Biotechnology Consultation – Note to the File
Biotechnology Notification File No. 000159

Date: August 1, 2018

From: Jianmei Zhu

To: Administrative Record, BNF No. 000159

Subject: CTC175-A Insect Resistant Sugarcane

Keywords: sugarcane; *Saccharum X officinarum*; Cry1Ab; *Bacillus thuringiensis*; sugarcane borer infestation; Event CTC175-A; Centro de Tecnologia Canavieira; NPTII

Purpose

This document summarizes the Food and Drug Administration's (FDA's, our) evaluation of biotechnology notification file (BNF) No. 000159. The Centro de Tecnologia Canavieira (CTC) submitted a safety and compositional assessment of raw and refined sugar from genetically engineered insect resistant sugarcane, transformation event CTC175-A (CTC175-A sugarcane), which we received on February 15, 2017. We received additional information from CTC on July 19, 2017. We evaluated the information in CTC's submissions to ensure that regulatory and safety issues regarding raw and refined sugar derived from CTC175-A sugarcane in human or animal food have been resolved prior to commercial distribution.

In our evaluation, we considered all information provided by CTC as well as publicly available information and information in the agency's files. Here we discuss the outcome of the consultation, 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 CTC175-A sugarcane is to confer resistance to certain lepidopteran pests including the sugarcane borer. CTC introduced the *cry1Ab* gene from *Bacillus thuringiensis* (Bt), which encodes the Cry1Ab protein that confers resistance to the sugarcane borer. CTC also introduced the *nptII* gene, which encodes neomycin phosphotransferase (NPTII), used as a selective marker.

Regulatory Considerations

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

The United States 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 in the produce thereof, and the genetic material necessary for 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 and the FD&C Act. Under

EPA's regulations, the Cry1Ab and NPTII proteins are PIPs and are subject to existing tolerance exemptions in 40 CFR 174.511 and 40 CFR 174.521. CTC states that both proteins comply with these tolerance exemptions.

CTC states that this sugarcane event is intended solely for cultivation in Brazil; only raw sugar and refined sugar processed using sugarcane from this event will be imported to the United States for use in food.

Inheritance and Stability

CTC characterized the insertion event and genomic stability of the DNA that was inserted at different locations in the sugarcane genome using DNA sequencing, Southern blots, quantitative polymerase chain reaction (qPCR) and bioinformatics analyses of the data. CTC used the Illumina X and PacBio sequencing platforms to generate the sequencing data with an average sequencing coverage of 80x fold. Based on the data, CTC estimates that CTC175-A sugarcane has seven insertions, which include three complete insertions, one incomplete insertion, and three small, non-functional insertions. CTC reports that there are six copies of the *cry1Ab* gene and nine copies of the *nptII* gene. CTC identified nine junction sequences[1] between the inserted DNA and the genome.

CTC performed Southern blots on DNA samples from two generations (greenhouse and field) and three additional samples spanning five generations to confirm the genomic stability of CTC175-A sugarcane.[2] CTC detected similar or identical banding patterns among these DNA samples and concluded that the inserts are stably transmitted across multiple generations.

Human and Animal Food Uses

Sugarcane "tops" are usually left in the field after harvest of stalks and can also be used as animal food. Key products from sugarcane processing are sugar (raw and refined) and ethanol; sugar is primarily used as a sweetener in human food. Sugarcane is also used to make syrup, artisanal sugars, candies, and alcoholic beverages. By-products from sugarcane processing, such as bagasse and molasses, are used in animal food rations for their high carbohydrate and fiber content. [3]

Composition

Scope of Analysis

CTC analyzed the composition of CTC175-A sugarcane and statistically compared it with that of a conventional CTC20 isoline (the control). CTC also qualitatively compared the results of compositional analyses for CTC175-A sugarcane with the range of values observed from a CTC20 tissue culture null segregant (CTC20 null) and three commercially available sugarcane cultivars in Brazil (CTC4, CTC15, and CTC9001) (reference varieties). In addition, CTC compared raw sugar extracted from CTC175-A sugarcane with raw sugar from the control. Components analyzed were based on OECD's recommendation for nutritional assessment of sugarcane;[4] only the results of sucrose analyses are summarized in this document, because raw sugar and refined sugar from CTC175-A sugarcane are the only products intended for import into the United States. Raw sugar was also analyzed for key

[1] Nine sequences of flanking inserts were isolated and corroborated using whole genome sequencing and the remaining putative five junctions were not isolated due to the complex nature and size of the sugarcane genome.
[2] Sugarcane is typically vegetatively propagated.
[3] CTC states that by-products from sugarcane processing are not intended for export to United States for use in human and animal food.
[4] OECD, 2011. Consensus document on compositional considerations for new varieties of sugarcane (*Saccharum* ssp. Hybrids): Key food and feed nutrients, anti-nutrients and toxicants. ENV/JM/MONO(2011)51.

parameters as described by the International Commission for Uniform Methods of Sugar Analysis (ICUMSA).

Study Design - Compositional Analyses

CTC states that CTC175-A sugarcane, the control, the CTC20 null, and reference varieties were grown at six locations in Brazil. CTC planted the sugarcane in a randomized block design with four individual replicated blocks per site; each block contained six treatment groups. For each treatment within each block, CTC collected a whole plant sample (primarily stalk) for compositional analyses and a whole plant sample that was further processed into juice for sucrose analysis.[5] Each of the whole plant samples is a composite sample generated from 10 tillers/treatment/block. [6]

CTC statistically compared sucrose levels in sugarcane juice collected from CTC175-A sugarcane with the levels in sugarcane juice collected from the control. The statistical analysis of the intra-site mean value for each site and parameter was conducted using the PROC MIXED procedure of SAS. Differences observed were considered statistically significant based on an alpha level of 0.05. In addition, CTC determined whether the sucrose values from CTC175-A sugarcane fell within the range of values obtained for sucrose in the CTC20 null and reference varieties. In addition, CTC evaluated and compared raw sugar extracted from CTC175-A sugarcane and the control using the ICUMSA recommended methodologies.

Results and Summary of Compositional Analyses

CTC found no statistically significant difference in sucrose levels in juice between CTC175-A sugarcane and the control. CTC reported the values to be within ranges observed for the CTC20 null and reference varieties. CTC also reported that the physiochemical characteristics of raw sugar extracted from CTC175-A sugarcane and the control were comparable and comply with COPERSUCAR specifications. [7, 8] Consequently, CTC concludes that raw sugar obtained from CTC175-A sugarcane is comparable to raw sugar from the control. Further, CTC concludes that raw and refined sugar produced from CTC175-A sugarcane is as safe for use in human and animal food as that produced from conventional sugarcane.

Proteins in Raw and Refined Sugar

CTC cites published studies that demonstrated that levels of protein (and DNA) in sugar are very low, because sugar processing involves steps that include extractions, heating, and evaporation, which removes most DNA and proteins from the raw sugar product. CTC reports that levels of Cry1Ab and NPTII in CTC175-A sugarcane were at or below the limit of quantification in stalks and were not detectable in clarified juice and raw sugar.

Conclusion

FDA evaluated CTC's submission to determine whether raw and refined sugar from CTC175-A sugarcane raises any safety or regulatory issues with respect to its uses in human or animal food. Based on the information provided by the company and other information available to the agency, FDA did

[5] The samples were harvested at a stage of maturity (i.e., 365 days after planting) that is commonly used for sugarcane processing and sugar production.
[6] CTC explains that juice was extracted from composite samples to both lessen the known variability in sampling (as sucrose concentrations accumulated up the stalk during plant maturation) and to allow an estimate of total stalk sucrose levels, which more closely relate to key parameters in sugarcane breeding and processing.
[7] Copersucar. 2015. Specifications for sugar and ethanol – 15/16 vintage (2015). São Paulo. In Spanish.
[8] CTC states that refined sugar processed from raw sugar contains about 99.9% sucrose, along with low levels of water, invert or reducing sugars (glucose and fructose), ash, color components, and other organic non-sugar compounds.

not identify any safety or regulatory issues under the FD &C Act that would require further evaluation at this time.

CTC has concluded that raw and refined sugar derived from its sugarcane borer-resistant variety CTC175-A are as safe as raw and refined sugar derived from conventional sugarcane varieties and are not materially different in composition from raw and refined sugar from other sugarcane varieties grown, marketed, and consumed. At this time, based on CTC's data and information, the agency considers CTC's consultation on raw and refined sugar from CTC175-A sugarcane to be complete.

Jianmei Zhu -S

Digitally signed by Jianmei Zhu -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Jianmei Zhu -S, 0.9.2342.19200300.100.1.1=2000799960
Date: 2018.08.01 14:57:01 -04'00'

Jianmei Zhu, PhD