

## Biotechnology Notification File No. 000175 Center for Veterinary Medicine Note to the File

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**To:** Administrative Record, BNF No. 000175

**Subject:** event DP 23211 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 000175. Pioneer Hi-Bred International, Inc. (Pioneer) submitted a safety and nutritional assessment for a genetically engineered corn, transformation event DP 023211-2 (hereafter referred to as DP23211 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 DP23211 corn have been resolved prior to commercial distribution. FDA's Center for Food Safety and Applied Nutrition summarizes its evaluation of uses of DP23211 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 first intended effect of the modification in DP23211 corn is protection against western corn rootworm. To confer this trait, Pioneer introduced DNA sequences containing inverted repeat nucleotide sequences of the *smooth septate junction protein 1* (*dvssj1*) gene from western corn rootworm (*Diabrotica virgifera virgifera*), which produces double-stranded ribonucleic acid (dsRNA) transcripts that trigger RNA-

mediated silencing mechanism.<sup>1</sup> Pioneer also introduced the *ipd072Aa* gene from *Pseudomonas chlororaphis* that encodes the IPD072Aa protein, which confers protection against certain coleopteran pests. Thirdly, Pioneer introduced a modified *pat* gene from *Streptomyces viridochromogenes* that was codon-optimized for expression in corn. This gene encodes phosphinothricin N-acetyltransferase (PAT), which confers tolerance to glufosinate ammonium herbicide. Finally, Pioneer introduced the *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 *dvssj1* dsRNA expression cassette and the *ipd072Aa* expression cassette in DP23211 corn and the resulting expression products are considered pesticidal substances, and the *pmi* expression cassette and PMI protein are considered to be inert ingredients. In addition, the genetic material and any potential expression products used to create a “landing pad”<sup>2</sup> for the two PIP expression cassettes and the *pmi* gene expression cassettes is considered an inert ingredient.

EPA also regulates herbicides under the FIFRA and the FD&C Act. Under EPA regulations, the herbicide residues in DP23211 corn are considered pesticidal residues.

## Genetic Modification and Characterization

### Introduced DNA and Transformation Method

Pioneer conducted two separate transformations to generate DP23211 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 PHP74643 using disarmed *Agrobacterium*-mediated transformation. The transfer-DNA (T-DNA) region within plasmid PHP74643 contained the following four expression cassettes between flippase recombination target sites as well as other non-coding genetic elements between right and left border sequences:

- Flippase recombination target site from *S. cerevisiae* (FRT1);
- *pmi* gene including the terminator region from the *Solanum tuberosum* proteinase inhibitor II gene and terminator region from the *Zea mays* 19-kDa zein gene;

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<sup>1</sup> The ingestion of *dvssj1* dsRNA by western corn rootworm causes the suppression of the smooth septate junction 1 protein in the intestinal lining. The reduction in DvSSJ1 protein leads to the disruption of the gut epithelial barrier and cellular deformities that are lethal to western corn rootworm.

<sup>2</sup> The landing pad is a specific DNA sequence incorporated into the genome of a host plant to facilitate targeted insertion of expression cassettes.

- *pat* gene from *Streptomyces viridochromogenes*, which was codon-optimized to improve expression of this protein in DP23211 corn, expression cassette with regulatory elements, including promoter and intron regions of the *actin* gene from *Oryza sativa* and 35S gene terminator region from cauliflower mosaic virus;
- *dvssj1* dsRNA expression cassette with regulatory elements, including promoter, 5' untranslated region (UTR), and intron from *ubiquitin 1* gene from *Z. mays* upstream of the dsRNA *dvssj1* construct and terminator region of *27-kDa gamma zein* gene from *Z. mays*, terminator region *ubiquitin 1* gene from the *Arabidopsis thaliana*, and terminator region of the *ln201* gene from *Z. mays*;
- *ipdo72Aa* expression cassette with regulatory elements, including promoter region from the banana streak virus and intron region of *calmodulin 5* gene from *Z. mays* and terminator region of putative *mannose-binding protein superfamily* gene from *A. thaliana*; and
- Modified flippase recombination target site from *S. cerevisiae* (*FRT87*).

Transient expression of the flippase<sup>3</sup> recombinase induces the removal of DNA sequences within the landing pad and insertion of the *pmi*, *pat*, *dvssj1* dsRNA, and *ipdo72Aa* expression cassettes into the landing pad.

Following transformation, plants were regenerated and grown to maturity. Pioneer characterized the DP23211 corn insertion event using Southern-by-Sequencing<sup>4</sup> and bioinformatics analyses. Based on its analysis, Pioneer concludes that the four intended gene cassettes were inserted into a single intended location in the corn genome and that there were no rearrangements or truncations in the inserted DNA. Additionally, Pioneer states that no unintended DNA sequences were present in DP23211 corn.

### Inheritance and Stability

Pioneer confirmed stability of the inserted DNA in DP23211 corn by conducting Southern blot analysis using genomic DNA obtained from five generations of DP23211 corn. Pioneer also assessed segregation of the intended DNA using event-specific quantitative polymerase chain reaction and herbicide tolerance phenotyping in homozygous and hemizygous plants treated with glufosinate ammonium herbicide. The segregation ratios of the inserted DNA and herbicide tolerance trait were analyzed by Chi-square analysis. Pioneer concludes that the desired genotypes, presence of *ipdo72Aa*, *pat*, and *pmi* genes and *dvssj1* dsRNA cassette, were stably integrated at a single loci and segregated according to expected 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.

<sup>3</sup> The PHP74643 plasmid contains a *flippase* recombinase gene outside of the two flippase recombination target sites. This *flippase* gene is not incorporated into the corn genome, but is transiently expressed during the transformation process.

<sup>4</sup> 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.

## Protein Safety

Pioneer summarized the information available on the safety of the PAT protein. Pioneer states that the PAT protein expressed in DP23211 corn is identical to the PAT protein expressed in other genetically engineered (GE) plant varieties that have been safely grown and used in the U.S. including in DP202216 corn, the subject of BNF No. 000171. 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 food. Pioneer also cites an article by Hérouet et al. (2005).<sup>5</sup> Pioneer concludes that there is reasonable certainty of no harm resulting from the inclusion of the PAT proteins in human food or animal food.

## Expression Levels of Proteins in DP23211 Corn

Pioneer states that expression levels of PAT protein in DP23211 corn were similar to or lower than the levels that were found in DP202216 corn for leaves, pollen, whole plants, and grain at maturity. The highest mean values were observed (9.2 nanograms/milligram tissue dry weight (DW)) in whole plant material at R1 maturity stage and decreased to 1.1 nanograms/milligram of dry weight by the R6 growth stage. The average concentration of PAT protein in grain at maturity was 5.1 nanograms/milligram tissue DW.

Pioneer notes that a weight of evidence approach was used to demonstrate that the PAT protein expressed in DP23211 corn is identical to the PAT protein that was expressed in other new plant varieties that have been safely grown and used in the U.S. Pioneer concludes that the risk of adverse effects from the PAT protein in DP 23211 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 an important source of energy, essential fatty acids, and some of the essential amino acids.

## Composition

### Scope of Analysis

Pioneer determined whether there were any unintended changes in the nutrient composition of forage and grain obtained from DP23211 corn when compared to a non-GE, near-isogenic corn (control), and 14 non-GE corn varieties (four at each site) that were grown and harvested under similar conditions (reference varieties). The selected

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<sup>5</sup> 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. 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*.

components were based on the Organisation for Economic Cooperation and Development (OECD) corn composition consensus document.<sup>6</sup>

### Study Design

Pioneer conducted field trials in 2018. 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 ( $\leq 7.6$  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 DP23211 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 of the values for a component were below the lower limit of quantification (LLOQ) for either DP23211 corn or control. Components were expressed on a dry matter basis, with the exception of fatty acids, prior to statistical analysis. Forage and grain moisture were 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 DP23211 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 DP23211 corn and control were compared with 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 2015 (described as 93 commercial corn lines and 88 unique environments). If the range of DP23211 corn contained individual values that fell outside this range, then these values were compared to the range of values in the public literature.

### Results of Analyses – Forage

Pioneer reports values for proximates (crude protein, crude fat, carbohydrates by calculation, and ash), 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 DP23211 corn and the control. Pioneer concludes that forage obtained from DP23211 corn is comparable to forage from conventional corn varieties.

### Results of Analyses – Grain

Pioneer measured proximates, fiber components (ADF, NDF, crude fiber, and total dietary fiber), 18 amino acids, nine minerals, 16 fatty acids (four of the fatty acids were

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<sup>6</sup> Organisation of Economic Cooperation 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.



not included in the statistical analyses because most of the values fell near or below the LLOQ<sup>7</sup>), 11 vitamins plus total tocopherols (for vitamin B<sub>2</sub>, beta-tocopherol, and gamma-tocopherol most or all of the values were below the LLOQ), four secondary metabolites (for furfural most of the values were below the LLOQ), and three anti-nutrients. Pioneer reports that there were no statistically significant differences between DP23211 corn and control in any of the proximates, fiber components, amino acids, and minerals. Although Pioneer reports a statistically higher concentration of oleic acid and lower concentrations of arachidic and eicosenoic 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 vitamin B<sub>6</sub> and alpha-tocopherol, whereas the false discovery rate adjusted probability values were not statistically different. Pioneer notes that there was considerable variability in the values obtained for vitamin B<sub>6</sub>, but the values fell within the range of values in the public literature. There were no statistically significant differences between DP23211 corn and control in any of the secondary metabolites or anti-nutrients, with the exception of *p*-coumaric acid. The false discovery rate adjusted probability for *p*-coumaric acid was not statistically significant and the mean values for each of the secondary metabolites and anti-nutrients fell within the range of values observed for the reference varieties. Pioneer concludes that DP23211 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 DP23211 corn. Pioneer concludes that DP23211 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 DP23211 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 DP23211 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 this time, based on Pioneer's data and information, CVM considers Pioneer's consultation on DP23211 corn for use in animal food to be complete.

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<sup>7</sup> These included myristic, heptadecenoic, eicosadienoic, and erucic acids.