

CONSOLIDATED REPORT OF BAYER CROPSCIENCE LLCOTTON25 APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING

EXECUTIVE SUMMARY

On November 22, 2016, Bayer CropScience Inc.'s submitted LLCotton25 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 LLCotton25 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 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 LLCotton25 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 LLCotton25.

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, Inc.

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.

A. STRP, PPSSD, BAI ASSESSMENT

After thorough review of the technical documents submitted by the applicant, the assessors' findings are as follows:

A. Host Organism

The assessors concurred cotton contains nutrients that are used in human and animal feeding, principally, oil and protein. Cottonseed contains several minerals, vitamins, amino acids and fatty acids. Cotton contains antinutrients such as cyclopropenoid fatty acids (CPFA), including sterculic, dehydrosterculic, and malvalic acids.

Cotton contains terpenoid phytoalexins. These phytoalexins are antibiotics found in cotton and it plays a role in resistance of the plant to diseases. Terpenoid phytoalexins include gossypol, hemigossypol, desoxyhemigossypol, 2,7-dihydroxy cadalene, hemigossypolone and heliocides H1 and H2. Gossypol is the most common and it is toxic to non ruminants and has male sterility properties. The free gossypol is the toxic form with toxicity of 24 mg per head per day. The maximum amount of gossypol that should be fed to dairy cows should be 18 mg. Both cottonseed oil and meal are not sources of allergens.

Raw cottonseed is not used for food. It needs to be processed first before it becomes available for consumption of both human and livestock. It is an important source of vegetable oil for making various food preparations. There is no data on Philippine consumption of cottonseed oil and meal.

B. Transgenic Plant

LLCotton25 has been approved for feed use in the USA, Taiwan, Canada, Japan, South Korea, Mexico, Australia/New Zealand, European Union, Colombia, China, South Africa and Brazil. Consumption patterns are not expected to change as a result of the introduction of the novel trait as cottonseed derived from GHB119 Cotton is nutritionally and substantially equivalent to cotton derived from conventional breeding practices.

C. Donor Organism

The glufosinate tolerant cotton event LLCotton25 contains a single genetic insert of the *bar* gene. The *bar* gene encodes an enzyme that confers the phenotype of tolerance to herbicides containing glufosinate-ammonium as their active ingredient. The *bar* gene isolated from *S. hygroscopicus* encodes the enzyme phosphinothricin acetyltransferase (PAT) which confers resistance to the phytotoxic activity of glufosinate-ammonium.

All inserted regulatory sequences have been adequately described. The developer provided sufficient information regarding the introduced expressible sequences. Southern blot hybridization show that the only expressible sequence is the *bar* gene from *S. hygroscopicus*. *Streptomyces hygroscopicus* is neither toxic nor allergenic.

The only new protein encoded by the *bar* gene from *S. hygroscopicus* is PAT. Several *in silico* findings showed that the protein associated with the pat/bar protein is neither toxic nor allergenic.

D. Transformation System

The transformation method is Agrobacterium-mediated. The target of modification was the nucleus of recipient variety Coker 312. The genetic

elements and their characteristics are well described. The map of the plasmid vector pGSV1 was presented including the qualitative PCR method for cotton event LLCotton25. No carrier DNA or helper plasmids were used in the process.

E. Inserted DNA

The schematic drawing of the LLCotton25 transgenic locus in relation with the wild type pre-insertion locus was presented demonstrating one insertion site. LLCotton25 transgenic locus and pre-insertion locus were determined. Pairwise alignment between the transgenic and pre-insertion locus sequence revealed no differences between the homologous sequences. The one insertion site was demonstrated sufficiently. Southern blot hybridization demonstrated that the transferred DNA corresponds to the DNA configuration as designed in the pGSV71 plasmid.

There were no truncations, deletions, rearrangements identified/determined. BLAST analysis of the LLCotton25 insertion locus sequence using the *Gossypium hirsutum* genomic reference database shows that the sequence originates from cotton chromosome D06.

Aside from cotton, the transgene has been approved in maize, rice, canola and soybean. Results of Southern blot analyses demonstrated the absence of vector backbone sequences in the transformation cotton event LLCotton25.

F. Genetic Stability

The stability of LLCotton25 was demonstrated by Southern blot analyses conducted in different genetic background, different environment and over different generations. The result obtained demonstrates the stability of LLCotton25 at the genomic level in multiple genetic backgrounds, over multiple generations when grown in different environments. Mendelian Principle of Inheritance was used to assess the segregation using multiple backcross generations.

G. Expressed Material

The amount of phosphinothricin acetyltransferase (PAT) protein in 4 plant parts (roots, stems, leaves, and pollen) of LLCotton25 was determined. Results are shown in Tabulated form indicating the averages and ranges in PAT protein contents. The novel protein has no metabolic role since acetylation of the PAT protein expresses tolerance to glufosinate ammonium containing herbicide and not as having herbicidal activity. The study used an *in vitro* simulated gastro-intestinal condition. However, there is also an *in vivo* study in mice to support the results obtained from the *in vitro* studies.

SDS PAGE and Western blot hybridization analyses showed that the PAT/bar protein was heat stable up to 60 min at 90°C. Homology search with general protein database showed mainly similarities with other acetyl transferases from various bacterial origin. None of the matches obtained from the toxin database can be considered biologically relevant. Results of acute toxicity test of PAT/bar protein in mice showed no adverse effects even administered at 2000 mg/kg body weight, demonstrating that PAT/bar protein from transgenic cotton event LLCotton25 is as safe as the conventional cotton counterpart.

Results of analytical test showed that the PAT/bar protein produced in *E. coli* is equivalent to PAT/bar protein produced in LLCotton25. Digestibility of PAT in SIF and SGF used the enzymes pancreatin at pH 7.5 and pepsin at pH 1.2, respectively. After digestion, no visible fragment was seen in SIF with pancreatin while a 10% remaining fragment was observed in SGF with pepsin. SDS-PAGE and Western Blot analysis showed similar results in half life at 30 seconds of incubation. PAT protein has no relevant homology with any known allergen.

No detectable PAT protein in both crude and food grade oil produced from LLCotton25 seed was observed. Serum screening was not performed.

H. Toxicological Assessment

Using the internationally recommended protocol in digestibility study, the test protein, PAT/bar protein from *E. coli* was tested for stability in human simulated gastric fluid (SGF) with pH 1.2 and for incubation times from 0.5 to 60 minutes at approximately 37°C. PAT/bar protein tested for heat stability was found to be heat stable when incubated up to 60 minutes at 90°C. The protein was examined after SDS-PAGE by Coomassie blue staining and by Western blot analyses using a specific polyclonal rabbit anti-PAT/bar protein antibody.

Homology search with general protein database showed mainly similarities with other acetyltransferases from various bacterial origin. None of the matches obtained from the toxin database can be considered biologically relevant. Results of acute toxicity test of PAT/bar protein in mice showed no adverse effects even administered at 2000 mg/kg body weight, demonstrating that PAT/bar protein from transgenic cotton event LLCotton25 is as safe as the conventional cotton counterpart. The test protein was produced from *Escherichia coli*.

I. Allergenicity Assessment

Digestibility of PAT in SIF and SGF used the enzymes pancreatin at pH 7.5 and pepsin at pH 1.2, respectively. After digestion, no visible fragment was seen in SIF with pancreatin while a 10% remaining fragment was observed in SGF with pepsin. SDS-PAGE and Western Blot analysis showed similar results in half life at 30 seconds of incubation. PAT/bar protein was heat stable up to 60 minutes at 90°C. The protein was examined after SDS-PAGE by Coomassie blue staining and by Western blot analyses using a specific polyclonal rabbit anti-PAT/bar protein anti-body. PAT protein has no relevant homology with any known allergen. PAT protein has no identified potential N-glycosylation site which indicates that it is not glycosylated.

For raw agricultural products, more than 99% of the PAT protein was found in the kernel fractions of cottonseeds. Seed cotton and delinted cottonseed contain an average of 32.1 ug/g and 114 ug/g of PAT protein, respectively. Serum screening was not performed.

J. Nutritional Data

There were no significant differences between the modified product and the conventional product and were more pronounced for protein and crude fiber. The developer provided information on the comparison

between the parent line Coker 312 and the GM LLCotton25. Treatments were grown in the same environmental conditions. Comparisons with ranges of commercial varieties were within the range. A comparison of the proximate and fibre results obtained from lint sample analyses was not possible, since no reference values were found.

The key nutrients were limited to minerals, vitamins, total amino acids and total fatty acids. No significant differences were found between conventional and modified product. The LLCotton25 was compared using the non-GM parent line Coker312. Most of the mean values for key nutrients were within the literature range except for the palmitic acid value of the transgenic plant analyzed by the Covance Lab which falls slightly below the literature range but still within the reference range of published values for cotton seed oil standards of Codex Alimentarius. The LLCotton25 nutritional values were statistically similar with the profile of the non-GM variety of cottonseed.

During the long history of use, no adverse effects to human and animal have been attributed to the residual toxicant (gossypol) in properly processed, refined, bleached and deodorized cottonseed oil, hence no health risk.

B. DENR BC (for Safety of Event to the Environment)

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 series of 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 product LLCotton25, 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; 0
3. It is suggested that the BPI ensure the following conditions in the biosafety permit:
 - a) Control and mitigating measures as identified in the Environmental Management Plan (EMP) must be complied to prevent unwanted release of GM seeds into the environment;
 - b) Implementation of the EMP by the traders/importers involved in the import, handling, processing and transport of viable cotton LLCotton25 seeds; and

Strict monitoring of the regulated article from the port of entry to the traders/importers storage/warehouse (Section 32 of JDC 1 s2016).

C. DOH-BC (for Environmental Health Safety)

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 LL cotton 25. The DOH-BC found 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. Find that the regulated article applied for Direct Use for FFP 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 and the environment while in transit, storage and processing.
2. 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.
3. 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 s.2016.
 - 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 be used as planting materials.

D. SEC Expert (for Socio-economic Consideration)

The SEC expert said there is no record of the volume of cotton being produced or imported into the country nor the GM product in question. There is no intention to produce the GM product in the country thus, will not affect the current patterns of production. However, there is the possibility that this may have an effect on the utilization of other sources of meal and oil.

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