Biotechnology Notification File No. 000194
CFSAN Note to the File

Date: May 17, 2024

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To: Administrative Record, BNF No. 000194

Subject: Maize (Corn) with transformation event EH913 (EH913 corn)

Keywords: Maize, corn, *Zea mays* L., insect resistance, fall armyworm, *Spodoptera frugiperda,* herbicide tolerance, glufosinate ammonium, Cry1Da, *Bacillus thuringiensis, bar* gene, Phosphinothricin N-acetyltransferase (PAT), *Streptomyces hygroscopicus*, *Agrobacterium tumefaciens*, Helix Sementes e Biotecnologia Ltda.,[1] EH913, OECD Unique Identifier EH-BRS913-2.

Summary

Helix Sementes e Biotecnologia Ltda. (Helix) has completed a consultation with the Food and Drug Administration (FDA) on food derived from EH913 maize (corn) genetically engineered to express the insecticidal protein Cry1Da for protection against fall armyworm (*Spodoptera frugiperda*) and phosphinothricin acetyltransferase (PAT) for tolerance to glufosinate-ammonium herbicides. PAT was also used as a selectable marker during transformation and in the breeding program. This document summarizes Helix's conclusions and supporting data and information that FDA's Center for Food Safety and Applied Nutrition (CFSAN, we) evaluated pertaining to human food uses. FDA's Center for Veterinary Medicine (CVM) summarizes its evaluation pertaining to animal food uses in a separate document.

Based on the safety and nutritional assessment Helix has conducted, it is our understanding that Helix 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 EH913 corn is comparable to and as safe as human food from other conventional corn varieties.

CFSAN evaluated data and information supporting these conclusions and considered whether EH913 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 EH913 corn.

[1] The developer informed FDA about its name change from "Helix Sementes e Mudas Ltda." to "Helix Sementes e Biotecnologia Ltda." on May 9, 2023.

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 EH913 corn, the Cry1Da protein is a PIP and the PAT protein, which was used as a selectable marker during transformation and breeding, is a PIP inert ingredient. Helix explained that an appropriate exemption from the requirements of a tolerance for Cry1Da protein will be obtained from EPA. EPA granted permanent exemption from the requirement of a tolerance for PAT when used as a PIP inert ingredient in all food commodities (40 CFR 174.522). The safety of Cry1Da and PAT proteins in EH913 corn is under EPA's purview and is therefore not addressed in this document.

Subject of the Consultation

Crop	Corn
Designation	EH913
Intended trait 1	Insect resistance
Intended trait 2	Herbicide tolerance
Developer	Helix Sementes e Biotecnologia Ltda.
Submission received	October 27, 2022
Amendment(s) received	May 1, 2023; May 9, 2023; September 27, 2023; November 28, 2023; December 15, 2023; January 26, 2024
Intended use	General use in human food
Transformation plasmid	Plasmid pEH001
Expression cassette 1	The *Cry1Da* cassette encodes a truncated Cry1Da protein from *Bacillus thuringiensis* that confers resistance to *Spodoptera frugiperda*.
Expression cassette 2	The *bar* cassette encodes the PAT protein from *Streptomyces hygroscopicus*. It confers tolerance to glufosinate-ammonium herbicides and was also used as a selectable marker.
Method for conferring genetic change	*Agrobacterium*-mediated transformation

Inheritance and Stability

EH913 corn was developed from the public hybrid Hi-II transformed with plasmid pEH001 using *Agrobacterium*-mediated transformation.

Helix used high throughput sequencing to examine the genetic stability of the inserted DNA in four generations of EH913 corn. Helix confirmed that no changes were observed within the T-DNA in any of the generations tested. Helix also evaluated the inheritance of the glufosinate tolerance trait in three successive backcrossing generations of EH913 corn. The results demonstrated a Mendelian pattern of inheritance in all the generations of EH913 corn tested as the observed segregation ratios were comparable to the expected segregation ratios. The phenotypic results were consistent with the genotypic characterization results from other generations examined. Helix therefore concluded that the T-DNA insert was inherited as a single insert at a single locus and segregated following Mendelian principles.

Human Food Nutritional Assessment

The intended traits in EH913 corn are not expected to alter the levels of key nutrients, antinutrients, or secondary metabolites. To ensure the absence of unintended changes to components relevant to safety or nutrition, Helix conducted a compositional analysis comparing EH913 corn to a non-GE conventional hybrid variety with similar genetic background (control) and nine non-GE conventional commercial corn hybrids.[2] The corn varieties were concurrently grown in seven field locations in Brazil during the 2020/21 season. The compositional analyses were conducted following recommendations from the Organisation for Economic Co-operation and Development (OECD) consensus document on compositional considerations for the assessment of new varieties of corn.[3] EH913 corn grain was analyzed for proximates (protein, fat, ash, moisture, and carbohydrates by calculation), acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber, amino acids, fatty acids, minerals, vitamins, anti-nutrients (phytic acid and raffinose), and secondary metabolites (ferulic acid, furfural, and p-coumaric acid).

Helix reported the results of its analysis, noting that a total of 31 fatty acids in the grain and the secondary metabolite, furfural, had more than 50% of their observations below the lower limit of quantitation and hence were not statistically analyzed. The remaining analytes showed no statistically significant difference between EH913 corn and the conventional control, except for total dietary fiber, protein, methionine, palmitic acid, linoleic acid, calcium, zinc, vitamin A, vitamin B2, Vitamin B3, and vitamin E. However, for these 11 analytes, Helix reported that the differences between EH913 corn means and the conventional control means were small, and the individual values were within the range of the concurrently grown commercial hybrid reference varieties. Helix explained that these results reflect the natural variation present in corn and therefore the statistical differences observed were not biologically meaningful. Helix concluded

[2] Four of the nine conventional commercial hybrids were grown at each of the seven locations.
[3] Organisation for Economic Co-operation and Development. 2002. Consensus document on compositional considerations for new varieties of maize (*Zea mays*): Key food and feed nutrients, anti-nutrients, and secondary plant metabolites. ENV/JM/MONO (2002) 25. OECD, Paris, France.

that EH913 corn is nutritionally and compositionally comparable to the conventional control and commercial corn hybrids currently on the market with a history of safe use in food.

Conclusion

Based on the information provided by Helix and other information available to CFSAN, we have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from EH913 corn. We consider the consultation with Helix on EH913 corn to be complete.

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