

# **ASSESSORS' CONSOLIDATED REPORT ON BAYER CROPSCIENCE INC'S APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF COTTON GHB614**

## **EXECUTIVE SUMMARY**

On November 22, 2016, Bayer CropScience Inc. submitted cotton GHB614 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 GHB614 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 GHB614 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 GHB614.

Furthermore, the Socio-economic, Ethical and Cultural (SEC) Considerations expert also recommended for the issuance of biosafety permit for this regulated article after assessing the socio-economic, social and ethical indicators for the adoption of Genetically Modified Organisms.

## **BACKGROUND**

In accordance with Article VII. Section 20 of the JDC, no regulated article, whether imported or developed domestically, shall be permitted for direct use as food and feed, or for processing, unless: (1) the Biosafety Permit for Direct Use has been issued by the BPI; (2) in the case of imported regulated article, the regulated article has been authorized for commercial distribution as food and feed in the country of origin; and (3) regardless of the intended use, the regulated article does not pose greater risks to biodiversity, human and animal health than its conventional counterpart.

The BPI Biotech Office provided the assessors, except for the SEC expert, the complete dossier submitted by Bayer. The SEC expert, on the other hand, was provided with a questionnaire on socio-economic, ethical and cultural considerations that have been addressed by Bayer in relation to their application.

Upon receipt of the individual reports from the assessors, the BPI Biotech staff prepared this consolidated risk assessment report for the information of the public.

## **STRP ASSESSMENT AND RECOMMENDATIONS**

Based on the documents submitted by the applicant:

## A. Host Organism

The STRPs agreed that the key nutrients of cottonseed are protein (21.8–34.2%dw) and (15.4 – 36.3%dw). Cottonseed crude oil contains about 2% monoglyceride materials which are most removed by processing. They also agree that it contains antinutrients, for example, the crude cottonseed oil contains about 2% non-glyceride materials. Cotton contains a number of terpenoid phytoalexins. Gossypol is the most notable of the terpenoid phytoalexins. Cotton also contains general cyclopropenoid fatty acid (CPFA).

They also concurred that gossypol is the main toxicant present in cotton. It has a role in plant resistance to potential pathogens and possible role as anti-viral and anti-carcinogenic drug. Gossypol has also anti-fertility properties. However, its long history of use, no adverse effects to humans have been attributed to the residual gossypol in properly processed refined, bleached and deodorized cottonseed oil

Lastly, cotton has been considered as an uncommon allergenic food. Cottonseed oil has been used for human consumption for many years and to date there has been no allergenic response reported resulting from the consumption of cottonseed oil.

## B. Transgenic Plant

The STRPs agreed that consumption pattern of cottonseed oil include the following data: about 56% of the oil is used for salad or cooking oil, 36% is used for baking and frying fats, and the remaining 8% is used in the manufacture of margarine and other uses.

Cottonseed flours is mixed with corn flour fortified with vitamins and minerals that served to combat protein deficiency. Processed food grade cotton linter is used as casing in meat products as sausages.

It is possible for the seeds from genetically modified GHB614 will be used as food and feed as its conventional counterparts.

## C. Donor Organism

The STRPs all agreed that maize, the donor organism has a long history of safe use therefore deemed safe as source of the gene. The 2mEPSPS protein is not toxic for mammals and do not possess any of the characteristics associated with food allergen, therefore can be concluded that no expected risk associated with 2mEPSPS protein consumption.

They also agreed that the references provided the potential safety concerns related to the transgenic 2m EPSPS protein confirming that it does not possess any of the properties associated with known toxins or allergens. The wild-type epsps gene used to generate the epsps gene was isolated from maize.

Lastly, inserted regulatory sequences (promoters, enhancers, termination signals etc.) has been adequately described by the applicant and documents are provided.

## D. Transformation System

The STRP all agreed that the applicant provided documents and sufficient information on the *Agrobacterium* transformation system and the protocols used in the transformation. They also agreed that the complete genetic elements used in the transformation process were described and documents were presented by the applicant.

They concurred that the genomic DNA sample extracted from leaves of plants have been analyzed using the methods and quality standards for cotton plants carrying event GHB614-Batch number 05 GA GH059 analysis confirmed the identity of the indicated event as evidenced by the series of scientific data presented.

#### E. Inserted DNA

The STRPs concurred that based on Southern blot and genetic segregation analysis, it was demonstrated that the DNA is integrated in a single genetic locus in the cotton nuclear genome. They also affirmed that the determination of inserted sequences in GHB614 cotton confirmed the presence of one copy of the 2mepsps gene cassette. The DNA sequence of the insert in GHB614 is completely identical to the corresponding transforming plasmid DNA sequence.

Lastly, BLASTn and BLASTx searches concluded that the probability that known sequences, like endogenous cotton genes and/or regulatory elements, interrupted by the insertion of the 2mepsps gene cassette into the cotton genome is negligible.

#### F. Genetic Stability

The STRPs all agreed that molecular stability was confirmed over multiple generations, in multiple genetic backgrounds and in different environments through Southern blot and segregation analyses.

They also agreed that all data and analyses indicate that the insert in GHB614 cotton behaves genetically as a single allele at one locus and that the field environmental conditions do not have any effect on the stability of the insert and flanking regions even seven generations after genetic modification.

#### G. Expressed Material

The STRPs agreed that 2mEPSPS protein was detected in all tissues of GHB614 line. The amounts of 2mEPSPS ranged from 0.16-4.04 µg/g fresh weight in root, stem, leaf and pollen and from 15.8-40.1 µg/g fresh weight in seed. The 2mEPSPS protein comprised an average of 0.0093% ± 0.0018% of total crude protein in fuzzy seed from unsprayed treatment and an average of 0.0100%±0.0019% of total crude protein in fuzzy seed from sprayed treatment.

They concurred that documents and papers regarding metabolic pathways of the expressed material were presented. The 2mEPSPS protein confers tolerance to the herbicide glyphosate. Glyphosate is a wide-spectrum herbicide that works by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms. The 2mEPSPS enzyme however is not inhibited by glyphosate and the expression is sufficiently high to provide a good level of specific activity and ensure glyphosate tolerance in GHB614 cotton.

#### H. Toxicological Assessment

The STRPs all agreed that the test for stability of 2mEPSPS protein (produced in *Escherichia coli*) in human simulated gastric fluid (SGF) showed the 2mEPSPS protein was degraded very rapidly in human SGF, within 30 seconds of incubation at 37°C in the presence of pepsin at pH1.2. The 2mEPSPS protein was also tested for stability in human simulated intestinal fluid (SIF) with pancreatin at pH 7.5. Results showed that 2mEPSPS proteins was degraded very rapidly in human SIF in less than 30 seconds of incubation in the presence of pancreatin at pH7.5.

They also agreed that the 2mEPSPS protein encoded by the 2mepsps gene was tested for stability at temperatures of 60, 75, 90°C for periods of 20, 30 or 60 minutes. The protein was examined by SDS-PAGE and by Western blot analysis. The results of the study showed that the 2mEPSPS protein is partially heat stable up to 90°C for 30 minutes and markedly degraded at 90°C for 60 minutes.

They concurred that results showed that overall homology search with general protein database show similarity with other enzymes from the EPSPS family from various organism, including food plants. There is no record of potential toxicity associated with these proteins. Treatment with 2mEPSPS protein at 2,000 mg/kg body weight via the oral route did not produce signs of systemic toxicants in the OF1 female mice.

Lastly, they also verified that the equivalence of 2mEPSPS protein expressed in GHB614 and the 2mEPSPS produced by *E. coli* was demonstrated by adequate/scientific procedure.

#### I. Allergenicity Assessment

The STRPs all agreed that the proteins that are rapidly degraded are considered less likely to be involved in eliciting an allergic response. The 2mEPSPS protein was subjected to digestibility studies using simulated human gastric fluid (SGF) containing pepsin and simulated intestinal fluid (SIF).

They also agreed that the 2mEPSPS is partially heat-stable up to 90°C for 30 minutes and markedly degraded at 90°C for 60 minutes and that it lack any significant amino acid sequence homology with known allergens supports that it is unlikely that the 2mEPSPS protein possesses allergenic properties (Rasclé, JB, 2011).

The main cotton product consumed by humans is the cottonseed oil. Since the 2mEPSPS protein is not detected in oil products derived from seeds of GHB614 cotton, an intake of this recombinant protein via cottonseed food grade oil or products containing this oil quality is not possible.

#### J. Nutritional Data

For most components no significant differences for the majority of the by-site components were observed between the GHB614 fuzzy seeds compared to their non-transgenic counterpart, Coker 312. Statistical significant differences were detected for a number of fatty acids, amongst them the anti-nutritional cyclopropenoid fatty acids. The statistical findings have no nutritional relevance since the absolute differences in the fatty acid contents are minimal, the amounts of cyclopropenoid fatty acid are lower in the transgenic samples, and all mean values are inside the reference ranges reported from literature.

#### K. 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

### **BPI-PPSSD ASSESSMENT AND RECOMMENDATION**

GHB614 was developed by Bayer CropScience, Inc. through the use of recombinant DNA technology, a glyphosate herbicide tolerant. The single event was developed through *Agrobacterium*-mediated transformation of cotton genome with plasmid vector pTEM2 carrying, 2mepsps gene encoding 2mEPSPS protein which provides tolerance to glyphosate herbicides..

### Host Organism (*Gossypium hirsutum*)

The role of cotton (*Gossypium hirsutum*) in the food supply chain has always been associated with the production of cotton by-products such as cottonseed oil which only takes 5-6% of the total domestic fat and oil supply in the United States, cottonseed meal and hulls. In the Philippines, there are still no extensive literature that can provide any consumption pattern of its by-products.

Primarily, oil is an excellent source of oil and protein suitable for human consumption and livestock feeding. Commercial range for proximates such as moisture, protein, ash, total fat, neutral detergent fiber (NDF), acid detergent fiber (ADF), crude fiber, total dietary fiber and nonfibrous carbohydrates has been reported. Anti-nutrients are also present in cotton. These includes terpenoids, phytoalexins, cyclopropenoid fatty acids, flavonoids, tannins and anthocyanin. Cotton also contains toxicants such as gossypol which is toxic to non-ruminants and has male anti-fertility properties. However, it has also been reported to have anti-viral and anti-carcinogenic property. However, cotton is being considered as a non-common allergenic food.

### Transgenic Plant

GHB614 has been reviewed and approved for food and/or feed use in many countries including Australia (Food, 2009; Feed, 2016), Brazil (Food and Feed, 2010), Canada (Food and Feed, 2008), Colombia (Feed, 2012), European Union (Food and Feed, 2011), Japan (Food and Feed, 2010), Malaysia (Food and Feed, 2017), Mexico (Food, 2008), New Zealand (Food, 2009), South Korea (Feed, 2010), Taiwan (Food, 2015) and United States of America (Food and Feed, 2009).

GHB614 was developed with 2mepsps genes derived from *Zea mays* L. Transformation of conventional cotton variety Coker312 into transgenic GHB614 was conducted through *Agrobacterium*-mediated transformation using plasmid vector pTEM2 that contains both left and right transfer-DNA (T-DNA) border sequences to facilitate transformation through integration of the 2mepsps gene expression cassette.

Genomic DNA was isolated from GHB614 and was subjected to Southern Blot Analysis using the complete T-DNA fragment and its different components of the transgene cassette as probes. The genomic DNA was digested with EcoRV, BglII, PvuII, AseI, NcoI, HindIII, AflIII, BamHI and KpnI. Southern Blot Analyses showed that single copies of 2mepsps gene was integrated at a single locus in the cotton genome with all expression elements intact and without plasmid backbone sequences present. This implies that the 2mepsps gene was the only expressible sequences introduced in GHB614.

A 17 bp target size deletion was detected through alignment of the sequences derived from the pre-insertion locus to transgenic locus. However, bioinformatics analyses illustrated the safety of GHB614 in terms of the potential for creating novel chimeric ORFs. Results of the analyses indicated that neither allergenic nor toxicological in silico findings were associated with the potential ORF polypeptides identified from GHB614 insertion site.

### Donor Organism

Corn, *Zea mays* L., was the donor organism for 2mepsps gene encoding 2mEPSPS protein. History of safe use was attributed to the donor organism as it has been widely consumed as staple food for centuries. Corn was not considered as a common allergenic food. This indicates that 2mEPSPS was derived from a non-allergenic source.

## Genetic Stability

Multigenerational stability of 2mepsps in GHB614 was demonstrated through Southern Blot Analyses of five (5) generations (T3, T4, T5, T6 and BC2F2) of GHB614 cotton. Results showed that the hybridization bands specific to the GHB614 were identical in lanes containing DNA from cotton grown from 5 generations. These showed the stability of the GHB614 insert inherited from one generation to the next.

Three generations of backcrosses (BC1F1, BC2F1 and BC2F2) were used to assess segregation of 2mepsps gene. Segregation ratio of the gene was determined. Chi-Square Analysis was performed to determine the segregation of 2mepsps gene in the generations of backcrosses including F1 and F2. Results showed that the inheritance of 2mepsps from one generation to the other follows the Mendelian Law of Segregation.

## Expressed Material

2mEPSPS protein is involved in the shikimic pathway of aromatic amino acid biosynthesis in all plants. This protein has lower binding affinity for glyphosate, thus conferring tolerance to glyphosate by allowing enzymatic activity in the plants for growth to continue even at the presence of glyphosate

Enzyme-linked Immunosorbent Assay (ELISA) was conducted to quantify the level of expression of 2mEPSPS in different plant parts and forms of GHB614 cotton. The concentration ranges of 2mEPSPS in different plant parts in four growth stages are 0.23 ug/g fresh weight – 14.89 ug/g fw in leaves, 0.62 ug/g fw – 2.55 ug/g fw in stem, 0.99 + 1.00 ug/g – 5.75 ug/g fw in roots, 5.1 ug/g fw – 5.6 ug/g fw in square, 5.25 ug/g fw – 5.69 ug/g fw in apex and 0.15 ug/g fw – 0.17 ug/g fw in pollen. The concentration of 2mEPSPS in different plant forms in four growth stages are 32.8 + 6.2 ug/g sample in kernel, 0.40 + 0.15 ug/g sample in lint coat, 6.63 ug/g sample in fuzzy cottonseed, 102 + 2 ug/g sample in delinted cottonseed, 6.93 + 0.40 ug/g sample in seed hulls, and 0.26 + 0.10 ug/g samples in defatted meal.

## Toxicity and Allergenicity Assessment

The safety of the novel protein, 2mEPSPS, in terms of toxicity and allergenicity was assessed through digestibility studies, heat inactivation, acute oral toxicity and amino acid sequence comparison. *Escherichia coli*-produced 2mEPSPS protein was used in the analyses. Equivalency report indicated the suitability of the microbially produced proteins as a surrogate test proteins for the toxicity and allergenicity assessment of the proteins derived from GHB614.

Sodium Dodecylsulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis showed that 2mEPSPS protein was degraded rapidly in human simulated gastric fluid within 0.5 minutes of incubation, in presence of pepsin, at pH 1.2. The protein was also rapidly degraded in simulated intestinal fluid, in presence of pancreatin, at pH 7.5 after 0.5 minutes.

The heat stability of 2mEPSPS was determined through incubation of 2mEPSPS samples at 4°C, 25°C, 37°C, 55°C, 75°C and 95°C for 30 minutes. SDS-PAGE and Western Blot Analysis were performed to determine the effect of temperature in the band intensity of 2mEPSPS. The primary structure of 2mEPSPS remained stable at all temperatures with minor degradation starting at 55°C.

Acute oral gavage is performed in mice with 2000 mg/kg body weight showing no mortalities, no treatment related clinical signs, no effect on body weight parameters, no effect on food consumptions, no macroscopic changes at necropsy were observed in mice treated with 2mEPSPS protein.

Bioinformatics analysis indicated that 2mEPSPS protein in GHB614 cotton has no homology to any known toxins and allergens based on NCBI Non-Redundant Protein Sequence Database, Bayer Toxin Database and FASTA searches.

Exposure potential of both proteins was also estimated. 2mEPSPS comprises only 0.0329% dry matter of crude protein in delinted seeds, 0.00478% dry matter of crude protein in hulls and 0.0000509% dry matter of crude protein in meals. The protein was not detected in lint, linters, toasted meal and oil samples from GHB614.

### Compositional Analysis

The compositional equivalence of GHB614 to conventional cotton was assessed through comparison with non-transgenic cotton variety, Coker 312, and comparison with range of literature values of commercial cotton varieties.

Most of the proximate, key nutrients (minerals, vitamins, amino acids and fatty acids) and anti-nutrients levels in GHB614 seeds were comparable to non-transgenic cotton. Values obtained from GHB614 seeds were mostly within the range of reported values from cotton literatures. Deviance from the literature ranges cannot be directly attributed to the genetic transformation since the same levels of composition were observed in non-transgenic Coker 312. These indicate that there are no statistical differences in the composition of GHB614 seeds that can be considered as biologically relevant.

### Conclusion

For the transgenic cotton, GHB614, there is enough evidence to support the equivalence of the GM crop, in terms of the nutritional composition, agronomic characteristics and food safety, with the conventional cotton other than the tolerance to glyphosate herbicides. After reviewing all the data and documents provided by the developer, it is therefore concluded that GHB614 is substantially equivalent to its conventional counterpart.

## **BAI ASSESSMENT AND RECOMMENDATIONS**

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

### A. Host Organism

BAI has affirmed that cotton seed is a source of key nutrients for both humans and animal consumption. The cottonseeds are processed into two major products from extraction, the oil for human and the oil-free meal containing proteins for livestock and fish. They also stated that cottonseed contains a few anti-nutrients such as cyclopropenoid fatty acids (CPFA) which include sterculic, dihydrosterculic, and malvalic acids. However, after undergoing processing, CPFA's in cottonseeds become negligible.

In addition, cottonseed is a source of toxicants. It contains terpenoid phytoalexins including gossypol which is toxic to non-ruminants and has male anti-fertility properties. No adverse effects however, has been observed to humans consuming properly processed cottonseed oil and there has been no reported health issue regarding its use.

Moreover, a number of cotton by-products are used to feed livestock. The meal produced after extracting oil from cottonseed is used more in feeding ruminants due to its free-gossypol content which affects the digestibility of lysine (a limiting amino acid). The whole cottonseed is feed to dairy

animals though less to beef and sheep animals. To improve the diet, a good quality source of protein could be added.

Lastly, in the country, there has been no direct information regarding consumption pattern. As reported by the National Cottonseed Products Association of US, the Philippines is not a major export destination of cottonseed products.

#### B. Transgenic Plant

BAI has listed the countries which approved this transgenic plant for food, from the references of the applicant, these are: Argentina, Australia/New Zealand, Brazil, Canada, EU, Japan, Korea, Mexico, Taiwan, and the USA.

BAI agree that the introduction of Cotton GHB614 is not expected to change consumption patterns as cottonseed derived from genetically modified cottonseed according to literatures is substantially and nutritionally equivalent to cottonseed derived from the conventional breeding practices.

#### C. Donor Organism

BAI stated that Zea mays is widely consumed as food and feed with no known pathogenic effect to humans and animals. 2mepsps, the gene has the same basic amino acids as any DNA from known food constituents in both human and animal diets. 2mEPSPS is not known to possess any property associated with toxicity or allergenicity, its presence in food and feeds does not present a potential risk. They also stated that all potentially inserted regulatory sequences were adequately described.

#### D. Transformation System

BAI agrees with the information provided by the applicant that the transformation method is Agrobacterium-mediated, and that the target is Nuclear DNA. They also agree that the experimental protocol is given and that Plasmid vector pTEM2 was used in the transformation event. A plasmid map and table of all genetic elements are provided.

#### E. Inserted DNA

BAI has concurred that data obtained from the Southern blot analysis indicate a single copy integration in event GHB614 and that the transferred DNA corresponds to the DNA configuration as designed in the pTEM2 plasmid. PCR with subsequent sequencing demonstrated the alignment of GHB614 with the T-DNA of plasmid vector.

They also concurred that sequence alignment analysis showed no differences between the inserted transgenic sequences and the pTEMs sequence. Flanking sequences were observed to be completely identical to the sequences present in the integration site before transformation. During transformation, 17 bp wild type sequences were deleted (target site deletion).

Lastly, they stated that 309 ORFs were identified through bioinformatics analysis and their sequences showed no biologically relevant sequence identities with known allergens and known toxins.

#### F. Genetic Stability

BAI confirmed that Southern Blot analysis was used to determine the structural stability of the trait. Probing DNA restriction digests of GHB614 DNA with the labeled T-DNA probe revealed the expected restriction fragments in all tested samples from all sites of cultivation. The results demonstrate the stability of the integrated DNA in 7 generations after the genetic modification.

They also concurred that segregation of resistance was assessed in BC2F1 materials and BC2F2 progenies. Results demonstrate Mendelian inheritance and confirms a single gene insert.

## G. Expressed Material

BAI agreed with the applicant that the levels of expression of 2mEPSPS protein were determined from the different tissues (leaf, stem, root, square, apex, and pollen) at four (4) different growth stages of the Cotton GHB614 plants.

They also agreed that based on the study conducted, the presence of 2mEPSPS protein was detected in all transgenic samples. Over a period of time, the protein content in leaf tissue decreased- higher at Stage Growth Number 1 and lowest at Growth Stage No 4,  $0.45 \pm 0.22$  from a high of  $11.16 \pm 3.73$  in Stage Growth No 1. Proteins were not detected from tissues of stem and roots at Stages Growth No 1 and 3, but were detected at Stage Growth no 2 and 4. The levels of expression almost remained constant.

Lastly, BAI affirmed that the novel protein has a metabolic role to play just like its equivalent wild type protein. It was described that the 2mEPSPS protein is a double mutant form of the wild type 5-enol pyruvylshikimate-3-phosphate synthase (EPSPS) which is an essential enzyme involved in the biosynthesis of aromatic amino acids in all plants, bacteria and fungi that when it is exposed to glyphosate herbicide, the activity is blocked.

## H. Toxicological Assessment

BAI concurred that in the test for stability of 2mEPSPS using human simulated intestinal fluid (SIF) with pancreatin at pH 7.5, the 2mEPSPS protein was also degraded very rapidly in less than 30 seconds of incubation, and that at the digestibility trial using human simulated gastric fluid (SGF) to test the stability of 2mEPSPS protein at approximately 37°C, at pH1.2 with pepsin, the 2mEPSPS protein was degraded very rapidly within the 30 seconds incubation period.

They also confirmed that majority of the 2mEPSPS proteins remained in the supernatant as soluble protein after temperature treatment at 55°C and above with slight degradation of the protein possibly occurring at elevated temperatures and that the overall search showed that there was no biologically relevant identity between the query sequences and any known allergenic proteins and so also with any toxic protein from the NCBI non-redundant database.

They concurred with the applicant that an acute oral toxicity study was performed to assess oral toxicity of 2mEPSPS protein in OF1 mice plus the acute oral toxicity of bovine serum albumin as negative control. NOEL is at 2000 mg/Ka body weight.

They also concurred that the source of test protein was *E. coli* and used criteria and methodologies used for demonstrating equivalence between the same protein produced in a bacterium and plant were summarized in Table 10, page 51 of the RAR.

## I. Allergenicity Assessment

BAI confirmed that in the test for stability of 2mEPSPS using human simulated intestinal fluid (SIF) with pancreatin at pH 7.5, the 2mEPSPS protein was also degraded very rapidly in less than 30 seconds of incubation, and that at the digestibility trial using human simulated gastric fluid (SGF) to test the stability of 2mEPSPS protein at approximately 37°C, at pH1.2 with pepsin, the 2mEPSPS protein was degraded very rapidly within the 30 seconds incubation period.

They agreed that majority of the 2mEPSPS proteins remained in the supernatant as soluble protein after temperature treatment at 55°C and above with slight degradation of the protein possibly occurring at elevated temperatures and that the overall search showed that there was no biologically relevant identity between the query sequences and any known allergenic proteins and so also with any toxic protein from the NCBI non-redundant database.

Lastly, they also agreed that there is no detectable or quantifiable 2mEPSPS protein amount in crude and food grade oil produced from cotton event GHB614 seeds.

#### J. Nutritional Data

BAI concurred that there are no significant differences compositionally and nutritionally between cotton event GHB614 and its non-GM counterpart, Coker 312. All mean values were also within the reference ranges from cotton literatures.

They also that there is no biological relevance on the differences of values obtained since these were within the range of variation among commercial varieties.

#### K. 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.

### **DENR ASSESSMENT AND RECOMMENDATION**

After thorough and scientific reviews and evaluation of the document provided by the Bureau of Plant Industry (BPI) to the DENR Biosafety Committee within the prescribed period pursuant to Joint Department Circular (JDC) No. 1 s2016 on the application of Bayer CropScience Inc. for direct use for feed, food or processing of Genetically Modified Cotton tolerant to glyphosate herbicide single trait product GHB614, the following are the observations and recommendations:

1. The effect of the regulated article on the environment depends largely on the viability of the product to be utilized for direct use. If the article is transported in a non-viable form, there is no danger to the environment;
2. Due to the absence of a specified Environmental Management Plan (EMP) by the trade/importers, the Committee would like to recommend that it be added to the requirements for the issuance of an import permit by the Bureau of Plant Industry (Section 26 of JDC No. 1 s2016);
3. It is suggested that the BPI ensure the following:
  - a) Development of guidelines on the EMP in coordination with DENR;
  - b) Implementation of the EMP by the traders/importers involved in the import, handling, processing and transport of viable corn DAS 59122-7 commodity products; and
  - c) Strict monitoring of the regulated article from the port of entry to the traders/importers storage/warehouse (Sec 23 of JDC 1 s2016).

Based on the above considerations and with the submitted sworn statement and accountability of the proponent, a biosafety permit may be issued to the proponent if the abovementioned recommendations are followed.

### **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 for Food and Feed or for Processing (FFP) of Cotton GHB614. I/We,

Find that the regulated article applied for Direct Use for Food and 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 provided references i.e. literatures show that the regulated article applied for Direct Use as FFP is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health and on the environment.
2. It is suggested that the BPI ensure the following:
  - a) Strict monitoring of the regulated article from port of entry to the traders/importers storage/warehouse as stated in Sec 32 of JDC 1 s2016
  - b) The BPI to include in the issuance of permit for release of this product the following conditions:
    - i. Any spillage (during unloading and loading/hauling and transport unloading and storage) shall be collected and cleaned up immediately.
    - ii. Transportation of the consignment from the port of entry to any destination shall be in closed containers.
    - iii. There shall be a clear instructions that the product is only for the purpose of direct use for FFP and is not to be used as planting materials.
3. Based on the above considerations and with the submitted sworn statement and accountability of the proponent, this recommendation is being submitted to the BPI related to the processing and issuance of a biosafety permit for Direct Use as FFP of Cotton GHB614.

#### **SEC ASSESSMENT AND RECOMMENDATIONS**

The SEC Expert stated that the issue is if the imported cotton that may contain GM cotton will be used as feed, fuel or food. . There is the possibility that cotton seeds, which can be milled to extract the oil, can also produce cotton oil meal. The meal can be used as feed ingredient. In other countries, this is used for cattle and sheep. The livestock in the Philippines is primarily of pigs and poultry. The oil seed meal can be processed into a feed ingredient but this is very unlikely. The technology is there but the economics of its use is not compelling. Cotton oil is not competitive with other forms of vegetable oil or even economical for industrial use. The country is not in the business of importing cotton to produce cotton oil, and therefore livestock producers would likewise be pushed to use cotton oil meal as feed ingredient on a regular basis. But there is always the possibility.

Therefore, the SEC Expert recommended for the approval and issuance of biosafety permit of the said GM product.