ASSESSORS' CONSOLIDATED REPORT ON BASF PHILIPPINES INC.'s APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF SOYBEAN A2704-12

EXECUTIVE SUMMARY

On June 14, 2018, BASF Philippines Inc. submitted soybean A2704-12 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 soybean A2704-12 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 A2704-12 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 A2704-12.

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

Soybean is a source of oil for human consumption. Glycerol, fatty acids, sterols and lecithin are derived from soybean oil. Soy protein isolate is used as a source of amino acids for infant formula and other food products. Soybean meal is rich in lysine and tryptophan which are important in animal diet. It also contains anti-nutrients. These include phytic acid, protease inhibitors, hemagglutinins, stachyose, raffinose and isoflavones. However, processing soybeans through heat treatment can inactivate or eliminate the harmful effects of these anti-nutrients.

It is not a source of toxicants according to OECD 2012, however, it accounts for 90% of IgE-mediated food allergies, which, in turn, is associated with 0.3 to 0.7% of the general population. Fatal allergic reactions, such as anaphylactic shock and death, though, are reportedly rare. Those allergenic proteins (such as glycinin and conglycinin) are associated with storage, enzymatic and protective functions. Depending on the degree of purity, soy lecithin may also contain allergenic proteins.

Soybean is a common food material. The components used are mainly the seeds and processed products such as oil, soy flour, and meal. Whole soybeans are used to produce soy sprouts, baked and roasted soybeans, full-fat soy flour and the traditional soy foods such as miso, soy milk, soy sauce, and tofu, among others. Soy protein concentrate is prepared from soybeans and incorporated in infant food formula. A large bulk of soybean seed mass is processed into high quality soya oil mainly used for human foods. Soybean meal is rich in amino acids like lysine and tryptophan which are required supplements in animal diets for optimum growth and health. Soybean meal is used in the diets of poultry, swine, dairy cattle, beef cattle and pets.

B. The Transgenic Plant

A2704-12 soybean had been granted food and feed approvals in 24 countries namely: Australia/New Zealand, US, Canada, Mexico, European Union, Russia, Turkey, Argentina, Brazil, Colombia, Uruguay, Paraguay, Japan, Korea, China, Taiwan, Philippines, Singapore, Malaysia, India, Vietnam, Thailand, and South Africa. Table 1 was provided to show the list of countries that have granted food and feed approval to A2704-12 soybean.

Consumption patterns by population subgroups are not changed as a result of introducing A2704-12 soybean. A2704-12 soybean is compositionally and nutritionally equivalent to its non-transgenic counterpart and to current commercial soybean varieties hence is consumed as conventional soybean. About 460 regulatory approvals in regulatory authorities in more than 20 different countries or regions including the European Union have been issued for food and feed uses of genetically engineered plants expressing the phosphinothricin acetyltransferase (PAT) protein, the introduced protein in A2704-12 soybean.

C. Donor Organism

The pat gene from *Streptomyces viridochromogenes* codes for PAT protein consisting of 183 amino acids. All potentially inserted regulatory sequences had been adequately described. These include the 35S terminator sequence from Cauliflower Mosaic Virus, synthetic pat gene from *S.*

viridochromogenes, 35S promoter sequence from Cauliflower Mosaic Virus, and sequence of vector pUC19 including the polylinker at position 1885-1843, the origin of replication at position 2257 and the β–lactamase (bla) gene at position 3876-3016.

The functional, expressible sequence introduced is the PAT-encoding sequence pat. The pat gene from *S. viridochromogenes* codes for the PAT protein consisting of 183 amino acids and have been used extensively in genetic engineering of crop plants.

The recombinant plasmid contains two open reading frames, bla and pat. The ß-lactamase gene *bla*, was isolated from pBR322, a plasmid of E. coli. It has regulatory signals recognized in bacteria but not functional in plant cells. Prior to transformation, the vector was digested with the PvuI restriction enzyme to disrupt the coding sequence of the *bla* gene, thus removing any remote possibility of its expression. Only the pat reading frame, therefore, is functional and intact in A2704-12 event.

The donor microorganism is *Streptomyces viridochromogenes*. This microorganism is widespread in nature. The genus *Streptomyces* also contains a few species reportedly pathogenic to man, such as *S. somaliensis, S. candidus, S. gedaensis, S. horton and S. willmorei*. Regardless, a two-step approach was employed to evaluate the safety of the proteins expressed in plants. The current conclusion is that both the genes and the donor microorganism are innocuous. The genes are not homologous with known toxins or allergens in existing databases.

Only the PAT protein is encoded by the expressible pat gene. An in silico search was conducted to identify any protein presenting 35% amino acid identity with PAT protein and known to present potential allergenicity concerns. Further, an allergen database search was conducted to identify proteins with 8 contiguous and matching amino acids. Both searches returned negative results.

C. Transformation System

The transformation method used was particle acceleration. The plasmid DNA is introduced into soybean tissue by the particle acceleration method. In this method DNA was precipitated onto microscopic gold or tungsten particles. The coated particles were spread onto a mylar carrier sheet which was then accelerated towards a stainless steel retaining screen. The screen stops the flight of the sheet but allows the continued flight of the DNA coated particles. The particles penetrate the target plant cells where the DNA is deposited and introduced into the cell genome. The cells were induced to produce shoots on plant tissue culture medium containing plant hormones. The shoots which developed from the transformed cells expressed the phenotype encoded by the genes on the introduced DNA. The expression of the introduced genes was used as evidence of transformation. Expression of the PAT enzyme was detected by spraying plantlets in axenic culture with glufosinate-ammonium (GA). Surviving plantlets were transferred to soil, grown in the greenhouse and then screened again for glufosinate resistance.

The target of the genetic modification is the nucleus to confer recipient organism and its progenies tolerance to herbicide glufosinate-ammonium.

The recombinant plasmid contains two open reading frames, bla and pat. Only the pat reading frame is functional and intact in the event A2704-12. The genetic components are as follows: 1) CaMV 35S promoter and terminator - the 35S promoter and terminator sequences are derived from CaMV. The promoter controls transcription initiation of the pat gene. The terminator ends transcription of the pat gene. CaMV is a double-stranded DNA caulimovirus with a host range

restricted primarily to cruciferous plants. The 35S promoter directs high level constitutive expression and is widely used as a promoter for high expression of genes. The CaMV sequences, as used in A2704-12, do not cause the soybean to become a plant pest. 2) pat - the pat gene is a synthetic version of the pat gene isolated from S. viridochromogenes, strain Tu 494. Since the native pat gene has a high G:C content, which is atypical for plants, a modified nucleotide sequence was synthesized using codons preferred by plants. The amino acid sequence of the enzyme remains unchanged. The pat genes encode the enzyme phosphinothricin acetyltransferase (PAT), which imparts resistance to the phytotoxic activity of glufosinate-ammonium. 3)bla - the □-lactamase gene was isolated from pBR322, a plasmid of E. coli. beta-lactamase genes are found throughout nature. The gene is expressed in bacteria where it is used in the selection of transformed bacteria which are then used to amplify the plasmid vector.

Genetic element RB is found in position 189-243 of the plasmid vector and has a size of 0.54 kb. It functions as the right border sequence of A. tumefaciens Ti plasmid pTiAch5. Genetic element P-35S is the cauliflower mosaic virus promoter of the 35S transcript. It is 0.54 kb in size and found in positions 461-1003 of the plasmid vector. The pat gene measures 0.55 kb and encodes for the synthetic glufosinate resistance gene. It is found in position 1012-1563 of the plasmid vector. T-35S is the 3'-nontranslated region of the 35S transcript. It measures 0.20 kb and found in position 1582 to 1784 of the plasmid vector. ori-pUC is the origin of replication of pUC18. It measures 0.55 kb in size and found in positions 2253-2803 in the plasmid vector. The bla gene encodes for ampicillin resistance from E. coli that expresses beta-lactamase only in bacteria. No helper plasmids were used.

D. Inserted DNA

Analyses confirmed the single insertion site of the A2704-12 transgenic locus. Verification through inheritance analyses and bioinformatics of the insertion locus support insertion at a single site. Segregation ratios determined for two successive generations of A2704-12 soybean confirmed that the A2704-12 insert is inherited in a predictable manner and as expected for a single nucleic insert. These data are consistent with Mendelian principles and support the conclusion that the A2704-12 event consists of a single insert integrated into a single chromosomal locus within the soybean nuclear genome.

Bioinformatics analysis on the A2704-12 soybean insertion locus sequence was used to identify the position of the insertion locus in the genome and to determine whether endogenous soybean genes were interrupted upon the insertion of T-DNA sequences. The bioinformatics analysis demonstrated that the A2704-12 soybean insertion locus is located on soybean chromosome 8.

Southern blot analyses were performed to examine the integrity of the inserted sequences in A2704-12 event. These analyses also serve to determine the nature and number of pat and bla gene insertions which occur in transformation event A2704-12.
The results of the analysis of soybean event A2704-12 indicates that two copies of the pat gene cassette were integrated into the plant genome in a 'head-to-tail' configuration. One copy of the 3' bla sequences and one copy of the 5' bla sequences were integrated in between the two pat cassettes. Both integrated parts of the bla gene do not reconstitute an intact bla gene as they are integrated in opposite orientation with the 5' bla sequences being integrated opposite to how they are placed on the plasmid pB2/35SAcK. The probes were specific to the introduced sequences in event A2704-12 since no hybridization was seen with non-transgenic soybean.

Examination of the junctions between the inserted sequences and the plant DNA genome showed 8 newly created Open Reading Frame (ncORF) nucleic acid sequences (ORF-1 to ORF-8). Putative translated amino acid sequences of ORF-1 to ORF-8 were analyzed in this study.

Query sequences were compared with sequences of known allergens contained in the public allergen database AllergenOnline (www.allergenonline.org), by using several in silico approaches: 1) An 8-mer search by using SeqMatchAll, which compared the query sequences subdivided into 8 amino acid blocks with sequences from the allergen database. The criterion indicating potential significant similarity to an allergen was a 100% identity over at least 8 amino acids. 2) An overall identity search by using FASTA algorithm, which compared the complete query sequence with sequences from the allergen database. The criterion indicating potential significant similarity to an allergen was a 35% identity over the full-length query sequence (for sequences of < 80 amino acids), or over at least 80 amino acids (for sequences of ≥ 80 amino acids).

In addition, query sequences were compared to all protein sequences present in the following public reference databases: Uniprot_Swissprot, Uniprot_TrEMBL, PDB, DAD and GenPept. The over-conservative E-value threshold of 0.1 was used. The relevance of hits was also assessed by examining the E-value, the bit score as well as the biological significance of the matched protein(s). The query sequences ORF-1 to ORF-8 showed no biologically significant sequence similarities with known allergens and known toxins. There are neither allergenic nor toxicological in silico findings associated with the presence of the putative ORF polypeptides.

It can be seen that a few short vector backbone sequences had been inserted but these are non-coding and not expressed at all in A2704-12. The pUC sequences in the plasmid include a β–lactamase (bla) gene and a bacterial origin of replication. Both genetic elements are originated from natural sources and are broadly used in modern molecular biology tools. The bla gene has regulatory signals recognized in bacteria so that expression of the gene confers resistance to certain β–lactam antibiotics, however, the regulatory sequences are not functional in transgenic soybean cells. Prior to transformation of A2704, the pB2/35SAcK vector was digested with Pvu1 to disrupt the coding sequence of the bla gene and thereby remove any remote possibility of its expression. Therefore, only the pat reading frame is functional in event A2704-12.

E. Genetic Stability

Plants of A2704-12 soybean were grown for 8 or 9 generations exposing them to a variety of environmental conditions, both in the field and in the greenhouse. For each one of the generations, the plants were grown at six geographic sites. All plants gave the expected DNA hybridization pattern in Southern blot analyses. The A2704-12 insert was also introduced into six diverse genetic backgrounds of soybean and grown under varying environmental conditions for 12 generations and finally in the field for 1 additional generation. These plants were grown in at least 3 different locations by 3 companies. All plants from all genotypes gave the expected DNA hybridization pattern in Southern blot analyses. The results showed the stability of the A2704-12 DNA insert at the genomic level in different genetic backgrounds grown for multiple generations and in the same genetic background grown in different locations.

A study was then conducted to determine the segregation ratios of A2704-12 in successive generations of A2704-12 soybean and to confirm that the A2704-12 soybean insert is inherited in a predictable manner according to the Mendelian inheritance pattern. The segregation ratios determined for two successive generations of A2704-12 soybean confirmed that the A2704-12

insert is inherited in a predictable manner and as expected for a single nucleic insert. These data conform with Mendelian principles and support the conclusion that the A2704-12 event consists of a single insert integrated into a single chromosomal locus within the soybean nuclear genome. In addition, the presence or absence of the pat gene was confirmed for the F2 and F3 plant samples.

F. Expressed Material

The presence of the PAT protein was detected by ELISA. The PAT ELISA is a sandwich immunoassay in which PAT specific polyclonal antibodies are used. This determined the amount of PAT protein present in leaves during the life cycle stages of transgenic soybean event A2704-12 which are most important for herbicide tolerance. The amount of PAT protein present in leaves of A2704-12 and its non-transgenic counterpart A2704 was measured at 4 vegetative stages.

The PAT protein content ranged from 0.04 μg/g to 35.8 μg/g fresh weight in individual samples of leaves. The average amount of PAT protein measured in the four growth stages and the different treatments ranged from about 8.5 μg/g to 28.2 μg/g in the transgenic leaf samples. The PAT protein content increases with the age of the plant during the vegetative stages analyzed in this study (V3 to V8). The total extractable protein (TEP) was also measured and varied through the life cycle without a clear trend. PAT as percent of the TEP also increased during the stages measured in said study.

The average PAT as percent of crude protein ranged from 0.010% to 0.035%. PAT as a percent of TEP ranged from 0.05% to 0.13%. No PAT protein was detected in the non-transgenic counterpart A2704. For estimates of exposure, the amount of PAT protein in the leaves of soybean event A2704-12 during the vegetative life cycle of the plant has an upper limit of approximately 36 μg/g fresh weight

In addition, the amount of PAT protein was measured in soybean seeds, hay and forage, as well as and in the soybean products. The average PAT protein constitutes 1243 ng/g fresh weight of seed (range 573 - 2138 ng/g fresh weight of seed), 1515 ng/g fresh weight of hay and 1054 ng/g fresh weight of forage. The PAT protein expression was found to be very low and was not detectable in toasted soybean meal, crude lecithin, refined oil, and refined bleached and deodorized oil.
PAT content in processed fractions of transgenic A2704-12 and of the corresponding conventional soybean were also determined. The PAT contents of the processed fractions of soybean was not detected in non-transgenic samples of study. The data showed that the PAT protein is present at very low levels in the analyzed processed commodities. No PAT protein was detected in toasted soybean meal, crude lecithin, refined oil, and refined bleached and deodorized oil (food grade soybean oil).

Lastly, the PAT enzyme does not have a metabolic role. The PAT enzyme acetylates phosphinothricin at the N-terminus. By acetylating the free amino group of the active herbicidal component phosphinothricin, the PAT enzyme prevents autotoxicity in the producing organisms and shows complete resistance towards high doses of phosphinothricin or glufosinate. N-acetyl phosphinothricin has no herbicidal activity, and resistance is therefore conferred through modification of the herbicide rather than the target of its activity.

G. Toxicological Assessment

The PAT protein solutions were incubated with human simulated gastric fluid (SGF) or human simulated intestinal fluid (SIF) for different periods of time (up to 60 min) and then analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) or Western blotting, respectively. The PAT/pat protein was degraded very rapidly with less than 10% residual protein visible at 30 seconds of incubation with SGF, in presence of pepsin, at pH 1.2. There was also no significant digestion at pH 1.2 in the absence of pepsin, showing that digestion requires pepsin. The PAT/pat protein was degraded very rapidly into fragments visible up to 5 minutes of incubation. The PAT/pat protein was completely degraded within 10 minutes of incubation with SIF, in presence of pancreatin, at pH 7.5.

The rapid degradation of the PAT proteins by human simulated gastric and intestinal fluids shows that these proteins cannot survive in the digestive tract. Therefore, it could not be absorbed into the body under normal conditions.

The PAT/pat protein (encoded by the pat gene, produced in E. coli) was also tested for heat stability at temperatures of 60, 75 and 90°C for periods of 10, 30 and 60 minutes. The stability of the protein was examined using Coomassie blue stained-SDS-PAGE and Western blot analysis using a specific polyclonal rabbit anti-PAT/pat protein antibody. The PAT/pat protein was heat stable when incubated up to 30 minutes at 90°C and slightly degraded when incubated 60 minutes at 90°C. This in vitro heat stability study has been conducted to evaluate if heat treatment causes structural changes to the PAT/pat protein, as can be detected by gel electrophoresis followed by a Coomassie blue staining or a Western blot analysis.
In conclusion, the PAT/pat protein was heat-stable when incubated up to 30 minutes at 90°C, and slightly degraded when incubated 60 minutes at 90°C.

No homology with known toxins were detected. A study evaluated the potential amino acid sequence homology of the PAT protein from the pat gene (PAT/pat) with known toxins. Two in silico approaches based on the FASTA algorithm associated with the BLOSUM50 scoring matrix were used to evaluate the potential amino acid sequence identity of the query protein with known toxins. Overall, no matches were considered toxicologically relevant. As expected based on the safety profile of the query protein, no match was found with proteins from the Bayer toxin database.

Acute oral gavage was then performed. A study was conducted to assess the acute oral toxicity of the PAT/pat protein in male and female C57BL/6J mice. A group of 10 male and 10 female C57BL/6J mice was administered the PAT/pat protein (batch number 1339_PATpat) by oral gavage at the limit dose level of 2000 mg/kg body weight. A similarly constituted group of 10 male and 10 female mice received vehicle alone administered in the same manner and acted as a control. All animals were observed for clinical signs daily for fifteen days whilst their body weights and food consumption were measured weekly. At termination of the study period, animals were weighed and subjected to a necropsy including a macroscopic examination. Tissues were retained for possible microscopic examinations. No mortality and treatment related clinical signs were observed.
No treatment-related clinical signs were observed in control or PAT/pat protein-treated animals throughout the study period. Animal weights were not affected. Food consumption and macroscopic changes at necropsy were not affected in C57BL/6J mice, after an acute oral administration of PAT/pat protein at NOEL =2000 mg/kg body weight.

The source of the test protein was Escherichia coli. It is commonly practiced to conduct safety studies with PAT proteins produced in E.coli because the expression level of the PAT proteins in

transgenic plants is extremely low. Producing the amount of purified protein required to conduct the studies from plant extracts would be technically impractical because the levels of PAT protein expression are low (typically <50mg/kg of plant material). The results of six analytical tests, namely: Analysis by N-terminal sequencing, SDS-PAGE, Western blotting, HPLC/Electrospray, Mass Spectrometry, Glycoprotein staining analysis, and enzymatic activity showed that the PAT/pat protein produced in E. coli is representative of PAT/pat protein produced in A2704-12 soybean. The results of these experiments indicate safety of the PAT/pat protein produced by A2704-12 soybean.

H. Allergenicity Assessment

The potential amino acid sequence homology of the PAT protein from the pat gene (PAT/pat) with known allergens was evaluated using several in silico approaches: a) overall identity search to compare the complete query sequence with all protein sequences present in the public allergen database COMPARE, b) 8-mer search to identify any short sequences of 8 amino acids or longer that share 100% identity to an allergenic protein. The study also considered the potential N-glycosylation sites by searching their known consensus sequences, potentially found in allergenic proteins. The overall identity search showed no biologically relevant identity between the query protein and any known allergenic proteins. The 8-mer search showed no 100% identity with known allergenic proteins.

Furthermore, the study considered the potential N-glycosylation sites by searching their known consensus sequences, potentially found in allergenic proteins.
The overall identity search showed no biologically relevant identity between the query protein and any known allergenic proteins. In addition, the 8-mer search showed no 100% identity with known allergenic proteins. No potential N-glycosylation sites were identified on the amino acid sequence of the query protein.

In conclusion, there are no allergenic in silico findings associated with the PAT/pat protein. The molecular weight of PAT is about 21 kDa, hence within the 10-70 kDa range.

A study was also conducted to determine PAT content in processed fractions of transgenic A2704-12 and of the corresponding conventional soybean. PAT protein was not detected in non-transgenic samples of study BK96B02 and study BK99B013 and is therefore not presented in the table. The limit of detection (LOD) of the immunoassay procedure is 0.4 ng/mL extract, equivalent to 2ng/g for samples from study BK96B02 or equivalent to 4ng/g for samples from study BK99B013 respectively. The PAT results from study BK96B02 are averages from 2 extracts assayed. PAT results from study BK99B013 are the means of four assays. Each sample was extracted twice and each extract was assayed twice.

Serum screening was not performed. Previous data indicate that the protein is extremely labile in the digestive tract suggesting minimal to no systemic exposure anticipated in food. Results do not suggest further test such as serum screening.

I. Nutritional Data

Comparisons made between A2704-12 and its non-transgenic counterpart A2704. Seed samples from A2704-12 and its conventional, non-transgenic counterpart A2704 were generated in 9 different trial sites in the USA and Canada in the years 1999 and 2000. The multi-site study design enable to compensate environmental effects such as soil fertility, temperature, and light or water

availability on the single site. The agronomic practices followed in growing the transgenic plants and their traditional counterparts were absolutely identical except for the treatment with the corresponding herbicide for herbicide-tolerant transgenic plants.

All mean values determined for all three treatment groups are inside the respective reference ranges, indicating that with respect to these compounds A2704-12 seeds provide the same nutritional value as commercial available soybean seeds. Based on the statistical evaluation of the analytical data and the nutritional relevance of the obtained results, the seeds from A2704-12 are found to be compositionally and nutritionally equivalent to their traditional, non-transgenic counterpart, the seeds from the soybean variety A2704. There is no impact on the nutritional value of the soybean seeds caused by the genetic modification.

J. 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

BAI AND BPI-PPSSD ASSESSMENT AND RECOMMENDATIONS

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

A. Toxicological Assessment

SDS-PAGE and western blot analysis showed that digestibility of PAT in simulated gastric fluid (SGF) with pepsin at pH 1.2 is within 30 seconds after incubation (Herouet et al., 2005; Rascle, 2009; Rouquie, 2015). Digestibility was also assessed by SDS-PAGE and western blot in simulated intestinal fluid (SIF) with pancreatinin at pH 7.5. Results showed that the protein was completely broken down within 10 minutes of incubation. Since PAT protein was easily digested in SGF and SIF, indicating unlikely to cause toxicological risk to human health.

The PAT/pat protein was then tested for heat stability at temperatures of 60, 75 and 90°C for periods of 10, 30 and 60 minutes. The stability of the protein was examined using Coomassie blue stained-SDS-PAGE and western blot analysis using a specific polyclonal rabbit anti-PAT/pat protein antibody. The PAT/pat protein was heat stable when incubated up to 30 minutes at 90°C and slightly degraded when incubated 60 minutes at 90°C. Therefore the test protein is resistant and cannot be degraded by heat at 90°C for 60 minutes.

Bioinformatics analyses using FASTA algorithm associated with the BLOSUM50 scoring matrix sequence alignment tool showed that no relevant structural similarities were observed between the PAT and human and animal tonxins. Further searches using the Bayer in-house database also showed that no relevant similarities was found to known toxins (Capt, 2017).

Further, an acute oral toxicity study conducted using E. coli-produced PAT protein in 10 male and 10 female C57BL/6J mice at a total dose level of 2000 mg/kg body weight (Blanck, 2014). Results showed no treatment-related effects on survival, clinical observations, body weight gain, food consumption or gross pathology. The NOAEL for PAT was considered to be 2000 mg/kg bw.

Studies designed to evaluate the Pat/pat protein for characteristics associated toxins were conducted using highly purified Pat/pat protein produced in Escherichia coli expressing the Pat/pat gene. Six analytical tests (Confirm identity of Pat/pat protein, Comparable immuno-

reactivity, Comparable molecular weight, Comparable peptide masses, Glycosylation profile, Comparable biological activity) show that the Pat/pat protein produced in E. coli is representative of Pat/pat protein produced in A2704-12 soybean. The results of these experiments indicate safety of the Pat/pat protein produced by A2704-12 soybean.

B. Allergenicity Assessment

Bioinformatics analyses using FASTA sequence alignment tool showed that no relevant structural similarities were observed between PAT and known protein allergens upon evaluation using BLOSUM50 scoring matrix and an E-value threshold of 10 (Capt, 2009). An 8-mer search was also provided identifying any short sequences of 8 amino acids or longer that share 100% identity to an allergenic protein. The search was performed using SeqMatchAll from the EMBOSS suite, which compared the query sequence with all known allergens present in the allergen database. Analyses showed that there were no observed similarity of PAT to any known allergen.

A panel of analytical tests were also established to show the equivalence of the physicochemical and functional properties between the A2704-12 produced PAT protein and E. coli-produced PAT protein (Currier and Hendrickx, 2006; Herouet et al., 2005): a)N-terminal sequencing was determined by Edman degradation. The result showed that PAT protein from A2704-12 misses the N-terminal methionine. Post-translational modifications such as removal of a methionine are often found in proteins from both prokaryotic and eukaryotic organisms like E. coli; b)SDS-PAGE analysis of both the A2704-12 and E. coli-produced PAT proteins demonstrated a band at (~24 kDa) and showed an electophoretic mobilities of 52 mm; c)Analysis by HPLC/Electrospray Mass Spectrometry showed that the calculated masses for the peptides for both proteins were identical. However, the N-terminal penta-peptide characterized for the PAT protein from E. coli was absent in the A2704-12 soybean protein; d)The Western blot analysis showed that immunoreactive bands were present in all lanes loaded with A2704-12 produced PAT and E. coli-produced PAT proteins demonstrating equivalent immunoreactivity of the two proteins; e) The molecular weight (MW) analysis showed that the intact A2704-12 produced PAT protein migrated to the same position on the gel as the E. coli-produced PAT protein and the apparent MW was calculated to be ~24 kDa; f) Enzymatic activity analysis demonstrated that PAT protein from A2704-12 and E-coli isolated protein generates free Coenzyme A sulfhydryl groups during the transfer of the acetyl group to phosphinothricin resulting in an increase of the absorption at 412 nm. This result showed that both proteins are biologically active; g) The glycosylation analysis showed that A2704-12 produced PAT protein are not glycosylated and is equivalent to that of the E. coli -produced PAT protein.

Additionally, a study was presented on the determination of PAT fragments/content in A2704-12 soybean and its conventional counterpart. Results showed that the protein is present at very low level in the analyzed, processed commodities and no PAT protein was detected in toasted soybean meal, crude lecithin, refined oil, and refined bleached and deodorized oil (food grade soybean oil).

C. Nutritional Data

Mean level of crude protein in A2704-12 was within the range of commercial soybean varieties. Mean levels of palmitic, stearic, oleic, linoleic, linolenic, arachidic and behenic acid in A2704-12 was also within the range of commercial soybean varieties. Mean level of raffinose in A2704-12 was also found to be within the range of commercial soybean varieties.Compositional equivalence analysis showed that the seeds from A2704-12 are found to be compositionally and nutritionally equivalent to their traditional, non-transgenic variety (Oberdoerfer, 2008).

D. Recommendation

Find 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

After a comprehensive review and evaluation of the documents including the scientific evidences from provided references and literature submitted by Bayer CropScience, Inc., on its application for Direct Use as FFP of Soybean (A2704-12), 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 biodiversity. The transgenic crop will not increase its weediness potential in case the seeds spill out into the environment because the PAT protein produced by the transgenic crop will degrade upon exposure to the natural environment and general conditions, that is high temperatures (90°C and above), varying pH, enzyme digestion, etc. (Rascle, 2009).

2. PAT protein has no amino acid sequence homology with putative toxins, examined through two in silico approaches, FASTA algorithm and BLOSUM50 scoring matrix (Capt, 2017). This protein is rapidly degraded in simulated gastric and intestinal fluid of mammals (Herouet, et al., 2005).

3. The project description report (PDR) discusses the specified environmental management plan indicating the possible risk and harm to the environment and biodiversity 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, soybeans generally are very highly domesticated and does not survive well without human intervention (FAO, 2014).

Based on the evaluation and review of the literatures cited, the DENR-BC considered the regulated article safe to the environment and biodiversity, and hereby submits the technical report relative to the application of Bayer CropScience, Inc. for Biosafety Permit for direct use as food, feed, or for processing of Soybean A2704-12.

DOH ASSESSMENT AND RECOMMENDATION

After a thorough review and evaluation of the documents provided by the proponent. Bayer CropScience. 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 Soybean A2704-12. I We.

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.

The following are the observations and recommendations:

1. Scientific pieces of evidence 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 in allergic reaction.

3. The regulated article is as safe as food or feed derived from conventional soybean varieties.

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

5. It is suggested that the Bureau of Plant Industry (BPI) ensure that there shall be clear instmctions that the product is only for the pui*pose 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 Food. Feed, or for Processing (FFP) of Soybean A2704-12

SEC ASSESSMENT AND RECOMMENDATIONS

The SEC expert has concurred with the information provided by the applicant as follows:

Soybean production, which constituted about 2 percent of the country's total supply, fluctuated from 2012 to 2014. From 576 metric tons in 2012, it increased to 828 metric tons in 2013. In 2014, it was down to 718 metric tons. Annually, production grew by an average of 16.73 percent. However, the country's soybean production exhibited a downtrend 544 metric tons in 2016. It registered negative growth rate averaged 10.88 percent per year for the period 2014-2016 where production averaged 595 metric tons.

Total net food available for soybean averaged 11.7 thousand metric tons from 2012-2014 and 24.8 thousand metric tons from 2014-2016. From 11.7 thousand metric tons in 2014, it increased to 22.4 thousand metric tons in 2015 and further expanded in 2016 to 40.2 thousand metric tons. The per capita net food disposable was highest in 2016 at 0.39 kilogram per year and lowest in 2013 at 0.08 kilogram. It averaged 0.24 kilogram per year during the period 2014-2016.

During the period 2012-2014, about 98.0 percent of the country's total supply of soybean was sourced from importation. The highest volume of imports was recorded in 2012 at 58.3 thousand metric tons and the lowest was in 2013 at 28.7 thousand metric tons. It was 43.6 thousand metric tons in 2014. Exports, meanwhile, increased from 4 metric tons in 2012 to 170 metric tons in 2013. By 2014, it went down to 31 metric tons. Exports averaged 68 metric tons during the same period.

During the period 2014-2016, soybean imports constituted about 99.36 percent of the country's total supply, averaged 93.0 thousand metric tons. The biggest import volume was recorded in 2016 at 151.3 thousand metric tons, while the lowest was in 2014 at 43.7 thousand metric tons. Exports of soybean were recorded only in 2014 and 2015 at 31 metric tons and 118 metric tons, respectively.

A2704-12 soybean is not expected to change drastically the current patterns of production, consumption/utilization and trade. The current application for A2704-12 is for direct use as food and feed or for processing (FFP) and not for cultivation in the Philippines.

Recommendation

The SEC expert has recommended for the approval and issuance of the biosafety permit of the GM product.