



Dietrich B. Conze, Ph.D.
Spherix Consulting Group, Inc.
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Rockville, MD 20852

Re: GRAS Notice No. GRN 000919

Dear Dr. Conze:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000919. We received the GRAS notice that you submitted on behalf of Jennewein Biotechnologie GmbH (Jennewein) on February 21, 2020, and filed it on May 12, 2020. Jennewein submitted an amendment to the notice on July 17, 2020, that clarified the intended use¹ and manufacturing.

The subject of the notice is lacto-N-neotetraose (LNnT) for use as an ingredient in non-exempt infant formula for term infants; in toddler formula² and other drinks for young children at a maximum level of 0.6 g/L; in beverages and beverage bases, dairy product analogs, milk (whole and skim), milk products, and processed fruits and juices at levels up to 2.5 g/L; and in grain products, pastas, and infant and toddler foods at levels up to 20 g/kg. The notice informs us of Jennewein's view that these uses of LNnT are GRAS through scientific procedures.

Jennewein provides information about the identity of LNnT, which is a tetrasaccharide consisting of D-galactose, N-acetyl-D-glucosamine, D-galactose, and D-glucose. Jennewein states that LNnT is a white to off-white powder containing $\geq 92\%$ LNnT. The chemical name for LNnT is β -D-galactopyranosyl-(1 \rightarrow 4)-2-acetamido-2-deoxy- β -D-glucopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 4)-D-glucopyranose, with an empirical formula of C₂₆H₄₅NO₂₁ and molecular weight of 707.63 Da.

Jennewein describes the method of manufacture, and states that LNnT is manufactured from D-glucose, fructose, sucrose, or glycerol in a 2-step fed-batch fermentation process

¹ Jennewein states that LNnT may be used individually or in combination with other human milk oligosaccharide (HMO) ingredients that have been the subjects of previous GRAS notices. Jennewein notes that infant formula manufacturers may use different combinations of HMOs and are ultimately responsible for meeting the requirements of section 412 of the Federal Food Drug & Cosmetic (FD&C) Act.
² While we do not have a regulatory definition for "toddler formula," we recognize it as formula intended for children >12 months of age. Formulas for older infants (e.g., 9-12 months of age) would be included in the category of infant formula and must comply with the infant formula regulations under Section 412 of the FD&C Act.

using two genetically engineered strains of *Escherichia coli* BL21 (DE3)³ called “PS-LNnT-JBT” and “DS-LNnT-JBT.” Jennewein describes the construction of “PS-LNnT-JBT” by stating that they deleted, inactivated, or ectopically overexpressed genes⁴ to allow the bacterium to grow on sucrose, produce high levels of uridine diphosphate-galactose, and synthesize lactose and LNnT. Jennewein describes the construction of “DS-LNnT-JBT” by stating that they inactivated a gene and ectopically overexpressed³ additional genes by transposition to allow the bacterium to hydrolyze lactose, cleave the *N*-acetylglucosamine from lacto-*N*-triose II (LNT II), and improve the degradation of the monosaccharide impurities *N*-acetylglucosamine and galactose. Jennewein states that all added genes are chromosomally integrated, the production strains do not contain plasmids, and all episomal vectors were removed from the genome. Jennewein states that the production strains do contain antibiotic resistance genes, but that they are chromosomally located and genetically stable.

Lactose is either synthesized by the *E. coli* BL21 (DE3) during fermentation under pH-controlled conditions or added to the growth medium prior to inoculation. Fermentation proceeds until most of the lactose is converted to LNnT. The resulting biomass is separated from the fermentation medium by centrifugation and discarded. The LNnT-containing supernatant is then further processed by chromatography, filtration, and decolorization with activated charcoal to remove any contaminants, such as ions, trace elements, peptides, amino acids, DNA, and carbohydrate by-products. The refined solution is concentrated under vacuum or by nanofiltration, filtered, and dried to generate the LNnT powder. Finally, the powder is packaged into polyethylene-lined paper bags, sealed, and stored under ambient conditions. Jennewein states that all raw materials, processing aids, and food contact substances are authorized for use and that LNnT is manufactured in accordance with good manufacturing practices.

Jennewein provides specifications for LNnT that include assay ($\geq 92\%$), and limits for sulfated ash ($\leq 0.4\%$), lactose ($\leq 3.0\%$), LNT II ($\leq 3.0\%$), *para*-lacto-*N*-neohexaose ($\leq 2.0\%$), other human-identical saccharides ($\leq 8.0\%$), protein ($\leq 100 \mu\text{g/g}$), moisture ($\leq 9.0\%$), lead ($\leq 0.02 \text{ mg/kg}$), and microorganisms, including *Salmonella* serovars (absent in 25 g) and *Cronobacter sakazakii* (absent in 10 g). Jennewein provides the results of five, non-consecutive batch analyses to demonstrate that LNnT can be manufactured to meet these specifications. Jennewein states that GRN 000547⁵ provided the results of a 36-month stability study conducted under ambient conditions (25 °C/60% relative humidity) and a 6-month study conducted under accelerated conditions (40 °C/75% relative humidity). Jennewein also states that data in GRN 000659⁵ indicate that LNnT is stable up to 2 years under accelerated conditions and up to 5 years under ambient conditions.

³ Jennewein states that *E. coli* BL21 (DE3) is a well-characterized, commercial food production organism and is non-pathogenic and non-toxicogenic.

⁴ All heterologous genes to *E. coli* BL21(DE3) were purchased as synthetic DNA constructs and amplified by PCR and flanked with antibiotic resistance genes. All homologous genes from *E. coli* BL21(DE3) and *E. coli* K12 were amplified from genomic DNA by PCR and flanked with antibiotic resistance genes.

⁵ LNnT was the subject of GRNs 000547 and 000659. We evaluated these notices and responded in letters dated October 2, 2015, and November 23, 2016, respectively, stating that we had no questions at that time regarding the notifier’s GRAS conclusions.

Jennewein states that they intend to use LNnT powder as a substitute for other forms of LNnT currently in the U.S. market and at the same use levels as in GRNs 000547 and 000659; therefore, Jennewein incorporates into the notice the exposure estimates from GRNs 000547 and 000659. Jennewein concludes that the resulting exposure to LNnT will not exceed that from other forms of LNnT.

Jennewein discusses the safety of LNnT and states that it is structurally identical to human milk LNnT and the subjects of GRNs 000547 and 000659; therefore, Jennewein incorporates into the notice the safety data and information from those notices.

Jennewein states that the final product contains LNnT, lactose, LNT II, and para-lacto-*N*-neohexaose, all of which are components of human milk. Jennewein notes that as one of the most abundant oligosaccharides in human milk, the proposed use level of its LNnT is intended to match LNnT levels consumed safely in human milk. Jennewein states that a literature search conducted through December 2019 did not reveal new studies since GRN 000659 that would suggest LNnT may be unsafe for consumption.

Jennewein states that all available data suggest that the majority of LNnT, like all HMOs, is resistant to digestive enzymes, poorly absorbed as it reaches the large intestine undigested, and is either mostly fermented by the microbiota or excreted intact in the feces. Jennewein summarizes the published repeat-dose oral toxicity and genotoxicity studies discussed in GRNs 000547 and 000659 to support the conclusion that LNnT is not genotoxic and is safe up to 5000 mg/kg body weight/day. Further, Jennewein discusses other published and unpublished oral toxicity studies to support the safety of LNnT. As supportive evidence, Jennewein discusses published human studies showing that infant formula containing LNnT is well-tolerated and results in anthropometric parameters similar to infant formula or breast-fed controls. Finally, Jennewein states that the estimated dietary exposure from the intended uses are below the safe or tolerable level of intake for LNnT determined using toxicological and supporting clinical studies.

Jennewein includes the statement of a panel of individuals (Jennewein's GRAS panel). Based on its review, Jennewein's GRAS panel concluded that LNnT is safe under the conditions of its intended use.

Based on the totality of the data and information, Jennewein concludes that LNnT is GRAS for its intended use.

Standards of Identity

In the notice, Jennewein states its intention to use LNnT 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.

Potential Labeling Issues

Under section 403(a) of the FD&C Act, a food is misbranded if its labeling is false or misleading in any way. Section 403(r) of the FD&C Act lays out the statutory framework for labeling claims characterizing a nutrient level in a food or the relationship of a nutrient to a disease or health-related condition (also referred to as nutrient content claims and health claims). If products containing LNnT bear any nutrient content or health claims on the label or in labeling, such claims are subject to the applicable requirements and are under the purview of the Office of Nutrition and Food Labeling (ONFL) in the Center for Food Safety and Applied Nutrition. The Office of Food Additive Safety did not consult with ONFL on this issue or evaluate any information in terms of labeling claims. Questions related to food labeling should be directed to ONFL.

Intended Use in Infant Formulas

Under section 412 of the FD&C Act, a manufacturer of a new infant formula must make a submission to FDA providing required assurances about the formula at least 90 days before the formula is marketed. Our response to Jennewein's GRAS notice does not alleviate the responsibility of any infant formula manufacturer that intends to market an infant formula containing LNnT to make the submission required by section 412. Infant formulas are the purview of ONFL.

Section 301(ll) of the 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 have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In our evaluation of Jennewein's notice concluding that LNnT 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 LNnT. Accordingly, our response should not be construed to be a statement that foods containing LNnT, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Jennewein provided, as well as other information available to FDA, we have no questions at this time regarding Jennewein's conclusion that LNnT is GRAS under its intended conditions of use. This letter is not an affirmation that LNnT 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 000919 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J.
Carlson -S

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