

Biotechnology Notification File No. 000188 HFP Note to the File

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To: Administrative Record, BNF No. 000188

Subject: Corn with transformation event PY1203 (PY1203 corn)

Keywords: Corn, Maize, *Zea mays*, PY1203, modified *appA* phytase (phy02opt) gene, Phy02 phytase, *manA* gene, phosphomannose isomerase (PMI), Agrivida, OECD Unique Identifier AGV-PY203-5, AGRN 21, AGRN 27, AGRN 32, NPC 00002, NPC 000015, BNF No. 000167

Summary

Agrivida, Inc. (Agrivida) has completed a consultation with the Food and Drug Administration (FDA) on food derived from PY1203 corn, which is genetically engineered to express the phytase enzyme Phy02 derived from *Escherichia coli* strain K-12 as a source of phytase for use in animal foods. Agrivida also introduced the *manA* gene from *E. coli* encoding the enzyme phosphomannose isomerase (PMI) for use as a selectable marker. This document summarizes Agrivida's conclusions and supporting data and information that FDA's Human Foods Program (HFP, we) evaluated pertaining to PY1203 corn if present in human food. FDA's Center for Veterinary Medicine (CVM) summarizes its evaluation pertaining to animal food in a separate document.

While not intended for use in human food, Agrivida assessed the safety of PY1203 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
- PY1203 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 PY1203 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 PY1203 corn if present in human food.

Subject of the Consultation

Crop	Corn
Designation	PY1203
Intended trait	Corn containing Phy02 phytase
Developer	Agrivida, Inc.
Submission received	January 25, 2022
Amendment(s) received	June 22, 2023; August 7, 2023; April 6, 2024
Intended use	While PY1203 corn is not intended for use in human food, Agrivida assessed the safety of PY1203 corn if present in human food.
Transformation plasmid	Plasmid pAG4916
Expression cassette 1	Modified <i>appA</i> phytase gene (Agrivida also refers to the gene as <i>phy02opt</i>) derived from <i>Escherichia coli</i> strain K-12 encoding for Phy02 phytase; the endosperm-specific expression of <i>phy02opt</i> is directed by rice-derived Glutelin-1 gene promoter (<i>GTL</i>).
Expression cassette 2	Modified <i>appA</i> phytase gene (Agrivida also refers to the gene as <i>phy02opt</i>) derived from <i>E. coli</i> strain K-12 encoding for Phy02 phytase; the endosperm-specific expression of the <i>phy02opt</i> is directed by corn-derived <i>Zc2</i> gene promoter (<i>ZmZ27</i>).
Expression cassette 3	<i>manA</i> gene, derived from <i>E. coli</i> K-12, encoding phosphomannose isomerase (PMI) enzyme for use as a plant selectable marker. Expression of <i>manA</i> gene is under the control of the corn Ubiquitin 1 gene promoter (<i>ZmUbi1</i>).
Method for conferring genetic change	<i>Agrobacterium</i> -mediated transformation

In BNF 000188, Agrivida relies on incorporating information from previous submissions to FDA to establish several aspects of its evaluation of the safety of PY1203 corn. Agrivida also provides additional information including field trial and crop composition data related specifically to this consultation.

Molecular Characterization

In 2019, Agrivida submitted Animal Food GRAS Notice (AGRN) 32 to CVM regarding the use of ground grain from PY1203 corn in feed for poultry and swine. In AGRN 32, Agrivida provided data and information to support the integrity and stability of the intended genetic changes in PY1203 corn. On April 15, 2020, CVM responded to Agrivida with a letter stating that it had no questions regarding Agrivida's conclusion that ground grain containing Phy02 protein from PY1203 corn is safe for its intended use in poultry feed and swine feed. In BNF 000188, Agrivida incorporated and summarized the data and information on the molecular characterization and genetic stability in PY1203 corn described in AGRN 32.

Introduced Proteins: Phy02 phytase and PMI

In 2015, Agrivida submitted a New Protein Consultation (NPC 000015) to FDA to address the safety of low levels of the Phy02 protein in human food. In NPC 000015, Agrivida provided data and information to support that Phy02 phytase is unlikely to be allergenic or toxic. On August 7, 2015, FDA responded that we had no questions about Agrivida's conclusions regarding the safety of low levels of the Phy02 protein in food. Subsequently, in 2018, Agrivida submitted BNF 000167 in which Agrivida incorporated the data and information from NPC 000015 to support its safety assessment of PY203 corn when present in human food.¹ Agrivida also submitted GRAS Notices AGRN 21 and 27, respectively for use in poultry and swine feed.² Agrivida notes that PY1203 corn expresses a modified *appA* phytase gene that is similar to the gene inserted in PY203 corn. However, in PY1203 corn the *appA* gene is codon optimized for expression in corn, resulting in higher phytase Phy02 expression compared to that from event PY203.

Because the Phy02 enzymes produced in corn events PY203 and PY1203 are similar, Agrivida incorporates data and information from NPC 15, AGRN 32, and BNF 000167 to support its safety assessment of PY1203 corn for presence in human food. To this end, Agrivida compared the phytase enzymes expressed by both events PY1203 and PY203, addressing enzyme specific activity, kinetic properties, thermostability, pH activity profile, molecular weight, immunoreactivity, predicted amino acid sequence, and N-terminal sequence analysis. Agrivida concludes that the phytase enzymes expressed in the corn derived from events PY1203 and PY203 are identical.

Agrivida notes that the presence of exogenous phytase from PY1203 corn might cause an increase in the level of bioavailable phosphorous in human diet due to hydrolysis of phytic acid. Agrivida provides a discussion on factors that would minimize such an occurrence. Agrivida states that the *E.coli*-derived phytase in PY1203 corn is not thermostable under normal cooking conditions. For example, the enzyme loses all activity when exposed to 75°C for 5 minutes. Agrivida also states that common methods used during food preparation of grains and pulses, such as milling and refining, blanching, boiling, soaking, germinating, fermenting, roasting, and cooking, reduce the phytic acid content of the foods. For example, Agrivida states that, depending on the staple, soaking of grains and pulses reduces phytic acid by as much as 55%; extrusion by as much as 44%; and depending on the staple and type of fermentation used, by as much as 80%. Agrivida also notes that because of its mechanism of action, during digestion of food, the phytase in PY1203 corn is not reasonably expected to release all phosphorous bound in dietary phytic acid. Agrivida states that because current food processing activities already remove large proportions of phytic acid found in raw staples, the presence of Phy02 phytase in PY1203 corn is not expected to affect food processing activities.

Agrivida used phosphomannose isomerase (PMI) protein as a selectable marker in the development of PY1203 corn. In BNF 000188, Agrivida states that the *manA* gene from *E. coli* K-12, which encodes PMI, and associated regulatory sequences introduced to PY1203 corn are identical to the genetic sequences submitted to FDA in NPC 000002 by Syngenta.³ Agrivida notes that the PMI protein in PY1203 corn has been widely used in genetically engineered corn and other crop species, and its safety has been

¹ On January 27, 2021, FDA issued a response letter to BNF 167 indicating that FDA had no questions concerning human or animal food derived from PY203 corn at that time.

² On May 23, 2017, and July 8, 2019, CVM issued response letters indicating that CVM had no questions at that time regarding the notifier's conclusion that ground corn grain containing Phy02 phytase derived from event PY203 is GRAS under its intended conditions of use in poultry and swine feeds, respectively.

³ 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.

characterized in scientific publications⁴ and assessed by the Environmental Protection Agency and by FDA.

Human Food Nutritional Assessment

To ensure the absence of unintended changes in components relevant to human food safety or nutrition, Agrivida analyzed the grain of PY1203 corn and a related control (a near isogenic non-transformed control hybrid, control) for key components. Agrivida measured levels of proximates (protein, moisture, crude fat, crude fiber, ash, and carbohydrates (by calculation)), amino acids, fatty acids, minerals, vitamins, and anti-nutrients (phytic acid, trypsin inhibitor, inositol, and raffinose,) and secondary metabolites (*p*-coumaric acid and ferulic acid). Overall, Agrivida observed that the levels of most components in PY1203 corn and the control were similar and were within ranges of variation reported in the International Life Sciences Institute Crop Composition Database (ILSI-CCDB⁵). The search parameters in the database were historic means and ranges for corn grain for all years and countries. Agrivida noted that levels of several components, including all proximates, some amino acids, some fatty acids, some minerals, some vitamins, and the anti-nutrient ferulic acid in PY1203 corn were statistically different from levels in the control but were still within the ranges reported in the database. The levels of phosphorous were statistically higher in the grain of PY1203 corn, when compared to the control, but were within the ILSI-CCDB range. This observation also applied to other minerals such as calcium, iron, magnesium, manganese, potassium, and zinc. Agrivida concludes that human food derived from PY1203 corn is as safe and nutritious as human food 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 PY1203 corn if present in human food. We consider the consultation with Agrivida on PY1203 corn to be complete.

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⁴ 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.

⁵ ILSI-CCDB (2020). Crop Composition Database, International Life Science Institute. Version 7.0; Generated 06/03/2020. Available at: <https://www.cropcomposition.org/query/index.html>. On May 1, 2020, the International Life Sciences Institute Crop Composition Database became known as the Agriculture and Food Systems Institute Crop Composition Database.