



## Biotechnology Notification File No. 000190 CFSAN Note to the File

**Date:** 1/22/2024

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**To:** Administrative Record, BNF No. 000190

**Subject:** Corn with transformation event DP910521 (DP910521 Corn)

**Keywords:** Corn, maize, *Zea mays* L., insect resistance, lepidoptera, herbicide tolerance, glufosinate-ammonium, *cry1B.34* gene, Cry1B.34 protein, *Bacillus thuringiensis*, *mo-pat* gene, phosphinothricin acetyltransferase (PAT), *Streptomyces viridochromogenes*, *pmi* gene, phosphomannose isomerase (PMI), *Escherichia coli*, Pioneer Hi-Bred International, Inc., DP910521, OECD unique identifier DP-910521-2

### Summary

Pioneer Hi-Bred International, Inc. (Pioneer) has completed a consultation with the Food and Drug Administration (FDA) on food derived from DP910521 corn with Cry1B.34 and phosphinothricin acetyltransferase (PAT) proteins to confer resistance to certain lepidopteran insects and tolerance to glufosinate-ammonium herbicides, respectively. 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 DP910521 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 DP910521 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 DP910521 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 DP910521 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 DP910521 corn, Cry1B.34 is a PIP while PMI is a PIP inert ingredient. Pioneer reported that it intends to submit a registration application to the EPA under FIFRA Section 3, including a petition for exemption from the requirement of a tolerance under the FD&C Act for the Cry1B.34 protein. Pioneer also reported that residues of PMI, the selectable marker, are exempt from the requirement of a tolerance when used as PIP inert ingredients in all food commodities, per 40 CFR 174.527. The safety of Cry1B.34 and PMI proteins in DP910521 corn is under EPA’s purview and is therefore not addressed in this document.

Pioneer also reported that the tolerances for the residues of glufosinate-ammonium herbicides in or on corn have been previously established by the EPA under 40 CFR §180.473.

## Subject of the Consultation

<b>Crop</b>	Corn
<b>Designation</b>	DP910521
<b>Intended trait 1:</b>	Insect resistance
<b>Intended trait 2:</b>	Herbicide tolerance
<b>Developer</b>	Pioneer Hi-Bred International, Inc.
<b>Submission received</b>	January 26, 2022
<b>Amendment received</b>	April 26, 2023; May 12, 2023; September 5, 2023; September 14, 2023
<b>Intended use</b>	General use in human food
<b>Transformation plasmid</b>	Plasmid PHP79620
<b>Expression cassette 1</b>	The <i>Cry1B.34</i> gene expression cassette encodes the Cry1B.34 protein from <i>Bacillus thuringiensis</i> . It confers resistance to susceptible lepidopteran pests.
<b>Expression cassette 2</b>	The <i>mo-pat</i> gene expression cassette, optimized for expression in corn, encodes the PAT protein from <i>Streptomyces viridochromogenes</i> . It confers tolerance to glufosinate-ammonium herbicides.
<b>Expression cassette 3</b>	The <i>pmi</i> gene expression cassette encodes the PMI protein from <i>Escherichia coli</i> . It was used as a selectable marker during transformation. It allows tissue growth using mannose as a carbon source.

**Method for** Site-specific integration using two sequential transformation steps: 1) insertion of the integration site (landing pad) and 2) insertion of intended expression cassettes, using microprojectile bombardment.

## Molecular Characterization

### Transformation methods and confirmation of genetic change

Pioneer conducted two sequential transformations to achieve the intended insertion in DP910521 corn using microprojectile bombardment. The purpose of the first transformation was to insert a landing pad at a specific location in the corn genome while the second transformation was aimed at inserting the intended expression cassettes into the landing pad. During the first transformation step, the landing pad sequence was inserted into the corn genome using a CRISPR-Cas9-mediated targeted insertion approach. The transiently expressed Cas9 protein (an RNA-guided DNA endonuclease) produced a double-stranded DNA break at the specified target location in the corn genome. The landing pad was then inserted at this location by homology-directed repair. Pioneer selected a transformant line that had the inserted landing pad sequence and no unintended plasmid sequences for use in the second transformation. The second transformation introduced *cry1B.34*, *mo-pat*, and *pmi* gene expression cassettes into the landing pad.

After each transformation, corn plants were regenerated, characterized, and selected plants were grown to maturity. Pioneer performed Southern by-Sequencing analysis of DP910521 corn to confirm the presence and intactness of the intended insertion, as well as the absence of vector backbone sequences in the genome. Pioneer determined that DP910521 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 DP910521 corn, consistent with the presence of a single intact insert.

### Inheritance and stability

Pioneer conducted Southern blot analysis on five generations of DP910521 corn to determine the stability of the inserted gene cassettes. Pioneer found single event-specific hybridization bands unique to the DP910521 corn insertion with each of the *cry1B.34*, *mo-pat*, and *pmi* gene probes. Therefore, Pioneer concluded that the single copy of T-DNA inserted in DP910521 corn was stable and equivalent across multiple generations in the breeding process.

Pioneer studied Mendelian inheritance of DP910521 corn in four segregating generations and one non-segregating generation of DP910521 corn. Genotypic results demonstrated a Mendelian pattern of inheritance in all five generations of DP910521 corn as the observed segregation ratios matched expected segregation ratios. Pioneer also reported that phenotypic results based on tolerance to glufosinate-ammonium herbicide were similar to the genotypic results, thereby confirming that the introduced traits were inherited in a Mendelian fashion.

## Open reading frame analysis

Pioneer assessed the insertion site of DP910521 corn and its flanking borders and found no disruptions or deletions of known corn genes or regulatory elements. When the identified open reading frames (ORFs) were evaluated for potential allergenicity and toxicity, they did not generate biologically relevant amino acid sequence similarities to known allergens, toxins, or other proteins that would be harmful to humans. Pioneer therefore concluded that the putative translated ORFs in the DP910521 corn insertion site do not raise allergenicity or toxicity concerns.

## Introduced Protein

<b>Intended trait</b>	Tolerance to glufosinate-ammonium herbicides
<b>Source organism</b>	<i>Streptomyces viridochromogenes</i>
<b>Intended function</b>	PAT catalyzes the acetylation of glufosinate-ammonium herbicides

Pioneer notes the PAT protein present in DP910521 corn is identical to the corresponding protein found in several authorized events across different crops that are currently commercialized and have a history of safe use. The PAT protein has been previously assessed for potential allergenicity and toxicity by numerous regulatory agencies and has been determined unlikely to present significant risk to human health.<sup>1, 2</sup> Pioneer conducted studies to demonstrate equivalence of the PAT protein in DP910521 corn to the PAT protein in previously authorized events. The results showed that PAT from DP910521 corn had the expected size and immunoreactivity. Bioinformatic analyses showed no alignment of the PAT protein amino acid sequences against databases of known or putative allergens or toxins. Taken together, Pioneer concluded that there was no concern regarding the safety of the PAT protein in DP910521 corn.

## Human Food Nutritional Assessment

The intended traits in DP910521 corn are not expected to alter levels of key nutrients or anti-nutrients.<sup>3</sup> To assess potential unintended changes in composition relevant to human safety or nutrition, Pioneer analyzed grain from DP910521 corn, a non-genetically engineered (non-GE) near-isoline control, and 18 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), fiber, fatty acid, amino acid, mineral, vitamin, secondary metabolite, and anti-

<sup>1</sup> CERA - ILSI Research Foundation (2016) A Review of the Food and Feed Safety of the PAT Protein. International Life Sciences Institute, Center for Environmental Risk Assessment

<sup>2</sup> Hérouet C, Esdaile DJ, Mallyon BA, Debruyne E, Schulz A, Currier T, Hendrickx K, van der Klis R-J, Rouan D (2005). Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the pat and bar sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants. Regulatory Toxicology and Pharmacology 41: 134-149

<sup>3</sup> DP910521 corn was developed by site-specific integration (SSI) into a pre-selected integration site in the corn genome to avoid disruptions or deletions of known corn genes or regulatory elements.

nutrient analytes. Data from DP910521 corn were compared to that of the non-GE control, the in-study reference ranges, and ranges from published literature and publicly available databases.<sup>4,5,6</sup> 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 analyses. In grain, four analytes (lauric acid, vitamin B2,  $\beta$ -tocopherol, and furfural) had all data values below the lower limit of quantitation for the methods used and therefore, were not statistically analyzed.<sup>7</sup> Pioneer reported that there were no statistically significant differences observed between DP910521 corn and the control corn for the remaining grain-derived analytes, except for grain moisture and phytic acid. However, after False Discovery Rate adjustment, the P-values for grain moisture and phytic acid were not significant, indicating that they were false positives. In addition, all values of moisture and phytic acid were within the tolerance interval, literature range, and/or in-study reference range. This further indicates the natural variation present in corn, making the statistical differences observed not biologically meaningful. Pioneer therefore concluded that the results of the composition assessment demonstrate that grain derived from DP910521 corn was compositionally comparable to that of conventional corn, represented by the non-GE near-isogenic control and the conventional commercial reference varieties.

## 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 DP910521 corn. We consider the consultation with Pioneer on DP910521 corn to be complete.

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<sup>4</sup> AFSI (2021) Crop Composition Database, Version 8.0. Agriculture & Food Systems Institute, <https://www.cropcomposition.org/>

<sup>5</sup> Codex Alimentarius Commission (2019) Codex Standard for Named Vegetable Oils. Codex Alimentarius, CXS 210-1999

<sup>6</sup> 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

<sup>7</sup> Both DP910521 corn and the control corn had 100% of samples below the LLOQ and hence no statistical analyses were performed.