ASSESSORS' CONSOLIDATED REPORT ON BASF PHILIPPINES INC.'s APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF COTTON GHB811

EXECUTIVE SUMMARY

On July 15, 2019, BASF Philippines Inc. submitted cotton GHB811 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 cotton GHB811 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 cotton GHB811 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 cotton GHB811.

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 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. These assessors were given thirty (30) days to submit their independent assessment to BPI Biotech Secretariat.

STRP'S ASSESSMENT

1. The Host Plant

a. Oil and proteins may be derived from cotton. After oil extraction, cotton seedcake and cotton meal are used as feeds because of its protein contents. Anti-nutrients such as Gossypol, Malvalic Acid, Sterculic Acid and Dihydrosterculic Acid are present in whole cottonseed, and cotton seed oil. Cottonseed meal contains only Gossypol.[1].

b. Food grade cottonseed oil is used as cooking oil, salad oil, as ingredients in snack foods, in margarine, pastries, baked products, mayonnaise and as shortening. It is also used as a cocoa butter substitute. About 56% of the oil is used for salad or as cooking oil. The processed linter pulp used as casings for sausages, etc. Cottonseed flour is mixed with corn flour, torula yeast and fortified with vitamins and fed to children in Central America in their first years of age to combat protein deficiency.[1].

c. Cottonseed meal is an excellent source of proteins for ruminants. Whole cottonseed is used as dairy feed. Cottonseed hulls are also used to feed ruminants in combination with corn silage and hay.[1].

2. The Transgenic Cotton

a. Cotton GHB811 is approved for food and feed on the following countries: Australia, New Zealand, Japan, USA, Argentina and Canada.[6][7].

b. Consumption patterns will not change due to introduction of new food because both food and feed products derived from GHB811 are found to be compositionally and nutritionally equivalent to their non-GM counterpart.[8].

3. Donor Organism

a. The following protein-encoding sequences are described adequately: *hppdPfW336-1Pa* coding sequence; *2mepsps* coding sequence.[9][10][11][12].

b. *Pseudomonas fluorescens* – a non-pathogenic saprophyte found in soil, water and plant surface environments and has a history of safe use.[10].

c. *Zea mays* – eaten by humans and animals and also has a long history of safe use, donor of the 2MEPSPS.[9][11].

d. HPPD W336 does not contain known allergens; it lacks amino acid sequence similar to known toxins and allergens.[18].

e. 2mEPSPS protein is innocuous; does not possess any of the properties associated with known allergens.[16].

4.Transformation System

a. *Agrobacterium*-mediated transformation using the vector pTSIH09 containing hppdPfW336-1Pa and 2mepsps expression cassettes.[12][25][26].

b. The target of genetic modification is the nucleus of the cotton plant is and its progenies tolerant to HPPD inhibitors and the glyphosate herbicides.[12][25][26].

c. The pTSIH09 vector has a size of 13099 base pairs. The schematic overview of the pTSIH09 plasmid is given in indicating the size, orientation and location of all the genetic elements, oligonucleotide primers used for PCR analysis, and the sites of any restriction endonucleases used in the analysis of the inserted DNA.[12][25][26].

d. The two plasmid components of the vector system are the non-oncogenic helper Ti-plasmid pEHA101 and the T-DNA region containing the vector pTSIH09.[12][25][26].

1. The Inserted DNA

a. There is only one insertion site. This was demonstrated by Southern Blot Analysis on the genomic DNA prepared from cotton leaf.[27].

b. Each base in the GHB811 cotton transgenic and insertion loci consensus sequences has been sequenced in both the forward and reverse direction, and in at least two independent PCR amplicons. The results of this study and that obtained by Dreesen indicated that the sequences contain an additional 'T' within a T-stretch located in the 5' flanking the genomic sequence regions of both the GHB811 cotton insertion and the transgenic loci.[27].

c. The bioinformatics study described demonstrated that the GHB811 cotton insertion locus originates from cotton chromosome A05. This indicates that it is unlikely that the insertion of T-DNA sequences in the GHB811 cotton insertion locus interrupt or alter the transcriptional or translational activity of the endogenous cotton genes.[27].

d. A search for the ORFs defined as the translation between two stop codons was performed. ORFs with a minimum size of 3 amino acids and crossing a junction or overlapping the inserted DNA were identified which rendered 549 ORFs sequences. The 126 ORFs sequences of ≥ 30 amino acids length were selected and used as query sequences in similar searches with allergen and toxin sequences. These ORFs sequences showed no relevant sequences identities with known allergens and toxins sequences.[27].

e. The Southern Blot Analysis results demonstrated that cotton GHB811 contains a single copy of the complete T-DNA, which consists of one copy of the hppdPfW336-1Pa gene cassette and one copy of the 2mepsps gene cassette, at a single locus. In addition, the Southern Blot results confirmed no plasmid vector backbone sequences were detected in cotton GHB811.[27].

2. Genetic Stability

a. Genomic DNA from individual cotton GHB811 plants from five generations was digested with the restriction enzyme combination PsiI/SapI and hybridized to the T-DNA probe. All cotton GHB811 samples showed the expected fragments. The structural stability of the cotton GHB811 transgenic locus was demonstrated in the T1, T3, T4, BC1F2 and BC2F3 generations.[30][31].

b. They were also tested for the genotype *hppdPfW336-1Pa* and *2mepsps* genes by PCR. The PCR results were used to calculate the segregation ratios of the genes contained within the GHB insert. Chi-square analysis of the segregated data for the 3 generations was performed. The analysis confirmed that the *hppdPfW336-1Pa* and *2mepsps* genes contained within the GHB811 insert are inherited in a manner that is predictable according to the Mendelian principles and is consistent with insertion into a single chromosomal lotus within the cotton nuclear genome.[30][31].

3. Expressed Material

a. ELISA was used to determine the expression levels of the novel proteins in different plant parts. The level of 2mEPSPS protein in GHB 811 in cotton leaf, root, square boll, whole plant and seeds ranged from 76.36 to 1762.54 ug/g dry weight. The level of 2mEPSPS protein in pollen ranged from 12.86 to 33.89 ug/g fresh weight.[32].

b. The levels of HPPD W336 protein in cotton leaf, root, square boll, whole plant and seeds ranged from 10.91 to 1673.89 ug/g dry weight. The level of HPPD W335 protein in pollen ranged from below the lower limit of detection to 0.69 ug/g fresh weight.[32].

c. The expression levels of both the 2mEPSPS and HPPD protein in all plant parts of the treated and non-treated (with trait specific herbicides) GHB811 cotton were similar. Both the HPPD W336 and 2mEPSPS proteins do not have metabolic roles.[32].

4. Toxicological Assessment

a. For the digestibility study in human simulated intestinal fluid, a porcine pancreatin solution at pH 7.5 was used. The HPPD W336 protein was degraded rapidly, in less than 30 seconds of incubation. Meanwhile, in human simulated gastric fluid, a pepsin solution at pH 1.2 was used. The HPPD W336 protein was also degraded rapidly, within 30 seconds of incubation.[20][21].

b. The HPPD W336 protein was heat stable up to 60 minutes at 90 deg C. The methods used were SDS-PAGE and Western Blot.[19].

c. A bioinformatics analysis was then done to evaluate the potential amino acid sequence identity of the single mutated HPPD W336 protein with known allergens and toxins. Based on this, there are neither allergenic nor toxicological in silico findings associated with the HPPD W336 protein.[18].

d. Acute oral toxicity was also performed in male and female C57BL/6J mice. There were no mortalities, no treatment-related clinical signs, no effects on the body weight and food consumption parameters as well as no macroscopic changes in necropsy in

C57BL/6J mice after acute oral administration of the HPPD W336 protein at 2000 mg/kg body weight.[15].

e. 2mEPSPS protein degraded very rapidly in human simulated intestinal fluid, in less than 30 seconds using pancreatin enzyme at pH 7.5. It was also very rapidly in human simulated gastric fluid, within 30 seconds using pepsin enzyme at pH 1.2. Both tests showed no fragments visible after SDS-PAGE.[23][24].

f. The 2mEPSPS protein is also partially heat-stable up to 90 deg C for 30 minutes and markedly degraded at 90 deg C for 30 minutes.[22].

g. Two in silico approaches using the FASTA algorithm with BLOSUM50 scoring matrix were used. And based on the bioinformatics analysis, there are no toxicological in silico findings associated with the 2mEPSPS protein. Acute oral toxicity was also performed in male and female C57BL/6J mice. There were no mortalities, no treatment-related clinical signs, no effects on the body weight and food consumption parameters as well as no macroscopic changes in necropsy in C57BL/6J mice after acute oral administration of the 2mEPSPS protein at 2000 mg/kg body weight.[16].

h. The 2mEPSPS and the HPPD W336 proteins were produced in a recombinant *E. coli* and were engineered to match the amino acid sequences of their counterparts expressed in GHB811. The equivalence of the *E-coli*-produced and the GHB811 cotton-produced proteins were evaluated and found that they are equivalent using analytical tests and assays including densitometry analysis of Coomasie-stained SDS-PAGE, Western Blot and glycostaining analysis, Mass Spectrometry, and N-terminal sequence analysis.[35][36].

5. Allergenicity Assessment

a. Two in silico approaches using the FASTA algorithm with BLOSUM50 scoring matrix were used to check HPPD W336 for homology with known allergens. Based on the bioinformatics analysis, there are no allergenic in silico findings associated with the HPPD W336 protein. Likewise, this was also done for 2mEPSPS protein, and based on the bioinformatics analysis, there are no allergenic in silico findings associated with the 2mEPSPS protein.[16][18].

b. The chronic dietary exposure of cottonseed oil consumption is to a maximum of 0.054g/kg body weight per day GHB811 cotton and 0.000671 g/kg body weight per day of the HPPD W336 protein while .000335 g/kg body weight per day of the 2mEPSPS protein. This estimate is low even under worst case assumptions and can be considered negligible.[32][38].

6. Nutritional Data

a. There are no significant differences between the GHB811 cotton (treated and not treated with trait-specific herbicides) and its GM-counterpart for moisture, ash, carbohydrates, crude fat, acid detergent fiber and total dietary fiber. For crude protein, statistical differences were observed for both the GHB811 cotton (treated and not treated with trait-specific herbicides) and its GM-counterpart. For neutral detergent

fiber, statistical difference was observed for the GHB811 cotton (treated with trait-specific herbicides) and its GM-counterpart. Nevertheless, the means for all entries (proximate and fiber analysis) were within the range of the reference varieties as well as the tolerance intervals.[8].

b. The means for all entries of the amino acids, fatty acids, minerals and alpha-tocopherol were within the range of the reference varieties as well as the tolerance intervals.[8].

c. The means for all entries of the anti-nutrients (free gossypol, total gossypol, dihydrosterculic acid, malvalic acid and sterculic acid) were within the range of the reference varieties as well as the tolerance intervals. Processes such as refining, bleaching and deodorizing of cottonseed oil have no effect on the concentrations of free gossypol, total gossypol, dihydrosterculic acid, malvalic acid and sterculic acid.[8].

STRP'S 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'S ASSESSMENT

1. Toxicological Assessment

a. SDS-PAGE and western blot analysis showed that digestibility of HPPD W336 in simulated gastric fluid (SGF) with pepsin at pH 1.2 is within 30 seconds after incubation.[21].

b. It was also assessed in simulated intestinal fluid (SIF) with pancreatinin at pH 7.5. Results showed that the 90% of the protein was completely broken down in less than 30 seconds of incubation.[20].

c. SDS-PAGE and western blot analysis also showed that HPPD W336 protein was heat-stable when incubated up to 60 minutes at 90°C.[19].

d. Bioinformatics analyses using FASTA algorithm associated with the BLOSUM50 scoring matrix sequence alignment tool showed that no relevant structural similarities were observed between the HPPD W336 and human and animal toxins. This indicates that HPPD W336 will not cause toxicity or health risk to human health. [18].

e. Further, acute oral toxicity assessment was then conducted using *E. coli*-produced HPPD W336 protein in 6 male and 6 female C57BL/6J mice at a total dose level of 2000 mg/kg body weight [5]. Based on the toxicity study, there were no treatment-related effects on survival, clinical observations, body weight gain, food consumption or gross pathology, thus the NOAEL for HPPD W336 was 2000 mg/kg bw, the highest dose tested. *Escherichia coli* was used as the source protein. The *E- coli*-produced HPPD W336 protein has been shown to be equivalent to the plant-produced HPPD W336

present in GHB811 cotton [6 and 7].Comparison analyses were done by SDS-PAGE, Western blot analysis, glycostaining analysis, mass spectroscopy, and N-terminal sequence analysis.[15].

f. Meanwhile, results showed that 2mEPSPS protein was degraded with no residual protein visible or no band was visible at 30 seconds of incubation in SGF. In SIF, at time zero of incubation, numerous bands (at about 31 kDa) corresponding to pancreatin can be seen. This shows the complete digestion of 2mEPSPS protein or the protein was degraded quickly in less than 30 seconds. Thus, implying that if the protein was consumed by the animal, it will not incorporate in its own genetic composition because it will be degraded upon digestion.[23][24].

g. Further, heated 2mEPSPS protein showed no significant changes at up to 60 degrees Celsius for 10 minutes. After incubation at 90 degrees Celsius for 60 minutes, a marked but incomplete degradation of the protein was observed. Using the data generated, it was determined that the 2mEPSPS protein was partially heat-stable up to 90 degrees Celsius for 30 minutes and is degraded at 90 degrees Celsius for 60 minutes.[22].

h. With the use of two in silico approaches using FASTA algorithm with BLOSUM50 scoring matrix, an overall identity search with all protein sequences in NCBI non-redundant and internal toxin databases with E-value threshold of 0.1 and 10, respectively was performed to assess if the double mutated 5-enol pyruvylshikimate-3-phosphate synthase (2mEPSPS) protein has a similarity with known toxins. Results in general protein database showed various proteins from different origins but has no potential hazard recorded. Also, in internal database, no similarities or identities were found with any toxin proteins. Thus, it is not likely to be toxic.[16].

i. In addition, 2mEPSPS protein was also administered through oral gavage to C57BL/6J male and female mice at limit dose levels of 2000 mg/kg body weight. For the controls, similar groups of 6 male and 6 female mice received vehicle alone administered in the same manner as the test protein. All samples were observed for 15 days to note the changes in body weights and food consumption.[13].

2. Allergenicity Assessment

a. This study considered the potential N-glycosylation sites by searching their known consensus sequences, potentially found in allergenic proteins. The overall identity search using the allergen database showed no significant identity between the query protein and any known allergenic protein. No relevant identities were found between the query protein and known allergens, based on a '35% identity over an 80-amino acid segment' matching criterion. No potential N-glycosylation sites were identified on the amino acid sequence of the query protein by using the N – X~{P} – [S, T] motif, and no potential N-glycosylation sites were identified by using N – X- C motif. In conclusion, there are no allergenic in silico findings associated with the HPPD W336 protein. [16][18].

b. No changes in consumption patterns are also expected since cotton varieties containing GHB811 event, food and feed products derived from them have been shown to be compositionally and nutritionally equivalent to their non-genetically

modified (non-GM) counterpart. Acute and chronic dietary exposure assessments were based on different international and national food consumption data and dietary exposure evaluation models. National surveys often lacked data on cottonseed and cottonseed oil consumption. In these cases, consumption data for vegetable oils, oil crops, or nuts and seeds were used. Although the event-specific proteins were not detectable in GHB811 cottonseed oil, the exposure assessments were performed including cottonseed oil consumption data.[32][38].

c. Using the high percentile consumption data from the WHO GEMS/Food Program, the acute dietary exposure to GHB811 cottonseed was estimated to be 0.05 g/kg bw on a daily basis for the general population and children (<6 years). The acute dietary exposure to GHB811 cottonseed oil was estimated to be 0.14 g/kg bw for the general population and for children (<6 years) on a daily basis. The acute dietary exposures to the event-specific proteins via consumption of cottonseed oil were at a maximum of 0.005125 ug/kg bw on a daily basis. They were considerably lower than the estimates based on the consumption of cottonseed, due to the HPPD W336 protein concentration being below the Lower Limit of Quantitation (LLOQ) in GHB811 RBD cottonseed oil.[32][38].

3. Nutritional Data

a. No significant differences were observed in the proximate analysis for moisture, ash, carbohydrates, crude fat, acid detergent fiber, and total dietary fiber between the non-GM counterpart and GHB811 cotton not treated or treated with trait-specific herbicides. However, for crude protein, statistically significant difference was observed between the non-GM counterpart and GHB811 cotton not treated or treated with trait-specific herbicides. Also, non-GM counterpart and GHB811 cotton neutral detergent fiber showed a statistically significant difference ($p < 0.05$).[8].

b. Even though there were statistical differences observed, all means of the proximates and fiber were within the range of the reference varieties and the tolerance intervals. Thus, it is unlikely that GHB811 cotton will pose a biologically relevant nutritional concern.[8].

c. Further, amino acids in cotton fuzzy seed of GHB811 cotton (not-treated and treated with trait-specific herbicides) and its non-GM counterpart were compared. Results showed that there was no statistically difference in alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine means between the non-GM counterpart and GHB811 cotton, both treated and not treated. However, statistically significant differences were observed for cysteine and methionine between non-GM counterpart and treated GHB811 cotton but the means for all amino acids were within the literature range and tolerance interval. Thus, the difference will not pose any significant change in the nutritional level of the GHB811 cotton and will not be considered as biologically relevant.[8].

d. For fatty acids, no statistically significant differences were observed between the non-GM counterpart and not treated/treated GHB811 cotton for myristic, palmitic, heptadecanoic, heptadecenoic, oleic, linoleic, linolenic, eicosenoic, behenic and lignoceric acids. However, there is a statistically significant difference in palmitoleic

and stearic acids means between the treated GHB811 and non-GM counterpart, but such differences were not biologically relevant as all means of the fatty acids are still within the reference range and tolerance interval. [8].

e. On the other hand, for minerals and a-tocopherol, results showed that all tested minerals shows no statistically significant differences while alpha tocopherol between not-treated/treated GHB811 cotton and non-GM counterpart were statistically different but within reference range and tolerance thus, it is not biologically relevant nor will pose any compositional change that will cause adverse effect on animals.[8].

f. Meanwhile, total and Free Gossypol between not treated/treated GHB811 cotton and its non-GM counterpart do not show any statistically significant differences. The values of the total and free gossypol for the three groups were similar or close to each other, also, they were all within reference range and tolerance interval. However, the levels of gossypol in cotton should be considered if will be used as cottonseed meal since it which exhibits a variety of toxic effects especially to non-ruminants and young ruminants.[8].

g. For CPFAs, only the mean of dihydrosterculic acid between the treated GHB811 cotton and non-GM counterpart showed a statistically significant difference but such difference will not be considered as biologically relevant as the mean were within the reference range and tolerance interval.[8].

BAI'S RECOMMENDATION

Find scientific evidence that the regulated article applied for direct use has no evidence of interaction on the resulting gene products.

BPI-PPSSD'S ASSESSMENT

1. Toxicological Assessment

a. SDS-PAGE and western blot analysis showed that digestibility of HPPD W336 in simulated gastric fluid (SGF) with pepsin at pH 1.2 is within 30 seconds after incubation. It was also assessed in simulated intestinal fluid (SIF) with pancreatinin at pH 7.5. Results showed that the 90% of the protein was completely broken down in less than 30 seconds of incubation. These results indicate an unlikely to cause toxicological risk to human health.[20][21].

b. SDS-PAGE and western blot analysis also showed that HPPD W336 protein was heat-stable when incubated up to 60 minutes at 90°C.[19].

c. Bioinformatics analyses using FASTA algorithm associated with the BLOSUM50 scoring matrix sequence alignment tool showed that no relevant structural similarities were observed between the HPPD W336 and human and animal toxins. This indicates that HPPD W336 will not cause toxicity or health risk to human health. Acute oral toxicity assessment was then conducted using *E. coli* produced HPPD W336 protein in

6 male and 6 female C57BL/6J mice at a total dose level of 2000 mg/kg body weight. Based on the toxicity study, there were no treatment-related effects on survival, clinical observations, body weight gain, food consumption or gross pathology, thus the NOAEL for HPPD W336 was considered to be 2000 mg/kg bw, the highest dose tested.[18].

d. *Escherichia coli* was used as the source protein. The *E- coli*-produced HPPD W336 protein has been shown to be equivalent to the plant-produced HPPD W336 present in GHB811 cotton. Comparison analyses were done by SDS-PAGE, Western blot analysis, glycostaining analysis, mass spectroscopy, and N-terminal sequence analysis.[34][35].

e. Meanwhile, *E. coli*-produced 2mEPSPS protein was used for digestibility study where it underwent incubation at human simulated gastric fluids (SGF) containing pepsin at pH 1.2 and simulated intestinal fluids (SIF) with pancreatin at pH 7.5 and was assessed using SDS-PAGE and Western blot analysis.[23][24].

f. Results showed that 2mEPSPS protein was degraded with no residual protein visible or no band was visible at 30 seconds of incubation in SGF. In SIF, at time zero of incubation, numerous bands (at about 31 kDa) corresponding to pancreatin can be seen. This shows the complete digestion of 2mEPSPS protein or the protein was degraded quickly in less than 30 seconds. Thus, implying that if the protein was consumed by the animal, it will not incorporate in its own genetic composition because it will be degraded upon digestion.[23][24].

g. Further, heated 2mEPSPS protein showed no significant changes at up to 60 degrees Celsius for 10 minutes. After incubation at 90 degrees Celsius for 60 minutes, a marked but incomplete degradation of the protein was observed. Using the data generated, it was determined that the 2mEPSPS protein was partially heat-stable up to 90 degrees Celsius for 30 minutes and is degraded at 90 degrees Celsius for 60 minutes.[22].

h. With the use of two In silico approaches using FASTA algorithm with BLOSUM50 scoring matrix, an overall identity search with all protein sequences in NCBI non-redundant and internal toxin databases with E-value threshold of 0.1 and 10, respectively was performed to assess if the double mutated 5-enol pyruvylshikimate-3-phosphate synthase (2mEPSPS) protein has a similarity with known toxins. Results in general protein database showed various proteins from different origins but has no potential hazard recorded. Also, in internal database, no similarities or identities were found with any toxin proteins. Thus, it is not likely to be toxic.[16].

i. In addition, 2mEPSPS protein was also administered through oral gavage to C57BL/6J male and female mice at limit dose levels of 2000 mg/kg body weight. For the controls, similar groups of 6 male and 6 female mice received vehicle alone administered in the same manner as the test protein. All samples were observed for 15 days to note the changes in body weights and food consumption.[13].

j. No Observed Effect Level (NOEL) for 2mEPSPS protein was at 2000 mg/kg body weight, with no signs of treatment-related clinical signs and no changes in food consumption as the applicant noted. The mean food consumption of the treated

samples was significantly lower in both sexes compared to control but the values were within normal range however, the differences can be attributed to increased consumption of the control samples not to the lowered consumption of the treated samples. Thus, it can be concluded that the usual consumption of GHB811 with the abovementioned protein will not cause any adverse effects to animals.[13].

k. Lastly, Using a panel of analytical tests and assays which includes densitometry analysis of Coomassie-stained SDS PAGE, Western blot, glycostaining, Mass spectroscopy, and N-terminal sequence analysis (Edman degradation), *E. coli*-produced 2mEPSPS protein has been shown to be equivalent to the plant-produced 2mEPSPS protein present in GHB811.[36][37].

2. Allergenicity Assessment

a. Bioinformatics analysis was performed to evaluate the potential amino acid sequence identity of HPPD W336 protein with known allergens by using several in silico approaches. This search evaluated the potential amino acid sequence identity of the query protein with known allergens by using in silico approaches.[18].

b. An overall identity search was carried out to compare the complete query sequence with each protein sequence present in the public allergen database COMPARE. The FASTA Algorithm was used, with the BLOSUM50 scoring matrix and E-value threshold of 10. The criterion indicating potential allergenicity was > 35% identity over at least 80 consecutive amino acids with an allergenic protein.[18].

c. An 8-mer search was carried out to identify any segment of 8 amino acids or longer that share 100% identity to an allergenic protein. This search was performed using SeqMatchAll from the EMBOSS suite, which compared the query sequence with each of the known allergens present in the allergen database.[18].

d. Furthermore, this study considered the potential N-glycosylation sites by searching their known consensus sequences, potentially found in allergenic proteins.[18].

e. The overall identity search using the allergen database showed no significant identity between the query protein and any known allergenic protein. No relevant identities were found between the query protein and known allergens, based on a '35% identity over an 80-amino acid segment' matching criterion. No potential N-glycosylation sites were identified on the amino acid sequence of the query protein by using the N – X~{P} – [S, T] motif, and no potential N-glycosylation sites were identified by using N – X- C motif. In conclusion, there are no allergenic in silico findings associated with the HPPD W336 protein.[18].

f. Likewise, using FASTA algorithm with BLOSUM50 scoring matrix, an overall identity search to compare 2mEPSPS against COMPARE database was performed. Results showed that there was no significant identity between 2mEPSPS and any known allergens. In addition, an 8-mer search was done using SeqMatchAll from EMBOSS suite which showed that 2mEPSPS does not have a segment sharing a 100% identity with known allergenic proteins. Overall, 2mEPSPS shows no structurally relevant identity with any allergenic proteins. Thus, 2mEPSPS will not pose any allergic

properties when consumed by the animal, as also supported by the digestibility study and study on the effects of heat.[16].

g. Further, using the high percentile consumption data from the WHO GEMS/Food Program, the acute dietary exposure to GHB811 cottonseed was estimated to be 0.05 g/kg bw daily for the general population and children (<6 years). The acute dietary exposure to GHB811 cottonseed oil was estimated to be 0.14 g/kg bw for the general population and for children (<6 years) daily. The acute dietary exposures to the event-specific proteins via consumption of cottonseed oil were at a maximum of 0.005125 ug/kg bw daily. They were considerably lower than the estimates based on the consumption of cottonseed, due to the HPPD W336 protein concentration being below the Lower Limit of Quantitation (LLOQ) in GHB811 RBD cottonseed oil.[32][38].

h. A chronic dietary exposure assessment was performed with chronic cottonseed oil consumption data from the WHO GEMS/ Food Program. The general population would be exposed to a maximum of 0.054 g/kg bw/d GHB811 cotton and 0.000671 ug/kg bw/d of the HPPD W336 protein. The chronic exposure estimate for the HPPD W336 protein is low even under worst-case assumptions and can be considered negligible.[32][38].

3. Nutritional Composition

a. No significant differences were observed between the proximates and fiber of GHB811 cotton and the conventional cotton. No significant differences were also observed on key nutrients and anti-nutrients content of GHB811 cotton and the non-transgenic cotton varieties and is within the tolerance interval or combined literature range.[8].

BPI-PPSSD'S RECOMMENDATION

Find scientific evidence that the regulated article applied for direct use has no evidence of interaction on the resulting gene products.

DENR BC'S ASSESSMENT

After a comprehensive review and evaluation of the documents including the scientific evidences from provided references and literature submitted by BASF Philippines, Inc. on its application for Direct Use as FFP of Cotton GHB811, here under 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 and biodiversity. The transgenic crop will not increase its weediness potential in case the seeds spill out into the environment because cotton has limited potential to survive outside agricultural settings, and the introduced genes are not expected to increase its ability to spread and persist.[40].

2. 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 considering that cottons have no potential to persist in an unfavorable environment.[41].

DENR BC'S RECOMMENDATION

Based on the evaluation and review of literatures cited, the DENR-BC considered the regulated article safe to the environment, particularly on biodiversity and non-target organisms.

DOH BC'S ASSESSMENT

After a thorough review and evaluation of the documents provided by the proponent BASF Philippines, Inc., through the Bureau of Plant Industry (BPI), in support of their application for approval for Direct Use as Food, Feed or for Processing (FFP) of Cotton GHB811. 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 cotton varieties.

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

DOH BC'S RECOMMENDATION

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.

SEC EXPERT'S ASSESSMENT

1. Importation of the cotton will have no significant impact on the Philippine market. A review of the data shows that it is very insignificant in terms of supply production that has been declining over the years while importation and domestic use has been increasing since 2013.

2. This GM product should have very negligible effect, if any, on the current patters of production, consumption/utilization and trade of cotton in the Philippines for food and feed uses.

SEC EXPERT'S RECOMMENDATION

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

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