APPLICATION FOR THE APPROVAL OF THE HUMAN-IDENTICAL MILK OLIGOSACCHARIDE MIXTURE OF 2'-FUCOSYLLACTOSE AND DIFUCOSYLLACTOSE 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 a mixture of 2'-fucosyllactose and difucosyllactose ("2'-FL/DFL mixture"). This mixture is obtained from microbial fermentation and is isolated as a mixture; thus, it is not a blend of separately produced compounds. The manufactured 2´-FL and DFL are identical in structure to the same molecules that are present in human milk and are therefore referred to as human-identical milk oligosaccharides (HiMOs). 2'-FL and DFL are structurally and biologically closely related since DFL is metabolically obtained from 2'-FL by the simple addition of a second fucose unit ("fucosylation"). Indeed, 2'-FL and DFL are always found together in human milk; never is 2'-FL present on its own, nor is DFL without the concurrent presence of 2'-FL. Constituting 2 of the major fucosylated human milk oligosaccharides (HMOs), the 2'-FL/DFL mixture represents an important and significant sub-fraction of the natural oligosaccharide fraction of human milk.

Glycom's 2'-FL/DFL ingredient is manufactured and purified in several steps to a well-defined and highly pure specification, containing not less than 85.0 % of the sum of 2'-FL and DFL (the gap to 100% is predominantly lactose) at an isolated ratio between 2'-FL and DFL that comes close to the natural ratio found in human milk. In stage 1 of the manufacturing process (upstream processing), D-lactose and D-glucose (or alternatively D-glycerol) are converted to 2'-FL and DFL by the adapted cellular metabolism of the 2'-FL/DFL production microorganism, which uses glucose (or glycerol) as an exclusive energy and carbon source and lactose as a substrate for 2'-FL and DFL biosynthesis. In stage 2 (downstream processing), a series of purification and isolation steps are used to generate the final high-purity 2'-FL/DFL mixture.

Proposed uses for 2'-FL/DFL are as an ingredient in infant formulas (up to 12 months) and formulated milks for young children and older populations at a use level of up to 1,600 mg/L of the ready-to-drink or reconstituted product. The maximum use level is proposed on the basis of providing a similar amount of 2'-FL and DFL as occurs naturally in mature human breast milk. The food-uses and use levels, in accordance to the food categorisation system from Codex, are presented in Table 1 below.

Table 1 Proposed Food Uses and Use Levels for 2'-FL/DFL mixture

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	2.0 g/L
1.2/1.3	Unflavoured fermented milk-based products	2.0 g/L beverages
		20 g/kg products other than beverages
1.4	Flavoured fermented milk-based products including heat-treated products	2.0 g/L beverages
		20 g/kg products other than beverages
7	**Bakery wares**	
7.2	Fine bakery wares. Cereal bars only	20 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	1.6 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer
13.1.2	Follow-on formula as defined in Regulation (EU) No 609/2013	1.2 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	1.2 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer
		10 g/kg for products other than beverages

Table 1 Proposed Food Uses and Use Levels for 2'-FL/DFL mixture

EU Food Category Number	Food Category Name	Proposed Maximum Use Level
13.1.4	Milk-based drinks and similar products intended for young children	1.2 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer
		10 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	4.0 g/L beverages
		40 g/kg products other than beverages
14	**Beverages**	
14.1.4	Flavoured drinks	2.0 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	4.0 g/day for general population

2'-FL/DFL = 2'-fucosyllactose and difucosyllactose; EU = European union; UHT = ultra-high temperature.

HMOs, including 2'-FL and DFL, do not undergo any significant digestion in the upper gastrointestinal tract, nor are they orally absorbed to any significant extent (a small portion of approximately 1 to 2 % of the total amount of ingested HMO is excreted unchanged in urine). Therefore, the absorption of the 2'-FL/DFL mixture would be limited and any level of 2'-FL and DFL that is absorbed would be no different to that resulting from infants consuming human breast milk and the potential absorption of the 2'-FL/DFL mixture 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 2'-FL and DFL also poses no safety concern for other age groups.

Glycom's 2'-FL/DFL mixture is neither mutagenic (as assessed in the bacterial reverse mutation test, at concentrations up to 5,000 µg/mL) nor clastogenic/aneugenic (as assessed in the *in vitro* mammalian cell micronucleus test, at concentrations up to 2,000 µg/mL). 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 5,000 mg/kg body weight/day 2'-FL/DFL mixture (the highest dose tested and maximum tolerated dose in neonatal animals, based on data for similar compounds). No measurable protein residues are detected in 2'-FL/DFL mixture and it is inherently non-allergenic.

In addition, the safety of the major component of 2'-FL/DFL mixture (*i.e.* 2'-FL), has previously been extensively investigated in a series of preclinical and clinical studies, which support the safety of the 2'-FL component of the 2'-FL/DFL mixture for its intended uses. The dataset on 2'-FL 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 2'-FL obtained by fermentation and on the basis of these studies, 2'-FL preparations obtained by microbial fermentation have also been authorised for use as novel foods in the European Union. Four clinical studies have been conducted to date with 2'-FL (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.