

Determination of the Safety of Monsanto's
Combined trait product corn: LY038 X MON 810
for Direct use Food, Feed, and Processing

Food and Feed Safety:

The product dossiers on Monsanto's combined trait product corn: LY038 x MON 810 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: LY038 X MON 810 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 August 9, 2006. The notification is valid for five years and shall expire on August 8, 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: LY038 X MON 810 in the Philippines. Food and Feed use of combined trait product corn: LY038 X MON 810 its by-products is therefore authorized as of August 9, 2006. The biosafety notification (No. 06-011) stated that combined trait product corn: LY038 X MON 810 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: LY038 X MON 810
Applicant:	MONSANTO PHILIPPINES, INC. 7 th Floor, Ayala Life-FGU Center Alabang-Zapote Road cor Acacia Avenue Madrigal Business Park Alabang, Muntinlupa City 1770
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:	Increased lysine level in grain and insect resistance
Trait Introduction Method:	Conventionally plant breeding
Donor Organisms:	<p><i>Corynebacterium glutamicum</i>, a widely distributed non-pathogenic, rod-shapes, non-sporulating, Gram positive species of coryneform bacteria, is a natural producer of glutamic acid. <i>C. glutamicum</i> is used by the chemical industry for the biotechnological production of the amino acid lysine, an essential amino acid in animal nutrition.</p> <p><i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> strain HD-1(<i>B.t.k</i>), a widely distributed non-pathogenic, sporulating, Gram positive species of <i>Bacillus</i> is the source of <i>cryIAb</i> gene which produces a crystal protein effective as insecticide against Lepidopteran insects.</p>
Pathogenicity:	<p><i>Corynebacterium glutamicum</i> is widely distributed in the environment. It is not a human or animal pathogen. The <i>cordapA</i> gene sequence encodes for the protein DHDPS which is naturally present in feed and food (e.g. corn, rice, soy and wheat) and has a history of safe use.</p> <p><i>Bacillus thuringiensis</i> var. <i>kurstaki</i> has been shown to be non-toxic to humans, other vertebrates and beneficial insects. <i>B.t.k.</i> based foliar insecticides have been registered for over 30 years and have a long history of safe use. The toxin produced by the <i>cryIAb</i> gene is known to act specifically on the gut of lepidopterous insects. There are no receptors for the protein 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.</p>
Proposed Use:	For direct use as food, feed or for processing

II. Background Information

Monsanto Philippines, has filed an application with attached technical dossiers to the Bureau of Plant Industry on May 18, 2006 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: LY038 X MON 810 which has been genetically modified for insect resistance and herbicide tolerance.

A safety assessment of combined trait product corn: LY038 x MON 810 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

Lysine maize LY038 contains the *cordapA* coding sequence isolated from *Corynebacterium glutamicum* that encodes the lysine-insensitive dihydodipicolinate synthase (cDHDPS) enzyme. The transcription of *cordapA* is under the control of the corn Glb1 promoter, which directs cDHDPS expression predominantly in the germ, resulting in accumulation of free lysine in grain. In *cordapA* cassette, the cDHDPS coding sequence is under the control of *Zea mays* globulin 1 (Glb1) promoter, which in wild-type corn directs expression of the most abundant embryo-specific protein in corn. The utilization of the Glb1 promoter for *cordapA* transcription results in the expression of cDHDPS and the accumulation predominantly in the germ portion of the grain. The intron-sequence following Glb1 promoter is derived from the *rice actin-1* gene, and the purpose of the element is to enhance DNA transcription. The 3' nontranslated region of the globulin 1 gene following the *cordapA* gene contains the polyadenylation signal that directs the termination and maturation of the *cordapA* gene transcript.

Corn Event MON 810 (Trade name: Yieldgard®) and all corn lines/hybrids derived from this event contain the *cry1Ab* coding sequence from *Bacillus thuringiensis* sp *kurstaki* strain. The *cry 1Ab* gene was modified to produce a protein with enhanced insecticidal activity against the lepidopteran pest, ACB,

The novel variety produces a truncated version of the insecticidal protein, Cry1Ab, derived from *Bacillus thuringiensis*. Delta-endotoxins, such as the Cry1Ab protein expressed in MON 810, act by selectively binding to specific sites localized on the brush border midgut epithelium of susceptible insect species. Following binding, cation-specific pores are formed that disrupt midgut ion flow and thereby cause paralysis and death. The insecticidal properties of Cry1Ab is applicable only to lepidopteran insects, and its specificity of action is directly attributed to the presence of specific binding sites in the target insects. There are no binding sites for delta-endotoxins of *B. thuringiensis* on the surface of mammalian intestinal cells, therefore, livestock animals and humans are not susceptible to these proteins.

A commercial inbred line with the inserted gene of LY038 is developed by the conventional backcrossing of LY038 and a conventional elite inbred line. The commercial inbred line with the inserted gene of LY038 is then crossed with another inbred line that contains the inserted gene of MON 810. The resulting seeds are stacked genes F1 hybrid

Safety of the Expressed Proteins

The likelihood of interaction between cDHDPS and Cry1Ab proteins is highly unlikely because of their different mechanisms of action. Thus, the production of a new allergen or a new toxin as a result of interaction is also highly unlikely.

The two gene products accumulate in different subcellular compartments of the corn plant cells. The cDHDPS protein is directed to the chloroplasts where lysine biosynthesis occurs. The *cordapA* gene cassette encoding the cDHDPS protein contains a transit peptide (mDHDPS CTP). The Cry1Ab protein, on the other hand, accumulates in the cytoplasm; it does not contain a transit peptide.

The mode of action of each gene product is different. The DHDPS enzymes is involved in the rate limiting step in the lysine biosynthetic pathway is controlled by a lysine feedback inhibition. The enzyme variant from *Corynebacterium glutamicum* (cDHDPS) is less sensitive to lysine feedback inhibition. Due to this insensitivity, the levels of lysine can be increased in the genetically altered corn encoding the cDHDPS protein compared to the conventional one.

The Cry1Ab protein, on the other hand, is a toxic protein specifically targeting the gut of specific Lepidopteran insects. The Bt proteins exert their insecticidal activity via receptor-mediated mechanisms that are highly selective in terms of organisms targeted. The products are not involved in the same metabolic pathway. There are no possible unexpected effects of the stacked genes on the metabolism of the plant based on previous safety assessments of individual transformation events.

The expression levels of the individually approved transformation events are the same as the individually approved transformation events. Both proteins are expressed at low levels in plants. The concentration of free lysine in stacked trait variety is comparable to that of the single trait variety. The marker genes were excised in the previous transformation events. Thus, no marker genes were transferred to the plants with stacked genes.

Based on the experimental results obtained from bioefficacy studies on Lysine Maize x YieldGard Corn Borer Corn, the activities of *cDHDPS* and *cry1Ab* genes are not affected by potential interactions with one another.

IV. Nutritional Composition (Compositional Analysis)

The efficacy performance of the Lysine maize and Yieldgard corn borer traits in the conventional bred F1 hybrid is confirmed in the case of the lysine maize trait by comparison of grain compositional data (specifically free lysine concentration) in the single trait LY038 grain with the free lysine concentration in grain from the combined trait LY038 x MON 810.

V. Anti-Nutritional Factors

No known anti nutritional factors for individual events. Thus, MON 810 x LY038 corn has no known anti nutritional factors.

VI. Regulatory Decision

After reviewing the scientific data and information relevant to the stacked trait corn LY038 x MON 810 application of Monsanto Philippines Inc. it is concluded that no interaction found between/among the combined 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.