**RISK ASSESSMENT REPORT OF THE FOOD SAFETY TECHNICAL TEAM (FSTT ) FOR GM MAIZE EVENT MIR162**

**I. Introduction**

MIR162 maize is the product of Syngenta claimed to provide benefit for resistance against Lepidoptera pest on corn. MIR162 maize produces Vip3Aa20 protein and phosphomannose isomerase (PMI) protein.

MIR162 maize has been used as a food and or feed in 8 countries namely Australia (2009), United States (2008), Brazil (2009), Canada (2010), Japan (2010), Philippines (2010), Taiwan (2009).

Food Safety Technical Team has conducted food safety studies for MIR162 maize based on genetic information and food safety information consisting of substantial equivalence, allergenicity, and toxicity according to the 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. Summary of assessment are specified below.

**II. Genetic Information**

**II.1 Genetic Element**

MIR162 maize contains two novel genes namely *vip3Aa20* gene and *pmi*  gene. *vip3Aa20* gene produces Vip3Aa20 protein responsible for resistance against *Ostrinia nubilalis*, *Helicoverpa zea*, *Spodoptera frugiperda*, *Agrotis ipsilon*, dan *Striacosta albicosta;* and *pmi* gene encodes PMI (*phosphomannose isomerase*) protein as selection marker. Promotor and terminator used for *vip3Aa20* genes are ZmUbilInt (*polyubiquitin Zea may*s) and CaMV-35S from 35S cauliflower mosaic virus. Promotor and terminator used for *pmi* gene are ZmUbilInt and NOS (nopaline synthase) from *Agrobacteria tumefaciens*.

**II.2 Gene Source**

1. *Vip3Aa20* gene is isolated from *Bacillus thuringiensis* strain AB88. *Bacillus thuringiensis* has been used commercially as safe bio-pesticides by farmers since 1958.
2. *pmi* gen is isolating from *Escherichia coli*

**II.3 Transformation Method**

Event MIR162 maize was produced through *Agrobacterium tumefaciens*-mediated transformation of immature embryos of *Z. mays* line NP2500 x NP2499. Plasmid pNOV1300 is used for as vector in the transformation of MIR162.

**II.4 Genetic Stability**

1. Molecular analysis using Southern blot is conducted to see the stability of inserted genes from generation to generation. The result shows that the inserted gene is stable up to the generation of back-cross 4 (BC4F1). Southern blot analysis using backbone specific probe revealed no hybridization bands in the genomic samples, demonstrating that MIR162 maize does not contain any backbone sequences from the transformation plasmid pNOV1300. *vip3Aa20* and *pmi* genes in MIR162 maize segregate according to the Mendelian inheritance Law. Data from Southern blot analysis revealed that MIR162 maize contains single copy of VIP3AA20 genes and PMI (*phosphomannose isomerase*) genes;

**II.5.** Based on genetic information study concludes that:

1. MIR162 maize contains single copy of *vip3Aa20* genes and *pmi* (*phosphomannose isomerase*) genes;
2. MIR162 maize does not contain any backbone sequences from the transformation plasmid pNOV1300;
3. Both gene of interest, *vip3Aa20* and *pmi*, introduced to MIR162 maize remain stable up to the generation of back-cross 4 (BC4F1);
4. Both gene of interest, *vip3Aa20*  and *pmi*, introduced to MIR162 maize are segregated according to Mendelian inheritance Law.

**II.6 References**

**Katie Pence. 2006.** *Stability of Vip3Aa20 and Phosphomannose Isomerase (PMI) Protein Expression Across Multiple Generations of Maize (Corn) Derived from Transformation Event MIR162. Protocol No. MIR162-05-02. Report No. SSB-002-06. Syngenta Biotechnology, Inc. Regulatory Science Post Office Box 12257 3054 East Cornwallis Road Research Triangle Park, North Carolina, USA 27709-2257.*

**III. Food Safety Information**

**III.1 Substantial Equivalence**

Complete substantial equivalence study for MIR162 maize is explained under company report No: 146-06 “*Compositional Analysis of Grain and Forage Derived from Event MIR162 Hybrid Maize Grown During 2005 in the USA”* (K. Launis, 2007). For the needs of composition analysis and chemical components in grain and forage, MIR162 maize and non-MIR162 maize were planted in the US during 2005.

Levels of 65 maize chemical components (proximate, mineral, vitamin, amino acid, fatty acid, secondary metabolite, and anti-nutrient) were measured in forage and grain from MIR162 maize hybrids and compared to levels in forage and grain from nontransgenic, near-isogenic maize hybrids.

Grain analysis shows statistically significant differences in ash. *Neutral Detergent Fiber* (NDF), starch, beta carotene, pyridoxine, and alfa tocopherol. While forage analysis show significant difference in the NDF. However, the differences observed were small and the average values observed for these component in MIR162 grain were within the ranges of values observed for the nontransgenic grain. Furthermore, the average values were within the ranges reported in the literature (ILSI, 2006; OECD, 2002).

Assessment on the substantial equivalence study above concludes that MIR162 maize is substantially equivalent with nontransgenic maize.

**III.2 Allergenicity**

Protein testing was carried out on recombinant proteins derived from Maize plants and recombinant proteins derived from *Escherichia coli*. This study was conducted in Syngenta laboratory in compliance with the relevant provisions of Good Laboratory Practice (GLP). Both proteins were extracted and purified using column chromatography

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 (Vip3Aa20 HD-1) produced by recombinant *E. coli* with which produced by MIR162 maize. Therefore, the test with *E. coli* protein can be made as a reference in concluding similar tests for protein produced by MIR162 maize.

Characterization of Vip3Aa20 protein and PMI protein includes immunoreactivity, molecular weight, glycosylation, and N-terminal amino acid sequences. The PMI protein produced in E. coli is different on 16 non-functional amino acids at N-terminal, however biochemical characteristics, immune reactions, biological activities and enzymatic reactions of both proteins (E. coli and plants) were the same.

Quantity and quality testing of protein were conducted according to the method described by Bradford, SDS-PAGE, ELISA, Western blot methods, enzymatic activity tests and bioassays, glycosylation analysis were analyzed using the DIG Glycan Detection Kit, Edman degradation and peptide map.

The levels of Vip3Aa20 and phosphomannose isomerase (PMI) protein expression in different tissue were analyzed over different stage and multiple generations of transformation Event MIR162-derived maize (field corn) plants. The mean Vip3Aa20 and PMI concentrations measured in leaves were 12 – 148 μg/g and 0,2 – 25 μg/g respectively. Vip3Aa20 and PMI protein remain stable up to four generations.

Homology sequence of amino acid was analyzed using a bioinformatics program of SWISSPROT, BLASTP, FASTA, and FARRP Data Base.

Allergen database includes various types of allergens including: food allergens, respiratory allergens, venom protein, contact allergens, gliadin, gluten. The result showed no homology among overall Vip3Aa20 proteins with allergen data toxin sequence in database except plant insecticidal proteins. The result showed that PMI proteins were not homolog with toxin sequence in database

Bioinformatics approach for the analysis of Vip3Aa20 sequence homology ( 789 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 known allergen data. 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 (110 amino acid described as *Rana* species). The specific amino acid sequence in common was “DLSDKETT,” which occurs at positions 327 – 334 of PMI and positions 77 – 84 of the allergen sequence.

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

Syngenta Bioinformatics analysis report were consist of:

* 1. Brian Harper. Vip3Aa20 (Entrez Accession Number ABG20429): Assessment of Amino Acid Sequence Homology with Known Allergens. Study completion date: July 16th 2007.
  2. Brian Harper. Phosphomannose Isomerase Protein (Entrez Acession No. AAA24109): Assessment of Amino Acid Sequence Homology with Known Toxins. Study completion date: July 13th 2007.

In vitro protein digestibility of Vip3Aa20 in simulated gastric fluid (SGF) showed that Vip3Aa20 protein was degraded rapidly in 1 minute. . Whereas the digestibility test of PMI protein in SGF (pH 1.2) and SIF (pH 7.5) which concentration was diluted to 0.001 times showed that PMI was degraded rapidly both in SGF and in SIF, whereas in dilution 0.0001 times after 10 minutes incubation did not detect the presence of protein and PMI activity (perfectly degraded).

The heat stability of Vip3Aa20 protein showed that this protein is not stable at 65 ° C for 30 minutes, even though it is stable at 37° C for 30 minutes. This result was reported in the company study report number SSB-039-06 entitled "Effect of Temperature on The Stability of Vip3Aa20 Protein" (Cheryl Stacy, 2007).

Heat stability test of PMI protein showed decreased immunoreactivity by 78.66% at 37.55 ° C and 22% at 65 ° C, while at 95 ° C for 30 minutes, the reactivity was 0 (zero). It can be concluded that the PMI protein is not stable at 37 ° C even though it is stable at 25 ° C for 30 minutes. Enzyme activity were decrease to 40% when the protein exposed to 55 ° C for 30 minutes; at 65 ° C it decreases to 3%; and at 95 ° C it drops to 0% (lost).

Result of heat stability study concluded that Vip3Aa20 and PMI proteins were not stable against heating. Based on the results of allergenicity study it can be concluded that the Vip3Aa20 protein and PMI protein do not show any potential allergic reactions.

**III.3 Toxicity**

Acute toxicity of Vip3Aa20 protein was conducted to mice and its result was reported as a company study report (MIR162 VIP3A-0106): “*Single Dose Oral Toxicity Study in Mice”*  by C. Draper. The research was conducted at Syngenta Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, United Kingdom, SK10 4TJ.

Test results showed that there were no deviant mortality and clinical signs in experimental animals (mice). Oral administration with a single dose of 1250 mg Vip3Aa20 protein per kg body weight in mice did not have a negative effect. Therefore Vip3Aa20 protein is considered non-toxic.

Acute toxicity of PMI protein was conducted to mice and its result was reported as a company study report study completion date August 11th 1999: 11 Agustus 1999, “Phosphomannose Isomerase (Sample PMI-0198): Acute Oral Toxicity Study in Mice” by Janice O. Kuhn. The research was conducted at STILLMEADOW Inc., 12852 Park One Drive, Sugar Land, TX 77478.

The test results showed that at a single dose of 5050 mg per kg body weight of mice, the PMI-0198 protein was included in the class of substances that were practically non toxic. From the results of the toxicity study it can be concluded that the Vip3Aa20 protein is considered non-toxic and PMI protein is included in the practically non toxic group of substances.

**IV. Conclusion**

According to the explanations on genetic information of *vip3Aa20* gene which isolated from *Bacillus thuringiensis* strain AB88 and *pmi* gene cloned from *Escherichia coli* that inserted in MIR162 maize; substantial equivalence analysis between the composition of MIR162 maize and nontransgenic maize; as well as allergenicity and toxicity study of Vip3Aa20 and PMI protein, it can be concluded that MIR162 maize is safe to be consumed as food.