Nicole Albrecht
c-LEcta GmbH
Perlickstr. 5
04103 Leipzig
GERMANY

Re: GRAS Notice No. GRN 001146

Dear Dr. Albrecht,

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001146. We received c-LEcta GmbH's (c-LEcta) notice on May 23, 2023, and filed it on September 5, 2023. c-LEcta submitted amendments to the notice on March 4, 2024, and May 14, 2024, that updated the notifier's name and provided clarifications on identity, specifications, dietary exposure, and the safety narrative.

The subject of the notice is nuclease enzyme preparation produced by *Bacillus amyloliquefaciens* expressing a nuclease from *Serratia marcescens* (nuclease enzyme preparation) for use as an enzyme at up to 143 mg total organic solids (TOS)/ kg microbial biomass in manufacturing of microbially-derived food ingredients. The notice informs us of c-LEcta's view that this use of nuclease enzyme preparation is GRAS through scientific procedures.

Commercial enzyme preparations that are used in food processing typically contain an enzyme component that catalyzes the chemical reaction, as well as substances used as stabilizers, preservatives, or diluents. Enzyme preparations may also contain components derived from the production organism and from the manufacturing process, e.g., constituents of the fermentation media or the residues of processing aids. c-LEcta's notice provides information about the components in the nuclease enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, nuclease is identified by the Enzyme Commission Number 3.1.30.2 and the Chemical Abstracts Service number 9025-65-4.[1] c-LEcta states that the primary sequence of nuclease consists of 245 amino acids with a calculated molecular weight of 26.7 kDa.

c-LEcta states that the *B. amyloliquefaciens* production organism is non-pathogenic and non-toxigenic and is a well-characterized production organism with history of safe use in the food industry. c-LEcta states that the *B. amyloliquefaciens* production strain GSB272 was produced by integration of an expression cassette carrying a native *B.*

[1] https://iubmb.qmul.ac.uk/enzyme/EC3/1/30/2.html

amyloliquefaciens promoter and terminator, a leader sequence from *Bacillus spp*., and the sequence encoding the nuclease from *S. marcescens* into one chromosomal locus of the recipient strain LE2B100.[2] c-LEcta states that they confirmed sequence integration by polymerase chain reaction (PCR) and whole genome sequencing. c-LEcta verified the genetic stability of the production strain by measuring enzyme activity and protein expression. c-LEcta also states that based on the results of whole genome sequencing, the production strain does not contain any functional antibiotic resistance genes.

c-LEcta states that the nuclease enzyme preparation is produced by a submerged fermentation of a pure culture of the *B. amyloliquefaciens* production strain under controlled conditions. The nuclease enzyme is secreted into the fermentation medium and then recovered by an optional pretreatment via flocculation, then separation via filtration or centrifugation. This is followed by concentration via multiple filtration steps. The resulting liquid enzyme concentrate is brown in color and is stabilized with glycerol. c-LEcta states that the fermentation media contains soy peptone but ensures that no soy allergens were detected above the LOQ of 2 mg/L in the final enzyme product using an ELISA-based methodology. c-LEcta states that the entire process is performed using food grade raw materials and in accordance with current good manufacturing practices.

c-LEcta has established food grade specifications and states that the nuclease enzyme preparation conforms to the specifications set in the Food Chemicals Codex (FCC, 12th edition, 2020)[3] and to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA, 2006). c-LEcta provides results from analyses of three non-consecutive batches of nuclease enzyme concentrate to demonstrate that the manufacturing acceptance criteria can be met, including the absence of the production organism.

c-LEcta intends to use nuclease enzyme preparation at a maximum use level of 143 mg TOS/ kg microbial biomass as the liquid form in manufacturing of microbially-derived proteins, cell extracts, and functional ingredients. c-LEcta states that the nuclease hydrolyzes single or double stranded DNA and RNA at the P-O3' bond, generating 5'-phosphate and 3'-OH products (mono- and oligonucleotides). c-LEcta notes that the final enzyme is inactivated or removed during processing. c-LEcta estimates a maximum dietary exposure to nuclease enzyme preparation to be 3.25 mg TOS/kg body weight per day (mg TOS/kg bw/d) from the intended uses with the assumption that all of the nuclease enzyme preparation will be active and remain in the final food.[4]

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[2] c-LEcta states that the production strain is deposited at the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures.

[3] Specifications for enzymes remain the same in the most recent edition of the Food Chemicals Codex (FCC, 13th edition, 2022).

[4] C-LEcta uses the Budget method to estimate the dietary exposure to nuclease enzyme preparation based on a maximum use levels of 76.5 mg TOS/kg in liquid foods and 107 mg TOS/kg in solid foods respectively, and consumption of 25 mL of non-milk beverages and 12.5 g of solid foods per kg body weight per day.

c-LEcta relies on published information that discusses the safety of the nuclease, the *B. amyloliquefaciens* production organism, and the safety of microbial enzyme preparations used in food processing. In support of the safety of the nuclease enzyme preparation, c-LEcta highlights published information that supports the history of safe use of nuclease in food and discusses the similarity between the nuclease enzyme preparation and other nucleases safely consumed in the human diet. c-LEcta states that any nuclease remaining in the final food will be digested and thus will not pose a safety risk. Additionally, c-LEcta states that a literature search did not identify any information that would contradict a general recognition of safety of the nuclease enzyme preparation. c-LEcta discusses several published toxicological studies using nuclease enzymes produced by *Penicillium citrinum*.[5] c-LEcta notes that the nuclease enzyme was not found to be mutagenic, and no treatment-related adverse effects were observed in a 90-day oral toxicity study. In the amendment dated February 20, 2024, c-LEcta discusses the similarities and differences between their nuclease enzyme preparation and the nuclease enzyme preparations from these published toxicological studies, noting that all the nucleases are similar in regard to their catalytic activity.

c-LEcta discusses publicly available literature to address potential allergenicity due to nuclease. Based on bioinformatic analysis, c-LEcta reports no significant matches between the amino acid sequences of the nuclease and the primary sequences of known food allergens based on the guidelines developed by the Codex Alimentarius Commission (Codex, 2009). Additionally, c-LEcta states that they do not expect soy allergens to be present in the final product from soy peptone due to consumption during fermentation. Based on the totality of information available, c-LEcta concludes that it is unlikely that oral consumption of nuclease enzyme preparation from the intended uses will result in allergic responses.

Based on the data and information summarized above, c-LEcta concludes that nuclease enzyme preparation is GRAS for its intended use.

Standards of Identity

In the notice, c-LEcta states its intention to use nuclease enzyme preparation in several food categories, including foods for which standards of identity exist, located in Title 21 of the CFR. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

Section 301(ll) of the Federal Food, Drug, and Cosmetic Act (FD&C Act)

Section 301(ll) of the FD&C Act prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of the FD&C Act, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations

[5] c-LEcta discusses three published toxicological studies using nuclease enzyme preparations produced in *P. citrinum* including Burdock et al. (2000), Kondo et al. (2001), and Okado et al. (2016).

have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In our evaluation of c-LEcta's notice concluding that nuclease enzyme preparation is GRAS under its intended conditions of use, we did not consider whether section 301(ll) or any of its exemptions apply to foods containing nuclease enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing nuclease enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that c-LEcta provided, as well as other information available to FDA, we have no questions at this time regarding c-LEcta's conclusion that nuclease enzyme preparation is GRAS under its intended conditions of use. This letter is not an affirmation that nuclease enzyme preparation is GRAS under 21 CFR 170.35. Unless noted above, our review did not address other provisions of the FD&C Act. Food ingredient manufacturers and food producers are responsible for ensuring that marketed products are safe and compliant with all applicable legal and regulatory requirements.

In accordance with 21 CFR 170.275(b)(2), the text of this letter responding to GRN 001146 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2024.06.12 17:06:51 -04'00'

Susan Carlson, Ph.D.
Director
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