U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

Biotechnology Notification File No. 000184
HFP Note to the File

Date: May 16, 2025

From: Charles Kanobe, Ph.D.; Biologist

To: Administrative Record, BNF No. 000184

Subject: Alfalfa with altered lignin profile (Altered lignin alfalfa)

Keywords: *Medicago sativa L.*, Alfalfa, altered lignin profile, [redacted], Transcription Activator-Like Effector Nuclease (TALEN), genome editing, syringyl (S) lignin, guaiacyl (G) lignin, Calyxt, Inc., Cibus, Inc.[1]

Summary

Cibus, Inc. (Cibus) has completed a consultation with the Food and Drug Administration (FDA) on food derived from altered lignin profile alfalfa (referred to as IQ alfalfa by Cibus; hereafter referred to as "altered lignin alfalfa") due to reduced S and G lignin. This document summarizes Cibus' conclusions and supporting data and information that FDA's Human Foods Program (HFP, we) evaluated pertaining to human food uses. FDA's Center for Veterinary Medicine summarizes its evaluation pertaining to animal food uses in a separate document.

Based on the safety and nutritional assessment Cibus has conducted, it is our understanding that Cibus concludes:

- it has not introduced into human food a new protein or other substance that would require premarket approval as a food additive, and
- human food from altered lignin alfalfa is comparable to and as safe as human food from other alfalfa varieties.

HFP evaluated data and information supporting these conclusions and considered whether altered lignin alfalfa raises other regulatory issues involving human food within FDA's authority under the Federal Food, Drug, and Cosmetic Act (FD&C Act). We have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from altered lignin alfalfa.

[1] On August 9, 2023, the developer informed FDA about their name change from "Calyxt, Inc." to "Cibus, Inc." because of change in company ownership on June 1, 2023.

Subject of the Consultation

Crop	Alfalfa (*Medicago sativa L.*)
Designation	Altered lignin profile alfalfa
Intended trait	Altered lignin profile
Developer	Cibus, Inc.
Submission received	August 31, 2021
Amendment(s) received	August 9, 2023a; August 9, 2023b; October 4, 2023; August 23, 2024
Intended use	While altered lignin alfalfa is intended primarily for use in animal feed, alfalfa is used in human food applications, including as sprouts, and in dietary supplements and herbal teas.
Intended genetic change	Loss of function mutations in [redacted] gene
Method for conferring genetic change	Transcription activator-like effector nucleases (TALENs) delivered by polyethylene glycol-mediated transformation.

Molecular Characterization

Confirmation of intended genetic change

Cibus used polymerase chain reaction (PCR) amplification followed by sequencing to characterize the TALEN-induced mutations in the target gene. Cibus reported that there were [redacted] frameshift mutations in the [redacted] gene in altered lignin alfalfa. If translated, the frameshift mutations were predicted to produce hypothetical truncated proteins of 14-22 amino acids. Cibus also reported that the [redacted] mutations resulted in the loss of both the dimerization and methyltransferase domains. As such, in the unlikely event that a protein is translated from the edited [redacted] gene, it would not be functional. Bioinformatic analysis of the hypothetical truncated proteins produced by the edited [redacted] gene did not show similarity to known allergens or toxins.

Cibus used whole genome sequencing and in silico analysis to confirm that there were no unintended TALEN-target mutations present in altered lignin alfalfa.

Absence of TALEN DNA

To confirm the absence of TALEN transformation plasmid DNA in altered lignin Alfalfa, Cibus examined the [redacted][2] generation of altered lignin alfalfa following genome editing using Illumina high throughput DNA sequencing and bioinformatic analysis. Cibus reported that no detectable TALEN T-DNA or transformation plasmid sequences were identified in the altered lignin alfalfa genome.

[2] [redacted] Alfalfa is naturally outcrossing and therefore is cultivated in heterogeneous, heterozygous (synthetic) populations.

Intended Human Food Uses

In the United States, alfalfa is used in human food primarily as sprouted seeds, and in dietary supplements and herbal teas. Consumption is typically occasional and in small quantities. According to Cibus, the intended use of altered lignin alfalfa is in animal food, the predominant use for alfalfa in the United States.

Human Food Nutritional Assessment

Characterization of intended trait

Cibus analyzed the lignin content of altered lignin alfalfa forage at bud stage compared to that of a conventional unedited commercial control and three commercial reference varieties grown in three locations in USA. Cibus states that while alfalfa contains S, G, and hydroxyphenyl (H) lignin, the H lignin comprises only a very minor amount of the total lignin and therefore was not measured. The results showed 18% reduction in each of the S and G lignin, and 9% reduction in acid detergent lignin (total lignin), in altered lignin alfalfa compared to the control. Cibus therefore concluded that the intended reductions in levels of S and G lignin were achieved in altered lignin alfalfa.

Analysis of key nutrients, anti-nutrients, and toxicants

The intended trait in altered lignin alfalfa is not expected to alter levels of key nutrients, anti-nutrients, or toxicants, other than lignin. To assess potential unintended changes in composition relevant to safety or nutrition, Cibus analyzed forage from altered lignin alfalfa, the control, and three reference varieties, grown in multiple locations in the United States. Cibus analyzed proximates, fiber (Acid Detergent Fiber and Neutral Detergent Fiber), amino acids, minerals, and secondary metabolites (sinapine, coumestrol, p-coumaric acid, total polyphenols and ferulic acid). Cibus reported that levels of these components in altered lignin alfalfa were within the range of the control and within ranges typical of alfalfa forage in published literature.

Conclusion

Based on the information provided by Cibus and other information available to the HFP, we have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from altered lignin alfalfa. We consider the consultation with Cibus on altered lignin alfalfa to be complete.

CHARLES KANOBE -S

Digitally signed by CHARLES KANOBE -S
Date: 2025.05.16 11:29:02 -04'00'

Charles Kanobe, Ph.D.