



Stella Si
Anchor Center for Certification
No. 1295 Chuan Qiao Road, Building 2, Suite 302
Pudong, Shanghai, China

Re: GRAS Notice No. GRN 001208

Dear Ms. Si:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001208. We received the notice that you submitted on behalf of Shandong Henglu Biotechnology Co., Ltd. (Henglu) on April 12, 2024, and filed it on October 15, 2024. Henglu submitted amendments to the notice on February 20, 2025, and March 12, 2025, that provided additional information on the chemical identity, manufacturing process, specifications, intended uses, dietary exposure, and safety narrative.

The subject of the notice is lacto-N-triose II (LNTr II) for use as an ingredient in various foods at the maximum use levels specified in Table 1.¹ The notice informs us of Henglu's view that these uses of LNTr II are GRAS through scientific procedures.

Table 1. Intended food categories and maximum use levels for LNTr II

Food Categories	Maximum Use Levels (g/kg or g/L)
Unflavored pasteurized and sterilized milk	0.05
Buttermilk	0.05
Flavored milk	0.05
Yogurt	0.5
Milk-based meal replacement beverages	0.1
Non-milk based meal replacement beverages	0.1
Sport and isotonic drinks	0.05
“Energy” drinks, including carbonated energy drinks	0.05
Fortified or enhanced waters, including carbonated water	0.05
Formula-type drinks for young children (1-3 years)	0.03
Other drinks for young children, including yogurt and juice drinks (1-3 years)	0.03
Foods for young children (1-3 years)	0.25
Nutrition bars (milk- and non-milk based), including meal replacements for weight management and general use	1.0

¹ Henglu states that LNTr II is not intended for use in infant formula and in products under the jurisdiction of the United States Department of Agriculture.

Food Categories	Maximum Use Levels (g/kg or g/L)
“nutrition bars”	
Cereal and granola bars	0.5

Henglu describes LNTr II as a white to off-white powder. LNTr II is a trisaccharide derived from lactose by addition of N-acetyl-D-glucosamine (GlcNAc) in a linkage-specific manner. The chemical name of LNTr II is 2-(acetylamino)-2-deoxy- β -D-glucofuranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 4)-D-glucose, the CAS Registry number is 75645-27-1,² the chemical formula is C₂₀H₃₅O₁₆N and the molecular mass is 545.487 g/mol. Structurally, LNTr II comprises a GlcNAc unit connected through a β -(1 \rightarrow 3) bond to D-galactose, which is further linked via a β -(1 \rightarrow 4) bond to the reducing end of D-glucose. The structure of LNTr II is supported by liquid chromatography ion trap/time-of-flight mass spectrometry, ¹H nuclear magnetic resonance (NMR) spectroscopy, and ¹³C NMR spectroscopy. Aside from LNTr II, the ingredient also contains small amounts of D-glucose, lactose, galactose, and lacto-N-triitol. Henglu notes that LNTr II is among the oligosaccharides present in human milk and that it is also a structural precursor for other human milk oligosaccharides (HMOs), including the tetrasaccharides lacto-N-neotetraose (LNnT)³ and lacto-N-tetraose (LNT).⁴

LNTr II is produced by controlled fermentation of a pure culture of the genetically engineered production organism *Kluyveromyces lactis* DSM 709-2-02, developed from the platform strain *K. lactis* DSM 70799.⁵ The production organism *K. lactis* DSM 709-2-02 is genetically engineered to overexpress a gene encoding β -1,3-N-acetylglucosaminyltransferase (an enzyme that transfers a GlcNAc unit from intracellular UDP-GlcNAc to the galactose residue in lactose) and eliminate the β -galactosidase gene Lac4 to prevent hydrolysis of D-lactose into D-glucose and D-galactose. Henglu states that the genome of the production organism has been sequenced and bioinformatic analysis shows that the introduced genes do not confer toxigenic or pathogenic properties.

Henglu describes the two-stage manufacturing process for LNTr II, which is produced in accordance with current good manufacturing practices using food-grade raw materials and processing aids that conform to applicable U.S. regulations or are GRAS for the

² FDA notes that we observed some ambiguity in the synonyms for LNTr II in the literature. LNTr II is distinct from lacto-N-triose I (LNTr I) (CAS Reg No. 64317-83-5), which has the same chemical formula and molecular mass as LNTr II.

³ LNnT (a tetrasaccharide abbreviated Gal β 1,4GlcNAc β 1,3Gal β 1,4Glc) was the subject of GRNs 000547, 000659, 000895, 000919, 001059, and 001067. We evaluated these notices and responded in letters dated October 2, 2015, November 23, 2016, December 3, 2020, October 30, 2020, December 2, 2022, and April 4, 2023, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

⁴ LNT (a tetrasaccharide abbreviated Gal β 1,3GlcNAc β 1,3Gal β 1,4Glc) was the subject of GRNs 000833, 000923, 001017, and 001068. We evaluated these notices and responded in letters dated October 7, 2019, February 2, 2021, August 23, 2022, and June 5, 2023, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

⁵ *K. lactis* DSM 70799 is deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany.

intended use. In the fermentation stage, the production organism is grown in a medium containing D-glucose as an energy and carbon source and D-lactose as a substrate for LNTr II biosynthesis. The LNTr II is secreted extracellularly, and the production strain is removed after completion of the fermentation process. In the second stage of the manufacturing process, LNTr II is purified through a series of steps including microfiltration and nanofiltration to remove minerals and biomolecules, ion exchange chromatography with anionic and cationic resins to remove salts and coloring matter, vacuum concentration, and crystallization in ethanol and acetone. The resulting crystals are separated by centrifugation and dried to remove water and residual solvent, resulting in the LNTr II powder.

Henglu provides specifications for LNTr II that include minimum levels of LNTr II ($\geq 88\%$ on a dry matter basis (DM)) and limits on D-lactose ($\leq 5\%$ DM), D-glucose ($\leq 4\%$ DM), galactose ($\leq 3\%$ DM), lacto-N-triitol⁶ ($\leq 0.5\%$ DM), moisture ($\leq 9\%$), ash ($\leq 1\%$), acetone (< 15 mg/kg), ethanol ($\leq 0.2\%$), heavy metals, including lead (≤ 0.05 mg/kg), protein (≤ 100 mg/kg), and microorganisms, including *Cronobacter sakazakii* (absent in 25 g), *Listeria monocytogenes* (absent in 25 g) and *Salmonella* serovars (absent in 25 g). Henglu provides the results from the analyses of five non-consecutive batches to demonstrate that LNTr II meets the stated specifications.

Henglu discusses the estimated dietary exposure to LNTr II from its intended uses. Using food consumption data from the 2017-2020 (pre-pandemic) National Health and Nutrition Examination Survey (NHANES), Henglu estimates the mean and 90th percentile eaters-only dietary exposures to LNTr II for children ages 1-3 years to be 0.04 and 0.085 g/person (p)/d (3.1 and 6.5 mg/kg bw/d), respectively. The mean and 90th percentile eaters-only dietary exposures to LNTr II for the U.S. population ages 1 year and older are reported to be 0.04 and 0.09 g/p/d (0.74 and 1.6 mg/kg bw/d), respectively. Henglu also discusses cumulative estimates of dietary exposure to LNTr II from its intended uses as well as its presence as a minor component (up to 10%) in the LNT that is the subject of GRN 000833. Henglu notes that the intended uses of LNTr II are more restrictive than those of LNT⁷ but does acknowledge possible contribution of LNTr II present in the LNT ingredients to the cumulative dietary exposure to LNTr II. Henglu estimates the cumulative eaters-only dietary exposure to LNTr II based on the intended uses described in GRN 001208, the presence of LNTr II at levels up to 10% in the LNT ingredients used in the same food categories, and food consumption data from the 2017-2020 NHANES. The highest mean and 90th percentile cumulative eaters-only dietary exposures to LNTr II were reported for young children (1-3 years), with mean

⁶ Henglu provides a CAS Reg. No 92919-31-8 for lacto-N-triitol, noting its chemical name is D-Glucitol, O-2-(acetylamino)-2-deoxy- β -D-glucoopyranosyl-(1 \rightarrow 3)-O- β -D-galactopyranosyl-(1 \rightarrow 4)-.

⁷ For comparison, in GRN 000833, Glycom provided estimates of dietary exposure to LNT based on its intended uses (including foods categories not listed in GRN 001208) and food consumption data from the 2013-2014 NHANES. Glycom states that the mean and 90th percentile eaters-only dietary exposure to LNT for the total population 1 year and older was estimated to be 0.83 and 1.77 g/p/day (d), respectively. The mean and 90th percentile dietary exposures for young children ages 1-3 years were estimated to be 0.90 and 1.88 g/p/d. If 10 percent of LNT is assumed to be LNTr II, based on the maximum level by specification, then the 90th percentile estimates for LNTr II from all LNT uses would be 0.18 g/p/d for the total population 1 year and older and 0.19 g/p/d for young children (1-3 years).

and 90th percentile estimates of 0.13 and 0.26 g/p/d (9.2 and 19.4 mg/kg bw/d), respectively. For the U.S. population (ages 1 year and older), cumulative eaters-only dietary exposures at the mean and 90th percentile are 0.12 and 0.28 g/p/d (2.2 and 4.8 mg/kg bw/d), respectively.

Henglu discusses publicly available data and information supporting their GRAS conclusion. Henglu states that LNTr II is a natural component in human milk, and LNTr II produced via fermentation is chemically identical to LNTr II in human milk. Henglu states that available data suggest that LNTr II is poorly absorbed, reaches the large intestine undigested, and subsequently serves as a substrate for gut microflora or is excreted in feces unchanged. Henglu discusses published toxicological studies with test articles similar to the notified substance as pivotal information for their GRAS conclusion. These include a Bacterial Reverse Mutation (Ames) Test and an *in vitro* Micronucleus Assay, showing that LNTr II is not clastogenic or aneugenic, as well as a 90-day repeat dose oral toxicity study in juvenile rats (introduced through gavage) with a 4-week recovery period. A No-Observed-Adverse-Effect level (NOAEL) was determined to be 5000 mg/kg bw/d, the highest dose tested in the 90-day study. Based on this NOAEL, Henglu determined a safe level for oral consumption of LNTr II to be up to 50 mg/kg bw/d, using a 100-fold safety factor. Henglu concludes that the intended uses of LNTr II are safe because estimates of the dietary exposure to LNTr II at the 90th percentile for the total population (ages 1 year and older) and for the population with the highest estimated dietary exposure based on age (young children 1-3 years old) are less than 50 mg/kg bw/d. To further support their GRAS conclusion, Henglu notes that the test article used for toxicological studies to partly support a GRAS conclusion of another HMO, LNT,⁴ contained LNTr II as an impurity. Given there were no toxicologically relevant adverse effects identified with these studies, Henglu concludes that these studies also support the safety of LNTr II. Henglu also notes that while no clinical studies have been conducted with LNTr II, clinical studies conducted with LNnT³ are relevant to assess tolerability of LNTr II, because: 1) LNnT is naturally catabolized to LNTr II through gut microbiota fermentation or acid hydrolysis, and 2) LNTr II is also present in the LNnT test article at approximately 3%.

Based on the totality of the data and information, Henglu concludes that LNTr II is GRAS for its intended use.

Standards of Identity

In the notice, Henglu states its intention to use LNTr II in several food categories, including foods for which standards of identity exist located in Title 21 of the 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, and Cosmetic Act (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 LNTr II 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 Nutrition Center of Excellence. The Office of Pre-market Additive Safety (OPMAS) 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. LNTr II 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 OPMAS. Questions related to food labeling in general should be directed to 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 Henglu’s notice concluding that LNTr II 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 LNTr II. Accordingly, our response should not be construed to be a statement that foods containing LNTr II, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Henglu provided, as well as other information available to FDA, we have no questions at this time regarding Henglu’s conclusion that LNTr II is GRAS under its intended conditions of use. This letter is not an affirmation that LNTr II 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 001208 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

**Susan J.
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