APPLICATION FOR THE APPROVAL OF THE HUMAN-IDENTICAL MILK OLIGOSACCHARIDE LACTO-*N*-TETRAOSE AS A NOVEL FOOD INGREDIENT FOR USE IN INFANT AND FOLLOW-ON FORMULAE AND IN FOODS

Regulation (EU) No 2015/2283 of the European Parliament and of the Council of 25 November 2015 Concerning Novel Foods and Novel Food Ingredients

Non-Confidential Summary of the Application

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Summary

The subject matter of this application is lacto-*N*-tetraose ("LNT"), which is obtained from fermentation and is isolated as a purified ingredient. The manufactured LNT is identical in structure to the same molecule that is present in human milk and is therefore henceforth referred to as human-identical milk oligosaccharide (HiMO)[1]. LNT is one of the most abundant individual oligosaccharides of the complex natural oligosaccharide fraction of human milk, and its chemical structure presents one of the most human-characteristic structural features of mammalian milk oligosaccharides.

Glycom's LNT ingredient is manufactured and purified in several steps to a well-defined and highly pure specification, containing not less than 93.0 % human-identical milk saccharides (HiMS) [predominantly LNT (≥78 %), with some levels of lactose (the principal raw material) and lacto-N-triose II]. In stage 1 of the manufacturing process (upstream processing), D-lactose and D-glucose (or alternatively D-glycerol) are converted to LNT by the adapted cellular metabolism of the LNT production microorganism, which uses glucose (or glycerol) as an exclusive energy and carbon source and lactose as a substrate for LNT biosynthesis. In stage 2 (downstream processing), a series of purification and isolation steps are used to generate the final LNT product.

The LNT product is intended for use in infant formulas (up to 12 months), follow-on formula, infant-specific foods and foods for young children at a use level of up to 0.8 g/L or 5 g/kg in ready-to-drink and reconstituted products. The LNT product is also intended for use in food and beverages targeted towards older population groups (up to 1.0 g/L or 10 g/kg), foods for special medical purposes (use level determined on a case-by-case basis), foods for total diet replacement for weight control (up to 2.0 g/L or 20 g/kg), and supplements (2.0 g/day in the general population). The maximum use levels are proposed on the basis of providing similar levels of LNT as those occurring on average in mature human breast milk and have been balanced for other age groups as well to achieve resulting intakes that stay well within the natural intakes from breast milk on a body weight basis.

Table 1 Proposed Food Uses and Use Levels for LNT Product

EU Food Category Number	Food Category Name	Proposed Maximum Use Level
1	**Dairy products and analogues**	
1.1	Unflavoured pasteurised and unflavoured sterilised (including UHT) milk	1.0 g/L
1.2/1.3	Unflavoured fermented milk-based products	1.0 g/L beverages
		10 g/kg products other than beverages
1.4	Flavoured fermented milk-based products including heat-treated products	1.0 g/L beverages
		10 g/kg products other than beverages
7	**Bakery wares**	
7.2	Fine bakery wares. Cereal bars only	10 g/kg
13	**Foods for Special Groups (FSG)**	
13.1	**Foods for infants and young children**	
13.1.1	Infant formula as defined in Regulation (EU) No 609/2013	0.8 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer

[1] Throughout this application, the term "human milk oligosaccharide (HMO)" is used to refer to the naturally occurring oligosaccharides in human breast milk, while the term "human-identical milk oligosaccharide (HiMO)" is used to refer to the manufactured counterparts of these substances.

Table 1 Proposed Food Uses and Use Levels for LNT Product

EU Food Category Number	Food Category Name	Proposed Maximum Use Level
13.1.2	Follow-on formula as defined in Regulation (EU) No 609/2013	0.6 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer
13.1.3	Processed cereal-based food and baby food for infants and young children as defined in Regulation (EU) No 609/2013	0.6 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer
		5 g/kg for products other than beverages
13.1.4	Milk-based drinks and similar products intended for young children	0.6 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer
		5 g/kg for products other than beverages
13.2	**Foods for special medical purposes as defined in Regulation (EU) No 609/2013**	
13.2	Foods for special medical purposes as defined in Regulation (EU) No 609/2013	On case-by-case basis
13.3	**Total diet replacement for weight control as defined in Regulation (EU) No 609/2013**	
13.3	Total diet replacement for weight control as defined in Regulation (EU) No 609/2013	2.0 g/L beverages
		20 g/kg products other than beverages
14	**Beverages**	
14.1.4	Flavoured drinks	1.0 g/L
17	**Food supplements as defined in Directive 2002/46/EC, excluding food supplements for infants**	
17	Food supplements as defined in Directive 2002/46/EC, excluding food supplements for infants	2.0 g/day for general population

LNT = lacto-*N*-tetraose; UHT = ultra-high temperature.

HMOs, including LNT, do not undergo any significant digestion in the upper gastrointestinal tract; however, HMOs are orally absorbed intact to a small extent, a small portion of which (approximately 1 to 2% of the total amount of HMO ingested) is excreted unchanged in urine. Therefore, the absorption of the LNT would be limited and any level of the LNT product that is absorbed would be no different to that exposed to by infants consuming human breast milk. Therefore, the potential absorption of LNT product from its consumption is not a safety concern for infants. Since infants comprise the most sensitive age group, it may be concluded that the absorption of LNT ingredient does not pose a safety concern for other age groups.

Glycom's LNT product is neither mutagenic (as assessed in the bacterial reverse mutation test) nor clastogenic/aneugenic (as assessed in the *in vitro* mammalian cell micronucleus test). In a 90-day toxicity study conducted in neonatal Sprague Dawley rats (dosed from Day 7 of age), the no-observed-adverse-effect-level was concluded to be 4,000 mg/kg body weight/day LNT (the highest dose tested and maximum feasible dose). No measurable protein residues are detected in the LNT product and it is inherently non-allergenic.

In addition, the safety of lacto-*N*-neotetraose (LNnT) a chemically and structurally similar isomer of LNT, has previously been extensively investigated in a series of preclinical and clinical studies, which further support the safety of the LNT product for its intended uses. The dataset on LNnT obtained by chemical synthesis has been reviewed by the European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies and it was concluded that the material would not pose any safety concerns under its intended conditions of use. Additional studies were subsequently conducted on LNnT obtained by

fermentation and on the basis of these studies, LNnT preparations obtained by microbial fermentation have also been authorised for use as novel foods in the European Union. Three clinical studies have been conducted to date with LNnT (either alone or in combination with other non-digestible oligosaccharides) and it was found to be generally well tolerated in all of them.

The totality of the presented data, including thorough safety assessments, highlights the safety and suitability of this ingredient for its proposed food uses.