

Biotechnology Notification File No. 000201

HFP Note to the File

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From: Matthew Fabian, Ph.D.

To: Administrative Record, BNF No. 000201

Subject: Corn with transformation event MZIR260 (MZIR260 corn)

Keywords: Corn, maize, *Zea mays*, insect resistance, fall armyworm, *Spodoptera frugiperda*, *Lepidoptera*, crystal protein, *eCry1Gb.1lg-03* gene, Cry1Gb, Cry1lg, *pmi-15* gene, eCry1Gb.1lg protein, PMI protein, phosphomannose isomerase, Bt, *Bacillus thuringiensis*, Syngenta Seeds, LLC., MZIR260 corn, OECD Unique Identifier SYN-ØØ26Ø-3

Summary

Syngenta Seeds, LLC. (Syngenta) has completed a consultation with the Food and Drug Administration (FDA) on food derived from MZIR260 corn. MZIR260 corn expresses the eCry1Gb.1lg protein, conferring enhanced resistance to fall armyworm (*Spodoptera frugiperda*), a Lepidopteran insect pest, as well as the protein phosphomannose isomerase (PMI), which permits growth of plant cells on mannose and was used as a selection marker. This document summarizes Syngenta's conclusions and supporting data and information that FDA's Human Foods Program (HFP, we) evaluated pertaining to human food uses of MZIR260 corn. FDA's Center for Veterinary Medicine summarizes its evaluation pertaining to animal food uses in a separate document. Syngenta did not identify any confidential business information in its submission to FDA.

Based on the safety and nutritional assessment that Syngenta has conducted, it is our understanding that Syngenta concludes:

- it has not introduced into human food a new protein or other substance that would require premarket approval as a food additive;
- human food from MZIR260 corn is comparable to and as safe as human food from other varieties of corn

HFP evaluated data and information supporting these conclusions and considered whether MZIR260 corn raises other regulatory issues involving human food within FDA's authority under the Federal Food, Drug, and Cosmetic Act (FD&C Act). We have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from MZIR260 corn.

The U.S. Environmental Protection Agency (EPA) evaluates and authorizes the use of plant-incorporated protectants (PIPs) under the FD&C Act and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). A PIP is defined in 40 CFR 174.3 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.” In MZIR260 corn, eCry1Gb.1lg is a PIP and PMI is a PIP inert ingredient. Syngenta notes that, per 40 CFR §174.527, EPA has exempted residues of PMI in or on food and feed commodities of all plants from the requirement of a tolerance when used as a PIP inert ingredient. The safety of the eCry1Gb.1lg and PMI proteins in MZIR260 corn is under EPA’s purview and is therefore not addressed in this document. Syngenta notes that they have filed a petition with EPA to establish a permanent exemption from the requirement of a tolerance for the eCry1Gb.1lg protein.

Subject of the Consultation

Crop	Corn (<i>Zea mays</i>)
Designation	MZIR260
Intended trait	Insect resistance
Developer	Syngenta Seeds, LLC.
Submission received	July 31, 2024
Intended use	For general use in human food
Transformation plasmid	pSYN24795
Expression cassette 1	<i>eCry1Gb.1lg-03</i> , a chimeric gene derived from <i>Bacillus thuringiensis</i> and conferring resistance to fall armyworm
Expression cassette 2	<i>pmi-15</i> , derived from <i>Escherichia coli</i> strain K-12 and used as a selection marker, permitting tissue growth on media containing mannose as a carbon source
Method for conferring genetic change	<i>Agrobacterium</i> -mediated transformation

Inheritance and stability

To generate MZIR260 corn, Syngenta utilized *Agrobacterium* to transform a proprietary, elite, inbred corn line, with plasmid pSYN24795, which contained two expression cassettes within the T-DNA region: *eCry1Gb.1lg-03*, encoding a codon-optimized chimera of proteins Cry1Gb and Cry1lg; and *pmi-15*, encoding a phosphomannose isomerase protein. To verify the transformation event, Syngenta utilized whole-genome sequencing to examine insert copy number, organization, generational stability, and the absence of plasmid backbone sequences. Analyzed samples were obtained from the MZIR260 T1 and T3 inbred generations and an F1 hybrid generation derived from a cross between MZIR260 and a separate, non-transgenic line. Syngenta observed single, identical recombination sites in the T1, T3, and F1 hybrid lines and concluded that the T-DNA insertional event is generationally stable. No alignments were identified between the pSYN24795 backbone sequence and reads derived from the T1, T3, and F1 hybrid lines, leading Syngenta to conclude that MZIR260 corn does not contain backbone sequences from pSYN24795.

To evaluate the inheritance of the T-DNA insertion in MZIR260 corn, Syngenta utilized quantitative real-time PCR and a Chi-squared test for statistical analysis of segregation ratios. Samples were obtained from the MZIR260 F2 hybrid generation derived from the cross previously described, as well as from two backcrossed generations derived from a cross between MZIR260 and an additional non-transgenic line.

Syngenta concluded that the T-DNA insert in MZIR260 corn exhibits a Mendelian inheritance pattern corresponding to an insertional event at a single locus.

Human Food Nutritional Assessment

The intended trait in MZIR260 corn is not expected to alter levels of key nutrients or anti-nutrients. To assess potential unintended changes in composition relevant to safety or nutrition, Syngenta analyzed grain tissue from the aforementioned MZIR260 F1 hybrid, a non-transgenic, near-isogenic control hybrid, and six reference hybrids, grown in eight U.S. locations during the 2022 growing season. At each site, MZIR260 F1 hybrid, the control hybrid, and three reference hybrid lines were planted in randomized complete blocks with four replicates per line. Grain samples were surveyed for analytes in accordance with the Organisation for Economic Co-operation and Development (OECD) Consensus Document for corn¹, with compositional analyses measuring proximates, fiber, starch, fatty acids, amino acids, minerals, vitamins, secondary metabolites, and antinutrients. Comparative statistical analyses were performed on analyte measurements from MZIR260 F1 hybrid and the control hybrid, and statistically significant differences (p-value < 0.05) between the two lines were identified. Reference ranges for each analyte were sourced from the in-study reference hybrid lines and the Agriculture and Food Systems Institute Crop Composition Database² (AFSI CCD) reference ranges for corn grain.

Via the reported p-values, statistically significant differences between MZIR260 F1 hybrid and control hybrid were identified for eleven analytes (α -tocopherol, β -carotene, pyridoxine, thiamin, copper, manganese, palmitoleic acid, heptadecanoic acid, eicosenoic acid, *p*-coumaric acid, and raffinose). However, the values recorded for MZIR260 samples fell within the ranges for corn grain reported in the reference hybrid lines and AFSI CCD. Overall, Syngenta concluded that food derived from MZIR260 corn is compositionally comparable to that of non-GE conventional corn with a history of safe use in food.

Conclusion

Based on the information provided by Syngenta and other information available to HFP, we have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from MZIR260 corn. We consider the consultation with Syngenta on MZIR corn to be complete.

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¹ OECD (2002) Consensus Document on Compositional Considerations for New Varieties of Maize (*Zea mays*): Key Food and Feed Nutrients, Antinutrients and Secondary Plant Metabolites, [https://one.oecd.org/document/env/jm/mono\(2002\)25/en/pdf](https://one.oecd.org/document/env/jm/mono(2002)25/en/pdf)

² AFSI (2023) Crop Composition Database, Version 9.1. Agriculture & Food Systems Institute, <https://www.cropcomposition.org>