Christoph H. Röhrig, Ph.D.
Glycom A/S
Kogle Allé 4
2970 Hørsholm
DENMARK

Re: GRAS Notice No. GRN 001059

Dear Dr. Röhrig:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001059. We received Glycom A/S (Glycom)'s notice on December 2, 2021, and filed it on June 8, 2022. Glycom submitted an amendment to the notice on September 30, 2022, that narrowed the targeted population to only term infants with cow milk protein allergy (CMPA) and provided additional information on the intended use, manufacturing, and literature search.

The subject of the notice is lacto-*N*-neotetraose (LNnT) for use as an ingredient in extensively hydrolyzed- and amino acid-based, exempt infant formula for term infants with CMPA at a level up to 600 mg/L as consumed. The notice informs us of Glycom's view that this use of LNnT is GRAS through scientific procedures.

Glycom provides information about the identity of LNnT,[1] which is a tetrasaccharide composed of D-galactose, *N*-acetyl-D-glucosamine, D-galactose, and D-glucose. Glycom states that LNnT is a white to off-white powder containing ≥ 92% LNnT that may also contain other carbohydrates, including lactose, lactose-*N*-triose II, *para*-lacto-*N*-neohexaose, and LNnT fructose isomer. LNnT (CAS Registry Number 13007-32-4) has the chemical name β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-D-glucose.

Glycom describes the two-stage manufacturing process for LNnT and incorporates information on the production organism from GRN 000659 into the notice.[2] The first

[1] Glycom notes that the identity and method of manufacture of LNnT are the same as in GRN 000659. LNnT is the subject of GRN 000659. We evaluated this notice and responded in a letter dated November 23, 2016, stating that we had no questions at that time regarding Glycom's GRAS conclusion.
[2] Glycom states that LNnT may be produced using either *E. coli* K-12 DH1 MDO MP572 or *E. coli* K-12 DH1 MDO MP572b, which are described in GRN 000659 and in the supplement to GRN 000659, respectively. Glycom states that the two production strains were deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig, Germany under deposition numbers DSMZ 32272 and DSMZ 33638, respectively.

stage involves the production of LNnT from D-lactose by fermentation using a production organism derived from *Escherichia coli* K-12 DH1 MDO, which secretes LNnT into the fermentation broth. After fermentation is complete, the microbial biomass is removed by filtration. In the post-fermentation stage, the LNnT is isolated, purified, and concentrated via a series of filtration, deionization, decolorization, and chromatography steps. The product is then crystallized in methanol and the crystalline LNnT is separated, washed, dried to remove methanol, and milled to obtain the final LNnT product. Glycom states that all materials used in the manufacturing process are food-grade and authorized for their respective uses in the U.S., and that LNnT is manufactured following current good manufacturing practices.

Glycom provides specifications for LNnT that include minimum levels of LNnT (≥ 92% on a dry matter basis (DM)) and total oligosaccharides (≥ 95% DM), and limits for D-lactose (≤ 1%), lacto-*N*-triose II (≤ 3%), *para*-lacto-*N*-neohexaose (≤ 3%), LNnT fructose isomer (≤ 1%), moisture (≤ 9%), ash (≤ 1.5%), lead (≤ 0.1 mg/kg), residual proteins (≤ 0.002%), casein (≤ 0.5 mg/kg), β-lactoglobulin (≤ 0.05 mg/kg), methanol (≤ 100 mg/kg), and microorganisms, including *Salmonella* serovars (absent in 25 g) and *Cronobacter sakazakii* (absent in 10 g). Glycom provides the results of seven non-consecutive batch analyses[3] to demonstrate that LNnT can be manufactured to meet the specifications.

Glycom estimates the dietary exposure to LNnT and incorporates information from GRN 000659 into the notice. Glycom estimates dietary exposure to LNnT based on the intended use in infant formula and previously notified uses in other conventional foods. The estimates are based on food consumption and body weight data from the 2011-2012 National Health and Nutrition Examination Survey. Glycom concludes that infants with CMPA are unlikely to consume greater quantities of infant formula than healthy term infants; therefore, consumption data for infant formula in healthy term infants is applicable to the intended use in GRN 001059. Glycom reports the cumulative mean and 90th percentile eaters-only dietary exposures to LNnT for infants up to 6 months of age to be 0.8 g/person (p)/d and 1.45 g/p/d, respectively. The cumulative mean and 90th percentile eaters-only dietary exposures to LNnT for infants 7 to 12 months of age are reported to be 1.18 g/p/d and 2.35 g/p/d, respectively.

Glycom discusses the safety of LNnT, and states that crystallized LNnT is of high purity and is identical to LNnT naturally present in human milk. Glycom notes that the dietary exposure to LNnT from the intended use is not expected to present safety concerns because of the safe consumption of human milk containing LNnT. Glycom incorporates into the notice all published information included in GRN 000659 and discusses published data cited therein for its safety assessment. Glycom states that all available data suggest that the majority of LNnT reaches the large intestine undigested, serves as a substrate for gut microflora, and/or is excreted intact in the feces. Glycom states that LNnT is neither mutagenic nor genotoxic, and published subchronic repeat-dose studies in rats showed no toxicologically relevant adverse effects at the highest dose tested.

[3] Four batches of LNnT were produced using *E. coli* K-12 DH1 MDO MP572 and three were produced using *E. coli* K-12 DH1 MDO MP572b.

Glycom states that no new animal toxicology studies were identified since GRN 000659; however, they discuss several infant clinical trials with endpoints related to the safe use of LNnT. Glycom discusses several interventional studies with infants suffering from CMPA that were fed either extensively hydrolyzed- or amino acid-based formula supplemented with both LNnT and 2′-fucosyllactose. Glycom concludes that these studies did not identify any safety concerns with LNnT supplementation for infants with CMPA. Glycom further provides evidence demonstrating that the conservative estimate of dietary exposure to potential residual milk proteins from the intended use of LNnT is sufficiently below the minimum level necessary to produce an allergenic response in infants, based on available data. Thus, Glycom concludes that any risk of allergic reactions in infants with CMPA would be very low based on the small potential dietary exposure to milk proteins from the use of LNnT in infant formula.

Based on the totality of the data and information, Glycom concludes that LNnT 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 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 (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 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 nine foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, soybeans, and sesame (effective January 1, 2023)) or a food ingredient that contains protein derived from one of those foods. LNnT derived from lactose may require labeling under the FD&C Act because it may contain protein derived from milk. Questions about petitions or notifications for exemptions from the food allergen labeling requirements should be directed to the Division of Food Ingredients in OFAS. Questions related to food labeling in general 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 Glycom'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 Glycom'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 Glycom provided, as well as other information available to FDA, we have no questions at this time regarding Glycom'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 001059 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2022.12.02 09:28:59 -05'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
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