CONSOLIDATED REPORT OF MONSANTO PHILIPPINES' COTTON MON15983 x MON88913 APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING

EXECUTIVE SUMMARY

On May 5, 2016, Monsanto Philippines applied the combined trait product cotton MON15985 x MON88913 as an original application under the DOST-DA-DENR-DOH-DILG Joint Department Circular No. 1 Series of 2016 (JDC No.1, S2016).

Under the JDC No.1, S2016, the assessors for Monsanto's MON15985 x MON88913 for direct use as food and feed or for processing were the following:

- One (1) member of the Scientific and Technical Review Panel (STRP) – for evaluation of the Applicant's submitted risk assessment report
- Department of Environment and Natural Resources (DENR) – for the determination of the environmental impact of the said application
- Department of Health (DOH) - for the determination of the environmental health impact of the said application
- Bureau of Animal Industry (BAI) – for the determination if the application is in compliance with feed safety standards
- Bureau of Plant Industry- Plant Products Safety Services Division (BPI-PPSSD)- for the determination if the application is in compliance with food safety standards
- Socio-economic, ethical and cultural (SEC) Expert – to evaluate SEC impact of the said application

After reviewing the documents submitted by the applicant, the STRP, BPI-PPSSD and BAI find scientific evidence that the regulated article applied for direct use as food and feed, or for processing, has no evidence of interaction on the resulting gene products while DOH, DENR, and SEC expert recommended for the issuance of Biosafety Permit for cotton MON15985 x MON88913.

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 Monsanto. The SEC expert, on the other hand, was provided with a questionnaire on socio-economic, ethical and cultural considerations that have been addressed by Monsanto 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

A. Gene Interaction

According to the STRP, it is very unlikely that the products of MON15985 and MON88913 will interact with each other and produce new allergens or toxins. The mode of action of the introduced genes are different from each other. MON15985 produces *cry1Ac* and *cry2Ab2* proteins which is effective against Lepidopteran insects while MON88913 produces CP4EPSPS

protein which confers tolerance to glyphosate. The aforementioned proteins have different structures and functions and are independent from each other. Also, they have different modes of action and binding sites, thus no interactive or synergistic effects on the metabolism of the plants is expected.

In addition, the STRP reported that accumulation of the gene products will occur at various sub-cellular compartments of the plant cell. CP4EPSPS protein, which belongs to the EPSP synthases, is involved in the biochemical shikimic pathway producing aromatic amino acids in the chloroplasts. Most plastid proteins, such as CP4EPSPS protein, are encoded by the nuclear genes, thus a transit peptide is necessary for the transport of the protein into the chloroplast where it accumulates. Cry2Ab2, just like CP4EPSPS, required a transit peptide for proper delivery to the chloroplast. On the other hand, Cry1Ac, which does not require a transit peptide, accumulates in the plant cell cytoplasm. Cry1Ac is an insect control protein that is toxic to the gut of specific lepidopterous insects.

B. Metabolic Pathways

The STRP found sufficient description on the mode of action of each gene product. Cry1Ac and Cry2Ab proteins are insect control proteins that are toxic in the gut of specific lepidopterous insects. According to Hernandez-Rodriguez et al. 2008, for Cry2Ab, there would be an essentially infinite number of binding sites available for binding (Fig. 3, page 7656). High affinity competition between Cry1Ac and Cry2A proteins in *H. armigera* and *H. zea* was not found (Fig. 3, page 7656). As shown in Fig. 3, none of the cry 2A proteins competed for binding with ^{125}I-Cry 1 Ac. This confirms the occurrence of different binding sites for cry1Ac and cry2A proteins. As shown in Fig. 4 (page 7657), ^{125} I-Cry 1 Ac showed no competition of Cry2Ab for cry 1Ac binding sites which indicates the existence of different binding sites for cy1Ab and cry 1Ac.CP4EPSPS protein belongs to the EPSPS synthase family. This enzyme is involved in the penultimate step of the biochemical shikimic pathway to produce aromatic amino acids in the chloroplasts. The native EPSPS is inhibited by glyphosate (the active ingredient of Roundup herbicide). CP4EPSPS is less sensitive to the inhibitory effects of glyphosate. This confers tolerance of the transgenic plants to glyphosate (Padgette et al., 1996, page 58-59).

The STRP reported that the gene products of the inserted novel genes are not involved in the same metabolic pathway. Similarly, they also have different mode of actions, description of which were completely provided by the applicants. The Cry proteins have been found toxic only in the gut of specific lepidopteran insect species and not to mammals (Betz et al., 2000), while CP4 EPSPS is a member of the EPSP synthase enzyme family, whose enzymes catalyze the second to the last step of shikimate pathway that produces aromatic amino acids, and is localized in plant chloroplasts.

Furthermore, the STRP reported that the modes of action of the gene products are different. Cry1Ac and Cry2Ab proteins exert its insecticidal activity in the midgut of specific lepidopterous insects such as cotton bollworm, tobacco budworm, pink bollworm and army worm via receptor-mediated mechanism. (Request for Review, May 2016). CP4EPSPS, meanwhile is involved in the penultimate step of the biochemical shikimate pathway producing aromatic amino acid in the chloroplasts of plants.

Since the modes of action of the proteins are highly specific, there is no possible unexpected effects of the stacked genes on the metabolism of the plant.

C. Gene Expression

The STRP reported that both proteins were expressed at low levels and the mean expression of all Cry proteins between MON15985 x MON88913 and MON15985 and the CP4EPSPS protein in MON15985xMON88913 and MON88913 is the same. In addition, the STRP stated that since

Crya1Ac, Cry2b2, NPT II, GUS and CP4EPSPS proteins were expressed in the single event and combined product MON 15985 x MON 88913, this would mean that inserted genes as well as the markers genes are inherited and functioning well in the MON 15985 x MON 88913.

Lastly, there is no possible interaction between the two genes, as reported by the STRP. Due to the fact that Cry1Ac, Cry2Ab2 and CP4EPSPS have different modes of action, as stated previously.

D. Recommendation

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

BPI-PPSSD ASSESSMENT AND RECOMMENDATION

A. Gene Interaction

The developer provided sufficient information that there is no possibility of interaction among the resulting products which could lead to the production of a new allergen or toxin due to the differences in the mode of action, metabolic pathway and the specificity of each protein.

CP4 EPSPS protein confers to tolerance to glyphosate while Cry1Ac and Cry2Ab confers toxic actions in the midgut of specific Lepidopteran insects and operate through independent, unrelated biochemical mechanisms (OGTR, 2006). Cry1Ac and Cry2Ab have related biochemical mechanisms and may have synergistic effects when ingested in combination, and potentially impact on toxicity for invertebrates. Hammond et al. (2013) and other literatures had indicated that exposure of mammals to Cry proteins has not been associated with additive or synergistic toxicity due to the lack of high affinity Cry protein receptors in mammals.

Toxicological and allergenicity assessment of GM cotton lines including MON 15985 x MON 88913 were conducted in Australia. No risk relating to production of toxins or allergens were identified as a result of growing cotton plants (OGTR, 2006).

Also, the gene products will not accumulate in the same subcellular compartments of the plant parts. Cry1Ac and Cry2Ab2 will accumulate in the cytoplasm since it has no cellular localization sequences for targeting a specific organelle. The *cp4 epsps* genes expressing the CP4 EPSPS protein are designed specifically to encode chloroplast transit peptides to direct the protein to the chloroplast.

B. Metabolic Pathways

The mode of action and the metabolic pathway of each protein is significantly different from one another.

Cry1Ac and Cry2Ab2 are proteins derived from the common soil bacterium *Bacillus thuringiensis subsp. kurstak*i. These are insecticidal proteins which confers protection to specific lepidopteran insects via toxic action in the gut of the insect.

Hernandez-Rodriquez et al (2008) elaborated the distinctiveness in the binding site of Cry2Ab2 and Cry1Ac; thus, indicating the difference in the mode of action of the two proteins. Binding assays with I-labeled Cry1Ac and Cry2Ab showed that Cry2A proteins binds saturably and with high affinity to specific sites in the Brush Border Membrane Vesicles (BBMVs) of *Helicoverpa armigera* and *H. zea*. This was shared among Cry2Aa, Cry2Ab and Cry2Ae but not with Cry1Ac.

CP4 EPSPS proteins are known to confer tolerance to glyphosate (Padgette et al, 1996). These proteins are responsible for the catalysis of the enolpyruvyl group from phosphoenol pyruvate (PEP) to the 5-hydroxyl of shikimate3-phosphate (S3P) which leads to the production of inorganic phosphate and 5 enolpyruvylshikimate-3-phosphate (EPSP) (Alibhai and Stallings,

2001). This was being blocked by glyphosate binding. CP4 EPSPS were known to have higher affinity for PEP thus allowing the mechanism to proceed even in the presence of glyphosate (Franz et.al., 1997).

The gene products are not involved in the same metabolic pathway since Cry proteins have no enzymatic activities while CP4 EPSPS is involved in the shikimate pathway of aromatic amino acid biosynthesis (Padgette et al., 1996).

C. Gene Expression

Based on the data provided by the developer, the protein expression level of Cry1Ac, Cry2Ab, GUS and NPTII in MON 15985 x MON 88913 is equivalent with its corresponding single event. The expression of marker genes does not pose food safety concern since there is a history of safe use attribute to both genes. The *uidA* gene expresses the GUS (β-D-glucuronidase) protein which catalyzes the hydrolysis of β-D-glucuronides. History of safe use was attributed to GUS (β-D-glucuronidase) which is known to exist in human tissues. Gilissen et al (1998) identified no toxic reaction of humans and animals upon ingestion of GUS-containing *E. coli*. No significant sequence homology to any known protein food allergens was also detected (Fuchs & Astwood, 1996). The nptII gene expresses the enzyme neomycin phosphotransferase type II (NPTII) which confers resistance to the antibiotics kanamycin and neomycin. Protein and DNA sequence comparisons indicated that NPTII does not have significant homology to any proteins listed as food toxins in Gen-Bank, EMBL, PIR29 and Swiss-Prot (FDA, 1994). No significant sequence homology to any known protein food allergens was also detected (Fuchs & Astwood, 1996).

Based on the documents provided by the developer, there is no likelihood of interaction among the genes that could affect the stability and expression level of either one of the genes due to their differences in mode of actions and metabolic pathways. The protein expression level of Cry1Ac, cry2Ab, GUS, NPTII and CP4 EPSPS was equivalent to the corresponding single events. This indicates that the genes are inherited and functioning properly in MON 15985 x MON 88913.

D. Recommendation

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

BAI ASSESSMENT AND RECOMMENDATIONS

A. Gene Interaction

BAI has stated that the proteins introduced in the plant have shown distinct modes of action specific only for the individual protein. Therefore, the previous safety reports for the allergenicity and toxicity assessments on the single event for each of the individual proteins are applicable to support a conclusion that the combined trait product are unlikely to allow interactions between and among the stacked traits that might lead to production of a new allergen or a new toxin.

They also stated that the gene products accumulate in different subcellular compartments of the plant parts.

B. Metabolic Pathways

BAI has concurred that the mode of action of Cry1Ac, Cry2Ab2, and CP4 EPSPS proteins has been completely described in the previous safety assessment on each of the individual single event products. They stated that Cry1Ac and Cry2Ab2 proteins, although derived from the same bacterium and has similar toxic action directed in the gut of specific insects, has

demonstrated different binding site for toxins based on heterologous-competition assays. While CP4 EPSPS protein confers tolerance to glyphosate and is involved in an enzymatic pathway producing aromatic amino acids in the chloroplasts of plants. This indicates that Cry1Ac, Cry2Ab2, and CP4 EPSPS proteins have different modes of biological action.

Moreover, they also stated that inserted genes were not designed to alter the plant metabolism and were shown to have been inherited and functioning properly when combined into the breeding stack as demonstrated in the protein expression analysis. Since the interaction between/among these products are highly unlikely because of the structural and functional differences and the different modes of action of each gene product and that each gene product functions independently as in single event, it is highly unlikely that there will be unintended effects of the combined traits on the metabolism of the plant.

In conclusion, they added that Cry1Ac, Cry2Ab2, and CP4 EPSPS proteins have different modes of biological action and are structurally and functionally different, therefore, each protein functions independently from each other and are not involved in the same metabolic pathway.

C. Gene Expression

BAI has concurred that results obtained from ELISA performed on seed tissues of MON 15985 X MON 88913, MON 15985 and MON 88913 collected from five field sites in United States show that the proteins were expressed properly in the combined trait product as in its relevant single events. The assessment generally showed almost similar levels of expression of all proteins in the single-event and stacked plants. They also concurred that the proteins were expressed at low level as demonstrated by ELISA. A detailed summary of the expression levels of proteins are shown in Table 2 of the Request for Review of MON 15985 X MON 88913.

Further, they stated that GUS and NPTII proteins used as selective markers were expressed in the combined product as shown in the results obtained from the protein expression analysis. AAD protein, which was used as a selectable marker, is under the control of a bacterial promoter which is only functional in prokaryotic cells and is not expected to be expressed in cotton tissue which is eukaryotic.

They also stated that due to the different modes of action of each protein, any form of interaction is unlikely, which means that the stability and expression levels of all the genes will not be significantly affected as shown in the protein expression level study.

D. Recommendation

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

DENR ASSESSMENT AND RECOMMENDATION

After thorough and scientific review and evaluation of the documents provided by the Bureau of Plant lndustry (BPl) to the DENR Biosafety Committee within the prescribed period pursuant to Joint Department Circular (JDC) No.1 s.2016 on the application of MONSANTO PHILIPPINES, lnc. for direct use for feed, food or processing of Genetically Modified Cotton resistant to insect pests and tolerant to glyphosate herbicide with Stacked trait product MON15985 x MON88913, the following are the observations and recommendations: The effect of the regulated article on the environment depends largely on the viability of the product to be utilized for direct use. lf the article is transported in a non-viable form, there is no danger to the environment; 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 lndustry (BPl) (Section 26 of JDC No.1 s.2016); It is suggested that BPI ensure the following:

a) Implementation of the EMP by the traders/importers involved in the import, handling, processing and transport of viable Cotton MON15985 x MON88913 commodity products; and
b) 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 ASSESSMENT AND RECOMMENDATION

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. On the description of the phases or stages of the biotechnology project, Monsanto Philippines, Inc. claimed that exposure to Cotton MON 15985 x MON 88913 is highly unlikely to give rise to an adverse effect and does not pose greater risks to biodiversity, human and animal health than its conventional counterpart when brought to unloading and loading, hauling and transport, loading and storage and during food and/or processing. It was further stated that safety of individual single products and proteins produced in the products has been extensively assessed through robust, comprehensive analyses and data packages including molecular characterization, nutritional and compositional analyses, toxicity studies and environmental assessment.

2. On the Risk to Health Matrix (Integration of the health consequence rating with incident potential rating), the Monsanto Philippines, Inc. rated the activities of the phases of project a Very Low Incident/Exposure Rating.

3. Scientific pieces of evidences from provided references i.e. literatures show that regulated article applied for direct use is as safe as its conventional counterpart and shall not pose any significant risk on human health, animal health and on the environment.

4. It is suggested that the Bureau of Plant Industry (BPI) ensure the following:

1) Strict monitoring of the regulated article from port of entry to the trader’s/importer’s storage/warehouse as stated in Section 32 of the DC No.1 series, 2016
2) The BPI to include in the issuance of permit for the release of this product the following conditions:
    a. Any spillage (during unloading and loading/hauling and transport unloading and storage) shall be collected and cleaned up immediately
    b. Transportation of the consignment from the port of entry to any destination within the country shall be in closed containers.
    c. There shall be clear instructions that the product is only for the purpose of direct use for food, 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, feed or processing of Cotton MON 15985 x MON 88913

SEC ASSESSMENT AND RECOMMENDATIONS

The SEC Expert has reviewed the answer of the applicant regarding the SEC Impact of cotton MON15983 x MON88913 and has found some queries. Monsanto has answered the query to which the SEC expert has approved and has recommended for the approval and issuance of biosafety permit for MON15985 x MON88913 for direct use as food and feed, or for processing.