Biotechnology Notification File No. 000167
CVM Note to the File

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To: Administrative Record, BNF No. 000167

Subject: Genetically Engineered Corn Producing a Phytase, Event PY203

Keywords: Corn, Maize, *Zea mays*, OECD Identifier AGV-PY203-4, *Escherichia coli*, altered *appA*, Phytase enzyme, Phosphomannose isomerase (PMI), Agrivida, Animal Food GRAS Notices (AGRN) 21 and 27, New Protein Consultation (NPC) 000015

Purpose

This document summarizes the Food and Drug Administration (FDA) Center for Veterinary Medicine's (CVM, we) evaluation of biotechnology notification file (BNF) number 000167. Agrivida, Inc. (Agrivida) submitted a safety and nutritional assessment for a genetically engineered (GE) corn, transformation event PY203 (PY203 corn) and additional information afterwards. Agrivida had previously submitted NPC 000015 and AGRN 21 regarding its conclusions about the safety of the novel phytase enzyme when potentially, inadvertently present in human or animal food at low levels and the use of grain from PY203 corn as a source of the enzyme in poultry diets, respectively.[1] CVM evaluated the information in Agrivida's submissions to ensure that regulatory and safety issues regarding animal food derived from PY203 corn have been resolved prior to commercial distribution. FDA's Center for Food Safety and Applied Nutrition summarizes its evaluation of PY203 corn in human food in a separate document.

In CVM's evaluation, we considered all of the information provided by Agrivida 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 effect of the modification in PY203 corn is to produce a phytase enzyme Phy02 in the GE corn variety.[2] To confer this trait, Agrivida introduced DNA sequences

[1] After Agrivida submitted BNF 000167, Agrivida submitted to CVM AGRN 27 regarding its conclusion about the use and regulatory status of the phytase enzyme in ground PY203 corn grain in swine diets.
[2] Agrivida states that the grain from PY203 corn will be ground to form a coarse corn meal, which will be added to animal diets as a source of the Phy02 phytase enzyme.

containing three copies of an altered *appA* phytase gene from *Escherichia coli* (*E. coli*) strain K12, described as the *phy02* phytase gene, each under the control of a different monocot-derived seed specific promoter. Agrivida also introduced the *E. coli manA* gene encoding the enzyme phosphomannose isomerase (PMI), which was used as a selectable marker.[3]

Regulatory Considerations

The purposes of this evaluation are (1) to assess whether Agrivida 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

Agrivida states that the *phy02* phytase gene was generated by creating nucleotide substitutions in another phytase gene that was generated from the native *E. coli appA* gene by site saturation mutagenesis.[4] Agrivida transformed immature corn embryo tissue using an *Agrobacterium* mediated transformation method. Within the transfer DNA (T-DNA) region, there are three copies of the *phy02 phytase* gene, each under the control of a different monocot derived promoter, whereas the termination of transcription of the genes is mediated by the terminator of the *nopaline synthase* gene (*nos*) from *Agrobacterium tumefaciens*. The T-DNA region also contains the *phosphomannose isomerase* gene (*manA*) from *E. coli*, which was used as a selectable marker.

Genomic DNA was isolated from the leaves of PY203 corn to confirm the presence and to determine the number of copies of the DNA insert. Additional breeding steps[5] were conducted to generate plants used in the genetic stability analyses and inheritance studies. A combination of Southern blot and DNA sequencing analyses were utilized to identify the DNA inserted into the PY203 corn genome. Consequently, Agrivida reports that a complete copy of the T-DNA was inserted into chromosome 8 (designated as locus 3293) and a partial copy[6] of the T-DNA was inserted into chromosome 2 (designated as locus 3507). Southern blot analysis was also used to demonstrate the absence of plasmid backbone sequences and the region outside the T-DNA borders in PY203 corn. Agrivida used genome walking and sequencing strategies to confirm the nucleotide sequences of both loci 3293 and 3507, and the flanking maize genomic DNA. A comparison of these sequences against the B73 maize genome identified a 24 base pair (bp) deletion in maize

[3] The PMI enzyme enables maize tissue to grow on mannose as a sole source of carbon.
[4] The information about the genetic modification and characterization of PY203 corn was provided in AGRN 21 by Agrivida.
[5] Agrivida states that the original PY203 T0 plant was crossed to an inbred background "E" to generate the PY203_F1E plant, which was subsequently backcrossed to "E" to generate up to four generations BC1E - BC4E.
[6] Agrivida states that the partial copy of the T-DNA fragment which was inserted into the chromosome 2 of PY203 corn includes only two of the three *phy02* genes from the complete copy of the T-DNA fragment. Subsequently, by using genome walking and sequencing of locus 3507, Agrivida indicates that the T-DNA elements, including the third, downstream copy of the *phy02* gene, much of the *Z. mays* globulin-1 promoter, and the *manA* gene, were lost during integration.

chromosome 8 and a 40 bp deletion in chromosome 2 at loci 3293 and 3507, respectively.

Agrivida evaluated the genetic stability of the two loci in four different backcross generations (BC1E - BC4E) using both polymerase chain reaction (PCR) and Southern blot analyses. Utilizing PCR and subsequent sequencing of the amplicons, Agrivida reports that "all four generations had identical insertion site sequences for both loci." The genetic stability of the two insertion loci was also assessed using Southern blot hybridization. Two hybridizing fragments with predicted sizes for loci 3293 and 3507 were observed in restriction digested genomic DNA from each of the four PY203 back-cross generations. Based on both PCR and Southern blot analyses, Agrivida concludes that the maize genomic DNA adjacent to both loci in transformed PY203 corn are unchanged among successive backcross generations and are stable. Agrivida also assessed inheritance of the two DNA inserts using event-specific PCR analyses in progeny of a cross between the original PY203 T_0 plant and inbred E (PY203_F1E). Agrivida analyzed the results of these analyses by Chi-square goodness-of-fit analysis and showed that the desired genotype segregated according to the expected Mendelian principles. These data also support the conclusion that PY203 corn contains two DNA inserts in its genome.

Agrivida also conducted an open reading frame analysis to determine whether any putative proteins of 30 amino acids or greater could be formed at the junctions of the integrated T-DNAs. There were three and three putative open reading frames associated with loci 3293 and 3507, respectively. There was no sequence homology between these putative proteins and proteins in the National Center for Bioinformatics Information (NCBI) protein database that are identified as toxins.

Protein Safety

Phytase proteins are a class of acid phosphatase enzymes that hydrolyze phosphate from phytate, thereby making the phosphorus nutritionally available to monogastric animals. Agrivida states that Phy02 protein differs from the native *E. coli* AppA phytase protein by 16 of the 410 total amino acid residues in the mature protein.

The Phy02 protein was extracted and enriched from grain of PY203 corn, and subsequently subjected to evaluation for its physicochemical and functional properties, including molecular weight determination, enzymatic activity, and glycosylation status.[7] Using SDS-PAGE and Western blot, Agrivida confirms that the Phy02 protein extracted from PY203 corn grain has the expected molecular weight of approximately 46 kilodaltons (kDa). Agrivida indicates that the Phy02 protein from PY203 corn is not glycosylated utilizing a commercial deglycosylation kit. Agrivida also states that the phytase activity of PY203 grain samples was assayed and exhibited the expected activity. Agrivida provides results of bioinformatics analysis of the Phy02 amino acid sequences, which showed no biologically significant similarities to known toxins in the NCBI protein database.[8] Agrivida concludes that the Phy02 protein is unlikely to be a toxin.

[7] The information about the physicochemical and functional properties of the Phy02 protein extracted from PY203 corn grain was provided in AGRN 21.
[8] Evaluation of the toxicity potential of the Phy02 protein was also provided in NPC 000015 by Agrivida.

The levels of Phy02 protein in PY203 corn were determined in tissue samples from plants grown at two locations (Indiana and Nebraska) in 2016. Different tissue samples were collected at three developmental states (V8, R1 and R6).[9] Agrivida analyzed the samples using an enzyme-linked immunosorbent assay and reports average Phy02 protein levels in kernels of PY203 corn were 6670 and 6258 microgram per gram (µg/g) dry weight (dw) at the Indiana and Nebraska field sites, respectively. Phy02 protein levels were either below the Limit of Detection (LOD) or close to the Limit of Quantification (LOQ) in all PY203 leaf, stem, and pollen samples and in the majority of root samples.[10]

Agrivida states that the PMI protein encoded by the *E. coli manA* gene has been used as a selectable marker in GE plant varieties that have completed FDA's consultation procedures. Agrivida also cites a scientific publication on the safety of PMI. Agrivida states the expressed PMI protein was detectable using commercially available test kits and concludes that the PMI protein expressed in PY203 corn presents no safety concerns based on a weight of the evidence approach.

Animal Food Use

Corn (*Zea mays* L.) is a commodity crop grown worldwide for various uses, including food and feed. In the United States, the world's leading producer of corn, several different types of corn are cultivated, including field corn (e.g., yellow dent, white dent), sweet corn, and popping corn. Corn is an important crop for animal feed. 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

Agrivida analyzed the nutrient composition of PY203 corn and a control variety (non-GE null segregant) that were grown and harvested under similar conditions. The firm indicates it analyzed the grain and forage of PY203 corn and the control variety.

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[9] Mueller, D., & Pope, R. (Eds.). (2009). Corn Field Guide: A reference for identifying diseases, insect pests, and disorders of corn. Ames: Iowa State University of Science and Technology.

[10] Agrivida states that two of eight root samples collected at the V8 stage (Indiana) demonstrated higher protein levels compared to other samples (5.004 and 1.433 µg/g dw); all other samples had less than 0.087 µg/g dw. One of the eight root samples collected at the R6 stage (Indiana) demonstrated higher levels of the Phy02 protein compared to other samples (1.028 µg/g dw); all other samples had less than 0. 175 µg/g dw.

Study Design

Agrivida harvested grain and forage from PY203 corn and the control varieties at five locations (Hamilton and Tipton counties in Indiana, Dallas county in Iowa, Brunswick and York counties in Nebraska) in 2016.

The studies were designed as randomized block studies with four replicate plots at each of the five sites. The entire above ground portion of one plant for each replicate plot was collected at the R4 stage for forage analysis. Additionally, at physical maturity grain from several plants was collected to yield a two-kilogram sample for each replicate plot. Forage and grain samples were analyzed using commercially available methods. Forage and grain were analyzed for proximates and grain was also analyzed for: amino acids, fatty acids, minerals, vitamins, and anti-nutritional components.

Agrivida calculated the mean, standard deviation, and range for each of the analytes. The firm bases its conclusions on comparisons of the means from its analytical results from PY203 corn with those of the control variety and also with the ranges published for conventional corn varieties in the International Life Sciences Institute Crop Composition Database (ILSI database), version 6.0.[11]

Results of analyses

Agrivida reports results for corn grain for the proximate analytes (moisture, crude protein, crude fat, crude fiber, ash, and carbohydrates by calculation), ten minerals, eight vitamins, eighteen amino acids, eight fatty acids[12], and anti-nutritional factors (phytic acid, trypsin inhibitor, raffinose, ferulic acid, *p*-coumaric acid, and inositol). The firm states that for the proximate analytes means for PY203 corn grain fell within the ranges for the control, except for crude fat.[13] Additionally, Agrivida states that these analytes were within the ranges present in the ILSI database. Agrivida states that the results for the analyzed amino acids, fatty acids[14], and minerals also fell within the ranges for these analytes reported in the ILSI database. The means for the vitamin content of PY203 corn grain fell within the ranges published in the ILSI database.[15] The firm states that analyzed levels of all anti-nutritional factors were within the ranges reported in the ILSI database.

Agrivida reports results for proximate and other analytes for corn forage (crude protein, crude fat, crude fiber, total dietary fiber, acid detergent fiber, neutral detergent fiber, ash, carbohydrates by calculation, calcium, and phosphorus). Agrivida states the results for PY203 forage were within the ranges for these analytes reported in the ILSI database.

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[11] The ILSI Crop Composition Database has become the Agriculture and Food Systems Institute Crop Composition Database and is available at www.cropcomposition.org.
[12] The firm reports that some fatty acids were below the analytical LOD.
[13] Values for carbohydrate content of PY203 grain also fell outside the range for the control variety.
[14] The ILSI database did not contain values for lignoceric acid.
[15] The firm states that there were differences in the analytical units reported for beta-carotene, but after conversion to the same units of measure, the beta-carotene content of PY203 corn grain fell within the range of the ILSI database.

Summary of Compositional Analyses

Agrivida states the mean composition values for PY203 corn fell within reference ranges of the ILSI database. Agrivida states that the results of the compositional analyses "demonstrate that the expression of phytase protein in event PY203 corn does not significantly affect the nutrient composition in the grain or forage." Agrivida concludes that PY203 corn is compositionally comparable to conventional corn varieties.

Animal Food Labeling Considerations

It is a producer's or distributor's responsibility to ensure that labeling of the foods it markets meets applicable legal requirements, including disclosure of any material differences in the food. In evaluating the common or usual name appropriate for animal food ingredients derived from PY203 corn, CVM considered that this new corn variety was genetically engineered to express the Phy02 phytase enzyme in its grain, and that Agrivida has concluded that the corn grain and forage are compositionally comparable to those from conventional corn varieties. When used in poultry or swine food according to the intended conditions of use described in AGRNs 21 or 27, respectively, CVM recognizes the name "phytase" as the common or usual name for the Phy02 phytase enzyme in PY203 corn grain. CVM recognizes that for all other uses of PY203 corn and derived products in animal food, "corn" is the appropriate name (for example, "flaked corn" and "dehydrated corn plant").

Conclusion

CVM evaluated Agrivida's submissions to determine whether PY203 corn raises any safety or regulatory issues with respect to traditional uses of corn in animal food. Based on the information provided by Agrivida 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.

Agrivida has concluded that PY203 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 conventional corn varieties. At this time, based on Agrivida's data and information, CVM considers Agrivida's consultation on PY203 corn for use in animal food to be complete.

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