Determination of the Safety of Syngenta's
Corn MIR604 (Insect Resistant corn)
for Direct use as Food, Feed, and for Processing

Food and Feed Safety:

The product dossier on Corn MIR604 were reviewed for safety and nutritional differences compared with the conventional corn. The focus of the review was on any new or altered expression trait and changes in composition and nutritional content or value relative to the conventional corn. At the end of the safety assessment, a conclusion was made that the Corn MIR604 is as safe as the conventional corn taking into account dietary impact of any changes in nutritional content or value.

A biosafety permit for Corn MIR604 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 October 8, 2007. The permit is valid for five years and shall expire on October 7, 2012 subject to the terms and conditions set forth in DA Administrative Order No. 8, Series of 2002, as amended by DA Administrative Order No. 22, Series of 2007. The said MIR604 Corn 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 Insect protected Corn MIR604 in the Philippines. Food and Feed use of MIR604 corn its by-products is therefore authorized as of October 8, 2007. The biosafety permit (No.07 -0026) stated that "Insect protected Corn" 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: Corn MIR604

Applicant: SYNGENTA Philippines, Inc.
Building 1-B, Sunblest Compound,
Km.23 West Service Road, Cupang
Muntinlupa City
Philippines

Plant Species:

Name: Corn (*Zea mays*)

Parent Material: Inbred corn lines (and/or isolines) developed and produced by Syngenta

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 (corn rootworm) resistance
Trait Introduction Method:	*Agrobacterium* - mediated transformation
Donor Organisms:	*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.
Pathogenicity:	*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. *Escherichia coli* is ubiquitous in the digestive system of vertebrates, including humans. GUS protein was originally isolated from *E. coli*. GUS activity has been detected in a large number of bacteria, mammals and plants including food plants such as potato and apple and constitutes a history of safe exposure.
Proposed Use:	For direct use as food, feed and for processing

II. Background Information

Syngenta Philippines, Inc submitted an application with attached technical dossiers to the Bureau of Plant Industry on May 23, 2007 requesting for biosafety permit under Administrative Order (AO) No. 8 Part 5 for corn MIR604 which has been genetically modified for insect resistance.

SYNGENTA Philippines, Inc. has provided data on the identity of corn MIR 604, a detailed description of the transformation method, data and information on the gene insertion sites, copy number and levels of expression in the plant, the role of the inserted genes and regulatory sequences in donor organisms and full nucleotide sequences. The novel proteins were identified, characterized and compared to the original bacterial proteins, including an evaluation of their potential toxicity to livestock and non-target organisms. Relevant scientific publications were supplied.

Extensive safety evaluation of Corn MIR 604 in terms of genetic stability, agronomic characteristics, food compositional analysis, and potential toxicity and allergenicity was undertaken by the concerned agencies [Bureau of Animal Industry (BAI), Bureau of Agriculture, Fisheries and Product Standards (BAFPS)] and a Scientific Technical Review Panel (STRP) following the Department of Agriculture's AO8 guidelines for the release of genetically modified organisms.

The Public Information Sheet (PIS) of the said application was published in two widely circulated newspapers: Manila Standard on June 29, 2007 and Malaya on June 29, 2007 for public comment/review. BPI received no comment on the petition during the 30-day comment period

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 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 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.

Transformation of mCry3A expressing maize event MIR604 was conducted using immature maize embryos derived from a proprietary *Zea mays* line via *Agrobacterium*-mediated transformation. The Event MIR604 derived hybrids have a single intact insertion of the transgene from the pZM26 vector. Southern hybridization data provide confirmatory evidence to support the Taqman PCR analysis that Event MIR604 contains a single copy of m*cry3A* gene and *pmi* gene.

Event MIR604 contains a single copy of the MTL and ZmUbint promoters, without any vector backbone sequences present in pZM26. Sequence analysis revealed that some truncation occurred at the RB (by 44 bp) and LB (by 43 bp) ends of the T-DNA insert during the transformation process. Truncations and deletions occurred but have no effect on the efficacy of the T-DNA insert. Three Base pair changes were noted: One occurred in the regulatory region and does not encode for a protein. Two other bas pair changes occurred within the coding region but these amino acid changes did not result in any apparent functional change in the new insert.

pZM26 vector backbone sequences were not present in event MIR 604 as demonstrated by Southern hybridization. Lack of hybridization with full length backbone probe proves the absence of any vector backbone sequence.

Expression of mCry3A protein was shown to be stable across four generations. The mCry3A protein was detected in all plant tissues, except the pollen which is below limit of detection. PMI protein levels in the leaves, roots, kernels, silk, pollen, whole plant, and silage ranged from not detectable to low levels. The levels were similar across 4 generations and appeared stably expressed. Expression of the PMI gene in transformed plants does not appear to adversely affect plant morphology, growth or agronomic characteristics

Safety of the Expressed Proteins

MCry3A expressed in transgenic plants were readily digested as conventional dietary protein under typical mammalian gastric conditions; No intact mCry3A or immunoreactive fragments were detected as assessed by SDS-PAGE and western blot analysis. MCry3A protein was completely inactivated at 95°C within 30 minutes incubation, reduction of bioactivity at 65°C and no effect at temperature up to 37°C. These results indicate that mCry3A protein is unaffected (active) in uncooked grains from corn event MIR604. Amino acid comparison was done with known toxins, and No homology was shown to any proteins identified as or known to be toxins.

Acute mouse toxicity studies showed that mCry3A-0102 sample was not acutely toxic to male and female mice given mCry3A protein at dosages at Ca2377 mg/ kg b.w. This is another proof that mCry3A protein will not cause detrimental effects on human and animal health as no evidence of treatment related effects was recorded considering mortality, clinical signs, body weight, and feed consumption. Similarly, no treatment related adverse effects were observed following macroscopic and microscopic examination of organs and tissues of experimental mice.

PMI in test substance MIR604-PMI-0105 was shown to be rapidly digested under typical mammalian gastric conditions. NO intact PMI or apparent degradation products were detected following digestion as assessed by SDS-PAGE and western blot. The PMI protein was found stable for at least 30 minutes at temperature up to 37°C but unstable at 55°C and above as measured by both enzymatic activity and immuno-reactivity. There was complete loss of immuno-reactivity at 65°C to 95°C at 30 minutes incubation, indicating that PMI in corn event MIR604 is stable in uncooked corn grain but completely destroyed in cooked corn grain. Amino acid comparison with known toxins showed no homology.

Results of evaluation of PMI for acute toxicity test in mice administered at a level of 5050 mg/kg. b.w. showed no mortality due to the test substance, no clinical signs, no adverse effects on body weight gain, no observed abnormalities indicating that PMI protein in corn event MIR604 is not a risk concern.

Feeding trials on 49-day-old broiler chickens showed no untoward or deleterious effects on the growth performance, feed conversion efficiency and overall carcass yield. Although mCry3A and PMI proteins in corn event MIR604 are readily degraded in SGF, not detrimental or toxic to mice even at high dosages and completely destroyed or inactivated at 95°C within 30 minutes incubation.

No intact mCry3A protein was detected following disposition in SGF for 2 minutes incubation as assessed by SDS-PAGE followed by Western Blot analysis, supporting conclusion that mCry3A expressed in transgenic plant will be readily destroyed or deactivated as conventional dietary protein under typical mammalian gastric condition, hence its presence in food and feed will not pose any adverse effects on both human and animal health.

In vitro digestibility studies on PMI showed that PMI protein was readily degraded in SGF in 1 minute exposure as assessed by SDS-PAGE and Western Blot, indicating that the presence of PMI in corn event MIR604 is not a risk concern Results of studies showed that PMI is stable for at least 30 minutes at temperature up to 37°C but unstable at 55°C and above, as measured by ELISA and enzymatic activity (bioassay). No immuno-activity was observed by ELISA at temperature ranging from 65°C to 95°C incubated for 30 minutes, indicating that PMI in corn event MIR604 will be degraded completely in cooked food but not in uncooked food or feed.

IV. Nutritional Composition (Compositional Analysis)

Proximate analysis of protein fiber, fat, ash, and carbohydrates of grains from corn event MIR604 and conventional corn grain are nutritionally, compositionally and substantially equivalent. All values obtained are within ranges reported in literature.

Fatty acid levels for transgenic and non-transgenic corn counterpart are within reported literature range, although statistically significant differences are obtained in certain hybrid grains.

Mineral composition of grains derived from corn event MIR604 are within reported literature range. Although significant statistical differences are noted in calcium and zinc but inconsistent across years (2002 and 2003 grain samples), indicating no risk concern.

Vitamin composition of grains obtained from transgenic and conventional corn was within literature range. Although scattered significant differences were obtained, the values obtained are within literature range. Vitamin composition of corn grains from corn event MIR604 is not a risk concern for both man and animals.

Amino acid composition of both transgenic and non-transgenic counterpart is within ranges reported in literature, hence not a risk concern.

V. Anti-Nutritional Factors

Some anti-nutrients are found in maize (phenolic acids, furfural, phytic acid, protease inhibitors, alpha-galactosidases of sucrose, phytosterols) but they have been found to be present in low levels and considered a minor health concern for both man and animals.

VI. Regulatory Decision

After reviewing the scientific data and information relevant to the application of Syngenta Philippines, Inc., it is concluded that Corn MIR604 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 and for processing is as safe and substantially equivalent to its unmodified counterpart, and is therefore approved for direct use as food, feed and for processing. Syngenta shall duly inform the public of this approval by way of publishing in any one (1) of the top three (3) leading newspapers in the country that imports of this product is covered by conditions for approval as provided in Department of Agriculture Memorandum Circular No. 8, Series of 2003.