FDA U.S. FOOD & DRUG ADMINISTRATION

Biotechnology Notification File No. 000174
CFSAN Note to the File

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From: Patrick Cournoyer, PhD

To: Administrative Record, BNF No. 000174

Subject: Z6 Snowden Potato

Keywords: *Solanum tuberosum*, potato, Snowden, lower levels of reducing sugars, late blight resistance, *Phytophthora infestans*, *Rpi-vnt1* gene, late blight resistance protein VNT1, vacuolar invertase (*VInv*), RNA interference (RNAi), *Solanum venturii*, Z6, V11, J.R. Simplot Company, OECD unique identifier SPS-ØØØZ6-5

Summary

J.R. Simplot Company (Simplot) has completed a consultation with the Food and Drug Administration (FDA) on food derived from potato transformation event Z6 (hereafter referred to as Z6 potato) with suppressed expression of the vacuolar invertase gene (*VInv*) to lower levels of reducing sugars and expressing the *Rpi-vnt1* gene encoding VNT1 to increase resistance to late blight.[1] This document summarizes Simplot's conclusions and supporting data and information that FDA's Center for Food Safety and Applied Nutrition (CFSAN, we) evaluated pertaining to human food uses. FDA's Center for Veterinary Medicine summarizes its evaluation pertaining to animal food uses in a separate document.

Simplot concludes:

- it has not introduced into food a new protein or other substance that would require premarket approval as a food additive.
- food from Z6 potato is as safe as and, except for the intended traits, is similar in composition to food from comparable potato varieties.

CFSAN evaluated data and information supporting these conclusions and considered whether Z6 potato raises other regulatory issues involving human food within FDA's authority under the

[1] Z6 potato was derived by modifying V11 potato with the genetic elements and traits that are the subject of this consultation, BNF 000174. V11 potato was the subject of consultation BNF 000152. V11 potato was developed by transforming the variety Snowden with RNAi expression cassettes intended to (1) suppress expression of asparagine synthetase 1 (*Asn1*) to reduce levels of free asparagine, (2) suppress expression of glucan water dikinase 1 (*R1*) to lower levels of reducing sugars, (3) suppress expression of phosphorylase-L (*PhL*) to lower levels of reducing sugars, and (4) suppress expression of polyphenol oxidase 5 (*Ppo5*) to reduce enzymatic darkening. See BNF 000152 for more information about these traits. The subject of this consultation is the additional traits present in Z6 potato.

Federal Food, Drug, and Cosmetic Act (FD&C Act). We have no further questions at this time about the safety, nutrition, and regulatory compliance of food from Z6 potato.

The U.S. Environmental Protection Agency (EPA) evaluates and authorizes the use of plant incorporated protectants (PIPs) under the FD&C Act and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). A PIP is defined in 40 CFR 174.3 as "a pesticidal substance that is intended to be produced and used in a living plant, or the produce thereof, and the genetic material necessary for the production of such a pesticidal substance," including "any inert ingredient contained in the plant, or produce thereof." In Z6 potato, VNT1 is a PIP. Simplot has applied for registration for VNT1 as a PIP in Z6 potato. EPA has issued a tolerance exemption for the VNT1 protein in potato (40 CFR 174.534). The safety of VNT1 in Z6 potato is under EPA's purview and is therefore not addressed in this document.

Subject of the Consultation

Crop:	Potato
Designation:	Z6
Intended Trait:	Lower levels of reducing sugars, which is intended to prevent excessive darkening during frying and to reduce the potential for acrylamide formation
Intended Trait:	Increased resistance to late blight (*Phytophthora infestans*)
Developer:	J.R. Simplot Company
Submission received:	April 4, 2019
Amendments received:	July 22, 2020; December 16, 2020; March 10, 2021
Intended use:	Uses customary for Snowden potato in human and animal food
Expression cassette 1:	RNAi gene suppression cassette targeting the vacuolar invertase gene (*VInv*). Invertase converts sucrose into the reducing sugars glucose and fructose. Suppression of *VInv* gene expression is intended to reduce conversion of sucrose to glucose and fructose during cold storage of tubers.
Expression cassette 2:	*Rpi-vnt1*-from *Solanum venturii* encoding the VNT1 protein for resistance to late blight
Transformation method:	Agrobacterium-mediated transformation of V11 potato

Molecular Characterization

Confirmation of intended genetic change

Simplot used Southern blot analysis to confirm the integrity and copy number of the insertion in Z6 potato. Using probes distributed across the T-DNA sequence and multiple restriction enzyme treatments of Z6 genomic DNA, Simplot observed banding patterns consistent with an intact, single-copy insertion. Droplet digital polymerase chain reaction (ddPCR) analysis of multiple

targets in the insertion showed signal intensity consistent with a single-copy insertion. Simplot used sequence capture with Z6 genomic DNA to enrich plasmid vector sequences followed by high-throughput sequencing to determine the sequence and structure of the insertion and the flanking genomic DNA.[2] High-throughput sequencing identified two junctions between inserted and potato genomic DNA, consistent with the presence of a single insertion site. The sequence of the insertion and flanking genomic DNA were confirmed by Sanger sequencing.

Absence of vector backbone DNA

Simplot performed Southern blot analysis of genomic DNA from Z6 potato using probes targeting vector backbone sequences. Simplot found no evidence of vector backbone sequences in Z6 potato.

Inheritance and stability

Commercial potatoes are propagated vegetatively and do not undergo meiotic recombination, therefore potatoes are expected to be genetically stable. Simplot performed Southern blot analysis on Z6 potato over four vegetative propagation cycles to assess the stability of the intended genetic change. Simplot observed consistent hybridization patterns in each sample, indicating a stable insertion.

Open reading frame analysis

Simplot identified potential open reading frames in the insertion and flanking genomic sequences from start codon to stop codon. Simplot analyzed open reading frames longer than 30 codons for sequence similarity to known allergens in the Food Allergy Research and Resource Program AllergenOnline database (Version 19) and to protein sequences identified using the NCBI Entrez Global Search query "toxin." Simplot did not identify translated open reading frames with similarity to proteins annotated as toxins. Simplot identified translated two open reading frames with sequence similarity to a vacuolar invertase from tomato, which is minor allergen. The open reading frames are part of the *VInv* silencing cassette, which is derived from a vacuolar invertase sequence already present in potato and is not designed to be translated into protein. Simplot concludes that in the unlikely event unintended open reading frames were to be expressed as proteins in food, they would not raise concerns of toxicity or allergenicity.

Human Food Nutritional Assessment

Uses in Human Food

The intended uses of Z6 potato are the same as those of Snowden potato. Snowden potato produces round tubers with high specific gravity, which are desirable traits for chipping.

Lower levels of reducing sugars

Simplot measured levels of sucrose and of reducing sugars (glucose and fructose combined) in Z6 and Snowden,[3] both in fresh tubers and in tubers that were stored at 7°C for six months.

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[2] Simplot determined the average high-throughput sequencing coverage of the Z6 insertion and flanking sequence was 183×.

[3] Simplot used Snowden as a comparator for Z6 potato because Snowden is the non-genetically engineered potato variety with a history of safe use from which Z6 was derived.

Simplot produced tubers for analysis at four locations in potato-growing regions in the United States using typical production practices for potato. Simplot analyzed four samples from each site and for each variety, with each sample consisting of 6 randomly selected tubers. In both fresh and cold stored tubers, Z6 had lower levels of reducing sugars than Snowden. For sucrose, levels in fresh tubers were slightly higher in Z6 than in Snowden tubers, whereas levels of sucrose were similar in both varieties after cold storage. Simplot notes that levels of reducing sugars in tubers are a measure of quality and do not impact safety or nutrition.

Analysis of key nutrients, anti-nutrients, and toxicants

To ensure the absence of unintended changes in components relevant to safety or nutrition, Simplot analyzed tubers from Z6 potato and from the parental variety Snowden[3] for proximates (ash, carbohydrate, fat, protein, moisture, crude fiber), calories, vitamins (vitamin B3, vitamin B6, vitamin C), minerals (copper, magnesium, potassium), amino acids, and glycoalkaloids (α-solanine and α-chaconine combined). Simplot produced and sampled tubers for analysis as described above. Ranges and means for each component were compared to values in the International Life Sciences Institute (ILSI) Crop Composition Database (2019)[4] and the OECD Consensus Document on Compositional Considerations for New Varieties of Potatoes (2002), with the exception of glycoalkaloids, which were compared to values in a published scientific study. Simplot observed differences in the levels of some components between Z6 potato and Snowden; however, the values for key components in Z6 potato were within the published ranges. Simplot concludes that Z6 potato is as safe and nutritious as other potato varieties that are safely consumed.

Food Labeling Considerations

It is a producer's or distributor's responsibility to ensure that labeling of the food it markets meets applicable legal requirements, including disclosure of any material differences in the food. It is our understanding that Z6 potato may be used in various food applications. Depending on the particular food application, differences between Z6 potato and conventional potato varieties may be considered material information requiring disclosure to the consumer under Section 201(n) and Section 403(a)(1) of the FD&C Act. Companies marketing Z6 potato or products containing Z6 potato are advised to consult with FDA's Office of Nutrition and Food Labeling, Food Labeling and Standards Staff to discuss any required or voluntary labeling, including statements relating to attributes of this potato and its potential to lower acrylamide levels during processing or any other type of claim.

[4] On May 1, 2020, the International Life Sciences Institute Crop Composition Database became known as the Agriculture and Food Systems Institute Crop Composition Database.

Conclusion

Based on the information provided by Simplot and other information available to CFSAN, we have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from Z6 potato. We consider the consultation with Simplot on Z6 potato to be complete.

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