U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

Biotechnology Notification File No. 000205
HFP Note to the File

Date: March 23, 2026

From: Dr. Matthew Fabian, Biologist

To: Administrative Record, BNF No. 000205

Subject: Potato with transformation event Kal91.3 (Kal91.3 potato)

Keywords: *Solanum tuberosum*, potato, reduced levels of reducing sugars, reduced cold-induced sweetening, silencing, RNA interference, RNAi, *VInv* gene, *nptII* gene, NPTII protein, neomycin phosphotransferase II, Michigan State University, MSU, Potato Breeding and Genetics Program, Kal91.3

Summary

The Michigan State University Potato Breeding and Genetics Program (MSU) has completed a consultation with the Food and Drug Administration (FDA) on food derived from Kal91.3 potato. Kal91.3 potato expresses 1.) an RNAi cassette to silence expression of the vacuolar invertase gene *VInv*, leading to lower levels of reducing sugars in tubers, thereby preventing cold-induced sweetening, and 2.) the NPTII protein, conferring resistance to the antibiotics kanamycin and neomycin and utilized as a selection marker. This document summarizes MSU's conclusions and supporting data and information that FDA's Human Foods Program (HFP, 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.

Based on the safety and nutritional assessment MSU has conducted, it is our understanding that MSU concludes:

- it has not introduced into human food a new protein or other substance that would require premarket approval as a food additive;
- human food from Kal91.3 potato is comparable to and as safe as human food from other potato varieties

HFP evaluated data and information supporting these conclusions and considered whether Kal91.3 potato raises other regulatory issues involving human food within FDA's authority under the Federal Food, Drug, and Cosmetic Act. We have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from Kal91.3 potato.

Subject of the Consultation

Crop	Potato (*Solanum tuberosum*)
Designation	Kal91.3
Intended trait	Lower levels of reducing sugars
Developer	Michigan State University Potato Breeding and Genetics Program
Submission received	September 15, 2025
Amendment(s) received	December 19, 2025
Intended use	For general use in human food
Transformation plasmid	pINVBP1
Expression cassette 1	RNAi cassette for silencing expression of the vacuolar invertase gene *VInv*, leading to lower levels of reducing sugars (fructose and glucose) and cold-induced sweetening in tubers
Expression cassette 2	*nptII* gene encoding the selection marker protein NPTII, which confers resistance to kanamycin and neomycin antibiotics
Method for conferring genetic change	*Agrobacterium*-mediated transformation

Molecular Characterization

Confirmation of intended genetic change

To generate Kal91.3 potato, MSU transformed internode explant tissue from the potato variety “Kalkaska” with the plasmid vector pINVBP1, derived from the pHELLSGATE8 RNAi plasmid, via *Agrobacterium*-mediated transformation. The T-DNA region of pINVBP1 possesses an RNAi expression cassette, containing a 508-bp region of *VInv* cloned in sense and antisense orientation, as well as the *nptII* cassette, which encodes the NPTII protein for selection on kanamycin. Insert copy number was determined via digital droplet PCR with probes amplifying *nptII*, which indicated two T-DNA insertions. To identify the insertion loci, MSU utilized next generation sequencing and Sanger sequencing and located two insertions, with individual positions on chromosomes 1 and 3. At the chromosome 1 insertional locus, MSU identified a 963-bp chromosomal deletion, as well as 454-bp truncated from the T-DNA left border element, resulting from T-DNA integration. Similarly, integration at chromosome 3 resulted in a 121-bp chromosomal deletion and a 323-bp left border element truncation.

Absence of vector backbone DNA

MSU performed PCR with primer sets spanning the pINVBP1 plasmid backbone, and gel electrophoresis indicated the absence of backbone DNA in Kal91.3. Additionally, Southern blot analysis, incorporating six probes spanning the pINVBP1 plasmid backbone, affirmed the absence of vector backbone DNA in Kal91.3.

Inheritance and stability

Potato is clonally propagated, and therefore the insertional event is expected to remain stable over clonal generations. To confirm generational stability, MSU utilized tuber samples from the third clonal generation to verify the presence of both T-DNA inserts via PCR and gel electrophoresis.

Open reading frame analysis

For both insertion loci, MSU surveyed all six potential open reading frames (ORFs) for translation products with homology to allergens. At each insertion junction, 1,000-bp of flanking sequence and 500-bp of adjacent T-DNA insert sequence were analyzed for potential ORFs, with translation products compared to allergen sequences via the COMPARE[1] and AllergenOnline[2] databases. MSU identified no significant homology to allergen sequences and thus concluded that ORFs arising from the insertional events of Kal91.3 potato raise no concerns of potential allergenicity.

MSU also analyzed ORF translation product sequences for homology to potential toxins via BLAST searches with the UniProt[3] database. While partial homologies to several proteins, including a 3-phosphoshikimate 1-carboxyvinyltransferase, PWWP domain-containing DNA repair factor 3A, and a leucine repeat-rich receptor kinase, were identified, no homology to toxin sequences was observed, leading MSU to conclude that ORFs arising from the insertional events of Kal91.3 potato raise no concerns of potential toxicity.

Introduced Protein: Neomycin phosphotransferase II (NPTII)

Intended trait	Resistance to aminoglycoside antibiotics, for use as a selectable marker
Source organism	*Escherichia coli*
Protein description	Aminoglycoside phosphotransferase
Intended function	Phosphorylation of aminoglycoside antibiotics

MSU utilized quantitative ELISA to survey NPTII levels in field-grown tuber samples from Kal91.3 and the control variety “Kalkaska.” MSU observed that levels of NPTII in Kal91.3 fell at or below levels observed for previously released, genetically engineered potato varieties. Furthermore, MSU noted that the safety of NPTII in human and animal food has been well characterized, highlighting published scientific literature as well as the use of NPTII in food from other genetically engineered plants that have been the subject of completed consultations with FDA. MSU concluded that there are no food safety concerns arising from the expression of NPTII in Kal91.3.

[1] https://comparedatabase.org/
[2] http://www.allergenonline.org/
[3] https://www.uniprot.org/

Characterization of the Intended Trait (reduced levels of reducing sugars)

Kal91.3 potato is characterized by lower levels of the reducing sugars glucose and fructose in tubers, leading to reduced cold-induced tuber sweetening, accomplished via RNAi-mediated silencing of the *VInv* gene, which encodes a vacuolar invertase. To affirm reduced expression of *VInv* in Kal91.3, MSU employed the NanoString nCounter platform for PCR-free analysis of field-grown tuber samples. While compositional analysis for sucrose, glucose, and fructose indicated no statistically significant differences between field-grown tuber samples of Kal91.3 and the control variety "Kalkaska", chip processing of tuber samples after 6-8 months of cold storage displayed reduced levels of browning for Kal91.3 samples, indicative of reduced levels of reducing sugars and cold-induced sweetening.

Human Food Nutritional Assessment

The intended traits in Kal91.3 potato are not expected to alter levels of key nutrients, anti-nutrients, or toxicants. To assess potential unintended changes in composition relevant to safety or nutrition, MSU analyzed tuber tissue samples from Kal91.3 potato and the control variety "Kalkaska", harvested from field trials conducted in Lakeview, MI USA in 2023 and 2024. In each field trial, plants were grown in four randomized complete blocks, and tubers were randomly sampled. Compositional analyses were performed for sugars (glucose, sucrose, and fructose), proximates (protein, total fat, ash, carbohydrates by calculation, and moisture), vitamins D2 and D3, and nitrogen. Differences between Kal91.3 potato and the control variety were assessed via ANOVA, with the exception of fructose, for which measurements for all samples fell below the lower limit of quantification. Additionally, measurements were compared to reference ranges sourced from published literature[4,5] and publicly available databases[6, 7], with the exception of vitamins D2 and D3 and nitrogen. MSU reported that no statistically significant differences were observed between Kal91.3 potato and the control variety, and all measurements were observed to fall within the cited reference ranges. Additionally, MSU surveyed glycoalkaloid levels in tuber samples of Kal91.3 potato and the control variety. Two samples from Kal91.3 and "Kalkaska" potato plants, cultivated during the 2024 field trial, were analyzed for the sum of α-solanine and α-chaconine levels, with no statistically significant difference observed, and with all values falling within cited reference ranges[6,8]. Overall, MSU concluded that food derived from Kal91.3 potato is compositionally comparable to that of conventionally bred, control potato with a history of safe use in food.

Conclusion

Based on the information provided by MSU and other information available to HFP, we have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from Kal91.3 potato. We consider the consultation with MSU on Kal91.3 potato to be complete.

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[4] Amrein, T.M., et al. (2003). Potential of acrylamide formation, sugars, and free asparagine in potatoes: a comparison of cultivars and farming systems. The Journal of Agricultural Food Chemistry. https://doi.org/10.1021/jf034344v

[5] Vivanti, V. et al. (2006). Level of Acrylamide Precursors Asparagine, Fructose, Glucose, and Sucrose in Potatoes Sold at Retail in Italy and in the United States. Journal of Food Science. https://doi.org/10.1111/j.1365-2621.2006.tb08886.x

[6] AFSI, 2025. Potato - Solanum tuberosum. https://www.cropcomposition.org/CCDB/SelectAnalytes

[7] OECD, 2021. Revised Consensus Document on Compositional Considerations for New Varieties of Potato (Solanum tuberosum): Key Food and Feed Nutrients, Toxicants, Allergens, Antinutrients and Other Plant Metabolites. Series on the Safety of Novel Foods and Feeds No. 33 ENV/JM/MONO. OECD, Paris.

[8] Kozukue, N., et al. (2008). Distribution of glycoalkaloids in potato tubers of 59 accessions of two wild and five cultivated Solanum species. The Journal of Agricultural Food Chemistry. https://doi.org/10.1021/jf802631t

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