U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

Biotechnology Notification File No. 000181
HFP Note to the File

Date: March 31, 2025

From: Jianmei Zhu, Ph.D.

To: Administrative Record, BNF No. 000181

Subject: Corn with transformation event FG259 (FG259 corn)

Keywords: Corn*, Zea mays*, maize, FG259, *AC1 glucanase* gene, AC1 beta-glucanase, *manA* gene, phosphomannose isomerase (PMI), Agrivida, OECD Unique Identifier AGV-FG259-5, AGRN 31, NPC 000002, NPC 000018

Summary

This Note to the File revises our Note to the File for BNF 181 signed on December 20, 2024. The purpose of this revised Note to the File is to correct a reference to the name of the variety on the first page.

Agrivida, Inc. (Agrivida) has completed a consultation with the Food and Drug Administration (FDA) on food derived from FG259 corn, which is genetically engineered to express AC1 beta-glucanase[1] enzyme as a source of AC1 beta-glucanase for use in animal foods and phosphomannose isomerase (PMI) for use as a selectable marker during transformation. This document summarizes Agrivida's conclusions and supporting data and information that FDA's Human Foods Program (HFP, we) evaluated pertaining to FG259 corn if present in human food.[2] FDA's Center for Veterinary Medicine (CVM) summarizes its evaluation pertaining to animal food uses in a separate document.

While not intended for use in human food, Agrivida assessed the safety of FG259 corn as if it were used as human food. Based on the safety and nutritional assessment Agrivida has conducted, it is our understanding that Agrivida concludes:

- the new proteins would not require premarket approval as a food additive if present in human food; and
- FG259 corn is comparable to and as safe as human food from other corn varieties.

HFP evaluated data and information supporting these conclusions and considered whether FG259 corn raises other regulatory issues involving human food within FDA's authority under the Federal Food, Drug, and Cosmetic Act. We have no further questions at this time about the safety, nutrition, and regulatory compliance of FG259 corn if present in human food.

[1] Agrivida referred to AC1 glucanase in the submission. FDA recognizes the name "beta-glucanase" as the common or usual name for the beta-glucanase produced by FG259 corn.
[2] Agrivida noted that FG259 corn grain will be processed in a closed loop system on a limited number of acres each year and is highly unlikely to be present in corn grain used for human food.

Subject of the Consultation

Crop	Corn
Designation	FG259
Intended trait	Corn containing the AC1 beta-glucanase
Developer	Agrivida, Inc.
Submission received	December 3, 2020
Amendment(s) received	February 22, 2021; August 30, 2022; May 11, 2023
Intended use	While not intended for use in human food, Agrivida assessed the safety of FG259 corn if present in human food.
Transformation plasmid	Plasmid pAG4588
Expression cassette 1	*AC1 glucanase* gene, derived from an environmental DNA library, encoding AC1 beta-glucanase (96% amino acid sequence identical to Cel5A glucanase from *Thermotoga maritima*)*;* seed specific expression directed by the rice glutelin promoter
Expression cassette 2	*manA* gene, derived from *Escherichia coli* K-12, encoding phosphomannose isomerase (PMI) enzyme for use as a plant selectable marker
Method for conferring genetic change	*Agrobacterium*-mediated transformation

In BNF 000181, Agrivida relies on incorporating information from previous submissions to FDA to establish several aspects of its evaluation of the safety of FG259 corn. Agrivida also provides more information including field trial and crop composition data related specifically to this consultation.

Molecular Characterization

In 2019, Agrivida submitted a GRAS notice (Animal Food GRAS Notice (AGRN) 31) to CVM regarding the use of ground grain from FG259 corn in food for poultry. In AGRN 31, Agrivida provided data and information to support the integrity and stability of the intended genetic changes in FG259 corn. On April 6, 2020, CVM responded to Agrivida with a letter stating that it had no questions regarding Agrivida's conclusion that ground grain containing AC1 beta-glucanase from FG259 corn is safe for its intended use in poultry feed.[3] In BNF 000181, Agrivida incorporated and summarized the data and information on the molecular characterization and genetic stability in FG259 corn described in AGRN 31.

[3] CVM responded with a letter stating that it had no questions to a second notice (AGRN 50) from Agrivida on the safety of the intended use of the ground grain from FG259 corn in swine feed on June 23, 2022.

Introduced Proteins: AC1 beta-glucanase and PMI

In 2017, Agrivida submitted a New Protein Consultation (NPC 000018) to FDA to address the safety of low levels of the AC1 beta-glucanase protein in human food. In NPC 000018, Agrivida provided data and information to support that AC1 beta-glucanase is unlikely to be allergenic or toxic. On February 21, 2018, FDA responded that it had no questions about Agrivida's conclusion that the potential inadvertent presence in the food supply of low levels of AC1 beta-glucanase would not raise food safety concerns. In BNF 000181, Agrivida incorporated the data and information from NPC 000018 to support its safety assessment of AC1 beta-glucanase if present in human food.

In addition, Agrivida described the enzyme activity of AC1 beta-glucanase and explained that the AC1 beta-glucanase produced in grain of FG259 corn does not react with starch, proteins, or oils in human food products. As a glucanase, AC1 beta-glucanase hydrolyzes certain glucan bonds in non-starch polysaccharides (NSP), producing oligo- and mono-saccharides. Agrivida noted that NSP makes up a very small proportion of the corn grain. Agrivida assessed the effects of purified AC1 beta-glucanase on corn grain fiber in *in vitro* experiments by measuring release of mono- and oligo-saccharides from the polysaccharides components, either alone or in combination with an enzyme mixture containing a variety of polysaccharide hydrolases. Agrivida reported the results of its assessment indicate that AC1 beta-glucanase is unlikely to have a significant effect on corn grain fiber. Agrivida concludes that AC1 beta-glucanase in FG259 corn does not affect the nutrition or safety of human food products derived from corn.

Agrivida used phosphomannose isomerase (PMI) protein as a selectable marker in the development of FG259 corn. In BNF 000181, Agrivida states that the *manA* gene from *E. coli* K-12, which encodes PMI, and associated regulatory sequences introduced to FG259 corn are identical to the genetic sequences submitted to FDA in NPC 000002 by Syngenta.[4] Agrivida notes that the PMI protein in FG259 corn has been widely used in genetically engineered corn and other crop species, and its safety has been characterized in scientific publications[5] and assessed by the Environmental Protection Agency and by FDA.

Human Food Nutritional Assessment

The intended traits in FG259 corn are not expected to alter levels of key nutrients, anti-nutrients, or toxicants. To ensure the absence of unintended changes in components relevant to human food safety or nutrition, Agrivida analyzed the grain from FG259 corn and a near-isogenic non-transformed control (the control), grown in three locations in the United States and one location in Argentina in 2018. Agrivida measured levels of proximates (protein, crude fat, crude fiber, ash, moisture, and carbohydrates (by calculation)), amino acids, fatty acids, minerals, and vitamins as well as anti-nutrients (phytic acid, trypsin inhibitor, inositol, and raffinose) and secondary metabolites (p-coumaric acid and ferulic acid). Agrivida found that the mean values of the tested components in FG259 corn are similar to the mean values in the control and within the historical ranges reported in the literature (ILSI-CCDB[6]), with the exception of Vitamin B5, inositol, and p-coumaric acid. Agrivida noted that while the ranges of these three components in both FG259 corn and the control were outside of the historical range as reported in the ILSI-CCDB, the

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[4] In 2005, Syngenta Seeds, Inc., submitted an NPC (NPC 000002) to FDA to address the safety of low levels of phosphomannose isomerase (PMI) in food. In NPC 000002, Syngenta provided data and information to support that PMI protein is unlikely to be allergenic or toxic. On February 10, 2009, FDA responded that we had no questions about Syngenta's conclusions regarding the safety of low levels of PMI in food.

[5] For example, in Privalle, L., M. Wright, J. Reed, G. Hansen, J. Dawson, E. M. Dunder, Y. Chang, M. L. Powell, and M. Meghji (2000). Phosphomannose Isomerase, A Novel Selectable Plant Selection System: Mode of Action and Safety Assessment. Proceedings of the 6th International Symposium on The Biosafety of Genetically Modified Organisms, Saskatoon, Canada.

[6] On May 1, 2020, the International Life Sciences Institute Crop Composition Database became known as the Agriculture and Food Systems Institute Crop Composition Database.

mean values in FG259 corn are nonetheless similar to the mean values in the control. Agrivida concludes that grain from FG259 corn is as safe and nutritious as that from conventional corn varieties.

Conclusion

Based on the information provided by Agrivida and other information available to HFP, we have no further questions at this time about the safety, nutrition, and regulatory compliance of FG259 corn if present in human food. We consider the consultation with Agrivida on FG259 corn to be complete.

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