FDA U.S. FOOD & DRUG ADMINISTRATION

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CFSAN Note to the File

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From: Carrie McMahon, Ph.D.

To: Administrative Record, BNF No. 000171

Subject: Corn with transformation event DP202216 (DP202216 corn)

Keywords: Corn; maize; *Zea mays; zmm28* gene; ZMM28 protein; MADS-box transcription factor; *mo-pat* gene; phosphinothricin N-acetyltransferase; PAT; *Streptomyces viridochromogenes*; Pioneer Hi-Bred International, Inc.; DP202216 maize (corn); Unique Identifier DP-2Ø2216-6

Summary

Pioneer Hi-Bred International, Inc. (Pioneer) has completed a consultation with the Food and Drug Administration (FDA) on food derived from DP202216 maize (corn) with enhanced grain yield potential and glufosinate herbicide tolerance.[1] 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. FDA's Center for Veterinary Medicine summarizes its evaluation pertaining to animal food uses in a separate document.

Pioneer concludes:

- it has not introduced into food a new protein or other substance that would require premarket approval as a food additive; and
- food from DP202216 corn is comparable to and as safe as food from other corn varieties.

CFSAN evaluated data and information supporting these conclusions and considered whether DP202216 corn raises other regulatory issues involving human food under the Federal Food, Drug, and Cosmetic Act. We have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from DP202216 corn.

[1] The United States Environmental Protection Agency (EPA) regulates herbicides under the Federal Food, Drug, and Cosmetic Act and the Federal Insecticide, Fungicide, and Rodenticide Act. Under EPA regulations, the herbicide residues in DP202216 corn are considered pesticide residues.

Subject of the Consultation

Crop:	Corn
Designation:	DP202216
Trait 1:	Enhanced grain yield potential
Trait 2:	Tolerance to glufosinate herbicides
Developer:	Pioneer Hi-Bred International, Inc.
Original submission received:	November 15, 2018
Amendments received:	November 27, 2018; July 2, 2019; November 5, 2019; January 23, 2020; April 13 and 14, 2020; February 2, 2021
Intended use:	General use in human and animal food
Transformation vector:	PHP40099 Plasmid
Expression cassette 1:	The *zmm28* expression cassette is intended to increase and extend expression of ZMM28. Moderate constitutive expression of the *zmm28* gene is driven by the promoter region of the *Z. mays* translation initiation factor *zm-gos2* gene and the intron region of the *Z. mays* ubiquitin 1 *ubiZM1*.
Expression cassette 2:	The *mo-pat* expression cassette encodes a phosphinothricin acetyltransferase gene from Streptomyces *viridochromogenes* that is codon-optimized for expression in corn.
Method for conferring genetic change:	Agrobacterium-mediated transformation of inbred line PH17AW

Molecular Characterization

Confirmation of intended genetic change

Pioneer performed Southern-by-Sequencing (SbS) analysis using probes designed for full-coverage of the PHP40099 plasmid to characterize the plasmid DNA present in the DP202216 corn genome. This technique involves the use of capture probes with homology to the plasmid to isolate inserted plasmid DNA from genomic DNA; the isolated DNA insert is then sequenced using high-throughput sequencing and analyzed using bioinformatic tools.[2] According to Pioneer, SbS analysis results for its positive control (genomic DNA from the inbred parental line spiked with PHP40099 plasmid) demonstrated that use of the full-coverage probe library is

[2] Zastrow-Hayes G.M., et al. (2015). Southern-by-Sequencing: a robust screening approach for molecular characterization of genetically modified crops. *The Plant Genome* **8**: 1-15.

capable of detecting PHP40099 plasmid sequences at a concentration equivalent to one copy of plasmid per copy of corn genome.

Pioneer reported the results of SbS analysis of DP02216 corn genomic DNA, which identified two unique junctions between plasmid T-DNA sequences and the corn genome. Junctions were not identified between non-contiguous regions of the plasmid T-DNA. Pioneer explained that these results are consistent with the presence of a single, intact copy (that is, without detectable rearrangements or internal truncations[3]) of the T-DNA insert in the DP202216 corn genome.

Absence of vector backbone DNA

Pioneer reported that SbS analysis, using probes targeting vector backbone sequences, did not identify junctions between PHP40099 plasmid backbone sequences and the DP202216 corn genome. Pioneer concludes that DP202216 corn does not contain vector backbone DNA.

Open reading frame analysis

Pioneer analyzed the DNA insert and flanking genomic sequences to identify potential open reading frames (ORFs) of equal to or greater than 30 codons at the insertion site. Pioneer reported that 45 ORFs were subsequently translated, and the potential polypeptides analyzed for similarity to known allergens and toxins. Bioinformatic analysis for potential allergens included comparison of amino acid sequences from the ORFs to known allergens in the Comprehensive Protein Allergen Resource database (COMPARE; February 2018) using FASTA alignment with an *E*-value threshold of 10^{-4} for the full-length ORF sequence. Pioneer also performed a separate bioinformatic analysis for exact matches of eight contiguous amino acids between the translated ORFs and known allergens. Bioinformatic analysis for potential toxins included comparison of the amino acid sequences of the ORFs to sequences in the Corteva Agriscience protein toxin database[4] using BLASTP alignment with an *E*-value threshold of 10^{-4}. Because none of the translated ORFs meeting the minimum frame size bear significant similarity to known allergens or toxins, Pioneer concludes that ORFs resulting from the insertion event do not raise allergenicity or toxicity concerns.

Stability over multiple generations

Pioneer examined the stability and inheritance of the DNA insert across multiple generations of DP202216 corn using Southern blot analysis, gene- and event-specific polymerase chain reaction (PCR), and determination of herbicide tolerance. Pioneer reported that all plants testing positive for the DP202216 event also tested positive for the presence of the inserted *zmm28* and *mo-pat* genes as well as the herbicide tolerance phenotype. Southern blot analysis also showed that the genomic borders of the DNA insert are stable in DP202216 corn across breeding generations. Pioneer used a chi-square test to analyze the inheritance pattern of the DNA insert and concludes that the DP202216 integration event is stable and that the genes segregate according to Mendelian rules of inheritance for a single genetic locus.

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[3] Minor truncations (less than 50 base pairs) of the T-DNA Right and Left Border regions were identified.
[4] Pioneer states that the Corteva Agriscience protein database was compiled from a subset of sequences (filtered by molecular function for terms associated with toxicity or adverse health effects) in the UniProtKB/Swiss-Prot database.

Introduced Protein: ZMM28

Intended trait	Enhanced grain yield potential
Source organism	*Zea mays*
Protein description	The ZMM28 protein is a MADS-box transcription factor that regulates transcription of genes linked to plant vigor, photosynthetic capacity, and nutrient utilization.
Intended function	Moderate constitutive expression of the introduced *zmm28* gene, relative to expression patterns for the native *zmm28* gene, is intended to increase and extend expression of the ZMM28 protein, thereby resulting in improved plant vigor, increased photosynthetic capacity, and enhanced nutrient utilization.[5]

ZMM28 protein safety assessment

Pioneer used Western blot analysis to measure the levels of ZMM28 protein in grain collected at the R6 stage of development when field corn is typically harvested and processed for use in human food. While ZMM28 protein was below the lower limit of quantitation (LLOQ < 0.0069 ng/mg tissue dry weight (DW)) in a non-genetically engineered, near-isoline control, it was present in DP202216 corn at a mean level of 0.012 ng/mg tissue (DW) with values ranging from below the LLOQ to 0.029 ng/mg tissue (DW).[6]

Pioneer estimated dietary exposure to ZMM28 protein from food containing DP202216 corn grain-derived products using the Dietary Exposure Evaluation Model – Food Commodity Intake Database (DEEM-FCID, Version 4.02)[7] and assuming a total replacement scenario where all consumed corn products were derived from DP202216 corn. For corn flour, meal, and bran products, Pioneer used the mean level of ZMM28 protein as measured in DP202216 corn grain. Citing published literature, Pioneer assumed protein was not present in corn oil, starch, and syrup products as a consequence of processing. Pioneer reported that the estimated mean annual and 95th percentile per capita daily exposures to ZMM28 protein are 2 ng/kg bw/d and 13 ng/kg bw/d, respectively, for the U.S. population. The estimated dietary exposure to ZMM28 protein was highest for the 'children ages 3-5 years' subgroup, with a mean annual exposure of 7 ng/kg bw/d and a 95th percentile per capita daily exposure of 27 ng/kg bw/d, respectively. Pioneer concludes that exposure to ZMM28 protein from consumption of DP202216 corn grain products would be very low.

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[5] Wu, J., et al. (2019). Overexpression of zmm28 increases maize grain yield in the field. *Proceedings of the National Academy of Sciences* **116**: 23850-58.

[6] The Western blot method used by Pioneer cannot distinguish between native and introduced ZMM28 proteins, which have identical amino acid sequences; consequently, the ZMM28 protein levels measured in DP202216 corn reflect the total of introduced and native ZMM28 proteins.

[7] DEEM-FCID is a dietary exposure model used by the U.S. Environmental Protection Agency to estimate exposure to substances in food.

Pioneer used a weight of evidence approach to assess the safety of ZMM28 protein in food containing DP202216 corn grain-derived products. In brief, Pioneer considered information about ZMM28 protein including its source (*Zea mays*), its function as a transcription factor, and the presence of native ZMM28 protein in conventional sweet corn kernels at the R3 growth stage (corresponding to the stage that sweet corn is typically harvested and consumed for food). Pioneer also considered the amino acid sequence similarity between ZMM28 protein and MADS-box transcription factors in commonly consumed crops with histories of safe use as food. In its original 2018 submission, Pioneer cited a manuscript later published by Anderson et al., (2019).[8]

Pioneer supported its rationale that ZMM28 protein has a history of safe consumption by comparing the estimated dietary exposure to ZMM28 protein from DP202216 corn grain-derived food products to dietary exposure from conventional sweet corn kernels. Based on data from DEEM-FCID (Version 4.02) and mean concentration values of native ZMM28 protein from select varieties of sweet corn, the mean annual and 95th percentile per capita daily exposures for the U.S. population are 0-3 ng/kg bw/d and 2-17 ng/kg bw/d, respectively. Pioneer concludes that the estimated dietary exposure to ZMM28 protein from DP202216 corn grain-derived food products (2 ng/kg bw/d and 13 ng/kg bw/d for the mean annual and 95th percentile per capita daily exposures, respectively) is comparable to current dietary exposure to ZMM28 protein from consumption of conventional sweet corn.

Based on the weight of this evidence, Pioneer concludes that consumption of the ZMM28 protein from DP202216 corn does not raise toxicity or allergenicity concerns.

Introduced Protein: Phosphinothricin-N-acetyltransferase (PAT)

Intended trait	Tolerance to glufosinate herbicides
Source organism	*Streptomyces viridochromogenes*
Intended function	PAT catalyzes the acetylation of phosphinothricin, the active component in glufosinate herbicides.

PAT protein safety assessment

Pioneer measured the levels of PAT protein in corn grain samples using quantitative ELISA. At the R6 stage of development, PAT protein is present at a mean level of 15 ng PAT/mg grain (DW) and ranges from 7.5 to 21 ng/mg grain (DW). Pioneer reported that the levels of PAT protein in DP202216 corn grain are comparable to the levels reported in other food crops expressing the PAT protein.

[8] Anderson, J.A., et al. (2019). Evaluation of the History of Safe Use of the Maize ZMM28 Protein. *Journal of Agricultural and Food Chemistry* **67**: 7466-7474.

Pioneer used a weight of evidence approach to assess the safety of the PAT protein in food from DP202216 corn based on its equivalence to PAT proteins studied in published peer-reviewed scientific literature and determined to have a safe history of use in food. Pioneer determined that the deduced amino acid sequence of the PAT protein from translation of the codon-optimized *mo-pat* gene is identical to those of PAT proteins from *S. viridochromogenes* as well as a number of previously evaluated new plant varieties. Pioneer used Western blot analysis to confirm the molecular weight and immunoreactivity of the PAT protein from DP202216 corn. Pioneer summarized the results of published digestibility and heat lability studies, and an unpublished acute oral toxicity study of PAT proteins.[9] These studies showed that PAT protein is rapidly degraded by pepsin in simulated gastric fluid (non-detectable at less than five seconds) and enzymatically inactivated at temperatures of 50°C or higher (after ten minutes incubation); no adverse effects were observed in the oral toxicity study. In addition to summarizing *in vitro* and *in vivo* study results, Pioneer reported that PAT protein does not have sequence similarities to known toxic or allergenic proteins. Based on the weight of this evidence, Pioneer concludes that presence of the PAT protein in human food from DP202216 corn does not raise toxicity or allergenicity concerns.

Human Food Nutritional Assessment

Analysis of key nutrients, anti-nutrients, and toxicants

To ensure the absence of unintended changes to components relevant to the safety or nutrition of human food from DP202216 corn, Pioneer measured the composition of DP202216 corn grain and compared it to grain from conventional, near-isoline (control) and non-genetically engineered commercial (reference) corn varieties grown concurrently in fields trials in 2017.[10] Pioneer stated that the compositional assessment included analytes based on the OECD consensus document on compositional considerations for new varieties of corn.[11] The analytes included proximates (ash, carbohydrate (by calculation), fat, protein, and fiber), fatty acids, amino acids, minerals, vitamins, and anti-nutrients (*p*-coumaric acid, ferulic acid, furfural, inositol, phytic acid, raffinose, and trypsin inhibitor). Results of the analysis were evaluated in the context of published literature values as well as tolerance intervals derived from Pioneer's proprietary accumulated data from non-genetically engineered commercial corn lines. Pioneer concludes that the levels of the key components in DP202216 corn grain fall within the range of natural variation for corn with a history of safe use in food.

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[9] Hérouet, C., et al., (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**: 134-49; Brooks K.J., (2000). PAT microbial protein (FL): acute oral-toxicity study in CD-1 mice. Dow AgroSciences LLC, Study No. 991249 (unpublished).

[10] Anderson, J.A., et al. (2019). Composition of forage and grain from genetically modified DP202216 maize is equivalent to non-modified conventional maize (*Zea mays* L.). *GM Crops & Food* **10**: 77-89.

[11] 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. Organisation for Economic Co-operation and Development, Paris, France.

Conclusion

Based on the information provided by Pioneer and other information available to FDA, we have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from DP202216 corn. We consider the consultation with Pioneer on DP202216 corn to be complete.

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