

**CONSOLIDATED REPORT OF COTTON GHB119
FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING**

EXECUTIVE SUMMARY

On December 07, 2016, Bayer CropScience's submitted cotton GHB119 for direct use as food and feed, or for processing to the Bureau of Plant Industry (BPI) 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 (BAI), concurred that cotton GHB119 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 that the conditions set by them 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 GHB119 will not pose any significant risk to 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 GHB119.

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 Bayer CropScience.

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

STRP, PPSSD and BAI ASSESSMENT

GHB119 was developed by Bayer CropScience, Inc. through the use of recombinant DNA technology, a glufosinate ammonium herbicide tolerant and lepidopteran insect resistant plant. The single event was developed through *Agrobacterium-mediated transformation* of cotton genome with plasmid vector *pTEM12* carrying, *cry2Ae* gene encoding *Cry2Ae* protein which provides resistance to lepidopteran insects and *bar* gene encoding PAT an enzyme involved in the inactivation of glufosinate ammonium through acetylation.

Host Organism (Cotton, 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, the assessors concurred that 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 (OECD, 2009). The STRPs, PPSSD and BAI added that anti-nutrients are also present in cotton. These includes terpenoids, phytoalexins, cyclopropenoid fatty acids, flavonoids, tannins and anthocyanin. Toxicant such as gossypol has been associated with cotton. However, cotton is being considered as a non-common allergenic food.

Transgenic Plant

The assessors noted that *GHB119* has been reviewed and approved for food and/or feed use in many countries including Australia, Canada, China, Japan, New Zealand, South Korea, Taiwan and United States.

According to the technology developer, *GHB119* was developed with *cry2Ae* genes derived from *Bacillus thuringiensis* subsp. *dakota* and *bar* genes from *Streptomyces viridochromogenes*. Transformation of conventional cotton variety Coker312 into transgenic *GHB119* was conducted through *Agrobacterium-mediated* transformation using plasmid vector pSYN15954 that contains both left and right transfer-DNA (T-DNA) border sequences to facilitate transformation through integration of the gene expression cassettes; *cry2Ae* and *bar*.

The results of sequence analyses showed that single copies of *cry2Ae* and *bar* genes are integrated at a single locus in the cotton genome with all expression elements intact and without plasmid backbone sequences present. This implies that the genes were the only expressible sequences introduced in *GHB119*.

Donor Organism

Bacillus thuringiensis subsp. *dakota* was the donor organism for *cry2Ae* gene encoding *Cry2Ae* protein. History of safe use was attributed to the donor organism as

it was known to be abundant, non-pathogenic and common in the environment. *B. thuringiensis* strains have been a source of biological pest control agents.

In addition, the *bar* genes encoding *PAT* proteins was derived from *Streptomyces hygrosopicus*, a common soil microbe. It is known to be non-pathogenic and non-toxic to human health.

Genetic Stability

On the other hand, the assessors agreed that multigenerational stability of *cry2Ae* and *bar* in *GH119* was demonstrated through Southern Blot Analyses of five (5) generations of *GHB119* cotton. Results showed that the hybridization bands specific to the *GHB119* insert were identical in lanes containing DNA from cotton grown from five (5) generations. Thus, STRPs, PPSSD and BAI concurred that these showed the stability of the *GHB119* insert inherited from one generation to the next.

Three generations were used to assess segregation of *cry2Ae* and *bar* genes. Segregation ratios of the genes were determine.. Chi Square Analysis of these segregation data was performed. The observed and expected frequencies of *cry2Ae* and *bar* in three (3) generations of *GHB119* cotton indicate that the segregation of both genes through generations follows the Mendelian principle.

Expressed Material

Cry2Ae protein confers resistance to certain lepidopteran insects through selective binding on midgut of target insect causing cotton-specific pores which eventually leads to death of insect.

PAT is an enzyme which inactivates glufosinate ammonium through acetylation. It catalyzes the transfer of acetyl group from acetyl coenzyme A to phosphinothricin. The released free thiol reacts with 5, 5'-dithiobis (2-nitrobenzoic acid) to form 2-nitro-5-thiobenzoate anion under mild alkaline conditions (pH 7 to 8).

Enzyme-linked Immunosorbent Assay (ELISA) was conducted to quantify the level of expression of *Cry2Ae* and *PAT* in different plant parts and forms of *GHB119* cotton.

Toxicity and Allergenicity Assessment

The safety of the novel proteins, *Cry2Ae* and *PAT*, in terms of toxicity and allergenicity was assessed through digestibility studies, heat inactivation, acute oral toxicity and amino acid sequence comparison. *Escherichia coli*-produced *Cry2Ae* and *PAT* proteins were used in the analyses. The assessors also noted that 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 *GHB119*.

Sodium Dodecylsulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis showed that *Cry2Ae* protein was degraded rapidly in human simulated gastric fluid within 2 minutes of incubation, in presence of pepsin, at pH 1.2. The *Cry2Ae* protein

was also partially degraded in simulated intestinal fluid, in presence of pancreatin, at pH 7.5. *PAT* protein is degraded very rapidly with no residual protein visible at 30 seconds of incubation with SGF, in presence of pepsin, at pH 1.2 and in less than 30 seconds of incubation with SIF, in presence of pancreatin, at pH 7.5. Food proteins that are rapidly degraded by gastric and/or intestinal processes are less likely to express any systemic immunogenicity or toxicity following digestion.

SDS-PAGE Analysis followed by Western Blot Analysis were conducted to determine the heat inactivation of *Cry2Ae* and *PAT* protein in *GHB119*. The proteins were subjected to heat treatment at 4°C, 60°C, 75°C and 90°C. Results showed that the band intensity of *Cry2Ae* decreased from 75°C after 10 minutes and disappeared at 90°C after 30 to 60 minutes. This indicates that *Cry2Ae* starts to degrade upon subject to processing at above 75°C after 10 minutes. *PAT* protein is heat stable up to 60 minutes at 90°C as it confirms different published results.

The assessors confirmed that 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 *Cry2Ae* and *PAT*.

Furthermore, bioinformatics analysis indicated that *Cry2Ae* and *PAT* protein in *GHB119* cotton has no homology to any known toxins and based on FASTA algorithm and BLOSUM50 scoring matrix.

Exposure potential of both proteins was also estimated. Cotton seed oil is the main dietary product obtained from cotton. The *Cry2Ae* and *PAT* is not detected in products derived from cotton event *GHB119* seeds thus; the intake will be significantly lower.

In terms of the interaction among the two novel proteins, no likelihood of interaction among the novel proteins, *Cry2Ae* and *PAT* since they are expressed independently of each other and their mode of actions and metabolic pathways are different from one another.

Compositional Analysis

The compositional equivalence of *GHB119* to conventional cotton was assessed through comparison with commercial cotton varieties, and comparison with range of literature values of commercial cotton varieties.

Most of the promixates, key nutrients (minerals, vitamins, amino acids and fatty acids) and anti-nutrients levels in *GHB119* seeds were not significantly different from non-transgenic cotton. Values obtained from *GHB119* seeds were within the range of reported values from cotton literatures. This indicates that there is no statistical differences in the composition of *GHB119* seeds that can be considered as biologically relevant.

Conclusion

For the transgenic cotton, GHB119, the STRPs, PPSSD and BAI concurred that 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 glufosinate ammonium herbicides and resistance to certain lepidopteran insects. After reviewing all the data and documents provided by the developer, it is therefore concluded that GHB119 is substantially equivalent to its conventional counterpart.

DENR RECOMMENDATION

After thorough and scientific review and evaluation of the documents provided by the Bureau of Plant Industry (BPI) to the DENR Biosafety Committee within the prescribed period pursuant to Joint Department Circular (JDC) No.1 s.2016 on the application of Bayer CropScience, Inc. for direct use for feed, food or processing of Genetically Modified Cotton tolerant to glufosinate ammonium herbicide Single trait product GHB 119, 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 traders/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 (BPI) (Article VIII, Section 26 of JDC No,1 s.2016);
3. It is suggested that 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 COTTON GHB 119 commodity products; and
 - c) Strict monitoring of the regulated article from port of entry to the trader's/importer's storage/warehouse (Section 32 of the JDC No. 1 s.2016).

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 RECOMMENDATION

After a thorough and scientific review and evaluation of the documents provided by the Bureau of Plant Industry (BPI) on the application of Bayer CropScience Inc., for direct use as food and feed or processing of Cotton GHB 119. I/we, Find scientific evidence that the regulated article applied for direct use as food and feed or processing is safe as its conventional counterpart and is not expected to pose any significant risk to human and animal health and environment.

The following are the observations and recommendations:

1. Find that the regulated article applied for Direct Use for Food, Feed and for Processing does not require changes in the usual practices in unloading, and loading, hauling, transport and storage and processing. As such, the regulated article is as safe as its conventional counterpart and is not expected to pose any significant risk to human and animal health and environment while in transit, storage and processing;
2. Scientific pieces of evidences from provided references i.e., literatures show that Regulated article applied for Direct Use for Food, Feed and or for Processing (FFP) is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health and on the environment.
3. It is suggested that the Bureau of Plant Industry (BPI) ensure the following:
 - a. Strict monitoring of the regulated article from port of entry to the trader's/ importers storage/warehouse as stated in Section 32 of the JDC No. 1 series, 2016.
 - b. The BPI to include in the issuance of permit for the release of this product the following conditions:
 - b. 1. Any spillage (during unloading and loading/hauling and transport unloading and storage) shall be collected and cleaned up immediately.
 - b. 2. Transportation of the consignment from the port of entry to any destination within the country shall be in closed containers.
 - b. 3. There shall be a clear labeling of the product from importation down to all levels of marketing stating that it is only for the purpose of direct use as food and feed or processing 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, this recommendation is being submitted to BPI related to the processing and issuance of a biosafety permit for direct use as food and feed or processing of Cotton GHB119.

SEC ASSESSMENT

The SEC expert agreed that the project specifically aims for the registration of GHB119 cotton for direct use in the Philippines so that any interested legal entity may be allowed to import cottonseed commodities having the GHB119 event for food and feed, or for processing uses in the country. The proponent itself does not import, nor is engaged in bringing in cottonseed commodities, and is not involved in importation and processing of any cotton commodity (whether or not having the GHB119 event) for aforementioned and for any other purpose. GHB119 cotton is not intended to be grown or planted in the Philippines, whether for field trial or for commercial propagation, hence GHB119 is of no significance in terms of production in the Philippines.

Furthermore, in cases where GHB119 cottonseed will eventually be imported into Philippines by some interested legal entities and considering current international trading practices, GHB119 cottonseed products are expected to be comingled with other cotton events as well as those from non-GM cultivars. GHB119 cottonseed is used and processed like any other commercially available

non-GM cottonseeds for direct use in the Philippines. From the data gathered from published reports, GHB119 is expected not to comprise a significant volume in terms of consumption and trade. Presented below are data on total trade of cottonseed products as a whole, i.e., not distinctly zeroing in on GHB119 alone for reasons described above.

Also, regarding the host organism cotton, extensive literature search had not resulted to direct information on consumption pattern of cottonseed oil and other products in the Philippines. Based on the latest cottonseed products (cottonseed, linters, cottonseed oil, cottonseed meal) export report compiled by National Cottonseed Products Association of US, Philippines is not among the major export destination for US cottonseed and products (<http://www.cottonseed.com/members/Statistics/exports/may15exp.asp>). If ever there are exports to Philippines, then these are likely very minor and insignificant.

Lastly, the SEC expert concurred that Philippines import data indicate insignificant or negligible influx of cottonseed products. From the latest update, 2 June 2015 Philippines oilseed trade statistics, Philippines Oilseeds Annual (<http://www.agrochart.com/en/news/news/020615/philippines-oilseeds-annual-apr-2015/>) there is even no mention of cottonseed oil. The report discusses only soybean and copra. Similarly with the latest 2014 Philippines Oilseeds Situation and Outlook, USDA-FAS GAIN report, http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Oilseeds%20and%20Products%20Annual_Manila_Philippines_6-5-2014.pdf) cottonseed oil is not mentioned. Only soybean and copra are reported. In addition, the Philippines Bureau of Agricultural Statistics has no record on importation for cottonseed oil and meal (<http://countrystat.bas.gov.ph/?cont=10&pageid=1&ma=G80TDVAI>). Instead, it presented an aggregated data for oilseeds and oleaginous fruits, animal and vegetable oils and fats, fixed vegetable oils and fats. These indicate the insignificant volume of cottonseed and cottonseed product consumption in the Philippines.

Based on the above considerations and supporting data and studies, the SEC expert recommend 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.