ASSESSORS' CONSOLIDATED REPORT ON BAYER'S APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF SOYBEAN A5547-127

EXECUTIVE SUMMARY

On April 26, 2016, Bayer CropScience Inc. submitted soybean A5547-127 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.

The said transformation event had obtained Biosafety Permit under the rules and regulations of the Department of Agriculture Administrative Order No. 8, Series of 2002 for direct use on June 23, 2011. The said Biosafety Permit for direct use expired last June 22, 2016, hence this application represents Bayer's submission for renewal of this permit under the JDC.

This application was assessed in accordance with the JDC Article VII. Direct Use of Regulated Articles for Food and Feed, or for Processing. This Article covers the basic biosafety policies, procedural requirements and guidelines in carrying out the risk assessment of plants carrying single transgene for direct use as food and feed, or for processing. Focus of the risk assessment is on food and feed safety of the GM product.

Under the JDC, the assessors for Bayer's soybean A5547-127 application for direct use are the following:

- Three (3) members of the Scientific and Technical Review Panel (STRP) – for evaluation of the risk assessment report
- Department of Environment and Natural Resources (DENR) – for the determination of the environmental impact
- Department of Health (DOH) - for the determination of the environmental health impact
- BPI Plant Products Safety Services Division (BPI-PPSSD) – for the determination of compliance with food safety standards
- Bureau of Animal Industry (BAI) – for the determination of compliance with feed safety standards
- Socio-economic, ethical and cultural (SEC) Expert – to evaluate SEC impact

After reviewing the documents submitted by the applicant, the three members of the STRP, BAI and BPI-PPSSD find scientific evidence that the regulated article applied for direct use as food and feed, or for processing is as safe for human and animal health, and the environment as its conventional counterpart, while DOH, DENR, and SEC expert recommended for the issuance of Biosafety Permit for soybean A5547-127.

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 Bayer 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 STRPs agreed that soybean (*Glycine max*) is a source of key nutrients, namely: glycerol, fatty acids, sterols and lecithins. It is also a good source of amino acids used in the production of infant food formula and other food products. It is rich in essential amino acids, particularly lysine and tryptophan, especially needed in animal diet. However, they also agreed that it is also a source of anti-nutrients, such as stachyose and raffinose oligosaccharides, phytic acid, isoflavones, lectins, and protease inhibitors.

Two of the STRPs agreed that soybean is a source of toxicants because it contains lectins, isoflavones, lecithins and sterols, while the third STRP says that when properly stored, soybean is not a source of toxicants. All of the STRPs also agreed that soybean is a source of allergen, accounting for 90% of all IgE-mediated food allergies (Taylor and Hefle, 2000), and affects 0.3-0.7% of the population. However, they also agreed that soybean and processed soybean products are not commonly eaten raw. Heat and processing treatments usually destroy soybean's allergenic properties.

All of the STRPs agreed that one form of soybean used for human consumption is for soybean oil, however, they also agreed that soybean may also be consumed through different other forms, fermented or non-fermented. They also approved on the information given by the applicant that the average consumption of soybeans in grams per person per day in regions, according to WHO were: 4.5 in Middle East, 2.0 in the Far East, 0.5 in Africa, and 0.1 in Latin America. Meanwhile, in Japan, it was reported that the daily intake of soy-based foods is between 63.2-70.2 g/person (Food Safety Commission of Japan, 2006), in Korea, is it estimated to be 21 g/person/day (Kim and Kwon, 2001). As for the yearly consumption of soybean oil per capita, 30 kg for Brazil, 4kg for China, 27 kg for US (Goldsmith, 2008). One of the STRPs also noted that consumption by vegetarians using soybean-based products as their protein source is higher than that of the typical Far East dietary pattern.

Furthermore, all of the STRPs concurred that soybean meal is widely used for animal feed. It is commonly mixed with other forms of meal in feed formulations and even pet food.

B. Transgenic Plant

The STRPs all concurred on the information provided by the applicant stating that countries such as Argentina, Australia/New Zealand, Brazil, Canada, China, Colombia, EU, Japan, Korea, Malaysia, Mexico, Philippines, Russian Federation, Singapore, Taiwan, US, Uruguay, and Vietnam have approved the said transgenic plant as food and feed. One of the STRPs even pointed out that Canada and China, two of the major suppliers of food soybean to the Philippines, have approved the use of soybean containing the said transformation event for food. They also agreed that the consumption patterns by population subgroups are unlikely to be changed as a result of the introduction of soybean A5547-27.

C. Donor Organism

All the STRPs agree that the information provided by the applicant is adequate and are described properly, and that the only protein encoding sequence that was in the original transformation cassette was the pat gene which encodes phosphinothricin acetyl transferase. They also agreed that all potentially inserted regulatory sequences were described properly by Moens, 2015, as per the said documents submitted. Moreover, they also approved the statement that all donor organisms of the said transgene, Cauliflower Mosaic Virus, *A. Tumefaciens*, *and S. viridochromogenes* have no significant sequence homology to known toxins or allergens by *in silico* analysis. The same way, they also agreed that the protein encoded by the expressible sequences, namely PAT, is neither allergenic nor toxic, based on *in silico* approaches.

D. Transformation System

All the STRPs approved the information submitted by the applicant reporting that the transformation method used was through particle acceleration. They also agreed that the target of the genetic modification is the nucleus and that the experimental protocol was completely provided by the applicant.

The STRPs also agreed that the genetic components used were adequately described and enumerated, including: sequence of vector pUC19, right border fragment of *A. tumefaciens* octopine plasmid pTiAch4, 35S promoter from CaMV vector pDH51, synthetic polylinker sequences, 35S terminator from CaMV vector pDH51, origin of replication, and *beta*-lactamase gene. The plasmid vector used was also adequately described by the applicant as follows: pB2/35SAcK has a size of 4067 bp, built from plasmid pUC19, therefore, it has the pUC 19 backbone. The plant codon-optimize pat gene was inserted as a cassette having the 35S promoter and T35S as the terminator from Cauliflower Mosaic Virus located upstream and downstream from the pat gene, respectively. The right border of the *A. tumefaciens* octopine plasmid pTi Ach5 was ligated upstream of P35S, which was added to enhance the expression of the inserted gene. The *beta*-lactamase (bla) gene and origin of replication was shown to be part of the pB2/35SAck and may have been part of pUC19. Synthetic polylinker sequences were also included to facilitate the insertion of the genetic elements.

Further, the STRPs all concurred that there were no carrier DNA and/or helper plasmids used in the transformation process.

E. Inserted DNA

All the STRPs agreed that there was only one insertion site, as determined by southern blot analysis by Verhaeghe, 2012 and De Pestel, 2009. Verhaeghe used fragments from different components of the transforming plasmid and the linearized transforming plasmids as probes. One hybridization product was evident for each of the probes, indicating only one copy of the inserted genetic element. Meanwhile, De Pestel used the same transforming plasmid, and probed with just 2 components of the transforming plasmid, i.e. sequences between bla and P5S and between T35S and bla. The genomic DNA from the transgenic A5547-127 and non-transgenic counterpart was cut with 5 different restriction enzymes. Only one hybridization band was seen in each of the genomic DNA-probe combinations, indicating one copy and one insertion site. All the STRPs also concurred that the demonstration of the characterization was sufficient.

All STRPs also agreed that based on *in silico* analysis, there were no interrupted, truncated, or re-arrangements that can be attributed to the transformation event. They also agreed that there is no indication for the presence of interrupted genes or regulatory elements in the A5547-127 insertion locus. They all agreed that this was satisfactorily demonstrated.

The STRPs are also in agreement on the results of *in silico* analysis showing it is unlikely for potential ORFs to code for proteins that may have allergenic and toxicological concerns. They were also in agreement that the transgene has also been expressed in other approved GM crops, as per information provided by the applicant, gathered from the Center for Environmental Risk Assessment, International Life Science Institute Research Foundation, and Bureau of Plant Industry. In addition, the STRPs are also in agreement that the determination if there was any plasmid backbone sequences present by southern blotting was efficient and that it was not a cause of concern.

F. Genetic Stability

The STRPs expressed their approval with the information provided by the applicant regarding the multigenerational stability assessment of the transgene. They are in agreement of the results using southern blot analysis done by De Beukeeleer in 1998, that a single copy of the pat gene inserted into the A5547-127 genome was found stable over three generations (R3, 4, and 5), using the 1329 bp EcoR1 fragment probe from transforming plasmid pB2/35S Ack that harbored the pat gene cassette. The STRPs also concurred that the information on segregation analysis, assessed in the field by Van Wert, 2009 at 19 sites in 1996 and 48 sites in 1997 in the United States and its territory, is sufficient and adequate. It was found that the pat locus has been inherited in a Mendelian fashion in A5547-127 homozygotes for a few generations.

G. Expressed Material

Two of the STRPs concurred that the information provided by the applicant on the level of novel protein expressed in the different plant parts of the host plant. It was found that the PAT protein content of the samples were measured by quantitative ELISA using antibodies that recognize the PAT. Results showed that on fresh weights basis, the average PAT protein at V3 and V8 stages increased from 18.40 to 26.22 ug/g but the change was not significant. The protein content of the stem was 39.19 ug/g at V3 and decreased to 13.85 ug/g at V9 stage. For the root samples, the average PAT content was 8.16 ug/g at the V3 growth stage and decrease to 3.6 ug/g. As expected, the PAT protein was not measurable in the non-transgenic controls.

Moreover, the level of expression of the novel protein was also measured in different plant forms, also using qualitative ELISA. PAT protein was measured in seed (10 ug/g fresh weight), soybean hulls (9.5 ug/gfw), defatted meal (0.07 ug/gfw), toasted meal (0.01 ug/gfw) and protein isolate (0.08 ug/gfw). Measurements of the PAT for oil samples were all below the limit of quantitation.

Meanwhile, one of the STRPs expressed that additional information should be given with regards to the information on the levels of the novel protein expressed in different plant forms. Bayer provided the said additional information which was approved by the said STRP.

H. Toxicological Assessment

The STRPs are in agreement that the applicant provided adequate information on the toxicological assessment of the novel protein (PAT) being expressed by the transgene. The digestibility study showed that using human simulated gastric fluid (SGF) with pepsin at pH 1.2 and human simulated intestinal fluid (SIF) with pancreatin at pH 7.5, the PAT protein was digested at 0.5 min at 37^{o}C for SGF and 10 min for SIF. Meanwhile, the heat inactivation study showed that the PAT protein is heat stable up to 30 min. at 90^{o}C.

The amino acid sequence of PAT protein showed no homology to known toxins based on bioinformatics analysis, wherein over-all identity search compared the PAT sequence with all proteins in available

databases, including those for allergens and toxins. There were also no biologically relevant identities found with any toxic proteins from the Bayer toxin database.

Moreover, acute oral toxicity for the PAT protein was tested using 10 male and 10 female C57BL/6J mice by oral gavage at the highest dose possible of 2000 mg/kg body weight. It was found that there was no treatment-related clinical signs, no adverse effects on body weight or food consumption, and no macroscopic changes in organs at necropsy, thus, PAT at 2000 mg/kg, body weight through oral route, did not result in systemic toxicity on the test subjects.

Further, the source of the test protein used for safety assessment was the recombinant PAT expressed in *E. coli*. This is because, the plant-expressed PAT gene product is very low in amount and was not enough to supply the testing requirements, however, equivalence between the PAT produced in the bacterium and those produced in plants were demonstrated using the following criteria: (a) same N-terminal amino acid sequence as determined by Edman degradation- theoretical sequence MSPERRP was found to be correct including the post-translational modification of removal of methionine; (b) same immunoreactivity to antibodies against PAT in Western-blot analysis; (c) same peptide masses generated from HPLC/electrospray mass spectrometry- 96.2% coverage of the protein mass; (d) same absence of glycosylation of amino acids in the PAT sequence as determined by Glycoprofile staining kit applied to PAT subjected to SDS-PAGE; and (e) same biological activity as measured by spectrophotometric enzymatic assay-the transfer on an acetyl group to PTT result in increase in absorbance at 412 nm.

I. Allergenicity Assessment

Similar to the above observations (H. Toxicological Assessment), the STRPs are in agreement that the applicant provided adequate information on the allergenicity assessment of the novel protein (PAT) being expressed by the transgene. The digestibility study showed that using human simulated gastric fluid (SGF) with pepsin at pH 1.2 and human simulated intestinal fluid (SIF) with pancreatin at pH 7.5, the PAT protein was digested at 0.5 min at 37^{o}C for SGF and 10 min for SIF. Meanwhile, the heat inactivation study showed that the PAT protein is heat stable up to 30 min. at 90^{o}C.

The amino acid sequence of PAT protein showed no homology to known toxins based on bioinformatics analysis, wherein over-all identity search compared the PAT sequence with all proteins in available databases, including those for allergens and toxins. There were also no biologically relevant identities found with any toxic proteins from the Bayer toxin database. In addition, it was also found out through homology search that glycosylation sequences yielded no potential N-glycosylation sites in the PAT query sequence.

Furthermore, it was also found out that the predicted dietary intake of PAT after consumption of A5547-127 soybean in regional diets ranges between 1.1 (Latin America) to 47.3 (Middle east) microgram per person per day, and that, after serum screening of soy extracted from two parental soybean lines and corresponding transgenic varieties, the results show that there was no significant difference in the level of native soy allergen in the transgenic soybean extracts as compared to the soy extracts from parental lines.

J. Nutritional Data

The STRPs concurred that the information submitted by the applicant regarding the nutritional data of soybean A5547-127 are adequate. It was found out through proximate analysis between event A5547-127 and its non-transgenic counterpart, and those from reported soybean varieties found in the literature, with the following parameters: crude fat, ash, total carbohydrate, ADF and NDF, that all the values between the transgene and non-transgene are within reference range, thus soybean seed from event A5547-127 have the same nutritional value as conventional soybean.

In addition, minerals and vitamins were also tested comparing soybean 15547-127 and its non-transgenic counterpart, with the values reported in literature. It was found out that the minerals and vitamin contents of the transgenic and non transgenic soybean were within reference range, except for one non-transgenic sample for iron. The analysis for iron was repeated and together with statistical analysis by site, the discrepancy was not found in the majority of the site.

Further, tocopherol and food grade oil were also analyzed in the transgenic soybean compared to its non-transgenic counterpart, and was found that the alpha, gamma and total tocopherol values were slightly higher in transgenic, glufosinate-treated soybean than non-transgenic counterpart. However, these were still within reported reference ranges.

Moreover, amino acids and fatty acids were analyzed comparing the transgenic soybean and its non-transgenic counterpart. It was found that all values of both transgenic and non transgenic soybean are well within reference range.

Anti-nutrients such as raffinose, stachyose, trypsin inhibitors, and isoflavones were also analyzed to complete the nutritional data of the transgenic soybean compared to its non-transgenic counterpart. The results showed that all the values were comparable and were all within literature range.

H. Recommendation

The STRPs unanimously find the scientific evidence provided by the applicant is sufficient in showing that the regulated article being applied for direct use is as safe for human and animal health, and the environment, as its conventional counterpart.

BPI-PPSSD ASSESSMENT AND RECOMMENDATION

Based on the documents submitted by the applicant, the BPI-PPSSD made the following assessment:

A. Host Organism

Soybean *(Glycine max L.*) has been grown world-wide as an important staple food for humans and feed ingredient for animals. Its major products are seeds, oil, and meal. Unprocessed soybeans are not suitable for food and their use for animal feed remains limited because they contain anti nutritional factors such as trypsin inhibitors and lectins which are inactivated by heat processing. Humans consume soybean mostly in processed form such as soy milk, soy sauce, tofu and other soybean products.

History of safe use was attributed to soybean. Based on OECD report, soybeans are commonly consumed in processed form and primary source of oil and protein. Heat processing eliminates the anti-nutritional factors in soybean. Toxicants are not commonly found in soybean.

B. Transgenic Plant

Soybean A5547-127 has been reviewed and approved for food and/or feed use in many countries including Argentina, Australia, New Zealand, Brazil, Canada, China, Colombia, European Union, Japan, Korea, Malaysia, Mexico, Philippines, Russian Federation, Singapore, Taiwan, United States, Uruguay and Vietnam.

Soybean A5547-127 was developed to produce phosphinothricin-acetyl-transferase (PAT) protein derived from *Streptomyces viridochromogenes* that confers tolerance to glufosinate ammonium, the active ingredient of Liberty® herbicides. The transformation method is through particle acceleration. Soybean tissue was obtained from shoot apices derived from the surface sterilized soybean seeds. The plasmid vector pB2/35SAck that contains a right border fragment from *Agrobacterium tumefaciens* Ti plasmid pTiAch5, and the synthetic *pat* gene from *Streptomyces viridochromogenes* that fused to 35S-promoter and 35S-terminator from Cauliflower Mosaic Virus. The pUC19 was modified by insertion of a DNA element containing the right border of the *Agrobacterium tumefaciens*octopine plasmid pTiAch5. The sequence (68 bp) was synthesized on a DNA synthesizer with *Ndel* sites at the ends. The right border element was ligated into the single *Ndel* site of the vector pUC19 to obtain pUC19RB2. The synthetic *pat* gene was introduced as an EcoR1 fragment into the vector pUC19RB2 to obtain pB2/35SAck. The DNA sequence preceding the ATG start codon was slightly modified by removing a *Sal*l site. The only expressible sequence is the synthetic *pat* gene from *Streptomyces viridochromogenes* at nucleotide position 1012 to 1563 in the transforming vector pB2/35SAck.

The results of molecular analyses showed that one copy of the *pat* gene cassette was inserted to the plant genome. This implies that no other expressible genes are within the inserted material in transformation event A5547-127.

Nutritional and compositional assessment of Soybean A5547-127 was conducted by Bayer to determine the substantial equivalence of the transgenic plant to its conventional counterpart. Results of the analyses proved that the modified Soybean A5547-127 was substantially equivalent to the produced non-transgenic soybean varieties and the comparable food and/or feeds derived from it.

C. Donor Organism (*Streptomyces viridochromogenes*)

Streptomyces viridochromogenes, a common soil saprophytic bacterium was the donor organism which is the source of *pat* gene that produces naturally occuring glufosinate ammonium tolerant PAT protein. According to Kutzner (1981), *Streptomyces* species has been isolated from animal or human sources and pathogenicity is not a typical property of this organism. It is not known to be human pathogen nor has it been associated with other properties like production of toxins that is known to affect human health.

Bayer also conducted an evaluation based on the recommendations provided by different international organizations and authorities with regards to the safety of the PAT proteins encoded by the *pat* and *bar* sequences that confer tolerance to glufosinate ammonium herbicide in transgenic plants. It was found that the coding region derived from *Streptomyces spp.* that encodes PAT protein are substantially homologous to other bacterial species and priori expected not to lead to the development of a pathogenic, toxic, allergenic transgenic plant. It was also found that the *pat* DNA sequence is considered to be safe as any other DNA in food that human usually consumed. Hence, conferring the *history of safe use*.

D. Expressed Material (PAT protein)

To confirm the identity and functions of the introduced protein, the detailed insert characterization of the transformation event soybean A5547-127 was subjected to a different method of analyses like Western and Southern blot analysis, sodium dodecyl sulfate polyacrylamide get electrophoresis (SDS-PAGE), N-terminal sequence analysis, glycosylation analysis. Through enzyme-linked immunosorbent assay (ELISA), the level of concentrations of the identified PAT protein in various tissues was quantified. Resulting data was used to calculate the dietary exposure and margins of exposure to PAT protein. Based on the results, the PAT protein was not detectable in the oil and an intake of this recombinant protein via soybean oil or products containing this quality would not be possible due to large margin of exposure. It indicates that the dietary exposure to food/feed derived from A5547-127 was *safe for human and animal health.*

Bioinformatics analyses was also performed using two *in silico* approach through BLAST, FASTA algorithm and other online sequence alignment tool. The 8-mer search to identify any short sequences of 8 amino acids or longer that 100% identity to an allergenic protein and overall identity search which compared each complete query sequence with all protein present on the online database. Results indicated that the expressed protein showed no 100% identity and has no biologically relevant identity to any known toxins and allergens. Therefore, *history of safe use* was addressed to PAT protein in terms of toxicity and allergenicity.

The PAT protein produced in *Escherichia coli* was tested for digestive stability in human simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) in presence of pepsin and pancreatin. Results show that the protein was degraded rapidly within 0.5 minutes of incubation in human SGF and 5 minutes of incubation in SIF. It indicates that food proteins that are rapidly degraded are less likely to express any systemic immunogenicity or toxicity (Herouet et al, 2005).

The results of the conducted acute oral gavage in mice showed no mortalities, no treatment related clinical signs, no effects on body weight parameters, food consumption and no macroscopic changes at necropsy in C57BL/6J mice. It indicated that there is no observed effect level (NOEL) after an acute oral administration of PAT protein at 2000 mg/kg body weight.

The effect of temperature on the structure of the PAT protein was examined by Bayer through SDS- PAGE and western blot analysis to determine its stability to heat processing. The two method of analysis showed one major band located around the molecular weight marker at 21.5 kDa, in accordance with the expected molecular weight of the PAT protein. The 10% PAT protein control was still visible although with a much lower intensity. After the various treatments, there were no visible changes to the PAT band with intensity similar to the unheated sample. The SDS-PAGE and western blot analysis has the same results, except for the final time point (60 minutes) at 90^0C where band intensity was slightly reduced. In the lanes with only buffer solution, there were no bands visible. Therefore, the PAT protein was not degraded or modified in a way that would affect their migration in SDS-PAGE at up to 90^0C for 60 minutes.

E. Conclusion

For the transgenic soybean A5547-127, enough evidence is provided to support the equivalence of the genetically modified crop, in terms of the nutritional composition, agronomic characteristics and food safety, with the conventional soybean other than the tolerance to Liberty® herbicides. After reviewing the provided material of Bayer Crop Science, Inc., it is therefore concluded that A5547-127 is substantially equivalent as its conventional counterpart.

BAI ASSESSMENT AND RECOMMENDATIONS

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

A. Host Organism

BAI agreed that soybean contains key nutrients such as proximates, amino acids, fatty acids, minerals and vitamins that is good for both human and livestock. However, they also agree that it also contains anti-nutrients such as protease/trypsin inhibitors, lectins, phytic acid especially at its raw state, limiting its

use for human and animal nutrition. It also a source of allergens, from which allergenic reactions from consuming soybeans are similar with those produced by other food allergens.

They also affirmed that soybean is used as food, and are consumed in fermented and non-fermented forms. Non-fermented forms include soymilk, veggie burgers, tofu, soy sprouts, soy flour, soy protein, among others. Fermented food include soy sauce, miso, tempeh, soy yoghurt etc. Soybean oil, soy protein isolate, and soy lecithin are used in infant formulas while soybean oil is used in a variety of food for humans. In addition, BAI also affirms that capita consumption of soybean oil are 30 kg in Brazil, 4 kg in China and 27 kg in US, and that soybean is also used to feed poultry, swine, cattle, other farm animals and pets.

B. Transgenic Plant

BAI agreed with the information submitted by the applicant that there were 18 countries listed with specific purpose and/or for environmental release/cultivation, food, feed safety certificate, 15 from which approved the regulated article for feed. The consumption pattern by population subgroups will not change using the principle of substantial equivalence.

C. Donor Organism

BAI reported that the applicant has adequately described and provided the information needed, including the protein-encoding sequences and regulatory sequences found in the original gene construct. They also agreed that CAMV and *S. viridochromogenes* are not known to be toxic or allergenic.

D. Transformation System

BAI stated that the applicant has provided adequate information on the transformation system of soybean A5547-127, namely microparticle technique/particle acceleration. Microparticle technique/particle acceleration was used to deliver foreign DNA into intact soybean embryo cells and stable transformants were recovered using a protoplast selection regimen.

E. Inserted DNA

BAI agreed that the applicant has adequately described and provided the information needed in terms of the inserted DNA. BAI added that there is indeed only one insertion site demonstrated by southern blot analysis. With the 258 newly created ORFs, there are neither allergenic nor toxicological *in silico* findings associated with these potential ORF polypeptides. In addition, the transgene has been expressed in 38 transformation events involving 8 plant species and that there were no plasmid backbone sequences present.

F. Genetic Stability

In terms of multigenerational stability, BAI confirms that the multi-generational stability was demonstrated by southern blot analysis tested over three generations. Field evaluations were also conducted at 67 sites to confirm segregation ratios, and BAI agrees that as expected, the Mendelian segregation of 3:1 for the pat locus was confirmed in 3 generations of backcrosses. This also supports that there is only a single insert.

G. Expressed Material

BAI reported that the information provided by the applicant in terms of the level of expression of novel protein in different plant parts are sufficient. Expression level was measured from leaf, stem and root at V3 and V8 growth stages. It was measured using ELISA.

H. Toxicological Assessment

BAI reported that the information on toxicological assessment was sufficient and adequately described by the applicant. The digestibility study used pepsin at pH 1.2 in human simulated gastric fluid (SGF) and pH 7.5 in simulated intestinal fluid (SIF). They agree with the results showing that the novel protein was degraded very rapidly in human SGF, within 5 minutes of incubation, with less than 10% of the PAT protein remaining. At 10 minutes of incubation with SIF, the protein was completely degraded. These were shown using coomassie blue stained SDS-PAGE and Western blot analysis.

Heat inactivation study was also done, and same with the results of the digestibility study, BAI are also in agreement with these results: No visible change was accounted on the novel protein after subjecting it to various heat treatments at 60, 75, 90 degrees Celsius from 10 to 60 minutes except at the final point of 60 minutes at 90 degrees Celsius where the band intensity was slightly reduced. It was also determined by coomassie blue stained SDS-PAGE and western blot analysis.

Results of the amino acid sequence comparison using the 8-mer search showed no 100% identity with known allergens were found. Acute oral gavage was also done to assess the acute oral toxicity of the novel protein in male and female mice. NOEL was at 2000 mg/kg.

I. Allergenicity Assessment

BAI reported that the information on toxicological assessment was sufficient and adequately described by the applicant. The digestibility study used pepsin at pH 1.2 in human simulated gastric fluid (SGF) and pH 7.5 in simulated intestinal fluid (SIF). They agree with the results showing that the novel protein was degraded very rapidly in human SGF, within 5 minutes of incubation, with less than 10% of the PAT protein remaining. At 10 minutes of incubation with SIF, the protein was completely degraded. These were shown using coomassie blue stained SDS-PAGE and Western blot analysis.

Heat inactivation study was also done, and same with the results of the digestibility study, BAI agreed with the following results: No visible change was accounted on the novel protein after subjecting it to various heat treatments at 60, 75, 90 degrees Celsius from 10 to 60 minutes except at the final point of 60 minutes at 90 degrees Celsius where the band intensity was slightly reduced. It was also determined by coomassie blue stained SDS-PAGE and western blot analysis.

BAI noted that the results of the amino acid sequence comparison using the 8-mer search showed no 100% identity with known allergens were found. Prevalence of the novel protein in food will be lower than 53.6 ug per person per day. Serum screening was also done and it demonstrated that there is no significant increase in the risk of allergenic activity of the genetically modified soy as compared to parental soy on soy-allergenic subjects, to which BAI also concurs.

J. Nutritional Data

BAI reported that the applicant provided sufficient information as evidence to prove that the regulated article is as safe as its conventional counterpart in terms of nutritional data. Proximate analysis, key nutrient analysis and anti-nutrient analysis were done to the regulated article. The results showed that in terms of proximates, key nutrient content and anti-nutrient content, soybean A5547-127 has no significant difference on its SE comparator. It was tested on five commercial varieties and were grown on same environmental condition.

H. Recommendation

Based on BAI Core Team's evaluation, all information or data provided were supported by a documented scientific evidence or study. Results observed in most studies conducted showed no significant differences between the transgenic and non-transgenic sources. The values of nutrients and anti-nutrients from the transgenic and traditional and other commercial varieties of soybean are comparable.

As a result of this evaluation, based on the documents submitted, the BAI poses no objection should the scientific panel recommends the approval of Bayer's soybean A5547-127 for direct use as food and feed, or for processing.

DENR ASSESSMENT AND RECOMMENDATION

After a thorough scientific review and evaluation of the documents provided by the Bureau of Plant Industry (BPI) to the DENR Biosafety Committee within the prescribed period pursuant to the Joint Department Circular (JDC) No. 1 S 2016 on the application of Bayer CropScience Inc. for direct use for feed, food or processing of Genetically Modified Soybean tolerant to glufosinate-ammonium containing herbicides with single-trait product A5547-127, along with the submitted sworn statement and accountability of the proponent, a biosafety permit may be issued to the proponent if the conditions set by DENR are followed.

DOH ASSESSMENT AND RECOMMENDATION

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

SEC ASSESSMENT AND RECOMMENDATIONS

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

A. Socio-economic issues

The SEC expert agreed that, in terms of production, consumption and trade, the Philippines is hardly a producer of soybeans, producing only about 1,000 tons per year, based on data provided by FAO from 2009 to 2013. In addition, the Philippines import about 57,000 tons of soybeans from suppliers, which grow mostly genetically modified soybeans, including those that manifest the trait in soybean A5547-127 (henceforth, GM product). Further, soybeans are important requirement in protein, carbohydrates and fats. Respectively, 0.28 grams of protein per day, 0.2 kcal of carbohydrates and 0.002 grams of fats are sourced from soybeans. The country consumes about 4,600 tons of soybean as food and process 50,400 tons of the bean to produce other food items derived from it.

The expert also agreed that the availability of the GM product will change drastically the consumption/use of soybeans in the country. Without the imported soybeans, the country will expect prices of soybeans to go up, which then reduce its consumption and trade of soybeans. The GM products is very likely to be even found in other shipments of the product, considering that all suppliers grow GM soybeans in their respective territories.

Further, the expert also expressed that not allowing imports of the GM product is equivalent to not allowing imports of soybeans at all, which would have tremendous effects on consumption and trade of soybeans. Sources of GM products in the world include North America, Brazil, Russia, India and China.

The expert also agreed that since the GM product will only be used for direct use and trade only, it will not have any impact on yields. The GM product is not grown in this country and indications are that the variety of soybeans containing the GM gene is not suitable to grow in this country in commercial quantities.

The expert added that allowing imports of the GM product becomes a concern if the country is rationing scarce foreign exchange, and it's not. Philippines' policy is for a freely floating exchange rate with occasional intervention in cases of extreme volatility in the value of currency. Nonetheless, the expert still does not see it as a concern since the amount that is imported is relatively low.

The expert also added that the GM product does not limit potential public institutions to pursue research that serves the interest of the poor. Research activities in public institutions respect IP rights, after all, the researchers here realize the importance of IP rights protection. If the research has to make use of the GM product, the researcher can always negotiate with the owner.

B. Social Issues

The expert agreed that since the GM product is imported for direct use and trade, and not for contained use, field trials or commercial propagation, there will be no perceived effects on health of producers, farming communities and consumers due to pesticide use. In contrast, the GM product will benefit the health of producers, farming communities and consumers from non-use of pesticides will not accrue since there is no local production thereof.
The expert also agreed that the concern on the risk of conflicts between people benefitting from GMO and those who do not is not a big issue since the concern of displacing local producers from allowing imported GM products does not arise because local production is only 1.7% imports. Local soybean production can expand with the appropriate variety suitable to the agronomic conditions in the country. The expert added that with a growing market of food derived from soybeans, the friction does not arise.

The expert then said that not allowing GM product will negatively affect basic human needs since presently, soybeans are important sources of the population's daily requirement in protein, carbohydrates and fats.

C. Ethical Issues

The expert agreed that there are no morality issues (ethical principles, ethical norms and values, ideals of human solidarity and equality)and minority's right violated of the population at large.

D. Recommendation

Based on the assessment of the above indicators, the SEC expert does not have any socio-economic, social, and ethical issues to raise regarding the approval of the applicant's application for biosafety permit for direct use as food and feed, or for processing of Soybean A5547-127. The expert recommends for the approval of said application.