in soil. Bt strains have been used commercially in the US since 1958 to produce microbial-derived products with insecticidal activity. The Cry3Bb1 protein encoded by the *cry3Bb1* sequence is known to act specifically on the gut of Coleopteran insects. There are no receptors for Cry3Bb1 on mammalian intestinal cell surfaces and humans are not susceptible to this toxin. Additionally, Cry3Bb1 denatures at elevated temperatures during food processing and rapidly degrades in the soil.

Bacillus thuringiensis var. *kurstaki*, the donor for the *cry1Ab* coding sequence, has been shown to be non-toxic to humans, other vertebrates and beneficial insects. Bt strains have been used commercially in the US since 1958 to produce microbial-derived products with insecticidal activity. The Cry1Ab protein encoded by the *cry1Ab* sequence is known to act specifically on the gut of lepidopteran insects. There are no receptors for Cry1Ab on mammalian intestinal cell surfaces and humans are not susceptible to this toxin. Additionally, Cry1Ab denatures at elevated temperatures during food processing and rapidly degrades in the soil.

The *cp4* gene was derived from the common soil bacterium *Agrobacterium* sp. Strain CP4 which encodes only for the naturally glyphosate-tolerant EPSPS protein. No other protein is produced or introduced which may bring about toxicity, allergenicity or may be anti-nutritional in nature. No known pathogenicity in humans and animals because of the absence of the shikimic acid pathway in animals.

Proposed Use: For direct use as food, feed or for processing

II. Background Information

Two inbreds with the same germplasm, but each developed to contain a different transgenic trait, can be sued to generate a stacked trait inbred by simply crossing these two inbreds, self pollinating the progeny, and then selecting for seeds that are homozygous for the transgenic traits of interest (Yieldgard Plus). This new stacked inbred is then crossed to a round up ready corn (NK 603) inbred to generate commercial stacked trait hybrids such as Yieldgard Plus x Roundup Ready.

Monsanto Philippines, Inc has filed an application with attached technical dossiers to the Bureau of Plant Industry on September 15, 2004 for a biosafety notification for direct use as food, feed and for processing under Administrative Order (AO) No. 8 Part 5 for combined trait product corn: MON 810 x NK 603 x MON 863 which has been genetically modified for insect resistance and herbicide tolerance.

A safety assessment of combined trait product corn: MON 810 x NK 603 x MON 863 was conducted as per Department of Agriculture Administrative Order No. 8 Series of 2002 and Memorandum Circulars Nos. 6 and 8, Series of 2004. 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

The Cry1Ab protein is an insect control protein and acts through a toxic action in the midgut of specific lepidopteran insects, such as the European corn borer (*Ostrinia nubilalis*). The *cry1Ab* gene was derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki*, and was introduced into corn to generate MON 810.

The Cry3Bb1 protein is an insect control protein and acts through a toxic action in the midgut of corn rootworm larvae, i.e., coleopteran species *Diabrotica virgifera virgifera*, *D. barberi*, and *D. virgifera zaeae*. The *cry3Bb1* gene was derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kumamotoensis*, and was introduced into corn to generate MON 863.

The CP4 EPSPS protein belongs to the family of EPSP synthases, which are enzymes involved in the penultimate step of the biochemical shikimate pathway producing aromatic amino acids in the chloroplasts of plants.

Results of the bioefficacy of *YieldGard*[®] *Plus x Roundup Ready*[®] corn showed levels of insect protection against target lepidopteran and corn rootworm species comparable to levels of insect protection observed in the single trait hybrids, MON 810 and MON 863. These data confirm the selectivity of the insecticidal action of the Cry1Ab and Cry3Bb1 proteins encoded and that the two proteins do not exhibit any interactive effects on the plants. Levels of tolerance to Roundup[®] herbicide (glyphosate), imparted by the continued enzymatic action of the CP4 EPSPS enzyme, in *YieldGard*[®] *Plus x Roundup Ready*[®] corn is comparable to that observed in the single trait NK 603 hybrid.

Crossing a MON 810 inbred to a non transgenic elite inbred develops a commercial inbred containing the inserted genes of MON 810. The progeny of this cross are then backcrossed to the elite parental inbred and then repetitively self pollinated. The resulting commercial MON 810 inbred is crossed to an inbred containing the inserted genes of MON 863. The seeds as *Yieldgard Plus* F1 hybrid variety (MON 810 x MON 863). The *Yieldgard Plus* (MON 810 x MON 863) and *Roundup Ready* (NK 603) stacked trait hybrid is produced in the same way except that an additional cross to an inbred containing the inserted genes of NK 603 is made.

Safety of the Expressed Proteins

Based on modes of action of CP4 EPSPS and the Bt proteins, Cry1Ab and Cry3Bb1, and location of these proteins' accumulation in the plant cells, the likelihood of interaction with one another to produce a new allergen or a new toxin is not considered to be significant.

CP4 EPS and the two Bt proteins accumulate in different subcellular compartments of the corn plant cells. In plant, EPSPS is localized in plastids. Most plastid proteins are encoded by nuclear genes and synthesized as higher molecular weight precursors. The additional size of precursor is due to an amino terminal extension called the transit peptide. Transit peptides are necessary and sufficient for transport of proteins into chloroplasts. The *cp4 epsps* gene contained in NK 603 and YieldGard® Plus x Roundup Ready® was designed to encode a chloroplast transit peptide so that the CP4 EPSPS protein is directed to the chloroplast, the site of all action for all EPSPS proteins.

The modes and sites of biological action are very different for the CP4 EPSPS and the Bt proteins. There is no known mechanism of action between these different classes of proteins that could lead to adverse effects in humans and animals.

There are possible unexpected effects of the stacked genes on the metabolism of plants, however, their probability is very low since the genes and their products are expressed in different parts of the cells.

The Cry1Ab protein in MON 810, Cry3Bb1 protein in MON 863 and CP4 EPSPS protein in NK603 are considered to have no effect on normal plant metabolism for assessment purposes. Regulatory approvals for all three parental traits have been granted. Production of the Cry1Ab, Cry3Bb1 and CP4 EPSPS proteins in the stacked trait maize does not produce interactive or synergistic effects on plant metabolism because of different modes of action and binding sites are involved for each protein.

IV. Nutritional Composition (Compositional Analysis)

In terms of compositional analyses dry weight [dw]% ash, dw % carbohydrates, dw % fat, dw % moisture and dw% protein), the combined trait product MON 810 x Nk603 x MON 863 is compositionally equivalent to its conventional counterpart.

Based on the principle of substantial equivalence and the fact that the product is already used in the US, Canada and Korea, the product can be considered safe to use as food, feed and for processing.

V. Anti-Nutritional Factors

No known anti nutritional factors for individual events. Thus, combined trait product MON 810 x NK 603 x MON 863 has no known anti nutritional factors.

VI. Regulatory Decision

After reviewing the scientific data and information relevant to the stacked trait corn MON 810 x NK 603 x MON 863 application of Monsanto Philippines Inc. it is concluded that no interaction found between/among the stacked traits, hence this plant product was found to be as safe as its conventional corn and can substitute for its traditional counterpart for direct use as food, feed and for processing. Monsanto is hereby notified that it may proceed with the activities for the above product for direct use as food and feed or for processing following all existing rules and regulations consistent with DA AO #8.