FDA U.S. FOOD & DRUG ADMINISTRATION
CENTER FOR VETERINARY MEDICINE

Memorandum

Biotechnology Notification File No. 000169
CVM Note to the File

Date: August 17, 2020

From: Lei Dai, Ph.D.

To: Administrative Record, BNF No. 000169

Subject: CTC91087-6 Insect Resistant Sugarcane

Keywords:
Sugarcane; Saccharum X officinarum; Cry1Ac; Bacillus thuringiensis; resistance to sugarcane borer; Event CTC91087-6; Centro de Tecnologia Canavieira; phosphinothricin N-acetyltransferase; bar; Streptomyces hygroscopicus

Purpose:
This document summarizes the Food and Drug Administration (FDA) Center for Veterinary Medicine's (CVM, we) evaluation of biotechnology notification file (BNF) number 000169. The Centro de Tecnologia Canavieira (CTC) submitted a safety and compositional assessment of raw and refined sugar from genetically engineered (GE) insect resistant sugarcane, transformation event CTC91087-6 (CTC91087-6 sugarcane) and additional information afterwards. CVM evaluated the information in CTC's submissions to ensure that regulatory and safety issues regarding the use of raw and refined sugar derived from CTC91087-6 sugarcane in animal food have been resolved prior to commercial distribution. FDA's Center for Food Safety and Nutrition summarizes its evaluation of raw and refined sugar from CTC91087-6 sugarcane for use in human food in a separate document.

In CVM's 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 for animal food use, but do not intend to restate the information provided in the final consultation in its entirety.

Intended Effect:
The intended effect of the modification in CTC91087-6 sugarcane is to confer resistance to certain lepidopteran pests including the sugarcane borer. CTC introduced the *cry1Ac* gene from *Bacillus thuringiensis* (Bt), which encodes the Cry1Ac protein that confers resistance to the sugarcane borer. CTC also introduced the *bar* gene from *Streptomyces*

hygroscopicus, which encodes phosphinothricin N-acetyltransferase (PAT/*bar*), which was used as a selectable marker.

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 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 Cry1Ac and PAT/*bar* proteins are PIPs and are subject to existing tolerance exemptions in 40 CFR 174.510 and 40 CFR 174.522. CTC states that the Cry1Ac and PAT/*bar* proteins expressed in CTC91087-6 sugarcane comply with the corresponding tolerance exemptions.

CTC states that CTC91087-6 sugarcane is intended solely for cultivation in Brazil; only raw and refined sugar processed from CTC91087-6 sugarcane will be imported to the United States for use in human food.[1]

Inheritance and Stability:

CTC characterized the insertion event and genomic stability of the insert in the sugarcane genome using Southern blots, DNA sequencing, and bioinformatics analyses of the data. Based on these data, CTC concludes that CTC91087-6 sugarcane has a single DNA insert containing the *cry1Ac* and *bar* expression cassettes.

CTC evaluated the stability and integrity of the insert in the initial CTC91087-6 sugarcane plant through six cuts using Southern blot analyses.[2] CTC detected similar banding patterns across these DNA samples and concluded that the insert is stably transmitted across multiple cuts.

---

[1] CVM notes that sugar is used in the diets of some animal species, and the by-products of raw sugar processing are used in animal food. For a product like bulk sugar (raw and refined) with uses in both human and animal food, it is impractical to maintain separate human food and animal food streams for raw and refined sugar from CTC91087-6 sugarcane, including the by-products from the refining of raw sugar. In its submission, CTC states that the sole food or food ingredient for consumers in the United States is refined sugar, and that CTC intends to import both raw and refined sugar into the United States. CVM notes that the by-products from the refining of raw sugar from CTC91087-6 sugarcane into refined sugar for human consumption are likely to enter the animal food supply.

[2] Sugarcane is typically vegetatively propagated.

Animal Food Use:

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 diets for their high carbohydrate and fiber content.[3]

Composition:

Scope of Analysis:

CTC analyzed the composition of CTC91087-6 sugarcane and statistically compared it with that of the conventional parental line CTC9001 (the control). CTC also qualitatively compared the results of compositional analyses for CTC91087-6 sugarcane with the range of values observed from six commercially available sugarcane cultivars in Brazil (CTC4, CTC20, CTC9002, RB855156, RB867515, and RB965902) (reference varieties). 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 CTC91087-6 sugarcane are the only products intended for import into the United States. In addition, CTC compared raw sugar extracted from CTC91087-6 sugarcane with raw sugar from the control, CTC20, and CTC175-A sugarcane as well as several types of commercial Brazilian raw sugar.[5] Raw sugar was analyzed for key parameters as described by the International Commission for Uniform Methods of Sugar Analysis (ICUMSA).

Study Design:

CTC states that CTC91087-6 sugarcane, the control, and reference varieties were grown at five locations in Brazil. CTC planted the sugarcane in a randomized block design with four individual replicated blocks per site; each block contained six varieties.[6] For each variety within each block, CTC collected a whole plant sample for compositional analyses and another sample (primarily stalk) for sucrose analysis.[7] CTC states that four plots of CTC91087-6 sugarcane were harvested and processed to produce four separate lots of raw sugar.[8]

---

[3] CTC states that by-products from sugarcane processing are not intended for export to United States for use in human or 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.

[5] Raw and refined sugar from CTC175-A sugarcane are the subject of BNF No. 000159.

[6] Four reference varieties were planted at each test site. Reference varieties were selected for each test site based on preferred environmental conditions for growth.

[7] The samples were harvested at a stage of maturity (i.e., 330 days after planting) that is near that commonly used for sugarcane processing and sugar production.

[8] CTC91087-6 stalk samples were harvested 330 days after planting and processed in a laboratory under conditions simulating industrial production of raw sugar. CTC states that stalk samples from the control and two other sugarcane varieties were processed under the same conditions.

CTC compared sucrose values from CTC91087-6 sugarcane with levels in the control, using R statistical software, and determined whether these values fell within the range of those obtained for sucrose in the reference varieties. The values were generated using a mixed linear model, and the statistical analysis used to compare CTC91087-6 sugarcane and the control was the t-test. Differences observed were considered statistically significant based on an alpha level of 0.05. In a separate analysis, CTC evaluated and compared raw sugar extracted from CTC91087-6 sugarcane using ICUMSA recommended methodologies to a reference range generated from other Brazilian raw sugars.

Results and Summary of Compositional Analyses:

CTC found no statistically significant difference in sucrose levels on a dry sugarcane stalk basis between CTC91087-6 sugarcane and the control. CTC reported the values to be within ranges observed for the reference varieties. CTC also reported that mean values for the physiochemical characteristics of raw sugar extracted from CTC91087-6 sugarcane were within the range of values for Brazilian raw sugar. Raw sugar from CTC91087-6 also complies with COPERSUCAR specifications.[9, 10] Consequently, CTC concludes that raw sugar obtained from CTC91087-6 sugarcane is comparable to commercial raw sugar. Further, CTC concludes that raw and refined sugar produced from CTC91087-6 sugarcane is as safe for use in food as raw and refined sugar produced from conventional sugarcane.

Proteins in Raw and Refined Sugar:

CTC cites published studies that demonstrated that levels of protein in raw sugar are very low, because sugar processing involves steps that include extractions, heating, and evaporation, which remove most proteins. CTC reports that levels of Cry1Ac and PAT/bar proteins in CTC91087-6 sugarcane were below the limit of detection in raw sugar.

Conclusion:

CVM evaluated CTC's submissions to determine whether raw and refined sugar from CTC91087-6 sugarcane raises any safety or regulatory issues with respect to its uses in animal food. Based on the information provided by the company 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.

CTC has concluded that raw and refined sugar derived from its sugarcane borer-resistant variety CTC91087-6 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, CVM considers CTC's consultation

[9] Copersucar. 2015. Specifications for sugar and ethanol – 15/16 vintage (2015). São Paulo. In Spanish.
[10] 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.

on raw and refined sugar from CTC91087-6 sugarcane for use in animal food to be complete.

Lei Dai -S Digitally signed by Lei Dai -S
Date: 2020.08.17 12:36:41 -04'00'

Lei Dai, Ph.D.
Molecular Biology Staff Fellow

Michaela G. Alewynse -S Digitally signed by Michaela G. Alewynse -S
Date: 2020.08.17 13:42:16 -04'00'

Michaela Alewynse, Ph.D.
Leader, Nutrition and Labeling Team II