



Biotechnology Notification File No. 000182 CFSAN Note to the File

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To: Administrative Record, BNF No. 000182

Subject: Maize (corn) with transformation event DP915635 (DP915635 corn)

Keywords: corn, maize, *Zea mays*, corn root worm (CRW), coleopteran pests, *ipdo79Ea* gene, IPDO79Ea protein, *Ophioglossum pendulum*, *mo-pat* gene, phosphinothricin acetyltransferase protein (PAT), *Streptomyces viridochromogenes*, *pmi* gene, phosphomannose isomerase protein (PMI), *Escherichia coli*, glufosinate-ammonium, site-specific integration, SSI, microprojectile bombardment, CRISPR-Cas9-mediated targeted insertion, *Agrobacterium*-mediated transformation, Pioneer Hi-Bred International, Inc., DP915635 maize, OECD Unique Identifier DP915635-4

Summary

Pioneer Hi-Bred International, Inc. (Pioneer) has completed a consultation with the Food and Drug Administration (FDA) on food derived from DP915635 corn genetically engineered to express the insecticidal protein IPDO79Ea for protection against susceptible corn rootworm (CRW) pests, 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, supporting data, and information that FDA's Center for Food Safety and Applied Nutrition (CFSAN, we) evaluated pertaining to human food uses of DP915635 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 DP915635 corn is comparable to and as safe as human food from other conventional corn varieties.

CFSAN evaluated data and information supporting these conclusions and considered whether DP915635 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 DP915635 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 DP915635 corn, IPD079Ea is a PIP and PMI is a PIP inert ingredient. Pioneer reported its intention to petition the EPA for an exemption from the requirement of a tolerance for the IPD079Ea protein and notes that a tolerance exemption exists for PMI (40 CFR 174.527). The safety of IPD079Ea and PMI in DP915635 corn is under EPA’s purview and is therefore not addressed in this document.

Subject of the Consultation

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| Crop | Corn |
| Designation | DP915635 |
| Intended trait | Resistance to certain coleopteran pests |
| Intended Trait | Tolerance to glufosinate-ammonium herbicides |
| Developer | Pioneer Hi-Bred International, Inc. |
| Submission received | March 5, 2021 |
| Amendments received | March 25, 2022; August 31, 2022; September 7, 2022 |
| Intended use | General use in human food |
| Transformation 1 plasmids | PHP73878, PHP70605 |
| Intended genetic change | <p>Insertion of a specific integration site sequence (landing pad) into the corn genome; the landing pad includes:</p> <ul style="list-style-type: none"> • <i>Zea mays</i> genomic recognition sequence sites for CRISPR-Cas9-mediated insertion of the landing pad by homology-directed repair (HDR) • flippase recombination target sites, FRT1 and FRT6, for use during recombinase-mediated cassette exchange • <i>nptII</i> expression cassette encoding neomycin phosphotransferase II for use as a selectable marker, with <i>ubiZM1</i> promoter, 5’ untranslated region to drive expression of selectable markers, and <i>pinII</i> terminator |
| Transformation 2 plasmid | PHP83175 |

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| Intended genetic change | <p>Insertion of gene expression cassettes into the landing pad through recombinase-mediated cassette exchange:</p> <ul style="list-style-type: none"> • <i>ipdo79Ea</i> expression cassette encoding IPDo79Ea from <i>Ophioglossum pendulum</i> for resistance to susceptible CRW pests • <i>mo-pat</i> encoding a maize-optimized version of the phosphinothricin acetyltransferase (PAT) protein from <i>Streptomyces viridochromogenes</i> for glufosinate-ammonium herbicide tolerance • <i>pmi</i> encoding the phosphomannose isomerase (PMI) protein from <i>Escherichia coli</i> used as a selectable maker |
| Method for conferring genetic change | <p>Site-specific integration using two sequential transformation steps: 1) insertion of an integration site sequence using microprojectile bombardment and CRISPR-Cas9 targeted insertion process; and 2) <i>Agrobacterium</i>-mediated transformation</p> |

Molecular Characterization

Transformation methods and confirmation of intended genetic change

Pioneer conducted two sequential transformations to achieve the intended insertion in DP915635 corn. The purpose of the first transformation was to insert a landing pad at a specific location in the corn genome. Pioneer used microprojectile bombardment to deliver components for site-specific integration of the landing pad sequence. During this transformation step, the landing pad sequence was inserted into the corn genome using a CRISPR-Cas9-mediated approach where 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 HDR. Pioneer selected a transformant line with only the inserted landing pad sequence and no unintended plasmid sequences for use in the second transformation. The purpose of the second transformation was to insert *ipdo79Ea*, *mo-pat*, and *pmi* expression cassettes into the landing pad. Pioneer used *Agrobacterium*-mediated transformation to deliver the T-DNA from plasmid PHP83175, which contains the three expression cassettes flanked by the FRT1 and FRT6 recombination sites. The T-DNA also contains the *mo-Flp¹* gene, located outside of the FRT1 and FRT6 recombination target sites, to facilitate recombinase-mediated cassette exchange (RMCE). Transient expression of the FLP recombinase leads to excision of the landing pad sequences (*nptII* and its *pinII* terminator) located between the FLP recombination target sites from the corn genome and replacement with the gene expression cassettes (the *pmi*, *mo-pat*, and *ipdo79Ea* cassettes).

After each transformation, Pioneer confirmed the genetic modification. Pioneer used Southern-by-Sequencing (SbS) to confirm the presence and intactness of the intended insertion, as well as the absence of vector backbone sequences in the genome. 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 DP915635 corn, consistent with the presence of a single intact insert, and confirmed that no plasmid backbone

¹ Pioneer modified the *mo-Flp* gene from *S. cerevisiae* for corn expression.

or other unintended sequences (e.g. CRISPR-Cas-9 DNA, *mo-flp*, *nptII* and *pinII* terminator) were inserted.

Inheritance and stability

Pioneer used Southern blot analysis to examine the inheritance and stability of the inserted DNA in five generations of DP915635 corn. Pioneer confirmed through segregation analysis that the inserted DNA was stably inherited across five generations in a Mendelian fashion.

Open reading frame analysis

Pioneer assessed the insertion site of DP915635 corn to identify potential open reading frames (ORFs) of 30 or more codons. Pioneer reported none of the putative translated ORFs returned alignments from searches using the Comprehensive Protein Allergen Resources (COMPARE) 2020 database and Pioneer's toxin database.² Pioneer concludes that the putative translated ORFs in the DP915635 corn insertion site do not raise allergenicity or toxicity concerns.

Introduced Protein: PAT

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

Pioneer notes the PAT protein present in DP915635 corn is identical to the corresponding protein found in previously authorized crops in many countries. PAT has been previously assessed for potential allergenicity and toxicity, with the conclusion that it is safe for food use.³ Pioneer cited a peer-reviewed publication⁴ supporting the safety of PAT proteins and concluded that the consumption of the PAT protein is unlikely to cause an adverse effect on humans.

Human Food Nutritional Assessment

The intended traits in DP915635 corn are not expected to alter levels of key nutrients, anti-nutrients, nor 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 DP915635 corn and the non-genetically engineered near-isoline corn line (control line), grown in 2019 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 (i.e., reference lines) from which it derived tolerance intervals for the key components analyzed.

² 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>).


³ BNF 000029, BNF 000055, BNF 000124, BNF 000136, BNF 000142

⁴ 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.

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 grain from DP915635 corn and the control line were similar. Pioneer further noted that the levels of all components from DP915635 corn grain were within the tolerance intervals or ranges reported in the literature. Pioneer concludes that DP915635 corn is compositionally and nutritionally comparable to control and commercial 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 DP915635 corn. We consider the consultation with Pioneer on DP915635 corn to be complete.

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