Joeri Beauprez, Ph.D.
Inbiose N.V.
Technologiepark Zwijnaarde 82 – bus 41
B-9052 Gent
BELGIUM

Re: GRAS Notice No. GRN 001067

Dear Dr. Beauprez:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001067. We received Inbiose N.V. (Inbiose)'s notice on January 21, 2022, and filed it on July 14, 2022. Inbiose submitted an amendment to the notice on January 4, 2023, that provided additional information about the literature search, the manufacture and safety of the production organism, the self-limiting nature of the substance, and the results of an unpublished 90-day rat oral toxicity study.

The subject of the notice is lacto-*N*-neotetraose (LNnT). The notice informs FDA of Inbiose's view that LNnT is GRAS, through scientific procedures, for use as an ingredient in non-exempt infant formula for term infants[1] at a level up to 0.6 g/L; in formula for young children (> 12 months) at up to 0.6 g/L; in drinks for young children at up to 0.58 g/L; in foods for infants and young children at up to 3 g/kg; in sports, isotonic, and "energy" drinks, milk (unflavored and flavored), imitation milk, buttermilk, and fruit juices and nectars at up to 0.58 g/L; in yogurt (including non-dairy yogurt) at up to 2.67 g/kg; in meal replacement drinks at up to 2.5 g/L; and in meal replacement bars at up to 20 g/kg.

Inbiose provides information on the identity and composition of LNnT. Inbiose describes LNnT as a white to off-white powder containing ≥ 80% LNnT. Inbiose reports that the final LNnT product may also contain other carbohydrates, including lactose, lactose-*N*-triose, fructo-lacto-*N*-neotetraose, and *para*-lacto-*N*-neohexaose. LNnT is a tetrasaccharide composed of D-galactose, *N*-acetyl-D-glucosamine, and D-glucose. LNnT has the CAS registry number 13007-32-4 and the chemical name β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-D-glucopyranose.

Inbiose describes the production organism, *Escherichia coli* K-12 strain LMBP 12728, used in the manufacture of LNnT. The production organism is genetically engineered from the parent strain, *E. coli* K-12 strain MG1655, to produce LNnT. Inbiose

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

constructed the production organism by insertion of synthesized, codon-optimized genes encoding enzymes for biosynthesis of LNnT from donor species[2] into the genome of the production organism and deletion of genes to improve efficiency and avoid production of metabolic byproducts. Inbiose states that all gene insertions and deletions were verified by polymerase chain reaction, Sanger sequencing, and whole genome sequencing. Inbiose states that the production organism does not contain any helper plasmids, antibiotic markers present on the helper plasmids, or antibiotic markers introduced into the genome. Inbiose states that *E. coli* K-12 strain LMBP 12728 is non-pathogenic and non-toxigenic and is deposited in the Belgian Co-ordinated Collections of Micro-organisms (BCCM) strain collection in Gent, Belgium with deposition number LMBP 12728.

Inbiose states that LNnT is manufactured in two main stages. In the fermentation stage, the production organism is grown in a medium containing sucrose as a carbon source and D-lactose as a substrate for the synthesis of LNnT. Inbiose notes that LNnT is partly secreted into the fermentation medium and the remaining intracellular LNnT is released following pasteurization. In the post-fermentation stage, the LNnT is isolated, purified and concentrated via a series of filtration, deionization, decolorization, and drying steps. Inbiose states that all materials used in the manufacturing processes are food-grade and are used in accordance with applicable U.S. regulations, are concluded to be GRAS for their respective use, or are the subject of an effective food contact notification, and that LNnT is manufactured following current good manufacturing practices.

Inbiose provides specifications for LNnT that include minimum levels of LNnT (≥ 80% on a dry matter basis (DM)) and total human milk-identical saccharides[3] (≥ 92% DM), and limits on D-lactose (≤ 10%), lacto-*N*-triose (≤ 5%), *para*-lacto-*N*-neohexaose (≤ 5%), fructo-lacto-*N*-neotetraose (≤ 1%), moisture (≤ 9%), ash (≤ 0.4%), lead (≤ 0.05 mg/kg), protein (≤ 100 mg/kg), and microorganisms, including *Cronobacter sakazakii* (absent in 25 g) and *Salmonella* spp. (absent in 25 g). Inbiose provides the results from the analyses of five non-consecutive batches to demonstrate that its LNnT meets the specifications. Inbiose incorporates into their notice the results of stability studies described in GRN 000547[4] that were conducted under ambient conditions (25 °C, 60% relative humidity) for up to five years, under accelerated conditions (40 °C, 75% relative humidity) for two years, and under the intended conditions of use that included infant formula and other food applications. Inbiose concludes that these studies demonstrate that LNnT is stable under the conditions tested.

Inbiose discusses the estimated dietary exposure to LNnT and states that the intended

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[2] Inbiose identifies the enzymes that are encoded and involved in the biosynthesis of LNnT and include lactose permease and sucrose permease from *Escherichia coli*, sucrose phosphorylase from *Bifidobacterium adolescentis*, fructokinase from *Zymomonas mobilis*, β-*N*-acetylglucosaminyltransferase and β-galactosyltransferase from *Neisseria meningitidis*, and β-galactosyltransferase from *Helicobacter pylori*.

[3] Inbiose states that the sum of human milk-identical saccharides includes LNnT, lactose, lacto-*N*-triose, and *para*-lacto-*N*-hexaose.

[4] LNnT is the subject of GRN 000547. We evaluated this notice and responded in a letter dated October 2, 2015, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

uses are the same as described in prior GRAS notices, and, therefore, Inbiose does not expect the dietary exposure to LNnT to change. Inbiose incorporates into their notice information on the estimated dietary exposure to LNnT from GRN 000547 and GRN 000659.[5] Inbiose discusses the estimates of dietary exposure to LNnT presented in GRN 000547 for infants and young children based on the intended uses and food consumption data from the 2009-2010 National Health and Nutrition Examination Survey (NHANES). Inbiose reports the mean and 90th percentile eaters-only dietary exposures to LNnT for infants up to 6 months of age to be 0.82 and 1.60 g/person (p)/d, respectively. The mean and 90th percentile eaters-only dietary exposures to LNnT for infants 7 to 12 months of age are reported to be 1.50 and 2.69 g/p/d, respectively. The mean and 90th percentile eaters-only dietary exposures to LNnT for children aged 1 to 3 years are reported to be 0.67 and 1.21 g/p/d, respectively. Inbiose also provides estimates of dietary exposure to LNnT presented in GRN 000659 for the total population based on the intended uses and food consumption data from the 2011-2012 NHANES. The mean and 90th percentile eaters-only dietary exposures to LNnT for the total population (all ages) are reported to be 304 and 646 mg/p/d, respectively.

Inbiose states that their LNnT is of high purity and is identical to LNnT naturally present within human milk. Inbiose notes that the exposure to LNnT from its intended use is not expected to present safety concerns because of the safe consumption of human milk. Inbiose further states that their LNnT is compositionally similar to other LNnT that have been previously concluded to be GRAS. Thus, Inbiose incorporates into their notice all publicly available data and information included in GRNs 000547, 000659, and 000919[6] and discusses pivotal published data cited therein for its safety assessment. Inbiose states that all available data suggest that the majority of LNnT reaches the large intestine undigested and serves as a substrate for gut microflora or is excreted intact in the feces. Inbiose 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. Inbiose also discusses unpublished toxicological studies on the subject of this notice, which included genotoxicity studies as well as acute, 21-day and 90-day oral toxicity studies in rats, to support the safety of LNnT.

Inbiose states that in their updated literature search through December 2022, no new animal toxicology studies were identified; however, Inbiose discusses several new published studies in humans with endpoints related to the safety of LNnT. These included studies ranging in length of 1 week to 12 months, with either LNnT alone or in combination with other human milk oligosaccharides, and with study populations of healthy full-term infants, healthy adults, adults with irritable bowel syndrome, pre-term infants, and infants suffering from cow milk protein allergy. Inbiose concludes from these studies that LNnT is safe and well-tolerated in infants and adults.

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[5] 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 the notifier's GRAS conclusion.

[6] LNnT is the subject of GRN 000919. We evaluated this notice and responded in a letter dated October 30, 2020, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

Based on the totality of information discussed above, Inbiose concludes that LNnT is GRAS for its intended use.

Standards of Identity

In the notice, Inbiose states their intention to use LNnT 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 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) 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 Inbiose'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 Inbiose'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 Inbiose provided, as well as other information available to FDA, we have no questions at this time regarding Inbiose'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 001067 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2023.04.04 15:09:08 -04'00'

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Director
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