**RISK ASSESSMENT REPORT OF**

**THE FOOD SAFETY TECHNICAL TEAM (FSTT ) FOR GM MAIZE EVENT BT11**

**I. Introduction**

Bt11 maize is the product of Syngenta claimed to provide benefit on resistance against Lepidoptera pest on corn. Bt11 maize produces Btk protein (Cry1A(b) protein) and phosphinothricin acetyl transferase (PAT) enzyme.

Bt11 maize has been used as a food and or feed in 18 countries namely United States (1996), Japan (1996), Canada (1996), Switzerland (1998), United Kingdom (1998), Europe Union (1998), Australia (2001), Argentine (2001), South Africa (2002), South Korea (2003), Philippines (2003), Russia (2003), Taiwan (2004), China (2004), Uruguay (2004), Mexico (2007), Brazil (2007), and Colombia (2008).

Food Safety Technical Team has conducted food safety assessment for Bt11 maize based on genetic information and food safety information consisting of substantial equivalence, allergenicity, and toxicity, according to the regulation of National Agency for Drug and Food Control (NADFC/BPOM) Number HK.00.05.23.3541 year of 2008 concerning Guidelines of Food Safety Study for Genetically Modified Products. Summary of assessments are specified below.

**II. Genetic Information**

**II.1 Genetic Element**

Bt11 maize contains two novel genes namely Btk gene and *pat* gene. Btk gene produces Btk protein responsible for resistance against corn borer. *pat* gene encodes PAT enzyme to be tolerant against phosphinothricin (glufosinate herbicide). Promotor and terminator used for both novel genes are CaMV-35S from 35S cauliflower mosaic virus and NOS (nopaline synthase) from *Agrobacteria tumefaciens*.

**II.2 Gene Source**

1. Btk gene is the full-length version of Cry1A(b) gene from *Bacillus thuringiensis* var. kurstaki HD-1. *B. thuringiensis* is Gram-positive aerobic soil bacteria which can form spore and produce crystal protein. The crystal protein will effectively functioned as insecticide after being consumed by specific insects which are sensitive to the crystal protein. *Bacillus thuringiensis* has been used commercially by farmers since 1958, as safe bio-pesticides. .
2. *pat* gene is cloned from soil microorganism *Streptomyces viridochromogenes* strain Tu494. *Streptomyces viridochromogenes* is Gram-positive soil bacteria spore-forming members of the family Actinomycetaceae. The bacteria produces PAT enzyme which protects itself from *phosphinothricyl-alanyl-aline* tripeptide (*phosphinothricin-tripeptide*), which also produces and exhibit broad spectrum phytotoxic activity.

**II.3 Transformation Method**

Plasmid pZO1502 is the vector used for the transformation of Bt11 maize through protoplast transformation /regeneration system using restriction fragment length polymorphism (RFLP) in long arm of chromosome 8. The plasmid pZO1502 contains two genes of interest namely *Btk* and *pat gene*s. Transformed plants with both genes are then crossed and back-crossed with Syngenta’s Elite line and used as parents for commercial hybrid.

**II.4 Genetic Stability**

Molecular analysis using Southern blot is conducted to see the stability of inserted gene from generation to generation. The result shows that the inserted gene is stable from three generations of back-cross 3 (BC3) to the generation of back-cross 6 (BC6). Btk and *pat* genes in Bt11 maize are closely linked and segregated together according to the principles of Mendelian inheritance law. The genetic stability of Bt11 maize was reported in study report of Syngenta Seed AG company: *Molecular Characterization of the Genetically Modified Bt11 Maize* by P. Ahl Goy in 2001.

Based on genetic information study it is concluded that:

1. Bt11 maize contains single copy of vector fragment which carry two *bt*k genes and *pat* genes;
2. Promotor and terminator used for two novel genes are CaMV-35S from 35S cauliflower mosaic virus and NOS (nopaline synthase) from *Agrobacteria tumefaciens*;
3. Two genes of interest, *Bt*k (Cry1A(b)) and *pat*, introduced to Bt11 maize remain stable from three generations of back-cross 3 (BC3) to the generation of back-cross 6 (BC6); and
4. Two genes of interest, *Bt*k (Cry1A(b)) and *pat*, introduced to Bt11 maize are inherited following Mendelian Inhetitance Law.

**III. Food Safety Information**

**III.1 Substantial Equivalence**

Assessment result of substantial equivalence study for Bt11 maize is obtained after considering four documents reported by Novartis Seeds, namely: (1) *Chemical Composition Analysis Done with Bt-11 Maize with a European Background* (Report of Novartis Seeds dated 3 September 1996), (2) *Chemical Composition Analysis Done with Bt-11 Maize with a US Background* (Report of Novartis Seeds sated 3 September 1996), (3) *Novartis Seed’s Genetically Modified Bt11 Maize: Further Determination of the Biochemical Composition of Kernels, including Determination of Anti-nutritional Factors* (P. Ahl Goy, Manager Regulatory Affairs, Novartis Seeds AG dated 6 April 1999), (4) *Comparison of Vitamin and Mineral Composition of Grain from Bt11 Maize and Non-Modified Maize Hybrids* (Novartis Seeds Biotechnology Report No. NSB-004-97 dated 11 July 1997).

For chemical composition analysis purposes, Bt11 maize and non-Bt11 maize were planted in 1995 in three locations namely Greenville, Ayden, and Wade located in North Carolina, USA. The corn samples are then analyzed at Illinois Crop Improvement Association Inc., Champaign, IL, USA. Data shows that the seeds of Bt11 maize are not different from nontransgenic maize in terms of density (g/cm3 from corn seeds in standard water level of 15.5%), weight of 100 corn seeds, seed size, % starch, % protein, % oil, and % fiber. The vitamin and mineral composition of Bt11 maize and nontransgenic maize planted in 1995 in three different locations of USA namely Janesville, Wisconsin, and Napoleon does not show significant difference.

With more hybrid corns being used, the composition test above is repeated by taking samples in several planting locations i.e. in Wisconsin (WI), Ohio (OH), and Iowa (IA), with 3 location each state and in Illinois (IL) and North Carolina (NC) with 3 location each state. In general, the results of tests regarding density, weight of 100 corn seeds, seed size, % starch, % protein, % oil, and % fiber and the profiles of fatty acid and amino acid show that Bt11 maize is not different from nontransgenic maize.

In 1998, the analyses of proximate, amino acid, fatty acid, phytic acid, and trypsin inhibitor in seeds of Bt11 maize and nontransgenic maize planted in three different locations of France have been conducted. The results of analysis show no compositional difference between Bt11 maize and nontransgenic maize related to genetic modification in corns.

The result of substantial equivalence study above concludes that Bt11 maize is substantially equivalent with nontransgenic maize.

**III.2 Allergenicity**

Homology sequence of amino acid was analyzed using a bioinformatics program of Basic Local Alignment Search Tool for Protein (BLASTP) version 2.2.19. Entry was compared to the protein database of National Center for Biotechnology Information (NCBI) Entrez Protein Database accessed on 12 February 2010. The result showed that 471 proteins were not homolog with toxin sequence in database, while 433 proteins showed amino acid sequence similarity using tested protein included in the category of delta endotoxin protein (Cry protein or insecticidal protein) derived from 8 species or which constitutes synthetic gene construction. Other proteins showing amino acid sequence similarity were included in the category of hypothetic protein with non-specific function from 7 species; translocation signal protein and protein from *B. thuringiensis* included in parasporin family with non-hemolytic, non-insecticidal natures and capable of inhibiting / killing cancer cells, but not providing effect to the normal cells of mammals including human.

Bioinformatics tests for the analysis of Cry1A(b) protein sequence homology (1471 amino acids) compares the overall sequences and peptides of 80 amino acids (Peptide 1: amino acid 1-80, peptide 2: 2-81, etc.) and searches 8 sequential amino acids commonly found in allergen protein. The result showed no homology among overall proteins, peptide of 80 sequential amino acids and segment of 8 sequential amino acids with allergen data.

Bioinformatics test was also conducted using FASTA program (FASTA SEARCH Algorithm version 3.45 1988) with allergen database stored in NCBI (in 2009) and FARRP Database. Result sequence comparison in bioinformatics analysis showed negative result so it it is concluded that Cry1A(b) protein does not have similarity with allergen protein and is not immunoreactive.

Equivalence test in terms of molecular weight; immunology response (data of Western blot and ELISA); trypsin; N-terminal sequence; glycosylation, and bioactivity test showed the equivalence of CrylA(b) protein (Btk HD-1) produced by recombinant *E. coli* with which produced by Bt11 maize. Therefore, the test with *E. coli* protein can be made as a reference in concluding similar tests for protein produced by Bt11 maize.

In-vitro protein digestibility test is conducted to PAT enzyme from Bt11 maize and its result was reported as a company study report (Study Number CAB-008-94): *In vitro Digestibility and Inactivation of the Bar Marker Gene Product Phosphinothricin Acetyltransferase (PAT) under Simulated Mammalian Gastric Conditions* by Laura Privalle. The research was conducted at CIBA Seeds Agricultural Biotechnology Research Unit, CIBA-Geigy Corporation, 3054 Cornwalls Road, Post Office Box 12257, Research Triangle Park, NC, USA 27709-2257.

The sensitivity of PAT enzyme against proteolytic degradation in simulated mammalian gastric fluid (SGF) containing pepsin enzyme was tested through visualization in SDS PAGE gel. The SDS PAGE data and enzyme activity quantification show that PAT enzyme was quickly hydrolyzed by pepsin, even if pepsin concentration is lowered by 0.01 times, hydrolysis occurs perfectly within 2 minutes. In 37oC temperature, enzyme was quickly damaged with or without pepsin. This study concluded that PAT enzyme will immediately lost their enzymatic activities and can be digested in mammal’s stomach like nontransgenic protein.

The result of allergenicity study concludes that Cry1A(b) protein and PAT enzyme do not indicate potentials to cause allergy.

**III.3 Toxicity**

A. Cry1A(b) Protein

Cry1A(b) protein acute toxicity study was conducted on mice and its result was reported as a company study report (Report No. MSL-11985, Job/Project No. ML-92-209/EHL 92069): *Acute Oral Toxicity of Btk HD-1 Tryptic Core Protein in Albino Mice* by MW Naylor dated 16 June 1992. The research was conducted at Environmental Health Laboratory, Monsanto Agricultural Group, 645 S. Newstead, St Louis, Missouri 63110.

Toxicity study using CD-1 mice divided into three groups (10 male mice and 10 female mice per group), based on the provision of *Bt*k HD-1 tryptic core protein (Cry1A(b) protein) with the dosages of 400, 1000, and 4000 mg/kg body weight. Two control groups were used (each consists of 10 male mice and 10 female mice); the first control group obtains 50-mm carbonate buffer solution with the dosage of 66.66 ml/kg body weight, while the second control group obtains bovine serum albumin (BSA) solution with the dosage of 4000 mg/kg body weight. Btk HD-1 tryptic core protein (Cry1A(b) protein) solution, buffer solution, and BSA solution were given through gavage. The experiment for 7 days, then on the 8th and 9th days, all living mice were dissected (necropsy). Selected several organs were taken to be observed microscopically.

The result of experiment showed no difference of body weight, food consumption, the number of survival mice, or the result of clinical observation and gross pathology. On the first day, 1 female mouse was dead from the group provided with BSA (control), right after oral gavage. It occurred **due to failure of intubation procedure (entry of “tube” for gavage)** and not due to poisonous effect of the tested material.

This study concludes that Btk HD-1 tryptic core protein (Cry1A(b) protein) does not cause toxic effect to mice at a dose level of 4000 mg/kg body weight.

B. PAT Enzyme

Acute toxicity study against PAT enzyme from Bt11 maize was conducted to mice and its result was reported as a company study report (Laboratory Study No. 1910-95): *Acute Oral Toxicity Study in Mice Using the Phosphinothricin Acetyltransferase* by Janice O Kuhn. The research was conducted from 22 March 1995 to 9 May 1995 in STILLMEADOW, Inc., 12852 Park One Drive, Sugar Land, Texas 77478.

Test using 8-12 weeks old. HSD:ICR mice was divided in to two groups namely control group (without PAT enzyme) and treatment group (treated with PAT enzyme with the dosage of 5050 mg.kg body weight through gavage of 19.42 ml/kg body weight). There are 5 male mice and 5 female mice per group. Material was dissolved in CMC 2% solution.

During the experiment, one mice from male treatment group were dead. On the first days of test, the dead mice showed activity degradation. On the 6th and 8th days, the dead mice showed piloerection and ptosis symptoms. Piloerection symptom was also detected on male mice from control group on the zero day of test. There is no other clinical sign shown by mice from both groups.

The death of a mice from treatment group was allegedly caused by clogged esophagus (according to necropsy observation) due to a solid and round material. The clogging material was not identified further, but the clogging allegedly obstructs the entry of food consumption and water into stomach. It allegedly causes the death of the mouse. The result of necropsy observation didn’t show organ abnormality in both experimental groups. Abnormality was only found in the dead mice.

The increase of mice’s body weights was not influenced by PAT enzyme, and didn’t indicate difference between treatment group and control group.

The result of test showed that PAT enzyme was not toxic on mice at the dose level of 5050 mg/kg body weight.

The result of toxicity study above concluded that Cry1A(b) protein was considered not toxic and PAT enzyme was included in the category of non-toxic substance (practically non-toxic).

**IV. Conclusion**

According to the explanations on genetic information of Btk gene which is the full-length version of Cry1A(b) gene from *Bacillus thuringiensis* var. kurstaki HD-1 and *pat* gene which is cloned from soil microorganism *Streptomyces viridochromogenes* strain Tu949 that inserted in Bt11 maize; substantial equivalence analysis between the composition of Bt11 maize and nontransgenic maize; as well as allergenicity analysis and toxicity test of Cry1A(b) protein and PAT enzyme, it can be concluded that Bt11 maize is safe to be consumed as food.

It is suggested that as long as Bt11 maize has yet to obtain environmental safety certificate, if corn seeds are found to grow (voluntary plant), they have to be terminated immediately. However, since it is related to environmental safety aspect, this suggestion may be ignored if the Bt11 maize has obtained environmental safety certificate.