

Determination of the Safety of Syngenta's
Stacked trait product corn: Bt11 x MIR 162 x GA21
for Direct use as Food, Feed, and Processing

Food and Feed Safety

The product dossiers on Syngenta's stacked trait product corn: Bt11 x MIR 162 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 stacked trait product corn: Bt11 x MIR 162 x GA21 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 Syngenta Philippines Inc. on July 28, 2010. The notification is valid for five years and shall expire on January 27, 2015 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 stacked trait product was included in the Lists of Approval Registry 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: Bt11 x MIR 162 x GA21 in the Philippines. Food and Feed use of combined trait product corn: Bt11 x MIR 162 x GA21 its by-products is therefore authorized as of July 28, 2010. The biosafety notification (No. 10-030) stated that combined trait product corn: Bt11 x MIR 162 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: Bt11 x MIR 162 x GA21

Applicant: SYNGENTA PHILIPPINES, INC.
12th Floor, Two World Square
#22 McKinley Rd., McKinley Town Center
Fort Bonifacio, Taguig City
Philippines

Plant Species:

Name: Corn (*Zea mays*)

Parent Material: Corn Bt11, Corn MIR 162 and Corn GA21

Center of Origin: Mexico and Central 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) is a potential toxicant but declines rapidly as the plant grows
Trait Description: Insect resistance and Herbicide Tolerance

Trait Introduction Method: Conventional breeding among the three genetically modified corn events (Bt 11, MIR 162 and GA21)

Donor Organisms: *Bacillus thuringiensis* var *kurstaki*, source of *cry1Ab* gene which produces crystal protein effective as insecticide against specific group of insects, and source of *vip3Aa20* gene which encodes a vegetative insecticidal protein (Vip) that controls several lepidopteran pests of maize including corn earworm, common cutworm, fall armyworm, and corn semi-looper.

| *Streptomyces viridochromogenes*, produces the *pat* gene encoding an enzyme, the phosphinotricin-N-acetyl transferase that detoxifies glufosinate ammonium.

| *Escherichia coli* (*E. coli*), source of the *phosphomannose isomerase (pmi)* gene which encodes the PMI protein for use as a selectable marker during the development of maize event MIR 162.

Zea mays, source of modified *epsps* gene which confers tolerance to herbicides

Pathogenicity: *B. thuringiensis* has no known pathogenicity and allergenicity to human, animals and non-target organisms.

Streptomyces viridochromogenes is ubiquitous in the soil and there have been no reports of adverse effects on humans, animals and plants.

E. coli has no known pathogenicity and allergenicity to humans, animals and non-target organisms.

Zea mays (the donor for mEPSPS) is generally recognized as safe (GRAS) and has a long history of safe consumption as human food and animal feed.

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

II. Background Information

Syngenta Philippines, Inc has filed an application with attached technical dossiers to the Bureau of Plant Industry on March 19, 2010 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: Bt11 x MIR 162 x GA21, which was developed by conventionally crossing three genetically modified corn events (Bt11, MIR 162 and GA21) for insect resistance and herbicide tolerance.

A safety assessment of combined trait product corn: Bt 11, MIR 162 and GA21 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 interaction among the three transgenes.

III. Description of Novel (Introduced) Traits

Bt 11 Corn and all corn lines/hybrids derived from this Event contain the *cry1ab* coding sequence derived from *Bacillus thuringiensis* var *kurstaki* which is a common soil bacterium. The *cry1ab* gene encodes for the production of Cry1Ab (Btk) protein. This corn event also contains the marker gene, *pat* derived from the soil bacterium, *Streptomyces viridochromogenes*. which encodes for the production of phosphinothricin acetyl-transferase (PAT) protein, that confers tolerance to glufosinate ammonium, an active ingredient in herbicide. The glufosinate ammonium inhibits the glutamine synthetase in plants, resulting in an accumulation of ammonia in plant tissues leading to its death.

Corn MIR 162 and all corn lines/hybrids derived from this Event express the *vip3Aa20*, from *Bacillus thuringiensis* for control of a number of Lepidopteran insects, namely common cutworm (*Spodoptera litura* Fabricius), corn semi-looper (*Chrysodeixis chalcites* [Esper] L.), true armyworm (*Mythimna separata* Walker) and corn earworm (*Helicoverpa armigera* Hubner). This corn event also expresses a *pmi* gene from *Escherichia coli*, that encodes the enzyme *phosphomannose isomerase* present as selectable marker, enabling transformed plant cells to utilize mannose as a primary carbon source.

Corn GA 21 and all corn lines/hybrids derived from this Event express a modified version of the endogenous maize 5-enol pyruvyl shikimate-3-phosphate synthase (EPSPS) protein. This modified enzyme, designated as mEPSPS, confers tolerance to glyphosate herbicide. The mepsps gene transformed into GA21 expresses a 576 amino acid protein which includes a 131 amino acid optimized transit peptide which is subsequently cleaved as demonstrated by the N- terminal sequence analysis (Graser, 2005) to generate the mature EPSPS protein, which contains 445 amino acids (47.5 kDa)

The transgenic traits from Event Bt 11, Event MIR162 and Event GA21 were combined through conventional breeding to produce the Bt 11 x MIR 162 x GA21. This stacked hybrid produces the five transgenic proteins present in Bt11, MIR162 and GA21 maize plants.

Safety of the Expressed Proteins

Using the Basic Local Alignment Search Tool for Proteins (BLASTP) search program, the expected sequence homology to other delta endotoxins, including the other Cry proteins, the *cry1ab* query sequence showed no significant sequence homology to any proteins identified or known to be toxins. By comparing the Cry1Ab amino acid sequence to the sequences in the SBI allergen Database, Bt 11 has any significant homology to known allergens,

Furthermore, The PAT query sequence showed no significant sequence homology to any proteins identified as or known to be toxins, BT 11 maize has any significant amino acid sequence homology to known toxins

The BLASTP search using the five shuffled versions of the VIP3A sequence identified several proteins that satisfied the conservative search criteria and returned E values that ranged from 0.043 to 10. The E values of the 12 entries identified in the GenBank as VIP3A polypeptides were extremely low and ranged from 0 to 7.0×10^{-30} , indicating they were identical (E value =0) or highly homologous to the VIP3A query sequence.

Vip3A as expressed in the transgenic Event MIR162 maize shows no amino acid sequence homology to known or putative allergenic proteins in the Genbank database, including any other proteins from *Bacillus thuringiensis* (the source of VIP3A protein) or any non VIP3A proteins identified as toxins.

The results of the BLASTP program search also show that the sequence of the phosphomannose isomerase protein (PMI) from *Escherichia coli* has no amino acid sequence homology to known protein toxins. Two different homology searches were performed by comparing the PMI amino acid sequence to the sequences in the Syngenta Biotechnology, Inc Allergen Database. The study found no new significant amino acid sequence homology to known or putative allergenic allergens.

The mEPSPS protein sequence was systematically compared to the latest posting of the National Center for Biotechnology Information (NCBI) Entrez Protein Database which show that MEPSPS query sequence has no significant sequence homology to any proteins or known to be toxins.

The five proteins (cry1Ab, PAT, Vip3Aa20, PMI and mepsps) show no homology to any known mammalian allergen or toxin. There is no evidence suggesting that the five proteins will interact to form some new allergen or toxin since each has distinct mode of action and are not likely to interact.

IV. Nutritional Composition (Compositional Analysis)

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

V. Anti-Nutritional Factors

There are no known anti nutritional factors for individual events. Thus, Bt11 x MIR 162 x GA21 corn has no known anti nutritional factors.

VI. Regulatory Decision

After reviewing the scientific data and information relevant to the combined traits product corn Bt11 x MIR 162 x GA21 application of Syngenta Philippines Inc., it is concluded that no interaction was 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 and is therefore approved for direct use as food, or feed or for processing. Syngenta 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.