CONSOLIDATED REPORT ON MONSANTO PHILIPPINES INC.'S COTTON MON 1445 APPLICATION FOR DIRECT USE AS FOOD AND FEED OR FOR PROCESSING (FFP)

EXECUTIVE SUMMARY

On April 08, 2019 Monsanto Philippines Inc.'s filed for application of Cotton MON 1445 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 MON 1445 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 MON 1445 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 the transformation event.

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

A. STRP, BPI-PPSSD, BAI (Safety Assessment)

After thorough review of the technical documents submitted by the applicants, the assesors' findings were as follows:

1. **Host Organism (***Gossypium hirsutum)*

The role of cotton (*Gossypium hirsutum*) in the food supply chain has 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 (OECD, 2009). 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. Cotton is being considered as a non-common allergenic food.

2. Transgenic Plant

MON 1445 has been reviewed and approved for food and/or feed use in many countries including Australia (Food), Canada (Food and Feed), New Zealand (Food) and United States of America (USA) (Food and Feed). (ISAAA, 2018).

Based on the documents provided by the applicant, the introduction of cotton MON 1445 in the market is not expected to change the consumption patterns by population subgroups.

3. **Donor Organism**

Agrobacterium sp. strain CP4 is the donor organism of CP4 EPSPS protein while *Escherichia coli* is the donor organism of *nptII* gene. Both donor organisms are ubiquitous in the environment and are not known to cause toxicity and allergenicity to humans. History of safe use is attributed to the donor organisms.

Toxicity studies indicated that the novel proteins derived from both donor organisms are not toxic nor allergenic to humans.

4. Transformation System

The event, MON 1445 was developed to express CP4 EPSPS derived from *Agrobacterium* sp. strain CP4, and NPTII protein derived from *E. coli*. The transformation method is through *Agrobacterium*-mediated transformation with plasmid vector PV-GHGT07. The plasmid vector, is composed of the gene expression cassettes for *cp4 epsps*, and *nptII*.

The developer provided a complete list of all genetic components used in the transformation of cotton MON 1445.

5. Inserted DNA Genetic Stability

Southern blot analyses were performed to characterize the transgenic insert of MON 1445 cotton. Results showed only a single fragment of the genetic components integrated in a single insertion site in cotton MON 1445 was detected. A single T-DNA insert of no more than 6.1 Kb, containing the CoMVb promoter region, *Arabidopsis* EPSPS CTP2 targeting sequence, *cp4 epsps* coding sequence, T-E9 transcription termination sequence, *aad* coding sequence, *nptII* cassette, and a portion of *ori*-V genetic element was transferred from PV-GHGT07 into MON 1445.

The order of the genetic elements within the T-DNA insert was confirmed by PCR analyses that amplified three overlapping regions of DNA, which span the entire length of the T-DNA insert. PCR and DNA sequence analysis indicated that the arrangement of elements within MON 1445 insert are similar with the arrangement of the elements within plasmid PV-GHGT07.

A 67 bp deletion was detected at the T-DNA insertion site of MON 1445. Analysis of this sequence indicated no homology with any cotton nuclear or mitochondrial coding sequence (Girault and McClain, 2008; McClain et al., 2007). PCR was performed on genomic DNA to verify the sequences at the 5' and 3' ends of the T-DNA insert in MON 1445 (Girault and McClain, 2008; McClain et al., 2007). Amplification and DNA sequencing confirmed the characterization of the insert indicating that the sequences flanking the insert are native to the cotton genome. Bioinformatics analyses indicating that any putative polypeptides or proteins possibly produced from ORFs in the insert or at the junction is unlikely to show allergenic, toxic or otherwise biologically adverse properties (Girault and McClain, 2008; McClain et al., 2007).

Southern blot analysis also demonstrated that the *aad* gene and a portion of *ori*-V genetic element were also transferred from PV-GHGT07 into MON 1445 the genome. However, the *aad* gene is under the control of a bacterial promoter, thus, the encoded protein is not expressed in MON 1445.

6. Genetic Stability

The multigenerational stability of the introduced traits was assessed through Southern blot analysis of the genomic DNA from three generations (R_3 through R_5). Results of the analysis showed that the inserted T-DNA in MON 1445 is stably inherited from one generation to the other (Kolacz and Higgins, 1994). Segregation data were determined through Chi-square analysis of R1 plants self-progeny of the initial transformant (R_0). Analysis indicated that the inserted T-DNA in MON 1445 segregates as a single locus following Mendelian law (Nida et al., 1996).

7. Expressed Material

CP4 EPSPS is involved in the shikimic pathway of aromatic amino acid biosynthesis catalysing the transfer of phosphoenol pyruvate (PEP) to the 5-hydroxyl of shikimate-3-phosphate (S3P) to form 5-enolpyruvylshikimate-3-phosphate (EPSP) and inorganic phosphate (Pi) (Alibhai and Stallings, 2001). NPTII functions as selectable marker in the initial laboratory stages of plant cell selection following transformation.

The levels of each protein in seeds and leaves were quantified using Enzyme-linked Immunosorbent Assay (ELISA). Results of ELISA showed that the mean CP4 EPSPS protein levels in MON 1445 leaf and seed were 0.052 µg/mg and 0.082 µg/mg, respectively, on a fresh

weight basis. As for the NPTII protein, the mean protein levels in MON 1445 leaf and seed were 0.045 µg/mg and 0.0067 µg/mg, respectively, on a fresh weight basis.

8. Toxicological and Allergenicity Assessment

The safety assessment of novel protein, CP4 EPSPS and NPTII, includes digestibility, heat inactivation, oral toxicity and amino acid sequence comparison studies to determine its potential to cause toxicity or allergenicity to humans. *Escherichia coli* is used as source of test protein for all novel proteins. The *E. coli*-produced proteins has been shown to be structurally and functionally equivalent to the MON 1445 produced proteins through amino terminal amino acid sequence comparison, apparent molecular weight and immunological recognition, glycosylation analysis and enzymatic activity characterization.

Digestibility study using Simulated Gastric Fluid (SGF) with pepsin and Simulated Intestinal Fluid (SIF) containing pancreatinin demonstrated that CP4 EPSPS protein was rapidly degraded in SGF and SIF at 15 seconds and 32 seconds, respectively (Padgette et al., 1993). NPTII was readily degraded upon incubation in SGF with pepsin for 10 seconds at approximately 37^0C. Complete degradation of NPTII in SIF with pancreatin was observed at 15 minutes of incubation at approximately 37^0C (Ream, 1993).

SDS-PAGE analysis indicated no effect on the band intensity of CP4 EPSPS protein upon heat treatment for 15 seconds or 30 minutes. However the functional stability assay showed a decrease in the relative activity of CP4 EPSPS by approximately 96% upon increase in temperature from 55^0C - 75^0C. At 75^0C and 95^0C, specific activity units/mg CP4 EPSPS was below limit of detection (LOD).

Bioinformatics analysis showed that CP4 EPSPS and NPTII protein has no homology to any known toxins and allergens (Hileman and Astwood, 1999; Tu, 2009; Monsanto, 1995).

Acute oral toxicity study of CP4 EPSPS provided by the developer showed that administration of CP4 EPSPS in mice have no treatment related adverse clinical observations and effect in body weight and food consumption (Naylor, 1993). The no observed effect level (NOEL) is 572 mg CP4 EPSPS/kg body weight. For NPTII protein, acute oral toxicity study indicated no treatment-related gross pathological observations, mortality, clinical observations and effect on body weight and food consumption upon administration of NPTII in mice (Naylor, 1992). The No Observed Effect Level (NOEL) of NPTII is 5000 mg/kg body weight.

CP4 EPSPS and NPTII constitutes 0.028% and 0.002% of the total protein in MON 1445 (Monsanto, 1995). Both proteins are expressed independently of each other and their functional activity was maintained. The expression cassette of *cp4 epsps* includes a chloroplast transit peptide (CTP) which directs the import of the newly translated protein into chloroplasts while the expression cassette of *nptII* does not involved a transit peptide and is likely to be expressed independently in the cytoplasm (Monsanto, 1995). The proteins have distinct mode of action and are not likely to interact.

Results of the toxicological and allergenicity assessment indicate that CP4 EPSPS and NPTII proteins being expressed in MON 1445 cotton are not toxic or allergenic to humans.

9. Nutritional Data

Compositional analysis provided by the developer indicating the nutritional data of MON 1445 in comparison with the non-transgenic cotton and range of literature values (Nida et al, 1993). Results of the analysis indicated that there is no differences in the proximate, amino acid, fatty acid and anti-nutrient levels of MON 1445 cotton and the non-transgenic cotton that can be considered biologically relevant.

10. Recommendation

According to the assesors, using the weight of evidences approach indicates that cotton MON 1445, is substantially equivalent with the conventional counterpart in terms of nutritional composition and food safety, other than the tolerance to glyphosate-containing herbicides. After reviewing the provided material of Monsanto Philippines, Inc. and other literatures, it is therefore concluded that cotton MON 1445 is as safe as its conventional counterpart.

B. DENR Biosafety Committee (Environmental Safety)

After a comprehensive review and evaluation of the documents provided by the applicant, the DENR-BC stated that the direct use of the regulated article whether for food, feed or for processing will not cause significant adverse effect to the environment (land and water) and biodiversity. The donor organism (*Agrobacterium sp.*) is ubiquitous to the environment and does not pose significant risk of pathogenicity to animals. The applicant's Project description Report discusses the specified enviromental management plan with its mitigating measures and contingency plan. The DENR-BC therefore considers the regulated article safe to the environment and biodiveristy.

C. DOH Biosafety Committee (Environmental Health Safety)

After a thorough review and evaluation of the documents provided by the applicant, the DOH-BC finds that the regulated article applied for direct use as food and feed or for processing is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health and environment. Furthermore that DOH-BC has stated that the through the scientific pieces of evidence from toxicity studies, they find that the regulated article will not cause significant effects to human and animal health and that the dietary exposure to the regulated article is unlikely to result to allergenic reaction. The regulated article is not materially different in nutritional composition from that of the conventional varieties of cotton.

D. SEC Expert

After thorough and scientific review and evaluation of the documents provided by the applicant, the SEC Expert recommends the approval and issuance of biosafety permit for cotton MON 1445. The SEC Expert finds that the applicant was able to provide timely and relevant supply and demand data from the USDA to support assertions that the GM cotton production in these areas are significant and may complement the Philippine governement's efforts to revitalize the local garments industry. The additional explanation of the applicant supported their assertion

that the regulated article will not drastically change the current patterns of production, utilization and trade in the country.