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

EXECUTIVE SUMMARY

On September 23, 2018, Monsanto Philippines Inc. submitted alfalfa KK179 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 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 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 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 KK179.

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.

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

Based on the documents submitted by the applicant:

A. Host Organism

Alfalfa has a long history as a good feed source for ruminant animals, and some non-ruminant animals. It is a key component of formulated livestock diets. For humans, Alfalfa has a history of minor uses as food, dietary supplements, or herbal remedies.

Alfalfa forage contains well described classes of anti-nutrient compounds including saponins, condensed tannins, and phytoestrogens. There are no reported cases of allergic reactions to alfalfa in humans. However, alfalfa forage contains saponins and canavanine. Saponins can produce toxic effects, primarily by medicagenic and zanhic acids. Symptoms include irritation to mouth and digestive tract, increased membrane permeability, and, in acute cases, hemolysis. Canavanine in alfalfa sprouts and seeds, in the form of L-canavanine, is a structural analog of L-arginine that can cause aberrant protein formation with potentially toxic effects in humans and animals.

Alfalfa has been used by humans as food, dietary supplements, and herbal remedies. It is prepared mainly in the form of alfalfa seedlings, also referred to as alfalfa sprouts. Another dietary supplement is alfalfa protein concentrate, which is high in protein and xanthophylls. The use of biotechnology-derived alfalfa seed for sprout production was restricted through signed agreements with seed purchasers. Thus, it is not normally intended to be used in the production of sprouts or other alfalfa-derived food products.

The consumption pattern is not expected to be changed as a result of introducing KK179 as KK179 is not materially different in composition, safety, or nutrition, other than the intended reduction in G lignin and total lignin compared to conventional alfalfa at the same stage of growth.

Alfalfa KK179 and its progenies are not significantly different from conventional alfalfa in terms of compositional, nutritional, and safety aspects, except for the introduced trait for reduced guaiacyl lignin subunits (G lignin) and total lignin. This product does not change nutrients relevant to feed and food consumption.

B. The Transgenic Plant

KK179 has been reviewed and approved for food and/or feed use in many countries including Australia/ New Zealand (Food, 2014), Canada (Feed, Environment, 2014; Food, 2014), Japan (Food, 2015; Feed, 2015; Environment, 2015), Korea (Food, 2016; Feed, 2015), Mexico (Food, Feed, 2015), Singapore (Food, Feed, 2017), United States (Food, Feed, 2013; Environment, 2014).

Collectively, the data summarized in this document support a conclusion that reduced lignin alfalfa KK179 is as safe and nutritious as its conventional alfalfa and does not pose greater risk to biodiversity, human and animal health than its conventional counterpart. Alfalfa has a history of minor uses by humans as food, dietary supplements, and herbal remedies. The consumption pattern is not expected to be changed as a result of introducing KK179 as KK179 is not materially different in composition, safety, or nutrition from conventional alfalfa, with the exception of the reduction in G lignin and total lignin (ADL).

C. Donor Organism

The T-DNA I suppression cassette present in KK179 contains a partial gene segment from CCOMT configured into an inverted repeat sequence. No evidence of human or animal pathogenicity is reported for any of the donor organisms of the coding and non-coding DNA sequences present in KK179. Double-stranded RNAs, which are commonly found in plants and other eukaryotes for endogenous gene suppression, are composed of nucleic acids. These plants are known be safe to consume and are considered GRAS by the US FDA, because there is no evidence of mammalian toxicity or allergenicity to RNA or DNA. Thus, the use of RNA-based suppression of endogenous CCOMT gene expression in KK179 poses no risks on exposure to expressed products of the DNA insert.

KK179 was developed by Agrobacterium tumefaciens-mediated transformation of conventional alfalfa R2336 leaf tissue using the plasmid vector PV-MSPQ12633. The insert present in KK179 contains a partial gene segment of CCOMT from Medicago sativa configured into an inverted repeat sequence. The CCOMT coding sequence is regulated by the Pal2 promoter and the nos 3′ untranslated region (UTR). The Pal2 promoter is the promoter for phenylalanine ammonia-lyase gene from Phaseolus vulgaris. It functions to direct transcription within vascular tissue, resulting in a pattern of expression that closely mirrors deposition of lignin as the plant matures. The nos 3′ UTR is the 3′ untranslated region of the nopaline synthase (nos) gene from Agrobacterium tumefaciens pTi encoding NOS that directs polyadenylation of the RNA transcripts.

KK179 does not contain any recombinant genes, which encode proteins. Analysis of dsRNA-encoding KK179 DNA segments indicates that protein production by the dsRNA encoded by the insert in KK179 is highly unlikely. No evidence of mammalian toxicity or allergenicity to RNA or DNA has been reported.

C. Transformation System

KK179 was developed through Agrobacterium tumefaciens-mediated transformation of alfalfa. Alfalfa R2336 (an FGI proprietary single alfalfa plant) leaf pieces were placed in a tissue culture media and co-cultured with Agrobacterium tumefaciens carrying the plasmid vector. After three days, explants were placed on selection medium containing the antibiotics kanamycin and timentin, to inhibit the growth of untransformed plant cells and excess Agrobacterium. The kanamycin-resistant calli are developed with somatic embryos. Somatic embryos were placed in media conducive to shoot and root development. Rooted plants (T0 plants) with normal phenotypic characteristics were selected and transferred to soil for growth and further assessment. The T0 plants were crossed to Ms208, a conventional male sterile plant selected from a population with a fall dormancy (FD4) phenotype, to produce F1 plants, in which the unlinked insertions of T-DNA I and T-DNA II were segregated. KK179 (P0)is the individual F1 plant produced from crossing T0 with Ms208. It has the reduced lignin phenotype without the T-DNA II.

V-MSPQ12633 was used to transform conventional alfalfa to KK179. It is approximately 10.6 kb and contains two T-DNAs, each delineated by Left and Right Border regions to facilitate transformation. The Left and Right Border regions were derived from Agrobacterium tumefaciens. These separate the T-DNA from the plasmid backbone region and are involved in the efficient transfer into the alfalfa genome. The first T-DNA designated as T-DNA I contains the CCOMT suppression cassette regulated by the Pal2 promoter and the nos 3′ UTR. The second

T-DNA, designated as T-DNA II, contains the nptII expression cassette regulated by the 35S promoter and the nos 3′ UTR.

Genetic elements that exist outside of the T-DNA border regions are those that are essential for the maintenance or selection of PV-MSPQ12633 in bacteria and are referred to as the plasmid backbone. Because these elements are outside the border regions, they are not expected to be transferred into the alfalfa genome.

Southern blot analyses were used to determine the number of copies, to characterize the insertion site of T-DNA I, as well as to assess the presence or absence of T-DNA II and plasmid vector backbone sequences, thus ensuring that all potential inserted segments would be identified. PCR and DNA sequence analyses of KK179, which complement the Southern blot analyses, determined the complete DNA sequence of the insert, confirmed the organization of the elements within the insert, and determined the 5′ and 3′ insert-to-plant junctions. The genomic organization at the KK179 insertion site was determined by comparing the 5′ and 3′ flanking sequences of the insert to the sequence of the insertion site in conventional alfalfa.

D. Inserted DNA

The copy number and insertion sites of T-DNA I sequences in the KK179 genome were evaluated by digesting the P0 generation of KK179 and the appropriate control genomic DNA samples with two sets of restriction enzymes, a combination of Xmn I and Dra III and a combination of Xba I and Swa I, and hybridized Southern blots with probes that span the T-DNA I. Each restriction digest is expected to produce a specific banding pattern on the Southern blots. Any additional copies and/or integration sites would be detected as additional bands.

The result demonstrated that a single copy of the T-DNA I sequences integrated into the alfalfa genome at a single integration locus.

To determine the integrity of the DNA insertion site in KK179, PCR and sequence analyses were performed on genomic DNA extracted from KK179 and the conventional parental control R2336. The PCR was performed with a forward primer specific to the genomic DNA sequence flanking the 5′ end of the insert paired with a reverse primer specific to the genomic DNA sequence flanking the 3′ end of the insert. Sequence alignments were performed between the conventional parental control sequence and the sequences flanking the 5′ and 3′ end of the KK179 T-DNA I insert.

The alignment between the sequence flanking the 5′ end of the KK179 insert and the conventional parental control sequence showed that the 5′ flanking sequence of the KK179 insert is identical to the conventional parental control sequence except for one base, which is a G within the 5′ flanking sequence of the KK179 insert and is a G/T heterozygote. The alignment between the 3′ end of the KK179 insert and the conventional parental control sequence showed that the conventional parental control sequence is identical to the sequence flanking the 3′ end of the KK179 insert except for one base, which is a G within the 3′ flanking sequence of the KK179 insert and is a A/G heterozygote. These two heterozygotes were most likely caused by single nucleotide polymorphisms segregating in the autotetraploid alfalfa population. The alignment analyses also indicated a 102 base pair deletion from the conventional genomic DNA occurred upon T-DNA I insertion in KK179. This deletion presumably resulted from double stranded break repair mechanisms in the plant during the Agrobacterium-mediated transformation process

Bioinformatics analyses support that no novel open reading frames (ORFs) or polypeptides were created. KK179 does not contain any detectable backbone sequences from the transformation vector PV-MSPQ12633.

E. Genetic Stability

Using genomic DNA obtained from four generations of KK179, the Southern blot analysis was performed. Genomic DNA that was isolated from each of the selected generations of KK179 was digested with the restriction enzymes Xmn I and Dra III and hybridized with Probe 2 and Probe 4. Probe 2 and Probe 4 are designed to detect both fragments generated by the Xmn I and Dra III digest at ≥2.2 kb and ~2.0 kb. Any instability associated with the insert would be detected as novel bands on the Southern blot. The molecular weight markers were used to estimate the band sizes present.

Chi square (χ^2) analysis over several generations was used to generate segregation data in order to assess the heritability and stability of the T-DNA I present in KK179. Three generations of backcrosses were tested. The $\chi 2$ value for three generations (two backcrossed generations and one synthetic self-cross generation) indicated no significant difference between the observed and expected segregation ratios. The results are consistent with the molecular characterization data that indicate KK179 contains a single, intact copy of the CCOMT suppression cassette that was inserted into the alfalfa genome at a single locus.

F. Expressed Material

The CCOMT suppression cassette encodes for dsRNA, which is extremely unlikely to encode for a protein.The assembled CCOMT gene segments produce a transcript with an inverted repeat sequence to form double-stranded RNA (dsRNA), which works via the RNA interference mechanism to suppress the endogenous CCOMT gene (Siomi and Siomi, 2009).

G. Toxicological Assessment

The CCOMT suppression cassette encodes for dsRNA, which is extremely unlikely to encode for a protein.The assembled CCOMT gene segments produce a transcript with an inverted repeat sequence to form double-stranded RNA (dsRNA), which works via the RNA interference mechanism to suppress the endogenous CCOMT gene (Siomi and Siomi, 2009).

H. Allergenicity Assessment

The CCOMT suppression cassette encodes for dsRNA, which is extremely unlikely to encode for a protein.The assembled CCOMT gene segments produce a transcript with an inverted repeat sequence to form double-stranded RNA (dsRNA), which works via the RNA interference mechanism to suppress the endogenous CCOMT gene (Siomi and Siomi, 2009).

I. Nutritional Data

Significant difference for ash was observed, with lower values in KK179 forage than the conventional control. However, the difference in ash in KK179 forage compared to the conventional control is not considered biologically meaningful from a feed/food safety and nutritional perspective

The following were analyzed for forage: carbohydrates by calculation, acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), minerals (Ca, Cu, Fe, Mg, Mn, P, K, Na, and Zn), and amino acids (essential and non-essential). There was no significant difference observed in key nutrients of KK179 and no biological significance when compared to non-transgenic alfalfa.

The forage were also analyzed for daidzein, glycitein, genistein, coumesterol, formononetin, biochanin A, saponins, and canavanine. There was no difference between KK179 and its conventional counterpart except for canavanine which is not biologically significant.

Fourteen different conventional commercial alfalfa varieties were included across the field production to provide data on the natural variability of each compositional component analyzed. All test values were within the 99% tolerance interval determined from the conventional commercial alfalfa varieties.

J. Recommendation

Find scientific evidence that the regulated article applied for human food and animal feed use is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health

BAI AND BPI-PPSSD ASSESSMENT AND RECOMMENDATIONS

Based on the documents submitted by the applicant, BAI made the following assessment:

A. Nutritional Data

Compositional analysis indicated no significant differences in the proximate levels for protein, fat and moisture in KK179 alfalfa and the non-transgenic alfalfa except for ash (Breeze, 2012). It also indicated no significant differences in the levels of fibers, minerals, amino acids and carbohydrates in KK179 and the non-transgenic alfalfa (Breeze, 2012).

Among the antinutrients evaluated, canavanine from KK719 has a mean level of 40.30 that has a computed p-value of 0.013, which is within the set significance level of $p<0.05$. This means that the values obtained from the compositional analysis have a significant difference compared to the control nevertheless, the said substance was lower in composition compared to the commercially available alfalfa. Canavanine is known to be a plant's defensive compound against herbivores and a lower level detected from this substance is desirable. The data from the analysis is still within the tolerance intervals and literature values. Hence, no biological significance is hereby rendered on the assessment.

14 conventional varieties were used for the comparison of proximate analysis with KK179 alfalfa. The acquired data from the analysis were within the tolerance values determined from the commercially available comparator. All mean values were also within the literature ranges (Breeze, 2012).

D. Recommendation

Find scientific evidence that the regulated article applied for animal feed use is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health

DENR ASSESSMENT AND RECOMMENDATION

After a comprehensive review and evaluation of the documents including the scientific evidence from references and literature submitted by Monsanto Philippines, Inc., on its application for Direct Use as FFP of Alfalfa (KK179), hereunder are the observations and appropriate actions:

1. The direct use of the regulated article whether for food, feed or for processing will not cause any significant adverse effect on the environment (land and water) and biodiversity. The inserted gene, an inverted repeat derived from the ccomt gene, forms dsRNA that suppresses the formation of G-lignin by inhibiting the expression of the ccomt gene via RNAi pathway. The inserted gene is a dsRNA, and like other forms of RNA, has no known cases of being an allergen or a toxin (FAO-WHO, 1991);

2. Based on the reproductive biology of alfalfa, the alfalfa bloom can only be pollinated once by a single pollinating insect (primarily bees) because of its nonreversible "tripping^ mechanism, which upon tripping, lodges the stigma into the groove of the standard petal preventing fertilization. Also, flowers do not shed its pollen grains to the wind (CFIA, 2012); and

3. The project description report (PDR) discusses the specified environmental management plan indicating the possible risk and harm to the environment and biodiversity as well as the mitigating measures and contingency plan. Furthermore, the chances of unintended release or planting of the regulated article is very minimal and will not cause any damaging and lasting effects because the receiving environment (areas near the port, roads, railways, etc.) is not conducive for plant growth. Also, alfalfa requires nutrients that are not commonly found in soil thus require human intervention for growth (Ottoman, 2010). Also, during ripening periods, rain causes poor seed quality and decrease in seed yield thus are suitable in prairies where rainy season is unlikely (CFIA, 2012).

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

DOH ASSESSMENT AND RECOMMENDATION

After a thorough review and evaluation of the documents provided by the proponent, Monsanto Philippines, Inc., through the Bureau of Plant Industry (BPI), in support of their application for approval for Direct Use as Food, Feed or for Processing (FFP) of Alfalfa KK179.I/We,

Find that the regulated article applied for Direct Use as Food, Feed or for Processing (FFP) is safe as its conventional counterpart and shall not pose any significant risk to human and animal health and environment.

The following are the observations and recommendations:

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 as safe as food or feed derived from conventional alfalfa varieties.

4. The regulated article is not materially different in nutritional composition from that of the non-transgenic alfalfa or the conventional alfalfa.

5. 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.

6. Based on the above considerations and with the submitted sworn statement and accountability of the proponent, we hereby submit our evaluation to BPI relative to the application of a Biosafety Permit for Direct Use as Food, Feed, or for Processing (FFP) of Alfalfa KK179.

SEC ASSESSMENT AND RECOMMENDATIONS

Based on SEC expert review of the SEC questionnaire answered by the applicant:

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.

Recommendation

The SEC expert has recommended for the approval and issuance of the biosafety permit of the GM product.