FDA U.S. FOOD & DRUG ADMINISTRATION

Biotechnology Notification File No. 000164
CFSAN Note to the File

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From: Patrick Cournoyer, Ph.D.

To: Administrative Record, BNF No. 000164

Subject: High Oleic Soybean FAD2KO

Keywords: High oleic acid oil, Soybean, *Glycine max* (L.) Merr., Transcription Activator-Like Effector Nuclease (TALEN), genome editing, *FAD2-1A*, *FAD2-1B*, FAD2KO, Calyxt, Inc.

Summary

Calyxt, Inc. has completed a consultation with the Food and Drug Administration (FDA) on food derived from high oleic soybean with mutations in the *FAD2-1A* and *FAD2-1B* genes (FAD2KO soybean). This document summarizes Calyxt'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.

Calyxt concludes:

- it has not introduced into food a new protein or other substance that would require premarket approval as a food additive
- food from FAD2KO soybean is comparable to and as safe as human food from other high oleic soybeans
- oil from FAD2KO soybean has a fatty acid profile consistent with criteria for "high oleic soybean oil" [1]
- "high oleic soybean oil" is an appropriate common or usual name for oil from FAD2KO soybean

We evaluated data and information supporting these conclusions and considered whether FAD2KO soybean raises other regulatory issues involving human food under the Federal Food Drug and Cosmetic Act. We have no further questions at this time about the safety, nutrition, and regulatory compliance of food from FAD2KO soybean.

[1] The composition of the oil is consistent with specifications for "high oleic soybean oil" in the Food Chemicals Codex, Edition 11, 2018.

Subject of the Consultation

Crop:	Soybean
Designation:	FAD2KO
Trait:	Increased levels of oleic acid
Developer:	Calyxt, Inc.
Original submission received:	November 14, 2017
Amendment received:	August 30, 2018
Intended use:	Oil in human food and defatted meal in animal food
Intended genetic change:	Deletion mutations in the fatty acid desaturase (*FAD*) genes *FAD2-1A* and *FAD2-1B*
Method for conferring genetic change:	A pair of transcription activator-like effector nucleases (TALENs) was designed to target sequences unique to *FAD2-1A* and *FAD2-1B*. DNA encoding the TALENs was delivered by *Agrobacterium*-mediated transformation. After identifying plants with the desired mutations, Calyxt self-pollinated them and identified progeny that inherited the desired mutations but did not inherit inserted TALEN DNA.

Molecular Characterization

Confirmation of intended genetic change

Calyxt used PCR amplification followed by direct sequencing to characterize the TALEN-induced mutations in the target genes. A 63-base pair (bp) deletion was identified in *FAD2-1A*. The mutation is in-frame and is predicted to result in a 21-amino acid deletion affecting the enzyme's active site. A 23 bp deletion was identified in *FAD2-1B*. This mutation results in a frame-shift and premature stop codon, which is predicted to truncate and inactivate the protein.

Calyxt considered the specificity of the genetic changes. Whole genome sequencing found no evidence of new mutations in the seven genes with greatest similarity to the target sites.

Absence of TALEN DNA

After using PCR to identify self-pollinated plants that inherited the desired mutations and did not inherit TALEN DNA, Calyxt confirmed the absence of TALEN transformation vector DNA using whole genome sequencing and bioinformatic analysis. Calyxt identified no junction sequences with both TALEN transformation vector sequence and soybean genomic DNA.

Human Food Nutritional Assessment

Characterization of intended trait

Calyxt measured levels of palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and arachidic acid in oil and in seed from FAD2KO soybean and from the parental soybean variety (the control). The principal fatty acid in FAD2KO soybean is oleic acid (75-80%). The remaining fatty acids primarily consist of palmitic acid (~7%), linoleic acid (~2-6%), stearic acid (2-4%), linolenic acid (3-5%), and arachidic acid (0.35-0.4%). FAD2KO soybean has increased levels of oleic acid and decreased levels of linoleic acid, palmitic acid, and linolenic acid compared to the control. Compensatory reductions in levels of these fatty acids were expected.

Calyxt states that FAD2KO soybean oil is similar to other high oleic oils that are safely consumed, such as olive oil, high oleic sunflower oil, canola oil, safflower oil, and other high oleic soybean oils. Calyxt refers to nutritional assessments in previous FDA consultations on genetically engineered high oleic soybeans (BNF 000110 and BNF 000121).[2]

Analysis of key nutrients, anti-nutrients, and toxicants

To ensure the absence of unintended changes in other components relevant to safety or nutrition, Calyxt analyzed seed for proximates (ash, carbohydrate, fat, protein, moisture, acid detergent fiber, and neutral detergent fiber), isoflavones (daidzein, genisten, and glycitein), anti-nutrients (soybean lectin, phytic acid, raffinose, stachyose, and trypsin inhibitor), lecithins (phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol), and amino acids. Calyxt found that levels of these components in FAD2KO soybean were similar to the control and to literature values.

Patrick M. Cournoyer -S
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Patrick Cournoyer, Ph.D.

[2] FDA also evaluated genetically engineered, high oleic soybean in BNF 000039.