ASSESSORS' CONSOLIDATED REPORT ON MONSANTO PHILIPPINES INC.'s APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF ALFALFA KK179 X J101

EXECUTIVE SUMMARY

On June 25, 2020, Monsanto Philippines filed for application of alfalfa KK179 x J101 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 alfalfa KK179 x J101 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 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 documents related to Environmental Health Impact, concluded that alfalfa KK179 x J101 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. 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. These assessors were given thirty (30) days to submit their independent assessment to BPI Biotech Secretariat.

STRP'S ASSESSMENT

1. Gene Interaction

a. The modes of action are very different for the RNA-based suppression and CP4 EPSPS protein and this suggests that the RNA and protein act independently. It would be very unlikely that there is any potential interaction with one another that would lead to production of a new allergen or toxin in the combined trait product. Also, there is no known mechanism of interaction among RNA-based suppression and CP4 EPSPS protein that could lead to adverse effects in humans, animals or the environment. [1].

b. The CP4 EPSPS protein would accumulate in the chloroplast of alfalfa cells because they are targeted specifically to these organelles [1].

2. Metabolic Pathways

a. Yes, there is a complete description of the mode of action of each gene product, and the description is described in the next question [1].

b. Yes, the mode of action is different for each gene product. The CCOMT suppression cassette present in KK179 alfalfa has a partial gene segment from CCOMT configure into an inverted repeat sequence. This CCOMT sequence from *Medicago sativa* CCOMT gene encodes the caffeoyl CoA 3-O-methyltransferase protein. The transcript with the inverted repeat produces dsRNA that, via an RNA interference, suppresses endogenous CCOMT RNA levels, which results in reduced biosynthesis of G lignin. As previously described in KK179 alfalfa safety assessment, production of a protein from the dsRNA encoded by the insert in KK179 alfalfa is highly unlikely. Thus, it is highly unlikely that the CCOMT suppression cassette produces a protein. [1].

c. The CP4 EPSPS protein belongs to the family of EPSP synthases. These are enzymes involved in the last step of the biochemical shikimic acid pathway producing aromatic amino acids in the chloroplasts of plants, present only in organisms that undergo photosynthesis. The *cp4 epsps* gene present in J101 alfalfa is derived from the *Agrobacterium* sp. strain CP4, a common soil-borne bacterium. Native plant EPSPS is inhibited by glyphosate and the CP4 EPSPS enzyme is far less sensitive to the inhibitory effects of glyphosate, hence conferring tolerance of the transgenic plants to glyphosate. [1].

3. Gene Expression

a. The products are not involved in the same metabolic pathway as explained above, under B1 and B2 [1].

b. CP4 EPSPS protein was expressed properly in the combined trait product Alfalfa KK179 x J101 [1].

c. Protein was expressed at low level. KK179 alfalfa does not contain any

recombinant gene which encodes a protein. The level of protein expression in J101 alfalfa has been previously reviewed and approved separately by Department of Agriculture - Bureau of Plant Industry (DA-BPI). The CP4 EPSPS level in J101 alfalfa typically represent a very small portion of total protein in the plant. Additionally, the level of the introduced CP4 EPSPS protein in the single event of Alfalfa KK179 x J101 is as expected, and is comparable to the level of the protein in the combined trait product.

d. There is no marker gene transferred and expressed also in the plants containing the stacked genes [1].

e. There is no likelihood of possible interaction and therefore no effect on stability or expression level of each gene as a result. This is supported by weight of evidences. The RNA-based suppression and CP4 EPSPS protein have distinct modes of action. RNA and protein act independently and it would be very unlikely there is any potential interaction with one another. The protein expression data demonstrates that CP4 EPSPS protein was expressed properly in the combined trait product Alfalfa KK179 x J101, indicating that the inserted gene, *cp4 epsps*, is inherited and functioning properly when combined into the breeding stack. Based on the totality of evidence described above, the conclusion is that there is no change in the introduced trait or interaction between and among the combined traits in Alfalfa KK179 x J101. The inserted genes in the single events are maintained and are functioning properly when combined into the breeding stack. The previous safety conclusions of the single products are directly applicable to the combined trait product and the commercial distribution of Alfalfa KK179 x J101 as food and feed, or for processing. This combined product does not pose greater risks to biodiversity, human and animal health than its conventional counterpart. [1].

STRP'S RECOMMENDATION

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. The gene products will not accumulate in the same subcellular compartments of the plant. The CP4 EPSPS protein would accumulate in the chloroplast, while the CCOMT partial gene would unlikely accumulate in any subcellular compartment because it is highly unlikely to produce proteins. [2][3].

2. Metabolic Pathways

a. There is a complete description of the mode of action of each gene product. 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 to reduced production of G lignin. This cassette is also highly unlikely to produce proteins. *Cp4 epsps* is 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 products are not involved in the same metabolic pathway. The CCOMT partial gene segment is involved in suppressing endogenous CCOMT RNA levels that results to reduced production of G lignin, while *cp4 epsps* is involved in shikimate pathway. [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 Alfalfa KK179 x J101 and J101 alfalfa were 500 µg/g dw and 530 µg/g dw, respectively. [6].

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 RECOMMENDATION

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. CCOMT suppression cassettes encode for dsRNA and not for a protein. Analysis indicates that it is highly unlikely for a dsRNA to produce a protein which can interact with CP4 EPSPS. [4].

b. The CP4 EPSPS protein is targeted to accumulate in the chloroplast via chloroplast transit peptide, while the CCOMT suppression cassettes encode for dsRNA and is not regulated by a transit peptide [1][4].

2. Metabolic Pathways

a. The mode of action of CP4 EPSPS has been described in published literature,

while CCOMT suppression cassettes does not encode for a protein [1][4][5].

b. CP4 EPSPS proteins are involved in the biochemical shikimic pathway producing aromatic amino acid in the chloroplasts. 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. 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). CCOMT suppression cassette does not encode for a protein. [4].

c. The products are not involved in the same metabolic pathway since the CCOMT suppression cassettes does not encode for a protein [1][4].

d. Compositional analysis indicates that the stacked genes have no biologically relevant effects on the composition of alfalfa. CP4 EPSPS expression in the stacked genes is comparable to the corresponding single event, J101. CCOMT suppression cassettes do not encode for a protein that can interact with CP4 EPSPS. [1][4][7].

3. Gene Expression

a. Protein expression analysis via ELISA showed that the expression of CP4 EPSPS in KK179 x J101 is comparable to the corresponding levels in single event, J101. CCOMT gene suppression cassette does not encode for a protein. [1].

b. The protein expression analysis provided by the developer indicated that CP4 EPSPS is expressed at low level in KK179 x J101 [1].

c. There are no marker genes transferred and expressed in KK179 x J101 [1].

d. Based on the protein expression analysis, compositional analysis and the differences in the modes of action and metabolic pathways of the novel proteins, the CCOMT suppression cassette and CP4 EPSPS will not likely to cause interaction that can affect the stability and expression level of either one of the genes. [1][4][7].

BPI-PPSSD'S RECOMMENDATION

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

After a comprehensive review and evaluation of the documents and scientific evidence from literature submitted by Monsanto Philippines, Inc. concerning its application for direct use for food, feed, or for processing of Alfalfa KK179 x J101, the DENR-BC considered that the regulated article poses no significant adverse effect to the environment on the following bases:

1. The regulated article is considered substantially equivalent to its conventional counterpart for its history of safe use for food, feed, and cultivation in Argentina and Japan, for food and feed in South Korea, and for food in Mexico. Also, the individual events Alfalfa KK179 and Alfalfa J 101 have both been previously approved for direct use as food and feed in the Philippines. The stacked trait was also achieved through conventional breeding methods. [8][9][10].
2. Compositional analyses determined that the regulated article is nutritionally equivalent to its conventional counterpart except for the intended reduction of G lignin and total lignin. The exposure of organisms to the expressed non-coding RNA of the CCOMT suppression cassette in alfalfa KK179 is less likely to have negative impacts. [8][9][10].
3. Gene flow from the regulated article to wild relatives is highly unlikely to occur in nature. Also, alfalfa hay does not contain viable plant materials. There is negligible risk for the regulated article to be invasive. [8][9][10].
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.

DENR BC'S RECOMMENDATION

Based on the evaluation and review of literature cited, the DENR-BC considered the regulated article safe to the environment, particularly on biodiversity, and hereby submits the technical report relative to the application of Monsanto Philippines, Inc. and for issuance of a Biosafety Permit for direct use as food, feed, or for processing of alfalfa KK179 x J101.

DOH BC'S ASSESSMENT

The DOH-BC, after a thorough review of the documents, find that the regulated article applied for Direct Use as Food, Feed or for Processing (FFP) is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health and environment.

1. Scientific pieces of evidence from toxicity studies and references find that the regulated article will not cause significant adverse health effects to human and animal health.
2. Dietary exposure to the regulated article is unlikely to result in allergic reaction.
3. The regulated article is not materially different in nutritional composition from

that of the non-transgenic alfalfa or the conventional alfalfa.

4. The regulated article is as safe as food or feed derived from conventional alfalfa varieties.

DOH BC'S RECOMMENDATION

It is suggested that the Bureau of Plant Industry (BPI) ensure that there shall be clear instructions that the product is only for the purpose of direct use for FFP and is not to be used as planting materials.

SEC EXPERT'S ASSESSMENT

1. As applicant manifested, the GM crop subject of the application is not locally produced in the Philippines. Currently, GM alfalfa authorized for direct use as food and feed, or for processing is limited to the three single event ones (J101, J163, and KK 179) from the same applicant. Alfalfa produced in the country is non-GMO grown for human consumption and for animal feed. Alfalfa transformed into meals and pellets as feed for cattle and other animals is indeed imported by the Philippines but applicant's information can be updated: 2018 imports of alfalfa meals and pellets totaling 808, 796 GK came from Australia, Canada, Spain, United States and Brunei. [16][17].

2. Applicant'strendobservationsontheyearlyimportsofalfalfacanlikewisebeupdated. Statistics from the Food and Agriculture Organization in Table 2 show a sustained upward trend in the volume total imports of alfalfa meals and pellets. It should be noted however that while the Philippines has been importing alfalfa for years, cows in the country feed on various sources of forage (like corn silage, cut grass) and roughage. The country imports a significantly higher volume of other animal feed such as soybean and corn meal. The comparatively lower use of alfalfa could be a function of the local population of cattle at around 2.5 million heads. This count includes less than 70,000 dairy cows supplying only 1% of the total dairy demand in the country. [18][19][20][21].

3. The subject application is only for direct use as food and feed, or for processing. Given the figures and trends discussed in item #1, drastic changes in patterns of production, consumption/utilization are not expected in the introduction of the GM crop. As applicant properly acknowledged, the subject crop is primarily utilized as cow feed. With the local herd population remaining limited for almost a decade and with imports supplying 99% of Filipinos' dairy consumption, the impact of the GM alfalfa, if any, is expected to be minimal. Any possible changes in production, consumption/utilization will be incremental. Additional supply of cow feed through the GM crop may be expected to boost the Philippine Department of Agriculture's commitment to strengthen the national dairy industry as well as the ongoing Herd Build- Up Program of the National Dairy Authority, which seeks to increase the number of dairy cows and improved milk production. Increasing imports of alfalfa noted in item#1 is also consistent with the expected 30-45% growth in dairy consumption of Filipinos towards 2025, especially of milk and milk-based drinks, butter and butter- products, and hard and soft cheese. For

2020, Australia expected a 21% increase in the country's imports of milk products. The Filipino diet COVID-19-related restrictions have slowed down economic activities and overall consumption, but the economy is expected to rebound when the pandemic is contained. [23][24][25][26][27].

4. The subject application is only for direct use as food and feed, or for processing. As mentioned in item 1, conventional alfalfa is grown in the Philippines, but cultivation is limited. This and other cattle feed sources have not been sufficient to supply full demand, especially during the dry season. Traditional, time-consuming, and labor-intensive cut-and-carry practices of providing forage to cows may be reduced by using alfalfa as alternative. But any such effect is not attributable to the subject GM crop alone but also to the use of all other alternative cattle feed -- whether GM or conventional, and whether imported or locally sourced. In any case, possible effects on the use of the GM crop for food, feed and processing on specific ethnic or cultural groups should be best understood in the context of engagement and participation. [29][30][31].

SEC EXPERT'S RECOMMENDATION

The SEC expert recommend for the approval and issuance of the Biosafety Permit of the GM product.

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