ASSESORS' CONSOLIDATED REPORT ON SYNGENTA'S STACKED HERBICIDE TOLERANCE (GLUFOSINATE TOLERANT AND MESOTRIONE HERBICIDE TOLETANT) APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF SOYBEAN SYHT0H2

EXECUTIVE SUMMARY

On October 21, 2016, Syngenta Philippines Inc. soybean SYHT0H2 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 soybean SYHT0H2 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 soybean SYHT0H2 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 soybean SYHT0H2

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:

A. Host Organism

The STRP concurred that soybean is a source of nutrients especially of vitamins K and E. the refining and processing causes partial loss of these vitamins. The panel concurred that soybean is not source of toxicants and that the antinutrients present such as trypsin inhibitors and lectins, stachyrose, raffinose oligosaccharides and phytic acids are degraded during processing.

Soybean contains recognized allergenic proteins but according to STRP 3, the relevant estimate of its allergenic potential is not complete. No significant level of amino acid homology exists between the PAT gene and any protein allergens.

STRPs 1 and 3 concurred that processed soybeans in its final form is consumed as food as sprouts, vegetable oils, flour, soy foods (miso, soy milk, soy sauce, tofu) and as feed specifically as soybean meal of livestock, poultry, swine and pets. STRP 1 mentions that the developer provided the list of countries that use SYHT0H2 as feed while STRP2 argues that "there is no document presented that the transgenic soybean (SYHT0H2) have been used as food in any form"

B. Transgenic Plant

STRP2 comments that although adequate information points that (whole, oil) is used in variety of processed foods (94% of soybean food ingredient are consumed by human) there is no document presented that the transgenic soybean (SYHT0H2) have been used as food in any forms. The expert also notes that only data on regional diets has been provided by the World Health Organization Global Environment-Food Contamination Monitoring & Assessment Program (GEMs/Food) that can be used to estimate the likely daily food consumption of soybean-based products in the Philippines (WHO, 2012). The expert has mentioned that although it was shown that 98% of soybean meal has been processed for use as feed, no data was provided to show that SYHT0H2 was used in animal feeds.

STRP1 presents the need to provide the document on approval of SYHT0H2 as food or feed in various countries listed in the Basic Info Sheet. STRPs 1 and 3 agreed that with the introduction of the novel food, it is probable that the consumption of the population will change depending on how acceptable the transgenic plant is.

C. Donor Organism

The panel confirms that SYHT0H2 contains that gene avhppd-03 derived from *Avena sativa* (common oat) which encodes the enzyme AvHPPD-03 and also pat gene derived from *Streptomyces viridochromogenes*. Oat does not contain proteins that are listed in the FAARP 2012 allergen database and is there fore non allergenic.

Reports showed that *S. viridochromogenes* does not have a toxic or allergenic effect on humans or animals. The genetic inserts have no known allergic properties.

The DNA insert in the transgenic soybean SYHT0H2 contains a single copy of avhppd-03, four copies of pat, a single copy of the avhppd-03 enhancer complex sequence, two copies of the 35S promoter, two copies of the CMP promoter, two copies of the TMV enhancer and five copies of the NOS terminator

Southern blot analysis and nucleotide sequencing were done for the extensive characterization of the DNA inserted in SYHT0H2 soybean. In addition, the soybean genome sequence flanking the SYHT0H2 inserted were identified and characterized. Results showed that the SYHT0H2 inserted did not disrupt the function of any known soybean gene. These data collectively demonstrate that no deleterious changes occurred in the SYHT0H2 soybean genome as a result of the DNA insertion

The PAT regulatory enzymes are reportedly not associated with food toxins and allergens backed up by the absence of a sequence homology with known allergens or toxins.

D. Transformation System

The transformation of immature soybean seed to produce herbicide tolerant soybean plants is mediated by *Agrobacterium tumefaciens*. The genetic element in the transformation plasmid pSYN1 used to produce SYHT0H2 soybean was adequately described.
The developer provided sufficient information that the target of modification is the Nuclear DNA Complete experimental protocol was provided.

E. Inserted DNA Genetic Stability

PCR has demonstrated the presence of one insertion site. Southern blot and nucleotide sequencing demonstrated the integrity and order of genetic elements. There were no extraneous DNA fragments from the soybean genome and transformation plasmid pSY N15954 FMV, enhancer and backbone sequences were absent.

Sequence analysis of the Event SYHT0H2 Soybean insertion site at the 5' and 3' shows deletion of 15bp of soybean genomic sequence after the integration of the SYHT0H2 Soybean into the soybean genome and 7 bp present in the 3' flanking region adjacent to the SYHT0H2 insert do not align to the sequence of the non-transgenic genome at the insertion site.

No plasmid backbone was present as demonstrated by Southern blot analysis and two restriction enzyme digestion strategies.

F. Genetic Stability

The absence of unexpected bands indicates no extraneous fragments were in the insert. Southern blot was performed to demonstrate the genetic stability of the SYHT0H2 insert overtime. Stability was observed in 3 generations as confirmed by the Southern blot analysis. Real time PCR was used to determine the segregation ratios of the 2 genes and the observed ratio confirms that plants in each generation are expected to carry the gene.

G. Expressed Material

ELISA was used to quantify AvHPPD-03 and PAT protein from the different plant parts, with both proteins having metabolic role. Although there were considerable variabilities observed in the concentrations of AvHPPD-03 and PAT, the applicant claimed that the variabilities of the performance of their herbicide tolerant traits, (which have been demonstrated in replicate efficiency field trials) could not be attributed to the study conducted, as several levels of bias control were implemented throughout the study. Furthermore, the data on the heat stability studies of AvHPPD-03 and PAT proteins strongly suggested the reduction through processing into food/feed products would render minimal dietary exposure to these proteins.

H. Toxicological Assessment

The protein used in the assessment of toxicity was microbially produced AvHPPD-03 from *Eschericia coli* and is reportedly non-toxic to mice as evidenced by the absence of mortality during two and 14-day observation. Sufficient data and analyses were provided by the developer indicating that the microbially produced AvHPPD-03 is biochemically and functionally equivalent to AvHPPD-03 produced in SYHT0H2 soybean and therefore is a suitable surrogate to evaluate the safety of AvHPPD-03 produced in SYHT0H2 soybean.

Safety evaluation supports the innocuousness of the PAT gene which does not possess characteristics of a toxin or allergen, no N-glycosylation sites, easily degraded by gastric and intestinal fluids and devoid of adverse effects in mice after IV injection of a high dose. Utilizing the NCBI Entrez Protein Database in search of the similarity of the PAT amino acid sequences shows that it has no homologies with known toxins.

Acute Oral Gavage tests were performed and reasonable certainty of safety is expected from the inclusion of PAT proteins inhuman food and animal feed.

I. Allergenicity Assessment

Both proteins were degraded by SGF and were inactivated at 65 ^{o}C and 55 ^{0}C respectively, no homology with any known or putative allergen, and there are reportedly no post-translational glycosylation in plant and microbiologically produced PAT proteins. There is a minimal chance of dietary exposure to the AvHPPD-03 and PAT protein via the transgenic soybean since they are estimated to be destroyed during processing when subjected to high temperatures.

J. Nutritional Data

Proximate Analysis concluded that there is no significant difference in key nutritional components of forage and seed from SYHT0H2 soybean were found to be similar in composition with the conventional soybean. The panel verified that the mean levels of calcium, magnesium and phosphorus did not differ from the control soybean while levels of iron and potassium differ significantly between the transgenic soybean and treated soybean and the control soybean at one location.

No unintended, adverse consequences of the transformation process or expression of the transgenes in SYHT0H2 were evident that will affect human health and no possible risk in animal health as shown in the poultry feeding study.

K. Recommendation

The three recommends 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 (Glycine max L.)

BPI-PPSD has verified that the developer provided sufficient information regarding the nutrients present in soybean which are proteins, fats, ash, carbohydrates, amino acids, minerals and vitamins. The developer also provided information on the antinutrients present in soybean meal such as stachyose, raffinose, trypsin inhibitors, lectins and phytic acid and that soybean is not associated with any toxicants. Soybean is on the list of common food that causes allergy as mentioned in Allergen Online.

Soybean is consumed as food as soy milk, milk curd/tofu, whole cooked seed, edible soy oil, soy protein concentrate, isolated soy protein, hydrolyzed vegetable protein, textured soy protein and soy protein fibers. Its is also used as feed in the form of seed, forage/silage, hay, meal and hulls.

Transgenic Plant (SYHT0H2)

The developer provided a complete list of countries that have approved SYNT0H2 which includes Canada, Russia, Belarus, Kazakhstan, South Africa, Mexico, Australia, New Zealand, Korea, Japan and Taiwan and as feed in Canada, Colombia, Russia, South Africa, Mexico and Korea. SYHT0H2 is not likely to cause a change in consumption pattern in population subgroups.

Donor Organisms (Avena sativa and Streptomyces viridochromogenes)

Avena sativa is the donor organism for the avhppd-03 gene encoding the protein of the same name and is not considered an allergenic food. The PAT genes were derived from *Streptomyces viridochromogenes*, a common non pathogenic soil bacterium. PAT proteins

and its homologues are not known to cause toxicity or allergenicity to humans or animals. History of safe use was attributed to both organisms.

Expressed Material (AvHPPD-03 and PAT proteins)
The enzyme AvHPPD expressed by avhppd-03 gene confers tolerance to commercial application rates of HPPD-inhibiting herbicides such as mesotrione. It is not known to be homologous to known toxin or allergen as supported by analyses provided by developer. PAT expressed by pat-09 genes is an enzyme involved in the inactivation of glufosinate ammonium through acetylation. History of safe use was attributed to PAT proteins which are not associated with any known toxins or allergens. The developer provided sufficient information that there is no possibility of any interaction of AvHPPD and PAT in a metabolic pathway.

Conclusion
After thorough evaluation of the documents provided by the applicant, Syngenta Philippines, Inc., the Plant Product Safety Services Division- Bureau of Plant Industry concluded that the regulated article, soybean SYHT0H2 is substantially equivalent to the conventional soybean.

BAI ASSESSMENT AND RECOMMENDATIONS

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

A. Host Organism
BAI has agreed that soybean is a source of key nutrients, such as vitamins K and E. The agency has identified soybean to be a source of antinutrients but not of toxicants. Soybean has been identified as a source of common food allergy with this event having 38 protein sequences of soybean allergens mentioned on the Allergen Online and is used as food and feed.

B. Transgenic Plant
BAI has stated that consumption patterns by population subgroups will not be changed as a result of introducing the novel food.

C. **Donor Organism**
BAI verifies that the synthetic gene avhppd-03,was derived from common oats which is not considered allergenic. The agency also verifies that pat genes were derived from *S. viridochromogenes* strain Tu494 which has not been reported as toxic or allergenic in humans or animal and that all potentially inserted regulatory sequences were adequately described.

D. Transformation System
BAI stated that *Agrobacterium tumefaciens*-mediated transformation of immature soybean seed of variety 'Jack' was used to produce SYHT0H2 soybean with nuclear DNA as the target of transformation.

E. Inserted DNA Genetic Stability

BAI confirms that the Southern blot analyses sufficiently confirmed a single integration site within the SYHT02 soybean genome and that the presence of the expected size insert was confirmed by sequencing. The likelihood that a novel protein would result from the putative ORFs of the insert was ruled out by bioinformatics analyses and showed no biological similarity to any known putative allergen or toxin. Backbone sequences were absent from the transformation plasmid.

BAI confirms that the avhppd-03 gene has only been expressed in soybean while the pat gene has been expressed in maize and cotton.

F. Genetic Stability

BAI states that the Southern Blot analyses confirms the inheritance of the introduced traits in all three generations studied and that segregation was observed by PCR analysis and is ratio was found to be within expected values for a gene inherited according to Mendelian principles. The results of segregation analysis confirm that SYHT02 soybean carries a single insert consisting of two partial copies of the T-DNA.

G. Expressed Material

BAI states that the applicant provided the novel protein's expression levels from different plant parts using ELISA. The metabolic activity of PAT and its homologues is highly specific and is limited to the acetylation of the glufosinate-ammonium herbicide, details of which were described.

H. Toxicological Assessment

BAI has concurred that all information relative to the toxicological assessment done to AvHPPD-03 and PAT are sufficiently described. They also agreed that the information on the digestibility studies, heat inactivation studies, Amino acid sequence comparison studies, acute oral gavage studies and protein equivalence to source proteins of the three expressed novel proteins are adequate. The two proteins are expressed independently of each other but functional activity is maintained.

I. Allergenicity Assessment

BAI states that bioinformatics comparisons did not find any significant level of similarity with proteins in the Allergen Protein Database. Dietary exposure to the proteins is considered minimal or non-existent.

J. Nutritional Data

BAI has concurred that there are no statistically differences identified between SYHT0H2 and the control soybean in terms of proximate analysis of grains and forage, levels of key nutrients and anti-nutrients and that there is no biological relevance in terms of safety.

K. Recommendation

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

DENR ASSESSMENT AND RECOMMENDATION

In the loading/unloading, transport, storage and processing of transgenic soybean SYNT0H2, the applicant identified accidental spillage or intentional planting during the abovementioned activities as the potential source of risk to the environment and according to the applicant, such spillage will occur in an industrial area with unsuitable conditions for seed germination and plant establishment to occur. Mentioning that the soybean will only be imported for direct use as food feed or for processing, their mitigating measure is to follow the rules and regulations on importation set by the agency.

After a thorough review by the DENR Biosafety Committee, they have observed that the effect of the regulated article on the environment depends largely on the viability of the product for direct use and when transported in non-viable form, poses no danger to the environment. The committee also noted that due to the absence of a specified Environmental Management Plan (EMP) by the traders/importers, the committee recommends the document as additional requirement for issuance of import permit by the BPI, (Section of JDC No.1 s2016).

It was suggested that BPI ensure the following:

a) Development of guidelines on the EMP in coordination with DENR;

b) Implementation of the EMP by the traders/importers involved in the import, handling, processing and transport of viable Soybean SYHT0H2 'Commodity products; and

c) Strict monitoring of the regulated article from port of entry to the trader's/importer's storage/warehouse (Section 32 of the JDC No. 1 s.2016);

The DENR-BC suggests that the above mentioned considerations be noted in addition to the submitted sworn statement and accountability of the proponent to be required prior to issuance of Biosafety Permit.

DOH ASSESSMENT AND RECOMMENDATION

In the loading/unloading, transport, storage and processing of transgenic soybean SYNT0H2, the applicant identified accidental spillage or intentional planting during the abovementioned activities as the potential source of risk to the environment and according to the applicant, such spillage will occur in an industrial area with unsuitable conditions for seed germination and plant establishment to occur. Mentioning that the soybean will only be imported for direct use as food feed or for processing, their mitigating measure is to seek the biosafety approval in the Philippines through the submission of applications prior to importation.

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

The SEC expert asked the applicant to indicate if there is a growing demand of the product in the local market and cite whether there is a growing demand for soybean as input if the animal feed market. The applicant then cited a 2017 PSA report which states that the Philippines produces only a small percentage (1-3%) of the soybeans and soybean products that are consumed each year, with 97% or more of the soybeans used for food, feed and processing being imported from 2010 to 2014. Syngenta then argued that the SYHT0H2 soybean, as raw materials for the food and feed industry will help meet the local requirements while maintaining the trade between Philippines and US and other trade partners. The SEC expert stated that the import substitute concern is not applicable in the case of Syngenta who claim that global competitiveness will not be affected by the introduction of this event.

After reviewing the responses of Syngenta to the queries sent by the SEC expert, the expert recommended the approval of the said event.