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

Food and Feed Safety

The product dossiers on Syngenta's combined trait product corn: Bt11 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 combined trait product corn: Bt11 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 January 23, 2007. The notification is valid for five years and shall expire on January 22, 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: Bt11 x GA21 in the Philippines. Food and Feed use of combined trait product corn: Bt11 x GA21 its by-products is therefore authorized as of January 23, 2007. The biosafety notification (No. 07-013) stated that combined trait product corn: Bt11 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 xGA21

Applicant: SYNGENTA PHILIPPINES, INC.
8th Floor, Salustiana Dee Ty Tower
104 Paseo de Roxas Avenue
Makati City
Philippines

Plant Species:

Name: Corn (*Zea mays)*

Parent Material: Corn Bt11 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:	Insect resistance and Herbicide Tolerance
Trait Introduction Method:	Conventional breeding between two genetically modified corn events (Bt11 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 *Streptomyces viridochromogenes* which produces the *pat* gene encoding an enzyme, the phosphinotricin-N-acetyl transferase that detoxifies glufosinate ammonium. *Zea mays,* source of *mepsps* gene
Pathogenicity:	Cry1Ab protein has been shown to be non-toxic to humans, other vertebrates and beneficial insects. *Bt*- based foliar insecticides have been registered for over 30 years and have a long history of safe use. *Streptomyces viridochromogenes* is ubiquitous in the soil and there have been no reports of adverse effects on humans, animals and plants. *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 October 31, 2006 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 GA21, which was developed by conventionally crossing two genetically modified corn events (Bt11 and GA21) for insect resistance and herbicide tolerance.

A safety assessment of combined trait product corn: Bt 11 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 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

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 crystal protein protects the plant from specific lepitopteran 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.

This corn event 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 GA 21 and all corn lines/hybrids derived from this Event contain the *epsps* coding sequence from corn The *epsps* gene codes for the synthesis of EPSPS enzyme, which is involved in the shikimic pathway for aromatic amino acid biosynthesis in plants and microorganisms (Steinrucken and Amrhein, 1980). The shikimic pathway is not present in animals, which contributes to the selective toxicity of glyphosate to plants. The modified maize *epsps* (*mepsps*) gene is completely sequenced and encodes a 47.7 kD protein consisting of 445 amino acids. It differs from wild – type maize *epsps* by two amino acid substitutions. This results in a protein with greater than 99.3 % sequence identity to that of the maize protein. The mEPSPS protein and the wild type EPSPS from corn are immunologically and functionally equivalent, except for their affinity to glyphosate, as anticipated by high sequence similarity.

Backcrossing was used to move the trait into an inbred background to generate a fixed inbred for each trait. The fixed transgenic inbreds are then crossed to produce a commercial hybrid containing both events, Bt 11 x GA21.

Safety of the Expressed Proteins

There is no interaction of the resulting products such that that a new allergen or a new toxin could be produced. On the issue of the homology of the three proteins produced by the *cry1Ab, pat* and *epsps* genes to mammalian allergens and toxins, Syngenta's search effort plus similar reports have shown no such homology.

As to the interaction effects to form a new type of allergen/toxin, no such phenomenon is in evidence. If any interaction effects are to be expected, it should be between the PAT and mEPSPS proteins since their respective genes both encode for herbicidal tolerance, but the biochemical pathways and principles involved are different and this probably explains why no such interaction is manifested.

The mEPSPS enzyme in the stacked Bt11 x GA21 hybrid is accumulated more in the chloroplasts in the leaves where it is transported by a transit peptide. With reference to the Bt 11 event that produces both the Cry1Ab and PAT proteins, both proteins are distributed in most parts of the plant including the endosperm of the grains that are utilized/processed as food or feed. However, the level of the PAT protein in the grains is very low or below the limit of quantitation.

Bacillus thuringiensis produces during sporulation crystal protein inclusions, also called "protoxins". These protoxins are solubilized by the alkaline gut juice and proteolytically cleaved in to a smaller active toxic fragment, the core fragment. The active core is tolerant to further digestion by proteases such as trypsin. The activated protein binds to brush border membrane vessicules in the insect midgut

inducing the formation of pores, affecting osmotic balance. The cells swell and lyse. Susceptible larvae stop feeding and eventually die.

GA21 event contains a modified *epsps* gene from corn (*Zea mays* L.). The mEPSPS protein has low affinity for glyphosate. Thus, when plants expressing the mEPSPS protein are treated with glyphosate, the plants are unaffected. The continued action of mEPSPS enzyme provides the plant's need for aromatic amino acids. Glyphosate kills plants by inhibiting the enzyme 5 enovylpyruvyshikimate-3- phosphate (EPSPS). This enzyme is a critical step in the shikimic pathway for the biosynthesis of aromatic amino acids in plants and microorganisms, and its inhibition leads to the death of the plants.

The modes of action of each gene product (Bt protein and mEPSPS protein) are different. The products of the *Bt* gene and *mepsps* gene are involved in two distinctly different metabolic pathways. The stacked genes do not pose any adverse effect on the metabolism of the plant as evidenced by the fact that the superiority in yield of the hybrid has been maintained.

Results of biochemical analyses have shown that the expression levels of the *cry1Ab, pat* and *mepsps* genes in the stacked hybrid are comparable with those of the counterpart genes in the single events. The proteins are expressed at low levels in the stacked hybrid but sufficiently effective in providing resistance to the attack of corn borer larvae and the effect of glyphosate application. It is for these reasons why the Bt 11 x GA21 hybrids has been commercialized in the US. The PAT gene is expressed in the stacked hybrids, but the amount is below the limit of quantitation.

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 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 GA 21 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.