Biotechnology Notification File No. 000190
CVM Note to the File

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To: Administrative Record, BNF No. 000190

Subject: Event DP910521 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 000190. Pioneer Hi-Bred International, Inc. (Pioneer) submitted a safety and nutritional assessment for a genetically engineered (GE) corn, transformation event DP-910521-2 (hereafter referred to as DP910521 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 DP910521 corn have been resolved prior to commercial distribution. FDA's Center for Food Safety and Applied Nutrition summarizes its evaluation of DP910521 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 DP910521 corn is to provide resistance to certain susceptible lepidopteran pests. To confer the insect resistance trait, Pioneer introduced the *cry1B.34* gene[1] from *Bacillus thuringiensis* that encodes for the Cry1B.34 protein. The second intended effect is to confer tolerance to glufosinate-ammonium herbicides. For this, Pioneer introduced the *mo-pat* gene, a corn-optimized

[1] The *cry1B.34* gene, a chimeric gene comprised of sequences from a *cry1B-class* gene, the *cry1Ca1* gene, and the *cry9Db1* gene, all derived from strains of *Bacillus thuringiensis*.

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) 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 Cry1B.34 protein and the genetic material used to express it in DP910521 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"[2] for the PIP are considered inert ingredients. Therefore, the safety assessment of these products falls under the regulatory purview of the EPA.

Genetic Modification and Characterization

Introduced DNA and Transformation Method

Pioneer conducted two separate transformations to generate DP910521 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 PHP79620 using microprojectile bombardment. The transfer-DNA (T-DNA) region within plasmid PHP79620 that was inserted in the genome of DP910521 corn consists of the following expression cassettes which lie between two flippase recombination target sites FRT1 and FRT87:

- *pmi* gene including the terminator regions from the *Solanum tuberosum proteinase inhibitor II* gene and the *Zea mays 19-kDa zein* gene;
- *mo-pat* gene, a codon-optimized version of the *pat* gene from *S. viridochromogenes* to improve expression of this protein in DP910521 corn, with regulatory elements, including promoter and intron regions from the *actin* gene from *Oryza sativa*, as well as terminator regions of the *35S* gene from the cauliflower mosaic virus, and the *ubiquitin* and *gamma-kafarin* genes from *Sorghum bicolor*.
- *cry1B.34* gene with regulatory elements, including enhancer region from the mirabilis mosaic virus (MMV) genome, promoter region from the lamium leaf distortion-associated virus genome, intron region from the *translation initiation*

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

factor 6 gene from *Z. mays*, as well as terminator regions from the *ubiquitin* gene from *O. sativa*.

Transient expression of the flippase recombinase induces the removal of specific DNA sequences within the landing pad and insertion of the *pmi*, *mo-pat*, and *cry1B.34* expression cassettes into the landing pad.

Following each of the transformations, plants were regenerated, grown to maturity, and characterized. Pioneer characterized the DP910521 corn insertion event using Southern-by-Sequencing[3] on an Illumina platform to a depth of at least 100x for the captured sequences. Based on its bioinformatic 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 DP910521 corn.

Stability and Inheritance

Pioneer confirmed stability of the inserted DNA in DP910521 corn by conducting Southern blot analysis using genomic DNA obtained from multiple generations of DP910521 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 for the inserted DNA, as measured by qPCR, to the expected segregation ratios for different generations. Pioneer concludes that the desired genotypes, presence of single copy of *pmi*, *pat*, and *cry1B.34* 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.[4]

Protein Safety

Pioneer summarized the information available on the safety of the PAT protein. Pioneer states that the PAT protein expressed in DP910521 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 food. Pioneer also cites an article by Hérouet *et al.* (2005), which summarized the safety of the PAT protein in GE plants.[5] In addition, Pioneer states that FDA had reviewed the

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[3] 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 were mapped against the sequences of the transformation plasmid and control corn genome using bioinformatics tools.

[4] 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.)".

[5] 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

safety of the PAT protein in previous submissions (BNF 29, BNF 55, BNF 124 and BNF 142) and did not identify any safety issues relevant to human and animal consumption.

Additionally, Western blot analysis was used to confirm that the PAT protein derived from DP910521 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 assessments and its bioinformatics analyses, Pioneer concludes that the PAT protein expressed in DP910521 corn is unlikely to raise safety concerns.

Expression Levels of Protein in DP910521 corn

Pioneer quantified the amounts of the PAT protein in DP910521 corn. Tissue samples were collected for leaf (V6, V9, R1, and R4 growth stages), root (V9, R1, and R4 growth stages), pollen (R1 growth stage), stalk with leaf sheath removed (R1 growth stage), forage (R4 growth stage), and grain (R6 growth stage) during the 2020 growing season at five sites in the United States and one site in Canada. Each site included DP910521 corn and a near-isoline control corn, 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, 110 nanograms (ng) of PAT protein /milligram (mg) dry weight (DW) in leaf, was obtained at R1 and R4 growth stages. The concentrations of the PAT protein in forage (R4 stage) and grain (R6 growth stage) were 70 ng/mg DW and 10 ng/mg DW, respectively.

Pioneer notes that a weight of evidence approach was used to demonstrate that the PAT protein expressed in DP910521 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 DP910521 corn is low.

Animal Food Use

Pioneer states DP910521 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. The typical uses of corn-derived human and animal food are well documented (OECD, 2002)[6], including human food use of the kernels for oil, starch, grits, meal, flour, and use of the kernels or whole plant silage for animal food. Production and different methods of processing are also described in detail in the OECD maize composition consensus document.

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by the *pat* and *bar* sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants. Regul. Toxicol. Pharmacol. 41:134-149.

[6] Organisation for Economic Co-operation and Development. 2002. Consensus document on compositional considerations for new varieties of maize (*Zea mays*): Key food and feed nutrients, anti-nutrients, and secondary plant metabolites. OECD ENV/JM/MONO 25. OECD, Paris, France.

Composition

Scope of Analysis

Pioneer analyzed the nutrient composition of forage and grain obtained from DP910521 corn, non-GE near-isogenic corn (control), and four non-GE corn varieties at each site that were grown and harvested under similar conditions (reference varieties).[7] The components selected for analyses were based on the OECD maize composition consensus document.

Study Design

Pioneer conducted field trials in 2020. 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 location. One forage sample (combination of three plants) was harvested at the R4 growth stage; plants were chopped into sections (≤ 7.6 centimeters in length) prior to pooling and sub-sampling, resulting in one sample per replicate plot at each location. Ears were husked and shelled and grain samples (R6 growth stage) from each replicate plot 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 DP910521 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 DP910521 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 DP910521 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 DP910521 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 2019 (described as 184 commercial corn lines and 185 unique environments). If the range of DP910521 corn contained individual values that fell outside these ranges, 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 (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

[7] Pioneer reports 18 total reference corn varieties.

DP910521 corn and the control. Pioneer concludes that forage obtained from DP910521 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[8], 15 fatty acids[9], seven vitamins plus total tocopherols[10], four secondary metabolites[11] and three anti-nutrients. Pioneer reports that there were no statistically significant differences between DP910521 corn and control in any of the proximates, fiber components, amino acids, fatty acids, minerals, secondary metabolites, and anti-nutrients, with the exception of moisture and phytic acid. Pioneer states that FDR adjusted P-values were not different. Pioneer concludes that grain from DP910521 corn is comparable in nutrient composition to grain from 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 DP910521 corn. Pioneer concludes that DP910521 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 DP910521 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 DP910521 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 DP910521 corn for use in animal food to be complete.

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[8] For copper, one or more sample values were below the assay LLOQ for both DP910521 corn and control. For sodium, one or more sample values were below the assay LLOQ for the control.

[9] For lauric acid, all of the values were below the LLOQ and hence was not included in the statistical analyses. For heptadecenoic acid, the values for DP910521 corn were below the LLOQ.

[10] For Vitamin B1, one or more sample values were below the LLOQ for the control. Vitamin B2 and beta-tocopherol, were not included in the statistical analyses because the majority or all of the values were below the LLOQ.

[11] For furfural, all of the values were below the LLOQ and hence was not included in the statistical analyses.