ASSESSORS' CONSOLIDATED REPORT ON SPS INTERNATIONAL INC.'S APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF POTATO Y9

EXECUTIVE SUMMARY

On February 1, 2019, SPS International Inc. submitted potato Y9 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 potato Y9 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 potato Y9 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 potato Y9.

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 SPS International 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

Potato is a source of key nutrients for the human diet, such as sugars and starch, essential amino acids, potassium and vitamin C. Starch is the major carbohydrate in potato and is composed of both amylose and amylopectin. Potato starch is about 20% amylose and 80% amylopectin (Storey, 2007, pp. 444-446).

Potato is a natural source of antinutrients such as protease inhibitors and lectins. However these are readily inactivated by heating/cooking. It is also a natural source of the toxicant class called glycoalkaloids, specifically alpha-chaconine and alpha-solanine.

In addition, potato is a source of, and an eliciting agent of allergy, specifically, patatin. It is widely known that potato belongs to the group of food allergens. However, allergic reactions are associated mostly in raw potato tubers that are being peeled, and tolerance is common starting 4 y.o. Patatin is a storage glycoprotein in the tuber and further processing or heating/cooking of raw tuber result to degradation and lower concentrations.

Further, it is a major source of food items, whether raw/fresh or processed. Per capita consumption of potato was verified in AIS, 2017, while decline in production and increase in potato imports was verified in AIS, 2018. Potato consumption in the Philippines as compared to other countries and region was verified in FAO, 2008. Only a small percentage of potato is used for feed for livestock animals. Potato is indeed not widely grown for animal feed but mostly for human consumption.

B. Transgenic Plant

Potato Y9 has been approved for food in Australia, United States, New Zealand and Canada, and for feed in Canada and United States. The consumption pattern will not change as Y9 will be used the same as the Atlantic and other conventional cultivars.

C. Donor Organism

All the protein-encoding sequences found in the original gene construct. The sequences encoding for VNT1 protein were also described with respect to its source organism, which is a wild relative of potato. The VNT1 protein, expressed in POTATO Y9, is indeed non-pathogenic nor allergenic based on the bioinformatic analyses that the proponents performed and presented herewith.

All potentially inserted regulatory elements and backbone sequences, have been adequately described. The regulatory elements were also clearly described, which are essentially cisgenes obtained from a sexually compatible wild relative of potato.

The donor organism is the potato plant itself and two other relatives, S. venturii and S. verrucusom. The genes and its expressed proteins do not have a potential toxicogenic or allergenic property in humans or animals, and have a long history of safe use in crop genetic improvement thru recombinant DNA technology.

D. Transformation System

The transformation method (Agrobacterium tumefaciens-mediated transformation) was stated using two- phased transformation, first with pSIM1278 (J3 event) and then with pSIM1678 (Y9 event).

In Event Y9, the pSIM1278 insert contains two cassettes designed to reduce expression of 1) asparagine synthetase (ASN) and polyphenol oxidase (PPO), and 2) water dikinase (R1) and phosphorylase L (PHL). The pSIM1678 insert contains a cassette designed to reduce the expression of vacuolar invertase (VInv) and to express the Rpi-vnt1 gene (VNT1). The down regulation of target transcripts occurs in tubers by using promoters primarily active in the tuber. The genetic modification down regulates enzymes in the cytoplasm, the amyloplast (starch granule), or the vacuole depending on the target enzyme and its cellular function.

ASN is a cytoplasmic enzyme that catalyzes the transfer of side-chain amines (NH2) from glutamine to aspartate to form asparagine and glutamate, a critical role in the transport and storage of nitrogen. PPO is located in plant cell plastids (Stensballe et al., 2008, pp. 1727-1729). When a plastid is damaged by cutting or rough handling of the tuber, PPO is released into the cytoplasm where it oxidizes phenolic compounds and forms dark pigments that result in black spot. R1 and PHL are located in the amyloplast (Stensballe et al., 2008, pp. 1727-1729). These enzymes function in the metabolism of starch and are bound to starch polymers within the amyloplast (known as a starch granule). Invertase is located in the vacuole where it degrades starch into fructose and glucose.

The expression of the Rpi-vnt1 gene is driven by its native promoter. The VNT1 protein is located in the cytoplasm where it recognizes pathogen-secreted effector proteins. Effector recognition activates a signaling pathway that leads to a plant hypersensitive response and protection against foliar late blight.

E. Inserted DNA

Based on Southern blots of BbsI digested genomic DNA from Atlantic (WT), J3, Y9, and Atlantic spiked with pSIM1278 plasmid (WT pl278), the characterization of the inserted DNA demonstrated that pSIM1278 and pSIM1678 T-DNA integrated at single genomic loci (Technical Dossier pp. 29-31,45-47). This was clearly demonstrated in the Southern blot analysis, coupled with PCR, Sanger and NGS analyses that the proponents performed and presented and expounded in the technical report. Indeed, only a single copy of the T-DNA is inserted in a single locus in the potato genome.

Based on the Southern blots, PCR, and sequencing data, the integrity and order of genetic elements within each insertion site were clearly demonstrated to confirm the structure of the insert found in Technical Dossier pp. 32-44, 47-55.

Truncated Rpi-vntl promoter (pVntl) on the left side and a partial VInv cassette on the right side nearly full- length pSIM1678 T-DNA was also defined (see Technical Dossier pp. 47-55). There was a deletion of part of the left border of the pSIM1678 insert (see Technical Dossier pp. 47-49), which included 36 bp of the Rpi-vnt1 promoter. This deletion did not affect the down regulation of the invertase gene or the expression of the VNT1 protein. This was clearly demonstrated in the PCR and DNA sequence analysis that the proponents performed and presented and expounded in the technical report. Indeed, these aberrations are common during Agrobacterium-mediated plant transformation

Results from trait efficacy studies demonstrated that fructose and glucose levels were decreased while sucrose levels were elevated in Y9 compared to Atlantic. This is an expected outcome due to down regulation of vacuolar invertase (see Technical Dossier pp. 95-96), which converts sucrose to frusctose and glucose.

Field efficacy trials demonstrated that Y9 potatoes were protected against foliar late blight infection (see Technical Dossier pp. 96-99), even though the VNT1 protein was undetectable (see Technical Dossier pp. 67-74).

These results demonstrate that the invertase down regulation and late blight protection traits are functioning as intended in Y9 potatoes, and that the small deletion from the LB end of the T-DNA did not affect the target traits. Southern blot analysis results with a series of probes spanning the plasmid backbone sequence, and the result clearly showed that plasmid backbone was not incorporated in the Y9 genome.

F. Genetic Stability

The stability of the insert in Y9 was confirmed through analysis of G0 and G3 plants following vegetative propagation and since the integrity of the insert was demonstrated via consistent banding pattern via Southern blots using probes that hybridize to genetic elements within T-DNA, it shows that the integrity and consistency of the insert (Technical Dossier pp. 58-63). Since potato, was propagated vegetatively, it will be genetically and phenotypically stable even after several propagations because as we know vegetative propagation will not result into genetic variation due to meiosis, recombination, and segregation (see Technical Dossier pp. 58- 63).

G. Expressed Material

The data result from liquid chromatography-mass spectrometry [LC-MS] and immunoblot assays were clearly showed, level of the VNT1 protein was estimated to be below 500 ppb (see Technical Dossier pp. 67-74).

VNT1, have no apparent metabolic role or is not directly involved in any metabolic activities in the potato plant. Plants encode multiple resistance genes (R-genes) that function in a well-defined role as receptors in the recognition of different pathogens, including bacteria, fungi, viruses, and oomycetes. The recognition of pathogen-secreted effector proteins by R-genes is a well-studied mechanism in plant defense response (Panstruga et al., 2009, pp. 978-978.e1). The plant immune system relies on R- genes for recognition of pathogen-secreted effector proteins and for signaling the plant immune response. The activated plant immune response results in resistance to disease through changes in levels of reactive oxygen species (ROS), transcription of pathogenesis-related (PR) genes, ion fluctuations, hormone accumulations, and programmed plant cell death (Baker et al., 1997, p. 731). The type and intensity of the response is dependent upon the invading pathogen and activated plant receptor.

The specificity of the interaction between the plant expressed R-gene and the pathogen-secreted effector protein together with the low concentrations of R-proteins in plant cells make the chance of indirect or secondary activities highly unlikely. Other than interacting with the Avr-vnt1 effector protein and initiating a plant immune response that prevents pathogen infection, there is no report of VNT1 being involved in other plant metabolic pathways.

H. Toxicological Assessment

Digestibility assessment of VNT1 is not possible due to its very low concentration in plant tissues. Heat inactivation assessment of VNT1 is also not possible due to the same reason. Instead, a threshold of toxicological concern (TTC) assessment was done by the proponents (Habig et al., 2018). The TTC assessment presented in the paper was convincing enough to support their claim regarding the improbable toxicogenic effect of VNT1 or R proteins in general.

The toxic potential of VNT1 was determined by a bioinformatics approach comparing the VNT1 amino acid sequence to the National Center for Biotechnology Information (NCBI) database queried for protein sequences annotated using the keyword "toxin". This means that protein sequences evaluated using this method may include actual toxins, proteins involved in the synthesis of toxins in a host, proteins that interact with toxins, proteins involved in the defense or response to infection, or non-toxic proteins from organisms known to produce a toxin. Their results were that they did not identify any toxins homologous to the Rpi-vntl (VNT1) coding sequence. However, VNT1 is similar to proteins involved in cellular responses to toxins or pathogens that contain the keyword "toxin" in their accession record. These matches included LOV1, RP3-likerTsnl, and RGA2-like proteins (Technical Dossier pp. 82-84).

Based on previous studies, it was found out that LOV1, RP3-like, and Tsnl are R-protein homologs that function in the protection of their host to fungal pathogens through recognition of effector molecules, i.e. victorin, Pc toxin, and ToxA. The good thing is that literature reviews confirmed that these R-proteins are, in fact, not toxins or substances with toxic properties (Faris et al" 2010, p. 13544; Lorang et al" 2007, p. 14861; Nagy and Bennetzen, 2008, p. 1918; Walton, 1996, p. 1727).

In addition, RGA2-like is a disease resistance protein from apple (Malus domestica) that contains a 65 amino acid region annotated as BrnT toxin. The overall identity between VNT1 and RGA24ike is low, and VNT1 is not homologous to the BrnT toxin region.

In general, we can say that no homology between the VNT1 protein and known toxins was found (Technical Dossier pp. 82-84).

I. Allergenicity Assessment

Although, there were seventy-six unique start-to-stop ORFs were identified between the pSIM1278 and pSIM1678 inserts, the analysis did not identify any ORFs homologous to the allergens in the AllergenOnline.org database. Since AllergenOnline.org database is peer-reviewed, and updated annually, and are subject to review for appropriateness by a panel of qualified food allergenicity experts, and the present version used (Version 18A) contained 2,093-deposited sequences, based on the searched data, the evaluation of the ORFs did not identify any matches to known allergens (Technical Dossier p. 85)

The percent share of VNT1 in total protein of potato is impossible to determine due to its very low concentration in plant tissues, which is well below the 500 ppb LOQ.

J. Nutritional Data

There were some observed statistical differences for protein, crude fiber, carbohydrates, calories wherein they are higher than the Atlantic variety. However, moisture significantly lower in Y9 than in Atlantic variety. The good thing is that the means were within the tolerance interval and

combined literature range for conventional varieties CLR combined literature range (CLR) (ILSI, 2016), which may imply that the observed differences were not nutritionally meaningful (Technical Dossier pp. 86-89).

There were no differences were observed for vitamins and minerals between Y9 and Atlantic potato variety except for potassium. But based also on the data, all mean values, even for potassium, were within the tolerance interval or combined literature range (CLR) (ILSI, 2016). Lower aspartic acid + asparagine and higher glutamic acid + glutamine were observed for Y9 compared to Atlantic. This was expected due to the downregulation of ASN. But the resulted amino acid levels are equivalent to those of conventional potatoes based on combined literature range (CLR) (ILSI, 2016; OECD, 2002, p. 16).(Technical Dossier pp. 89-92) so there's no reason for safety concern.

Potassium and some amino acid levels significantly differed between Y9 and Atlantic variety. Although there were significantly higher potassium level and lower aspartic acid + asparagine and higher glutamic acid + glutamine were observed for Y9 compared to Atlantic potato variety, the mean and range values for all the analyzed vitamins, minerals, and amino acids fell within the tolerance interval or combined literature range, meaning that Y9 was comparable to conventional potato varieties (Technical Dossier pp. 89-92) so there's no worry here for safety concern.

The free amino acid analysis was already expected, that is, the down regulation of ASN was caused the reduction of free asparagine in tubers. Although the results showed that Y9 tubers had reduced free asparagine, which was compensated for by an increased level of free glutamine compared to Atlantic, (see Technical Dossier pp. 89-92), the only relevant question here is because of this change in amino acid profile, will there be any biological significance that will arise from the changed in the amino acid composition of potato especially in efficiency of protein synthesis? And so it might also change the diet trend or pattern to compensate for the changed amino acid profile.

Glycoalkaloid levels were not statistically different between Y9 and Atlantic, and below the safe level of 20 mg per 100 g fresh weight tuber [OECD, 2002, p. 19). All mean values fell within the tolerance interval or combined literature range (Technical Dossier p. 93). Protease inhibitors and lectins are inactivated during heating, and adverse effects do not result from consumption of cooked potato (OECD, 2002, p. 21).

Considering the maximum generally accepted level of 20 mg per 100 g fresh weight tuber of glycoalkaloid levels(OECD, 2002 ; p. 19) , and since the glycoalkaloid levels were similar between Y9 and Atlantic potato, there will be no worries for safety concern.

H. 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. Toxicological Assessment

Digestibility study using the full-length VNT1 was not possible because they require purification of large amounts of protein from the host plant or equivalent proteins expressed and purified from heterologous systems. Due to an inability to obtain functional and purified VNT1 protein in sufficient quantities, digestibility experiment was not performed (SPS, 2019, pp. 60-65).

Bioinformatics analyses comparing the VNT1 amino acid sequence to the National Center for Biotechnology Information (NCBI) showed no relevant structural similarities to actual toxins, proteins involved in the synthesis of toxins in a host, proteins that interact with toxins, proteins involved in the defense or response to infection, or non-toxic proteins from organisms known to produce a toxin. This indicates that VNT1 will not cause toxicity or health risk to human health (SPS, 2019, pp. 65-72).

B. Allergenicity Assessment

Digestibility study using the full-length VNT1 was not possible because they require purification of large amounts of protein from the host plant or equivalent proteins expressed and purified from heterologous systems. Due to an inability to obtain functional and purified VNT1 protein in sufficient quantities, digestibility experiment was not performed (SPS, 2019, pp. 60-65).

In addition, heat inactivation study using the full-length VNT1 was also not possible because they require purification of large amounts of protein from the host plant or equivalent proteins expressed and purified from heterologous systems. Due to an inability to obtain functional and purified VNT1 protein in sufficient quantities, Heat Inactivation experiment was not performed (SPS, International, pp. 60-65).

However, bioinformatics analysis provided using the full-length sequence, an 80-mer sliding window and 8-mer exact match in AllergenOnline.org database did not yield significant homology of VNT1 to any known allergen (SPS, 2019, pp. 75-76). VNT1 has an approximate molecular weight of 102 kDa (SPS, 2019, p. 28).

C. Nutritional Composition

Mean values of the proximates in Y9 were all within the tolerance interval and combined literature range. A statistical difference was observed in crude fiber, carbohydrates, calories, and moisture between Y9 and Atlantic but within the tolerance interval and combined literature range which indicates that the difference was not nutritionally meaningful.

No differences were also observed for vitamins and minerals between Y9 and Atlantic, except for potassium but all mean values including potassium were within the tolerance interval and combined literature range.

Moreover, due to the down regulation of Asn1, low aspartic acid + asparagine and high glutamic acid + glutamine levels were observed for Y9. Statistical differences were observed in amino acid levels measured in Y9 tubers (see Table 10-4, p. 92 of Technical Dossier) but these were all within tolerance interval and combined literature range.

Potassium and amino acid levels were statistically different between Y9 and Atlantic. But all test values of nutrients including potassium and amino acids were within the tolerance interval established from the commercial varieties which indicates that Y9 is comparable to the conventional counterpart.

Y9 glycoalkaloid level was found to be below the safe level of 20 mg per 100g fresh weight tuber, similar to Atlantic Also, mean values were within the tolerance interval and combined literature range. This indicates that the glycoalkaloid levels in Y9 were unchanged and were within recommended safety limits. During heating, anti-nutritional factors like protease inhibitors and lectins are inactivated but does not result any adverse effects from consumption.

There were no statistical differences between the antinutrient content of Y9 potato and non-transgenic potato that can be considered biologically relevant. All values are not significantly different and within the range of literature values (SPS, 2019, pp. 89-99).

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 SPS International, Inc., on its application for Direct Use as FFP of Potato (Y9), 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 inserted genes, inverted repeats derived from various Solarium species (S. tuberosum, and S. vermcosurn}, form dsRNAs that suppress the expression of the targeted genes (Ppo, Asn, PhL, and Rl) via RNAi pathway. The expression of late blight resistance is also due to the insertion of inverted repeats, VInv, and Rpi-vntl, derived from S. venturii. The inserted genes are 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 potato, terminal pores of anthers must be vibrated (buzz pollination) to release the pollen grains (Plaisted, 1980) frequently in S. tuberosum^ thus the spread of pollen grains via insect pollination is also reduced (Vasil, 1964). The role of wind in pollination is also minor thus the spread of pollen grains via wind pollination is unlikely (White, 1983); and

3. The project description report (PDR) stated that “Potato products, including chips, are not viable for purposes of cultivation. Mitigating measures for unintended release or unauthorized planting are therefore not needed.” Thus, the chances of unintended release or planting of the regulated article is minimal and will not cause any damaging effects. Also, Solartum sp. rarely exist as a wild plant and are cultivated in areas with adequate rainfall or irrigation due to its sensitivity to drought stress (Canadian Food Inspection Agency, 2015).

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 SPS International, Inc. for Biosafety Permit for direct use as food, feed, or for processing of Potato Y9.

DOH ASSESSMENT AND RECOMMENDATION

After a thorough review and evaluation of the documents provided by the proponent SPS International, 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 Potato Y9. 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 potato varieties.

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

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 Potato Y9.

SEC ASSESSMENT AND RECOMMENDATIONS

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

Potato is also grown in the Philippines and its components are used as raw materials for the food processing industry. However, local production cannot meet the requirements of the food processing industry, thus, we import a significant volume of white potato.

The approval to import Y9-7 potatoes may not affect our domestic production since we have been importing potato for manufacturing purposes. Likewise, consumption will not be affected since Y9-7 it will just be used for chips manufacturing and chips consumption is very insignificant to the total diets of the Filipinos. In addition, food industries have been importing potato as raw material for chips production. I strongly believe that it will not significantly affect domestic production and trade of white potatoes.

Allowing this event to enter our domestic market may help stabilize prices of potato chips thus may lower prices of the said processed product.

Recommendation

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