

Determination of the Safety of Monsanto's
Combined trait product corn: NK 603 x MON 863
for Direct Use as Food, Feed, and Processing

Food and Feed Safety:

The product dossiers on Monsanto's Combined trait product corn: 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 stacked trait product corn: 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 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: NK 603 x MON 863 in the Philippines. Food and Feed use of combined trait product corn: NK 603 x MON 863 its by-products is therefore authorized as of November 16, 2004. The biosafety notification (No. 04-004) stated that combined trait product corn: 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)

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| Designation: | Combined trait product corn: NK 603 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: Herbicide tolerance and insect resistance

Trait Introduction Method: Conventional breeding

Donor Organisms: *Bacillus thuringiensis* subsp. *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.

Agrobacterium sp. Strain CP4, source of *cp4epsps* gene which encodes only for the naturally 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.

The *cp4 epsps* gene was derived from the common soil bacterium *Agrobacterium* sp. Strain CP4 which encodes for the naturally occurring glyphosate-tolerant CP4 EPSPS protein. No other protein is known to be produced 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

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, Inc. 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 combined trait product corn: NK 603 x MON 863, which has been genetically modified for insect resistance and herbicide tolerance.

A safety assessment of combined trait product corn: 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

Corn event NK 603 (Trade name: Roundup-Ready Corn) and all corn lines/hybrids derived from this Event contain the CP4 EPSPS coding sequence from *Agrobacterium* sp CP4 strain. The CP4 EPSPS sequence encodes for the production of the naturally-occurring CP4 EPSPS protein that renders the corn NK 603 tolerant to glyphosate, a herbicide known to be environmentally-compatible.

Corn Event MON 863 (Trade name: Yieldgard Corn Rootworm) and all corn lines/hybrids derived from this event contain the *cry3Bb1* coding sequence from *Bacillus thuringiensis* sp *kumamotoensis* strain. The *cry3Bb1* gene was modified to produce a protein with enhanced insecticidal activity against the coleopteran pest, CRW, and was codon optimized for expression in monocotyledonous plants.

A commercial inbred line with the inserted genes of MON 863 is developed by the conventional backcrossing of MON 863 and a non transgenic elite inbred line. The resulting commercial inbred line with the inserted genes of MON 863 is then crossed with another inbred line, which contains the inserted genes of Corn NK 603. The resulting seeds are stacked traits F1 hybrid variety.

Safety of the Expressed Proteins

Based on modes of action of CP4EPSPS and Cry3Bb1 proteins 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. NPTII protein is not detectable in grain.

CP4EPSPS and Cry3Bb1 proteins accumulate in different sites of NK 603 or MON 863 x NK 603 corn plant cells. NPTII protein is not detectable in grain. In plants, EPSPS is localized in plastids. Most plastids proteins are encoded by nuclear genes and synthesized as higher molecular weight precursors. The additional size of the 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 cp4epsps gene contained in NK 603 and MON 863 x NK 603 corn was designed to encode a chloroplast transit peptide (CTP) so that the proteins would be directed to the chloroplast, the site of action of all EPSPS proteins. As shown by N terminal sequencing of the expressed CP4EPSPS protein, the chloroplasts transit peptide is removed, indicating that the protein has been deposited in the plastid.

Proteins destined for the plant cell cytoplasm do not require amino terminal transit peptides. Since Cry3Bb1 expressed in MON 863 and MON 863 x NK 603 corn is an insect control protein, having toxic action in the gut of specific corn rootworms, it was necessary to design the *cry3Bb1* gene to include a transit peptide. Therefore, unlike CP4EPSPS, the Cry3Bb1 protein does not contain a transit peptide and accumulates in the plant cell cytoplasm.

No interactions or synergistic effects are expected since there are different modes of action and binding sites of the proteins produced, i.e., CP4EPSPS, Cry3Bb1 and even the marker for NPTII. The biological activities of CP4EPSPS and Cry3Bb1 are different and there is no possibility in terms of conceivable mechanisms that will produce interaction of the two introduced proteins. CP4EPSPS is involved in the shikimate pathway in the chloroplasts and Cry3Bb1 is an insect protein produced in the cytoplasm. Due to the difference in the involvement of the two proteins or gene products in the metabolic processes of the corn plants, an unintentional secondary effect in metabolism will be expected.

The expression levels of the individual protein products are the same as the individually approved transformation events. Protein levels are comparable in the stacked trait and single trait products. Both proteins are expressed at low level compare to other endogenous corn proteins. The proteins are present in the parts per million range, marker gene is not expected to produce harmful effects; as expressed, its product occurs in low amount.

The introduced genes act differently and since there is no interaction between them, the expression and stability of either one will not be affected.

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, NK 603 x MON 863 corn has no known anti nutritional factors.

VI. Regulatory Decision

After reviewing the scientific data and information relevant to the combined trait corn NK 603 x MON 863 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.