



## Biotechnology Notification File No. 000196 CVM Note to the File

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**From:** Ramavati Pal, Ph.D.

**To:** Administrative Record, BNF No. 000196

**Subject:** Event DP51291 Corn

**Keywords:** Corn, *Zea mays L.*, *ipd072Aa* gene, IPD072Aa protein, *Pseudomonas chlororaphis*, Insect resistance, *Phosphinothricin acetyltransferase (maize optimized-pat)* gene, PAT protein, *Streptomyces viridochromogenes*, Glufosinate-ammonium tolerant, Herbicide tolerance, *Phosphomannose isomerase (pmi)* gene, PMI protein, *Escherichia coli*, OECD Identifier DP-Ø51291-2, Pioneer Hi-Bred International, Inc.

### Purpose

This document summarizes the Food and Drug Administration (FDA) Center for Veterinary Medicine's (CVM, we) evaluation of biotechnology notification file (BNF) number 000196. Pioneer Hi-Bred International, Inc. (Pioneer) submitted a safety and nutritional assessment for a genetically engineered (GE) corn, transformation event DP-51291-2 (hereafter referred to as DP51291 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 DP51291 corn have been resolved prior to commercial distribution. FDA's Center for Food Safety and Applied Nutrition summarizes its evaluation of DP51291 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

One of the intended effects of the modifications in DP51291 corn is to provide resistance to certain susceptible corn rootworm (CRW) pests. To confer the insect resistance trait, Pioneer introduced the *ipd072Aa* gene from *Pseudomonas chlororaphis* that encodes for the IPD072Aa protein. The second intended effect is to confer tolerance to glufosinate-ammonium herbicides. For this, Pioneer introduced a corn-optimized version of the *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). Pioneer states that both PAT and PMI proteins serve as selectable markers.

### Regulatory Considerations

The purpose of this evaluation is to determine whether use of the new plant variety in animal food raises safety or regulatory issues under 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 IPDO72Aa protein and the genetic material used to express it in DP51291 corn are considered pesticidal substances, and the PMI and PAT proteins and the genetic material used to express them are considered to be inert ingredients. Therefore, the safety assessment of these products falls under the regulatory purview of EPA.

### Stability and Inheritance

Pioneer characterized the insertion event and genomic stability of the insert in the DP51291 corn genome using Southern-by-Sequencing method. To confirm genomic stability, Pioneer performed whole genome sequencing on multiple breeding generations of DP51291 corn. Pioneer detected two identical junction sequences<sup>1</sup> in each of the generations tested, indicating a single site of insertion, and no junction sequences were observed in the control lines.

In addition, Pioneer assessed inheritance of the inserted transfer DNA in DP51291 corn in both inbred and outbred lines using southern blot, polymerase chain reaction (PCR) (real time quantitative PCR and qualitative PCR), and herbicide tolerance phenotype. The results of Chi-square analysis of the segregation data from five generations show that the segregation pattern of the insert is consistent with Mendelian principles of inheritance for a single locus. Pioneer concludes that the insert in DP51291 corn was integrated at one locus and is stably transmitted across multiple generations.

### Animal Food Use

Pioneer states DP51291 corn is expected to be grown for the same uses as currently commercialized corn, and no new or specialty human or animal food uses are anticipated. Pioneer references the Organisation for Economic Co-operation and Development (OECD) consensus document on compositional considerations of maize<sup>2</sup> and states that corn (*Zea mays L.*) is a commodity crop grown worldwide for various

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<sup>1</sup> Zastrow-Hayes, Gina M et al. “Southern-by-Sequencing: A Robust Screening Approach for Molecular Characterization of Genetically Modified Crops.” *The plant genome* vol. 8,1 (2015): eplantgenome2014.08.0037.

<sup>2</sup> OECD (2002) Consensus Document on Compositional Considerations for New Varieties of Maize (*Zea Mays*): Key Food and Feed Nutrients, Anti Nutrients and Secondary Plant Metabolites. Organisation for Economic Cooperation and Development, ENV/JM/MONO(2002)25

uses, including human and animal food. Pioneer notes that corn has a long history of safe use.

## Composition

### Scope of Analysis

Pioneer analyzed the nutrient composition of DP51291 corn, a non-GE corn variety with a similar genetic background (control), and non-GE commercial corn varieties. All were harvested under similar agronomic field conditions. Compositional analyses on grain and forage samples were reported for components listed in the OECD corn composition consensus document.

### Study Design

Pioneer conducted field trials in 2021 at eight sites in the United States and Canada. A randomized complete block design with four replicate plots at each field site was used. Each block included DP51291 corn, non-GE near isoline control corn, and four non-GE commercial corn reference lines and were grown under normal agronomic field conditions for their respective regions. Pioneer harvested grain and forage from each replicate within each site for composition analysis. Grain was harvested at physiological maturity (R6 growth stage). Forage samples were harvested at R4 growth stage. Both grain and forage samples from the field sites were placed on dry ice and transferred to a freezer. The samples were shipped frozen from each replicate at each site to analytical lab for nutrient compositional analyses.

For statistical analysis, Pioneer combined composition data for each component from DP51291 corn and the control across locations using a linear mixed model and Fisher's exact test with site and replicate as random factors. Mixed model analyses were used to test at the level of  $P < 0.05$  for differences between DP51291 corn and control. Fisher's exact test was used if analytes did not meet criteria for enough observations above the LLOQ<sup>3</sup>. Additionally, false discovery rate (FDR) was used to conclude a significant difference between DP51291 corn and control. Pioneer states that when a statistically significant difference was identified, the respective range of individual values from DP51291 corn was compared to a tolerance interval<sup>4</sup>. If the range of DP51291 corn contained individual values outside the tolerance interval, it was then compared to the respective literature range. These literature ranges included values for conventional corn varieties in the Agriculture and Food Systems Institute (AFSI) Crop Composition Database (CCDB)<sup>5</sup> and Codex<sup>6</sup> or in the scientific literature<sup>7</sup>. Results were all expressed

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<sup>3</sup> Lower limit of quantification

<sup>4</sup> Pioneer states the tolerance intervals were derived from Pioneer and Dow AgroSciences accumulated data from non-GE maize lines, which were grown in commercial maize growing regions between 2003 and 2020 in the United States, Canada, Chile, Brazil, and Argentina.

<sup>5</sup> AFSI (2022) Crop Composition Database, Version 9.0. Agriculture & Food Systems Institute, <https://www.cropcomposition.org>

<sup>6</sup> Codex Alimentarius Commission (2019) Codex Standard for Named Vegetable Oils. Codex Alimentarius, CXS 210-1999

<sup>7</sup> Cong B, Maxwell C, Luck S, Vespestad D, Richard K, Mickelson J, Zhong C (2015) Genotypic and Environmental Impact on Natural Variation of Nutrient Composition in 50 Non Genetically Modified Commercial Maize Hybrids in North America. *Journal of Agricultural Food Chemistry* 63: 5321-5334

on a dry matter basis prior to statistical analyses except for fatty acids, which were expressed on a percent of total fatty acids basis.

### Results of Analyses

For 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 significant differences between the control and DP51291 corn in these components and, therefore, Pioneer concludes there are no biologically meaningful differences from an animal food safety perspective.

For grain, Pioneer chemically analyzed proximates, fiber (crude fiber, ADF, NDF, and total dietary fiber), moisture, 18 amino acids, 15 fatty acids, nine minerals, eight vitamins, four anti-nutrients and three secondary metabolites. Pioneer noted that four of the fatty acids and furfural were not statistically analyzed because all the observations fell below the LLOQ. Pioneer reports statistically significant differences between the control and DP51291 corn in the levels of five components (oleic acid, eicosenoic acid, lignoceric acid, copper and ferulic acid) after FDR adjustment. For these components, the mean difference between DP51291 corn and the control was less than the range of values for the control and within the mean ranges of the non-GE varieties with a history of safe use. Pioneer concludes that the differences in these components between DP51291 corn and the control are not biologically meaningful from an animal food safety perspective.

### Summary of Compositional Analyses

Pioneer states based on the results from the compositional analyses that forage and grain obtained from DP51291 corn are not biologically different from those of the control and reference varieties. Pioneer concludes that these results support the conclusion that forage and grain obtained from DP51291 corn are compositionally comparable to the control in the levels of key nutrients, anti-nutrients, and secondary metabolites.

### Conclusion

CVM evaluated Pioneer's submissions to determine whether DP51291 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 DP51291 corn and the animal foods derived from it are as safe as and are not materially different in composition or any other relevant parameter from other corn varieties now grown, marketed, and consumed. At this time, based on

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OECD (2002) Consensus Document on Compositional Considerations for New Varieties of Maize (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.

Watson SA (1982) Corn: Amazing Maize. General Properties. In IA Wolff, ed, CRC Handbook of Processing and Utilization in Agriculture, Vol 2. CRC Press, Boca Raton, pp 3-29

Pioneer's data and information, CVM considers Pioneer's consultation on DP51291 corn for use in animal food to be complete.

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