ASSESSORS' CONSOLIDATED REPORT ON SYNGENTA PHILIPPINES' APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF CORN MZIR098

EXECUTIVE SUMMARY

On April 2, 2018, Syngenta Philippines Inc. submitted corn MZIR098 for direct use as food and feed, or for processing, as original application 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- Biotech Team (BAI-BT), concurred that corn MZIR098 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 the conditions set by DENR 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 MZIR098 will not pose any significant risk to the 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 MZIR098.

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, except for the SEC expert, the complete dossier submitted by Syngenta Philippines. The SEC expert, on the other hand, was provided with a questionnaire on socio-economic, ethical and cultural considerations that have been addressed by Syngenta in relation to their application.

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

STRP ASSESSMENT AND RECOMMENDATIONS

Based on the documents submitted by the applicant:

A. Host Organism

Corn is a source of essential nutrients. It contains macronutrients such as starch/carbohydrates, amino acids, and fatty acids, micronutrients such as beta-carotene and B-vitamins, and minerals like calcium and potassium. Few anti-nutrients are reported to occur in corn. Phytic acid reduces some minerals. It also contains other antinutrients such as raffinose and trypsin inhibitor.

Corn leaves and root tissues contain a potential toxicant. This is 2-4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one or DIMBOA. According to OECD (2002), the levels of DIMBOA vary among corn varieties and its amount declines as the plant matures. Corn is also not considered to be a common allergenic food. Corn has been listed as a "less common allergenic food".

In addition, corn (Zea mays L.) is a commodity crop grown worldwide for various uses, including food and feed. In the United States, the world's leading producer of corn, several different types of corn are cultivated, including field corn (e.g.,yellow dent, white dent), sweet corn, and popping corn. Corn may be consumed as whole grain but is primarily used in food in the form of processed products such as high fructose corn syrup, cereals, oil, meal, flour, starch, and grits. Corn grain can be processed either by wet milling, dry milling or alkali treatment. Corn oil is rich in polyunsaturated fatty acids and is used as a salad oil, as cooking oil, and in margarine.It is also an important crop for animal feed. Corn grain and by-products of corn processing may be included in diets for most animal species. Corn silage is a readily digestible, high-energy, and fermented forage product. It is fed primarily to ruminants (e.g., cattle, sheep and goats). For animal nutrition, corn is considered to be an important source of energy, essential fatty acids and some of the essential amino acids.

The estimated consumption of corn of the Cluster G09 (where Philippines is included) is 16.736 g/kg bw/day for the general population and for the children, it is 32.518 g/kg bw/day (WHO, 2012)

B. Transgenic Plant

MZIR098 corn has food approval in the U.S. (2016), Australia and New Zealand (2016), Japan (2017), and Canada (2016). MZIR098 corn is not materially different in composition, safety, and other relevant parameters from the conventional corn in the market. It also has approval for sale as feed in the US and Canada. Syngenta has concluded that its corn variety, MZIR098 corn, and the feeds derived from it are as safe and are not materially different in composition or any other relevant parameter from the conventional corn in the market.

It is anticipated that the introduction of MZIR098 maize will replace some of the maize in existing food and feed products but the food consumption pattern is not expected to change significantly with the introduction of MZIR098.

C. Donor Organism

The source and potential pathogenic or allergenic properties of the transgenes ecry3.1Ab, mcry3A and pat-08 contained in MZIR098 were adequately described. Transgenes ecry3.1Ab and mcry3A that codes for insecticidal proteins were derived from B. thuringiensis, which is a ubiquitous bacterium in the soil. The native Cry1Ab protein originated from Bacillus thuringiensis subsp. kurstaki while the Cry3A protein originated from Bacillus thuringiensis subsp. tenebrionis. Insecticidal Cry Bt proteins have no potential pathogenic or allergenic properties. These have been used and consumed safely for decades (Koch et al.,2015)

The transgene pat-08 came from Streptomyces viridochromogenes, which is a common nonpathogenic bacterium in the soil. Streptomyces species are naturally occurring and found

predominantly in soil and decaying vegetation; few are associated with pathogens. Streptomyces species that express PAT or its homologues have no toxic or allergenic effects on humans or animals. Moreover, PAT has no homology to known toxic proteins and does not possess characteristics typical to allergenic proteins (Karenlampi, 1996)

E. Transformation System

The method used to produce corn MZIR098 was Agrobacterium tumefaciens – mediated transformation of immature embryos of a proprietary corn line.The genetic modification targets to integrate two insecticidal genes (ecry3.1Ab and mcry3A) from Bacillus thuringiensis and one transgene (pat-08) from Streptomyces viridochromogenes into the nuclear genome of corn. This was done in order to produce MZIR098 corn, which has dual modes of action for corn rootworm control in a single event. In additon, MZIR098 maize plants are also tolerant to glufosinate-ammonium herbicide products since it has the gene pat-08, which encodes for the PAT enzyme. This acetylates glufosinate-ammonium, inactivating it and conferriing resistance to glufosinate-ammonium herbicides.

The experimental protocol was completely provided. The details of the transformation system used were provided. These include the protocol for the Agrobacterium tumefaciens-mediated transformation, development of the MZIR098 corn, production and quality control of test and control seeds and genetic elements (potentially inserted regulatory sequences). Particulars of the genetic characterization aspect were also provided. This step ensures that only the genes of interest were introduced and no extraneous DNA fragments occur elsewhere in the MZIR098 corn genome. Finally, the protocols for PCR, sequence alignment and Southern blot analyses were also provided.

F. Inserted DNA

Southern blot analyses were done to determine the number of plasmid pSYN17629 T-DNA integration sites and showed that MZIR098 corn contained a single T-DNA insert. Genetic characterization studies, using nucleotide sequence analyses, demonstrated that the MZIR098 corn contained, at a single locus within the corn genome, a single copy of each of the following functional elements: ecry3.1Ab, mcry3A, pat-08, NOS-02 enhancer, CMP-04 promoter, Ubil-18 promoter, NOS-20 terminator, 35S-04 promoter, and two copies of the NOS-05-01 terminator.

A single T-DNA specific probe that detected every base pair of the pSYN17629 T-DNA expected to be transferred and integrated into the corn genome was also used. This was useful in determining the number of T-DNA integration sites within the MZIR098 corn genome as well as the number of copies of the T-DNA at each integration site within the MZIR098 corn genome. The methods employed are sufficient to demonstrate the integration sites.

Sequence analysis of the MZIR098 insertion site showed that the 24-bp from the corn genomic seqience was deleted during the integration of the MZIR098 insert. Truncation also occurred at the right and left border ends of the T-DNA. Specifically, the right border, along with 10 base pairs of non-coding sequence and 10 bp from the left border were truncated. However, these truncations have no effect on the functionality of the T-DNA.

eCry3.1Ab and mCry3A proteins have been expressed and approved in only GM corn plants, whereas PAT proteins have been expressed and approved in GM corn, canola, cotton and soybean. No plasmid backbone sequences were present. Southern blot analyses were done to confirm this. The presence or absence of the plasmid backbone was determined using two backbone-specific probes that together covered the sequences of pSYN17629 outside the T-DNA.

H. Expressed Material

The genetic stability of the introduced traits in MZIR098 was assessed using two methods: Southern blot analyses over five generations namely, F1, F2, F3, F4, and F5; and examination of inheritance patterns of the three genes across three generations, specifically at BC2F1, BC3F1, and BC4F1. Using a probe that spans the MZIR098 insert, three different restriction enzymes and the appropriate controls, Southern blot analyses consistently showed the expected banding patterns across the five generations.

Three generations of MZR098 corn were individually analyzed for the presence of ecry3.1Ab, mcry3A, and pat-08 by real-time PCR analysis (Ingham et al., 2001). The results from the real-time PCR analysis were used to determine the segregation ratios of ecry3.1Ab, mcry3A, and pat-08. Hemizygous MZIR098 corn plants of the F2 generation were crossed with nontransgenic corn line NP2391. The resulting F1 generation was backcrossed with the nontransgenic recurrent parent (NP2391) to yield the BC1F1 generation. MZIR098 corn plants from the BC1F1 generation were backcrossed three more times with the nontransgenic recurrent parent (NP2391) to yield the BC2F1, BC3F1, and BC4F1 generations analyzed in the study. The expected segregation ratio for each gene was 1:1 in each generation (i.e., 50% of the plants in each generation were expected to carry the genes). Ch-square analysis of the segregation data was performed to test the hypothesis that the MZIR098 insert is inherited in a predictable manner according to Mendelian principles and consistent with insertion into a chromosome within the corn nuclear genome. The expected and observed segregation ratios are shown in Table 5 – Observed and expected frequencies of ecry3.1Ab, mcry3A, and pat-08 in three generations of MZIR098 corn.

I. Toxicological Assessment

The eCry3.1Ab protein was rapidly digested upon exposure to the pepsin enzyme in SGF at pH 1.2 in less than 30 seconds. No fragments were detected by western blot analysis after 30 seconds of exposure to SGF. For SIF, no intact eCry3.1Ab protein was detected following the incubation in SIF fluid for 1 minute. Some immunoreactive fragments of eCry3.1Ab protein with molecular weights of approximately 56, 40, and 5 kDa were present at the conclusion of the 48-hour time course of the study. These findings indicate that eCry3.1Ab protein are sensitive to proteolysis by pepsin and pancreatin and is rapidly degraded to constituent peptides.

The temperature stability of eCry3.1Ab protein was evaluated by incubation of aliquots of an aqueous solution of microbially produced eCry3.1Ab test substance at 40C (control), 250C, 370C, 370C, 650C and 950C for 30 minutes and assessing the immunoreactivity using an enzyme-linked immunosorbent assay (ELISA) and determination of the the loss of insecticidal activity in a bioassay with larvae of the Colorado potato beetle (Leptinotarsa decemlineata), a sensitive coleopteran species.

There were no sequence homology structural alerts for potential toxicity of the eCry3.1 Ab Protein. The acute oral toxicity of eCry3.1Ab is performed in mice. Microbially-produced eCry3.1Ab was administered orally by gavage in a single dose of 2000 mg protein/kg body weight of five male and five female mice. NOEL is 2000 mg eCry3.1Ab protein/kg body weight.

The recombinant Escherichia coli was the source of test protein. The result demonstrate that the microbially produced eCry3.1 Ab protein is biochemically and functionally equivalent to the eCry3.1Ab protein produced in MZIR098 corn when compared with respect to identity, integrity and insecticidal activity. Hence, The microbially produced eCry3.1 Ab protein is a suitable surrogate to evaluate the safety of of eCry3.1Ab produced in MZIR098 corn.

On the other hand, the mCry3A protein was rapidly degraded upon exposure to the pepsin enzyme in SGF at pH 1.2. No intact mCry3A or mCry3A- derived fragments were immunologically detected by western blot analysis after 2 minutes of exposure to SGF. For SIF, no intact eCry3.1Ab protein was detected following the incubation in SIF fluid for 5 minutes.The findings support the conclusion that mCry3A is sensitive to proteolysis by pepsin and pancreatin and is rapidly degraded to constituent peptides.

The immunoreactivity and bioactivity of mCry3A is lost upon heating at 950C. The temperature stability of mCry3A was evaluated by ELISA, while its bioactivity was determined by an insecticidal assay using D. virgifera virgifera.

Results of database comparisons (with NCBI and Sygenta database) confirmed that mCry3A is not a toxic protein in mammals, nor does mCry3A shared significant sequence similarity with other known or putative protein toxins.

Microbiologically produced mCry3A was administered as a single oral dose via gavage to groups of 5 male and 5 female at a dose level of ca.2377 mg active ingredient/kg bodyweight.Results showed no evidence of toxicity resulting from the administration of microbiologically produced mCry3A.

In order to assess the biochemical and functional equivalence of mCry3A protein derived from MZIR098 corn; it was compared with mCry3A recombinant E. coli expression system. The results demonstrate that the microbially produced mCry3A protein is biochemically and functionally equivalent to mCry3A produced in MZIR098 corn. Specifically, the Western blot revealed mobility and immunoreactivity equivalence. The microbially produced mCry3A protein is a suitable surrogate to evaluate the safety of mCry3A produced in MZIR098 corn.

Meanwhile, the digestibility of PAT was investigated in vitro in simulated mammalian gastric fluid (SGF) using enzyme pepsin and simulated mammalian intestinal fluid (SIF) using enzyme pancreatin. For SGF, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western Blot analyses were used to evaluate the in vitro digestibility of PAT in SGF over a 60-minute time course at 37oC. For SIF, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western Blot analyses were used to evaluate the in vitro digestibility of PAT in SIF over a 48-hour time course at 37oC.

No intact PAT or PAT-derived fragments were immunologically detected by western blot analysis after 1 minute of exposure to SGF. For SIF, no intact PAT proteins were detected upon sampling of the reaction at 5 minutes.

Microbially-produced PAT was exposed to different temperatures (4 °C – control, 25 °C, 37 °C, 65 °C, and 95 °C) for 30 min. Loss or decrease of PAT immunoreactivity was measured by ELISA and assessment of specific enzymatic activity by a continuous spectrophotometric assay measuring the formation of 2-nitro-thiobenzoate anion during acetylation pf phospinothricin. The PAT immunoreactivity was below the limit of detection when incubated at 95 °C. Its enzymatic activity was below the limit of detection at 65 °C. From the results of the experiments, it can be concluded that PAT had loss its functional activity at 65 °C and its immunoreactivity at 95 °C.

PAT protein sequence matched with 14 out of 15 protein sequence belonging to GNAT toxin-antitoxin (TA) system. However, the information provided gave sufficient evidences justifying the sequence homology: 1) GNAT superfamily of proteins is universally distributed in nature thus the possibility of sequence homology is possible; 2) These GNAT proteins catalyze the transfer of acetyl group from acetyl coenzyme A to a variety of substrates, including proteins and small molecules. In bacteria, GNATs are involved in a variety of functions, including antibiotic resistance; 3) These GNAT

domains are the source of the alignments to the PAT protein, which is also an acyltransferase; acyltransferases are not toxic proteins; and 4) PAT catalyzes a very specific reaction, not recognizing even close structural homologs of its actual substrate; therefore, the applicant claimed that " PAT is unlikely to act on the same substrates as the TA system components, and un-intended acetylation reactions catalyzed by PAT are unlikely to occur". These justifications support the conclusion that PAT is not a toxic protein nor does PAT share significant sequence similarity with other known or putative protein toxins.

Microbially-produced PAT was administered orally by gavage in a single dose of 2000 mg protein/kg body weight of five male and five female mice. NOEL is 2000 mg PAT protein/kg body weight.

In order to assess the biochemical and functional equivalence of PAT protein derived from MZIR098 corn, it was compared with PAT recombinant E. coli expression system. The results demonstrate that the microbially produced PAT protein is biochemically and functionally equivalent to PAT produced in MZIR098 corn. The microbially produced PAT protein is therefore a suitable surrogate to evaluate the safety of PAT produced in MZIR098 corn.

J. Allergenicity Assessment

Two bioinformatics comparisons (using the Comprehensive Protein Allergen Resource) were done to determine whether eCry3.1Ab had biologically relevant amino acid sequence similarity to known or putative allergens. No significant sequence similarity was observed between the eCry3.1Ab amino acid sequence and any entry in the database. The FASTA search returned 3 alignments with E-values less than 10, none of which exceeded the minimum significance criteria of >35% shared identity over a minimum of 80 amino acids of alignment length. Also, no matches between any sequence of eight or more contiguous amino acids of eCry3.1Ab and any entry in the database were found.

Glycosylation analysis indicates that no glycosylation of eCry3.1Ab protein produced in MZIR098 had occurred in planta. Based on Western Blot mobility, the molecular weights were 74.8kDa and 73.8kDa, respectively, which were consistent with the predicted molecular weight for microbially-produced and plant-produced eCry3.1Ab. Using maximum protein expression levels in the kernels of MZIR098, eCry3.1Ab constitutes ~0.00372% of the total kernel weight and assuming that it constitutes ~10% of the total kernel weight, then, eCry3.1Ab comprises ~0.0059% total protein in the kernels.

On the other hand, a full-length sequence search using FASTA, and a separate search for exact matches of eight or more contiguous amino acids, were used to compare mCry3A to each of the known or putative allergen sequences. Together, these results support the conclusion that mCry3A shares no biologically relevant amino acid sequence similarity to known or putative protein allergens.

The mCry3A proteins from both sources (microbially produced and plant produced) were demonstrated to have the predicted molecular weight of ~67.7 kDa and immunologically cross-reacted with the same antibodies. The mCry3A produced in MZIR098 corn was analyzed to ensure that no post-translational glycosylation of the protein(s) had occurred in planta. The Western blot mobility of mCry3A proteins was consistent with the predicated molecular weight for microbially produced and plant-produced mCry3A (approximately 67.7 kDa). Lastly, mCry3A comprises around 0.002283% of the total weight in kernels.

Meanwhile, the deduced amino acid sequence of PAT was subjected to full length sequence search using FASTA and a separate search for exact matches of eight or more contiguous amino acids against known or putative allergens in the Comprehensive Protein Allergen Resource (COMPARE)

database version 2017. Results of the search showed that PAT shares no significant sequence similarity to known or putative protein allergens.

Glycosylation analysis indicates that no glycosylation of PAT protein produced in MZIR098 had occurred in planta. The mCry3A proteins from both sources (microbially-produced and plant-produced) were demonstrated to have the predicted molecular weight for microbially-produced and plant-produced PAT (approximately 20.5 kDa).

Assuming that the protein constitutes ~10% of the total kernel weight (ILSI, 2014), then the PAT comprises ~0.000025% of the total protein in kernels.

K. Nutritional Data

In both MZIR098 corn and the nontransgenic control corn, the mean levels of all proximates and minerals were within the ranges for the reference varieties and the ranges reported in the ILSI database. Forage from MZIR098 is not materially different in nutrient composition from forage of the nontransgenic, near isogenic comparator or of conventional field corn. There are also no significant differences between MZIR098 and its non-transgenic, near-isogenic counterpart in terms of calcium and phosphorus in the forage.

In statistical comparisons between MZIR098 maize and the nontransgenic control maize, no significant differences were observed in the levels of any proximates and starch in the grain. No significant differences were observed in the levels of protein, fat, ash, carbohydrates, ADF, TDF in the comparisons between the test + Trait Specific Herbicide (TSH) and the control. The levels of NDF were significantly higher in the test + TSH than in the control maize and the starch was significantly lower.

There were also no significant differences observed in the levels of iron, magnesium, manganese, phosphorus, or zinc. The levels of calcium, copper and potassium were significantly higher in MZIR098 maize than in the control maize. No statistically significant differences were observed in the levels of calcium, copper, iron, magnesium, manganese, phosphorus and zinc in comparisons between the MZIR098 corn (Test) + Trait Specific Herbicide (TSH) and the control. The levels of potassium were higher in the Test + TSH corn than in the control corn. For selenium and sodium, low levels below the LOQ precluded calculation of the means and statistical comparisons across locations and treatments.

Additionally, no statistically significant differences were observed in levels of Vitamins A, B1, B2, B3, B6, B9 between the MZIR098 corn (Test) + TSH than in the control corn. The levels of vitamin A (beta-carotene) and Vitamin E (alpha-tocopherol) were higher in MZIR098 corn (Test) + Trait Specific Herbicide (TSH) than in the control corn. There were also no statistically significant differences observed in levels of 17 amino acids between the MZIR098 maize and the nontransgenic control maize. The level of lysine was significantly lower in the test corn than in the control corn.

Furthermore, no statistically significant differences were observed in levels of 18 amino acids between the MZIR098 corn (Test) + Trait Specific Herbicide (TSH) than in the control corn. There were also no statistically significant differences observed in proportions of 16:0 palmitic, 16:1 palmitoleic, 18:3 linolenic, 20:1 eicosenoic, and 22:0 behenic fatty acids between MZIR098 (Test) or MZIR098 (test) + TSH and the control. In both the test and test + TSH corn, the proportions of 17:0 heptadecanoic and 18:2 linoleic fatty acids were significantly higher than in the control corn and proportions of 18:0 stearic, 18:1 oleic, and 20:0 arachidic fatty acids were significantly lower. The levels of other fatty acids were below the LQQ for all replicates.

Meanwhile, no statistical difference were observed in the levels of of ferulic acid, p-coumaric acid, inositol, phytic acid, trypsin inhibitor, and raffinose between the test and the test+TSH and the control maize. For furfurals, levels below the limit of quantification (LQQ) precluded calculation of the means and statistical comparisons across locations.

L. Recommendation

Find scientific evidence that the regulated article applied for human food and animal feed use is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health

BPI-PPSSD and BAI ASSESSMENT AND RECOMMENDATION

A. Host Organism

Corn contains protein, fat, carbohydrates, and dietary fiber but is not typically consumed for specific nuntrients (OECD, 2002). It contains only few anti-nutrients as reported. Phytic acid reduces the availability of phosphorus, especially in mono-gastric animals. Other anti-nutrients such as raffinose and trypsin inhibitor are not considered nutritionally significant in corn (OECD, 2002).

No significant native toxins are reported to be associated with the genus Zea (International Food Biotechnology Council, 1990). 2-4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA) has been described as potential toxicants in corn leaves and roots tissues. DIMBOA levels vary by orders of magnitude among corn varieties, and declines rapidly as plants grow (OECD, 2002). It is also not a common allergenic food, although in some case-studies, allergenic reactions were reported. Based on the OECD report (2002) and information provided by the developer, maize has been described as a food that is likely to have low allergenicity. Gastrointestinal and respiratory allergenic reactions were reported in some cases.

Most of the human consumption of corn is in the form of corn-based ingredients such as high fructose corn syrup, starch, sweeteners, cereals, oil and alcohol, while field corn is used mainly for animal feed and for processing and is preferred in livestock production as a processed whole grain, as a by-product of the milling industry, or as silage. The estimated consumption of corn of the Cluster G09 (where Philippines is included) is 16.736 g/kg bw/day for the general population and for the children, it is 32.518 g/kg bw/day (WHO, 2012).

B. Transgenic Plant

MZIR098 Corn has been approved as food for direct use and processing in countries such as Australia, Canada, New Zealand and USA last 2016. It was also approved as feed for direct use and processing in countries such as Canada, and USA last 2016. Consumption pattern by population subgroups is not expected to change with the introduction of MZIR098 corn.

C. Donor Organism

A complete description of ecry3.1Ab gene encoding eCry3.1Ab protein, mcry3A gene encoding mCry3A protein derived from B. thuringiensis, and pat gene encoding PAT protein derived from Streptomyces viridochromogenes (Syngenta, unpublished) was provided. These donor organisms has a long history of safe use.

Cry and PAT proteins have a long history of safe use in food crops and no proteins have been reported as toxic or allergenic in humans or animals (Kutzner, 1981). This was being supported by

the bioinformatics analyses provided by the developer indicating that all protein encoding sequences in MZIR098 do not share structurally or immunologically relevant amino acid sequence similarity with known allergen.

Based on the information provided by the proponent the donor organisms, Bacillus thuringiensis, a ubiquitous soil bacterium, and Streptomyces viridochromogenes, a common nonpathogenic soil bacterium, are not known to be toxic or allergenic (Taylor et al, 2001; Kutzner, 1981). History of safe use is attributed to the donor organisms.

E. Transformation System

The transformation method is Agrobacterium tumefaciens-mediated transformation (Negrotto et al. 2000). Nuclear DNA is the target of genetic modification (Syngenta, n.d., Section V, Inserted DNA, pp. 31-41)

A complete experimental protocol for the transformation of Z. mays to MZIR098 corn is provided(Komari et al., 1996; Negrotto et al., 2000; Xing et al., 2008). They also provided a complete list of all genetic components used in the transformation of corn MZIR098 (Syngenta, unpublished, Section IV, Table 2 pp 27-30).

The developer provided a complete descrition of the plasmid vector pSYN17629 used to produce MZIR098 corn by A. tumefaciens–mediated transformation of immature corn embryos (Syngenta, unpublished, Section V, Table 2, pp. 27-30). The DNA region between the left and right borders of the transformation plasmid included gene-expression cassettes for ecry3.1Ab, mcry3A, and pat-08. The ecry3.1Ab expression cassette consisted a CMP promoter from cestrum yellow leaf curling virus (CMP-04), nopaline synthase (NOS) terminator sequence from A. tumefaciens (NOS-05-01) and NOS enhancer sequence (NOS-02). The mcry3A expression cassette consisted of a corn ubiquitin promoter (Ubi1-18) and NOS terminator (NOS-20). The pat-08 expression cassette consisted of the 35S promoter from cauliflower mosaic virus (35S-04) and NOS terminator (NOS-05-01).

F. Inserted DNA

The MZIR098 insert contains a single copy of each of the functional elements ecry3.1Ab, mcry3A, pat-08, NOS-02 enhancer, CMP-04 promoter, Ubi1-18 promoter, NOS-20 terminator, 35S-04 promoter and two copies of the NOS-05-01 terminator as expected.

Southern blot analyses were performed to characterize the transgenic insert of MZIR098 corn by determining the number of plasmid pSYN17629 T-DNA integration sites and the presence or absence of pSYN17629 backbone sequence or additional extraneous fragments of T-DNA. The Southern blot analyses also demonstrated that the hybridization bands specific to the MZIR098 insert were identical in all lanes containing genomic DNA extracted from MZIR098 corn plants of the generations tested. These results support the conclusion that the MZIR098 insert is stably inherited from one generation to the next and that MZIR098 corn contains a single T-DNA insert. No unexpected bands were detected, indicating that the MZIR098 corn genome contains no extraneous DNA fragments of the insert.

The number of T-DNA integration sites within the MZIR098 corn genome and the number of copies of the T-DNA at each integration site within the MZIR098 corn genome were determined through the use of a single T-DNA-specific probe that covered every base pair of the pSYN17629 T-DNA expected to be transferred and integrated into the corn genome.

There were no plasmid backbone sequences present in the genome of corn line MZIR098 demonstrated in Southern blot analysis provided by the developer. Hybridization bands were not detected confirming the absence of plasmid DNA in MZIR098 corn (Syngenta, unpublished).

G. Genetic Stability

The multigenerational generational stability of the insert was demonstrated by Southern blot analysis over five generations of MZIR098. Further stability of the insert was assessed by examining the inheritance patterns of the transgenes over three generations of MZIR098 corn (Syngenta, unpublished, Section VI. pp. 42-52). Results indicated that the introduced trait is stably inherited from one generation to the other during conventional breeding.

Segregation data were determined through detecting the presence of presence of ecry3.1Ab, mcry3A, and pat-08 by real-time PCR analysis in three generations of MZIR098 corn (Ingham et al. 2001). Hemizygous MZIR098 corn plants of the F2 generation were crossed with nontransgenic corn line NP2391. The resulting F1 generation was backcrossed with the nontransgenic recurrent parent (NP2391) to yield the BC1F1 generation. MZIR098 corn plants from the BC1F1 generation were backcrossed three more times with the nontransgenic recurrent parent (NP2391) to yield the BC2F1, BC3F1, and BC4F1 generations analyzed in this study. The expected segregation ratio for each gene was 1:1 in each generation. Chi-square analysis of the segregation data indicated that the MZIR098 insert is stably inherited following Mendelian law.

H. Expressed Material

The concentrations of eCry3.1Ab, mCry3A and PAT proteins in various MZIR098 corn tissues were quantified by enzyme-linked immunosorbent assay (ELISA) to establish an expression profile for these proteins as produced in MZIR098 corn. The tissues analyzed were leaves and roots at four growth stages (V6, R1, R6, and senescence)

The metabolic roles of the eCry3.1Ab, mCry3A and PAT proteins have been described.PAT acetylates glufosinate-ammonium, thus inactivating it and conferring tolerance to glufosinate-ammonium in herbicide products. Glufosinate-ammonium (L-phosphinothricin) inhibits glutamine synthetase, an enzyme in the nitrogen assimilation pathway. PAT is a highly specific enzyme for acetylation of glufosinate-ammonium, and it does not acetylate glutamate or other L-amino acids. The engineered protein eCry3.1Ab is a chimera of mCry3A and Cry1Ab that is active against D. virgifera virgifera and other related pests of maize. The native Cry3A from the soil bacterium B. thuringiensis subsp. tenebrionis is active against certain coleopteran pests. The native Cry1Ab from B. thuringiensis subsp. kurstaki is active against certain lepidopteran pests; however, the portion of Cry1Ab included in eCry3.1Ab has not preserved the activity of Cry1Ab against lepidopterans. eCry3.1Ab and mCry3A are not enzymes and therefore, do not affect plant metabolism.

eCry3.1Ab, mCry3A, and PAT are not expected to interact within, nor affect together the metabolism of the corn plant.

I. Toxicological Assessment

The eCry3.1Ab protein was rapidly degraded in SGF containing pepsin at pH 1.2. No intact eCry3.1Ab or eCry3.1Ab derived fragments were immunologically detectable by western blot analysis after 30 seconds of exposure to SGF. It was also evaluated in simulated mammalian intestinal fluid (SIF) containing pancreatin. SDS-PAGE and western blot analyses were used to evaluate the in vitro digestibility of microbially produced eCry3.1Ab in SIF over a 8-hour time course at 37ºC.

At 65ºC and 95ºC, the immunoreactivity of eCry3.1Ab was below the limit of detection for the ELISA. eCry3.1Ab was not immunodetectable following incubation for 30 minutes at 65ºC and above when analyzed by ELISA, indicating the loss of its immunoreactivity. At 95ºC, microbially produced eCry3.1Ab was incubated for 30 mins which resulted too low mortality to allow calculation of LC50 values and 95% confidence intervals.

The result of bioinformatics analysis provided by the developer using BLASTP showed that eCry3.1Ab protein has no homology to any known toxins in the toxin databases, and, based on the acute oral toxicity assessement conducted, the No Observable Effect Level (NOEL) for eCry3.1Ab is 2,000 mg protein/kg bodyweight. No treatment-related mortality, clinical signs of distress or impairment and anatomical pathology was observed upon administration of eCry3.1Ab in mice

Escherichia coli is used as source of test protein. The E. coli-produced eCry3.1Ab has been shown to be structurally and functionally equivalent to the plant-produced eCry3.1Ab present in MZIR098 through comparison of molecular weights and immunological properties via Western blot analysis, glycosylation analysis, determination of insecticidal activity and peptide mass coverage analysis.

On the other hand, microbially derived mCry3A was evaluated in simulated mammalian gastric fluid (SGF) containing pepsin and simulated mammalian intestinal fluid (SIF) containing pancreatin. Using SDS-PAGE and Western blot, no intact mCry3A or mCry3A-derived fragments were immunologically detectable after 2 minutes of exposure to SGF. In SIF, no intact mCry3A was detected at 5 minute point. A faint band of the same molecular weight as mCry3A was detected on SDS-Page after 5 minutes in SIF but was not detected using the anti-Cry3A antibodies. Thus, mCry3A protein is rapidly degraded by pepsin and pancreatin.

In addition, upon heating at 95ºC, mCry3A has lost it immunoreactivity and bioactivity. This was determined using ELISA and insecticidal assay using Diabrotica virgifera virgifera. The bioinformatics analysis using BLASTP showed that mCry3A protein has no homology to any known toxins in the toxin databases.

As for the acute oral toxicity assessement conducted, the No Observable Effect Level (NOEL) for mCry3A is 2,377 mg protein/kg bodyweight. No treatment-related effects were observed in mice upon acute oral dose of 2632 mg mCry3A/ kg body weight (~2377 mg/kg) (Syngenta, unpublished, Appendix 14).

Meanwhile, results of Western blot analysis provided by the developer indicated that the PAT protein is rapidly degraded in SGF and SIF with pepsin and pancreatinin, respectively, within one (1) minute. Upon heating at 65ºC, PAT has lost it functional activity and immunoreactivity. This was determined using ELISA and insecticidal assay using continuous spectrophotometric assay (modified from the method described by Thompson et al. [1987] and D'Halluin et al. [1992]

Further, it was evaluated by incubation of aliquots of an aqueous solution of microbially produced PAT test substance at 4ºC (control), 25ººC, 37ººC, 65ººC, and 95ººC for 30 minutes and assessing immunoreactivity using an enzyme-linked immunosorbent assay (ELISA). Complete loss of immunoreactivity was observed at 95ºC after 30 minutes. The No Observable Effect Level (NOEL) for PAT is 2,000 mg protein/kg bodyweight. No treatment-related effects were observed in mice upon acute oral dose of 2000 mg mCry3A/ kg body weight (Syngenta, unpublished, Appendix 16).

J. Allergenicity Assessment

Using FASTA, a full-length sequence search and a separate search for eight or more contiguous amino acids, eCry3.1Ab, mCry3A, and PAT shares no biologically relevant amino acid sequence similarity to known or putative protein allergens.

The microbially produced and plant produced eCry3.1Ab proteins, mCry3A protein, and PAT protein have molecular weight of ~74.8 kDa, ~67.7 kDa and ~20.5 kDa respectively and immunologically cross-reacted with the same antibodies.

Based on the documents provided by the developer, the eCry3.1Ab comprises ~0.00372% of the total protein in kernels (Section IX.A.7. pp. 76-77), the mCry3A comprises ~0.01451% of the total protein in kernels and PAT comprises <0.000025% of the total protein in kernels.

K. Nutritional Data

Based on the compositional analysis, there is no significant differences between the levels of moisture, protein, ash, fat, carbohydrates, ADF and NDF of MZIR098 forage and the non-transgenic control. There is also no significant differences between the levels of moisture, protein, ash, fat, carbohydrates, ADF, NDF, TDF and starch of MZIR098 grains and the non-transgenic control

In addition, there is no significant differences between the levels of calcium and phosphorus of MZIR098 forage and the non-transgenic control, also there is no significant differences between the levels of minerals of MZIR098 grains and the non-transgenic control except for calcium, copper and potassium. MZIR098 treated with TSH has significantly higher potassium level compard to the non-transgenic control.

Further, there is no significant differences between the levels of vitamins of MZIR098 grains and the non-transgenic control except for Vitamin A. MZIR098 treated with TSH has significantly higher level of Vitamin A and E compared to the non-transgenic control. There is also no significant differences between the levels of amino acids of MZIR098 grains and the non-transgenic control except for lysine, additionally there is no significant differences between the levels of fatty acids of MZIR098 grains treated and untreated with TSH and the non-transgenic control except for heptadecanoic, streatic, oleic, linoleic, arachidic.

Meanwhile, there is no significant differences between the levels of anti-nutrients and secondary metabolites of MZIR098 grains treated and untreated with TSH and the non-transgenic control.

L. Recommendation

Upon evaluation of the documents provided by the proponent and scientific literature search conducted for the food safety risk assessment of corn MZIR098, the following assessments were made:

- History of safe use is attributed on the host organism (Zea mays L.) as well as the donor organism, Bacillus thuringiensis and Streptomyces viridochromogenes, which are not know to be toxic or allergenic to humans and animals based on the toxicity and allergenicity studies.
- Safety of the novel proteins, eCry3.1Ab, mCry3A and PAT, in MZIR098 were assessed based on the digestibility, heat inactivation, amino acid sequence comparison and oral toxicity studies provided by the developer. Results of the analyses indicated that the novel proteins are digested rapidly in mammalian gastric fluid, a characteristics of dietary proteins, are being inactivated by induction of heat which is normally occurring during processing and cooking, and do not cause toxicity on mice via acute oral gavage. Amino acid sequence

analysis indicated that eCry3.1Ab, mCry3Ab and PAT has no significant homology to any toxins or allergens.

- Safety assessment based on the nutritional data indicates that there is no significant difference between the proximate, fiber, vitamin, mineral, amino acid, fatty acid, anti-nutrient and secondary metabolite levels of MZIR098 corn and conventional corn that can be considered biologically relevant.

Weight of evidences approach indicates that the single event, corn MZIR098, is substantially equivalent with the conventional counterpart in terms of nutritional composition and food safety, other than the protection to certain lepidopteran insects and tolerance to glufosinate-ammonium-containing herbicides. After reviewing the provided material of Monsanto Philippines, Inc. and other literatures, it is therefore concluded that corn MZIR098 is as safe as its conventional counterpart.

DENR ASSESSMENT AND RECOMMENDATION

After a comprehensive review and evaluation of the documents including the scientific evidences from provided references and literature submitted by Syngenta Philippines, Incv on its application for Direct Use as FFP of Corn MZIR098. hereunder are the observations and appropriate actions:

1. The direct use of the regulated article whether for food, feed or for processing will not cause any significant adverse effect on the environment (land, and water〕 and non-target organisms. The transgenic crop will not increase its weediness potential in case the seeds spill out into the environment because eCry3.1Ab, and mcry3A protein product produced by the transgenic crop will degrade upon exposure to the natural environment and general conditions that is high temperatures (950C and above for both proteins〕， varying pH, enzyme digestion, etc. (Song, 2010〕 and (Joseph, 2003).

2. Cry proteins, when consumed, are converted to active toxin in an alkaline gut, and binds to specific Cry protein receptors present on the midgut of lepidopteran insects. Mammals do not possess alkaline guts and Cry protein-binding receptors which makes them invulnerable to toxicity (Hoffmann, Vanderbruggen, Hofte, Rie, & Mellaert, 1988〕 and (Shai & Aronsol, 2001). Moreover, mammalian digestive environment contains pepsin, which readily digests Cry and PAT proteins (FSANZ, 2003). Mammalian gastric environment is also similar with the physiology of digestion of avian gastrointestinal tract, in terms of pH and type of enzyme secreted (Privalle, 1994).

3. The project description report (PDR) discusses the specified environmental management plan indicating the possible risk and harm to the environment and non-target organisms as well as the mitigating measures and contingency plan. Furthermore, the chances of unintended release or planting of the regulated article is very minimal and will not cause any damaging and lasting effects because the receiving environment (areas near the port, roads, railways, etc.) is not conducive for plant growth. Also, corn is a highly domesticated plant that requires human intervention for it to persist in the environment (Organization for Economic and Development (OECD〕， 2003〕 and (Raybould, et alv 2012〕

4. The Bureau of Plant Industry (BPI〕 shall ensure the proper and secure packaging of the regulated article for transport and the safety and durability of the transport vehicle, for prevention of any possible spillage or unintended release during transport/import based on BPI's inspection in the port area.

Based on the review and evaluation, the DENR-BC considered the regulated article safe to the environment and non-target organisms and hereby submits the technical report relative to the application of Syngenta Philippines, Inc. Corn MZIR098 for Biosafety Permit for direct use as food, feed, or for processing

DOH ASSESSMENT AND RECOMMENDATION

After a thorough review and evaluation of the documents provided by the proponent, Syngenta Philippines Inc., through the Bureau of Plant Industry (BPI), in support of their application for approval for Direct Use for Food and Feed or for Processing (FFP) of Corn MZIR098. I/We,

Find that the regulated article applied for Direct Use for Food and Feed or for Processing (FFP) is safe as its conventional counterpart and shall not pose any significant risk to human and animal health, and environment.

The following are the observations and recommendations:

1. Scientific pieces of evidence from provided toxicity studies and references find that the regulated article will not cause significant adverse health effects to human and animal health.

2. Dietary exposure to the regulated article is unlikely to result allergic reaction.

3. The regulated article is as safe as food or feed derived from conventional corn varities.

4. The regulated article is not materially different in nutritional composition from that of the non-transgenic corn or the conventional corn.

5. It is suggested that the BPI ensure that there shall be clear instructions that the product is only for the purpose of direct use for FFP and is not to be used as planting materials.

6. Based on the above considerations and with the submitted sworn statement and accountability of the proponent, we hereby submit our evaluation to BPI relative to the application of a Biosafety Permit for Direct Use as Foof, Feed or for Processing (FFP) of Corn MZIR098.

SEC ASSESSMENT AND RECOMMENDATIONS

Based on SEC expert review of the SEC questionnaire answered by the applicant:

The SEC expert has expressed that the applicant was able to explain their assertion and support it with relevant data from the Philippine Statistics Authority. The SEC expert has recommended for the approval and issuance of biosafety permit of the said GM product