



Christoph H. Röhrig, Ph.D.  
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Re: GRAS Notice No. GRN 000833

Dear Dr. Röhrig:

This letter corrects our response letter to GRN 000833 signed on October 7, 2019. The purpose of this revised letter is to correct a typographical error in the units shown for “20 g/L in meal replacement bars” in the second paragraph from g/L to g/kg. Additionally, we included a new Footnote 6 to clarify the *Cronobacter sakazakii* specification in paragraph five of our October 7, 2019, response letter.

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000833. We received Glycom A/S (Glycom)’s notice on January 28, 2019, and filed it on March 20, 2019. Glycom submitted amendments to the notice on May 22, 2019, July 17, 2019, and October 4, 2019, that clarified the intended use,<sup>1</sup> manufacturing, specifications, dietary exposure, and safety data.

The subject of the notice is lacto-*N*-tetraose (LNT) for use as an ingredient at levels up to 0.8 g/L in non-exempt, cow’s milk-based infant formula for term infants; 0.6 g/L in drinks for young children (including toddler formulas); 5 g/kg in foods for infants and young children (including toddler foods); 10 g/kg in yogurt; 1 g/L in fluid milk (flavored and unflavored); 2 g/L in meal replacement drinks; 20 g/kg in meal replacement bars; 10 g/kg in cereal and granola bars; and 1 g/L in soft drinks, fruit-based -ades, sports drinks, energy drinks, and enhanced waters. The notice informs us of Glycom’s view that these uses of LNT are GRAS through scientific procedures.

Glycom describes LNT as a white to off-white powder and a tetrasaccharide derived from lactose by addition of N-acetylglucosamine (GlcNAc) and galactose in a linkage-specific manner. The chemical name for LNT is  $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-D-glucopyranose (CAS Registry Number 14116-68-8). Glycom states that the LNT product contains small amounts of lactose and lacto-*N*-triose II (a precursor of LNT). Glycom notes that LNT is structurally similar to lacto-*N*-neotetraose (LNnT), differing only by

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<sup>1</sup> Glycom states that LNT may be used individually or in combination with other human milk oligosaccharide (HMO) ingredients that have been the subjects of previous GRAS notices. Glycom 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.

the terminal linkage and sharing a common precursor (lacto-*N*-triose II).<sup>2, 3</sup>

Glycom notes that LNT's method of manufacture is similar to that in GRNs 000650<sup>4</sup> and 000659,<sup>2</sup> and incorporates information from these notices into GRN 000833. Glycom describes the two-stage manufacturing process for LNT. First, LNT is produced by fermentation of a pure culture of the genetically engineered production organism *E. coli* (K-12 DH1 MDO) MP813<sup>5</sup> in media containing lactose derived from cow's milk and is secreted into the fermentation media. After fermentation is complete, the LNT is purified by a series of ultrafiltration and nanofiltration steps to remove the production microorganism, water, minerals, and other small molecules. The LNT is further treated with ion exchange resin to remove trace metals or salts. The resulting product is subsequently decolorized with activated charcoal and subjected to additional filtration to further purify and concentrate LNT. The resulting solution is spray dried or freeze dried to obtain the final product. Glycom states that LNT is manufactured in accordance with current good manufacturing practices using permitted food-grade materials.

Glycom provides specifications for LNT that include minimum levels of LNT ( $\geq 70\%$  on dry matter (DM) basis) and total saccharides ( $\geq 90.0\%$  DM), and limits on D-lactose ( $\leq 12.0\%$ ), lacto-*N*-triose II ( $\leq 10.0\%$ ), *para*-lacto-*N*-hexaose-2 ( $\leq 3.5\%$ ), LNT fructose isomer ( $\leq 1.0\%$ ), sum of other carbohydrates ( $\leq 5.0\%$ ), moisture ( $\leq 6.0\%$ ), sulfated ash ( $\leq 0.5\%$ ), lead ( $\leq 0.1$  mg/kg), residual proteins ( $\leq 0.01\%$ ), *Salmonella* serovars (absent in 25 g), and *C. sakazakii* (absent in 10 g).<sup>6</sup> Glycom provides the results of five, non-consecutive batch analyses to demonstrate that LNT can be manufactured to meet these specifications. Glycom discusses the stability of LNT and states that LNT is stable for up to 3 years under ambient storage conditions and for up to 1.5 years under accelerated conditions.

Glycom provides estimates of dietary exposure to LNT based on the intended use and food consumption data from the 2013-2014 National Health and Nutrition Examination Survey (NHANES). Glycom states that the mean and 90<sup>th</sup> percentile dietary exposures to LNT for the total population 1 year and older (consumers only, 93.8% consumers) are estimated to be 0.83 and 1.77 g/person (p)/day (d) (17.2 and 33.6 mg/kg body weight (bw)/d), respectively. The mean and 90<sup>th</sup> percentile dietary exposures for toddlers ages

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<sup>2</sup> 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 notifiers' GRAS conclusions.

<sup>3</sup> The IUPAC name of LNnT is  $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-D-glucopyranose.

<sup>4</sup> 2'-O-fucosyllactose was the subject of GRN 000650. We evaluated this notice and responded in a letter dated November 23, 2016, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

<sup>5</sup> *E. coli* K-12 is a non-pathogenic, non-toxicogenic, and safe production strain when used in accordance with good manufacturing processes (55 FR 10932 at 10934; March 23, 1990). The modifications to the MDO parent strain to produce MP813 include transformation with exogenous genes, which are well characterized and not associated with pathogenicity or toxigenicity, encoding  $\beta$ -1,3-*N*-acetylglucosaminyltransferase from *Neisseria meningitidis* and  $\beta$ -1,3-galactosyltransferase from *Helicobacter pylori*.

<sup>6</sup> Glycom's *C. sakazakii* specification is intended for use of LNT in powdered infant formulas. We note that a comparable limit for LNT for use in liquid infant formulas that require a retort step is not needed.

1-3 years (98.5% consumers) are estimated to be 0.90 and 1.88 g/p/d (70.2 and 141 mg/kg bw/d), respectively.

Glycom provides estimates of dietary exposure to LNT for infants 0-6 months and infants 7-12 months of age based on the maximum intended use levels in infant and toddler foods, non-exempt infant formula for term infants, toddler formula, and consumption data from the 2013-2014 NHANES. Glycom reports the mean and 90<sup>th</sup> percentile dietary exposures to LNT for infants 0 to 6 months of age (consumers only, 80.1% consumers) to be 1.21 and 2.20 g/p/d (176 and 301 mg/kg bw/d), respectively. The mean and 90<sup>th</sup> percentile dietary exposures to LNT for infants 7 to 12 months of age (99.9% consumers) is 1.75 and 3.28 g/p/d (197 and 352 mg/kg bw/d), respectively.

Glycom discusses the safety of LNT. Glycom states that the final product contains LNT, lactose, and lacto-*N*-triose II, all of which are components of human milk, and that its LNT is structurally identical to LNT in human milk. Glycom notes that the use level of LNT is targeted to match LNT levels consumed safely in human milk. Glycom states that all available data suggest that the majority of LNT reaches the large intestine undigested and serves as a substrate for gut microflora or is excreted intact in the feces.

Due to LNT's structural similarity to LNnT, Glycom incorporates into the notice data and information discussed in GRNs 000547 and 000659,<sup>2</sup> including summaries of published repeat-dose oral toxicity and genotoxicity studies. In addition, Glycom discusses published toxicological studies on LNT that is the subject of this notice, including 14- and 90-day repeat dose toxicity studies in neonatal rats, a bacterial reverse mutation assay, and an *in vitro* mammalian micronucleus assay. Glycom states that when LNT was administered daily by gavage to neonatal rats for 14- and 90-days, no test substance-related adverse effects were observed up to 4,000 mg/kg bw/d, the highest dose tested. Glycom discusses published studies showing that LNT with or without metabolic activation is not genotoxic or clastogenic. Additionally, as supportive evidence, Glycom discusses published human studies showing that infant formula containing LNnT was well-tolerated and resulted in anthropometric parameters similar to formula- or breast-fed controls.

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

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

### **Standards of Identity**

In the notice, Glycom states its intention to use LNT in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. 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.

## **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 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 may require labeling under the FD&C Act because the final product may contain protein derived from cow’s milk. Questions about petitions or notifications for exemptions from the food allergen labeling requirements should be directed to the Division of Food Ingredients in the Office of Food Additive Safety (OFAS). Questions related to food labeling in general should be directed to the Office of Nutrition and Food Labeling (ONFL) in the Center for Food Safety and Applied Nutrition.

## **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). These claims are subject to the applicable requirements and are under the purview of ONFL. 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.

## **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 LNT’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 Glycoms’ 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(l).

### **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 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 000833 is accessible to the public at [www.fda.gov/grasnoticeinventory](http://www.fda.gov/grasnoticeinventory).

Sincerely,  
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 Digitally signed by Susan J.  
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