ASSESSORS' CONSOLIDATED REPORT ON BASF PHILIPPINES INC.'s APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF OILSEED RAPE MS11

EXECUTIVE SUMMARY

On October 12, 2018, BASF Philippines Inc. submitted oilseed rape MS11 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 oilseed rape MS11 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 oilseed rape MS11 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 oilseed rape MS11.

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 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

Oilseed rape contains significant amounts of protein and lipids that account for more than 60% of the seed weight The lipids are primarily oleic acid and linoleic acid. Faccty acids serve as important sources of energy as well as source of essential fatty acids that are essential constituents of the cell membrane. It also contains other essential nutrient such as Vitamin K (phylloquinone), Vitamin E (tocopherols) and sterols. Rapeseed meal, a by-product that remains after extraction, is a rich source of protein. Oilseed rape is also a source of a few anti-nutrients. Glucsinolates, phenolic compounds like sinapine, phytic acid, and tannins are considered anti-nutritional factors. However, these are in very low amounts in current low erucic acid and low glucosinolate rapeseed meal. Moreover, processing steps including heat treatment eliminates or inactivates anti-nutritional factors.

Oilseed rape is not a source of toxicants. Rare cases of respiratory symptoms were reported among adults not due to erulic rapeseed oil but due to other factors such as occupational exposure or proximity to areas of intense canola cultivation. Food allergy to low erulic acid rapeseed oil by the general population is likely low as it is not a source of protein.

Low erulic acid rapeseed oil is commonly consumed globally. It is used in food processing as well as for home and baking. It is used in both salad and cooking oil products as well as in hydrogenated products such as margarine and shortenings. Though consumption data are not extensively available, the usage of oilseed rape- derived products in the food chain is well known. It is widely used in both salad and cooking oil products as well as in hydrogenated products such as margarine and shortenings. Low erucic acid rapeseed oil is used by all population subgroups in all food products requiring an oil source.

B. The Transgenic Plant

MS11 had been granted food approvals in 3 countries namely: Australia/New Zealand (December 7, 2017), United States (October 20, 2017), and Canada (January 30, 2018).While, it had been granted feed approvals in 2 countries namely: United States (July 26, 2017), and Canada (January 30, 2018).

Oil from MS11, just like any conventionally produced rapeseed oil, is used across all ages and consumer groups. It is to be noted also that MS11 is male sterile hence is not a stand-alone line. Therefore, no MS11 product (as a single event) enters the food chain.

C. Donor Organism

The source and potential pathogenic or allergenic properties of the protein-encoding sequences contained in MS11 oilseed rape were adequately described.

Both the barnase and barstar genes were derived from *Bacillus amyloliquefaciens*. Expression of the barnase gene in the anther results in the absence of viable pollen and male sterility. Barstar gene, on the other hand, encodes for the barstar protein, which is a known inhibitor of the Barnase protein and was utilized in this event to enhance the transformation frequency. Both genes are

non-allergenic and have no biologically relevant identities with any toxic proteins from the Bayer's toxin database (Deltort, 2018).

The bar gene, on the other hand, was isolated from *Streptomyces hygroscopicus*, a common microbe in the soil. This gene encodes for phosphinothricin acetyltransferase (PAT/bar) protein, which confers tolerance to glufosinate ammonium. Bar and its homologues have no record of being toxic or allergenic in humans or animals. Food and feeds that made use of genetically engineered plants expressing the PAT protein, either by itself or together with other traits have been approved in more than 20 countries (Herouet, et al., 2005).

C. Transformation System

MS11 oilseed rape was produced by Agrobacterium-mediated transformation using the plasmid vector pTCO113. This contains the barnase- barstar gene casette causing male sterility and bar gene cassette, which confers tolerance to the herbicide glufosinate ammonium. The hypocotyl segments from the germinated seedlings of Brassica napus (variety N90-740) were dissected and subjected to callus induction. Small clumps of embryogenic calli were transformed using the vector system consisting of an Agrobacterium strain C58C1Rif and two plasmid components, non-oncogenic helper Ti-plasmid pGV4000 and T- DNA cloning vector pTCO113. The non-oncogenic helper Ti-plasmid pGV4000, with a deleted T- region, carries the vir genes necessary to deliver an artificial T- DNA cloned on the second plasmid into the plant genome. The transformed calli were selected using a medium with glufosinate ammonium. Somatic embryogenesis was induced, which was followed by the regeneration of plantlets. These were transferred to a greenhouse where characterization of flowers, seeds and other traits was performed. The target of genetic modification is the nucleus to render the recipient organism and its progenies male sterile and to confer tolerance to herbicide glufosinate-ammonium.

The recombinant plasmid contains three transgenes: barnase, barstar, and bar. Barnase gene expression cassette: Pta29: promoter of the anther-specific gene TA29 of Nicotiana tabacum coding sequence of the barnase gene of B. amyloliquefaciens, 3' untranslated region of the barnase gene of B amyloliquefaciens and 3' nos: 3' untranslated region of the nopaline synthase gene derived from the T-DNA o pTiT37; Barstar gene expression cassette: Pnos promoter region of the nopaline synthase gene of Agrobacterium tumefaciens, coding sequence of the barstar gene of B. amyloliquefaciens and 3' untranslated region of the TL DNA gene 7 of the Agrobacterium tumefaciens octopine Ti plasmid; Bar gene expression cassette: PssuAt: promoter region of the ribulose-1,5-bisphosphate carboxylase smal subunit gene of Arabidopsis thaliana, coding sequence of the phosphinothricin acetyltransferase of S hygrocopicus and 3' untranslated region of the TL-DNA gene 7 of the Agrobacterium tumefaciens octopine T plasmid.

The pTCO113 vector is 13540 bp in size. A non- oncogenic helper Ti-plasmid pGV4000, whose T-region was deleted, was used. This helper plasmid also carries the vir genes, in order to transfer the artificial T-DNA from pTCO113 into the plant genome.

D. Inserted DNA

There was only one insertion site in MS11 oilseed rape. This was confirmed using Southern blot analysis wherein the genomic DNA of MS11 was digested using restriction enzymes. The hybridization fragments obtained after the MS11 genomic DNA was digested with the restriction enzymes BclI, NdeI, HpaI, KpnI, EcoRV, HindIII, and EcoRI and then hybridized with various probes, fit the expected fragment size. In addition, the fragment size of the 3' integration fragment

(2.76 kb) and internal fragment (2.48 kb) obtained using BclI and AflIII digestions are nearly the same Additional 5' and 3' flanking sequences of MS11 were determined to obtain at least 1 kb of both host flanking regions and the corresponding insertion locus sequence of MS11. Afterwards, these sequences were compared and merged with the sequences reported in the paper of Habex (2008).

Genomic DNA from both MS11 oilseed rape and the non- transgenic variety N90-740 plant material were used as templates for PCR amplification. Amplicons were sequenced, assembled, compared and merged with the previously determined sequences. The final MS11 transgenic locus is 2471 bp long, which included 1129 bp of the 5' flanking sequence, 1302 bp of the 3' flanking sequence and a target site deletion (TSD) of 40 bp. Furthermore, the flanking sequences obtained at the transgenic locus were identical to the corresponding sequences obtained from the insertion locus. These results showed that the inserted transgenic sequence of MS11 consisted of one copy of the transforming T-DNA and that the transferred DNA in the plant corresponds to the original DNA configuration of the pTCO113 plasmid.

There were no truncations, deletions, rearrangements in the transgenic sequences. Sequence alignment revealed no differences between the flanking sequences in the pre -insertion locus and in the transgenic locus. This showed that the flanking sequences of MS11 correspond to the oilseed rape genome in its original order and that all inserted transgenic sequences originate from the plasmid pTCO113.

The barnase, barstar and bar genes have also been expressed in genetically modified oilseed rape events, MS8 and RF3 which have been approved globally in 12 countries worldwide. In addition, the PAT protein has been expressed in other engineered crops such as corn, canola, cotton, and soybean.

There were no plasmid backbone sequences present in the MS11 oilseed rape. Southern blot analysis and PCR were done to determine the presence of any backbone sequences of the transformation vector used. Genomic DNA from the MS11 and non-transgenic plants were isolated and subjected to Southern blo analysis using four vector backbone probes, covering the vector backbone sequences of the pTCO113 vector, except for the barstar sequences. No hybridization fragments were detected in the transgenic MS11 oilseed rape confirming the absence of background hybridization. The absence of the barstar gene, as part of the vector backbone, was confirmed using PCR. No amplification fragmen from the primer pair MDB469-MDB470, targeting the barstar gene from the vector backbone, was visible in the MS11 sample. The results of the Southern blot analysis and PCR were sufficient to confirm the absence of plasmid backbone sequences in MS11 oilseed rape.

E. Genetic Stability

Southern blot analysis was used to demonstrate the genetic stability of the insert and the absence of plasmid backbone sequence in five generations (T2, T3, F1, BC1, and BC2) of MS11 oilseed rape genome. Results showed that the hybridization bands specific to the MS11 insert were identical in all lanes containing genomic DNA extracted from MS11 oilseed rape plants of the generations tested. Furthermore, the genomic DNAs of the T2, T3, F 1, BC1, and BC generations of MS11 oilseed rape were digested with the restriction enzyme, EcoRV. The analyses showed similarity in the profiles of the generations tested showing that MS11 insert is stably inherited from one generation to the next.

Massengil (Annex 21: 2016 M-545765-01-1) determined the segregation ratios of the introduced genes in multiple generations of the MS11 to confirm that the MS11 insert is inherited in a predictable manner according to the Mendelian inheritance pattern. Genomic DNA from individual plants of five MS11 generations was tested for the absence or presence of event MS11 by polymerase chain reaction (PCR) analysis. The results from event-specific PCR analysis (Table 12) were used to calculate the segregation ratios of the MS11 insert. Chi-square analysis of the segregation data for five generations confirmed that the MS11 insert is inherited in a manner that is predictable according to Mendelian principles. These data support the conclusion that the MS11 event consists of a single insert integrated at a single chromosomal locus within the B. napus nuclear genome (Annex 21: Massengil (Annex 21: 2016 M-545765-01-1).

F. Expressed Material

The level of PAT/bar expression in treated (with trait-specific herbicide) and untreated MS 11 oilseed rape matrices ranged from below the lower limit of quantification (LLOQ) to 74.44µg/g dry weight. Root (BBCH 30-39 and BBCH 57 65 growth stages) and grain matrices all exhibited lower mean PAT/bar dry weight expression levels relative to mean dry weight values for the other matrices of MS11. The mean PAT/bar dry weight expression levels were highest in whole plant samples of both untreated and treated MS11 at BBCH 30-39 and BBCH 57-65 growth stages, respectively Meanwhile, the level of barstar expression in untreated and treated MS11 matrices ranged from <LLOQ to 1.0 µg/g dry weight. These low expressions of the barstar protein were consistently detected in the root samples (BBCH 30-39 and BBCH 57 -65 growth stages). The level of barnase expression was also <LLOQ in all untreated and treated MS11 matrices.

The levels of expression of barnase and barstar are below the lower limit of quantification (LLOQ) in MS11 grain and in all the processed fractions. Meanwhile, PAT/bar protein's level of expression ranged from 0.25 to 0.31 µg/g dry weight in MS11 grain, press cake, and solvent extracted meal; and below the lower limit of quantification (LLOQ) in MS11 toasted meal, crude oil, and RBD oil.

The levels of expression of the PAT/bar, barnase and barstar proteins were measured using sandwich enzyme-linked immunosorbent assay (ELISA) using the following sample matrices: whole plant during leaf development (BBCH 13 16), stem elongation (BBCH 30-39), and inflorescence (BBCH 57-65); root during stem elongation and inflorescence raceme during inflorescence; and grain at maturity. ELISA is a plate-based assay teachnique designed for detecting and quantifying substances such as proteins, antibodies, peptides and hormones. An antigen is immobilized on a solid surface and then complexed with an antibody that is linked to an enzyme. Detection was done by evaluating the conjugated enzyme activity via incubation with a substrate to produce a measurable product.

Quantification of the PAT/bar protein was done using EnviroLogix QualiPlate™ Kit for LibertyLink® PAT/bar Meanwhile, quantification of the barstar and barnase protein was performed using EnviroLogix Barstar Plate Kit and a EnviroLogix Barnase Plate Kit, respectively.

Barnase expressed in the tapetum cells during anther development leads to lack of viable pollen and male sterility while Barstar is an inhibitor of Barnase (but was used to enhance transformation frequency). Bar encodes for phosphinothricin acetyltransferase (PAT), which acetylates glufosinate to its inactive form conferring tolerance to glufosinate-ammonium herbicide.

G. Toxicological Assessment

The PAT/bar protein was degraded very rapidly in human simulated gastric fluid (using pepsin at pH 1.2) and human simulated intestinal fluid (using pancreatin at pH 7.5), within 0.5 minutes and subsequent incubation times). At time 0 incubation, the intact protein band was not visible anymore and some degradation bands were clearly visible At approximately 6kDa. No bands were visible during these incubation periods which indicates a complete digestion of of the PAT/bar protein in less than 30 seconds. The PAT/bar protein was also tested for heat stability at temperatures of 60, 75 and 900C for periods of 10, 30 and 60 minutes. The stability was examined using Coomassie blue stained-SDS-PAGE and Western Blot Analysis using specific polyclonal rabbit anti- PAT/bar protein antibody. The PAT/bar protein was heat-stable when incubated up to 30 minutes at 900C, and slightly degraded when incubated at 900C for for 60 minutes.

There are no in silico toxicological findings associated with the PAT/bar protein from the toxin database. There was also an acute oral gavage study for the PAT/bar protein which was done at a dose level of 2000mg/kg body weight for 15 using 20 C57BL/6J, 10 females and 10 males. No signs of systemic toxicity were noted.

Microbially-produced PAT/BAR protein and plant produced PAT/BAR protein showed functional and structural equivalence.

On the other hand, Human simulated gastric fluid (using pepsin at pH 1.2) and human simulated intestinal fluid (using pancreatin at pH 7.5) were also used in the digestibility study of Barnase protein. Based on the Western blot, all bands were not visible within 30 seconds which indicates complete digestion of Barnase protein within 30 seconds. Heat stability study was also done and SDS-PAGE and Western blot analyses were performed. Samples of Barnase were incubated for 30 minutes at 4°C, 25°C 37°C, 55°C, 75°C, and 95°C followed by SDS-PAGE and Western blot analyses. The SDS -PAGE results suggest tha Barnase degrades upon heating at 55°C and, therefore, not stable upon heating at 55°C.

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. Barnase protein showed a high degree of similarity with other proteins of its respective ribonuclease family. However, all matches were considered not toxicologically relevant either in terms of sequence homologies, or in terms of toxicological concerns as no records were found on potential risk associated with this protein family once ingested. With the Bayer toxin database, a total of 2 matches, with an E-value <10, were obtained but based on the safety profile of the query protein, none of the matches were biologically relevant because of the poor alignments (e.g., low % identity, short alignment, presence of gaps, high E -value). Therefore, no biologically relevant identities were found between the query protein and any toxic proteins from the toxin database.

An acute oral toxicity test with the Barnase protein was also done at 2000 mg/kg body weight via the oral route. It did not produce any signs of systemic toxicity in the male (10) and female (10) C57BL/6J mice. Peptide mapping of the microbially-produced protein demonstrated 100% coverage against the theoretical amino acid sequence of the Barnase protein and was 100% identical to the amino acid sequence predicted from the nucleotide sequence of the MS11 insert.

Meanwhile, human simulated gastric fluid using pepsin at pH 1.2 and a pancreatin solution at pH 7.5 at incubation times 0.5 to 60 minutes was used in the digestibility study for the Barstar protein. Based on the Western blot using pepsin, all bands were not visible within 30 seconds

which indicates complete digestion of Barstar protein within 30 seconds. For the intestinal fluid using pancreatin, >90% of the Barstar protein was degraded within 10 minutes of incubation.

The Barstar protein was also tested for heat stability by incubating for 30minutes at 4°C, 25°C, 37°C, 55°C, 75°C, and 95°C followed by SDS-PAGE and Western blot analyses.SDS -PAGE and Western blot analyses showed that at 25°C, 37°C, and 55°C, the majority of Barstar remained in the supernatant; at 75°C and 95°C, a small amount of Barstar in the pellet but the majority still remained in the supernatant and at 95°C, Barstar forms soluble oligomers upon heating at 95°C and the native form of Barstar was not stable upon heating at 95°C.

In addition, based on FASTA algorithm and BLOSUM50 scoring matrix and Bayer Toxin database, no biologically relevant identities were found between Barstar protein and any toxic proteins.

The acute oral toxicity test with the Barstar protein at 2000 mg/kg body weight via the oral route did not produce any signs of systemic toxicity in the male (6) and female (6) C57BL/6J mice. Microbially-produced protein was used. Barstar in MS11 was produced as intended and that the microbially-produced Barstar protein can be considered as a surrogate for the Barstar expressed in MS11.

H. Allergenicity Assessment

The 80-mer sliding window search as well as the overall identity search against the COMPARE database showed no biologically relevant identities between the query sequence and 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/bar, Barnase and Barstar proteins. The Barnase and the Barstar proteins both has an approximate molecular weight of 12.4 kDa and 10.3 kDa respectively, and therefore within the 10 – 70 kDa range.

Oilseed rape (OSR) grain is only used in the human diet after processing into food grade vegetable oil. Oil from low glucosinolate and erucic acid rapeseeds (canola quality) is used in a variety of food applications including: salad oil, frying fat, baking shortening and table spreads (margarine). Beside the oil there are no other products from OSR that enter the food chain. Because virtually no protein is present in the oil extracted from the seed, the potential for human exposure is exceedingly low. Since only the processed oil from OSR is available for human consumption, and the processing removes proteinaceous material, there are no additional toxicity or allergenicity concerns regarding this product compared with conventional canola. Table 17 in Section VII.2 shows that PAT/bar is <LLOQ in crude and RBD oil.

It is to be noted also that MS11 is male sterile hence is not a stand-alone line. Therefore, no MS11 product (as single event) enters the food chain. No serum screening was performed. The no likelihood that PAT protein is allergenic is based on weight of evidence.

I. Nutritional Data

MS11 is male sterile, having flowers but lacking anthers. MS11 provides the male sterile line as part of a hybridization system and will not be marketed as a single event. It is not a standalone line, any grain/seed it produces results from pollination by pollen from pollen-fertile plants and therefore not anymore true MS11 as it already is a mix of different genetic backgrounds. Analyses

using these grains/seeds are not appropriate to evaluate the MS11 event per se. Hence, no nutritional data are available.

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

The results of the SDS-PAGE and Western blot assays demonstrate that PAT protein is rapidly degraded in both simulated gastric fluid containing pepsin and simulated intestinal fluid containing pancreatin within 30 seconds. No degenerative bands were observed due to digestion (Rascle, 2009; Herouet et al., 2005, Rouquie, 2016). The heat stability analysis using SDS-PAGE and western blot analysis provided by the developer indicated that PAT protein is heat-stable when incubated up to 30 minutes at 90°C, and slightly degraded when incubated 60 minutes at 90°C. The activity is significantly impacted by heat treatment (Herouet et al., 2005; Rascle, 2009).

Moreover, the results of bioinformatics analysis provided by the developer using FASTA sequence alignment tool showed that PAT protein has no homology to any known toxins in the NCBI non-redundant database and in-house Bayer toxin database. The evidence indicates that the PAT protein does not share any significant sequence similarity with the database of known sequenced protein toxins (Deltort, 2018).

In addition, an acute oral gavage study for the PAT/bar protein was performed. Treatment with PAT/bar protein at 2000 mg/kg body weight via the oral route did not produce any signs of systemic toxicity in male and female C57BL/6J mice. Escherichia coli is used as source protein (Raybould et al., 2013; Vandermarliere, 2015). E- coli-produced PAT recombinant protein batch 1215_PAT/bar protein has been shown to be equivalent to the plant-produced PAT present in MS11.

Meanwhile, the Barnase protein was tested for stability in human SGF and SIF with pepsin and pancreatin at pH 1.2 and ph7.5 respectively at 0.5 to 60 minutes. The samples were analyzed for presence of the test protein and potential stable protein fragments by Coomassie blue-stained sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot. The Barnase protein was completely digested within 30 seconds when subjected to SGF, on the other hand the protein was not completely digested when subjected to SIF at 60 minutes.
In conclusion the Barnase protein is stable on vitro digestibility in human simulated intestinal fluid.

In addition, to assess the heat stability of the microbially produced Barnase protein, SDS-PAGE and Western blot analyses were performed
The SDS-PAGE results suggest that Barnase degrades upon heating at 55°C and therefore, is not stable upon heating at 55°C. The Western results suggest that Barnase degrades upon heating at 55°C and therefore, is not stable upon heating at 55°C.

Moreover, an overall identity search with all protein sequences present in the NCBI non-redundant database. The Barnase protein showed a high degree of similarity with other proteins of its respective ribonuclease family. Overall, all matches were considered not toxicologically relevant either in terms of sequence homologies, or in terms of toxicological concerns as no records were found on potential risk associated with this protein family once ingested.

The developer also provided sufficient information regarding the acute oral toxicity study conducted (Totis, 2014). Barnase protein was administered at a single dose levels of up to 2000 mg/kg body weight to 6 male and 6 female C57BL/6J mice. There were no treatment-related effects on survival, clinical observations, body weight gain, food consumption and gross pathology. The NOAEL for Barnase was considered to be 2000 mg/kg.

Lastly, the results of the SDS PAGE and western blot assays demonstrate that Barstar protein is rapidly degraded in simulated gastric fluid containing pepsin within 30 seconds while it is >90% degraded within 10 minutes of incubation in simulated intestinal fluid containing pancreatin (Lautraite, 2012; Rouquie, 2016).

In addition, to assess the heat stability of the microbially produced Barnase protein, SDS-PAGE and Western blot analyses were performed. Barstar forms soluble oligomers upon heating at 95°C and the native form of Barstar is not stable. The results of bioinformatics analysis provided by the developer that Barstar protein has no homology to any known toxins using NCBI non-redundant database and in-house Bayer toxin database with FASTA algorithm (Deltort, 2018).

Moreover, the Barnase protein was evaluated for acute oral toxicity in male and female C57BL/6J mice.The treatment with the Barnase protein at 2000 mg/kg body weight via the oral route did not produce any signs of systemic toxicity in the male and female C57BL/6J mice.

Escherichia coli is used as source protein. E- coli-produced Barstar protein has been shown to be equivalent to the plant-produced Barstar present in MS11. These are demonstrated by comparing molecular weight, immuno-reactivity, glycosylation status, N-terminal sequence, and biological activity (Bushey et al., 2014; Habex, 2008; Vigeolas, 2017).

B Allergenicity Assessment

The results of bioinformatics analysis provided by the developer that PAT protein has no homology to any known allergens using 80-mer sliding window search, overall identity search, and 8-mer search with COMPARE database with FASTA algorithm. Equivalence study provided by the developer indicated the N-terminal sequence, molecular mass, peptide map and enzyme activity of PAT protein in MS11. No potential N-glycosylation sites were identified on the amino acid sequence of the query protein.The molecular weight of PAT protein is calculated to be 21 kDa.

In addition, the results of bioinformatics analysis show that Barnase protein has no homology to any known allergens using 80-mer sliding window search, overall identity search, and 8-mer search with COMPARE database with FASTA algorithm. No potential N-glycosylation sites were identified on the amino acid sequence of the query protein. he molecular weight of Barnase protein is calculated to be 12.4 kDa.

Moreover, Barstar protein also has no homology to any known allergens using 80-mer sliding window search, overall identity search, and 8-mer search with COMPARE database with FASTA algorithm. No potential N-glycosylation sites were identified on the amino acid sequence of the query protein. The molecular weight of Barstar protein is calculated to be 10.2 kDa.

Because virtually no protein is present in the oil extracted from the seed, the potential for human exposure of PAT, Barnase and Barstar proteins in MS11 is exceedingly low since virtually there is no protein that is present in the oil extracted from the seed. The PAT, Barnase and Barstar proteins are <LLOQ in crude and RBD oil. Also there is no MS11 product as single event enters the food chain because MS11 is male sterile hence is not a stand-alone line. Serum screening was also not performed. Bioinformatics analysis, digestibility assay, and heat studies are enough to conclude that the protein will not pose allergenicity concerns

C. Nutritional Data

MS11 is male sterile, having flowers but lacking anthers. MS11 provides the male sterile line as part of a hybridization system and will not be marketed as a single event. It is not a standalone line, any grain/seed it produces results from pollination by pollen from pollen-fertile plants and therefore not anymore true MS11 as it already is a mix of different genetic backgrounds. Analyses using these grains/seeds are not appropriate to evaluate the MS11 event per se. Nevertheless, relevant nutritional/compositional data will be provided when commercial breeding stacks involving MS11 will be filed as appropriate.

D. Recommendation

History of safe use is attributed on the host organism (Brassica napus) and donor organisms (Bacillus amyloliquefaciens and Streptomyces hygroscopicus) which is not known to be toxic or allergenic to humans and animals.

Safety of the novel proteins, PAT, Barnase and Barstar, in MS11 canola were assessed based on the digestibility, heat inactivation, amino acid sequence comparison and oral toxicity studies and other related scientific literatures provided by the developer. Results of the analyses indicated that the novel proteins are being digested rapidly in mammalian gastric fluid, a characteristics of dietary proteins, and do not cause toxicity on mice via acute oral gavage. Amino acid sequence analysis indicated that PAT, Barnase and Barstar have no significant homology to any known toxins or allergens.

Safety assessment based on the nutritional data is not applicable since MS11 is male sterile. For MS11 canola, weight of evidences approach indicates the substantial equivalence of the single event in terms of nutritional composition and food safety, with the conventional canola other than the tolerance to glufosinate-containing herbicides and male sterility. After reviewing the provided material of BASF Philippines, Inc. and other literature, it is therefore concluded that canola MS11 is as safe as its conventional counterpart.

DENR ASSESSMENT AND RECOMMENDATION

After a comprehensive review and evaluation of the documents including the scientific evidence from references and literature submitted by BASF Philippines, Inc., on its application for Direct Use as FFP of Oilseed Rape (MS1H, 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 be able to transfer its pollen grains to its wild relatives due to the expression of Barnase-barstar system, which was due to the insertion of the bar and barstar genes. Baxnase expression causes the lack of viable pollen grains and male sterility in the transgenic crop while barstar inhibits barnase expression causing fertility restoration (Singh et al., 2015);

2. Bacillus amyloliquefaciens, a bacterium used in brewing, bread-making, food industry, and is frequently used as a source of industrial enzymes, has no recorded cases of infections or intoxications (de Boer & Diderichsen, 1991);

3. Streptomyces hygroscopicus has been used for the production of validamycin, an antifungal agent, for more than 40 years (Wu et al" 2012). S. hygroscopicus has no recorded case of being a human, animal, or plant pathogen and is not associated with any other properties known to affect human health (Tresner & Backus, 1956); and

4. 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. Oilseed rape seedlings are also sensitive to weather changes, early growing conditions, and seedbed conditions thus need human intervention (AHDB Cereals & Oilseeds, 2009).

Based on the evaluation and review of 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 BASF Philippines, Inc. for Biosafety Permit for direct use as food, feed, or for processing.

DOH ASSESSMENT AND RECOMMENDATION

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 oilseed rape varieties.

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

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

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 Oilseed Rape MS11.

SEC ASSESSMENT AND RECOMMENDATIONS

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

The SEC expert does not believe that the current volume of consumption/utilization will be influential enough to affect production, consumption/utilization and trade given that the MS11 oilseed rape is not intended for cultivation in the Philippines but rather for importation only.

Recommendation

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