CONSOLIDATED REPORT OF SYNGENTA'S CORN MZHG0JG
APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING

EXECUTIVE SUMMARY

On October 28, 2016, Syngenta Philippines Inc.'s submitted corn MZHG0JG for direct use as food and feed, or for processing to the Bureau of Plant Industry (BPI) under the DOST-DA-DENR-DOH-DILG Joint Department Circular (JDC) No. 1 Series of 2016. After reviewing the Risk Assessment Report and attachments submitted by the applicant, the assessors namely: Scientific and Technical Review Panel (STRP), BPI- Plant Products Safety Services Division (BPI-PPSSD) and Bureau of Animal Industry (BAI), concurred that corn MZHG0JG is as safe for human food and animal feed as its conventional counterpart.

The Department of Environment and Natural Resources – Biosafety Committee (DENR-BC), after a thorough scientific review and evaluation of the documents related to Environmental Risk along with the submitted sworn statement and accountability of the proponent, recommended the issuance of a biosafety permit for this regulated event provided that the conditions set by them are complied.

Also, the Department of Health – Biosafety Committee (DOH-BC), after a thorough scientific review and evaluation of documents related to Environmental Health Impact, concluded that corn MZHG0JG will not pose any significant risk to health and environment and that any hazards could be managed by the measures set by the department. DOH-BC also recommended for the issuance of biosafety permit for corn MZHG0JG.

Furthermore, the Socio-economic, Ethical and Cultural (SEC) Considerations expert also recommended for the issuance of biosafety permit for this regulated article after assessing the socio-economic, social and ethical indicators for the adoption of Genetically Modified Organisms.

BACKGROUND

In accordance with Article VII. Section 20 of the JDC, no regulated article, whether imported or developed domestically, shall be permitted for direct use as food and feed, or for processing, unless: (1) the Biosafety Permit for Direct Use has been issued by the BPI; (2) in the case of imported regulated article, the regulated article has been authorized for commercial distribution as food and feed in the country of origin; and (3) regardless of the intended use, the regulated article does not pose greater risks to biodiversity, human and animal health than its conventional counterpart.

The BPI Biotech Office provided the assessors the complete dossier submitted by Syngenta Philippines, Inc.

Upon receipt of the individual reports from the assessors, the BPI Biotech Office prepared this consolidated risk assessment report for the information of the public.

STRP, BAI, BPI-PPSSD ASSESSMENT AND RECOMMENDATIONS

Based on the documents submitted by the applicant:

A. Host Organism
The assessors concurred that corn is a good source of carbohydrates, fats, calcium and phosphorus. It has long been domesticated and grown worldwide and is the second most important cereal crop in the Philippines. Corn has phytic acid which reduces the availability of phosphorus in monogastric animals. Raffinose and trypin inhibitors are also present in corn. A potential toxicant in roots and leaves, DIMBOA (2-4-hydroxy-7 methoxy-2H-1,4-benzoxacin-3 (4H)-one, has been reported and its level among corn varieties has been varied and its amount decreases as the corn grows. Amino acid sequence alignment did not match with any protein toxicant in the NCBI and Syngenta database.

B. Transgenic Plant
The corn event has been approved as food and feed in US and Canada, and as food in New Zealand. The assessors agreed with the report that the transgenic plant is not a source of allergens. This was further confirmed by the results of gastric fluid digestibility studies, SDS-PAGE analysis and Western blot analysis. Consumption of corn as food may not drastically change due to the availability of transgenic corn varieties like MZHG0JG corn. Assessment from these countries proved the transgenic corn is not materially different in composition, safety, and other relevant parameters from corn-derived food and feed found in the market.

C. Donor Organism
The two protein-encoding genes namely, *mepsps-02* (modified 5-enol pyruvylshikimate-3-phosphate synthase gene) and *pat-09* (phosphinothricin acetyltransferase gene) have been properly described with respect to source and potential pathogenic and allergenic properties. The gene *mepsps-02* from *Zea mays* encodes the enzyme mEPSPS, while *pat-09* gene was from *Streptomyces viridochromogenes*, a common soil bacterium. All genetic elements that make up the gene cassette has been elaborately described by the proponent. The MZHG0JG insert and its flanking sequences are intact with no changes in base pairs.

D. Transformation System
The transformation method used was *Agrobacterium tumefaciens* mediated transformation, producing MZHG0JG. Genetic elements within the border regions of the T-plasmid were integrated into the plant genome, while those outside the borders were not transferred. The target of modification was nuclear DNA and the complete details of the experimental protocol was provided, including a complete description of the plasmid vector. Neither carrier DNA nor helper plasmids were detected.

E. Inserted DNA Genetic Stability
The integrity of the genetic elements within the insert was also evaluated in succeeding generations of transgenic corn plants. Stability of the genetic elements in the insertion sites were evaluated through Southern blot analysis and application of the probe for the plasmid backbone utilizing two corn generations. The absence of extraneous T-DNA fragments in the genome and in the backbone sequences of the transformation plasmid vector of the transgenic MZHG0JG was described supporting the integrity of the genetic elements within the insert.

Open reading frames were not identified for comparison with allergen and toxin data base. These data conform with the conclusion that no amino acid translations of the start-to-stop junction open reading frames share relevant amino acid sequence similarity to known or putative protein allergens and toxins.

The mEPSPS integrated in the MZHGOJG corn has been expressed in other transgenic corn varieties like GA21corn. The similarity of the *mepsps-02* genes that encode for the mEPSPS protein in MZHGOJG corn and GA21 corn were validated by nucleotide sequencing.

F. Genetic Stability

Stability of the insert has been validated by Southern blot analysis which demonstrated identical hybridization bands for the MZHGOJG insert in lanes where the DNA extracts of transgenic plants grown from 5 corn generation

The presence of the *mepsps-02* and the *pat-09* genes in three generations of the transgenic corn was confirmed by the data generated from Real-time PCR. Chi-square analysis was used to validate the heritability of the MZHG0JG insert in a predictable manner in line with Mendelian principles and the results generated validated the previous hypothesis.

G. Expressed Material

Concentrations of proteins mEPSPS and PAT in MZHG0JG leaves, roots, whole plants, pollen, and kernels were measured by Enzyme Linked Immunosorbent Assay (ELISA). The two proteins are both enzymes and therefore participate in specific metabolic pathways.

The native EPSPS in corn catalyzes the synthesis of 5-enoylpyruvylshikimate-3-phosphate (EPSP) from shikimate-3-phosphate (S3P) and phosphoenolypyruvate (PEP). This reaction is one of the steps in the synthesis of aromatic amino acids. The herbicide glyphosate inhibits the action of the native enzyme making the native corn incapable of producing aromatic amino acids which leads to its death. On the other hand, the modified EPSP (mEPSP) in MZHG0JG differs with the native enzyme in that it contains two amino acid substitutions that make the modified enzyme lose its affinity with glyphosate thereby making the transgenic corn tolerant to the herbicide. Simulated gastric fluid at pH 1.2 and the T50 is practically none because all got inactivated at 1 minute. Determination was done using SDS-PAGE.

Pepsin was used in simulated gastric fluid studies and an intact mEPSPS was not detected after digestion for one minute, while faint diffused bands with low molecular weight were visible in SDS-PAGE analysis of the digested protein. The bands with low molecular weight did not cross-react with anti-mEPSPS antibody when assessed with Western blot. These reported observations demonstrate that mEPSPS in transgenic maize or in recombinant *E. coli* readily degrade under simulated mammalian gastric conditions. The quick degradation was proven by the absence of intact mEPSPS upon sampling even at 1 min and that no immuno-reactive protein of mEPSPS is detected. These imply that mEPSPS is readily digested as a conventional dietary protein in normal mammalian gastric environment.

H. Toxicological Assessment

The assessors confirmed that pepsin was used to simulate gastric fluid digestibility studies and an intact mEPSPS was not detected following digestion for one minute. SDS PAGE revealed the presence of faint diffuse bands of lower molecular weight over time and western blot analysis showed that this band did not cross react with the anti-mEPSPS antibody.

It was reported that subjecting the microbially-produced and the MZHG0JG mEPSPS proteins at lower temperature (25 to 37°C) for 30 minutes reportedly caused minimal reduction in mEPSPS immunoreactivity (96 and 92%, respectively). Incubation of the sample at higher temperature (65 to 95°C) for 30 min lead to non-stability of mEPSPS and complete loss of immunoreactivity when assessed by ELISA. Data demonstrate that mEPSPS is stable for 30 min when heated up to 37°C. At temperature above 65°C, which are often applied in the processing and cooking of corn, minute and negligible amounts of intact and functional mEPSPS protein in foods and feed may be expected.

The assessors also reported that systematic comparison of the mEPSPS amino acid sequence with those of the sequences of known or putative protein toxins in the NCBI Entrez Protein database (NCBI version 2016) and in the Syngenta Toxin database (version 2016) with the use of BLAST confirmed that mEPSPS is neither a toxic protein nor it shares significant sequence similarity with other known or putative toxins.

Administration of mEPSPS in mice at a dose of 2000 mg/kg body weight through oral gavage was not acutely toxic. Treatment-related effects such as alteration in food consumption, hematology parameters, blood clinical chemistry parameters, post-mortem alteration of weights of organs were not significantly different between mice in the treated (mEPSPS-gavaged groups) and control groups.

The assessors also confirmed that the susceptibility of PAT to proteolytic degradation was evaluated in simulated gastric fluid (SGF) containing pepsin and the results showed that PAT is readily digested in SGF in less than 1 minute as assessed by SDS-PAGE and Western blot.

There are no fragments left after digestion in SGF as assessed by SDS-PAGE and Western blot analyses. It can therefore be concluded that PAT is readily digested by the mammalian gastric enzyme, pepsin.

It was also reported that immunoreactivity of PAT is no longer detectable after incubation for 30 minutes at 95°C.

The results of BLASTP showed no significant homology of PAT to any known toxin. NCBI Database Alignments identified 1000 sequences with potentially significant similarity to the PAT amino acid sequence. Of these, 651 proteins were identified as PAT proteins; 275 were identified as acyl transferase proteins; 73 proteins were identified as hypothetical/ unknown proteins. One protein was identified as a DNA alkylation repair protein from *Streptococcus suis*.

The toxicity of the PAT protein administered 2 times a day by oral gavage in mice at a total dose level of 2000 mg/kg was evaluated. A 14-day observation was well-tolerated with no evidence of toxicity. Animals that received the gavage survived until the scheduled time for necropsy. No test substance-related clinical effects on body weight, food consumption, gross and macroscopic findings were reported.

The assessors affirmed that the test protein was produced in *Escherichia coli* expression system and is therefore microbially-produced. Equivalency was demonstrated through Western blot, glycosylation, and peptide mass coverage analyses. The microbially-produced

protein was compared to plant-produced PAT. Western blot analysis showed that the two proteins exhibit a single immunoreactive band consistent with the predicted molecular weight of 20.5 kDa. Glycosylation analysis showed no evidence of post-translational glycosylation for both proteins Peptide mass coverage analysis mapping confirmed the identity of the proteins from both sources as PAT.

Furthermore, the assessors have reported that mEPSPS and PAT are expressed independently of each other to retain their functional properties. The mEPSPS and PAT are integrated within the corn nuclear genome. Expression of the proteins were validated and confirmed by a series of tests that applied Southern blot, nucleotide sequencing of the nuclear genome and examination of inheritance pattern of the transgene over 3 generations of the transgenic MZHG0JG corn. The assessors confirmed that the expressed proteins do not interact with each other. They act as independent system conferring tolerance to herbicides of glufosinate or glyphosate composition

I. Allergenicity Assessment

The assessors confirmed that the Digestibility studies that used pepsin (2600units/ml) was performed at a ratio of 10 pepsin activity units/µg EPSPS. No intact mEPSPS (ca. 47.4 kDa) was detected following 1 min digestion in SGF. Faint diffused bands that registered low molecular weight were visible in SDS-PAGE over time. The bands did not cross-react with anti-mEPSPS-antibody in Western blot. These data demonstrate the fast degradation of the mEPSPS expressed in a consumed transgenic corn by mammalian gastric conditions.
There was loss of immune reactions for mEPSPS at 250C and 370C incubation for 30 mins with 92-96% loss of immune reactivity and complete loss at 95 degrees incubation at 30min. This was determine through ELISA.

It was reported that the mEPSPS protein showed no sequence similarity with any known allergen. The mEPSPS protein is not homologous with known allergens as demonstrated by the absence of similar sequences (indicated by greater than 35% shared identity over 80 or more amino acids)when compared with the amino acid sequences of any allergen in the FARRP allergen online database. The absence of similar alignments of 8 or more contiguous amino acids between the mEPSPS and sequences of allergens in the FARRP allergen database.

On the other hand, the digestibility of PAT to pepsin was done, which showed rapid digestion in less than 1 minute, as assessed by SDSPAGE and Western blot. As the digestion appeared complete, there were no fragments detected by SDS-PAGE and Western blot.

A diminishing immuno-reactivity is reported in PAT at 55.2%, 11.2% and 5.4% relative to its incubation at 25, 37 and 65°C, respectively. Incubation at 95°C for 30 min contributed to the low detection of immune-reactivity of PAT when evaluated by ELISA. Data indicate that a complete loss of detectable immune reaction of PAT is contributed by heating at high temperature which parallels possible inactivation of PAT through heating and cooking of corn.

The FASTA search did not show homology of greater than 35% of shared identity for the 80 aa bases. No biologically relevant match was found for allergens. PAT proteins are microbiologically derived for the transgenic event. These have not been shown to be glycosylated. Comparison of the plant and microbial derived PAT shows the same mol. wt within the range of 10-70 kDa range.

J. Nutritional Data

For the proximate analysis, the assessors confirmed that the comparison of the transformed corn forage and conventional counterpart showed equivalency of moisture, protein, fat, ash, acid detergent fiber (ADF) and neutral detergent fiber (NDF). However the transformed plant has a higher carbohydrate relative to the conventional corn. Furthermore, six non-transgenic commercial corn varieties were included in the comparison. All the control, MZHG0JG, MZHG0JG + trait specific herbicide (TSH), and the six commercial varieties were grown in ten corn-growing locations in the US in June 2013. The assessors reported that all the data on forage proximates derived from the transgenic lines are within the observed range.
The assessors confirmed that based on the results of the analysis provided by the developer, no statistical differences in the proximates of forage corn were observed that can be considered as biologically relevant.

On the other hand, the assessors confirmed that the applicant provided sufficient information regarding the proximate analysis of MZHG0JG grains in comparison with the non-transgenic corn. Levels of all proximates in MZHG0JG showed no significant difference with the non-transgenic corn except for NDF which was significantly lower than the control. In terms of MZHG0JG corn treated with TSH, no significant differences were observed in the levels of protein, fat, ash, carbohydrates, ADF, TDF, NDF and starch in comparison with the non-transgenic corn. Moreover, it was reported that in MZHG0JG corn, and MZHG0JG corn + TSH, the mean levels of all proximates were within the ranges for the reference varieties.

It was confirmed that while there was statistical significance on NDF, the difference was minimal in favor of better forage. Overall, the values fall within the range of commercial and ILSI values which provide proof of equivalency in the proximate analysis results.

In key nutrients analysis of forage, a corresponding non-transgenic, near-isogenic control corn was used as a comparator. Statistically, there are no significant differences between MZHG0JG, MZHG0JG + TSH and the control in terms of the amounts of calcium and phosphorus in forage. Furthermore, in reference to ILSI Database, mean levels of calcium and phosphorus in MZHG0JG corn and MZHG0JG corn + TSH were also within the ranges. Again, it was confirmed that based on the results of the analysis, no statistical differences in mineral composition of forage MZHG0JG were observed that can be considered as biologically relevant.

In key nutrients analysis of grain, A corresponding non-transgenic, near-isogenic control corn was used as a comparator. Statistically, there are no significant differences between MZHG0JG, MZHG0JG + TSH and the control in terms of the amounts of calcium, magnesium, manganese, phosphorus, potassium, selenium, sodium, and zinc. The amounts of copper and iron were significantly lower in the two transgenic plants compared to the control.

Furthermore, between MZHG0JG corn and the non-transgenic control corn, levels of vitamins B1, B2, B3, and B9 has no significant differences; level of vitamin A was significantly higher than in the control; and levels of vitamins B6 and E were significantly lower than in the control. Between MZHG0JG corn + TSH and control, no statistically significant differences were observed in the levels of B2, B3, B6 and B9; levels of vitamin A was higher than in the control; on the other hand, levels in B1 and E was lower than in the control. The assessors reported that based on the results of the analysis provided by the developer, no statistical differences between the mineral and vitamin content of MZHG0JG grains were observed that can be considered as biologically relevant.

In addition, the assessors reported that no significant differences were observed in the levels of 15 amino acids between MZHG0JG corn and the non-transgenic control corn. The levels of aspartic acid, arginine, and tryptophan were significantly lower in MZHG0JG corn than in the control corn. Between MZHG0JG corn and control corn, no statistically significant differences were observed in levels of 14 amino acids. The levels of aspartic acid, lysine, arginine, and tryptophan were lower in MZHG0JG corn + TSH than in the control corn.

Furthermore, as shown in the results of the analysis, there is no significant difference between the proportions of 16:0 palmitic, 16:1 palmitoleic, 18:0 stearic, 18:1 oleic, 18:2 linoleic, 20:0 arachidic, 20:1 eicosenoic, and 22:0 behenic fatty acids in MZHG0JG compared to the non-transgenic comparator. The proportions of 17:0 heptadecanoic acid and 18:3 linoleic fatty acid were significantly higher in MZHG0JG than in the non-transgenic comparator.

In terms of the treatment with TSH, MZHG0JG showed higher proportions of 17:0 heptadecanoic and 18:3 linolenic fatty acids and lower proportions of 16:0 palmitic fatty acids compared to non-transgenic corn.

The assessors concluded that based on the results of the analysis provided by Syngenta regarding the amino and fatty acid levels of MZHG0JG and non-transgenic comparator, statistical differences observed were not considered as biologically relevant.

For the analysis of antinutrients, there is no significant differences between the levels of ferulic acid, inositol, phytic acid, trypsin inhibitor and raffinose in MZHG0JG compared to the non-transgenic comparator. The level of ρ-coumaric acid observed was significantly higher in MZHG0JG than the non-trangenic corn. The same was observed in MZHG0JG + TSH including inositol which also exhibited significantly higher level than the non-transgeninc comparator. In the MZHG0JG corn, and MZHG0JG corn + TSH, the mean levels of anti-nutrients were within the ranges for the reference varieties. In reference to ILSI Database, mean levels of anti-nutrients in MZHG0JG corn and MZHG0JG corn + TSH were within the ranges. Based on the results of the analysis, no statistical differences between the levels of anti-nutrients of MZHG0JG and the non-transgenic corn were observed that can be considered as biologically relevant.

K. Recommendation

After a thorough scientific review and evaluation of the documents provided by the Bureau of Plant Industry (BPI) to the STRP, BAI, and BPI-PPSSD, the assessors found scientific evidence that the regulated article applied for human food and animal feed is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health.

DENR ASSESSMENT AND RECOMMENDATION

After thorough and scientific reviews and evaluation of the document provided by the Bureau of Plant Industry (BPI) to the DENR Biosafety Committee within the prescribed period pursuant to Joint Department Circular (JDC) No. 1 s2016 on the application of Syngenta Philippines, Inc. for direct use for feed, food or processing of Genetically Modified Corn MZHG0JG, along with the submitted sworn statement and accountability of the proponent, a biosafety permit may be issued to the proponent if the conditions set by DENR are followed.

DOH ASSESSMENT AND RECOMMENDATION

After a thorough scientific review and evaluation of the documents, DOH find sufficient evidence that the regulated article applied for direct use will not pose any significant risk to health and environment and that any hazards could be managed by the measures set by DOH.

SEC Assessment and Recommendation

According to the SEC expert, the country has become a marginal importer of yellow corn and in fact has been self-sufficient in its production. He reported that the importation of corn MZHG0JG may contribute a significant share in agricultural trade as a contributing factor to current corn supply and prices. In terms of consumption, importation of this event will increase the consumption of yellow corn for food, feed and processing due to lowered prices. Its importation will also drastically improve the patterns of consumption and it will also improve the patterns of production of livestock, poultry and aquaculture.

The SEC expert also noted that the importation of this corn MZHG0JG will help stabilize the prices of yellow corn, which will result to increase utilization of corn for food, feed production and processing. With the stabilization of feed prices, it will encourage more poultry and livestock production in the rural areas. With the increase in production, more employment will be generated by the poultry and livestock sub-sector. In addition, the stabilization of prices of yellow corn and feeds due the importation of this event will also results to increase employment in the related industries.

In terms of competitiveness, the assessor reported that the importation of MZHGOJG corn will affect country's foreign trade. More imports (holding other factor constant) will result to decrease of the foreign earnings and may affect our foreign exchange rate.

On the issue of Intellectual Property Rights, the expert has noted that the Philippines had promulgated substantial safety net policies and laws to protect the interest and welfare for both the consumers and farmers as well as the researchers. On the issue of Social Cohesion, the expert remarked that a) the importation of this event will not cause any negative effects on the social structures in the rural areas, b) doesn't have any negative impact today's and future human generations c) not negatively affect the basic human needs. In fact, it may positively affect due stable prices of yellow corn.

There were no evidence from the different studies conducted on GM products in general and on this event in particular that adaptation of the event will alter ethical norms in marketing. There are also no studies that point out to potential risk of conflict with the ideals of human solidarity and equality.

With the above scenario and observed consequences of importing MZHGOJG corn – stable yellow corn and feed prices, I recommend for the approval of permit for importation of MZHGOJG corn.