



Joeri Beauprez, Ph.D.
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BELGIUM

Re: GRAS Notice No. GRN 001068

Dear Dr. Beauprez:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001068. We received Inbiose N.V. (Inbiose)’s notice on January 21, 2022, and filed it on August 16, 2022. Inbiose submitted amendments to the notice on February 15, 2023 and May 2, 2023, that revised the specifications and provided additional details about the batch analyses, production organism, methods of production and purification, intended use, safety studies, literature search, and results of an unpublished 90-day subchronic toxicological study.

The subject of the notice is lacto-*N*-tetraose (LNT) for use as an ingredient in non-exempt infant formula for term infants¹ at a maximum level of 0.8 g/L as consumed, and in other food categories at maximum levels shown in Table 1. The notice informs us of Inbiose’s view that these uses of LNT are GRAS through scientific procedures.

Table 1: Intended food categories and use levels for LNT²

Food Categories	Serving Size	Maximum use levels (g/serving)	Maximum use levels (g/kg or g/L)
Meal replacement drinks, for weight management	240 mL	0.48	2.0
Sports, isotonic, and “energy” drinks	360 mL	0.36	1.0
Soft drinks	360 mL	0.36	1.0
Enhanced or fortified waters	360 mL	0.36	1.0
Fruit-based ades	360 mL	0.36	1.0
Term infant formula	100 mL	0.08	0.8
Formula-type drinks for young children	100 mL	0.06	0.6

¹ Inbiose states that the use of LNT in infant formula is not restricted to any specific protein base (e.g., cow milk-based, soy-based).

² Inbiose states that LNT is not intended in foods for which standards of identity do not permit its addition, and LNT is not intended in foods under the jurisdiction of the United States Department of Agriculture.

Other drinks for young children	120 mL	0.07	0.6
Baby foods for infants and young children	7 to 170 g	0.04 – 0.85	5.0
Meal replacement bars, for weight management	40 g	0.8	20
Cereal and granola bars	40 g	0.4	10
Unflavored pasteurized and sterilized milk	240 mL	0.24	1.0
Buttermilk	240 mL	0.24	1.0
Flavored milk	240 mL	0.24	1.0
Yogurt	170 g	1.7	10

Inbiose provides information on the identity and composition of LNT (CAS Registry Number 14116-68-8). Inbiose describes LNT as a white powder that is $\geq 80\%$ LNT on a dry matter (DM) basis, with small amounts of lactose, lacto-*N*-triose, and other related saccharides. The molecular formula of LNT is $C_{26}H_{45}NO_{21}$ and the molecular weight is 707.63 g/mol. LNT is an oligosaccharide, found in human milk, that is comprised of galactose, glucose, and *N*-acetylglucosamine. The chemical name of LNT is β -D-galacto-hexopyranosyl-(1 \rightarrow 3)-2-acetamido-2-deoxy- β -D-gluco-hexopyranosyl-(1 \rightarrow 3)- β -D-galacto-hexopyranosyl-(1 \rightarrow 4)-D-gluco-hexopyranose.

Inbiose describes the production organism used to produce LNT. The organism, *Escherichia coli* K12 strain INB-LNT_01, is deposited in the Belgian Co-ordinated Collections of Micro-organisms (BCCM) strain collection in Ghent, Belgium as LMBP 12730. Inbiose constructed the production organism through deletion and insertion³ of genes in the host microorganism genome, *E. coli* K-12 strain MG1655, to obtain efficient production of LNT. Inbiose states that the production organism is non-pathogenic and non-toxicogenic and that genetic modifications were verified by polymerase chain reaction, Sanger sequencing, and whole genome sequencing.

Inbiose states that LNT is produced in accordance with current good manufacturing practices. First, a pure culture of the production organism is inoculated into a fermentation medium that contains lactose and a carbon source (sucrose), resulting in production of LNT, which is secreted into the medium. After fermentation is complete, the fermentation biomass is pasteurized and the remaining intracellular LNT is released. The production organism is then removed, along with cell components and large molecules (i.e., protein, DNA, lipopolysaccharides), by successive microfiltration and ultrafiltration steps. The LNT is then further purified and concentrated by a series of ion exchange chromatography, nanofiltration, decolorization (activated charcoal), evaporation, sterile filtration, and drying steps. Inbiose states that all raw materials, processing aids, and food contact articles are used in accordance with U.S. regulations, have previously been concluded to be GRAS, or are the subject of an effective food

³ Inbiose states that the modifications to the parent strain, *E. coli* MG1655, to produce INB-LNT_01 include transformation with exogenous genes, which are well characterized and produced by DNA synthesis, encoding lactose permease, sucrose permease, sucrose phosphorylase, fructokinase, β -N-acetylglucosaminyltransferase, and β -galactosyltransferase.

contact notification for their respective uses.

Inbiose provides specifications for LNT that include the minimum amount of LNT ($\geq 80\%$ DM) and sum of lacto-*N*-triose, lactose, and LNT ($\geq 90\%$ DM), and limits for other carbohydrates⁴ ($\leq 5\%$, % area), lactose ($\leq 10\%$ DM), lacto-*N*-triose ($\leq 5\%$ DM), *para*-lacto-*N*-hexose ($\leq 3.5\%$ DM), and LNT-fructose ($\leq 1\%$ DM). Inbiose also provides specifications for protein (≤ 100 $\mu\text{g/g}$), ash ($\leq 0.1\%$), moisture ($\leq 9\%$), heavy metals, including lead (≤ 0.02 mg/kg), and microorganisms, including *Salmonella* serovars (absent in 25 g), and *Cronobacter sakazakii* (absent in 25 g). Inbiose provides the results from the analyses of five nonconsecutive batches to demonstrate that the product can be manufactured to meet the stated specifications.

Inbiose states that LNT is similar to the subjects of GRNs 000833 and GRN 000923.⁵ Inbiose concludes that, based on the stability studies incorporated into the notice from GRNs 000833 and 000923, LNT has a shelf-life of 2 years from the date of production when stored under ambient conditions (25 °C, 60% relative humidity).

Inbiose states that its intended uses of LNT in infant formula and conventional foods are substitutional for the intended uses in GRN 000833. As such, Inbiose incorporates into the notice estimates of dietary exposure to LNT from GRN 000833. Using food consumption data from the 2013-2014 National Health and Nutrition Examination Survey (NHANES), Inbiose reports the mean and 90th percentile eaters-only dietary exposures for infants 0 to 6 months to be 1.21 g/person (p)/day (d)⁶ and 2.20 g/p/d, respectively, and for infants 7-12 months to be 1.75 g/p/d and 3.28 g/p/d, respectively. Inbiose also reports the mean and 90th percentile eaters-only dietary exposures for young children ages 1-3 years to be 0.90 g/p/d and 1.88 g/p/d, respectively. For the U.S. population aged 1 year and older, Inbiose reports the mean and 90th percentile eaters-only dietary exposures to be 0.83 g/p/d and 1.77 g/p/d, respectively.

Inbiose discusses data and information supporting the safety of LNT and states that a literature search conducted through February 2023 did not identify any new data that would contradict its GRAS conclusion. Inbiose states that LNT is chemically identical to the LNT in human milk and compositionally similar to other LNT ingredients concluded to be GRAS. Therefore, Inbiose incorporates into the notice published data discussed in GRNs 000833 and 000923 including absorption, distribution, metabolism, and excretion data, as well as genotoxicity tests and 14-day and 90-day repeated dose

⁴ Inbiose describes “other carbohydrates” as comprised of glucose, N-acetylglucosamine, LNT-fructose, and *para*-lacto-*N*-hexaose.

⁵ LNT is the subject of GRNs 000833 (infant formula and conventional food uses) and 000923 (infant formula use only). We evaluated these notices and responded in letters or corrected letters dated April 13, 2020, and February 2, 2021, respectively, stating that we had no questions at that time regarding the notifiers’ GRAS conclusions.

⁶ FDA notes the dietary exposure estimates, expressed on a body weight basis, from GRN 000833 (Table 3.2.2-2) and its May 22, 2019, amendment (Table 3) are as follows: mean and 90th percentile eaters-only dietary exposures are, respectively, 176 and 301 mg/kg body weight (bw)/d for infants 0 to 6 months, 197 and 352 mg/kg bw/d for infants 7-12 months, and 70.2 and 141 mg/kg bw/d for young children ages 1-3 years. For the U.S. population aged 1 year and older, mean and 90th percentile eaters-only dietary exposures are 17.2 and 33.6 mg/kg bw/d, respectively.

toxicity studies using LNT. Inbiose also incorporates into the notice corroborative genotoxicity tests and safety studies in rats and piglets in which LNT was tested as part of a mixture of human milk oligosaccharides (HMO). Inbiose discusses unpublished safety data with the article of commerce including genotoxicity tests, single dose acute, 21-day range finding, and 90-day repeated dose studies, all in rats, which showed no evidence of toxicity. Inbiose summarizes the results of published clinical studies in which LNT was demonstrated to be safe and well tolerated when consumed by healthy term infants as part of an HMO mixture.

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

Standards of Identity

In the notice, Inbiose states their 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 (21 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 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 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) or a food ingredient that contains protein derived from one of those foods. LNT 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 Inbiose'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 Inbiose'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 Inbiose provided, as well as other information available to FDA, we have no questions at this time regarding Inbiose'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 001067 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

**Susan J.
Carlson -S**

Susan J. Carlson, Ph.D.
Director

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

Digitally signed by Susan J.
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