Determination of the Safety of Syngenta's
Combined Trait Product Corn: 3272 x Bt11 x MIR604 x GA21
For Direct use as Food, Feed, or Processing

**Food and Feed Safety**

The product dossiers on Syngenta's combined trait product corn: 3272 xBt11 x MIR604 x GA21 were reviewed for safety and nutritional differences compared with the conventional cornThe 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: 3272 xBt11 x MIR604 x GA21 and all progenies derived from crosses of the product with any conventionally bredcorn and corn containing approved-biotech events for direct use as food, feed or for processing was issued to Syngenta Philippines Inc. on July 28, 2010. The notification is valid for five years and shall expire on July 27, 2015subject 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 List 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: 3272 x Bt11 x MIR604 x GA21 in the Philippines. Food and Feed use of combined trait product corn:**3272 x** Bt11 x MIR604 x GA21 andits by-products is therefore authorized as of July 28, 2010. The biosafety notification (No. 10-032) stated that combined trait product corn:3272 x Bt11 x MIR604 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: 3272 xBt11 x MIR604 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 3272, Corn Bt11, Corn MIR604 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) a potential toxicant but declines rapidly as the plant grows

**Trait Description:** Modified alpha-amylase**,** insect resistance and herbicide tolerance

**Trait Introduction Method:** Conventional breeding among and betweenfour genetically modified corn events (3272, Bt11, MIR604 and GA21)

**Donor Organisms***:* *Thermococcales*, source of *amy797E* gene responsible for the increased thermostability and activity during the high temperatures required for starch hydrolysis in corn processing.

*Bacillus thuringiensis*var*kurstaki*, source of *cry1Ab* gene which produces crystal protein effective as insecticide against specific group of insects and *Streptomyces viridochromogenes* which produces the *pat* gene encoding an enzyme, the phosphinotricin-N-acetyl transferase that detoxifies glufosinate ammonium.

*Bacillus thuringiensis*subsp*tenebrionis,* source of modified *cry3A* gene conferring the resistance to rootworm and *Escherichia coli,* source ofphosphomannoseisomerase *(pmi)* gene,encodes the enzyme phosphomannoseisomerase (PMI) that allows the plants to utilize mannose as a carbon source and is used as a selectable marker.

*Zea mays,* source of *mepsps* gene

**Pathogenicity:** *Thermococcales spp.*(donor of *amy797E* gene) is not known to be sources of allergenicproteins.

*Bacillus thuringiensis var. kurstaki* (the donor for *cry1Ab* gene) has been shown to be non-toxic to humans, other vertebrates and beneficial insects. *B.t.k.* based foliar insecticides have been registered for over 30 years and have a long history of safe use.

*Bacillus thuringiensis* subsp. *tenebrionis i*s a common soil bacterium that has a long history of safe use as a microbial insecticide with no reported allergenic and toxic responses, establishing basis for the lack of allergenic or toxic concern for the Cry3A protein. Bt-based products have shown that the proteins produce toxic effects only in the gut of chewing insects and are not activated in human digestive tracts.

*Zea mays,* source of *mepsps* gene, has been traditionally used as food and feed for many years and has shown no adverse effect on human/animal health.

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

## II. Background Information

Syngenta Philippines has developed a combined trait product corn: 3272 x Bt11 x MIR604 x GA21 with modified alpha-amylase, insect resistance and herbicide tolerance trait.

On April 22, 2010,Syngenta Philippines Inc., has filed an application with attached technical dossiers to the Bureau of Plant Industry for a biosafety notification for direct use as food, feed or for processing under Administrative Order (AO) No. 8 Part 5 for stacked trait product corn: 3272 x Bt11 x GA21, which was developed by conventionally crossing four genetically modified corn events (Bt11, MIR604 and GA21) for insect resistance and herbicide tolerance. Relevant scientific publications were also supplied.

A safety assessment of combined trait product corn: 3272 x Bt11 x MIR604 x 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 between the six transgenes*: cry1Ab, pat, mcry3A, pmi, mepsps and amy797E.*

Review of results of evaluation by the BPI Biotech Core Team completed the approval process.

## III. Description of Novel (Introduced) Traits

Corn 3272 contains a synthetic thermostable alpha amylase protein,AMY797E produced by *amy797E* gene derived from *Thermococcales*. Alpha amylases are enzymes that catalyze the hydrolysisof starch into soluble sugars.

Corn event Bt11 contains the c*ry1ab* coding sequence derived from *Bacillus thuringiensis*var*kurstaki*which isa common soil bacterium. The c*ry1ab* gene encodes for the production of Cry1Ab (Btk) protein. This crystal protein protects the plant from specific lepidopteran insect damage. When eaten by the larva, the *Btk* protein is broken down by digestive enzymes in the larva's alkaline intestine, generating a shorter protein that binds to the wall of the intestine. This damages the larva's cell membrane, making it leaky, and stops the larva from feeding that leads to its eventual death.

Corn Bt11 also contains the marker gene *pat* derived from the soil bacterium *Streptomyces viridochromogenes*. The *pat* coding sequence encodes for the production of phosphinothricin acetyl-transferase (PAT) protein. This protein gives the plant 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 event GA21 contains a double mutated 5-enol pyruvylshikimate-e-phosphate synthase (*mepsps*) gene that confers tolerance to glyphosate. The mEPSPS protein shows no significant amino acid sequence homology to known or putative allergenic proteins.

Maize plants derived from the transformation event MIR604 containsa modified Cry3A (*mcry3A*gene,from*Bacillusthuringiensis subsp. tenebrionis,* which encode for control of corn

rootworm*and*, a phosphomannoseisomerase (*pmi*)gene from *Escherichia coli*, as a selectable marker trait which was employed only during selection of transformed plant cells in culture to utilize mannose as a primary carbon source.

BT11 x MIR604 x GA21 maize was produced by combining Bt11, MIR604 and GA21 maize transgenic traits through conventional breeding.

**Safety of the Expressed Proteins**

3272 x BT11 x MIR604 x GA21 maize produces the six transgenic proteins (AMY797E, Cry1Ab,PAT, mCry3A, PMI and EPSPS. The six proteins show no homology to any known mammalian allergen or toxin. There is no evidence that the six proteins will interact to form some new allergen or toxin since each has distinct mode of action and are not likely to interact.

None of the transgenic proteins produced by3272 x Bt11 x MIR604 x GA21 maize (AMY797E, Cry1Ab, PAT, mCry3A, PMI, MIR604 PMI and mEPSPS) come fromdonors known to be a significant cause of food allergy. All proteins have no biologically significant amino acid homology to known allergens, are readily degraded in *in vitro* digestibility assays and all show no acute oral toxicity in mammalian studies.

## 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, 3272 x BT11 x MIR604 x GA21 maize has no known antinutritional factors.

## VI. Regulatory Decision

After reviewing the scientific data and information relevant to the combined traitsproduct 3272 x BT11 x MIR604 x GA21 maize 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 Philippines, Inc. 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.