



Biotechnology Notification File No. 000191

CFSAN Note to the File

Date: January 22, 2024

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To: Administrative Record, BNF No. 000191

Subject: Corn with transformation event DAS1131 (DAS1131 corn)

Keywords: Corn, maize, *Zea mays* L., insect resistance, lepidoptera, herbicide tolerance, glyphosate, Cry1Da2, *Bacillus thuringiensis*, DGT-28 EPSPS, *Streptomyces sviveus*, Pioneer Hi-Bred International, Inc., DAS1131, OECD unique identifier DAS-Ø1131-3

Summary

Pioneer Hi-Bred International, Inc. (Pioneer) has completed a consultation with the Food and Drug Administration (FDA) on food derived from DAS1131 maize (corn) genetically engineered to express Cry1Da2 and DGT-28 EPSPS proteins to confer resistance to certain lepidopteran insects and tolerance to glyphosate herbicide, respectively. DGT-28 EPSPS was also used as a selectable marker during transformation. This document summarizes Pioneer'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 of DAS1131 corn. 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 Pioneer has conducted, it is our understanding that Pioneer concludes:

- it has not introduced into human food a new protein or other substance that would require premarket approval as a food additive, and
- human food from DAS1131 corn is comparable to and as safe as human food from other corn varieties.

CFSAN evaluated data and information supporting these conclusions and considered whether DAS1131 corn raises other regulatory issues involving human food within FDA's authority under the 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 human food from DAS1131 corn.

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 DAS1131 corn, the Cry1Da2 protein is a PIP and the DGT-28 EPSPS protein, which was used as a selectable marker during transformation, is a PIP inert ingredient. Pioneer explained that it will seek an exemption from the requirement of a tolerance for Cry1Da2 and for DGT-28 EPSPS in or on food derived from DAS1131 corn. The safety of Cry1Da2 and DGT-28 EPSPS in DAS1131 corn falls under EPA’s purview and therefore is not addressed in this document.

Pioneer also reports that the tolerances for the residues of the glyphosate herbicide in or on corn have been previously established by the EPA under 40 CFR §180.364.

Subject of the Consultation

Crop	Corn
Designation	DAS1131
Intended trait 1:	Insect resistance
Intended trait 2:	Herbicide tolerance
Developer	Pioneer Hi-Bred International, Inc.
Submission received	January 26, 2022
Amendments received	May 3, 2023; May 24, 2023; September 5, 2023; September 14, 2023
Intended use	General use in human food
Transformation plasmid	Plasmid PHP88492
Expression cassette 1	The <i>cry1Da2</i> gene cassette encodes the Cry1Da2 protein from <i>Bacillus thuringiensis</i> . It confers resistance to susceptible lepidopteran pests.
Expression cassette 2	The <i>dgt-28 epsps</i> gene cassette encodes the DGT-28 EPSPS protein from <i>Streptomyces sviveus</i> . It confers tolerance to glyphosate herbicides and was used as a selectable marker.
Method for conferring genetic change	<i>Agrobacterium tumefaciens</i> -mediated transformation

Inheritance and stability

DAS1131 corn was developed from a public inbred line transformed with plasmid PHP88492 using *Agrobacterium tumefaciens*-mediated transformation. Pioneer performed Southern-by-Sequencing analysis of DAS1131 corn to confirm the presence and intactness of the intended T-DNA insertion, as well as the absence of vector backbone sequences in the genome. Pioneer determined that DAS1131 corn contains a single copy of the inserted DNA with the expected

organization and no additional insertions or plasmid backbone sequences. Pioneer identified only two unique genome-insertion junctions (one at each end of the intended insertion) and no unexpected junctions between non-contiguous regions of the intended insertion in DAS1131 corn, consistent with the presence of a single intact insert. Pioneer performed Southern blot analysis on five generations of DAS1131 corn to determine the stability of the inserted gene cassettes. Pioneer reported single event-specific hybridization bands unique to the DAS1131 corn insertion with each of the *Cry1Da2* and *dgt-28 epsps* gene probes. Therefore, Pioneer concluded that the single copy of T-DNA in DAS1131 corn was stable and equivalent across multiple generations in the breeding process.

Pioneer also studied Mendelian inheritance of the DAS1131 trait in three segregating generations and two non-segregating generations of DAS1131 corn. Genotypic results demonstrated a Mendelian pattern of inheritance of the DAS1131 T-DNA in all generations as the observed segregation ratios were similar to the expected segregation ratios. Pioneer also reported that phenotypic results, based on tolerance to glyphosate herbicide, were similar to the genotypic results, thereby confirming that the introduced traits segregated as a single locus and were inherited in a Mendelian fashion.

Human Food Nutritional Assessment

The intended traits in DAS1131 corn are not expected to alter levels of key nutrients or anti-nutrients. To assess potential unintended changes in composition relevant to safety or nutrition, Pioneer analyzed grain from DAS1131 corn, a non-genetically engineered (non-GE) near-isoline control, and 16 conventional commercial corn reference lines (4 reference lines were planted per location), grown in multiple locations in the United States and Canada in 2020. Grain samples were assessed for proximate (crude protein, crude fat, ash, moisture, and carbohydrates (by calculation)), fiber, fatty acid, amino acid, mineral, vitamin, secondary metabolite, and anti-nutrient analytes. Data from DAS1131 corn were compared to that of the non-GE control, the in-study reference ranges, and ranges from literature.^{1,2,3} Comparing of results to literature ranges provides context of natural variation of plant composition resulting from a combination of genetic diversity and environmental conditions at time of production.

Pioneer reported the results of its analysis, noting that seven analytes (lauric acid, myristic acid, heptadecenoic acid, eicosadienoic acid, vitamin B12, delta-tocopherol and furfural) had all data values for the non-GE control and DAS1131 corn were below the lower limit of quantitation and therefore were not statistically analyzed. Pioneer reported that there were no statistically significant differences observed between DAS1131 corn and the non-GE control for the remaining grain-derived analytes except for stearic acid, manganese, zinc, calcium, and inositol. However, after False Discovery Rate (FDR) adjustment, the P-values were not significant for

¹ AFSI (2021) Crop Composition Database, Version 8.0. Agriculture & Food Systems Institute, <https://www.cropcomposition.org/>

² Codex Alimentarius Commission (2019) Codex Standard for Named Vegetable Oils. Codex Alimentarius, CXS 210-1999

³ OECD (2002) Consensus Document on Compositional Considerations for New Varieties of Maize (Zea Mays): Key Food and Feed Nutrients, Anti-Nutrients and Secondary Plant Metabolites. Organisation for Economic Co-operation and Development, ENV/JM/MONO(2002)25

stearic acid, zinc, calcium, and inositol, indicating that they were false positives. In addition, all values for stearic acid, zinc, calcium, and inositol were within the tolerance interval, in-study reference range, and/or literature range. While the raw and FDR-adjusted P-values for manganese were significant, all values for manganese in DAS1131 corn were within the tolerance interval, literature range, and/or in-study reference range. Pioneer explained that these results reflect the natural variation present in corn and that the observed statistical differences are therefore not biologically meaningful. Pioneer concluded that the results of the nutrient composition assessment demonstrate that grain derived from DAS1131 corn is compositionally comparable to that of corn with a history of safe use in food.

Conclusion

Based on the information provided by Pioneer 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 DAS1131 corn. We consider the consultation with Pioneer on DAS1131 corn to be complete.

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