Dietrich B. Conze, Ph.D.
Spherix Consulting Group, Inc.
11821 Parklawn Drive, Suite 310
Rockville, MD 20852

Re: GRAS Notice No. GRN 000923

Dear Dr. Conze:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000923. We received the GRAS notice that you submitted on behalf of Jennewein Biotechnologie GmbH (Jennewein) on March 23, 2020, and filed it on May 14, 2020. Jennewein submitted amendments to the notice on October 19, 2020, November 3, 2020, November 5, 2020, and January 21, 2021, which reduced the intended use level, removed the use of cobalt chloride in the fermentation medium, provided additional details on the dietary exposure estimate, literature search, production organism, specifications, and corrected typographical errors.

The subject of the notice is lacto-*N*-tetraose (LNT) for use as an ingredient in cow milk-based, non-exempt infant formula for term infants at a level of 0.8 g/L. The notice informs us of Jennewein's view that this use of LNT is GRAS through scientific procedures.

Jennewein provides information on the identity and composition of LNT (CAS Registry Number 14116-68-8). Jennewein describes LNT as a white to ivory-colored powder that is ≥ 75% LNT on a dry weight basis with a molecular formula of $C_{26}H_{45}NO_{21}$ and molecular weight of 707.632 Da. The chemical name of LNT is β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-D-glucopyranose.

Jennewein describes the production organism used in the three-stage manufacturing process for LNT. The non-pathogenic and non-toxigenic production organism, *Escherichia coli* BL21 (DE3) strain DSM 33494, is genetically engineered to produce LNT. This strain is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) strain collection in Braunschweig, Germany, and is a modification of the host strain, *E. coli* BL21 (DE3).[1] Jennewein constructed the production strain by

[1] Jennewein states that the safety of *E. coli* BL21 (DE3) is summarized in GRNs 000485 and 000571. Beta-galactosidase enzyme preparation and 2′-fucosyllactose are the subjects of GRNs 000485 and 000571, respectively. We evaluated these notices and responded in letters dated April 15, 2014, and November 6, 2015, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

making five insertions of genes encoding functions for sugar metabolism from three donor species to optimize the production of LNT. Jennewein states that all gene insertions were verified by polymerase chain reaction.

Jennewein states that the manufacturing process for LNT is the same process as described in GRN 000571, except for certain process parameters, and incorporates information from GRN 000571. First, the production strain is inoculated into a fermentation medium that contains lactose and a carbon source, resulting in the secretion of LNT into the fermentation medium. After fermentation is complete, the production strain is removed and LNT is purified and concentrated by a series of filtration, ion exchange, electrodialysis, and decolorization steps. The LNT is then spray-dried to a powder. Jennewein states that all raw materials, processing aids, and medium components are used in accordance with U.S. regulations or are previously determined to be GRAS for their respective uses.

Jennewein provides specifications for LNT that include minimum levels of LNT (≥ 75% dry weight (w/w)) and limits on other carbohydrates[2] (≤ 25%, % area), lactose (≤ 5%, % area), lacto-*N*-triose II (≤ 5%, % area), para-lacto-N-hexose (≤ 5%, % area), glucose/galactose (≤ 5%, % area), protein (≤ 100 µg/g), ash (≤ 1% (w/w)), moisture (≤ 9% (w/w)), lead (≤ 0.02 mg/kg), *Salmonella* serovars (absent in 25 g), and *Cronobacter sakazakii* (absent in 10 g). Jennewein provides the results from three non-consecutive lots to demonstrate that the product can be manufactured to meet the stated specifications.

Jennewein states that their LNT is similar to the subject of GRN 000833.[3] Jennewein concludes that based on the stability studies incorporated by reference from GRN 000833 and their stability study in combination with other human milk oligosaccharides (HMOs), Jennewein's LNT has a shelf-life of 2 years from the date of production when stored under ambient conditions.

Jennewein estimates the mean and 90th percentile exposures to LNT based on published data on the daily energy intakes for infants, a typical caloric content for infant formula of 670 kcal/L, and a use level of 0.8 g LNT/L. Jennewein estimates that the exposure for LNT for infants from birth to 6 months of age ranges from 0.11 to 0.13 g/kg bw/d at the mean and from 0.13 to 0.17 g/kg bw/d at the 90th percentile. Jennewein notes that these estimates assume that infants will consume the LNT-containing infant formula as the sole source of nutrition. Jennewein further notes that LNT exposure for infants from birth to 5.9 months represents the most conservative scenario and that infants from 6 to 11.9 months will consume less LNT than those infants from birth to 5.9 months. Jennewein states that their LNT will be substitutional for other sources of LNT and, therefore, there will be no increase in the overall exposure to LNT.

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[2] As specified in Jennewein's amendment dated October 19, 2020, "other carbohydrates" are comprised of N-acetylglucosamine (GlcNAc); lacto-N-biose (LNB); galactosyllactose; glucosyllactose; LNT-$(LNB)_n$, which includes LNT-$(LNB)_2$, also known as para-lacto-N-octaose (pLNO); LNT-$(LNB)_n$-N-GlcNAc, which includes LNT-$(LNB)_0$, also known as LNT-GlcNAc; and unspecific impurities.

[3] The subject of GRN 000833 is LNT. We evaluated GRN 000833 and responded in a letter dated October 7, 2019 stating that we had no questions at that time regarding the notifier's GRAS conclusion.

Jennewein provides data and information supporting the safety of LNT and states that a literature search was conducted through March 2020. Jennewein states that HMOs, including LNT, are highly resistant to digestive enzymes, with most ingested HMOs remaining unabsorbed and passing through the gut, where they are either fermented by the resident microbiota or excreted unchanged in the feces. Jennewein states that their LNT is structurally identical to the LNT present in human breast milk, and qualitatively and quantitatively similar to the LNT that is the subject of GRN 000833. Jennewein notes that the intended use level of their LNT in infant formula is within the range of reported means for LNT in human milk and identical to the infant formula use level in GRN 000833. Jennewein incorporates the published subchronic toxicity and genotoxicity studies that were discussed in GRN 000833 to support the safe use of their LNT in infant formula. Jennewein summarizes the results of these studies, noting that there was no toxicologically significant adverse effect in the subchronic study in neonatal rats, and no indication of genotoxicity. Jennewein also discusses published safety studies on their LNT conducted as part of an HMO mixture.[4] In addition, Jennewein states that with the results of the bioinformatic analysis of the genome of the production organism and their protein specification of less than ≤ 0.01%, allergic reactions are not expected.

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

Based on the totality of data and information included in their notice, Jennewein concludes that their LNT is GRAS for its intended use.

Potential Labeling Issues

Under section 403(a) of the Federal Food, Drug, & Cosmetic (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 LNT 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 (OFAS) 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.

Allergen Labeling

The FD&C Act requires that the label of a food that is or contains an ingredient that

[4] We did not evaluate the use of LNT in combination with other HMO ingredients during our evaluation of GRN 000923.

contains a "major food allergen" declare the allergen's presence (section 403(w)). The FD&C Act defines a "major food allergen" as one of eight foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans) or a food ingredient that contains protein derived from one of those foods. LNT requires labeling under the FD&C Act because it contains protein derived from milk.

Intended Use in Infant Formula

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 LNT 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 LNT 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 LNT. Accordingly, our response should not be construed to be a statement that foods containing LNT, 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 LNT is GRAS under its intended conditions of use. This letter is not an affirmation that LNT 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

000923 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2021.02.02 17:34:00 -05'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
Office of Food Additive Safety
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