U.S. FOOD & DRUG ADMINISTRATION

Biotechnology Notification File No. 000169
CFSAN Note to the File

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From: Jianmei Zhu, Ph.D.

To: Administrative Record, BNF No. 000169

Subject: Raw and Refined Sugar from CTC91087-6 Insect Resistant Sugarcane

Keywords: Sugarcane, *Saccharum officinarum*, Cry1Ac, *Bacillus thuringiensis*, PAT protein, *bar* gene, resistance to sugarcane borer, Centro de Tecnologia Canavieira

Summary

Centro de Tecnologia Canavieira (CTC) has completed a consultation with the Food and Drug Administration (FDA) on raw and refined sugar derived from genetically engineered insect resistant sugarcane, transformation event CTC91087-6 (CTC91087-6 sugarcane). This document summarizes CTC'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 summarizes its evaluation pertaining to animal food in a separate document.

CTC concludes that:

- food from CTC91087-6 sugarcane is comparable to and as safe as conventional sugarcane used in human food
- there is no detectable DNA or protein in raw sugar produced from CTC91087-6 sugarcane
- raw and refined sugar from CTC91087-6 sugarcane meet commercial and international purity standards and specifications for sugar

We evaluated data and information supporting these conclusions and considered whether CTC91087-6 sugarcane raises other regulatory issues involving use in human food under the Federal Food, Drug, and Cosmetic Act. We have no further questions at this time regarding the safety, nutrition, and regulatory compliance of CTC91087-6 sugarcane for use in human food.

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 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 FD&C Act and the Federal Insecticide, Fungicide,

and Rodenticide Act. Under EPA regulations, the *cry1Ac* gene in CTC91087-6 sugarcane and the resulting expression product are considered pesticidal substances, and the *bar* gene and resulting expression products are considered inert ingredients.

Subject of the Consultation

Food:	Sugar from Sugarcane
Designation:	CTC91087-6
Trait:	Resistance to sugarcane borer
Developer:	Centro de Tecnologia Canavieira (CTC)
Original submission received:	October 1, 2018
Amendment received:	March 27, 2019; May 7, 2020; July 16, 2020
Intended use:	Refined sugar in human food
Intended genetic change:	Cry1Ac protein derived from *Bacillus thuringiensis* was introduced for insect control and PAT protein encoded by the *bar* gene was introduced as a selective marker
Method for conferring genetic change:	A vector containing both the Cry1Ac and PAT coding sequences on the same T-DNA is transformed to CTC9001. Both coding sequences are under the control of maize *ubi-1* promoter and *nos* 3' polyadenylation signal. Transformants were selected for tolerance to glufosinate.
Transformation vector:	pCTC524
Transformation method:	*Agrobacterium*-mediated transformation

Molecular Characterization

Confirmation of intended genetic change

CTC characterized the insertion event and genomic stability of the sugarcane genome using Southern blot and DNA sequence analyses. CTC concludes that there is a single copy of the intended transgene integrated at a single locus. CTC also concluded that the vector backbone sequence is absent. CTC performed Southern blots on DNA samples spanning seven successive generations to confirm the genomic stability of CTC91087-6 sugarcane.

EPA granted exemptions from the requirement of a tolerance for Cry1Ac and PAT proteins (40 CFR 174.510 and 40 CFR 174.522, respectively). CTC states that CTC91087-6 sugarcane event is intended solely for cultivation in Brazil; only raw and refined sugar processed using this sugarcane will be imported to the United States for human food use.

DNA and Proteins in Raw and Refined Sugar

CTC cites published studies that demonstrate levels of protein and DNA in refined sugar are not detectable; this is due to steps during sugar processing such as extractions, heating, and evaporation that remove most DNA and proteins from raw sugar. CTC reports that levels of Cry1Ac and PAT DNA and protein were below the limits of detection of the methods employed (PCR analysis and ELISA, respectively), in raw sugar produced from CTC91087-6 sugarcane. CTC states that these results imply absence of protein and DNA from the corresponding refined sugar as a result.

Human Food Nutritional Assessment

CTC states that CTC91087-6 sugarcane, the control CTC9001 sugarcane and six commercial varieties were grown at five locations in Brazil during the 2017/18 season. CTC planted the sugarcane in a randomized complete block design with four replicates per block of each event, control and four reference varieties. Stalk and whole plant samples were collected per plot at the mature stage used in sugarcane processing and sugar production. These samples were subjected to compositional analyses of proximates and sugars (glucose, fructose, and sucrose) according to OECD recommendations for sugarcane nutritional assessment. CTC concludes that CTC91087-6 sugarcane is compositionally similar to the control and within the range of values observed for commercially grown sugarcane varieties in Brazil.

In a separate analysis, CTC analyzed raw sugar from four plots of CTC91087-6 sugarcane and compared the data to raw sugar from the control and reference varieties. CTC concluded that raw sugar from CTC91087-6 sugarcane is comparable to raw sugar from the control and reference varieties. Further, CTC concluded that raw and refined sugar from CTC91087-6 sugarcane is chemically indistinguishable from raw and refined sugar from other sources, meeting U.S. and international specifications, and is as safe for human consumption as that produced from conventional sugarcane.

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