TECHNICAL ASSESSMENT OF SIMPLOT PLANT SCIENCE INTERNATIONAL'S POTATO X17 APPLICATION FOR DIRECT USE AS FOOD AND FEED OR FOR PROCESSING (FFP)

EXECUTIVE SUMMARY

On February 1, 2019, SPS International submitted Potato X17 application for direct use 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 STRP, BAI, and BPI-PPSSD found scientific evidence that Potato X17 is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health.

The Department of Environment and Natural Resources – Biosafety Committee (DENR-BC), after a thorough scientific review and evaluation of the accomplished Project Description Report (PDR) and Environmental Risk Assessment (ERA) form along with the submitted sworn statement and accountability of the proponent, reported that the direct use of the regulated article will not cause any adverse effect on the environment (land and water) and biodiversity.

The DOH-BC, after a thorough scientific review and evaluation of documents related to Environmental Health Impact, found scientific evidence that the GM application will not cause significant adverse effects to human and animal health, is unlikely to result in allergenic reaction, and is as safe as food or feed derived from conventional varieties.

Furthermore, the Socio-economic, Ethical and Cultural (SEC) expert, after reviewing thoroughly the accomplished SEC questionnaire, also recommended for the issuance of biosafety permit.

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 SPS International. Upon receipt of the individual reports from the assessors, the BPI Biotech Secretariat prepared this consolidated risk assessment report for the information of the public.

Annex II

Information on Applied Event

Agrobacterium tumefaciens-mediated transformation was used to generate X17. X17 was developed as a new potato variety with reduced black spot, lower reducing sugars, low acrylamide potential, and late blight protection. Improved quality in X17 potatoes is the result of DNA sequences introduced to down regulate the expression of the polyphenol oxidase (PPO), asparagine synthetase (ASN), phosphorylase L (PHL), water dikinase (R1), and invertase (INV) enzymes in tubers, and introduction of the *Rpi-vnt1* gene conferring late blight protection.

X17 has been deregulated by the United States Department of Agriculture (USDA) on 28 October 2016, approved by the United States Food and Drug Administration (FDA) 21 February 2017, and registered with the United States Environmental Protection Agency (EPA) on 19 January 2017. X17 has also received regulatory approval in Canada, Australia, and New Zealand.

Source: *Public Information Sheet of Potato X17 for direct use as food and feed, or for processing*

Annex III

STRP, BPI-PPSSD, BAI (Safety Assessment)

1. **Host Organism** *(Solanum tuberosum)*

Potato is a staple food crop in many countries, with over two thirds of global potato production consumed directly by the people (FAO, 2008). Potatoes are processed into fries, chips, or dehydrated flakes, and are used for starch production. Due to consumer demand, potatoes are increasingly supplied in processed form

Potatoes are sources of key nutrients, including carbohydrates, protein, minerals, and vitamin C. Sugars and starches are important components of the total carbohydrate fraction in potatoes. Starch is the most important and abundant carbohydrate.

According to OECD (2002) the protein in potato has nutritional value as a source of essential amino acids including lysine, methionine, threonine, and tryptophan. Potatoes are also important sources of potassium, contributing up to 30% of the recommended daily intake. Furthermore, potato is an important source of vitamin C, which contributes up to 40% of the recommended amount.

Potato tubers contains trypsin, chymotrypsin, other protease inhibitors, and lectins. It also naturally contains several types of alkaloids which includes glycoalkaloids. 95% of the total glycoalkaloids in tubers consists of α-chaconine and α-solanine.Tubers also contains small amount of calystegines, nortropane alkaloids with glycosidase inhibitory activity (OECD, 2002).

Potato also contains several heat-labile proteins which can cause hypersensitivity reactions upon consumption of raw potatoes (OECD, 2002).

2. Transgenic Plant

Potato X17 has been approved as food in Australia, Canada, New Zealand, and United States of America while it was approved as feed in Canada and the United States of America.

X17 will be used the same as Ranger Russet and other conventional potatoes and will not change potato consumption in the overall population or any population sub-groups.

3. **Donor Organism**

X17 potato possesses the transgene *Rpi-vnt1* which encodes the VNT1 protein consists of 891 amino acids. The VNT1-encoding sequence found in the original construct has been fully described in terms of source and potential allergenic or toxic properties. The Rpi-vnt1 gene including its promoter and terminator is from *S. venturii*, a wild Solanum species. *S. venturii* is sexually compatible with cultivated potato, *S. tuberosum.* There is no available evidence that *S. venturii* is allergenic or toxic to humans. Its tubers are small at 5-10 mm in diameter and are not used as food source in modern times. Therefore, direct evidence of its safe consumption is unavailable.

The possible allergenic properties of VNT1 protein in potato X17 was determined bioinformatically by looking at sequence homology of X17 VNT1 against the entries in the

AllergenOnline.org database. In addition, sequence comparisons between VNT1 and the entries in the NCBI database (Entrez query: Toxin) was performed. The results indicate that the VNT1 in X17 does not share homology or similarity with known allergens and toxins.

Regulatory sequences include non-coding spacer elements and promoter sequences. The spacer sequences and ADP glucose pyrophosphorylase (Agp) and granule-bound starch synthase (Gbss) promoter sequences are from *S. tuberosum.* The VNT1 promoter and terminator sequences are from *S. venturii*.

X17 contains an insert from pSIM1278 intended to reduce expression of the following potato enzymes: 1) polyphenol oxidase (PPO), which decreases PPO levels in tubers and contributes to reduced black spot; 2) asparagine synthetase (ASN), which decreases free asparagine levels in tubers and contributes to reduced acrylamide potential; 3) water dikinase (R1), to lower reducing sugars in tubers, which contributes to reduced acrylamide potential; and 4) phosphorylase L (PHL), to lower reducing sugars in tubers, which contributes to reduced acrylamide potential. The expressible sequences consist of inverted repeat sequences from two potato species, *S. tuberosum* and *S. verrucosum*. *S. verrucosum* is a wild, diploid species that has been used to improve potato, including *S. tuberosum*. The inverted repeat sequences are derived from the asparagine synthetase, phosphorylase L, and water dikinase genes from *S. tuberosum* and from the polyphenol oxidase gene from *S. verrucosum*

X17 also contains an insert from pSIM1678 intended to express the *Rpi-vnt1* gene and reduce expression of the invertase (INV) enzyme. The expressible sequences are from two potato species, *S. tuberosum* and *S. venturii*. The inverted repeat sequence is derived from the vacuolar invertase (V*Inv*) gene from *S. tuberosum*. The *Rpi-vnt1* gene, including the native promoter and terminator, is from *S. venturi*

4. Transformation System

Agrobacterium tumefaciens-mediated transformation was used to generate X17. Potato X17 was the product of two separate transformations. Ranger Russet potato was first transformed with pSIM1278. The pSIM1278 gene cassette includes inverted repeat sequences derived from four genes namely, asparagine synthetase (*Asn1*), phosphorylate L (*PhL*), water dikinase (*R1*), and polyphenol oxidase (*Ppo5*) genes. These inverted repeats are for RNA interference (RNAi), designed to down regulate the expression of ASN, PHL, R1, and PPO enzymes which will result in reduced free asparagine (for ASN), lower reducing sugars (for PHL and R1), and reduced black spot (for PPO). Reduced free asparagine and lower reducing sugars will contribute to low acrylamide potential.

A specific product of the first transformation called event F10 was subjected to a second transformation, this time with pSIM1678. The pSIM1678 gene construct includes the late blight resistance gene (*Rpi-vnt1*) and inverted repeats derived from the vacuolar invertase gene (*VInv*). Introduction and expression of Rpi-vnt1 in the transformed plant will confer resistance to late blight. On the other hand, expression of VInv will down regulate INV enzyme resulting in lower reducing sugars.

5. Inserted DNA

The number of insertion site from pSIM1278 was sufficiently determined through Southern blot analysis. A series of seven overlapping probes covering the pSIM1278 T-DNA was used to analyze

X17 genomic DNA digested with Bbs I, which does not cleave within the T-DNA of pSIM1278. Since pSIM1278 and pSIM1678 share common elements including the LB and RB regions and the Agp and Gbss promoters, the probes designed to analyze pSIM1278 were also used to detect elements within the pSIM1678 insert. The F10 DNA sample was included in the Southern blots to serve as a control that allows bands from pSIM1278 and pSIM1678 to be distinguished from each other. A single band of consistent size was observed in the F10 and X17 samples with each pSIM1278 probe, indicating a single insert locus for the pSIM1278 T-DNA. Another single band of consistent size, and corresponding to the pSIM1678 insert, was observed in X17 samples for each 1278 probe. This observation indicated that there was a single insert locus for pSIM1678 T-DNA. Another set of Southern blots which used pSIM1678-specific probes confirmed the insertion of pSIM1678 T-DNA at a single site.

The integrity and order of genetic elements were checked and demonstrated using southern blotting, PCR, and sequencing.

6. Genetic Stability

The stability of the insert in potato X17 was assessed via Southern blot analysis on G0 and G2 plants using a total of six probes that hybridize to genetic elements of the T-DNAs of both pSIM1278 and pSIM1678. DNA samples from Ranger Russet and X17 plants were digested with Eco RV. The same banding pattern was observed in all Southern blots for each of the vegetative propagated X17 plants, indicating that the integrity of the insert is stable and maintained.

7. **Expressed Material**

The novel protein which is expected to be produced in potato X17 is VNT1. The level of expression of VNT1 in potato X17 was determined by using two methods: immunoblot assay and liquid chromatography-mass spectrometry (LC-MS). VNT1 protein levels were determined to be undetected in three biological replicates of X17 leaf and tuber tissues based on western blot analysis using the α-VNT1-602 antibody. On the other hand, the LC-MS results showed that VNT1 was not detected in W8 or W3. Both the immunoblot assay and LC-MS are sensitive methods which can determine protein levels as low as 500 pbb. Therefore, the amount of VNT1 protein in X17 must be less than 500 pbb.

8. **Toxicological and Allergenicity Assessment**

Based on the documents provided by the developer, digestibility, heat inactivation, andacute oral gavage studies using the full-length VNT1 were 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 and heat inactivation 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). Furthermore, 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).

9. Nutritional Data

X17 is comparable to Ranger Russet. Statistical differences in means were observed in the levels of some proximates in X17 compared to Ranger Russet. However, the observed levels were all within the tolerance interval and/or combined literature range for conventional potatoes, demonstrating that the nutritional content of X17 lies within the normal range for conventional potatoes. These results indicate that X17 potatoes are compositionally equivalent to conventional potatoes. The nutritional assessment demonstrated that X17 is as safe as conventional potato varieties, which have a history of safe consumption.

Statistical difference was observed for vitamin C and potassium between X17 and Ranger Russet but the mean values 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 X17. Statistical differences were observed in amino acid levels measured in X17 tubers but these were all within tolerance interval and combined literature range.

Vitamin C, potassium, and amino acid levels differed between X17 and Ranger Russet. However, the mean and range values for all vitamins, minerals, and amino acids fell within the tolerance interval or combined literature range, indicating that X17 was comparable to conventional potato varieties.

Glycoalkaloid levels in X17 and Ranger Russet were similar and below the safety level of 20 mg per 100 g fresh weight tuber (OECD, 2002).

10. Recommendation

BPI-PPSSD, BAI and STRPs find scientific evidence that the regulated article applied for direct use as food and feed or for processing is as safe as its conventional counterpart and shall not pose greater risk to human and animal health.

Annex IV

DENR Biosafety Committee (Environmental Safety)

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 (X17), hereunder is the DENR-BC's observation:

- 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, Solanum 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).

Annex V

DOH Biosafety Committee (Environmental Health Safety)

The DOH-BC, after thorough review of the documents, 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 of the DOH-BC:

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

Annex VI

SEC Expert (Socio-Economic Considerations)

According to the SEC Expert, the approval to import potato X17 may not affect our domestic production since food processing and institutional food chain were already importing white potatoes. In addition, since the event will not be propagated domestically, there will be no significant effects to the domestic production and trade of white potatoes.

It was reported that Potato X17 will not drastically affect the current patterns of production, consumption/ utilization and trade. Current production pattern will not be affected since Philippine had been importing a significant volume of white potatoes from the USA and X17 potato will not be produced locally. In addition, since X17 is only about 0.1% of the total potato production in the USA, allowing importation of this event will not alter the patterns of trade with the USA. On the other hand, allowing X17 potato to enter into the country for utilization in the food industry may stabilize prices thus, current consumption of products containing potato (e.g chips, french fries) as an ingredient may improve. With regards to the current consumption, I think it would not be affected with the entry of X17 potato.

Thus, X17 potato may have insignificant effect on the current pattern of consumption among Filipinos.

The entry of X17 will not affect the cultural practices of Filipino potato farmers since it will not be cultivated locally.

After a thorough and scientific review and evaluation of the documents provided by SPS international Inc., relevant to Potato X17, the SEC Expert recommends the approval and issuance of biosafety permit for direct use as food and feed or for processing.