ASSESSORS' CONSOLIDATED REPORT ON MONSNTO PHILIPPINES INC.'S APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF ALFALFA J101 X J163

EXECUTIVE SUMMARY

On June 25, 2020, Monsanto Philippines Inc. submitted alfalfa J101 x J163 for direct use, 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 Scientific and Technical Review Panel (STRP) concurred that alfalfa J101 x J163 is as safe for human food and animal feed as its conventional counterpart.

The Department of Health – Biosafety Committee (DOH-BC), after a thorough scientific review and evaluation of documents related to Environmental Health Impact, concluded that alfalfa J101 x J163 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 alfalfa J101 x J163.

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.

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, except for the SEC expert, the complete dossier submitted by Monsanto Philippines Inc. 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 Philippines Inc. in relation to their application.

STRP's Assessment

1. **Gene Interaction**
   a. There is no plausible interaction among the novel proteins when produced in the alfalfa plant.
   b. The probability of such interactions in which a new allergen or a new toxin could be produced, is very improbable and thus, no adverse effects in humans, animals and the environment are foreseen [1].
   c. The resulting novel proteins will accumulate in the chloroplast of the stacked event [1].

2. **Metabolic Pathways**
   a. The resulting novel protein products are involved in the same metabolic pathway. Moreover, these novel proteins do not share any intermediate metabolites in the biochemical pathways that the proteins act on or interfere with [1].
   b. There are no unintended nor unexpected effects on the metabolism of the alfalfa plant when the novel genes are introduced in it. The experiments done by the proponents and the data analyses that they performed are scientifically and statistically sound. The data presented herein indeed depicts that the various agronomic characteristics of the stacked trait plant is not statistically significantly different if compared to the conventional untransformed counterpart [1].

3. **Gene Expression**
   a. The expression levels of the novel protein were not biologically different between the stacked transgenic plant under evaluation and its parental genotypes.
   b. The measurements done by the proponents and subsequent statistical analysis clearly demonstrated that there is indeed no significant difference among the expression levels of the novel proteins [1].
   c. There is an expected low expression of the novel proteins in the regulated stacked event under evaluation, similar to what is recorded in its parental genotypes [1].
   d. The selectable marker genes used in the development of the individual transgenic events, are not transferred, and expressed in the regulated stacked event [1].
   e. The proponents were also able to comprehensively demonstrate the stability of the genome/partial genome of the parentals in the stacked transgenic alfalfa. The genetic material of the parentals, containing the novel genes, were indeed stably incorporated in Alfalfa J101 × J163 [1].

STRP's Conclusion

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

BAI's Assessment

1. **Gene Interaction**
   a. There is no known interaction of the resulting products such that a new allergen or a new toxin could be produced. The modes of action of both genes (partial coding sequence of the *Medicago sativa CCOMT* gene and *cp4 epsps*) are different from each other and will function independently [2][3].
   b. The gene products will not accumulate in the same subcellular compartments of the plant. The CP4 EPSPS would accumulate in the chloroplast while the *CCOMT* partial gene would be unlikely to accumulate in any subcellular compartment because it is highly unlikely to produce proteins [2].

2. **Metabolic Pathways**
   a. Partial coding sequence of the *Medicago sativa CCOMT* gene (from KK179) encodes caffeoyl CoA 3-O-methyltransferase protein and suppresses endogenous CCOMT RNA levels that results in reduced production of G lignin. This cassette is also highly unlikely to produce proteins. *cp4 epsps* involved in the shikimate pathway producing aromatic amino acids in the chloroplasts of plants and its presence results to tolerance of the plant to glyphosate [2][3].
   b. The mode of action of each gene product is different. The products are not involved in the same metabolic pathway. The *CCOMT* partial gene segment is involved in suppressing endogenous *CCOMT* RNA levels that results in reduced production of G lignin while CP4 EPSPS is involved in shikimate pathway [2][3].
   c. Unexpected effects of the stacked genes are highly improbable since the *CCOMT* partial gene segment and CP4 EPSPS have different modes of action and metabolic activity [2][3].

3. **Gene Expression**
   a. CP4 EPSPS protein is expressed properly in the combined trait product. The mean CP4 EPSPS protein levels in the forage of KK179 × J101 alfalfa and J101 alfalfa were 500 µg/g dw and 530 µg/g dw, respectively [1].
   b. Since the protein levels of CP4 EPSPS in the stacked and individual events were comparable, CP4 EPSPS protein was expressed at low level. KK179 alfalfa, on the other hand, does not contain any recombinant gene that encodes a protein [3].
   c. The marker gene was subsequently removed by traditional alfalfa breeding methods and meiotic segregation [1].
   d. Possible interaction is very unlikely since the *CCOMT* partial gene segment and CP4 EPSPS have different modes of action and metabolic activity. Thus, stability and expression level of the genes is not affected [2][3].

BAI's Conclusion

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

BPI PPSSD's Assessment

1.Gene Interaction

a. The stacked gene both contains CP4 EPSPS proteins. Protein expression analysis indicates no significant differences between the levels of CP4 EPSPS in J101 x J163 and its corresponding single events.[1].
b. The CP4 EPSPS protein is targeted to accumulate in the chloroplast via chloroplast transit peptide.[1].

2. Metabolic Pathways

a. CP4 EPSPS proteins are involved in the biochemical shikimic pathway producing aromatic amino acid in the chloroplasts [3]. 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 [5]. 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. This enzyme catalyzes the reaction wherein the enolpyruvyl group from phosphoenol pyruvate (PEP) is transferred to the 5-hydroxyl of shikimate-3-phosphate (S3P) to form 5-enolpyruvylshikimate-3-phosphate (EPSPS) and inorganic phosphate (Pi) [3].
b. The expression of the proteins in J101 x J163 is similar to the corresponding levels in single events. Results indicated that the proteins are expressed similarly to the combined trait product as in its corresponding single events [1].

3. Gene Expression

a. Protein expression analysis via ELISA showed that the expression of CP4 EPSPS in J101 x J163 is comparable to the corresponding single events. CP4 EPSPS is being expressed at low levels in the plant [1].
b. There is no marker gene used for the production of J101 x J163 and there is also no possible interaction since only CP4 EPSPS is present in J101 x J163.[1].

BPI-PPSSD's Conclusion

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

DENR-BC'S Assessment

1. The regulated article is considered substantially equivalent to its conventional counterpart for its history of safe use for food, feed, and cultivation in Japan, for food and feed in South Korea, and for food in Mexico [6]. Also, the individual events Alfalfa J101 and Alfalfa J163 have both been previously approved for direct use as food and feed in the Philippines. The stacked genes were also introgressed through conventional plant breeding methods, which has a history of safe use;
2. The crop was found to be compositionally equivalent and considered as safe as conventionally grown alfalfa in terms of its amino acids, fibers, and mineral constituents [9]. It was also considered safe in relation to food safety and toxicology [8].
3. Gene flow from the regulated article to wild relatives is highly unlikely to occur in nature [6]. Moreover, alfalfa hay does not contain viable plant materials. The potential to be invasive or weedy is less likely; and
4. The project description report (PDR) discusses the specified environmental management plan indicating the possible risk and harm to the environment particularly on biodiversity, as well as the mitigating measures and contingency plan [5][6][7][8].

DENR BC's Conclusion

Based on the evaluation and review of literatures cited, DENR-BC considered that the regulated article poses no significant adverse effect to the environment.

DOH-BC's Assessment

1. *Agrobacterium* sp. strain CP4 as well as other bacteria and some soil fungi, are resistant to the action of glyphosate for possessing the EPSPS enzyme, little sensitive to the inhibition of the herbicide. Further, this donor bacterium is not a food source but is related to microbes commonly present in the soil and in the rhizosphere of plants. All plant, microbial, and fungal food sources contain EPSPS proteins, therefore, this enzyme and its activity are not novel to the food supply.
2. Based on the sequence alignment run through FASTA and BLASP, CP4 EPSPS does not match with any known toxic protein. Most of the matches corresponded to sequences from the CP4 EPSPS family from various origins. Further, no matches of greater than 35% identity to known allergens were found based on the AllergenOnline database.
3. No. J101 and J163 alfalfa produce the same CP4 EPSPS protein and there is no known mechanism of interaction leading to adverse effects in humans, animals, or environment.

DOH-BC's Conclusion

Based on the evaluation of available literature and dossier documents presented, Alfalfa J163 x J101 is as safe as its conventional counterpart for Direct Use as Food, Feed or for Processing (FFP). Use of this event in its usual context is not expected to pose any new or additional risk to human and animal health and environment.

SEC Expert's Assessment

1. The crop is not grown in the Philippines and thus rely on imports to meet the feed requirements of the animal industry. It should have no effect on production, consumption, and trade. The importation of the product is very important to the livestock industry.
2. Since this refers only to the importation of FFP (agricultural commodities) and not intended to be commercially grown and marketed for propagation and cultivation, the cultural practices of specific ethnic and cultural groups should not be affected.

SEC EXPERT's Recommendation

The SEC expert recommends the approval and issuance of the biosafety permit of the GM product.

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