Biotechnology Notification File No. 000175
CFSAN Note to the File

Date: June 23, 2022

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To: Administrative Record, BNF No. 000175

Subject: Insect-resistant and herbicide-tolerant DP23211 corn[1]

Keywords: Corn, maize, *Zea mays*, insect resistance, western corn rootworm, *Diabrotica virgifera virgifera*, herbicide tolerance, glufosinate ammonium, DvSSJ1 dsRNA, RNA interference, IPD072Aa, *Pseudomonas chlororaphis*, phosphinothricin acetyltransferase (PAT), *Streptomyces viridochromogenes*, phosphomannose isomerase, *Escherichia coli*, Pioneer Hi-Bred International, Inc., OECD Unique Identifier DP-023211-2

Summary

Pioneer Hi-Bred International, Inc. (Pioneer) has completed a consultation with the Food and Drug Administration (FDA) on food derived from DP23211 corn genetically engineered to express a DvSSJ1 double-stranded RNA (dsRNA) and the insecticidal protein IPD072Aa for protection against western corn rootworm, phosphinothricin acetyltransferase (PAT) for tolerance to glufosinate ammonium herbicide, and phosphomannose isomerase (PMI) for use as a selectable marker. 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 this corn. FDA's Center for Veterinary Medicine (CVM) 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 that:

- 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 DP23211 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 DP23211 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 DP23211 corn.

[1] This document uses the word "corn" instead of "maize," in accordance with common practice in the United States.

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 DP23211 corn, DvSSJ1 dsRNA and the IPD072Aa protein are considered PIPs and the PMI protein is an inert ingredient. Pioneer reported its intention to petition EPA for an exemption from the requirement of a tolerance for the IPD072Aa protein and notes that the DvSSJ1 dsRNA is exempt from the requirement of a tolerance under 40 CFR 174.507. Pioneer also notes an existing EPA tolerance exemption for PMI (40 CFR 174.527).

Subject of the Consultation

Crop:	Corn
Designation:	DP23211
Trait:	Insect resistance and herbicide tolerance
Developer:	Pioneer Hi-Bred International, Inc.
Original submission received:	May 31, 2019
Amendments received:	July 1, 2019; April 26, 2021
Intended use:	General use in human food
Transformation 1 Vector:	Plasmid PHP56614
Intended genetic change	Insertion of a specific integration site sequence (landing pad) into the corn genome. The landing pad includes: • *Zea mays* recognition sequences flanking the genome location targeted for I-CreI-endonuclease-mediated insertion of the landing pad • flippase recombination target sites, FRT1 and FRT87, for use during recombinase-mediated cassette exchange • *nptII expression cassette* encoding neomycin phosphotransferase II for use as a selectable marker • *ubi*ZM1 promoter and 5' untranslated region to drive expression of selectable markers
Transformation 2 Vector:	Plasmid PHP74643

Intended genetic change	Insertion of gene expression and suppression cassettes into the landing pad through recombinase-mediated cassette exchange. • *pmi* expression cassette encoding phosphomannose isomerase (PMI) from *Escherichia coli* for use as a selectable marker • modified pat (mo-pat) expression cassette encoding PAT protein from *Streptomyces viridochromogenes* for tolerance to glufosinate-ammonium herbicide • DvSSJ1 fragment cassette encoding the DvSSJ1 dsRNA for protection against western corn rootworm through suppression of DvSSJ1 protein expression in the rootworm mid-gut • *ipd072Aa* expression cassette encoding the insecticidal protein IPD072Aa from *Pseudomonas chlororaphis* for protection against certain coleopteran pests
Transformation methods:	Microprojectile bombardment (Plasmid PHP56614) *Agrobacterium*–mediated transformation (Plasmid PHP74643)

Molecular Characterization

Transformation methods and confirmation of intended genetic change

Pioneer conducted two sequential transformations to achieve the intended insertion in DP23211 corn. The purpose of the first transformation was to insert a landing pad at a specific location of the corn genome. Pioneer used microprojectile bombardment with plasmid PHP55614, which contains an I-*Cre*I gene[2] located outside of the corn genome recognition sequences on landing pad sequences. Transient expression of I-CreI recombinase produced a double strand break in the corn genome at the targeted location. The break was repaired through insertion of the landing pad by homologous recombination. The landing pad contains the FTR1 and FRT87 recombination target sites and the *nptII* gene. Pioneer selected a line with the inserted landing pad and no unintended insertions for use in the second transformation. The purpose of the second transformation was to insert the gene expression and suppression cassettes into the landing pad. Pioneer used *Agrobacterium*-mediated transformation with the T-DNA from plasmid PHP74643, which contains a *mo-Flp* gene[3] located outside of the FRT1 and FRT87 recombination target sites, to facilitate recombinase-mediated cassette exchange (RMCE). Transient expression of the FLP recombinase leads to excision of the landing pad sequences located between the FLP recombination target sites (the *nptII* gene and its terminator) from the corn genome and replacement with the gene expression and suppression cassettes (the *pmi*, *mo-pat*, DvSSJ1, and *ipd072Aa* cassettes), which are flanked by the FLP recombination sites on the plasmid PHP74643 T-DNA.

After each transformation, Pioneer used Southern-by-Sequencing (SbS) to confirm the intended insertion and the absence of backbone sequences in the genome. Pioneer identified two unique

[2] Pioneer modified the gene for I-CreI endonuclease from *Chlamydomonas reinhardtii* for corn expression and to target its nuclease activity to a specified corn genome location.
[3] Pioneer modified the *mo-Flp* gene from *S. cerevisiae* for corn expression.

genome-insertion junctions in DP23211 corn, consistent with the presence of a single, intact insert, and confirmed that no plasmid backbone sequences were inserted.

Open reading frame analysis

Pioneer assessed the insertion site of DP23211 to identify potential open reading frames (ORFs) of 30 or more codons. Pioneer reported none of the putative translated ORFs returned alignments from the search against the Comprehensive Protein Allergen Resource (COMPARE) 2019 and from the search against Pioneer's toxin database.[4] Pioneer concludes that the putative translated ORFs in the DP23211 corn insertion site do not raise allergenicity or toxicity concerns.

Inheritance and stability over multiple generations

Pioneer used Southern blot analysis to examine the inheritance and segregation of the inserted DNA in five generations of DP23211 corn. Pioneer confirmed that the inserted DNA was stably inherited across five generations in a Mendelian fashion.

Introduced Protein: PAT

Intended trait	Tolerance to glufosinate ammonium herbicides
Source organism	*Streptomyces viridochromogenes*
Intended function	PAT catalyzes the acetylation of glufosinate ammonium herbicide

Pioneer notes the PAT protein present in DP23211 corn is identical to the corresponding protein found in previously authorized crops in many countries, and it has been previously assessed for potential allergenicity and toxicity and concluded to be safe for food use. Pioneer states that the expression levels of PAT protein in DP23211 corn were lower or similar to the PAT protein levels expressed in DP202216 corn, which FDA assessed in BNF 000171. Pioneer cited a peer-reviewed publication[5] supporting the safety of PAT proteins and concluded that there was no concern regarding the safety of PAT protein in DP23211 corn.

Human Food Nutritional Assessment

The intended traits in DP23211 corn are not expected to alter levels of key nutrients, anti-nutrients, and secondary metabolites (key components). To ensure the absence of unintended changes in key components relevant to safety or nutrition, Pioneer analyzed the composition of grain from DP23211 corn and the non-genetically engineered near-isoline corn line (control

[4] The pioneer toxin database was compiled from a subset of protein sequences (filtered for molecular function for terms associated with toxicity or adverse health effects) in UniProtKB/Swiss-Prot (http://www.uniprot.org/).

[5] Hérouet et al., Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the *pat* and *bar* sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants. Regul Toxicol Pharmacol. 2005, 41(2):134-49.

line), grown in 2018 at seven locations in the United States and one location in Canada. Pioneer also grew and collected grain from non-genetically engineered commercial corn lines to generate tolerance intervals for the key components analyzed. Pioneer measured levels of proximates, fiber, fatty acids, amino acids, minerals, and vitamins, secondary metabolites, and anti-nutrients (phytic acid, raffinose, and trypsin inhibitor). Pioneer determined that the levels of most key components in DP23211 corn grain and the control line were similar. Pioneer further noted that the levels of all components from DP23211 corn grain were within the tolerance intervals or ranges reported in the literature. Pioneer concludes that DP23211 corn is compositionally and nutritionally comparable to control and commercial, i.e., conventional corn 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 DP23211 corn. We consider the consultation with Pioneer on DP23211 corn to be complete.

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