Determination of the Safety Syngenta's
Combined trait product corn: Corn Bt11 x MIR604 x GA21
For Food, Feed, and Processing

Food and Feed Safety:

The product dossiers on Syngenta's Combined trait product corn: Corn Bt11 x MIR604 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 MIR604 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 March 3, 2008**.** 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 MIR604 X GA21 in the Philippines. Food and Feed use of Stacked trait product corn: Bt11 x MIR604 x GA21 its by-products is therefore authorized as of March 3, 2008. The biosafety notification (No. 08-018) stated that combined trait product corn: 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: Bt11 x MIR604 x GA21
Applicant:		SYNGENTA PHILIPPINES, INC. Building 1-B, Sunblest Compound, Km.23 West Service Road, Cupang, Muntinlupa City
Plant Species:		
	Name:	corn (*Zea mays*)
	Parent Material:	Inbred corn lines (and/or isolines) developed and produced by Syngenta Philippines
	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 amounts; 2,4-dihydroxy-7-methoxy-2H-1, 4 benzoxacin (DIMBOA) is a potential toxicant in corn but this declines rapidly as the plant grows.

Trait Description: Insect resistance and herbicide tolerance

Trait Introduction Method: Conventional breeding

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.

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

Zea mays L. a source of *mepsps* gene

Pathogenicity:

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.

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

Bacillus thuringiensis subsp. *tenebrionis* is 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.

Corn grain is a source of food for humans and animals and industrial applications for thousand of years. Animals consume silage that derives 50% of its nutritional value for grain. Corn as a food source for humans and animals has a history of safe use.

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

I. Background Information

Syngenta Philippines, Inc has filed an application with attached technical dossiers to the Bureau of Plant Industry on December 3, 2007 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 MIR604 x GA21 which has been genetically modified 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 interactions among the 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.

II. Description of Novel (Introduced) Traits

Bt 11 corn and all corn lines/hybrids derived from this Event contain the c*ry1ab* coding sequence derived from *Bacillus thuringiensis* var *kurstaki* which is a common soil bacterium. The c*ry1ab* gene encodes for the production of Cry1Ab (Btk) protein. This crystal protein protects the plant from insect damage. When eaten by the insects and corn pests, 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 cell membrane, making it leaky, and stops the larva in its tracks.

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 MIR604 contains a single copy of the *mcry*3A gene encoding the MCry3A protein and the *pmi* gene. The gene for mCry3A has been modified to incorporate a cathepsin-G serine protease recognition site within the expressed protein. The modification increases the toxicity to target pests. The PMI gene as the selectable marker encodes for a protein that catalyzes the reversible inter-conversion of mannose-6-phosphate and fructose-6-phosphate. Its reaction is specific and plant cells expressing the PMI gene are capable of survival and growth in the presence of mannose as the only carbon source.

Corn GA 21 and all corn lines/hybrids derived from this Event contain the EPSPS coding sequence from maize. 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 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 all three events, Bt11 X MIR604 x GA21.

Safety of the Expressed Proteins

The five (5) genes are independent of each other and their products are not interacting. So far, no evidence shows that the five (5) proteins synthesized by the genes in transgenic events exhibited significant amino acid sequence homology with any identified toxic proteins or known/putative allergenic proteins.

The gene products will be accumulated in different subcellular compartments of the corn plants. The products of cry1Ab and pat genes in Bt 11 as well as those of mcry3a and pmi genes in MIR 604 will be accumulated in the cytoplasm. On the other hand, the products of modified epsps gene and GA 21 will be accumulated in the chloroplast.

Bt 11		
Genes	Products	Modes of Action
Cry1Ab	Delta endotoxin protein	Protein binds to localized specific sites of the brush border midgut of borers causing cation-specific pores which disrupt ion flow resulting to paralysis and death of insects.
PAT	Phosphinothricin acetyltransferase	Enzyme detoxifies through acetylation of the phosphinothricin. The active ingredient of the Basta herbicide. (glufosinate ammonium) which inhibits the glutamine synthetase causing accumulation of ammonia leading to plant death.
MIR 604		
mCRy3A	Cry protein	Protein binds to receptors in midgut cells of rootworm and form ion-selective channels in the cell membrane. The cells swell due to an influx water causing cell lysis and insect death
PMI	Phosphomannose isomerase	Protein converts mannose to a readily metabolized compound, fructose-6-phosphate which improves the energy of cells
GA 21		
mepsps	modified 5-enol pyruvylshikimate -3-phosphate synthase	The modified protein has low affinity for Glyphosate which kills plants by inhibiting the enzyme epsps (a critical step in the Shikimic Acid Pathway for the biosynthesis of aromatic amino acids and for growth in plants).

The modes of action of the gene products indicate their differences. The genes *cry1Ab* and *mcry3A* are specific and distinct genes contained respectively by two different maize events, the Bt 11 and MIR604. Also the two genes specifically work against two different species of insects (corn borer and rootworm), thus definitely, they have different modes of action. The modes of action of the gene products indicate different functions.

Documented reports of studies on the effects of stacked genes on plant metabolism indicate no detectable unexpected effects. Based on the study reports, the phenotypic expression of the efficacy of stacked hybrids are simply equivalent to those of the stand alone transgene products. There was no other additional phenotypic expression indicative of the possible interactions of gene products in the cytoplasm.

Protein synthesis occurs in the cytoplasm. Phenotypic expression, in this case insect resistance and glyphosate tolerance, is an indication that there is no interaction if the stacks

behave in a similar manner as the single events. The site of protein synthesis for *cry1Ab*, *mcry3A* and *pmi* is in the cytoplasm but they are not likely to interact because these proteins are not involved in the same metabolic pathways: both Bt proteins (Cry1Ab and mCry3A) do not have enzymatic activity while in the case of PMI, there is no endogenous substrate for PMI in the maize plant except when the plant is exposed to mannose.

Considering the Syngenta-generated data, the expression levels of the individual protein products are the same as the individually approved transformation events and all proteins are effective even at low levels in plant.

The data and results of the comparative southern analyses of Bt 11 x MIR 604 x GA 21 maize with the individual events Bt 11 maize, MIR 604 maize and GA 21 maize indicate that the marker genes were transferred and expressed also in the plants containing the stacked genes.

III. 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

No known anti nutritional factors for individual events. Thus, Bt11x MIR604 x GA21 has no known antinutritional factors.

VI. Regulatory Decision

After reviewing the scientific data and information relevant to the combined trait corn Bt11 and MIR604 application of Syngenta 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 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.