

Memorandum

Biotechnology Notification File No. 000164 CVM Note to the File

Date: February 22, 2019

From: Rial Christensen

To: Administrative Record, BNF No. 000164

Subject: FAD2KO, high oleic acid soybean

Keywords:

Soybean, soybean meal, Glycine max (L.) Merr., Transcription Activator-Like Effector Nuclease (TALEN), genome editing, gene knock out, FAD2-1A, FAD2-1B, high oleic acid oil, Agrobacterium-mediated transformation, FAD2KO, Calyxt

Purpose:

This document summarizes the Food and Drug Administration (FDA) Center for Veterinary Medicine's (CVM, our) evaluation of biotechnology notification file (BNF) No. 000164. In a submission dated November 13, 2017, Calyxt, Incorporated (hereafter referred to as Calyxt) submitted to FDA a safety and nutritional assessment for a high oleic acid soybean variety (FAD2KO soybean). Calyxt provided additional information in a submission dated August 29, 2018. We evaluated the information in Calyxt's submissions to ensure that regulatory and safety issues regarding the use of meal derived from the new plant variety in animal food have been resolved prior to commercial distribution. FDA's Center for Food Safety and Applied Nutrition summarizes its evaluation pertaining to human food in a separate document.

In CVM's evaluation, we considered all information provided by the notifier 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 modifications in FAD2KO soybean is to alter the fatty acid composition of the oil obtained from the new plant variety. The developer accomplished the intended effect by deletion of nucleotide sequences within the microsomal omega-6 fatty acid desaturase (FAD) 2-1A and FAD2-1B gene homologues, which results in the inactivation of these two genes. The FAD2 enzymes, which are primarily expressed in

developing soybean seeds¹, are responsible for the conversion of oleic acid precursors to linoleic acid precursors.

Regulatory Considerations:

The purposes of this evaluation are (1) to assess whether the developer has introduced into animal food a substance requiring premarket approval as a food additive and (2) to determine whether use of meal derived from the new plant variety in animal food raises other regulatory issues with respect to the Federal Food, Drug, and Cosmetic Act (FD&C Act).

Genetic Modification and Characterization:

Explants from a soybean variety were transformed using *Agrobacterium*-mediated plant transformation.² The Transcription Activator-Like Effector Nuclease (TALEN)³ vector was delineated by left and right border sequences and contained a TALEN expression cassette, with two TALEN recognition sequences and nuclease monomers, and a selectable marker gene cassette, each under the control of well characterized promoter and terminator sequences. Subsequently, Calyxt states the explants were cultivated on selection media to identify T₀ transformed plants, which were then grown to maturity. Seed from the T₀ plant was harvested for production of T₁ plants. Calyxt used PCR-amplification of the TALEN target site, nucleotide sequencing, and alignment to wild-type DNA sequences to identify mutations in the FAD2-1A and FAD2-1B genes. One T₁ plant was identified that was homozygous both for a deletion in FAD2-1A and a deletion in FAD2-1B. This plant was self-pollinated to obtain T₂ seed and the resultant progeny were genotyped to identify those that did not carry any of the transfer DNA (T-DNA) or plasmid backbone sequences (null segregant).

In addition to Sanger sequencing, Calyxt performed whole genome sequencing on genetic material obtained from T₂ plants using Illumina HiSeq paired-end sequencing methodology. Calyxt performed quality control analysis on the data and collected sufficient data to achieve at least 67-fold read coverage for each of the FAD2 genes. Bioinformatics was used to verify the nucleotide sequence for the edited sequences and the absence of the TALEN expression vector sequences (T-DNA and backbone nucleotide sequences). Calyxt also verified the specificity of the transcription activator-like effector sequences to determine if there were unintended off-target changes using bioinformatics analysis. Calyxt concludes there were no changes in the nucleotide sequences in the seven gene sequences that are closely related to the TALEN targeted FAD2 genes.

¹ Schlueter, J.A., I.F. Vasylenko-Sanders, S. Deshpande, J. Yi, M. Siegfried, B.A. Roe, S.D. Schlueter, B.E. Scheffler, and R.C. Shoemaker. 2007. The FAD2 gene family of soybean: Insights into the structural and functional divergence of a paleopolyploid genome. *Crop Sci.* 47:S14-16.

² Haun, W., A. Coffman, B.M. Clasen, Z.L. Demorest, A. Lowry, E. Ray, A. Retterath, T. Stoddard, A. Juillerat, F. Cedrone, L. Mathis, D.F. Voytas, and F. Zhang. 2014. Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. *Plant Biotech. J.* 12:934-940.

³ A TALEN is a sequence of amino acids that form DNA binding motifs that specifically recognize target DNA sequences. In vivo, each pair of TALENS functions as a dimer to cleave the target DNA within the spacer sequence between the two TALEN recognition sites.

Calyxt monitored the stability and integrity of the intended modification across two self-pollinated generations (comparing the nucleotide sequence at the two edited loci in genomic DNA obtained from seeds obtained from T₁ and T₂ plants). Calyxt concludes that the desired genotype segregated according to the expected Mendelian principles.

Safety of Proteins:

FAD2 family of proteins catalyzes the conversion of the monounsaturated fatty acid, oleic acid, to the polyunsaturated fatty acid, linoleic acid. The soybean genome has at least five genes that are part of the FAD2 gene family. Among them, FAD2-1A and FAD2-1B are expressed primarily in developing seeds and, thus, their activity is likely to determine oleic acid levels in soybean seed. High oleic acid soybean varieties have been produced through conventional breeding and genetic engineering approaches aimed at reducing the activity of these two proteins.

Calyxt determined that deletions within the two targeted FAD2 genes encode for proteins that would be 147 and 366 amino acids, compared to the native proteins which both contain 387 amino acids. Further, Calyxt concludes that these deletions would result in deletion of the enzyme's catalytic site from the FAD2-1A protein and premature truncation of the FAD2-1B protein. Calyxt used bioinformatic analysis to identify potential new open reading frames (ORFs) related to the edited sequences and to assess whether the edited proteins and other potential putative peptides could be toxic. Calyxt compared the protein sequences for the edited proteins and other potential putative peptides to sequences in the National Center for Bioinformatics Information non-redundant protein sequence database with a keyword filter for "toxin". No unique matches were recovered when the edited proteins and other putative peptides were used as query terms. Calyxt notes that the FAD2 proteins are membrane-bound and states that membrane-bound proteins are a minor component of the total protein found in soybeans seeds. Calyxt concludes that there is no increased safety risk compared to wild-type soybean.

Animal Food Use:

Soybean (*Glycine max* (L.) Merr.) is grown around the world for a variety of human and animal food and industrial uses. Most soybean seeds are processed into oil and meal. Soybean oil is commonly used as a human food ingredient. The preponderance of soybean meal is used in animal food, primarily in poultry, swine, and beef and dairy cattle diets. Soybean meal is processed in moist heat to inactivate trypsin inhibitors and lectins, which are anti-nutrients occurring in raw soybeans. Calyxt states that it does not expect the use of whole soybean seeds, oil or forage from FAD2KO soybean in animal food.

Composition:

Scope of Analysis:

Calyxt analyzed the nutrient composition of FAD2KO soybean and conventional soybean (control) that were grown and harvested under similar conditions. Calyxt selected components for analysis from those recommended in the Organization for

Economic Cooperation and Development (OECD) consensus document for soybean composition.⁴

Study Design:

Calyxt conducted field trials during the 2016 growing season with four replicate plots at each of three field sites. Calyxt presents mean values and ranges for proximates (moisture, crude protein, crude fat, ash, and carbohydrates by calculation), fiber (acid detergent fiber and neutral detergent fiber), 18 amino acids, six fatty acids, three isoflavones (daidzein, genistein, and glycitein), four lecithins, and five anti-nutrients (lectin, phytic acid, raffinose, stachyose, and trypsin inhibitor) in whole seed. In addition, FAD2KO soybean seed was harvested from six sites during the 2016 growing season and processed into oil that was analyzed for six fatty acids, as well as lecithins (data were not provided for this analyte). The across-locations means for components were compared with ranges published for conventional soybean varieties in the International Life Sciences Institute (ILSI) Crop Composition Database and in the scientific literature.

Results of analyses:

Calyxt reports that the mean values for each of the five proximates, two fiber analytes, 18 amino acids, three isoflavones, and five anti-nutrients in FAD2KO soybean seed were similar to the mean values for these components in the control and the mean values, in almost every case, fell within the ranges of values for the control grown under similar conditions. In each case, the mean values for these components fell within ranges for these components in the ILSI database and/or scientific literature. Calyxt states that mean values for lecithins in FAD2KO soybean seed fell within the range of published values. Calyxt also reports that gamma linolenic acid levels in FAD2KO soybean seed and oil from the seed were analyzed and found to be nondetectable (less than 0.01%). Calyxt states that the low linoleic acid levels, which is an essential fatty acid for most animal species, in FAD2KO soybean would not lead to a nutritional deficiency because meal obtained from FAD2KO soybean would be mixed with other feed ingredients that provide linoleic acid, such as corn, to deliver a suitable diet.

Intended Compositional Changes:

Calyxt evaluated FAD2KO soybean seed and oil obtained from processed seed for the expected changes in fatty acid profile. Calyxt reports lower mean values for palmitic (7.76 versus 12.01 as percent of total fatty acids), stearic (3.48 versus 3.95%), linoleic (5.04 versus 53.15%), and linolenic (4.78 versus 9.16%) acids in FAD2KO soybean seed when compared to control. Calyxt reports higher mean values for oleic (77.13 versus 20.17%) and arachidic (0.36 versus 0.31%) acids in FAD2KO soybean seed when compared to control. Calyxt reports similar results for these fatty acids in oil from processed seed. Calyxt concludes that mean values for palmitic, stearic, linolenic, and arachidic acids fall within the range of values in the scientific literature and the concentration of oleic acid is higher and linoleic acid is lower than the range of values in the scientific literature (which are the intended effects).

⁴ Organization for Economic Cooperation and Development. 2001. Consensus document on compositional considerations for new varieties of soybean: key food and feed nutrients and anti-nutrients. OECD ENV/JM/MONO(2001)15.

Summary of Compositional Analyses:

Calyxt states that the genetic modifications (inactivation of the FAD2-1A and FAD2-1B proteins, which are primarily expressed in developing seeds) do not meaningfully affect composition and nutrition of the meal derived from FAD2KO soybeans except for the intended changes in the levels of specific fatty acids. Calyxt concludes that FAD2KO soybean and derived byproducts are as safe and nutritious as soybean and derived products currently on the market.

Conclusion:

CVM evaluated Calyxt's submissions to determine whether meal derived from FAD2KO soybean raises any safety or regulatory issues with respect to its uses in animal food. Calyxt states that it does not expect use of whole soybean seed, oil or forage from FAD2KO soybean in animal food, although Calyxt expects that uses of these products from FAD2KO soybean in animal food would not raise safety concerns. Based on the information provided by Calyxt 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 of meal derived from FAD2KO soybean at this time.

Calyxt has concluded that FAD2KO soybean meal and the animal foods derived from it are as safe as and, with the exception of fatty acid profile, are not materially different in composition or any other relevant parameter from meal derived from other soybean varieties now grown, marketed, and consumed. At this time, based on Calyxt's data and information, CVM considers Calyxt's consultation on meal derived from FAD2KO soybean for use in animal food to be complete.

Rial A.
Christensen -S

A red digital signature scribble is positioned over the text "Christensen -S".

Digitally signed by Rial A.
Christensen -S
Date: 2019.02.22 10:58:41 -05'00'

Rial Christensen, Ph.D.
Animal Scientist