FACT SHEET

APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF EVENT SPS-ØØE12-8 (E12) POTATO FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK (S) 602-1/1/39

The objective of the Biosafety Act is to protect human, plant and animal health, the environment and biological diversity. Under the Biosafety Act, the National Biosafety Board (NBB) is currently assessing an application for approval submitted by SPS International Inc. (Malaysia Branch).

1. What is the application for?

The application is for import and release of E12 potato and its products for supply or offer to supply for sale or placing in the market.

2. What is the purpose of the import and release?

The aim of the import is to supply or offer to supply for sale/placing on the market – for direct use as food, feed and processing (FFP) of E12 potato and its products. The said potato event is not intended for cultivation in Malaysia.

3. How has E12 potato been modified?

Using standard *Agrobacterium*-mediated transformation, the conventional Russet Burbank potato variety was transformed with the genetic construct pSIM1278 to create the E12 event. The insert from pSIM1278 down regulates polyphenol oxidase, asparagine synthetase, and phosphorylase L transcripts in the potato plant using RNA interference and is made up of genetic elements only from potato. The transformation resulted in a reduction of endogenous enzymes, but no new proteins are expressed as a result of the transformation.

4. Characteristics of E12 potato

a) Details of the parent organism

The scientific name for cultivated potato is *Solanum tuberosum*. Potato is classified in the *tuberosum* subspecies, within the *tuberosa* series, and within the *potatoe* subsection of the Solanaceae plant family. Plants in the *tuberosum* subspecies are cultivated worldwide.

Taxonomic position of *S. tuberosum* subs. *tuberosum*

Taxonomic Rank	Latin Name
Family	Solanaceae
Genus	*Solanum*
Section	*Petota*
subsection	*potatoe*
Series	*Tuberosa*
Species	*tuberosum*
subspecies	*tuberosum*

The Solanaceae family contains several well-known cultivated crops, such as tomato (*S. lycopersicum*), eggplant (*S. melogena*), and pepper (*Capsicum annuum*). Potatoes are known as poor competitors that do not thrive in non-cultivated environments.

Potatoes have a long history of safe use as food and feed.

b) Details of the donor organism

The sequences transferred into E12 potato are from *Solanum tuberosum* and *Solanum verrucosum*. *S. tuberosum* has a long history of safe use. *S. verrucosum* likewise is used in potato breeding programs and has provided genes for new potato varieties.

c) Description of the trait(s) and characteristics which have been introduced or modified

E12 potato is a new potato event developed by transforming a conventional potato variety, Russet Burbank, with a plasmid containing potato DNA sequences. The pSIM1278 T-DNA was designed to down regulate polyphenol oxidase, asparagine synthetase, phosphorylase L, and water dikinase (R1) transcripts in the potato using RNA interference. The transformation resulted in a reduction of endogenous enzymes but no new proteins were expressed.

The traits resulting from the transformation are: (1) reduced acrylamide potential, which results in less acrylamide, a potential health concern, when potatoes are cooked at high temperatures; and (2) reduced black spot and bruising, which results in less food waste for consumers and processors.

Summary of Genes, Traits, and Benefits

Gene Sequence	Mechanism	Trait	Benefit
Asn1: asparagine synthetase-1	RNAi down regulation	Reduced free asparagine	Contributes to low acrylamide potential[1]
R1: water dikinase	RNAi down regulation	Lower reducing sugars	Contributes to low acrylamide potential[1]
PhL: phosphorylase L	RNAi down regulation	Lower reducing sugars	Contributes to low acrylamide potential[1]
Ppo5: polyphenol oxidase-5	RNAi down regulation	Reduced polyphenol oxidase	Reduces black spot, which improves potato quality and reduces waste

[1]Acrylamide is formed primarily from free asparagine and reducing sugars heated at temperatures above 120 °C. According to some global regulators, acrylamide presents a potential health risk for consumers.

5. Modification Method

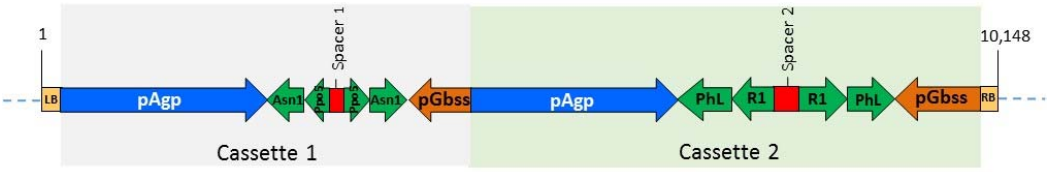
a) Characteristics of the modification

The Russet Burbank variety was transformed with pSIM1278 to create E12 potato. The plasmid pSIM1278 T-DNA contains two cassettes:

- The first cassette was designed to down regulate asparagine synthetase and polyphenol oxidase transcripts in tubers of transformed potatoes using sequences from *Asn1* and *Ppo5,* respectively. The inverted repeat is comprised of *Asn1* and *Ppo5* fragments, separated by a spacer element (Spacer-1) and arranged between two convergent potato promoters: the Agp promoter of the ADP glucose pyrophosphorylase gene (*Agp*), and the Gbss promoter of the granule-bound starch synthase gene (*Gbss*). Both promoters are active in tubers.
- The second cassette was designed to down regulate phosphorylase L and water dikinase transcripts in tubers using sequences from *PhL* and *R1*. The inverted repeat is comprised of *PhL* and *R1* fragments, separated by a spacer element (Spacer-2) and arranged between two convergent potato promoters: the Agp promoter of the ADP glucose pyrophosphorylase gene (*Agp*), and the Gbss promoter of the granule-bound starch synthase gene (*Gbss*). Both promoters are active in tubers.

The inverted repeats were separated by a non-coding 157-bp conventional potato nucleotide spacer element. The LB and RB regions were designed to

be similar to and function like T-DNA borders from *Agrobacterium tumefaciens*.



Design of pSIM1278 T-DNA
T-DNA region showing the flanking Left and Right Borders and the two down-regulation cassettes.

b) Safety of the expressed protein

These T-DNA sequences are non-coding and do not produce proteins. No new polypeptides are produced from this construct; rather, the small interfering RNA catalyzes the degradation of specific mRNA to down regulate target genes within the plant.

6. Assessment of Risks to Human Health

Potato has a long history of safe consumption. Potatoes (*S. tuberosum*) do not display pathogenicity. The primary toxin, allergy, and anti-nutrient concerns in potato are glycoalkaloids, patatin, protease inhibitors and lectins, respectively. The level of glycoalkaloids in E12 potato is well below the accepted safety limit of 20 mg/100 g in tubers. Consumption of patatin is managed through dietary choice, while cooking inactivates the anti-nutrients.

a) Nutritional Data

Compositional analysis was performed on field-grown tubers to compare nutritional and anti-nutritional analytes and determine if any nutritionally relevant differences exist between E12 potato and conventional potatoes. E12 potato was found to be substantially equivalent to Russet Burbank and other conventional potatoes.
Due to the presence of non-digestible starch and anti-nutrient proteins, potato tubers are traditionally consumed after cooking, e.g. frying, boiling, or baking. During heating, the starch is converted and the proteinase inhibitors and lectins are inactivated.

b) Toxicology

Glycoalkaloids are toxins commonly found in solanaceous crops, including potato. Together, σ-solanine and σ-chaconine make up 95% of the total glycoalkaloids in potato tubers (OECD, 2002). The widely accepted safety limit for total glycoalkaloids in tubers for human consumption is 20 mg/100 g fresh weight (Smith et al., 1996). Levels of glycoalkaloids in E12 potato was below this limit.

A bioinformatics analysis identified all start-to-stop open reading frames (ORFs) in the insert and crossing the junctions with the potato genome. The ORFs were screened for homology with known toxins by querying the NCBI database for protein sequences annotated with the keyword “toxin”. While two of the ORFs share homology with asparagine synthetases expressed in bacteria, the identification resulted from the word “toxin” in the accession record. Evaluation of the ORFs did not identify any matches to known toxins.

c) Allergenicity

Potatoes are not among the “Big Eight” group of foods that account for about 90% of all food allergies in the United States (FARRP, 2014). Patatin (Sol t 1) has been identified as the primary allergen associated with potato (Astwood et al., 2000). Because potato protein naturally contains a relatively large proportion of patatin, any change in patatin levels in E12 potato would be unlikely to affect allergenicity enough to alter consumption patterns for people allergic to potatoes. In addition, patatin levels vary considerably between commonly consumed potato varieties.

A bioinformatic analysis was conducted using the 2017 AllergenOnline.org database, available through the Food Allergy Research and Resource Program (FARRP) via the University of Nebraska (http://www.allergenonline.org/databasefasta.shtml). Comparisons were made using the full-length ORF sequence, an 80-mer sliding window, and an 8-mer exact match. The searches were conducted to identify matches between the protein query sequences and known allergens. None of the searches identified any significant homology.

There are no new polypeptides produced from the inserted DNA in E12 potato, and open reading frame analysis identified no allergen-based safety concerns.

d) Small interfering RNA (siRNA) Safety

The insert from pSIM1278 produces siRNA that catalyze the degradation of specific mRNA to down regulate target transcripts within the plant. Since siRNA are the product of the transformation, their safety was assessed. This assessment indicated that there is a long history of safe consumption of small RNA. Inserts designed to down regulate host genes for quality traits do not present an increased risk to consumers when compared to currently cultivated food crops.

7. Assessment of Risks to the Environment

This application does not cover an environment release (cultivation) of E12 potato in Malaysia. The release is intended only to cover the import of E12 potato and its products that may enter Malaysia as foodstuff, feed or for further food processing.

8. What is the Emergency Response Plan?

a) First Aid Measures

No special first aid measures are necessary for exposure to this product. Safety assessments have shown E12 potato be as safe as other conventional potato varieties (USDA, FDA, CFIA, Health Canada, and FSANZ).

b) Accidental Release Measures

Under the Simplot Closed Loop Stewardship Plan, E12 tubers do currently not enter export markets outside of countries where they are approved. Any whole conventional potato tubers imported into Malaysia would need to fulfill quarantine requirements and can be tested for presence of E12.

Furthermore, E12 is male sterile, so living E12 potato plants will not produce pollen or transfer traits to sexually compatible species. Safety assessments have not identified adverse effects related to release of E12.

SPSII's Closed Loop Stewardship plan details the steps to be taken should an accidental release occur. Response to unintended release would be carried out in consultation with the Malaysian regulatory authorities.

c) Handling and Storage

There are no specific requirements for the handling and storage of this event. E12 potato is handled and stored in the same way as conventional potato varieties.

d) Disposal Considerations

E12 potato is disposed of in the same way as conventional potato varieties.

9. How can I comment on this application?

Any member of the public may submit their comments or queries on publicly notified information about the application. Before submission of comments or queries, the person should review the information provided. Comments and queries on any possible impacts/risks to the health and safety of the people and the environment that may be posed by the proposed release are appreciated. The submission of comments or queries should be prepared carefully as it will be given the same scrutiny as the application by the NBB. The submission of comments and clarifications or queries should contribute to the NBB's assessment. Even if the submission is not science-based, and focuses on cultural or other values, it should still be developed in the form of a well-founded argument.

Please note that the consultation period closes on **25 October 2017** and written submissions are required by that date. Submissions must be addressed to:

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Please include your full name, address and contact details in your submission.