

Biotechnology Notification File No. 000194 CVM Note to the File

Date: April 15, 2024

From: Lei Dai, BVSc, Ph.D.

To: Administrative Record, BNF No. 000194

Subject: Event EH913 Corn

Keywords: Corn, Maize, *Zea mays* L, *cry1Da*, *Bacillus thuringiensis*, Cry1Da, Insect resistance, *bar*, *Streptomyces hygroscopicus*, Phosphinothricin-N-acetyltransferase (PAT), Herbicide tolerance, Glufosinate ammonium, OECD Unique Identifier EH-BRS913-2, Helix Sementes e Biotecnologia Ltda.¹

Purpose

This document summarizes the Food and Drug Administration (FDA) Center for Veterinary Medicine's (CVM, we) evaluation of biotechnology notification file (BNF) number 000194. Helix Sementes e Biotecnologia Ltda. (Helix) submitted a safety and nutritional assessment for a genetically engineered (GE) corn, transformation event EH913 (hereafter referred to as EH913 corn), and additional information afterwards. CVM evaluated the information in Helix's submissions to ensure that regulatory and safety issues regarding animal food derived from EH913 corn have been resolved prior to commercial distribution. FDA's Center for Food Safety and Applied Nutrition summarizes its evaluation of uses of EH913 corn in human food in a separate document.

In CVM's evaluation, we considered all of the information provided by Helix 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 EH913 corn is to confer resistance to certain lepidopteran pests including fall armyworm and sugarcane borer. To confer insect resistance, Helix introduced the *cry1Da* gene from *Bacillus thuringiensis* that encodes the Cry1Da protein. Helix also introduced the *bar* gene from *Streptomyces hygroscopicus* that encodes the PAT protein which confers tolerance to the herbicide glufosinate and was used as a selectable marker.

¹ On May 9, 2023 the developer informed FDA about its name change from "Helix Sementes e Mudas Ltda." to "Helix Sementes e Biotecnologia Ltda.".

Regulatory Considerations

The purpose of this evaluation is to determine whether use of the new plant variety in animal food raises safety or regulatory issues under the Federal Food, Drug and Cosmetic Act (FD&C Act).

The Environmental Protection Agency (EPA) defines a plant-incorporated protectant (PIP) 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” (40 CFR 174.3). EPA regulates PIPs under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the FD&C Act. Under EPA’s regulations, the Cry1Da protein and the genetic material used to express it in EH913 corn are considered pesticidal substances, and the PAT protein and the genetic material used to express it are considered inert ingredients. Therefore, the safety assessment of these products falls under the regulatory purview of EPA.²

Stability and Inheritance

Helix characterized the insertion event and genomic stability of the insert in the EH913 corn genome using bioinformatics techniques based on data obtained from whole genome sequencing (WGS). Helix estimated that it collected sufficient data for WGS to cover the corn genome in each EH913 corn plant obtained from four different generations and the corresponding non-GE conventional controls. Helix performed WGS on a total of four generations to confirm the genomic stability. Helix detected identical T-DNA and junction sequences in all four generations tested. Helix evaluated the inheritance pattern in three generations by evaluating the glufosinate tolerance phenotype which is conferred by the PAT protein. Helix concluded that the inserted nucleotide sequence was stably integrated at a single locus and segregated according to expected Mendelian principles.

Animal Food Use

The developer states that EH913 corn is intended for the same uses in animal food as conventional corn, with no new animal food uses anticipated. The typical uses of maize-derived food and feed are well documented (OECD, 2002),³ including food use of the kernels for oil, starch, grits, meal, flour, and use of the kernels or whole plant silage for feed. By-products that result from the processing of kernels are also used in animal feed. Production and different methods of processing are also described in detail in the OECD maize composition consensus document.

² Helix states that EPA has established a tolerance exemption for PAT protein (40 CFR 174.522). Helix also states that it will ensure that there is a tolerance exemption for Cry1Da protein.

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. OECD ENV/JM/MONO 25. OECD, Paris, France.

Composition

Scope of Analysis

Helix analyzed the nutrient composition of forage and grain from EH913 corn and a non-GE corn hybrid variety with a similar genetic background (control) and four non-GE conventional corn varieties⁴ that were grown and harvested under similar conditions. Compositional analyses of grain and forage samples were reported for components listed in the OECD maize composition consensus document (OECD, 2002).³

Study Design

Helix conducted field trials in the 2020-21 season at seven locations in Brazil. A randomized block design with four replicate plots was used at each field site. Forage samples were harvested at the R4-R5 growth stage and were immediately frozen before shipping. Grain samples were collected at the R6 stage and shipped at ambient temperature. Forage samples were maintained at no more than -60 °C during shipment to the analytical laboratory for composition analysis. Helix harvested grain and forage from each experimental plot within each site for composition analysis.

Helix statistically compared each component of EH913 corn and the control across locations using a mixed model analysis of variance (ANOVA) with replicates and location as random factors. All components except moisture were expressed on a dry matter basis prior to statistical analysis. Helix excluded components from statistical analysis if more than 50% of the observed values were at or below the limit of quantitation (LOQ). T-test analyses were used to test at the level of $P \leq 0.05$ for differences between EH913 corn and control. When a statistically significant difference in a component was detected between EH913 corn and control, Helix assessed whether the difference was biologically meaningful including comparisons of the EH913 corn means with ranges of reference varieties grown concurrently at the various locations. If the range of EH913 corn contained individual values that fell outside this range, then these values were compared to the range of values in the public literature or the ILSI Crop Composition Database (ILSI-CCDB).

Results of Analyses

For forage, Helix reports statistical values for proximates (crude protein, moisture, crude fat, ash, and carbohydrates by calculation), fiber (acid detergent fiber (ADF), neutral detergent fiber (NDF), and crude fiber), calcium, phosphorus, and one anti-nutrient (phytic acid). Helix found no statistically significant differences between the control and EH913 corn in these components.

For grain, Helix chemically analyzed proximates, fiber (ADF, NDF, and total dietary fiber), 18 amino acids, 37 fatty acids, nine minerals, 11 vitamins, two anti-nutrients and three secondary metabolites. Helix noted that 31 of the fatty acids and furfural were not statistically analyzed because more than 50% of the observations fell below the lower LOQ. Helix reports statistically significant differences between the control and EH913 corn in the levels of 11 components (total dietary fiber, crude protein, palmitic acid,

⁴ For comparison, Helix included a total of nine non-GE conventional corn hybrids reference lines, with four of the nine grown at each of the seven locations.

linoleic acid, methionine, calcium, zinc, and vitamins A, B2, B3, and alpha-tocopherol). For these components, the mean difference between EH913 corn and the control was less than the range of values observed in the concurrently grown non-GE conventional corn varieties.

Summary of Compositional Analyses

Helix states based on the results from the compositional analyses that forage and grain obtained from EH913 corn are not meaningfully different from those of the control and reference varieties. Helix concludes that these results support the conclusion that forage and grain obtained from EH913 corn are compositionally comparable to the control and reference varieties in the levels of key nutrients, anti-nutrients, and secondary metabolites.

Conclusion

CVM evaluated Helix's submissions to determine whether EH913 corn raises any safety or regulatory issues with respect to its uses in animal food. Based on the information provided by Helix 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.

Helix concludes that EH913 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 corn varieties now grown, marketed, and consumed. At this time, based on Helix's data and information, CVM considers Helix's consultation on EH913 corn for use in animal food to be complete.

Lei Dai -S Digitally signed by Lei Dai -S
Date: 2024.04.15 09:49:35
-04'00'

Lei Dai, BVSc, Ph.D.
Biologist