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CENTER FOR VETERINARY MEDICINE

Biotechnology Notification File No. 000209
CVM Note to the File

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To: Administrative Record, BNF No. 000209

Subject: Event COR1591 Soybean

Keywords: Soybean, Glycine max (L.) Merr., PPO-1.5 protein, *ppo-1.5.1* gene, *Amaranthus tuberculatus*, DGT-28 EPSPS protein, *dgt-28 epsps.1* gene, *Streptomyces sviceus*, AAD-12 protein, *aad-12.1* gene, *Delftia acidovorans*, DSM-2 PAT protein, *dsm-2 pat* gene, *Streptomyces coelicolor*, Tolerance to glyphosate, glufosinate, phenoxy auxin herbicides, and PPO-inhibiting herbicides, herbicide tolerance, OECD identifier COR-Ø1591-7, Pioneer Hi-Bred International, Inc., Corteva Agriscience

Purpose

This document summarizes the Food and Drug Administration (FDA) Center for Veterinary Medicine's (CVM, we) evaluation of biotechnology notification file (BNF) number 000209. Pioneer Hi-Bred International, Inc. (Pioneer) submitted a safety and nutritional assessment for a genetically engineered (GE) soybean, transformation event COR1591 (hereafter referred to as COR1591 soybean), and additional information afterwards. CVM evaluated the information in Pioneer's submissions to ensure that regulatory and safety issues regarding animal food derived from COR1591 soybean have been resolved prior to commercial distribution. FDA's Human Foods Program summarizes its evaluation of uses of COR1591 soybean 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 COR1591 soybean are to confer tolerance to several herbicides. Pioneer states that the parental soybean variety was transformed with the *ppo-1.5.1* gene from *Amaranthus tuberculatus*, which encodes the PPO-1.5 protein that confers tolerance to PPO-inhibiting herbicides. To confer tolerance to glyphosate, Pioneer introduced the *dgt-28 epsps.1* gene from *Streptomyces sviceus* that

encodes 5-enolpyruvylshikimate-3-phosphate synthase (DGT-28 EPSPS). Additionally, COR1591 soybean expresses a copy of the *dsm-2 phosphinothricin N-acetyltransferase* (*pat*) gene from *Streptomyces coelicolor*, which encodes the DSM-2 PAT protein that confers tolerance to glufosinate herbicide. Pioneer also introduced the *aryloxyalkanoate dioxygenase* (*aad-12.1*) gene, derived from the *Delftia acidovorans*, that expresses the AAD-12 protein to confer tolerance to certain phenoxy auxin herbicides such as 2,4-D.

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).

Genetic Modification and Characterization

Introduced DNA and Transformation Method

Pioneer transformed [redacted] with vector [redacted] using [redacted] Pioneer states that the transfer-DNA (T-DNA) region within the plasmid contained expression cassettes between the left (LB) and right (RB) border sequences. These include:

- [redacted]

[redacted]. Following transformation, [redacted] After a successful transformant was selected, [redacted] These plants [redacted] were screened for the presence of T-DNA, absence of vector backbone sequences and phenotypic assessments.

Pioneer characterized the number of T-DNA inserts, the number of insert junctions, the absence of vector backbone sequences, and organization and intactness of each insert in COR1591 soybean using whole genome sequencing (WGS). The control [redacted]

[redacted], was used as the comparator in these analyses. Pioneer reports a minimum average read depth of [redacted]. Pioneer reports that a single copy of the T-DNA sequence was inserted into the soybean genome and that COR1591 soybean does not contain sequences from the vector backbone.

The stability of the inserted T-DNA sequences in COR1591 soybean across multiple breeding generations were evaluated by WGS followed by bioinformatics analyses. Pioneer reports that the single locus of integration was characterized in all breeding generations of the COR1591 soybean. Within multiple breeding generations, Pioneer collected genotypic and phenotypic segregation data to assess inheritance pattern, and these data were analyzed using Chi-square analysis. Pioneer concludes that the inserted DNA segregated as a single locus in accordance with the principles of Mendelian inheritance.

Pioneer performed bioinformatics analyses using the nucleotide sequences obtained for the T-DNA insertion and flanking genomic regions to determine whether insertion of the introduced DNA created any potential open reading frames (ORF) that could encode for putative polypeptides. This analysis included all translated stop codon-bracketed frames of length greater than eight amino acids. Pioneer evaluated the putative polypeptides against the NCBI non-redundantprotein database to determine the similarity of these putative polypeptides to known toxins. Pioneer reports that none of the putative polypeptides are known to be harmful to animals. Based on the results of bioinformatics analyses, Pioneer concludes that the T-DNA insertion does not lead to the production of putative polypeptides that would raise animal food safety concerns.

Protein Safety

Pioneer highlights that the AAD-12 protein expressed in COR1591 soybean is identical to the AAD-12 protein present in BNFs that have completed FDA's Biotechnology Consultations on Food from GE Plant Varieties and includes by reference information on safety of AAD-12 proteins in these crops.[1]

COR1591 soybean was genetically engineered to express the PPO-1.5 protein derived from the *protoporphyrinogen oxidase* (*PPX2L*) gene *A. tuberculatus*. PPO-1.5 is an enzyme that catalyzes the oxidation of protoporphyrinogen IX to protoporphyrin IX, thereby conferring tolerance to PPO-inhibiting herbicides in COR1591 soybean by maintaining the oxidation of protoporphyrinogen IX to protoporphyrin IX. To conduct a safety assessment of PPO-1.5 protein, Pioneer produced the PPO-1.5 protein in a microbial protein expression system. Pioneer confirmed the identity and biochemical equivalence of the PPO-1.5 protein expressed in microbial expression system and COR1591 soybean, by comparing results from amino acid sequence, molecular weight and immunoreactivity and glycosylation. Pioneer used a weight of evidence approach to assess the safety of the PPO-1.5 protein. Pioneer highlights that the donor organism, *A. tuberculatus*, commonly known as rough-fruited water-hemp, is a dicotyledonous plant

[1] Pioneer notes that AAD-12 protein produced in COR1591 soybean is identical to the AAD-12 protein expressed in DAS-44406-6 soybean (BNF No. 000133), DAS-81910-7 cotton (BNF No. 000142).

native to North America. Some species of *Amaranthus* have been cultivated with a long history of human and animal consumption. Pioneer demonstrated that the PPO-1.5 protein was rapidly digested with pepsin and pancreatin and rapidly lost activity at 75 °C. Pioneer carried out bioinformatics analysis and concluded that PPO-1.5 protein in COR1591 soybean does not share similarity with any sequences known to be toxic in the NCBI nr protein database. Pioneer states that there was no evidence of acute toxicity in mice that were dosed orally at 2000 milligrams of PPO-1.5 protein/kilogram of body weight. Taken together, Pioneer concludes that dietary exposure to PPO-1.5 protein in COR1591 soybean poses no meaningful risk to animal health.

COR1591 soybean was genetically engineered to express the DGT-28 EPSPS protein derived from *S. sviceus*. Pioneer demonstrated that the DGT-28 EPSPS protein was rapidly digested with pepsin and pancreatin and rapidly lost activity at 75 °C. Pioneer carried out bioinformatics analysis and concluded that DGT-28 EPSPS protein in COR1591 soybean does not share similarity with any sequences known to be toxic in the NCBI nr protein database. Pioneer states that there was no evidence of acute toxicity in mice that were dosed orally at 2000 milligrams of DGT-28 EPSPS protein/kilogram of body weight.

COR1591 soybean was also genetically engineered to express the DSM-2 PAT protein derived from *S. coelicolor*. To conduct safety assessment of the DSM-2 PAT protein, Pioneer produced the DSM-2 PAT protein in a microbial protein expression system. Pioneer confirmed the identity and biochemical equivalence of the COR1591 soybean-derived and microbially derived DSM-2 PAT proteins, by comparing results from amino acid sequence, molecular weight and immunoreactivity and glycosylation. Pioneer used a weight of evidence approach to assess the safety of the DSM-2 PAT protein. Pioneer highlights that the donor organism, *S. coelicolor*, commonly found in the soil and not known to be toxin. Pioneer demonstrated that the DSM-2 PAT protein was rapidly digested with pepsin and pancreatin and rapidly lost activity at 75 °C. Pioneer carried out bioinformatics analysis and concluded that DSM-2 PAT protein in COR1591 soybean does not share similarity with any sequences known to be toxic in the NCBI nr protein database. Pioneer states that there was no evidence of acute toxicity in mice that were dosed orally at 5000 milligrams of DSM-2 PAT protein/kilogram of body weight. Taken together, Pioneer concludes that dietary exposure to DSM-2 PAT protein in COR1591 soybean poses no meaningful risk to animal health.

Protein Expression Level

Pioneer quantified PPO-1.5, DGT-28 EPSPS, AAD-12, and DSM-2 PAT protein levels in various tissues of COR1591 soybean. The study included six field trial sites with four replicate plots at each site. Flowers, forage, seed, leaf, and root samples were collected from each replicated plot at all field sites. Samples were prepared and analyzed using Enzyme-linked immunosorbent assay.

Pioneer reports that the mean PPO-1.5 level in COR1591 soybean across all sites was highest in leaf at 330 nanograms/microgram of tissue dry weight (DW) and lowest in roots and seed at <lower limit of quantitation (LLOQ). The mean DGT-28 EPSPS protein level in COR1591 soybean across all sites was highest in leaf at 60

nanograms/microgram DW and lowest in root at 6 nanograms/microgram DW. The mean AAD-12 protein level in COR1591 soybean across all sites was highest in leaf at 290 nanograms/microgram DW and lowest in root at < LLOQ. The mean DSM-2 PAT protein level in COR1591 soybean across all sites was highest in seed at 84 nanograms/microgram DW and lowest in leave at 37 nanograms/microgram DW.

Animal Food Use

The developer states that COR1591 soybean is expected to be grown for the same uses as currently commercialized soybean, and no new uses in animal food are anticipated. Pioneer references the Organisation for Economic Co-operation and Development (OECD) consensus document[2] for the typical uses of soybean-derived food and feed. Most soybean seeds are processed into oil and meal. Soybean oil is commonly used as a human food ingredient. The preponderance of soybean meal is used in animal food, primarily in poultry, swine, and beef and dairy cattle diets. Soybean meals are processed in moist heat to inactivate trypsin inhibitors and lectins, which are anti-nutrients occurring in raw soybeans.

Composition

Scope of Analysis

Pioneer analyzed the nutrient composition of COR1591 soybean, control soybean and four reference soybean lines that were grown and harvested under similar conditions. Compositional analyses of soybean samples were reported for components listed in the OECD soybean composition consensus document.

Study Design

Pioneer conducted field trials in 2024 at multiple locations in the United States and Canada. A randomized complete block design with four replicate plots was used at each field site. Pioneer harvested samples from each replicate within each site for composition analysis. Forage samples were harvested at the R3 growth stage, and grain samples were harvested at the R8 growth stage.

Pioneer statistically compared each component for COR1591 soybean and the control across locations using a linear mixed model analysis of variance. For a given analyte in the mixed model analysis, if a statistical difference (p-value < 0.05) was observed between COR1591 soybean and the control soybean, the False Discovery Rate (FDR)-adjusted P-value was examined. Fisher's exact test was used if analytes did not meet criteria for enough observations above the LLOQ. Pioneer states that the respective range of individual values from COR1591 soybeans were compared to tolerance intervals, the range of values in the public literature, and in-study reference range when a statistically significant difference was identified.

Results of Analyses

For forage, Pioneer reports values for moisture, crude fat, ash, crude fiber, acid

[2] Organisation for Economic Co-operation and Development. 2012. Revised consensus document on compositional considerations for new varieties of soybean [*Glycine max* (L.) Merr.]: Key food and feed nutrients, anti-nutrients, toxicants and allergens. Series on the Safety of Novel Foods and Feeds No. 25. ENV/JM/MONO 24. OECD, Paris

detergent fiber (ADF), and neutral detergent fiber (NDF). Pioneer reports statistically significant differences between COR1591 soybeans and the control for crude protein and carbohydrates. However, Pioneer notes that the individual values for all of these components were within the reference ranges. Additionally, the FDR-adjusted P-values for crude protein and carbohydrates are not statistically significant.

For grain, Pioneer measured proximate, fiber, fatty acid, amino acid, mineral, vitamin, isoflavone, and anti-nutrient. Pioneer reports statistically significant differences between COR1591 soybeans and the control for crude fat, stearic acid [C18:0], eicosenoic acid [C20:1], behenic acid [C22:0], calcium, and vitamin K1. However, Pioneer notes that the individual values for all of these components were within the reference ranges. Additionally, the FDR-adjusted *p*-values for crude fat, eicosenoic acid [C20:1], and calcium are not statistically significant. Pioneer concludes that the differences in these components between COR1591 soybean and the control are not biologically meaningful from an animal food safety perspective.

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 COR1591 soybean. Pioneer concludes that COR1591 soybean is comparable to soybean varieties that are currently used in animal food in the United States.

Conclusion

CVM evaluated Pioneer's submissions to determine whether COR1591 soybean 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 COR1591 soybean 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 cotton varieties now grown, marketed, and consumed. At this time, based on Pioneer's data and information, CVM considers Pioneer's consultation on COR1591 soybean for use in animal food to be complete.

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