**RISK ASSESSMENT REPORT OF THE BIOSAFETY COMMITTEE THE FOOD SAFETY TECHNICAL TEAM (FSTT ) FOR GM MAIZE EVENT 3272**

**I. Introduction**

3272 maize is the product of Syngenta claimed to provide benefit for the source of amylase enzyme in the dry-grind ethanol process, replacing the addition of microbially produced enzyme. 3272 maize produces AMY797E alpha-amylase enzyme and PMI (*phosphomannose isomerase*). 3272 maize has been used as a food and or feed in 6 countries namely United States (2006), Australia (2008), Philippines (2008), Canada (2008), Mexico (2008), Russian (2010).

Food Safety Technical Team has conducted food safety studies for 3272 maize based on genetic information and food safety information consisting of substantial equivalence, allergenicity, and toxicity, according to Regulation of Indonesia’s National Agency for Drug and Food Control (NADFC/BPOM) Number HK.00.05.23.3541 of 2008 concerning Guidelines of Food Safety Study for Genetically Modified Products as specified below.

**II. Genetic Information**

**II.1 Genetic Element**

3272 maize contains two novel genes namely *amy797E* gene and *pmi* gene. *AMY797E* gene produces AMY797E protein a thermostable alpha-amylase protein, which retains its activity during the high temperatures required for starch hydrolysis in dry-grind ethanol production; and *pmi* gene encodes (*phosphomannose isomerase*) protein as selectable marker. Promotor and terminator used for *amy797E* genes are GZein from the *Zea mays* and 35S from *cauliflower mosaic virus.* Promotor and terminator used for PMI gene are ZmUbilInt from the *Zea mays* and NOS (nopaline synthase) from *Agrobacteria tumefaciens*.

**II.2 Gene Source**

*amy797E* gene is isolated from hyperthermophilic microorganisms of the archael order *Thermococcales.* and *pmi* is isolated from *Escherichia coli* [Negrotto, D., Jolley, M., Beer, S., Wenck, A. R., Hansen, G., 2000, “*The Use of Phosphomannose-Isomerase as a Selectable Marker to Recover Transgenic Maize Plants (Zea mays L.) via Agrobacterium Transformation”*, Plant Cell Rep. 19: 798-803].

**II.3 Transformation Method**

Transformation of Syngenta’s AMY797E-expressing maize Event 3272 was conducted using immature maize embryos derived from a proprietary *Zea mays* line, via *Agrobacterium*-mediated transformation. By this method, genetic elements within the left and right border regions of the transformation vector plasmid pNOV7013 are efficiently transferred and integrated into the genome of the plant cell, while genetic elements outside these border regions are generally not transferred. Regenerated plantlets were tested for the presence of both the *pmi* and *amy797E* genes, as well as for the absence of the spectinomycin antibiotic resistance gene (*aadA*), by TaqMan® PCR analysis. Plants positive for both the *pmi* and *amy797E* genes, and negative for *aadA*, were transferred to the greenhouse for further propagation.

**II.4 Genetic Stability**

Molecular analysis using Southern blot is conducted to the see the stability of inserted gene from Generation to generation representing the backcross one (BC1), backcross two (BC2), backcross four (BC4) and backcross five (BC5) generations of Event 3272. The result shows that the TDNA inserted gene of pNOV7013 is stable up to the generation of back-cross 5 (BC5). Protein level of AMY797E and PMI are also stable over four successive backcross generations. The gene inserted in the 3272 maize, *amy797E* and *pmi*, are segregated according to the Mendelian Inheritance Law.

The Southern blot hybridization data provided confirmation that 3272 maize contained single intact insertion of the *amy797E* gene and *pmi* gene. A map of the 3272 maize transformation plasmid pNOV7013 indicating the location of the pNOV7013 backbone-specific probe. No hybridization bands were detected for the genomic samples, demonstrating that 3272 maize does not contain any backbone sequences from the transformation plasmid pNOV7013. Genetic stability of 3272 maize data was provided by Syngenta study report: Kim H. 2005. Stability of Amy797e α-Amylase and Phosphomannose Isomerase (PMI) Expression Over Multiple Generation in Maize (Corn) Derived from Event 3272” (Protocol No. AMY-04-02).

**II.5.** Based on genetic information data it can be concludes that:

1. 3272 maize contains 1 copy of vector fragment which carried two *amy797E* genes and *pmi* genes;
2. Promotor and terminator used for *amy797E* genes are GZein from the *Zea mays* and 35S from *cauliflower mosaic virus.* Promotor and terminator used for *pmi* gene are ZmUbilInt from the *Zea mays* and NOS (nopaline synthase) from *Agrobacterium tumefaciens*.
3. 3272 maize does not contain any backbone sequences from the transformation plasmid pNOV7013;
4. Two novel genes, *amy*797E and *pmi*, introduced to 3272 maize remain stable up to the generation of back-cross four;
5. Two novel genes, *amy797E* and *pmi*, introduced to 3272 maize are inherited following Mendel Law.

**III. Food Safety Information**

**III.1 Substantial Equivalence**

The result of substantial equivalence assessment for 3272 maize is obtained after considering three documents reported (1) Food safety assessment application event 3272, (2) Event 3272 Maize: Application for Direct Use as Food, Feed and for Processing (Syngenta Philippines, Inc., 2008), dan (3) Compositional Analysis of Grain and Forage from Transgenic Maize Event 3272 with an Introduced Alpha-Amylase (AMY797E) Enzyme (Philip Brune, 2010), Syngenta Seeds Biotechnology Report No. SSB-101-05 A1. This third document replaces the company study report issued earlier on August 1, 2005.

Composition of kernels and all parts of corn plants (forage) of 3272 maize and nontransgenic were obtained from corn grown during 2003 in 7 different locations and during 2004 in 6 different location in the USA. Location for 2003 planting were Bondville IL, Bloomington IL, Shirley IL, Stanton MN, Fairbault MN, and Glidden IA while location for 2004 planting were at Brookings SD, Stanton MN, Janesville WI, Glidden IA, Washington IA, Bondville IL, dan Bloomington IL. In 2004, across locations except Bloomington IL, grains and all parts of corn plants (forage) were harvested for the purpose of substantial equivalence study. Whereas in Bloomington IL, only all parts of the corn plant (forage) were harvested for animal feed purposes in the 2004

All samples in maize grain and forage were analyzed by Covance Laboratories Inc., Madison, WI. This study was conducted in compliance with the relevant provisions of Good Laboratory Practice Standards. The following is a composition analysis carried out for grain: proximate levels (water, protein, carbohydrate, fat and ash), starch, acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber (TDF), minerals (Ca, Cu, Fe, Mg, Mn, P, K, Na, Zn, and Se), amino acid composition, fatty acid composition, cryptoxanthine, vitamins (beta-carotene, folic acid, B1, B2, niacin, B6, C, and E), inositol, secondary and anti-nutrient metabolites including ferulic acid and p-coumaric acid , furfural, phytic acid, raffinose, and trypsin inhibitors. The composition of the forage was analyzed as follows: proximate levels (water, protein, carbohydrates, fats and ash), starch, acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber (TDF), and minerals ( Ca, Cu, Fe, Mg, Mn, P, K, Na, and Zn).

Statistical data of the composition analysis of event 3272 maize grain and forage and its nontransgenic were reported in “Application for Direct Use as Food, Feed and Processing (Syngenta Philippines, Inc., 2008), contained in Appendix 5, Table 1 (proximate, ADF and NDF of corn plants), Table 2 (mineral), Table 3 (proximate, ADF, NDF, TDF, and starch), Table 4 (mineral), Table 5 (vitamins), Table 6 (fatty acids), Table 7 (amino acid), and Table 8 (anti-nutrient and secondary metabolites of corn seeds). All results of this statistical analysis are compared with the database of food composition from ILSI (2006, International Life Sciences Institute Crop Composition Database Version 3.0. Http://www.cropcomposition.org) and OECD [2002, Consensus on Compositions Consideration for New Varieties of Maize (Zea mays): Key Food and Feed Nutrients, Anti-Nutrients and Secondary Plant Metabolites. Publication No. 6, 2002. ENV / JM / MONO (2002)].

The proximate and fiber analysis (ADF, NDF, and TDF) of grain on several components showed significant differences in some of the hybrid pairs, but still within the range of compositions reported by ILSI (2006) and OECD (2002). Likewise for the other components. The results of the analysis of anti-nutrient components and secondary metabolites generally fell within the range of ILSI (2006) and OECD (2002) data. The results of the analysis of the composition of forage fell into the range of data reported by ILSI (2006).

The result of substantial equivalence study above concludes that 3272 maize is substantially equivalent with nontransgenic maize.

**III.2 Allergenicity**

Maize event 3272 express a thermostable alpha-amylase enzyme (AMY797E) in grain for use in the dry-grind fuel ethanol process.

**Amino Acid Sequence Homology with Known Allergen**

The source of AMY797E enzyme are not known to be allergens. The donor organism from the AMY797E enzyme originates from the hypertermophilic microorganism *Thermococcales spp*. which is not known as an allergen source. (Richardson, TH, Tan, X., Frey, G., Callen, W., Cabell, M., Lam, D., Macomber, J., Short, JM, Robertson, DE and Miller, C., 2002, A Novel, High Performance Enzyme for Starch Freedom, Journal of Biological Chemistry Vol. 277, No. 29: 26501-26507).

The *amy797E* gene includes the fusion of the 797GL3 amylase with a 19 amino acid N-terminal maize gamma-zein signal sequence (GZein ss) and a C-terminal SEKDEL endoplasmic reticulum retention signal (ER rs) (Lanahan, M.B., Basu, S.S., Batie, C.J., Chen, W., Joyce, C. and Kinkema, M. 2003. Plant and Plant Parts US Patent Application Publication Number US2003 / 0135885 A1).

The maize gamma-zein signal sequence and the ER retention signal provide for protein targeting to and retention in the endoplasmic reticulum of the cell, respectively. The N-terminal maize gamma-zein signal sequence is cleaved from the precursor protein to yield the mature alpha-amylase protein. The alpha-amylase coding region of the *amy797E* gene was synthesized to accommodate the preferred codon usage for corn. The alpha-amylase enzyme catalyses the hydrolysis of starch by cleaving the internal α-1,4-glucosidic bonds into dextrins, maltose and glucose.

The results of the analysis carried out at Syngenta Biotechnology, Inc., USA, showed that the AMY797E enzyme had 93% amino acid identification of the alpha amylase enzyme from BD5088 isolates which had been stated as generally recognized as safe (GRAS) based on the US FDA category.

**III.2.2 AMY797E Enzyme Protein Concentration and PMI Enzyme**

AMY797E and PMI protein extraction were carried out from whole plants and pollen. Protein quantification was analyzed by ELISA using polyclonal antibodies from goats. (De Fontes J., and Kramer, C., 2005, A Quantification of AMY797E and PMI Proteins in Transgenic Maize (Corn) Tissues and Whole Plants Derived from Event 3272, Syngenta Seeds Biotechnology Report # SSB-028-04 A1. Unpublished).

The highest concentration of AMY797E enzyme is found in grain. This gene expression was stable for 4 generations in the range 1147-1369 mcg/g wet weight. The PMI enzyme was detected in various tissues and growth phases with a range of 8 - 8.5 mcg/g wet weight and highest in pollen, while the average concentration in seeds was 0.4 mcg/g wet weight. The expression of PMI enzymes is stable for 4 generations at 6.6 - 8.6 mcg / g wet weight. Enzyme analysis methods refer to Freeze, H.H., 2002, Phosphomannose Isomerase. In .: Handbook of Glycosyltransferase and Related Genes. Edition 1. Taniguchi, N., Honke, K. and Fukuda, M., **III.2.3 Bioinformatics Study**

The amino acid sequences of the AMY797E enzyme and PMI enzyme were compared to the Allergen Database (version 4.0) Syngenta Biotechnology Inc. (SBI), the SBI Allergen Database contains information on amino acid sequences known as protein allergens, including gliadin (SBI Allergen version 4.0, 2005). The whole homology was also tested by comparing 80 amino acid peptide sequences from the AMY797E enzyme sequence and PMI enzyme. In addition, screening of AMY797E enzyme sequences and PMI enzymes was carried out to match the presence of eight or more adjacent amino acids as regions that showed the location or identity of the common epitope binding IgE. The results of the analysis showed that there was no significant similarity between one 80 amino acid peptide sequence from AMY797E enzyme and PMI enzyme with the data in the SBI allergen database. There is one sequential homology area of ​​eight identical amino acids (contiguous amino acids) between AMY797E and Per-3 protein (specific allergens) from American cockroaches. The binding epitope of IgE Per a 3 was successfully identified and there was no overlap between the IgE binding epitope and the homology sequence region of the AMY797E enzyme. Thus, the sequence between the AMY797E and Per a 3 enzymes is biologically irrelevant, so the AMY797E enzyme does not have the potential to cause allergies. (Wu, C.H., Lee, M.L. and Tseng, Y., 2003, IgE-Binding Epitopes of the American Cockroach by 3 Allergen, Allergy 58: 986-992).

PMI sequence also showed no homology result among overall proteins with any known allergenic protein. There was one sequence identity match of eight contiguous identical amino acids between PMI and a known allergen, α-parvalbumin (described as *Rana* species CH2001-unidentified edible frog). Hilger *et al*. (2002) proceeded to identify the causative agent of this anaphylactic response as α-parvalbumin using PMI produced from an *Escherichia coli* overexpression system. The allergic patient’s serum IgE indicate no cross-reactivity to related parvalbumins from *Rana esculenta* and it does not recognize any portion of PMI as an allergenic epitope. This study supports the conclusion that PMI shows no biologically relevant amino acid sequence similarity to any known or putative protein allergens (Hilger, C., Grigioni, F., Thill, L., Mertens, L. and Hentges, F. (2002), *Severe IgE Mediated Anaphylaxis Following Consumption of Fried Frog Legs : Definition of* *-Parvalbumin as the Allergen in Cause*, Allergy 57:1053-1058).

**III.2.4 *In vitro* Digestibility of AMY797E enzyme dan PMI enzyme**

The AMY797E enzyme sensitivity to proteolytic degradation was evaluated by simulated mammalian gastric fluid (SGF) containing pepsin. In SGF, the AMY797E enzyme degraded within 5 minutes so that it is estimated to be readily digested in the digestive conditions of mammals. (De Fontes, J. and Kramer, C. 2005, In vitro Digestibility of AMY797E-Amylase (Test Substance AMY797E-0104) Under Simulated Mammalian Gastric Conditions, Syngenta Seeds Biotechnology Report # SSB-034-04 A1. Unpublished). After incubation in SGF, the PMI enzyme decomposes immediately within 2 minutes. So that it is estimated to be immediately digested in the digestive conditions of mammals.

**III.2.5 Protein Heat Stability of AMY797E Enzyme and PMI Enzyme**

AMY797E Enzyme was chosen for corn event 3272 as it is thermostable and still active at high temperatures. This is needed to hydrolyze starch in processing corn, especially for ethanol production. Although the AMY797E enzyme is a protein that is stable high, it is not the only indication that a protein has a risk of causing allergies. Allergic proteins (allergens) in food are usually stable in high temperature, however not all proteins that are stable in high temperature are allergens. There are several characteristics that confirm that the AMY797E enzyme is not allergenic: the source of protein (*Thermococcales spp*.) is not an organism known to cause allergies; Amino acid sequences had homology with allergenic protein; small concentration in GM corn; and this protein is rapidly degraded in digestive enzymes. In addition, the AMY797E enzyme will not be consumed directly because it is only used as a catalyst for hydrolysis of starch (which will then be processed into ethanol), and the by-products will be given to animals. The AMY797E enzyme is known to have 17% similarity in terms of amino acid sequences and has no resemblance in terms of eight amino acid peptides (IgE binding epitope) with alpha amylase *Aspergillus oryzae* used in the bakery industry which is reported to cause some allergic reactions in certain people. Another additional information is that this protein has no evidence of post-translational glycosylation. [Kramer, C. 2005. Characterization of Lyophilised Amlyase Test Substance (AMY797E-0104) and Certificate of Analysis. Syngenta Seeds Biotechnology Report # SSB-027-04. Unpublished] The PMI enzyme is stable for at least 30 minutes at 25oC, but becomes unstable at temperatures> 37oC. At a temperature of 65ºC, very small enzymatic activity was found and at 95ºC, all enzymatic activity was lost. (Stacy, C., Li, X., and G. Graser. 2006. "Effect of Temperature on Stability of Phosphomannose Isomerase (PMI) from PMI-0198 Substance Test", Syngenta Seeds Biotechnology Report # SSB-154-06. Unpublished).

The PMI enzyme does not contain the sequence of amino acids needed for the glycosylation, the expression is not directed to the glycosylation pathway. The PMI enzyme produced by E. coli cannot be glycosylated because bacteria are not able to carry out this reaction. (Taylor, SL. And Hefle, SL. (2001) Will Genetically Modified Foods be Allergenic? Journal of Allergenicity and Clinical Immunology 107(5):765-771. FAO/WHO (2001). Evaluation of Allergenicity of Genetically Modified Foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology. 22-25 January 2001. Rome, Italy).

Based on the results of the allergenicity study it can be concluded that:

1. The AMY797E enzyme is not from a known source of protein allergens. The donor organism of the AMY797E enzyme is *Thermococcales spp*. what is not known as a source of allergens.
2. The PMI gene comes from *E. coli* which is not as a source of allergens. These bacteria have no history of allergenicity. PMI enzymes are not consumed directly by humans.
3. AMY797E enzyme and PMI enzyme revealed no glycosylation.
4. The AMY797E enzyme and PMI enzyme do not have a sequence of amino acid homologies sequence with known protein allergens.
5. The PMI enzyme is very unstable towards heating and becomes inactive at 95ºC.
6. The AMY797E enzyme and PMI enzyme are sensitive to gastric fluid and will rapidly degraded in simulated gastric fluid containing pepsin. Thus if ingested, the AMY797E enzyme and PMI enzyme will immediately degraded in the digestion of mammals as well as other proteins.

**III.3 Toxicity**

**III.3.1 Amino Acid Sequence Homology with Known Toxins**

1. AMY797E enzyme

AMY797E enzyme Amino acid homology sequence analysis was conducted to AMY797E enzyme and its result was reported as a company study report: “*AMY797E: Assessment of Amino Acid Sequence Homology with Known*” by Brian Harper. Study completion date: February 18th 2009. The research was conducted at Syngenta Biotechnology, Inc., Regulatory Science, USA. This study was conducted in Syngenta laboratory in compliance with the relevant provisions of Good Laboratory Practice (GLP)

The BLASTP program (Altschul et. Al, 1997) was used to find NCBI Entrez Protein databases with AMY797E precursors as query sequences. The AMY797E enzyme is called a synthetic alpha-amylase protein. The search tools results using NCBI Entrez Protein, identified 2057 amino acid sequences similar to the AMY797E enzyme, and a number of 2053 sequences were proteins related to carbohydrate metabolism of 585 species or the results of synthetic construction. This shows that none of the proteins identified as protein toxins, so it can be concluded that the amino acid sequence of the AMY797E enzyme has no homology sequence with protein toxin. (Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs. Nucleic Acids Res 25: 3389–3402).

1. PMI Enzyme

PMI amino acid homology sequence analysis was conducted to PMI enzyme and its result was reported as a company study report (Report No: SSB-125-09): “*Phosphomanose Isomerase (Entrez Database Accession No. AAA24109): Assessment of Amino Acid Sequence Homology with Known Toxins*” by Brian Harper. Study completion date: February 18th 2009. The research was conducted at Syngenta Biotechnology, Inc., Regulatory Science, USA. This study was conducted in Syngenta laboratory in compliance with the relevant provisions of Good Laboratory Practice (GLP).

The BLASTP program is used to search NCBI Entrez Protein databases with PMI as a query sequence. The search tools results using NCBI Entrez Protein, identified 580 amino acid sequences similar to the PMI enzyme, but none of them were known as protein toxins, so it can be concluded that the amino acid sequences of PMI enzymes are not homologous with protein toxins.

**III.3.2 Acute toxicity in mice**

1. Enzim AMY797E

Acute toxicity of AMY797E enzyme was conducted to mice and its result was reported as a company study report (Report No: CTL Study number: AM7506; Document number: CTL/AM7506/REG/REPT REV 001): “*CT/AM7506/Regulatory/Report Revision 001. AMY797E-0104: Single Dose Oral Toxicity Study in the Mouse*” by E. Barnes. Study completion date: August 2nd 2009. The research was conducted in Central Toxicology Laboratory, UK in compliance with the relevant provisions of Good Laboratory Practice (GLP).

Experiment was using Alpk strain mice: APfCD-1, 8-12 weeks old, were divided into two groups, with the number of mice per group each 5 male mice and 5 female mice (a total of 20 mice). Mice were placed in cages, each of which contained 5 mice, and the placement of male mice and female mice was separated. The test substances material in the form of white AMY797E-0104 flour containing 42% AMY797E enzyme, 0.5% CMC solution was used as the protein solvent tested. Diet CTI powdered (Special Diet Services Ltd., Witham, Essex, UK) and mains water provided came from tap water. Tests were carried out for 15 days against two groups of mice, namely the control group and the treatment group. The AMY797E enzyme was not administered to Mice in the control group. The treatment group was administered by AMY797E-0104 flour as much as 3600 mg / kg body weight which is equivalent to the AMY797E enzyme dose 1511 mg / kg body weight. Mice were administered orally by gavage with a control solution and a treatment solution of 2.0 ml/100 g each, on the first day of the experiment and only one time dose. Diet and mains water, supplied by an automatic system were available *ad libitum.*

The experimental results showed that there were no clinical signs caused by the administration of the AMY797E enzyme; there was no difference between the control group and the treatment group in terms of mice weight, food consumption, hematological parameters, clinical blood chemistry, and organ weight. In microscopic observations, a small number of lesions (wounds) were present in all groups, and this was considered spontaneous, so it was not related to treatment. This study concluded that the AMY797E enzyme had no toxic effect on mice in the administration of a single dose of 1511 mg / kg body weight.

1. PMI Enzyme

Acute toxicity of PMI enzyme was conducted to albino mice and its result was reported as a company study report (Report No:4708-98): “*Phosphomannose Isomerase (Sample PMI-0198) Acute Oral Toxicity in Mice*” by Janice O. Kuhm. Study completion date: August 11th 1999. The research was conducted in Stillmeadow, Inc., 12852 Park One Drive, Sugar Land, TX 77478. in compliance with the relevant provisions of Good Laboratory Practice (GLP).

Tests using mice, HSD: ICR, 1 month old, with 13 male mice and 11 female mice (nulliparous and non-pregnant). Mice were placed in cages made of polycarbonate which was covered with woven wire, each cage contains 1 (one) mouse, and the placement of male mice and female mice was separated. The test material in the form of white PMI enzyme flour, the material used as a control was 0.5% CMC solution. The diets obtained from Purina Mills Inc., Formulab # 5008 and mains water, supplied by an automatic system were available *ad libitum.* Acute toxicity testing was carried out for 14 days in two groups of mice, namely the control group and the treatment group. The treatment group consisted of 7 male mice and 6 female mice; while the control group consisted of 6 male mice and 5 female mice. Mice in the control group were administered orally by gavage at a 0.5% CMC solution with a dose of 25.25 ml / kg body weight. The treatment group was given a solution of PMI enzymes were administered orally by gavage at a dose of 5050 mg / kg body weight dissolved in 0.5% CMC solution to obtain a concentration of about 20% (weight / volume), as much as 25.25 ml / kg body weight. Due to the volume, the administration of test and control materials is carried out twice with an interval of about 1 hour.

The experimental results showed that there were no mice that died due to the consumption of PMI enzyme. One male mice from the control group died and three female mice from the test group died, all due to evidence of gavage error on day 0. There were no clinical signs of toxicity during the trial. Necropsy results in animals at the end of the experiment did not show any abnormalities in the internal organs (liver, spleen, kidneys, including the brain). The LD50 value calculated from the trial of acute toxicity is greater than 5050 mg / kg body weight.

From the results of the toxicity study it can be concluded that the PMI enzyme is considered non-toxic and included in the practically nontoxic group of substances.

**III.3.3 Evaluation in Chicken Broiler Study**

Evaluation in chicken broiler was conducted and its result was reported as a company study report (Report No:4708-98): “*Evaluation of Event 3272 Transgenic Maize (Corn) in Broiler Chickens*” by John T. Brake. Study completion date: August 22nd 2005. The research was conducted in Department of Poultry Science, North Carolina State University, Chicken Educational Unit, Lake Wheeler Road Field Laboratory, 4108 Lake Wheeler Road, Raleigh, North Carolina, USA 27603 in compliance with the relevant provisions of Good Laboratory Practice (GLP).

This study aims to evaluate the adverse effects of chicken feed consumption made by adding corn event 3272 to broiler chickens (male and female). The study used three sources of maize grain fed: The transgenic maze grain (Event 3272 positive), hybrid maize (event 3272 negative) and near-isogenic control maize designated Event 3272 Negative (NC 2004, NC = North Carolina). The chickens used were broiler Ross 344 strain male and Ross 308 female, 1 day old. All chickens used amounted to 900 (450 male and 450 female). The chicken is divided into 36 cages, each cage contains 25 chickens (male and female separated). The trial was carried out for 49 days; Body weight data was measured on the 1st, 21st, 35th and 49th days. Diet and main water are given in ad libitum.

The results showed that the growth of chickens was very good. On the 49th day, the average weight of roosters reaches 3613 grams, while the hens reach 2825 grams. The average weight of chickens fed containing corn event 3272 grain had no different from chickens fed by hybrid maize, but chickens given near-isogenic were slightly heavier (around 68 grams) compared to the two groups. However, based on the ratio of conversion of feed consumption to body weight until the 49th day, there was no difference between the 3 groups of diets. Until the 49th day there were no dead chickens from the three groups of feed consumption. The results of the carcass analysis of hens showed no difference between the three groups of rations, but the group of roosters given hybrid corn seed rations weighed more lightly compared to the group given both transgenic maize and near-isogenic maize grain. But this is considered not too influential and inconsistent with other data. This study concluded that rations containing transgenic event 3272 maize could support the growth of broiler chickens, with very low mortality (zero), good conversion rates, and did not affect carcass quality and yield. From this study it was proved that transgenic event 3272 maize did not have an adverse effect on broiler chickens. From the results of the toxicity assessment above it can be concluded that the AMY797E enzyme and PMI enzyme had no homology sequence with known protein toxins; AMY797E enzyme is non-toxic and PMI enzyme belongs to a class of substances that is not toxic (practically non toxic); and PRG event 3272 corn did not has no adverse effect on broiler chickens.

**IV. Conclusion**

According to the explanations about genetic information of *amy*797E gene (isolated from hyperthermophilic microorganisme *Thermococcales spp*)and *pmi* gene (cloned from *Escherichia coli*) inserted in 3272 maize; substantial equivalence analysis between the composition of 3272 maize and nontransgenic maize; as well as allergenicity and toxicity study of AMY797E and PMI protein, it can be concluded that 3272 maize is safe to be consumed as food.

It is suggested that as long as 3272 maize has not yet to obtain environmental safety certificate, if corn seeds are found to grow (voluntary plant), they have to be eliminated immediately. However, since it is related to environmental safety aspect, this suggestion may be ignored if the 3272 maize has obtained environmental safety certificate.