Biotechnology Notification File No. 000182
CVM Note to the File

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To: Administrative Record, BNF No. 000182

Subject: Event DP915635 Corn

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Purpose

This document summarizes the Food and Drug Administration (FDA) Center for Veterinary Medicine's (CVM, we) evaluation of biotechnology notification file (BNF) number 000182. Pioneer Hi-Bred International, Inc. (Pioneer) submitted a safety and nutritional assessment for a genetically engineered (GE) corn, transformation event DP-915635-4 (hereafter referred to as DP915635 corn) and additional information afterwards. CVM evaluated the information in Pioneer's submissions to ensure that regulatory and safety issues regarding animal food derived from DP915635 corn have been resolved prior to commercial distribution. FDA's Center for Food Safety and Applied Nutrition summarizes its evaluation of DP915635 corn in human food in a separate document.

In CVM's evaluation, we considered all of the information provided by Pioneer as well as publicly available information and information in the agency's files. Here we discuss the outcome of the consultation for animal food use, but do not intend to restate the information provided in the final consultation in its entirety.

Intended Effects

The intended effects of the modifications in DP915635 corn are protection against corn rootworm pests and tolerance to glufosinate ammonium herbicides. To confer the insect resistance trait, Pioneer introduced the *ipd079Ea* gene from *Ophioglossum pendulum* that encodes the IPD079Ea protein, which confers resistance against certain coleopteran pests. To confer tolerance to glufosinate ammonium herbicides, Pioneer introduced a modified *pat* gene from *Streptomyces viridochromogenes* that encodes phosphinothricin N-acetyltransferase (PAT). Finally, Pioneer introduced the

phosphomannose isomerase (*pmi*) gene from *Escherichia coli* that encodes phosphomannose isomerase (PMI) that serves as a selectable marker.

Regulatory Considerations

The purposes of this evaluation are: (1) to assess whether Pioneer has introduced into animal food a substance requiring premarket approval as a food additive and (2) to determine whether use of the new plant variety in animal food raises other regulatory issues with respect to the Federal Food, Drug, and Cosmetic Act (FD&C Act).

The Environmental Protection Agency (EPA) defines a plant-incorporated protectant (PIP) 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" (40 CFR 174.3). EPA regulates PIPs under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the FD&C Act. Under EPA regulations, the IPD079Ea protein and the genetic material used to express it in DP915635 corn are considered pesticidal substances, and the PMI protein and the genetic material used to express it are considered to be inert ingredients. In addition, the genetic material and any potential expression products used to create a "landing pad"[1] for the PIP are considered inert ingredients. Therefore, the safety assessment of these products falls under the regulatory purview of EPA.

EPA also regulates herbicides under the FIFRA and the FD&C Act. Under EPA regulations, the herbicide residues in DP915635 corn are considered pesticidal residues.

Genetic Modification and Characterization

Introduced DNA and Transformation Method

Pioneer conducted two separate transformations to generate DP915635 corn. The purpose of the first transformation was to create a landing pad for insertion of expression cassettes. One of the characterized lines from the first transformation was then transformed with plasmid PHP83175 using *Agrobacterium*-mediated transformation. The transfer-DNA (T-DNA) region within plasmid PHP83175, that was inserted in the genome of DP915635 corn, consists of the following expression cassettes which lie between two flippase recombination target sites FRT1 and FRT6:

- *pmi* gene including the terminator regions from the *Solanum tuberosum proteinase inhibitor II* gene and the *Zea mays 19-kDa zein* gene;[2]
- *pat* gene from *Streptomyces viridochromogenes*, which was codon-optimized to improve expression of this protein in DP915635 corn, with regulatory elements, including promoter and intron regions from the *actin* gene from *Oryza sativa*, as well as terminator regions of the *cauliflower mosaic virus 35S* gene, the *Sorghum bicolor ubiquitin* gene, and the *Sorghum bicolor* gamma-*kafarin* gene;
- *ipd079Ea* gene with regulatory elements, including enhancer region from the *Sorghum bicolor root cortical RCc3* gene, promoter region upstream of a *Zea*

[1] The landing pad is a specific DNA sequence incorporated into the genome of a host plant to facilitate targeted insertion of expression cassettes.

[2] The promoter sequence for the *pmi* gene was inserted into the genome when the landing pad was created.

mays PCO118362 mRNA sequence, intron region from the *Zea mays* ortholog of an *Oryza sativa hypothetical protein (zm-HPLV9)* gene, as well as terminator regions from the following genes: *Sorghum bicolor subtilisin-chymotrypsin inhibitor 1B*, the *Zea mays* W64 line *27-kDa gamma zein*, the *Arabidopsis thaliana ubiquitin 14*, and the *Zea mays In2-1*.

Transient expression of the flippase[3] recombinase induces the removal of specific DNA sequences within the landing pad and insertion of the *pmi*, *pat*, and *ipd079Ea* expression cassettes into the landing pad.

Following transformation, plants were regenerated and grown to maturity. Pioneer characterized the DP915635 corn insertion event using Southern-by-Sequencing[4] and bioinformatics analyses. Based on its analysis, Pioneer concludes that a single copy of the inserted DNA containing the three intended gene expression cassettes is present in the corn genome without rearrangements. Additionally, Pioneer states that no unintended DNA sequences were present in DP915635 corn.

Stability and Inheritance

Pioneer confirmed stability of the inserted DNA in DP915635 corn by conducting Southern blot analysis using genomic DNA obtained from multiple generations of DP915635 corn. Pioneer also assessed segregation of the intended DNA using event-specific quantitative polymerase chain reaction (qPCR) and herbicide tolerance phenotyping. Chi-square statistical analysis was carried out to compare observed segregation ratios generated by qPCR to the expected segregation ratios for different generations. Pioneer concludes that the desired genotypes, presence of *pmi*, *pat*, and *ipd079Ea* genes, were stably integrated at a single locus and segregated according to Mendelian principles.

Pioneer performed bioinformatics analyses using the nucleotide sequences obtained for the inserted DNA and their corresponding flanking genomic junction sequences to determine whether insertion of the introduced DNA created any potential open reading frames (ORFs) that could encode for putative polypeptides. Pioneer reports that none of the putative polypeptides had alignments with proteins in its toxin database.[5]

Protein Safety

Pioneer summarized the information available on the safety of the PAT protein. Pioneer states that the PAT protein expressed in DP915635 corn is identical to the PAT protein expressed in other GE plant varieties that have been safely grown and used in the United States. Pioneer refers to authorizations by regulatory authorities in 20 different countries and/or regions relating to the presence of PAT protein in human and animal

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[3] The transformation plasmid contains a recombinase gene outside of the two recombination target sites. This recombinase gene is not incorporated into the corn genome but is transiently expressed during the transformation process.

[4] Southern-by-Sequencing technique utilizes probes that are homologous to the transformation plasmid to capture DNA sequences that hybridize to the probe sequences. The capture DNA is then sequenced using whole genome sequencing and the results are analyzed using bioinformatics tools.

[5] Pioneer states its internal toxin database is updated annually and contains a collection of protein sequences from UniProtKB/Swiss-Prot that are filtered for “function by keywords that could imply toxicity or adverse health effects (e.g., toxin, hemagglutinin, vasoactive, etc.)”.

food. Pioneer also cites an article by Hérouet et al. (2005), which summarized the safety of the PAT protein in transgenic plants.[6,7]

Additionally, Western blot analysis was used to confirm that the PAT protein derived from DP915635 corn has the expected molecular weight, and same immunoreactivity as microbially-derived PAT protein. Pioneer also conducted an *in silico* analysis using the amino acid sequence for the PAT protein to determine whether there were any potential polypeptides that align with sequences in its toxin database and concluded that the amino acid sequence for PAT protein did not align with any sequences in its database. Based on previous risk assessment and its bioinformatics analyses, Pioneer concludes that the PAT protein expressed in DP915635 corn is unlikely to raise safety concerns.

Expression Levels of Protein in DP915635 Corn

Pioneer quantified the amounts of the PAT protein in DP915635 corn. Tissue samples were collected for root (V6, V9, R1, and R4 growth stages), leaf (V9, R1, and R4 growth stages), pollen (R1 growth stage), forage (R4 growth stage), and grain (R6 growth stage) during the 2019 growing season at six sites in the United States and one site in Canada.[8] Each site included DP915635 corn and a near-isoline control maize, which were planted in a randomized complete block design containing four blocks. The amounts of the PAT protein present in the samples were determined by enzyme linked immunosorbent assay. Pioneer reports that the highest concentration of the PAT protein, 80 nanograms (ng) of PAT protein/milligram (mg) dry weight (DW) in pollen, was obtained at R1 growth stage. The concentrations of the PAT protein in leaf samples ranged from 3.8 to 7.5 ng/mg DW at R4 and R1 growth stages, respectively. The concentrations of the PAT protein in forage (R4 stage) and grain (R6 growth stage) were 9.3 ng/mg DW and 6.4 ng/mg DW, respectively.

Pioneer notes that a weight of evidence approach was used to demonstrate that the PAT protein expressed in DP915635 corn is identical to the PAT protein that was expressed in other new plant varieties that have been safely grown and used in the United States. Pioneer concludes that the risk of adverse effects from the PAT protein in DP915635 corn is low.

Animal Food Use

Corn (*Zea mays* L.) is a commodity crop grown worldwide for various uses, including human and animal food. Corn is an important crop for animal food. Corn grain and by-products of corn processing may be included in diets for most animal species. Corn silage is a readily digestible, high energy, fermented forage product. It is fed primarily to ruminants (e.g., cattle, sheep and goats). For animal nutrition, corn is considered to be

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[6] Hérouet, C., D.J. Esdaile, B.A. Mallyon, E. Debruyne, A. Schulz, T. Currier, K. Hendrickx, R.-J. van der Klis, and D. Rouan. 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. Regul. Toxicol. Pharmacol. 41:134-149.

[7] Hérouet and coworkers (2005) addresses the safety of PAT proteins, which are encoded by the *pat* gene from *Streptomyces viridochromogenes* and *bar* gene from *Streptomyces hygroscopicus*.

[8] Pioneer states all of the root R1 samples and some of the leaf R1 and root R4 samples were affected due to a lyophilizer issue during sample processing, thus data for the affected samples were not reported.

an important source of energy, essential fatty acids, and some of the essential amino acids.

Composition

Scope of Analysis

Pioneer analyzed the nutrient composition of forage and grain obtained from DP915635 corn, non-GE, near-isogenic corn (control), and 20 non-GE corn varieties (four at each site) that were grown and harvested under similar conditions (reference varieties). The selected components were based on the Organisation for Economic Cooperation and Development (OECD) corn composition consensus document.[9]

Study Design

Pioneer conducted field trials in 2019. There were eight locations, with seven locations in the United States and one in Canada. The corn varieties were planted using a randomized complete block design with four replicate plots at each field site. One forage sample was harvested at the R4 growth stage; plants were chopped into sections (≤ 7.6 centimeters in length) prior to pooling and sub-sampling. Ears were husked and shelled and grain samples (R6 growth stage) from each replicate at each location were pooled prior to sub-sampling. Forage and grain samples were transported in chilled containers and then stored frozen until compositional analysis was performed.

Pioneer statistically compared each component for DP915635 corn and the control across locations using a linear mixed model analysis of variance. The false discovery rate (FDR) method was also used to control for false positive outcomes across all components analyzed using linear mixed models. Fisher's exact test was utilized when 50% or more (but less than 100%) of the values for a component were below the lower limit of quantification (LLOQ) for either DP915635 corn or control. Components were expressed on a dry matter basis, with the exception of fatty acids, prior to statistical analysis. Forage moisture was not included in the statistical analyses. When a value for a component was less than the LLOQ for the analytical method, a value equal to half the LLOQ was assigned to this sample. Differences between DP915635 corn and control with a $P \leq 0.05$ in the mixed model or Fisher's exact test were considered to be statistically different. For each component, means, ranges, and non-adjusted and FDR adjusted P-values were reported. Any observed differences in a component between DP915635 corn and control were compared with the range of values obtained for the reference varieties grown under the same conditions and values obtained for non-GE corn varieties that were grown in the United States, Canada, and South America between 2003 and 2018 (described as 167 commercial corn lines and 171 unique environments). If the range of DP915635 corn contained individual values that fell outside this range, then these values were compared to the range of values in the public literature.

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[9] Organisation for Economic Co-operation and Development. 2002. Consensus Document on Compositional Considerations for New Varieties of Corn (*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. OECD, Paris.

Results of Analyses – Forage

Pioneer reports values for proximates (crude protein, crude fat, carbohydrates by calculation, and ash), fiber (crude fiber, acid detergent fiber (ADF), and neutral detergent fiber (NDF)), calcium, and phosphorus. Pioneer found no statistically significant differences between the mean values for these components in forage from DP915635 corn and the control. Pioneer concludes that forage obtained from DP915635 corn is comparable to forage from conventional corn varieties.

Results of Analyses – Grain

Pioneer measured proximates, fiber components (crude fiber, ADF, NDF, and total dietary fiber), 18 amino acids, nine minerals, 15 fatty acids (four of the fatty acids were not included in the statistical analyses because all of the values fell below the LLOQ[10]), 11 vitamins plus total tocopherols (for vitamin B_2, beta-tocopherol, and delta-tocopherol, the majority or all of the values were below the LLOQ), four secondary metabolites (for furfural, all of the values were below the LLOQ), and three anti-nutrients. Pioneer reports that there were no statistically significant differences between DP915635 corn and control in any of the proximates, fiber components, minerals, secondary metabolites, and anti-nutrients. Although Pioneer reports statistically higher concentrations of palmitoleic, stearic, and linoleic acids and lower concentrations of oleic and lignoceric acids, the false discovery rate adjusted probability values for these fatty acids were not statistically significant. Pioneer also states that the mean values for these fatty acids fell within the range of values for the reference varieties. Pioneer reports statistically significant differences in methionine and alpha-tocopherol, whereas the false discovery rate adjusted probability values were not statistically different. Pioneer also states that the mean values for methionine and alpha-tocopherol fell within the range of values for the reference varieties. Pioneer concludes that DP915635 corn is comparable in nutrient composition to conventional corn varieties.

Summary of Compositional Analyses

Pioneer highlights that the genetic modification does not meaningfully affect nutrient composition and nutritional value of forage and grain derived from DP915635 corn. Pioneer concludes that DP915635 corn is comparable to corn varieties that are currently used in animal food in the United States.

Conclusion

CVM evaluated Pioneer's submissions to determine whether DP915635 corn raises any safety or regulatory issues with respect to its use in animal food. Based on the information provided by Pioneer and other information available to the agency, CVM did not identify any safety or regulatory issues under the FD&C Act that would require further evaluation at this time.

Pioneer concludes that DP915635 corn and the animal food derived from it are as safe as and are not materially different in composition or any other relevant parameter from conventional corn varieties grown, marketed, and consumed in the United States. At

[10] These included lauric, myristic, eicosadienoic, and heptadecenoic acids.

this time, based on Pioneer's data and information, CVM considers Pioneer's consultation on DP915635 corn for use in animal food to be complete.

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