Biotechnology Notification File No. 000196
CFSAN Note to the File

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From: Charles Kanobe, Ph.D.

To: Administrative Record, BNF No. 000196

Subject: Corn with transformation event DP51291 (DP51291 corn)

Keywords: Corn, maize, *Zea mays* L., insect resistance, corn rootworm, *ipd072Aa* gene, IPD072Aa protein, *Pseudomonas chlororaphis,* herbicide tolerance, glufosinate-ammonium, maize-optimized phosphinothricin acetyltransferase (*mo-pat*) gene, PAT protein, *Streptomyces viridochromogenes*, phosphomannose isomerase (*pmi*) gene, PMI protein, *Escherichia coli*, Pioneer Hi-Bred International, Inc., DP51291, OECD unique identifier DP-Ø51291-2

Summary

Pioneer Hi-Bred International, Inc. (Pioneer) has completed a consultation with the Food and Drug Administration (FDA) on food derived from DP51291 corn with IPD072Aa and phosphinothricin acetyltransferase (PAT) proteins to confer resistance to susceptible corn rootworm pests and tolerance to glufosinate-ammonium herbicides, respectively. PAT was also used as a selectable marker during the breeding process. Phosphomannose isomerase (PMI) was 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 DP51291 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 DP51291 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 DP51291 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 DP51291 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." Pioneer states that in DP51291 corn, IPD072Aa is a PIP while PMI and PAT, both used as selectable markers during transformation and event characterization, respectively, are PIP inert ingredients. Pioneer stated that it intends to submit a registration application to the EPA under FIFRA Section 3, and has petitioned EPA for exemption from the requirement of a tolerance under the FD&C Act for the IPD072Aa protein. Pioneer also reported that residues of PMI and PAT, the selectable markers, are exempt from the requirement of a tolerance when used as PIP inert ingredients in all food commodities, per 40 CFR 174.527 and 40 CFR §174.522, respectively. The safety of IPD072Aa, PAT, and PMI proteins in DP51291 corn is under EPA's purview and is therefore not addressed in this document.

Subject of the Consultation

Crop	Corn
Designation	DP51291
Intended trait 1:	Insect resistance
Intended trait 2:	Herbicide tolerance
Developer	Pioneer Hi-Bred International, Inc.
Submission received	February 21, 2023
Amendment received	June 21, 2023; July 11, 2023; September 5, 2023; March 7, 2024
Intended use	General use in human food
Transformation plasmid	Plasmid PHP74638
Expression cassette 1	The *ipd072Aa* expression cassette encodes the IPD072Aa protein from *Pseudomonas chlororaphis*. It confers resistance to susceptible corn rootworm pests.
Expression cassette 2	The *mo-pat* expression cassette encodes the PAT protein from *Streptomyces viridochromogenes*. It confers tolerance to glufosinate-ammonium herbicides. PAT was also used as a selectable marker.
Expression cassette 3	The *pmi* expression cassette encodes the PMI protein from *Escherichia coli*. It was used as a selectable marker during transformation. It allows tissue growth using mannose as a carbon source.

Method for conferring genetic change	*Agrobacterium*-mediated transformation

Inheritance and stability

Pioneer used *Agrobacterium*-mediated transformation to achieve the intended insertion in DP51291 corn. After transformation, corn plants were regenerated and characterized. Selected plants were grown to maturity. Pioneer performed Southern-by-Sequencing analysis and determined that DP51291 corn contains a single copy of the inserted T-DNA without rearrangements, deletions, or duplications. Pioneer identified only two unique genome-insertion junctions consistent with a single T-DNA insert. Pioneer also determined that there were no plasmid backbone nor other unintended plasmid sequences incorporated in DP51291 corn. Pioneer therefore concluded that DP51291 corn contains a single intact T-DNA insert with the expected organization and no additional insertions or plasmid backbone sequences.

Pioneer conducted Southern blot analysis on five generations of DP51291 corn to determine the stability of the inserted gene cassettes. From the results, Pioneer reported single event-specific hybridization bands unique to the DP51291 corn insertion with each of the *ipd072Aa, mo-pat*, and *pmi* gene probes in each of the generations tested. Therefore, Pioneer concluded that the single copy of T-DNA inserted in DP51291 corn was stable and equivalent across multiple generations in the breeding process.

Pioneer conducted segregation analysis on five generations of DP51291 corn to determine the inheritance pattern of the inserted T-DNA during the breeding process. The results of genotypic analysis demonstrated observed segregation ratios were statistically similar to expected segregation ratios. Pioneer also reported that phenotypic results based on tolerance to glufosinate-ammonium herbicide aligned with the genotypic results. Pioneer therefore concluded that the inserted T-DNA in DP51291 corn segregated as a single locus in accordance with Mendelian principles of inheritance for a single locus.

Human Food Nutritional Assessment

The intended traits in DP51291 corn are not expected to alter levels of key nutrients or anti-nutrients. To assess potential unintended changes in composition relevant to human safety or nutrition, Pioneer analyzed grain from DP51291 corn, a non-genetically engineered (non-GE) near-isoline control, and 20 non-GE commercial corn reference lines (4 reference lines were planted per location), grown in multiple locations in the United States and Canada in 2021. Grain samples were assessed for proximate (crude protein, crude fiber, crude fat, ash, moisture, and carbohydrates), acid detergent fiber, neutral detergent fiber, total dietary fiber, fatty acid, amino acid, mineral, vitamin, secondary metabolite, and anti-nutrient analytes. Data from DP51291 corn were compared to those of the non-GE control, the in-study reference ranges, tolerance intervals,[1] and when appropriate, ranges from published literature and publicly

[1] The tolerance intervals were derived from Pioneer and Dow AgroSciences proprietary accumulated data from 196 non-GE commercial corn lines, grown in commercial corn growing regions in United States, Canada, Chile, Brazil, and Argentina between 2003 and 2020.

available databases.[2,3,4] Comparison of DP51291 corn values to literature ranges provides context for natural variation in plant composition resulting from a combination of genetic diversity and environmental conditions at time of production.

From the results of the analysis, Pioneer reported that five analytes (lauric acid, myristic acid, heptadecenoic acid, eicosadienoic acid, and furfural) had all data values below the lower limit of quantitation for the methods used and were not further analyzed. Pioneer also reported that there were no statistically significant differences observed between DP51291 corn and the non-GE control corn for the remaining grain-derived analytes, except for palmitic acid, stearic acid, oleic acid, eicosenoic acid, lignoceric acid, copper, and ferulic acid. However, after False Discovery Rate adjustment, the P-values for palmitic acid and stearic acid were not significant, indicating that they were false positives. In addition, for these seven analytes, the range of values for DP51291 corn were within the tolerance interval, literature range, and/or in-study reference range. Pioneer concluded that the results of the nutrient composition assessment demonstrate that grain derived from DP51291 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 DP51291 corn. We consider the consultation with Pioneer on DP51291 corn to be complete.

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[2] AFSI (2022) Crop Composition Database, Version 9.0. Agriculture & Food Systems Institute, https://www.cropcomposition.org/

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

[4] Organisation for Economic Co-operation and Development. 2002. Consensus document on compositional considerations for new varieties of maize (Zea mays): Key food and feed nutrients, anti-nutrients, and secondary plant metabolites. ENV/JM/MONO (2002) 25. OECD, Paris, France.