ASSESSORS' CONSOLIDATED REPORT ON SYNGENTA'S APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF COMBINED TRAIT PRODUCT CORN BT11 X DAS59122-7 X MIR604 X TC1507 X GA21

EXECUTIVE SUMMARY

On June 9, 2016, Syngenta Philippines Inc. applied the stacked trait product corn BT11 x DAS59122-7 x MIR604 x TC1507 x GA21 for direct use as food and feed, or for processing as an original application under the DOST-DA-DENR-DOH-DILG Joint Department Circular No. 1 Series of 2016 (JDC No.1, S2016).

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 eight (8) proteins (Cry1Ab, Cry35Ab1, mCry3A, Cry1F, PAT, PMI and mEPSPS) is unlikely because their modes of action are different. In addition, allergen and toxicity analyses of the eight (8) proteins have shown that these proteins show no homology to any known mammalian allergen or toxin. Furthermore, the assessors concurred that there are no possible unintended effects of stacked genes on the metabolism of the plant based on the previous assessments of individual transformation events.

After a thorough scientific review and evaluation of Syngenta's 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 BT11 x DAS59122-7 x MIR604 x TC1507 x GA21 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 BT11 x DAS59122-7 x MIR604 x TC1507 x GA21 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, except for the SEC expert, the complete dossier submitted by Syngenta 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 Syngenta 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 report thatthe likelihood of interaction among the eight proteins (Cry1Ab, Cry35Ab1, mCry3A, Cry1F, PAT, PMI and mEPSPS) is unlikely because of their different modes of action. Based on the Allergy and Toxin homology analyses, the STRP reported that the abovementioned proteins show no homology to any known mammalian allergen or toxin and there is no evidence of interaction among them.

B. Metabolic Pathways

The STRP confirmed the complete description of the mode of action of each gene product in the submitted application of Syngenta Philippines.

Furthermore, the STRP validated the report that Cry1Ab, Cry34Ab1, mCry3A, Cry1F protein acts by selectively binding to specific sites in the midgut epithelium and induce the formation of pores in the membrane of susceptible cells. The *pat* gene encodes an enzyme that detoxifies glufosinate ammonium which inhibits the glutamine synthetase in plants resulting in an accumulation of ammonia and plant death.

The STRP also verified the report that the plant cells that produce PMI can convert mannose to fructose-6-phosphate and mannose do not accumulate. In addition, the STRP described that plant expressing the mEPSPS or modified protein when treated with glyphosate are unaffected.

C. Gene Expression

The STRP verified the report that the expression levels of the individual products are the same as the individually approved transformation events and that data on expression levels are provided for Bt proteins, PAT, PMI, and mEPSPS.

As concurred by the STRP, the proteins were expressed at different levels in plants and significant differences in levels were observed. Further, it was reported that PAT levels were higher compared to single event hybrids.

The marker genes,on the other hand, were reportedly transferred and expressed in the plants containing the stacked genes. Further, it was confirmed that likelihood of interaction among the eight proteins is unlikely.Stability and expression levels of any of these genes maynot be affected.

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 sufficient information and references which support that the presence of eight proteins will not interact to produce any known mammalian allergen or toxins. This is due to the different mode of action of each protein which are not likely to interact.

Cry proteins are not known to induce toxicity or allergenicity to mammals. Hammond et. al. (2013) and other literatures had indicated that dietary exposure of mammals to Cry proteins, individual or mixed, has not been associated with additive or synergistic toxicity. This is due to the lack of high affinity Cry protein receptors in mammals. Cry1 and Cry3 proteins are known to induce toxicity specifically to Lepidopteran and Coleopteran insects, respectively.

The BPI-PPSSD added that Cry1Ab in Bt11, PAT (phosphinothricin acetyltransferase) in Bt11, DAS-59122-7, and TC1507, Cry34Ab1 in DAS-59122-7, Cry35Ab1 in DAS-59122-7, mCry3A in MIR604, PMI in MIR604, Cry1F in TC1507, and mEPSPS (5-enol pyruvylshikimate-3-phosphate synthase) in GA21 have been assessed individually using different bioinformatics analyses and showed that these proteins have no significant homology and similarity to any known allergen and toxins that could lead to potential adverse effect on human and animal health.

The expression of *cry1Ab* and *pat* gene (for BT11), *cry34Ab1* gene (for DAS-59122-7), *mcry3A* gene (for MIR604), *cry1F* and *pat* gene (for TC1507) is driven by each promoter and are intended to accumulate in cytoplasm since there is no presence of cellular localization sequences.

Modified EPSPS enzyme (in GA21) is identified to accumulate in the chloroplast. The gene product for this enzyme encodes chloroplast transit peptide that directs the protein to the chloroplast. The transit peptide is cleaved off the protein upon import to the chloroplast. Hence, only the cleaved off version of the protein can be found upon isolation from the plant indicating that the protein is immediately directed to the chloroplast upon synthesis

B. Metabolic Pathways

As per BPI-PPSSD, all proteins expressed in the combined trait product have different mode of actions and metabolic pathways.

According to them, Cry1 proteins are known to confer toxicity to lepidopteran insects while Cry3 proteins are known to target Coleopteran insects (Hofte et.al.).

Cry1Ab protein selectively binds to a specific site localized on the brush border midgut epithelium of susceptible insect species followed by the formation of cation-specific pores. These pores disrupt the midgut ion flow thereby causing paralysis and death.

Cry1F proteins binds selectively to a specific site on the lining of the midgut of susceptible insect species. Upon bounding, the toxin molecules form oligomers which creates pores in the membrane leading to osmotic destabilization and death.

mCry3A protein, has the same pattern of action but with a specificity in binding reception. The formation of ion-selective channels in the cell membrane leads to cell lysis and death of the insect.

Cry34Ab1 and Cry35Ab1 also has the same pattern of action but with specificity in binding reception. Following binding is the formation of pores which disrupt the midgut ion flow causing paralysis and death of the larvae.

Phosphinothricin-N-acetyl transferase detoxifies glufosinate ammonium, the active ingredient in the herbicide Basta ®, through acetylation of the phosphinotricin to N-acetyl-glufosinate (NAG) and 2 further metabolites, 3-methylphosphinicopropionic acid (MPP) and 3-methylphosphinicoacetic acid (MPA).

PMI was used as a selective marker during the selection of transformed cells in culture of the plants. This allows the plants to convert mannose to fructose-6-phosphate which improves the energy status of the cells avoiding the accumulation of derivatized mannose.

mCP4 EPSPS proteins are involved in the biochemical shikimic pathway producing aromatic amino acid in the chloroplasts (Padgette et.al., 1996). It 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 (Alibhai and Stallings, 2001). This mechanism is being inhibited with glyphosate binding which blocks the binding of EPSPS to PEP. CP4

EPSPS, on the other hand, has higher affinity for PEP thus allowing the catalysis to proceed even in the presence of glyphosate (Franz et.al., 1997).

Furthermore, the BPI-PPSSD reported that all of these proteins except for mCP4 EPSPS has no cellular localization sequences and are likely to accumulate in the cytoplasm. mCP4 EPSPS, on the other hand, is known to have chloroplast transit peptide which directs the protein into the chloroplast, the site of all EPSPS action.

C. Gene Expression

Enzyme-Linked Immunosorbent Assays (ELISA) was used to quantify and determine the level of proteins. Results showed that the concentrations of Cry1Ab, PAT, Cry34Ab1, Cry35Ab1, mCry3A, MIR604 PMI, Cry1F and mEPSPS in tissues of the Bt11 x DAS-59122-7 x MIR604 x TC1507 x GA21 maize hybrid were similar to those of the corresponding single event maize hybrids, Bt11, DAS-59122-7, MIR604, TC1507, and GA21. As expected, PAT concentrations in tissues of the stacked-trait were higher than those from each of the component single event hybrid due to the presence of 3 copies of the *pat* gene in the stacked-trait hybrid, while Bt11, DAS-59122-7, and TC1507 maize hybrids each contain 1 copy of the *pat* gene.

Southern Blot Analysis was conducted by the developer to determine the expression level and stability of the genes in the combined trait product. Results showed that the marker genes, PAT (for Bt11, DAS-59122-7 and TC1507) and PMI (for MIR604) are stably inherited and are likewise expressed in the combined trait product. This was also observed in the Cry proteins and mCP4 EPSPS. a

Based on the documents submitted, BPI-PPSSD has found no interaction on the resulting gene products of the regulated article applied for direct use based on scientific evidences provided.

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

The data gathered from different studies affirmed that there are no interactions found within and among the different proteins in the host plant which could produce a new allergen or a new toxin.

Further, the BAI concluded that all other gene products accumulated in the cytoplasm except for the modified EPSPS enzyme which is known to accumulate in the chloroplast.

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.

In addition, the gene products especially those that have enzymatic activities, such as PAT protein, modified EPSPS, and PMI are not involved in the same metabolic pathway.

C. Gene Expression

It was verified by BAI that the transgenic protein content of plant tissues from three growth stages harvested from three separate field trial locations was determined by ELISA with observations as follows:

- Cry1Ab concentration was generally higher in the stacked plant;
- PAT concentration was generally higher in the stacked plant than in single Bt11, DAS59122-7 and TC1507;
- Cry34Ab1 concentration was similar between DAS59122-7 and the stacked plant;
- Cry35Ab2 concentration was generally higher in the stacked than in DAS59122-7;
- mCry3A concentration was similar in MIR604 and the stacked plant;
- MIR604PMI concentration was similar in the single trait and stacked plants;
- Cry1F concentration was similar in TC1507 and the stacked plant; and
- mEPSPS concentration was generally higher in the single event GA21 than in the stacked plant

Overall, eight (8) significant differences were noted in the study out of fifty-eight (58) statistical comparisons but these were not consistently observed across tissue types or growth stages.

Furthermore, the BAI concurred that the evaluation of different plant tissue types in three (3) growth stages generally showed similar levels of expression of all proteins in the single-event and stacked plants. Marker genes: pat from Bt11, DAS59122-7 and TC1507 and PMI from MIR604 were confirmed to be incorporated and expressed in Bt11 x DAS59122-7 x MIR604 x TC1507 x GA21.

Based on the documents submitted, BAI has found nointeraction 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 provided by the Bureau of Plant Industry (BPI) to the DENR Biosafety Committee within the prescribed period pursuant to the JDC No.1 S2016 on the application of Syngenta Philippines Inc. for direct use as food and feed, or processing of Genetically Modified corn Bt11 x DAS59122-7 x MIR604 x TC1507 x GA21(resistant to insect pest and tolerant to glyphosate and glufosinate

herbicides) along with the submitted sworn statement and accountability of the applicant, a biosafety permit may be issued to Syngenta Philippines Inc. if the conditions set by DENR are followed.

DOH-BC ASSESSMENT AND RECOMMENDATION

After a thorough scientific review and evaluation of the documents, DOH find sufficient evidence that the regulated article applied for direct use will not pose any significant risk to the health and environment and that any hazards could be managed by the measures set by DOH.

SEC EXPERT ASSESSMENT AND RECOMMENDATIONS

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

A. Socio-economic Issues

In terms of production, consumption, and trade, the SEC expert stated that the GM product can be significant given the increasing trend in domestic demand for corn in the Philippines since 2010 and the decline in domestic corn outputs early this year.

In addition, the SEC expert reported that given the Philippine's history of corn importation vis a vis trends in consumption/utilization, drastic changes are not expected, and even modest changes in use for food and feed do not seem attributable to imports alone.

In terms of competitiveness, the SEC expert noted that the GM product along with other corn imports, are intended to help meet overall domestic demand for corn.

B. Social Issues

In terms of health concerns, the SEC stated that new information regarding perceived health effect of the GM products is subjected to applicable laws under the JDC No.1, S2016.

It was also reported by the SEC expert that the GM crop's introduction is not expected to reduce society's adaptability such as in problem solving skills related to corn farming. The recent decline in domestic production has been attributed to "extreme weather" and not to crop pests.

C. Ethical Issues

When asked if there is a risk of conflict with the ideals of human solidarity and equality (e.g. risk of the effects on the weaker groups of societies), the STRP responded that the issues of equity and equality are best understood in the context of engagement and participation, hence evaluation of applicant's response should be done alongside results of the procedures for Public Participation for Direct Use under Sec. 22 of JDC 1-2006.

Recommendation

Based on the assessment of the above indicators, the SEC expert does not have any socio-economic, social, and ethical issues to raise regarding the approval of the applicant's application for biosafety permit for direct use as food and feed, or for processing of corn Bt11 x DAS59122-7 x MIR604 x TC1507 x GA21. The expert recommends for the approval of said application.