

Determination of the Safety of Syngenta's  
Corn 3272 (Corn for optimised bioethanol production)  
for Direct use as Food, Feed, and for Processing

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**Food and Feed:**

The product dossiers on Corn 3272 were reviewed for safety and nutritional differences compared with the conventional corn. The focus of the review was on any new or altered expression of traits and changes in composition and nutritional content or value relative to the conventional corn. At the end of the safety assessment, the following conclusions were made: Corn 3272 is as safe as the conventional corn taking into account dietary impact of any changes in nutritional content or value.

Biosafety permit for Corn 3272 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 was issued to Syngenta Philippines Inc. on February 7, 2008. The permit for direct use is valid for five years and shall expire on February 6, 2013, 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 Corn 3272 was included in the Lists of Approval Registry (Delisting) prepared by the Department of Agriculture- Bureau of Plant Industry.

*This approval is for use as food, feed and for processing of Corn 3272 expressing an Alpha-amylase protein in the Philippines. Food and feed use of Corn 3272 and its by-products is therefore authorized as of February 7, 2008. The biosafety permit (No. 08-028) for direct use stated that "Corn expressing an AMY797E Alpha Amylase Protein 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)**

<b>Designation:</b>	AMY797E expressing corn Event 3272
<b>Applicant:</b>	<b>SYNGENTA PHILIPPINES, INC.</b> Building 1-B, Sunblest Compound, Km.23 West Service Road, Cupang Muntinlupa City
<b>Plant Species:</b>	
Name:	Corn ( <i>Zea mays</i> )
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), is a potential toxicant but it declines rapidly as the plant grows
<b>Trait Description:</b>	Corn expressing $\alpha$ – amylase
<b>Trait Introduction Method:</b>	<i>Agrobacterium</i> -mediated transformation
<b>Donor Organisms:</b>	<i>Thermococcales</i> , source of <i>amy797E</i> gene responsible for the increased thermostability and activity during the high temperatures required for starch hydrolysis in corn processing.  <i>Escherichia coli</i> , source of phosphomannose isomerase ( <i>pmi</i> ) gene as selectable marker
Pathogenicity:	AMY797E has no homology to known toxins and allergens and is rapidly degraded.

PMI has no homology to known toxins and allergens and is rapidly degraded.

**Proposed Use:** For direct use as food, feed and for processing and for propagation.

## **II. Background Information**

On September 10, 2007, Syngenta Philippines, Inc. submitted applications to the Bureau of Plant Industry, requesting for biosafety permit under Administrative Order (AO) No. 8 for Corn 3272 for direct use as food, feed, or for processing. Corn 3272 has been transformed with a synthetic *amy797E* gene whose expression produces the thermostable AMY797E alpha amylase protein.

Syngenta Philippines Inc has provided data on the identity of Corn 3272, 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.

Corn 3272 has been evaluated according to BPI's safety assessment by concerned agencies [Bureau of Animal Industry (BAI), Bureau of Agriculture, Fisheries, and Product Standards (BAFPS)] and a Scientific Technical Review Panel (STRP). The process involves an intensive analysis of the nature of the genetic modification together with a consideration of general safety issues, toxicological issues and nutritional issues associated with the modified corn

The petitioner/applicant published the application for direct use on two widely circulated newspapers: Manila Standard and Manila Times on October 25, 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 plants derived from Corn 3272 express a synthetic thermostable alpha amylase protein, AMY797E containing the *amy797E* gene. Alpha amylases are enzymes that catalyze the hydrolysis of starch into soluble sugars. The *amy797E* gene is a chimeric gene derived from sequences of three alpha-amylase genes originating from three hyperthermophilic microorganisms of the archaeal order *Thermococcales*.

The plants also express the selectable marker phosphomannose isomerase (PMI) containing the *pmi* gene. This gene represents the *manA* gene from *Escherichia coli*. Corn expressing *pmi* can utilize mannose as a primary carbon source.

### **Safety of the Expressed Proteins**

AMY797E is a thermostable protein. While heat stability of a protein generally represents an allergenic potential, not all heat stable proteins are allergens. No single protein characteristic is fully predictive of allergenicity. In the case of *amy797E*, the potential allergenicity of the protein was evaluated using an extensive weight of evidence approach. A decision tree for allergenicity assessment was followed. Based on these, it was concluded that AMY797E alpha amylase is very unlikely to represent a potential allergen in food.

Additionally, based on intended use (dry grind for ethanol production), there is low potential for dietary exposure to this protein via human consumption. Analysis of the AMY7979E alpha amylase protein as expressed in Event 3272corn revealed no evidence of post-translational glycosylation. SDS-PAGE analysis showed the presence of bands corresponding to the intact AMY7979E protein and derived polypeptides. These were confirmed by Western blot detecting the presence of intact immunoreactive protein and polypeptide fragments. There is low potential for dietary exposure to AMY7979E protein via human consumption of this corn because it is intended for dry grind ethanol processing, not for eating.

Using the latest version of Syngenta Biotechnology, Inc. Allergen Database version 4.0, the *amy797E* protein has no significant homology with known or putative allergenic proteins.

AMY797E has no effects on clinical conditions, body weights, macroscopic or microscopic pathology food consumption of the animals. Acute oral mouse toxicity study of alpha-amylase showed no toxicity to mice. A comparison of sequential 80 amino acid peptides of AMY797E alpha amylase protein sequence to known allergen sequences showed no significant similarity of AMY797E with any entries in the SBI Allergen Database.

The AMY797E alpha amylase protein sequence was also screened for matches of eight or more contiguous amino acids between AMY797E and allergen sequences to screen for short, local regions of amino acid identity that may indicate presence of common IgE-binding epitopes. This comparison showed one region of sequence homology of eight contiguous identical amino acids between AMY797E alpha amylase and a known species-specific allergen Per a 3 from American cockroach. The IgE-binding epitopes of Per a 3 however, showed no overlap between these binding epitopes and the region of sequence homology with alpha-amylase. Therefore, the observed sequence identity between AMY797E alpha-amylase and Per a 3 is not biologically relevant.

*In-vitro* digestibility study of AMY797E in SGF containing pepsin showed that AMY797E was rapidly degraded within 5 minutes and can be expected to be digested under typical mammalian gastric conditions as conventional dietary protein.

PMI has no significant sequence homology to any proteins known to be toxins. Acute oral mouse toxicity study of PMI protein showed no toxicity to mice. There was no significant similarity between any of the sequential 80-amino acid peptides and entries in the SBI allergen database. There was one region of homology of eight contiguous identical amino acids between PMI and a known allergen, alpha parvalbumin from *Rana* species edible frog. However, sensitive serum screening methodology demonstrated no cross-reactivity between PMI and the serum from a single individual known to have IgE-mediated allergy to this specific alpha-parvalbumin. Thus, the sequence identity between PMI protein and alpha parvalbumin from *Rana* species CH2001 is not biologically relevant.

PMI protein was rapidly degraded in simulated gastric fluids and can be expected to be digested under typical mammalian gastric conditions as conventional dietary protein. PMI is degraded within 2 minutes in simulated intestinal fluid. PMI is stable to heat treatment for at least 30 minutes up to 25 C, but becomes unstable at 37 C and above. Very little enzymatic activity remained at 65 C, with complete loss of activity at 95 C, even for immunoreactivity.

Event 3272 maize is targeted for dry grind fuel ethanol production industry in the USA. Co-products after removal of ethanol by distillation from yeast fermentation include distillers grains and solubles which are commonly used in animal feed, but not in food. Therefore, based on intended use, there is very low potential for dietary exposure to AMY797E protein via human consumption of Event 3272 maize-derived corn or corn by-products.

#### **IV. Nutritional Composition (Compositional Analysis)**

Compositional analyses that established substantial equivalence of Event 3272 with non-transgenic counterparts suggest that expression of AMY797E and *pmi* genes do not alter the basic maize metabolism. Expression of amy797E and PMI do not adversely affect plant morphology, growth nor agronomic characteristics: no changes in glycoprotein profiles, nor differences levels of sucrose, glucose or fructose were observed.

Proximate composition of hybrid Event 3272-derived grain and the corresponding non-transgenic controls showed sporadic significant differences but are not consistently associated with the presence of the transgene. Average values obtained from proximate analyses in grain were within ranges reported in literature. .

Levels of the free fatty acids (linoleic, oleic, palmitic, stearic and linolenic acids) in Event 3272-derived grains and the non-transgenic control grain showed no statistically differences.

Nine minerals were compared namely: Calcium, Copper, Iron, Magnesium, Manganese, Phosphorous, Potassium, Selenium and Zinc. No statistically significant differences were observed in levels of any mineral analyzed in forage or grain, except manganese in the grain. Although there is statistical difference in the results for manganese, the average level in forage and grain is within the ranges reported in the literature and is therefore not biologically relevant.

Vitamin analyses indicate that there are no consistent differences observed for maize plant derived from event 3272. Likewise, all values were within the literature ranges.

Amino acid levels were noted to be higher in transgenic hybrids than in non transgenic controls. However, the magnitude of the difference was small, and all levels of amino acids were within

published ranges. In as much as the levels reported are within ranges found in literature, the statistical difference is not biologically relevant.

**V. Antinutritional Factors**

There is no statistical difference between the levels of trypsin inhibitor and p-coumaric acid in the transgenic and non-transgenic samples.

**VI Regulatory Decision**

After reviewing the scientific data and information relevant to the application of Syngenta Philippines, Inc., it is concluded that Corn 3272 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 or feed or for processing is as safe and substantially equivalent to its unmodified counterpart, and is therefore approved for direct use as food, or feed or for processing. Syngenta shall duly inform the public of the approval for direct use 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 as amended by as amended by DA Administrative Order No. 22, Series of 2007.