

**CONSOLIDATED REPORT OF MONSANTO PHILIPPINES' CORN MON88017 X MON810
APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING**

EXECUTIVE SUMMARY

On April 22, 2016, Monsanto Philippines Inc. applied for the issuance of Biosafety Permit for Direct Use their combined trait product corn MON88017 x MON810.

After reviewing the Risk Assessment Report and attachments submitted by the applicant; the Scientific and Technical Review Panel (STRP) member, Bureau of Animal Industry (BAI), and BPI-Plant Products Safety Services Division (BPI-PPSSD) has found no interaction of the resulting gene product of the regulated article applied for direct use as food and feed, or processing based on scientific evidences provided.

The STRP, BAI, and BPI-PPSSD concurred that the likelihood of interaction among the three (3) proteins (Cry1Ab, Cry3Bb1 and CP4 EPSPS) is unlikely because their modes of action are different. The gene products will not accumulate in the same subcellular compartments of the plant parts. The gene constructs encoding the Cry1Ab and Cry3Bb1 proteins has no sequences for targeting its transport to a specific organelle and would accumulate in the cytoplasm of maize cells. CP4 EPSPS, on the other hand, will accumulate in the chloroplast. The cp4 epsps genes expressing CP4 EPSPS are designed specifically to encode chloroplast transit peptides to direct the protein to the chloroplast, the site of action of all EPSPS proteins.

After a thorough scientific review and evaluation of Monsanto Philippines' duly accomplished Environmental Risk Assessment (ERA) and Project Description Report (PDR) forms, the Department of Environment and Natural Resources – Biosafety Committee (DENR-BC), recommended for the issuance of a biosafety permit for this regulated event provided that the conditions set by DENR are complied. Also, the Department of Health – Biosafety Committee (DOH-BC), after a thorough scientific review and evaluation of the accomplished Environmental Health Risk Assessment form, concluded that corn MON810 x MON88017 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. Hence, the DOH-BC also recommended for the issuance of biosafety permit for the stacked trait product. Lastly, after assessing that there will be no negative socio-economic, ethical and cultural concerns that will arise from the adoption of Genetically Modified Organisms, the Socio-economic, Ethical and Cultural (SEC) expert recommended for the approval and issuance of biosafety permit of corn MON88017 x MON810 for direct use as food and feed, or for processing.

BACKGROUND

In accordance with Article VIII, Section 20 of the JDC No.1, S2016, 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. The SEC expert, on the other hand, was provided with a separate questionnaire on socio-economic, ethical and cultural considerations that have been addressed by Monsanto Philippines 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 RECOMMENDATION

After a thorough review of the documents submitted by the applicant, the STRP made the following assessment and recommendation:

A. Gene Interaction

The STRP agreed with the applicant that there is no likelihood of interaction among the Cry (Cry1 Ab and Cry3Bb1) and the CP4 EPSPS proteins that will produce new allergens and new toxins based on their distinct mode of action.

The STRP reported that Cry proteins are contained in the plant tissue in microgram levels and are produced at low levels in the pollen (Betz, 2000) while the CP4 EPSPS proteins are localized in the chloroplasts or plastids (Padgett, 1996).

B. Metabolic Pathways

The STRP confirmed that the complete description of the mode of action of each gene product is provided by Monsanto Philippines.

Furthermore, the STRP validated the report that the mode of action is different for each gene product. The Cry1Ab and Cry3Bb1 proteins are insect control proteins and act through a toxic action in the midgut of specific lepidopteran insects, such as the European corn borer (*Ostrinia nubilalis*) and the midgut of coleopteran species, such as corn rootworm (*Diabrotica virgifera virgifera*), respectively. The cry1Ab and cry3Bb1 genes were derived from the common soil bacterium *Bacillus thuringiensis* (Bt) subsp. *kurstaki* and subsp. *kumamotoensis*, respectively. *Bacillus thuringiensis* (Bt) proteins exert their insecticidal activity via receptor-mediated mechanisms and are highly selective in terms of the organisms they affect.

On the other hand, the CP4 EPSPS protein belongs to the family of EPSP synthases, which are enzymes involved in the penultimate step of the biochemical shikimate pathway producing aromatic amino acids in the chloroplasts of plants.

C. Gene Expression

The STRP reported that ELISA was used to validate the levels of the 3 proteins in the plants tissues in the combined trait product MON 88017 X MON 810. The results

showed that Cry 1Ab, Cry 3 Bb1 and CP4 EPSPS proteins are expressed in the combined product MON 88017 X MON 810 as in its single events.

The STRP also reported that Cry1Ab, Cry3Bb1 and CP4 EPSPS proteins are expressed at low levels in MON 88017 x MON 810.

Based on the documents submitted and research conducted, the STRP found 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

After a thorough review of the documents submitted by the applicant/proponent, BPI-PPSSD made the following assessment and recommendation:

A. Gene Interaction

According to BPI-PPSSD, the developer provided supporting documents indicating that any likelihood of interaction among the resulting products in MON 88017 x MON 810 could not lead to the production of new allergen or toxin. This is due to the difference in the mode of actions of the expressed proteins (Cry1Ab, Cry3Bb1 and CP4 EPSPS), genetic stability and low protein expression levels. There is no known mechanisms of interaction among these proteins that could lead to adverse effects in humans and other organisms in the environment.

- Toxicological and allergenicity analyses also indicated that MON 88017 x MON 810 did not produce any new toxins or allergens. Compositional analysis also showed that the combined trait product is substantially equivalent with the conventional corn (EFSA, 2009).
- Each single event, MON 88017 and MON 810, have been approved for Biosafety for food and feed, or for processing by the Bureau of Plant Industry.

According to the PPSSD, the gene products will not accumulate in the same subcellular compartments of the plant parts. The gene constructs encoding the Cry1Ab and Cry3Bb1 proteins has no sequences for targeting its transport to a specific organelle and would accumulate in the cytoplasm of maize cells. CP4 EPSPS, on the other hand, will accumulate in the chloroplast. The *cp4 epsps* genes expressing CP4 EPSPS are designed specifically to encode chloroplast transit peptides to direct the protein to the chloroplast, the site of action of all EPSPS proteins.

B. Metabolic Pathways

As per BPI-PPSSD, the developer provided a complete description of the mode of action of each gene product. Each single event, MON 88017 and MON 810, have been approved for Biosafety for food and feed, or for processing by the Bureau of Plant Industry.

They added that the reference provided by the applicant, Höfte and Whiteley (1989), indicated the difference in the actions of Cry1Ab and Cry3Bb1. Cry1 proteins are known to be selectively toxic to Lepidopterans while Cry3 proteins are known to be specific for Coleopterans. Cry1Ab proteins confer toxicity in the midgut of specific lepidopteran insects such as the European corn borer (*Ostrinia furnacalis*) while Cry3Bb1 proteins confer toxicity in the midgut of specific coleopteran insects such as the corn root worm (*Diabrotica virgifera virgifera*).

CP4 EPSPS proteins belongs to the family of EPSPS synthases which are known to confer tolerance to glyphosate. These proteins are involved in the shikimic acid pathway of aromatic amino acid biosynthesis (Padgett et.al., 1996). These proteins catalyzes the transfer of enolpyruvyl group from phosphoenol pyruvate (PEP) to the 5-hydroxyl of shikimate3-phosphate (S3P) producing 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 PPSSD confirmed that based on the documents provided by the developer and other literatures searched, there is no possibility of any unexpected effects of the stacked genes on the metabolism of the plant since there are no interactions detected among the resulting products, Cry1Ab, Cry3Bb1 and CP4 EPSPS. This was being supported by the Southern Blot Analyses which indicated that the structures of the single events MON 88017 and MON 810 were also expressed in MON 88017 x MON 810. Both single events were previously approved for direct use as food and feed or for processing by the Bureau of Plant Industry (BPI) in 2007 (MON 810) and 2011 (MON 88017). Evaluation of each single events indicates its equivalence to the conventional corn and each protein is not designed to alter corn plant metabolism.

C. Gene Expression

The PPSSD reported that the developer provided data and analysis on the expression levels of the individual protein products through ELISA. The results of analysis in corn grains produced in the United States (2002) and Argentina (2003-2004) field trials showed that the levels of the proteins, Cry1Ab, Cry3Bb1 and CP4 EPSPS, in MON 88017 x MON 810 is not significantly different from the single events MON 88017 and MON 810.

In addition, they confirmed that the proteins of the said event are expressed at low levels and that there were no marker genes transferred and expressed in the plant.

Furthermore, it was reported that there is no possible interaction among the resulting products that would affect the stability and expression level of either one of the genes due to the difference in mode of action and metabolic pathway.

Based on the documents submitted and research conducted, the PPSSD found scientific evidence that the regulated article applied for direct use has no evidence of interaction on the resulting gene products.

BAI ASSESSMENT AND RECOMMENDATION

After a thorough review of the documents submitted by the applicant/proponent, BAI made the following assessment and recommendation:

A. Gene Interaction

A new allergen or new toxin is unlikely to be produced out of the combined genes as there are no known mechanism of interaction between these different classes of protein. Each class of protein differs in structure and function from each other, such that any likelihood of interaction with one another is not considered to be significant to effect the stability and expression level of each gene nor could their interaction lead to production a new allergen or toxin in the combined trait product.

In addition, the BAI confirmed that the gene products are contained in different subcellular compartments of the plant parts. The Cry proteins are said to accumulate at the cytoplasm while the CP4 EPSPS protein are expected to accumulate at the chloroplast of the corn cells.

B. Metabolic Pathways

BAI confirmed that the mode of action of each gene product was properly described in the documents presented by the applicant and that other scientific materials attached supported the finding that the mode of action of each gene product is different from each other.

They also confirmed that each gene product has a different mode of action and are highly selective in terms of the organism they attach. Both cry proteins, Cry1Ab and Cry3Bb1 are insect control proteins derived from the common soil bacterium *Bacillus thuringiensis* (Bt) subspecies Kurstaki and Kumamotoensis, respectively. Cry1Ab toxin action act through the midgut of a specific lepidopteran insects, such as the European corn borer. On the other hand, the Cry3Bb1 toxin action acts also on the midgut but of a coleopteran species, such as corn rootworm. The CP4 EPSPS protein used in the event is from *Agrobacterium* sp strain CP4 also a common soil bacterium. It belongs to the family of EPSPS synthases, which are enzymes involved in the shikimate pathway producing aromatic amino acids in the chloroplasts of plants. The CP4 EPSPS enzyme confers tolerance of transgenic plants to glyphosate.

The BAI validated the applicant's claim that since the modes of action of the introduced proteins are different, it is not expected that there would be unintended adverse effects that will occur.

C. Gene Expression

The BAI confirmed that the evaluation of seed tissues showed low level expression of all proteins and that no marker genes were transferred.

The BAI has found no interaction on the resulting gene products of the regulated article applied for direct use based on scientific evidences presented.

DENR-BC ASSESSMENT AND RECOMMENDATION

After a thorough scientific review and evaluation of the documents including the Project Description Report and Environmental Risk Assessment forms provided by the Bureau of Plant Industry (BPI) along with the submitted sworn statement and accountability of the applicant, the DENR Biosafety Committee recommended that a biosafety permit may be issued to Monsanto Philippines Inc. given that the conditions set by DENR are followed.

DOH-BC ASSESSMENT AND RECOMMENDATION

After a thorough scientific review and evaluation of the documents including the Environmental Health Impact Assessment form, the DOH find sufficient evidence that the regulated article applied for direct use will not pose any significant risk to the health and environment. The Committee also suggested conditions which can also be included in the Biosafety Permit to be issued to Monsanto.

SEC EXPERT ASSESSMENT AND RECOMMENDATIONS

The SEC Expert has reviewed the answer of the applicant regarding the SEC Impact of corn MON88017 X MON810 and has found some queries. Monsanto Philippines has answered the query to which the SEC expert has approved and has recommended for the approval and issuance of biosafety permit for MON88017 X MON810 for direct use as food and feed, or for processing.