CONSOLIDATED TECHNICAL REPORT ON SYNGENTA'S GM CORN PRODUCING ALPHA-AMYLASE 3272 APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING

EXECUTIVE SUMMARY

On August 18, 2017, , Syngenta Philippines Inc.'s application for Corn 3272 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 3272 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 3272 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. The DOH-BC also recommended for the issuance of biosafety permit for soybean Corn 3272

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 Bayer. The SEC expert, on the other hand, was provided with a questionnaire on socio-economic, ethical and cultural considerations that have been addressed by Pioneer 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:

Host Organism

Corn is a source of key nutrients such as carbohydrates, proteins, fats and dietary fiber and is not typically consumed for specific nutrient. Anti nutrients were found to be present with STRP 3 remarking that these are not nutritionally significant in corn and no native toxins and allergens were reported with the organism.

Transgenic Plant

The STRPs confirmed that the corn event has been approved as food and/or feed in United States, Australia, New Zealand, Brazil, Canada, China, Colombia, Indonesia, Japan, Korea, Malaysia, Mexico, Philippines, Russian Federation and Taiwan. The approval of the corn event is not expected to change the consumption pattern in the market.

Donor Organism

The STRPs has stated that all protein encoding sequences found in the original gene construct have been described with respect to the source and potential pathogenic or allergenic properties. The amy797gene was derived from sequences of 3-alpha-amylase genes originating from *Thermococcales/Pyrococcus* and the pmi gene coming from Escherichia coli K-12 strain. Both donor organisms were not known to be sources of toxic or allergenic proteins. All potentially inserted regulatory sequences have been adequately described

Transformation System

The STRPs note that the nuclear DNA was the target of genetic modification using *Agrobacterium tumafaciens* on immature corn embryos derived from proprietary corn lines. All genetic components of the transformation plasmid which were present, was adequately described by the applicant.

Inserted DNA Genetic Stability

The experts have stated that the molecular characterization indicated that the transformed corn event line had single intact insertion of amy797e and pmi genes and was demonstrated by the results of the Southern analysis and T-DNA insert sequencing. Truncations were present at the right and left border portion of the T-DNA insert with no

effects on its efficacy. According to the STRP, this phenomenon has been adequately described in *Agrobacterium* transformation.

The pmi gene has been expressed in corn MIR 162 and corn 5307, both of which have been approved for use direct use as food, feed or processing in the Philippines. Southern blot analysis demonstrated the absence of the plasmid backbone of the vector sequence during the transformation process.

Genetic Stability

The multi generational stability of the corn event was analyzed through Southern blot analysis and show that hybridization patterns over several generations of the corn event was generations was identical using the probe specific to the gene demonstrating that the insert from the plasmid is stable across generations.

Expressed Material

The concentration of the AMY797E and PMI protein was determined by ELISA in several plant tissues at five growth stages and a relatively high level of the AMY979E protein was measured in the kernels while quantifiable levels of the protein were detected in some whole plant samples at whorl stage while the protein was absent in the pollen. PMI levels were generally similar with the hybrids at each time point for each tissue type.

Toxicological Assessment

The SDS PAGE from Simulated gastric fluid studies of the AMY979E protein indicates that AMY797E was completely digested after one minute incubation showing rapid and complete digestion of the protein. The alpha amylase protein is confirmed to be unstable upon heating at 120 ^{o}C the immunoreactivity of the protein was measured using ELISA at different temperatures stable at 25^{o}C 37^{o}C and 65^{o}C .

The comparison of the amino acid sequences yielded no similarities with any known or putative toxin. Acute Oral Gavage was performed and the NOEL was reported at 1511 mg/kg bodyweight of mice and shows that the protein is not toxic to humans as shown in single dose acute toxicity tests. the protein was used was purified and freeze dried from Event 3272 lines.

PMI protein was rapidly degraded in simulated mammalian gastric fluid studies and showed that it will be degraded in both gastric and intestinal environment. Data showed that PMI was unstable upon heating at 65 oC and above as demonstrated by immunoreactivity assays using ELISA. Comparison with amino acid sequences shows that PMI shares no biologically relevant sequences with any known or putative toxins. Acute oral gavage of microbially produced and equivalent PMI was performed and yielded a 5050mg/kg body weight NOEL value. No mortality due to substance ingestion was noted.

Allergenicity Assessment

AMY979E was evaluated for proteolytic degradation under SGF containing pepsin and results shows that no intact immunoreactive fragments were detected comparison of amino acid sequence yielded no similarity with any known or putative protein. the AMY979E protein comprises 0.1573% of the total weight in kernels and that serum antibody screening of the protein. there were no matches of 8 or more contiguous amino acids which would indicate the possible presence of common IgE binding epitopes or antigenic determinant. Furthermore, Serum Screening showed that there was lack of cross-reactivity of both proteins with AMY979E and the protein does not share any biologically relevant amino acid sequence similarity with any known or putative allergen.

PMI shares no biologically relevant amino acid sequence similarity to known or putative allergens. the protein comprises about 0.00007% of total protein in kernels. Serum screening was performed and showed that there is no cross -reactivity between PMI and the alpha-parabulmin protein using serum from single individual known to have demonstrated IgE mediated allergy to this specific alpha-parabulmin from *Rana* species.

Nutritional Data

The mean levels of TDF were within the range of literature and the expected variations can be attributed to gene by environment interaction. The differences observed were within the range in literature, except for starch which was lower than reported values for both hybrids in 2004. The difference are not biologically relevant for safety and quality of the grains and forage as food, feeds or for processing.

All proximate levels for all hybrids were within levels reported in literature. The transgenic enzyme expressed in event 3272 does not influence starch levels in the grain. There was no consistent statistical difference observed on forages' vitamins and amino acids fatty acids and minerals

Recommendation

Both genes are not expected to interact within ir affect the metabolism of the corn plant. Data regarding the morphology, nutrient composition and genetic stability studies show that the expression of both proteins have no adverse effect on the growth and performance of the transgenic corn. the transgenic and non transgenic corn are substantially equivalent in terms of safety and quality.

After a thorough and scientific review and evaluation of the documents provided by SYNGENTA relevant to 3272 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 ASSESSMENT AND RECOMMENDATION

Host Organism (*Zea mays* L.)

BPI-PPSD has verified that that Corn is a source of starch, proteins, oil, minerals, vitamins and organic acids and is a source of daily caloric intake and is typically not consumed for a specific nutrient and, that it also contains anti nutrients such as trypsin inhibitors, lectins, stachyose, raffinose and phytic acid. They have confirmed that corn is not a source of toxicants and allergens. It is also used as food, feed and in processing.

Transgenic Plant (3272)

The agency has listed the United States, Australia, New Zealand, Brazil, Canada, China, Colombia, Indonesia, Japan, Korea, Malaysia, Mexico, Philippines, Russian Federation and Taiwan as countries where the transgenic plant has been approved as food. BPI-PPSSD has noted that the introduction of Corn 3272 will not likely change the consumption patterns by Filipinos.

Donor Organisms (*Thermococcales/Pyrococcus, Escherichia coli* **)**

BPI noted that the amy797E genes was isolated from *Thermococcales/Pyrococcus* while the pmi genes was isolated from *E.coli.*, and that a history of safe use was attributed to both donor organisms. The proponent was able to provide sufficient description of regulatory sequences and emphasized on the presence of an endosperm specific promoter in corn. History of safe use was attributed to both donor organisms with both being not known to be sources of allergens. The other expressible sequences were examined by the panel and was not known to have similarity with allergens or toxins.

After reviewing the provided material of Syngenta Philippines, Inc. and other literatures, the BPI-PPSSD has therefore concluded that 3272 corn is as safe as its conventional counterpart

Expressed Material (AMY797E , PMI**)**

The agency confirmed that the developer provided sufficient documents to show the different levels of protein expresion of mCry3A and PMI protein in different plant parts with use of ELISA. Compositional analyses that established substantial equivalence of Event 3272 with non-transgenic counterparts suggest that gene expressions of amy797E and pmi do not alter the basic corn metabolism. Expression of the amy797E and pmi gene in the transformed plants does not appear to adversely affect plant morphology, growth or agronomic characteristics.

Conclusion

BPI states that a history of safe use is attributed to the host and donor organisms which are not known to be allergenic or toxic. The safety of the novel proteins. AMY79E and PMI were assessed and the different assessment on toxicity and allergenicity indicate that these novel proteins are being digested rapidly by mammalian gastric fluid and neither did they have significant homology with known toxins or allergens. It was concluded by the agency that there is no significant difference between the transgenic plant and its conventional counterpart in terms of composition.

BAI ASSESSMENT AND RECOMMENDATIONS

Based on the documents submitted by the applicant, BAI made the following assessment:

Host Organism

BAI states the corn is a source of starch, proteins, oil, fiber, minerals, vitamins, fatty acids and organic acids as well as antinutrients such as phytic acid, DIMBOA, raffinose, and trypsin and chymotrypsin inhibitors. Corn is mostly consumed in the form of corn-based ingriedients and can be directly consumed at different environmental stages as food and feed. There were no significant toxicants and allergens reported to be associated with the organism.

Transgenic Plant

BAI confirms that the transgenic organism has been previously approved for use as food and feed in 14 countries including the Philippines and that consumption patterns are not expected to change with the introduction of this novel grain.

Donor Organism

The amy797E gene is a chimeric gene derived from sequences of three alpha-amylase genes originating from three hyperthermophilic microorganisms of the order Thermococcales. T The PMI gene was derived from E. Coli. A history of safe use was attributed to the source organisms haing not known to be a source of allergenic protein.

Transformation System

BAI noted that Nuclear DNA was the target of Agrobacterium mediated transformation and that the developer disclosed sufficient documents regarding the used protocol including the map of the plasmid vector.

Inserted DNA Genetic Stability

AMY79E (alpha-amylase enzyme) and PMI (phosphomanose isomerase)

TaqMan PCR and Southern blot analysis confirms that Event 3272 contains a single insertion of the amy797E and the pmi genes, while sequencing of the insert demonstated the integrity of genetic elements, the presence of truncations and absence of an open reading frame (ORFs) between the plasmid and the Zea mays genomic sequences. There were no backbone sequences in the corn event as shown by southern blot analysis. The alpha amylase encoding gene has been expressed in corn as bth a single and stacked event, while pmi has been expressed in GM corn.

Genetic Stability

Southern analysis demonstrated the stability of the introduced gene in throughout generations as reflected in their studies. the examination of individuals plants from four backcross generations were tested for the presence of the gene and results confirm the presence of a single copy insertion.

Expressed Material

The levels of protein expression was measured using double sandwich ELISA. The BAI has reported that the protein does not have a metabolic role and that the enzymatic action of PMI allows for plant survival and growth in the presence of mannose, which is the primary carbon source during selection of transformants.

Toxicological Assessment

SDS PAGE and Western Blot analysis was used to demonstrate complete digestion of AMY797E after SGF with pepsin. A 43% decrease in immunoreactivity was observed at incubation at 1200 oC and at temperatures above 1500 oC there was complete loss of immunoreactivity. There is no biologically relevant sequence similarities to any known or putative toxins upon examination of the AMY797E amino acid sequence. The tests substance derived from event 3272 and was prepared through high temperature extraction procedures and was used in Acute Oral Gavage which reported a 1511 mg/kg NOEL level.

The PMI protein demonstrated complete and rapid digestion observed through SGF and SIF analysis and immunoreactivity was reduced to 6% at 65 oC and complete loss of reactivity at 95 oC as determined by ELISA. Comparison of its amino acid sequence showed no biologically relevant similarity with any known or putative toxins. Acute oral gavage was performed and a 5050 mg/kg body weight NOEL was reported and equivalence of test protein from recombinant E. coli was reported to be functionally and biochemically equivalent.

Allergenicity Assessment

BAI reports that no intact AMY797E or immunoreactive fragments were detected after SGF with pepsin was conducted and at 1500 oc incubation immunoreactivity was lost as measured by sandwich ELISA. comparison of the protein's amino acid sequence showed no similarity with any known or putative protein allergens. Serum antibody screening demonstated that there is no recognition for an allergenic epitope in the protein. PMI was

not detectable after subjecting to two minutes of SGF and SIF and incubation at 65oC resulted in 94% loss of immunoreactivity as measured by ELISA.

Nutritional Data

When compared with the non-transgenic near-isogenic control hybrid, the proximate composition of protein, fat, ash carbohydrates, ADF, NDF, TDF as well the levels of vitmins and minerals and antinutrient levels in the grains and forage were determined and no statistically significant differences were observed. All reported values were within the ranges reported in literature and statistical differences were reported to be not biologically relevant.

Recommendation

After a thorough and scientific review of the documents provided by the applicant relevant to corn event 3272, the BAI finds scientific evidence that the regulated article applied for direct use has no evidence of interaction on the resulting gene products

DENR ASSESSMENT AND RECOMMENDATION

After a thorough and scientific review and evaluation of the documents provided by the Bureau of Plant Industry (BPI) on the application of Syngenta Philippines, Inc. for Direct Use as Food and Feed or for Processing of Corn 3272, here under are the observations and appropriate actions:

1. From the evaluation of the application submitted by the proponent, including the scientific evidences from provided references and literature, as well as other related studies, the Committee finds that the direct use of the regulated article whether for food, feed and or for processing will not cause any significant adverse effect on the environment (land_ air_ and water) and non-target organisms, to wit:

    a) Genetic stability in the transaenic crop is ensured such that no unintended horizontal gene transfer shall occur to unrelated species;

    b)The protein product produced by the transgenic crop will degrade upon exposure to the natural environment and general conditions (i,e. high temperatures (60 C and above), varying pl-I, enzyme digestion, etc.); and

    c) The protein product will not increase the weediness potential of the transgenic crop.

The data evaluated support the conclusion that the regulated article is as safe as its conventional counterpart

2. The project description report (PDR) discuss 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 of the proponent. Upon evaluation of the submitted PDR and environmental risk assessment

(ERA), the Committee notes that 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/germination.

3. 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 as per BPI' s inspection in the port area.

The DENR-BC finds scientific evidence that the regulated article applied for Direct Use as Food and Feed or Processing is safe as its conventional counterpart and is not expected to pose any significant risk to the environment and to non-target organisms. Based on the above considerations and with the proponent's sworn statement of accountability, we hereby submit our evaluation relative to Syngenta Philippines, Inc. 3272 application for biosafety permit for food, feed, and/or 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 as Food, Feed or for Processing (FFP) of CORN 3272, the DOH Biosafety Committee find that the regulated article applied for Direct Use as Food, 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. They have also forwarded the following bservations and recommendations :

1.Scientific pieces of evidences from 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 varieties.
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 Bureau of Plant Industry (BPI) ensure the following :
a. Clear labeling of the regulated article from the source down to all levels of marketing stating that it is only for the purpose of direct use as food, feed or processing 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, this recommendation is being submitted to BPI related to the processing and issuance of a Biosafety Permit for Direct Use as Food. Feed or for Processing (FFP) of Corn 3272.

SEC Assessment and Recommendation

The SEC expert has noted that the use of imported corn for food, feed and processing will not be affected by by the importation of this event and no drastic trade is expected. Worldwide conditions will continue to be factors for international corn prices and trade. The expert recommends the approval of this event for direct use as food, feed, or for processing.