Glycom A/S Kogle Allé 4 2970 Hørsholm, Denmark

21 September 2021

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

Dear Dr. Gaynor:

Re: GRAS Notice of 2'-Fucosyllactose (2'-FL) for Use in Exempt Infant Formula

In accordance with 21 CFR §170 Subpart E consisting of §§ 170.203 through 170.285, Glycom A/S [Kogle Allé 4 2970 Hørsholm, Denmark], as the notifier, is submitting one hard copy and one electronic copy (on CD), of all data and information supporting the company's conclusion that 2'-Fucosyllactose (2'-FL) produced by derivatives of *Escherichia coli* K12 DH1 MDO (as per GRN 650), is GRAS on the basis of scientific procedures, for use in exempt term infant formula and is therefore not subject to the premarket approval requirements of the *Federal Food, Drug and Cosmetic Act*. Information setting forth the basis for Glycom's GRAS conclusion are enclosed for review by the agency.

Should you have any questions or concerns regarding this GRAS notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

[redacted]

Christoph H. Röhrig, Ph.D.
Senior Scientist
Head of Regulatory & Scientific Affairs
Glycom A/S

GRAS NOTICE OF 2'-FUCOSYLLACTOSE (2'-FL) FOR USE IN EXEMPT INFANT FORMULA

SUBMITTED TO:
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

SUBMITTED BY:
Glycom A/S
Kogle Allé 4
2970 Hørsholm
Denmark

DATE:
13 September 2021

Glycom A/S a wholly owned indirect affiliate of DSM Nutritional Products Ltd, a company with registered address at Wurmisweg 576, 4303 Kaiseraugst, Switzerland

GRAS Notice of 2'-Fucosyllactose (2'-FL) for Use in Exempt Infant Formula

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GRAS Notice of 2'-Fucosyllactose (2'-FL) for Use in Exempt Infant Formula

Glycom A/S[1] (Glycom), a manufacturer of human identical milk oligosaccharides, has previously concluded that 2'-fucosyllactose (2'-FL) is Generally Recognized as Safe for (GRAS) use in non-exempt term infant formula and in select food and beverage products across multiple categories. These conclusions were notified to the offices of the United States Food and Drug Administration (FDA) and filed by the Agency without objection under GRN 650 (U.S. FDA, 2016a). Supplemental information pertaining to strain changes were submitted to the Agency in 2020. Glycom intends to expand the current GRAS uses of 2'-FL as described in GRN 650 to also include use in hypoallergenic exempt infant formula for infants with cow's milk protein allergy (CMPA) or multiple food allergies. These formulas may also be appropriate for infants with non-allergenic gut impairment and malabsorptive conditions. Glycom notes that its 2'-FL is manufactured by using milk-derived lactose as a substrate for 2'-FL biosynthesis. Accordingly, food uses of 2'-FL are subject to the allergy labeling requirements of the *Food Allergen Labeling and Consumer Protection Act of 2004* (FALCPA) (U.S. FDA, 2018). As the labeling of 2'-FL with allergy statements "contains milk" would be conflicting with food uses in exempt hypoallergenic formula for infants with CMPA, Glycom has filed a petition with the FDA in accordance with 21 U.S.C. 343(w)(6) for exemption of 2'-FL from the allergy labeling requirements of FALCPA. This petition has been filed under FDA docket No. FDA-2020-FL-1865 (FALCPA No. 005) and is currently under review by the Agency (U.S. FDA, 2020a). Where applicable, data and information supporting conclusions that 2'-FL is absent of detectable milk allergic proteins and would not cause an allergenic response that poses a risk to human health are incorporated by reference to FALCPA No. 005.

[1] Glycom A/S a wholly owned indirect affiliate of DSM Nutritional Products Ltd, a company with registered address at Wurmisweg 576, 4303 Kaiseraugst, Switzerland.

Part 1. § 170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285 (U.S. FDA, 2020b), Glycom A/S (Glycom) hereby informs the United States (U.S.) Food and Drug Administration (FDA) that 2'-fucosyllactose (2'-FL) as described in GRN 650 (U.S. FDA, 2016a), is not subject to the premarket approval requirements of the *Federal Food, Drug, and Cosmetic Act* based on Glycom's view that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Section 1.3 below. In addition, as a responsible official of Glycom, the undersigned hereby certifies that all data and information presented in this Notice represents a complete, representative, and balanced submission, and considered all favorable, as well as unfavorable, information known to Glycom and pertinent to the evaluation of the safety and GRAS status of 2'-FL as a food ingredient for addition to exempt infant formula, as described herein.

Signed

21 Sept 2021

Christoph Röhrig, Ph.D.
Head of HMO Regulatory Affairs
Glycom A/S
Christoph.roehrig@dsm.com

Date

1.1 Name and Address of Notifier

Glycom A/S
Kogle Allé 4
2970 Hørsholm
Denmark
Tel: +45 8830 9500
Fax: +45 4593 3968

1.2 Common Name of Notified Substance

Common Name: 2'-Fucosyllactose (2'-FL)

Trade Name: GlyCare™ 2FL 9000 HA

1.3 Conditions of Use

2'-FL is intended to be added to exempt term infant formula targeted to infants with cow's milk protein allergy (CMPA) or multiple food allergies. These formulas may also be appropriate for infants with non-allergenic gut impairment and malabsorptive conditions. Uses of this ingredient in exempt infant formula (*i.e.*, infants up to 12 months) will provide a use level of 2'-FL of 2.4 g/L in the exempt formula (see Table 1.3-1). The maximum use levels are proposed on the basis of providing similar levels of 2'-FL, on a body weight basis, as those consumed by breastfed infants (see Section 3.1). Example products to which 2'-FL may be added include extensively hydrolyzed infant formula (EHF) for infants with CMPA, such as Gerber Extensive (Nestlé), which is lactose free and contains probiotics and medium chain triglycerides

(MCT). Addition of 2'-FL to amino acid-based formula such as Alfamino (Nestlé) would represent products targeted to infants not responding to EHF or for infants with moderate to severe CMPA, including those with anaphylaxis, food protein-induced enterocolitis syndrome (FPIES), multiple food protein allergy of infancy [non-immunoglobulin E (IgE)-mediated], or eosinophilic esophagitis.

Table 1.3-1 Summary of the Individual Proposed Food Uses and Use Levels for 2'-FL in Exempt Infant Formula the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2020b)	Proposed Food Use	Target Population	RACC[a] (g or mL)	Proposed Maximum Use Level[b] (g/RACC)	Proposed Maximum Use Level[b] (g/kg or g/L)
Exempt Term Infant Formulas	Extensively hydrolyzed formula (EHF) (*e.g.*, Gerber Extensive)	Cow's milk protein allergy (CMPA) Cow's milk protein intolerance Cow's milk-induced food protein-induced enterocolitis syndrome (FPIES) Soy protein sensitivity Fat malabsorption	100 mL[c]	0.24	2.40
	Amino acid-based formula (*e.g.*, Alfamino)	CMPA – Symptoms that persist after use of an EHF Multiple food allergies Eosinophilic gastrointestinal disorders FPIES Short-bowel syndrome (SBS) Malabsorption	100 mL[c]	0.24	2.40

2'-FL = 2'-fucosyllactose; CFR = *Code of Federal Regulations*; RACC = Reference Amounts Customarily Consumed per Eating Occasion; U.S. = United States.

[a] RACC based on values established in 21 CFR §101.12 (U.S. FDA, 2020b). When a range of values is reported for a proposed food use, particular foods within that food use may differ with respect to their RACC.

[b] Use level expressed on a 2'-FL basis in the final food, as consumed.

[c] RACC not available; 100 mL employed as an approximation.

1.4 Basis for GRAS

Pursuant to 21 CFR §170.30 (a)(b) of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2020b), Glycom has concluded, on the basis of scientific procedures, that 2'-FL is GRAS for addition to exempt term infant formula, as described in Table 1.3-1.

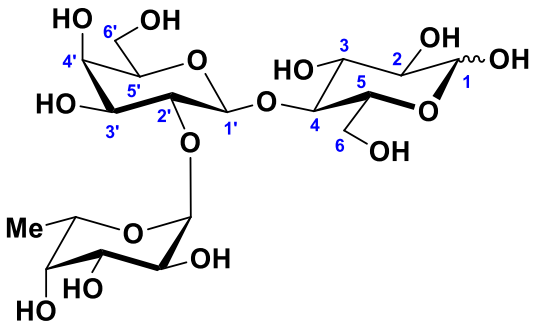
Part 2. Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

2'-FL is a naturally occurring trisaccharide that is present in some mammalian milks with the highest concentrations found in human milk from lactating women. Based on the significant content of 2'-FL in human milk, it is often categorized as a human milk oligosaccharide (HMO). 2'-FL is comprised of L-fucose, D-galactose, and D-glucose. Alternatively, the molecular constitution can be described as consisting of the monosaccharide L-fucose and the disaccharide D-lactose, which are linked by an alpha(1→2) bond to form the trisaccharide. 2'-FL is a chemically defined trisaccharide that occurs only as one specific constitutional isomer. 2'-FL as manufactured by Glycom has been characterized using ^{1}H and 2D-nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry, and X-ray crystallography, and was demonstrated to be qualitatively identical to 2'-FL that is present in human milk form lactating women. For additional data and information supporting the chemical characterization of 2'-FL, see Section II.A.2 of GRN 650 (Glycom A/S, 2016a).

Common Name:	2'-Fucosyllactose
Common Abbreviation:	2'-FL (2'FL, 2-FL, 2FL)
Trade Name:	GlyCare™ 2FL 9000 HA
International Union of Pure and Applied Chemistry (IUPAC) Name:	α-L-Fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucose
Alternative Denotations:	2'-*O*-Fucosyllactose; 2'-Fucosidolactose; 2'-*O*-L-Fucosyl-D-lactose; Fucosyl-α-1,2-galactosyl-β-1,4-glucose; Fuc-α-(1→2)-Gal-β-(1→4)-Glc
Chemical Abstracts Service (CAS) Registry Number:	41263-94-9
Chemical Formula:	$C_{18}H_{32}O_{15}$
Molecular Weight:	488.44

Structural Formula:



2.2 Manufacturing

2.2.1 Description of the Production Microorganism

2'-FL is produced by a derivative of *Escherichia coli* K-12 DH1 MDO, a platform strain from which other human-identical milk oligosaccharide (HiMO) production strains have been derived including several GRAS ingredients such as 2'-FL; lacto-*N*-neotetraose (LNnT); 2'-fucosyllactose/difucosyllactose (2'-FL/DFL); lacto-*N*-tetraose (LNT); 6'-sialyllactose (6'-SL) sodium salt; 3'-sialyllactose (3'-SL) sodium salt; 3-fucosyllactose (3-FL); and lacto-*N*-fucopentaose I/2'-fucosyllactose (LNFP-I/2'-FL). The characteristics of this parental host strain (K-12 DH1 MDO) have been described previously and are incorporated by reference to Sections II.B.1.1 through II.B.1.3 of GRN 650. Genetic modifications for expression of enzymes necessary for the biosynthesis of 2'-FL have been revised from the original production strain (SCR6) described in GRN 650 (Glycom A/S, 2016a; U.S. FDA, 2016a), and Glycom currently uses strain *E. coli* K-12 (DH1) MAP1001d (*i.e.*, DSM 32775), a strain that has been optimized for 2'-FL biosynthesis (U.S. FDA, 2016a).

2.2.2 Description of the Production Process

Glycom's 2'-FL is manufactured in compliance with current Good Manufacturing Practice (cGMP) and the principles of Hazard Analysis Critical Control Point (HACCP). The manufacture of 2'-FL is largely comparable to the production processes previously evaluated for other HiMOs with GRAS status (see GRNs 650, 659, 815, 833, 880, and 881) (U.S. FDA, 2016a,b, 2019a,b, 2020c,d). All additives, processing aids, and food contact articles used during manufacturing are permitted by federal regulation, have been previously determined to be GRAS for their respective uses, or have been the subject of an effective Food Contact Notification.

The manufacture of 2'-FL includes upstream (fermentation) and downstream (purification) stages as described in GRN 650 (Glycom A/S, 2016a).

In Stage 1 [upstream processing (USP)], D-lactose and D-glucose[2] are converted to 2'-FL by the adapted cellular metabolism of the production microorganism, which uses D-glucose as an energy and carbon source and D-lactose as a substrate for 2'-FL biosynthesis. The production microorganism is removed from the fermentation medium at the end of the fermentation process.

[2] Alternatively, D-sucrose or glycerol.

In Stage 2 [downstream processing (DSP)], a series of purification, isolation, and concentration steps are used to generate the final high-purity 2'-FL product. Production of 2'-FL includes a crystallization step (with methanol) to generate a higher-grade ingredient with further minimization of impurities.

A schematic overview of the manufacturing process for 2'-FL is presented in Table 2.2.2-1 below.

Table 2.2.2-1 Summary of the Overall Manufacturing Process for 2'-FL

Stage	Step No.	Process Step	Purification
Upstream Processing (USP)	01	Media Preparation	-
	02	Propagation	-
	03	Seed Fermentation	-
	04	Fermentation	Production of 2'-FL
	05	Ultrafiltration/Diafiltration (UF/DF)	Removal of cells and large biomolecules (*e.g.*, protein, nucleic acids and lipopolysaccharides)
Downstream Processing (DSP)	06	Nanofiltration (NF)	Concentration; Reduction of water, minerals, and very small biomolecules
	06a	*Optional Microfiltration*	*Removal of potential microbiological contamination*
	06b	*Optional Ion Removal (e.g., ion-exchange/adsorption resin)*	*Removal of small charged molecules and salts (e.g. trace metals)*
	06c	*Optional Pre-concentration (e.g., evaporation or nanofiltration)*	-
	07	Decoloration (*e.g.*, charcoal filtration)	Removal of color and impurities by adsorbent
	08	Microfiltration	Removal of potential microbiological contamination
	09	Pre-concentration (*e.g.*, evaporation or nanofiltration)	-
	10	Crystallization (from water with acetic acid)	Highly efficient removal of micro-impurities (traces of protein and DNA, amino acids, carbohydrate-type impurities, trace elements, *etc.*)
	11	Solid-Liquid-Separation (SLS)	
	12	Washing	
	13	Drying	Removal of water and acetic acid
	14	Sampling and Packaging	-
	15	Quality Control	Parameters of specifications are tested and CoA issued
	16	Batch Release	-

2'-FL = 2'-Fucosyllactose; CoA = Certificate of Analysis.

2.2.3 Quality Control

The manufacture of 2'-FL by microbial fermentation is conducted in accordance with cGMP and HACCP principles. Considering the chemically well-characterized principal raw materials and final products, the whole production process can be followed in detail by a range of analytical techniques. These techniques are applied either as in-process controls or at batch release (by Certificate of Analysis) to allow full control of the production process (refer to Table 2.2.2-1).

Both manufacturing stages (USP and DSP) are controlled by a HACCP plan that includes specifications for equipment, raw materials, product, and packaging materials. Master operating instructions are followed, batch records are kept, a number of in-process controls are applied, and the isolated product is controlled by Certificates of Analysis and batch release routines. The HACCP plan for both manufacturing stages also includes in-process controls to reduce potential impurities to the lowest level technically possible. Glycom's production process (including all processing aids, raw materials, unit operations, and filter aids) and the food safety management system comply with the Food Safety Systems Certification (FSSC) 22000 and International Organization for Standardization (ISO) 9001.

Incorporation of sterile filtration units throughout the manufacturing process of the HiMOs, ensures high microbiological sterility and therefore the absence of the production microorganism in the final product. The production organism is efficiently removed in the ultrafiltration step, which is applied directly following fermentation. In addition, several additional purification steps are carried out in the DSP stage to help achieve a highly purified 2'-FL, which is free from bacterial cells and residual fermentation by-products. The absence of the microorganisms can be measured by analysis for *Enterobacteriaceae* in the final product according to an internationally recognized method (ISO 21528-2). This specification for *Enterobacteriaceae* is set at "≤ 10 colony-forming units per gram" of test article, which also ensures the absence of the production microorganism as *E. coli*-K12 belongs to the *Enterobacteriaceae* family. As further assurance of the absence of viable production organism in the finished products, batches of 2'-FL have been tested for *E. coli* in accordance with ISO 16649-2. The results have confirmed the absence of enumerable *E. coli* in all tested batches of 2'-FL (results available upon request).

As discussed above, 2'-FL that is the subject of this Notice is the same ingredient described in GRN 650 (Glycom A/S, 2016a). This production process has been determined to produce a high-purity crystallized ingredient that is free of allergenic milk protein. The effectiveness of the production process to produce 2'-FL that is free of allergic milk protein is described in Petition No. FDA-2020-FL-1865 exempting 2'-FL from allergy labeling requirements of the *Food Allergen Labeling and Consumer Protection Act of 2004* (FALCPA) (U.S. FDA, 2018). Although the production process for 2'-FL as described in GRN 650 does not result in the transfer of allergenic milk protein to 2'-FL, the following quality control checks are used as control points to ensure that residual milk proteins originating from the production media are not transferred to the 2'-FL ingredients that will be added to exempt infant formula:

1. To limit the introduction of allergenic milk protein to the production process, an internal specification of < 100 ppm total protein is applied to all lots of lactose used during fermentation. This specification is applied exclusively to lots of 2'-FL that are intended for use in exempt infant formula.

2. The specification of 2'-FL intended for use in exempt infant formula will also comply with a stricter level for residual lactose (1 w/w %)

3. The specification for total protein in the final lots of 2'-FL is reduced from 0.01% (100 ppm) to non-quantifiable (*i.e.*, < 17 ppm) using a validated modified Bradford method developed by Glycom.

4. Final lots of 2'-FL intended for use in exempt infant formula will be tested for allergenic milk protein using a sensitive enzyme-linked immunosorbent assay (ELISA) assay for β-lactoglobulin (Euroclone BLG). This assay has a limit of detection (LOD) of 1.5 ppb, a limit of quantitation (LOQ) of 10 ppb and has been validated for sensitivity for detecting β-lactoglobulin in milk protein within the 2'-FL matrix by third-part experts (Neotron, Italy).

2'-FL ingredients passing the above quality control criteria are labeled “GlyCare™ 2FL 9000 HA” for differentiation of the ingredient from other lots of 2'-FL that have not been subjected to the extended quality control verification analyses.

2.3 Product Specifications and Batch Analyses

2.3.1 Specifications

Food-grade specifications for 2'-FL are presented in Table 2.3.1-1 below. All methods of analysis are either internationally recognized methods that are “fit-for-purpose” or developed by Glycom using validated in-house methods. The 2'-FL ingredient is specified as a crystallized white to off-white powder with a purity of at least 94% based on high-performance liquid chromatography with charged aerosol detection (HPLC-CAD) analysis. Upper limits have been established for the raw materials and processing aids used in the manufacturing (*e.g.*, D-lactose, acetic acid), the carbohydrates formed during the fermentation (*e.g.*, L-fucose, difucosyllactose, 2'-fucosyl-D-lactulose), chemical impurities, heavy metals, and microbiological parameters, to ensure the purity of the final product (See Section 2.4.1).

Table 2.3.1-1 Product Specifications for 2'-FL

Parameter	Specification	Method
Appearance	Powder or agglomerates	MSZ ISO 6658:2007
Color	White to off-white	MSZ ISO 6658:2007
Identification	RT of main component corresponds to RT of standard ± 3%	Glycom method HPLC-202-2C4-002
2'-FL Assay by HPLC (water free)	≥ 94.0%	Glycom method HPLC-202-2C4-002
D-Lactose	≤ 1.0 w/w%	Glycom method HPLC-206-2C4-001
L-Fucose	≤ 1.0 w/w%	Glycom method HPAEC-206-001
Difucosyllactose	≤ 1.0 w/w%	Glycom method HPAEC-206-001
Total human-identical milk oligosaccharides[a]	≥ 96.0%	Glycom methods HPLC-202-2C4-002, HPLC-206-2C4-001, and HPAEC-206- 001
2'-Fucosyl-D-lactulose	≤ 1.0 w/w%	Glycom method HPLC-206-2C4-001
pH (20°C, 5% solution)	3.2 to 5.0	Ph. Eur. 2.2.3
Water	≤ 5.0%	Karl-Fischer (Ph. Eur. 2.5.32)
Ash, sulfated	≤ 1.5%	Ph. Eur. 6.7 04/2010:20414
Acetic acid (as free acid and/or sodium acetate)	≤ 1.0%	Megazyme K-ACETRM 07/12
Residual proteins	≤ 0.002%	Bradford Assay; Glycom method UV-001
β-Lactoglobulin	≤ 0.05 mg/kg	ELISA
Casein	≤ 0.5 mg/kg	ELISA
Heavy Metals		
Lead	≤ 0.1 mg/kg	ICP-MS by EPA 6020A:2007
Microbiological Parameters		
Salmonella	Absent in 25 g	MSZ-EN-ISO 6579:2006
Aerobic mesophilic total plate count	≤ 500 CFU/g	MSZ-EN-ISO 4833-1:2014
Enterobacteriaceae	Absent in 10 g	ISO 21528-1:2004, MSZ ISO 21528-2:2007
Cronobacter (Enterobacter) sakazakii	Absent in 10 g	ISO-TS 22964:2006

Table 2.3.1-1 Product Specifications for 2'-FL

Parameter	Specification	Method
Listeria monocytogenes	Absent in 25 g	MSZ-EN-ISO 11290-1:1996/A1:2005, MSZ EN ISO 11290-1:1998
Bacillus cereus	≤ 50 CFU/g	MSZ-EN-ISO 7932:2005
Yeasts	≤ 10 CFU/g	MSZ-ISO 7954:1999
Molds	≤ 10 CFU/g	MSZ-ISO 7954:1999

2'-FL = 2'-fucosyllactose; CFU = colony forming units; ELISA = enzyme-linked immunosorbent assay; EPA = Environmental Protection Agency; HPAEC = high-performance anion-exchange chromatography; HPLC = high-performance liquid chromatography; ICP-MS = inductively couples plasma mass spectrometry; ISO = International Organization for Standardization; Ph. Eur. = European Pharmacopeia; RT = retention time; UV = ultraviolet.
[a] Human-identical milk oligosaccharides is defined as the sum of 2'-FL, lactose, difucosyllactose, and fucose.

2.4 Batch Analysis

The analytical results of three independent production batches of 2'-FL are summarized in Table 2.4-1. The stability of 2'-FL has been previously determined to be at least 5 years when protected from light and stored at room temperature and ambient humidity (see Section II.D of GRN 650 – Glycom A/S, 2016a).

Table 2.4-1 Batch Analysis of 2'-FL Produced by Fermentation

Specification Parameter	Specification Limit	2'-FL from SCR6		2'-FL from MAP1001d
		[redacted]		
Appearance	Powder, agglomerates, or powder with agglomerates	Powder	Powder	Powder with agglomerates
Color	White, white to off white, off white	White	White	White
Identification	RT of standard ± 3%	Complies	Complies	Complies
Assay (water free) – HiMS[a] (w/w %)	≥ 96.0	99.2	96.9	98.3
Assay (water free) – 2'-FL (w/w %)	≥ 94.0	98.9	97.5	97.8
D-Lactose (w/w %)	≤ 1.0	0.28	0.29	0.20
L-Fucose (w/w %)	≤ 1.0	< 0.03	< 0.03	< 0.03
Difucosyllactose (w/w %)	≤ 1.0	0.07	0.34	0.29
2'-Fucosyl- D-lactulose (w/w %)	≤ 1.0	0.18	0.48	0.29
pH (20°C, 5% solution)	3.2 to 5.0	3.7	3.7	3.6
Water (w/w %)	≤ 5.0	0.12	0.11	0.06
Ash, sulfated (w/w %)	≤ 1.5	0.10	< 0.10	< 0.10
Acetic acid (w/w %)	≤ 1.0	0.23	0.27	0.34
Residual proteins (w/w %)	≤ 0.002	< 0.0017	< 0.0017	< 0.0017
β-Lactoglobulin (mg/kg)	≤ 0.05	< 0.01	< 0.01	< 0.01
Casein (mg/kg)	≤ 0.5	< 0.2	< 0.2	< 0.2
Lead (mg/kg)	≤ 0.1	< 0.05	< 0.05	< 0.05
Salmonella (in 25 g)	Absent	Absent	Absent	Absent
Total plate count (CFU/g)	≤ 500	< 10	< 10	< 10
Enterobacteriaceae (in 10 g)	Absent	Absent	Absent	Absent

Table 2.4-1 Batch Analysis of 2'-FL Produced by Fermentation

Specification Parameter	Specification Limit	2'-FL from SCR6	[redacted]	2'-FL from MAP1001d
Cronobacter (Enterobacter) *sakazakii* (in 10 g)	Absent	Absent	Absent	Absent
Listeria monocytogenes (in 25 g)	Absent	Absent	Absent	Absent
Bacillus cereus (CFU/g)	≤ 50	< 10	< 10	< 10
Yeasts (CFU/g)	≤ 10	< 10	< 10	< 10
Molds (CFU/g)	≤ 10	< 10	< 10	< 10
Residual endotoxins (E.U./mg)	≤ 10	< 0.00025	< 0.00024	< 0.00025

2'-FL = 2'-fucosyllactose; CFU = colony forming units; E.U. = endotoxin units; HiMS = human-identical milk saccharides; LOQ = limit of quantitation; RT = retention time.
[a] LOQ = 0.0017%.

2.4.1 Manufacturing By-products, Impurities, and Contaminants

Carbohydrate-type by-products (*e.g.*, L-fucose, difucosyllactose, 2'-fucosyl-D-lactulose) are the main manufacturing impurities present in 2'-FL. These compounds are detectable, and levels are limited by appropriate specifications. Glycom also has established internal quality control measures that include microbial endotoxins and residual proteins and precautionary analyses demonstrating the absence of deleterious levels of several other potential residual compounds and trace elements that may originate from fermentation. These include amino acids and biogenic amines, trace elements, and the presence/absence of genes characteristic for the production microorganism. These by-products, impurities, and contaminants are confirmed to be absent at any relevant levels of safety concern, and, as such, are not proposed for addition to the product specifications (see Sections II.C.3 and II.C.4 of GRN 650 – Glycom A/S, 2016a).

2.4.1.1 Control Point Analyses for Protein and Allergenic Milk Protein

As discussed in Section 2.2.3 above, the production process and downstream purification steps used for the manufacture of crystallized 2'-FL are sufficient to ensure that the transfer of allergenic milk protein—originating from the milk derived lactose used during fermentation—are not present in 2'-FL at levels that would cause a risk to human health among individuals with milk allergy. The absence of protein in crystallized 2'-FL has been verified by analyses of multiple lots of the ingredient using Glycom's modified Bradford assay at a detection limit of 17 ppm. The absence of allergenic milk protein against multiple milk antigens was demonstrated using four ELISA assays against casein, milk protein, and β-lactoglobulin; the detection limits of these assays ranged from 1.5 ppb to 1.0 ppm. Additional highly sensitive and indiscriminate analyses for milk protein was conducted using liquid chromatography with tandem mass spectrometry (LC-MS/MS) proteomic analyses with a limit of quantitation of 20 ppb. All of the aforementioned assays have been validated by third-party experts for use on 2'-FL and an incorporated appropriate spiking methodology for verification of the assay sensitivity. This analytical data and validation work establishing the suitability of 2'-FL for use in hypoallergenic infant formula were reviewed by the U.S. FDA during the Agency's review of FALCPA Petition No. FDA-2020-FL-1865 exempting 2'-FL from the allergy labeling requirements of FALCPA (U.S. FDA, 2018, 2020a). Based on findings from Glycom's milk protein analysis, it was concluded with a high degree of confidence that 2'-FL does not contain milk protein above a detection limit of 20 ppb as established from the proteomics analyses. This value is considered conservative, as no milk proteins have been detected in Glycom's 2'-FL samples in any assay, including

investigational proteomic analyses with an extrapolated detection limit of 5 ppm and ELISA analyses with a detection limit sensitivity of 1 ppb for β-lactoglobulin.

Based on the above protein analyses work conducted on Glycom's 2'-FL ingredient, it was concluded that 2'-FL as described in GRN 650 is of suitable purity for consumption by infants with milk allergy (see Section 6.3 for the risk assessment analyses) (Glycom A/S, 2016a). Although it is Glycom's view that inclusion of further protein analyses in the ingredient specification is not necessary for ensuring safety for use in hypoallergenic exempt formula, for conservative reasons, 2'-FL samples used in exempt infant formula will be lot-selected for samples with total protein levels below the detection limit of Glycom's modified Bradford method (*i.e.*, < 17 ppm total protein). These samples will be subjected to an additional quality control analyses to ensure the absence of detectable milk protein using an ELISA assay for β-lactoglobulin (Euroclone; LOD = 1 ppb; LOQ = 10 ppb). Analyses of five lots of 2'-FL demonstrating the absence of protein/milk protein are presented below in Table 2.4.1.1-1 below.

Table 2.4.1.1-1 Detection of Milk Residues in 2'-FL

Lot	Modified Bradford (LOQ = 17 ppm)	Euroclone ELISA against β-Lactoglobulin (LOQ = 10 ppb)
[redacted]	< LOQ	< LOQ
[redacted]	< LOQ	< LOQ
[redacted]	< LOQ	< LOQ
[redacted]	< LOQ	< LOQ
[redacted]	< LOQ	< LOQ

2'-FL = 2'-fucosyllactose; ELISA = enzyme-linked immunosorbent assay; LOQ = limit of quantitation; ppb = parts per billion; ppm = parts per million.

Part 3. Dietary Exposure

3.1 Background Consumption of 2'-FL in Human Breast Milk

The concentration of 2'-FL in human milk has been measured and reported by numerous investigators. Comprehensive discussions on the background intakes of 2'-FL from breast milk are summarized in Section 3.1.3 of GRN 815 (Glycom A/S, 2018). The concentration of 2'-FL in human milk has been measured and reported to date in at least 28 independent publications [reviewed recently by Thurl *et al*. (2017)]. Table 3.1-1 below summarizes the levels of 2'-FL that have been reported in breast milk across these various studies independently. The data demonstrate that 2'-FL is the most abundant HMO of pooled human milk, though approximately 20% of women (termed "non-Secretors") do not express the α-1,2-fucosyltransferase enzyme in their mammary glands and thus their milk does not contain 2'-FL (Castanys-Muñoz *et al.*, 2013; Austin *et al.*, 2016). This enzyme is responsible for the fucosylation of oligosaccharides and lactose at the 2-position of galactose, resulting in the production of 2'-FL, among others. In this context it is noted that the diversity ("polymorphism") of the female population in regard to the Secretor genotype appears to have been maintained over evolutionary times due to a "parent-offspring conflict" (Springer and Gagneux, 2016). While the non-Secretor phenotype (*i.e.*, the resulting absence of characteristic cell-surface glycans) appears to provide a net benefit to the mother to escape some infectious agents (Marionneau *et al.*, 2005; Lindén *et al*., 2008; Carlsson *et al.*, 2009), there is a growing body of evidence that the breastfed infant actually benefits from the inverse situation: the Secretor phenotype as expressed into milk (*i.e.*, presence of the same characteristic glycans as free oligosaccharides in milk) (Morrow *et al*., 2004; Newburg *et al*., 2004; Lewis *et al*., 2015; Smith-Brown *et al*., 2016).

Table 3.1-1 2'-FL Concentration in Human Milk after Full-Term Birth

Lactation Time	Key Findings	References
Pooled Milk		
Days 1 to 4 ("colostrum")	**Reported Range: 1.0 to 8.4 g/L** Average: 3.2 g/L	Erney *et al.* (2000); Musumeci *et al.* (2006); Asakuma *et al.* (2008); Spevacek *et al.* (2015); Ma *et al.* (2018); Nijman *et al.* (2018)
Days 5 to 14 ("transitional milk")	**Reported Range: 1.0 to 2.8 g/L** Average: 2.2 g/L	Erney *et al.* (2000); Spevacek *et al.* (2015); Austin *et al.* (2016); Ma *et al*. (2018); Borewicz *et al.* (2020); Ferreira *et al.* (2020)
Days 10 to 60 ("mature milk")	**Reported Range: 0.7 to 3.9 g/L** Average: 1.9 g/L	Chaturvedi *et al.* (1997, 2001a); Nakhla *et al.* (1999); Erney *et al.* (2001, 2000); Morrow *et al.* (2004); Spevacek *et al.* (2015); Austin *et al.* (2016); McGuire *et al*. (2017); Sprenger *et al*. (2017); Ma *et al.* (2018); Nijman *et al*. (2018); Borewicz *et al.* (2020)
After 2 months ("mature milk")	**Reported Range: 0.7 to 2.3 g/L** Average: 1.1 g/L	Erney *et al.* (2000); Smilowitz *et al.* (2013); Austin *et al.* (2016); Azad *et al.* (2018); Ma *et al.* (2018); Borewicz *et al.* (2020)
Secretor Milk		
Days 1 to 4 ("colostrum")	**Reported Range: 1.1 to 6.1 g/L** Average: 3.7 g/L	Coppa *et al.* (1999); Thurl *et al.* (2010); Bao *et al*. (2013); Aakko *et al.* (2017); Kunz *et al.* (2017); Austin *et al.* (2019)
Days 5 to 14 ("transitional milk")	**Reported Range: 2.7 to 5.6 g/L** Average: 3.7 g/L	Coppa *et al.* (1999); Kunz *et al.* (2017); Austin *et al.* (2019)
Days 10 to 60 ("mature milk")	**Reported Range: 0.7 to 7.8 g/L** Average: 3.0 g/L	Thurl *et al.* (1996, 2010); Coppa *et al.* (1999, 2011); Leo *et al.* (2009); Galeotti *et al.* (2012, 2014); Bao *et al.* (2013); Hong *et al.* (2014); Olivares *et al.* (2015); Kunz *et al.* (2017); McGuire *et al*. (2017); Sprenger *et al.* (2017); Austin *et al.* (2019); Ferreira *et al.* (2020)

Table 3.1-1 2'-FL Concentration in Human Milk after Full-Term Birth

Lactation Time	Key Findings	References
After 2 months ("mature milk")	**Reported Range: 1.4 to 3.1 g/L** Average: 2.3 g/L	Coppa *et al.* (1999); Sprenger *et al.* (2017); Azad *et al.* (2018); Lefebvre *et al.* (2020)

2'-FL = 2'-fucosyllactose.

The average levels of 2'-FL in pooled milk are highest in colostrum (3.2 g/L), followed by transitional milk (2.2 g/L), and in mature milk (1.9 g/L) and continue to decline in mature milk from a lactation stage later than 2 months (1.1 g/L). In the context of relative abundance, 2'-FL ranks first with approximately 15 to 20 w/w % (corresponding to 24 to 30 mol %) of the total HMO biomass (Castanys-Muñoz *et al*., 2013). In milk from Secretor mothers, the corresponding levels are significantly higher, with average levels reported at 3.7 g/L in colostrum, 3.7 g/L in transitional milk, 3.0 g/L in mature milk and 2.3 g/L in mature milk from a lactation stage later than 2 months. 2'-FL concentrations between different mothers is highly variable, with reported levels reaching beyond 5 g/L (8.4 g/L as the highest level reported).

3.2 Current Food Uses of 2'-FL in Exempt Infant Formula

Glycom identified a GRAS Notice on the U.S. FDA's GRAS Notice inventory describing a GRAS conclusion from Jennewein Biotechnology GmbH (Jennewein) that the use of 2'-FL produced from lactose by microbial fermentation was GRAS for use in exempt infant formula at a use level of up to 2.0 g/L. This Notice was submitted to the offices of the FDA under the agency's voluntary GRAS notification program and filed without objection under GRN 929 (U.S. FDA, 2021). Within the Notice, Jennewein present data and information characterizing the protein content of their 2'-FL ingredient, which included analyses of total protein using a modified Bradford method, analyses using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) with silver staining, size exclusion chromatography and ELISA assays for casein and whey. The notifier reported that protein could not be detected in any of the assays for total protein, and one positive hit for milk protein against casein was detected using an ELISA assay suggestive of casein at a level of 25 ppm (detection limit of 9 ppm).

3.3 Estimated Intake of 2'-FL from Proposed Uses

Dietary intake of 2'-FL from use in infant formula has been estimated previously during Glycom's GRAS evaluation of 2'-FL for use in non-exempt infant formula for term infants. The intake estimations were conducted using statistical modeling software and food consumption data from the U.S. National Center for Health Statistics' 2011-2012 National Health and Nutrition Examination Surveys (NHANES) (USDA, 2014; CDC, 2015). A detailed description of the methodology and results are reported in Section IV.A of GRN 650 (Glycom A/S, 2016a). As infant formula consumption is not expected to change over time, results of the dietary intake estimates for 2'-FL calculated using the 2011-2012 NHANES data were used for estimating dietary intake of 2'-FL among infant consumers of exempt infant formula. These dietary intake estimates would be considered conservative as infants with atopic gastrointestinal diseases/disorders are unlikely to consume greater quantities of infant formula than healthy term infants. A summary of the estimated dietary intake of 2'-FL from the proposed and existing GRAS uses [*i.e*., use in exempt infant formula and conventional foods described in GRN 650 (Glycom A/S, 2016a) are shown below in Section 3.3.1. Dietary intake estimates from infant formula consumption alone are presented in Section 3.3.2.

3.3.1 Dietary Intake of 2'-FL by Infant Consumers from Exempt Formula and Background Diet

A summary of the estimated daily intake of 2'-FL by infant consumers from background food uses of 2'-FL as described in GRN 650 (Glycom A/S, 2016a) in conjunction with dietary intake of 2'-FL from exempt infant formula is provided in Table 3.3.1-1. On an absolute basis, the mean and 90th percentile consumer-only intakes of 2'-FL from all food uses were determined to be 3.2 and 5.8 g/person/day, respectively, among infants aged 0 to 6 months. The mean and 90th percentile consumer-only intakes of 2'-FL were estimated to be 4.7 and 9.1 g/person/day, respectively, among infants aged 7 to < 12 months.

Table 3.3.1-1 Summary of the Estimated Daily Intake of 2'-FL from Food Uses of 2'-FL in the U.S. by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Months)	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to 6	2.6	5.4	81.5	173	3.2	5.8
Infants	7 to < 12	4.7	8.9	99.5	127	4.7	9.1

2'-FL = 2'-fucosyllactose; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

On a body weight basis, the mean and 90th percentile consumer-only intakes of 2'-FL from all food uses among infants aged 0 to 6 months, were determined to be 462 and 796 mg/kg body weight/day, respectively. The mean and 90th percentile consumer-only intakes of 2'-FL were estimated to be 511 and 949 mg/kg body weight/day, respectively, among infants aged 7 to < 12 months (see Table 3.3.1-2).

Table 3.3.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from All Proposed Food Uses in the U.S. by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Months)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to 6	377	756	81.5	173	462	796
Infants	7 to < 12	508	949	99.5	127	511	949

2'-FL = 2'-fucosyllactose; bw = body weight; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

3.3.2 Dietary Intake of 2'-FL by Infants from Exempt Infant Formula Only

A summary of the estimated daily intake of 2'-FL from infant formula use only is provided in Table 3.3.2-1 on an absolute basis (g/person/day). To understand dietary intakes among heavy consumers of infant formula 99th percentile intake data was included in the evaluation. On an absolute basis, the mean and 99th percentile consumer-only intakes of 2'-FL from infant formula only were determined to be 2.1 and 5.8 g/person/day, respectively, among infants aged 0 to 6 months. The mean and 99th percentile consumer-only intakes of 2'-FL were estimated to be 1.8 and 3.5 g/person/day, respectively, among infants aged 7 to < 12 months. Due to the overestimation of intakes that occurs when 99th percentile intake estimates are used across multiple food use categories it was not considered appropriate to evaluate dietary intakes to 2'-FL by 99th percentile consumers of infant formula and conventional foods to which 2'-FL may be added.

Table 3.3.2-1 Summary of the Estimated Daily Intake of 2'-FL from Infant Formula Only in the U.S. by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Months)	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	99th Percentile	%	n	Mean	99th Percentile
Infants	0 to 6	1.6	5.8	76.0	161	2.1	5.8
Infants	7 to < 12	1.3	3.4	75.8	94	1.8	3.5

2'-FL = 2'-fucosyllactose; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

Part 4. Self-Limiting Levels of Use

No known self-limiting levels of use are associated with 2'-FL.

Part 5. Experience Based on Common Use in Food Before 1958

Not applicable.

Part 6. Narrative and Safety Information

6.1 Introduction

The use of 2'-FL, as manufactured by Glycom as an ingredient in infant formula, has been the subject of multiple safety reviews by various qualified experts and authoritative bodies (*e.g.*, GRN 650, 815 – U.S. FDA, 2016a, 2019a; FSAI, 2016; EFSA, 2019; FSANZ, 2019). Crystallized 2'-FL produced by Glycom is of high purity and has been demonstrated to be qualitatively identical to 2'-FL naturally present within human breast milk. Concentrations of 2'-FL in human milk are subject to significant interindividual variation and concentrations may vary by two orders of magnitude based on the Secretor phenotypes of the mother. Concentrations of up to ~8 g/L, several fold higher than those proposed for use in infant formula, have an established safe history of consumption through breast milk by healthy infants as well as infants with CMPA and other food allergy disorders (see Section 3.1.3.2 of GRN 815 – Glycom A/S, 2018). Accordingly, the use of 2'-FL in infant formula at concentrations within the upper mean percentiles of levels naturally present in human milk provides prima facie evidence of safety. To date, Glycom's 2'-FL ingredient has market access to over 160 countries for use in term infant formula[3].

Glycom intends to expand the use of 2'-FL to include exempt infant formula. The U.S. FDA defines exempt infant formula as:

> "[…] *an infant formula intended for commercial or charitable distribution that is represented and labeled for use by infants who have inborn errors of metabolism or low birth weight, or who otherwise have unusual medical or dietary problems*" (21 CFR §107.3 – U.S. FDA, 2020b).

The dietary condition for which 2'-FL is intended for use includes infants with CMPA or multiple food allergies. The purpose of this GRAS Notice is therefore to provide generally available data and information supporting Glycom's conclusion that the use of 2'-FL in hypoallergenic exempt infant formula would be concluded to be GRAS by qualified experts. Background information on CMPA and multiple food allergies is presented in Section 6.2.

As discussed, 2'-FL is manufactured using a modified strain of *E. coli* K-12 expressing biosynthetic enzymes that catalyze the addition of fucose to the 2' position of lactose. As the lactose used during fermentation is typically derived from cow's milk, small quantities of allergic milk protein are introduced during the manufacturing process. Although the potential introduction of milk allergens during 2'-FL manufacturing represents a potential hazard for use by infants with milk allergy, these levels are firstly significantly diluted during fermentation and secondly 2'-FL preparations produced by various manufacturers are subjected to significant DSP to further remove/reduce such impurities; however, the efficiency of the purification process will vary by production process and therefore are specific to each manufacturer. Glycom has therefore provided analytical data demonstrating that the company's manufacturing process controls are sufficient to ensure that allergic milk protein is not detected in the ingredient at levels that would cause an allergenic response that poses a risk to human health. This safety standard was established in a manner that is consistent with the petition requirements under 21 U.S.C. 343(w)(6) for exemption of 2'-FL from the allergen labeling requirements of FALCPA. Data and information supporting the safety of 2'-FL for use in hypoallergenic infant formula are therefore incorporated by reference to FALCPA Petition No. 005. The results of validated analytical data demonstrating the absence of milk protein in 2'-FL is discussed in brief in

[3] For a brief historical overview of the current commercial uses of Glycom's 2'-FL in infant formula, see Section 3.1.4 of GRN 815.

Section 2.4.1.1 along with confirmatory batch analyses obtained for multiple lots of 2'-FL subjected to Glycom's extended analytical allergen control processes that have been implemented for lots of 2'-FL used in infant formula.

Recognizing the challenges of demonstrating that an ingredient is wholly "absent" of milk protein, a risk-based approach (see Section 6.3) to the safety assessment of using 2'-FL in infant formula is presented that leverages a numerical detection limit for milk protein in 2'-FL obtained using multiple qualitatively distinct validated methods against generally recognized threshold levels for milk allergenicity reported by scientific experts of the Voluntary Incidental Trace Allergen Labeling (VITAL) program of the Allergen Bureau of Australia & New Zealand (ABA) (Allen *et al.*, 2014; Taylor *et al.*, 2014; Remington *et al.*, 2020). Using a detection limit of 25 ppb for potential residues of milk protein in 2'-FL and a maximum use level of 2.4 g/L of 2'-FL in exempt infant formula, a possible dietary intake of up to 73 ng/day can be estimated for infant consumers 0 to 6 months of age from exempt infant formula. This value is 2740-fold below the elicited dose (ED) that would protect 99% of milk allergic individuals from developing any objective reaction (ED_{01}; 0.2 mg).

In addition to the risk-based approach for assessing the safety of 2'-FL for use in exempt infant formula, additional supporting clinical data evaluating the safety and tolerance of Glycom's 2'-FL ingredient in hypoallergenic infant formula was evaluated in randomized controlled cross-over study in infants with confirmed CMPA (Nowak-Wegrzyn *et al*., 2019a). The study was conducted in accordance with the American Academy of Pediatrics (AAP) statement guidance on hypoallergenic infant formulas, and the base formula was validated for hypoallergenicity. The authors reported that 2'-FL was safe and well tolerated by infants with CMPA and no differences in incidences of adverse responses to the formula were observed between the groups. This study provides strong supporting information that 2'-FL manufactured by Glycom is safe for use in infants with CMPA and corroborates conclusions of the risk-based assessment. An additional non-randomized single-group multicenter study was identified in the literature where the safety and tolerance of 2'-FL in infant formula was evaluated in infants with CMPA in a 2-month feeding trial (Ramirez-Farias *et al*., 2021). The primary outcome was maintenance of weight for age z-score during the study. The authors concluded that the study formula was well tolerated, safe and supported growth in the intended population. Glycom notes that the 2'-FL used in the test formula was not manufactured by Glycom and due to expected differences in the manufacture and control of residual milk protein in the 2'-FL, the findings from this study are not relevant to the GRAS evaluation.

For the purposes of identifying new data relevant to the safety of 2'-FL published since the most recent 2'-FL GRAS conclusion notified to the U.S. FDA (*i.e.*, GRN 897; DuPont Nutrition and Health, 2019; U.S. FDA, 2020e), a comprehensive search of the published scientific literature was conducted on 27 August 2021 spanning the period of September 2019 to August 2021. The search was conducted using the electronic search tool, ProQuest, with several databases, including Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS reviews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile®. A discussion of all newly available published and unpublished studies is presented below.

Corroborating information to support the safety of 2'-FL in infant formula is provided by clinical trials in healthy term infants administered 2'-FL alone or in conjunction with other oligosaccharides such as LNnT, galacto-oligosaccharides (GOS), and short-chain fructo-oligosaccharides (Marriage *et al.*, 2015; Alliet *et al.*, 2016; Goehring *et al.*, 2016; Kajzer *et al.*, 2016; Steenhout *et al.*, 2016; Puccio *et al.*, 2017). See Section 6.4 of GRN 815 (Glycom A/S, 2018) for additional background information on the study designs and reported findings. The updated literature search identified 7 new interventional clinical trials in which endpoints related to the safety of 2'-FL were identified and these studies are reviewed in Section 6.4.2 below.

Supporting animal toxicity studies of Glycom's 2'-FL ingredients have been conducted using a sensitive repeat-dose neonatal rodent toxicity study conducted in under current Good Laboratory Procedures and in accordance with Organisation for Economic Co-operation and Development (OECD) Test Guideline 408 (Coulet *et al.*, 2014; Phipps *et al.*, 2018). The no-observed-adverse-effect level (NOAEL) values of 5,000 mg/kg body weight per day were derived from the studies. Other investigators have reported similar findings for 2'-FL in animal toxicity studies (Jennewein Biotechnologie GmbH, 2015; Penard, 2015) and tolerance studies in piglets (Hanlon and Thorsrud, 2014), where 2'-FL was concluded to be safe at the highest doses administered in the studies. The updated literature search identified additional safety and investigative studies of 2'-FL in animal models that further corroborate that 2'-FL is of low toxicity potential; these studies are discussed further in Section 6.5 below. Overall, there have been no findings in animal safety studies to suggest that the proposed uses of 2'-FL in infant formula at levels matching human breast milk would be unsafe.

Based on the findings from Glycom's risk-based assessment combined with supporting clinical data evaluating Glycom's 2'-FL ingredient in infants with CMPA it can be concluded that the ingredient is GRAS for use in hypoallergenic exempt infant formula.

6.2 Metabolic Fate

Reviews of published data and information characterizing the absorption, distribution, metabolism and excretion of 2'-FL have been the subject of previous comprehensive evaluations, and this information is incorporated herein by reference to Section IV.B.4 of GRN 546 (U.S. FDA, 2015). In brief, it is generally recognized that HMOs, including 2'-FL, are highly resistant to hydrolysis by digestive enzymes under conditions simulating the infant gastrointestinal tract (Engfer *et al*. 2000; Gnoth *et al*. 2000). Gnoth *et al*. (2001) have suggested that small quantities of 2'-FL may be transported transcellularly across the intestinal epithelium by receptor-mediated transcytosis, and/or by paracellular means, and low quantities of 2'-FL have been detected unchanged in the urine of breastfed infants (Rudloff *et al*., 1996, 2012; Obermeier *et al.*, 1999; Chaturvedi *et al*., 2001b; Dotz *et al*., 2014). However, data from infant studies analyzing HMO digestion by intestinal bacterial microflora and HMO fecal excretion indicate that the proportion of 2'-FL that may be absorbed would be relatively small. Therefore, the intended uses of 2'-FL as described herein will not be a safety issue to infants with malabsorptive conditions.

6.3 Cow's Milk Protein Allergy (CMPA) and Multiple Food Allergies

Food allergy is defined as an adverse health effect arising from a specific immune mediated response that occurs reproducibly from the ingestion of specific foods. Food allergy can be segregated into one of two major categories: IgE-mediated and non-IgE-mediated, based upon the immunological response. A third category of mixed IgE- and non-IgE-mediated food allergy responses also has been characterized. IgE-mediated reactions are the most well characterized and are easily diagnosed by the presence of specific serum IgE or a positive skin prick test response to a food antigen challenge (Calvani *et al*., 2021). The prevalence of milk allergy in the developed world is 2 to 3% and is the most common type of food allergy in the pediatric population (Lifshitz and Szajewska, 2015). Milk allergy occurs most frequently in the first years of life and produces a range of symptoms from acute urticaria of the skin, gastrointestinal reactions (pain, discomfort, diarrhea, vomiting) to the most severe reactions of anaphylaxis affecting multiple organ systems and potentially leading to cardio-respiratory collapse and death (Høst and Halken, 2014). Non-IgE-mediated food allergy disorders are believed to represent up to 40% of milk protein allergy in infants and young children and include FPIES, food protein-induced allergic proctocolitis (FPIAP), food protein-induced enteropathy, Heiner's syndrome (pulmonary hemosiderosis), and cow's milk protein-induced iron deficiency anemia. Mixed IgE- and non-IgE-mediated allergic disorders also have been characterized for milk and include eosinophilic esophagitis and eosinophilic gastroenteritis (Nowak-Węgrzyn, 2015).

The major allergens responsible for IgE-mediated milk allergy belong to the casein (αs1-, αs2-, β-, and κ-casein) and whey (β-lactoglobulin, and α-lactalbumin) fractions of milk; co-sensitization with soy is known to occur in some infants (Lifshitz and Szajewska, 2015). Although milk is one of the most frequent food triggers for non-IgE-mediated allergic reactions, non-IgE-mediated allergy to soy, egg, and cereals (wheat, rice, and oats) are also relatively common. Depending on the geographical region, non-IgE-mediated reactions to other dietary food proteins such as fish, pulses, poultry, and nuts have been identified. FPIES is typically caused by a single food in most children (65 to 80%), with milk and soy being the most common. Approximately 5 to 10% of infants with FPIES are allergic to more than three foods (Nowak-Węgrzyn, 2015).

Extensively hydrolyzed formula (EHF) is typically recommended as a first line intervention for infants with CMPA. When an EHF is provided for the first time to infants with CMPA, it should be provided under supervision of a physician experienced and equipped to treat anaphylaxis (Zeiger, 2003). Per definition by the AAP, to be labelled hypoallergenic, an infant formula needs to be tolerated by at least 90% of infants with CMPA (with 95% statistical confidence) (AAP, 2000). EHFs derived from bovine casein or whey are tolerated by approximately 95% of infants with CMPA (Bahna, 2008); however, some infants display very high sensitivities to low levels of intact/partially intact milk protein and cannot tolerate EHFs. Infants unable to ingest EHFs are then typically provided elemental formulas containing amino acids as a source of protein.

Consistent with the fact that most infants with CMPA respond favorably to EHF, the addition of 2'-FL to infant formula is expected to be well tolerated by these infants. The fact that a majority of infants with CMPA can tolerate lactose, which is derived from cow's milk tends to support this conclusion (Heine *et al*., 2017). Notwithstanding these conclusions, a small population of infants with CMPA will be expected to be highly sensitive to low levels of intact/partially intact milk proteins, and there remains a possibility that low-level milk protein residues in 2'-FL originating from the use of lactose during fermentation could induce an allergic response in these individuals should small quantities of milk protein be present at sufficiently high levels. The safety of 2'-FL for use in hypoallergenic exempt infant formula will therefore be determined on the basis of establishing the absence of detectable milk allergenic protein in the ingredient and through evidence demonstrating that the current detection limits for residual milk protein in the HMO ingredient added to infant formula is safely below the minimum level deemed necessary to produce an allergenic response that represents a safety risk to the infant based on available data.

6.4 Risk-Based Safety Assessment

The use of a risk-based evaluation procedures can be applied to evaluate the safety of 2'-FL as an ingredient for use in hypoallergenic infant formula. Risk-based procedures incorporates statistical findings from a large dataset of clinical trials to derive threshold doses for allergenic responses to milk protein. The advantage of this approach is the quality and robustness of the data that is provided and the ability to pool findings on thresholds for the most sensitive sub-populations of milk-allergic infants. The advantages of using a risk-based safety approach have been discussed previously by the U.S. FDA (Buchanan *et al*., 2008).

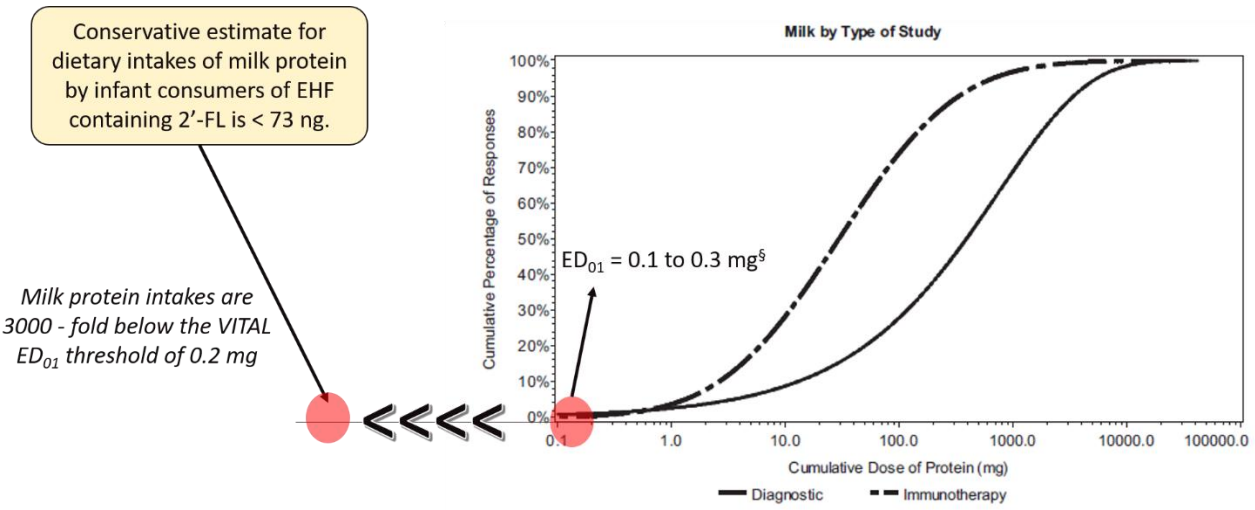
One of the most comprehensive risk-based evaluations of milk protein allergy thresholds was conducted in 2011 by an Expert Panel assembled as part of the VITAL program of the ABA (Taylor *et al*., 2014). The Panel applied statistical approaches described previously by Crevel *et al*. (2007) to model the dose distribution of allergy thresholds from oral clinical challenge studies. The authors incorporated the concept of a predicted population ED, where ED_p, refers to the dose of allergen that is predicted to produce a response in (100-p)% of the allergic population. Food challenge studies used for dose-modeling were selected based upon criteria outlined previously (Taylor *et al*., 2009) and placed an emphasis on low-dose oral challenge studies with a preference for double-blind placebo-controlled studies (except for data from infants and young children where double-blinding was not considered necessary). Data were modeled using both discrete and cumulative dose-response effects, and the lowest-observed-effect level was selected based on the first reported objective symptoms of an allergic response; the NOAEL was then set at the previous dose.

For characterization of milk allergy hazard, the VITAL Expert Panel used a clinical data set that included 17 published studies and 2 unpublished studies, containing a total of 351 subjects reporting objective symptoms. Objective symptom reporting favored children, with 323 objective symptoms reported for children, 25 from adults, and 3 from subjects where the age was uncertain. Data for both discrete and cumulative dosing was modeled, with discrete dosing considered the most conservative approach; however, the authors reported that little difference existed between the ED values based on the discrete *versus* cumulative doses for any of the parametric models. The data for milk allergy was sufficiently robust for calculation of an ED_{01}, a dose that would protect 99% of milk allergic individuals from developing any objective reaction. The Expert Panel recognized that an ED_{01} would imply that a small percentage of the population (*i.e*., 1%) of allergic individuals may elicit objective reactions at this dose; however, as adverse reaction experiences by individuals in the low-dose trials were characterized as mild to moderate, and never resulted in provocation of severe reactions, the risk of individuals developing severe reactions that would pose a risk to human health would be very low. Based on the dose-response modeling an ED_{01} of 0.1 mg was

established by the Panel. Additional qualitative analyses of the VITAL reference doses according to different statistical modeling techniques and analyses of the effects of age, geographic origin, nature of the challenge materials and dosing regimen were reported by Allen *et al*. (2014). The authors reported that the heterogeneity of the dataset for milk thresholds did not impact conclusions on the ED_{01} of 0.1 mg for milk. The reference doses were recently updated in 2020 and included new clinical data on milk allergy thresholds and re-analyses of the dose-threshold distributions using newly developed Stacked Model Averaging statistical modeling techniques (Remington *et al*., 2020). Using an updated dataset of 450 individuals, the authors reported a model averaged ED_{01} for discrete dosing[4] of 0.2 mg [95% confidence interval (CI) = 0.1, 0.5].

The ED_{01} threshold can be used to evaluate the risk of using 2'-FL in exempt infant formula by comparing theoretical dietary intakes of milk protein from the use of 2'-FL in infant formula to the ED_{01} value of 0.2 mg. 2'-FL is intended for use in non-exempt term infant formula at a use level of up to 2.4 g/L. The estimated 90th percentile intake of 2'-FL by infant consumers of term formula were reported to be 2.91 g/day (infants 0 to 6 months) and 2.63 g/day (infants 7 to 12 months). Using a detection limit for total milk protein of 25 ppb measured using validated LC-MS/MS proteomic analyses (see FALCPA No. 005), dietary intakes of milk protein would be 73 ng and 66 ng in infants aged 0 to 6 and 7 to 12 months, respectively. These intake levels are 2,740- and 3,030-fold below the ED_{01} threshold of 0.2 mg for infants 0 to 6 months and 7 to 12 months respectively (see Figure 6.4-1). This margin of safety is considered sufficiently high to protect the most highly sensitive population of infants with IgE-mediated food allergy.

Figure 6.4-1 Comparison of Estimated Dietary Intake of Milk Protein to Allergy Thresholds Using Risk-Based Assessment



Note: Dose-distribution models for individual thresholds (expressed as milligrams of protein) based on allergic patients for diagnostic studies, threshold studies, and immunotherapy studies (Modified from Allen *et al*., 2014).

§ ED_{01} value reported as 0.1 mg (Allen *et al*., 2014; Taylor *et al*., 2014). ED_{01} increased to 0.2 and 0.3 mg for discrete and cumulative dosing based on updated analyses by Remington *et al*. (2020).

[4] Discrete dosing schemes are reported as the mg protein amount of each separate dose within a food challenge.

With respect to the use of 2'-FL as an ingredient in exempt amino acid-based infant formula for sensitive subpopulations of infants with non-IgE-mediated milk allergy, thresholds for severe reactions by sensitive subpopulations have not been established; however, as reported by Munblit *et al*. (2020), "[…] *available data suggests that thresholds of reactivity in infants with non-IgE mediated CMA* [cow's milk allergy] *are usually higher than thresholds of reactivity for IgE-mediated CMA*". Therefore, the margin of safety between the ED_{01} values for IgE milk allergy and potential exposure to milk protein from the use of 2'-FL in infant formula strongly suggest that any risk of allergic reactions in sensitive infants with severe non-IgE food allergy would be very low. It also is noteworthy that children with non-IgE food allergy are not at risk for anaphylaxis (Calvani *et al*., 2021).

Glycom's 2'-FL is produced using fermentation technology that utilizes a modified strain of *E. coli* K12 expressing genes required for the synthesis of 2'-FL from lactose. The HMO is then purified through a variety of downstream processes such as micro-filtration, chromatographic separation, and crystallization to produce high-purity ingredients that are free of fermentation contaminants and contain virtually no detectable protein. 2'-FL manufactured by Glycom have GRAS status for use in term infant formula and therefore data and information characterizing the identity, quality, manufacturing, and safety of 2'-FL for use as infant formula ingredients can be incorporated by reference to GRN 650 (U.S. FDA, 2016a). 2'-FL intended for use in hypoallergenic infant formula will meet specifications set forth as described in GRN 650 and will be manufactured using the same methods and purification techniques described in the Notice. Glycom has conducted extensive analytical testing for residues of protein in 2'-FL and has demonstrated the absence of detectible milk protein in the ingredients using four validated ELISA kits for casein, total milk, and lactoglobulin. Lactose is produced from whey and therefore the major milk protein that could be transferred from lactose into 2'-FL is expected to be lactoglobulin. Using the most sensitive ELISA assay available for lactoglobulin, Glycom has demonstrated the absence of lactoglobulin at a detection limit of 10 ppb. As lactoglobulin comprises approximately 50% of the total protein content of whey (Regester and Smithers, 1991), a detection limit of 10 ppb total lactoglobulin would correspond to a detection limit for total milk whey protein of 20 ppb. Proteomics analyses of 2'-FL using LC-MS/MS have demonstrated the absence of milk protein fragments at a detection limit of 25 ppb based on findings from validated spiking assays. It also is noteworthy that findings from additional proteomic analyses on samples of 2'-FL using LC-MS/MS were able to detect low-level quantities of *E. coli* protein from the fermentation organism at an extrapolated detection limit of 5 ppb corroborating the sensitivity of the assay; however, no milk protein fragments have ever been detected in any 2'-FL samples that have been analyzed to date. Due to technical challenges with spiking and sample preparation at such low concentrations, validation of the 5 ppb detection limit was deemed impractical; however, the totality of evidence from ELISA assays and proteomic analyses provide support that no milk protein is present in the Glycom's 2'-FL. Based on findings from the proteomics assay a detection limit of 25 ppb was used for risk assessment purposes (*i.e*., it was assumed that 25 ppb of milk protein will be present in 2'-FL). The 25 ppb detection limit from the proteomics assay was preferred over the ELISA as the proteomic analyses is a more robust method that is not impacted by potential protein hydrolysis or denaturation of the milk protein. The totality of information characterizing the protein content of 2'-FL obtained using the most sensitive analytical methods available to date, have demonstrated that milk protein cannot be detected in 2'-FL under the conditions of manufacture as described in GRN 650 (U.S. FDA, 2016a). Although the current production controls have been deemed sufficient to ensure that milk protein will not be transferred to 2'-FL above a detection limit of 25 ppb, Glycom has applied additional quality control limits to production process for 2'-FL batches that will be used for hypoallergenic infant formula; these controls include limits for total protein on the incoming lactose used for production of 2'-FL and two ELISA-based assays for β-lactoglobulin and casein demonstrating the

absence of detectable milk protein in the ingredients. The strong congruence between findings from the ELISA and LC-MS/MS data support a conclusion that the ELISA assay is appropriate for this purpose.

6.5 Infant Studies

6.5.1 Infants with Cow's Milk Protein Allergy (CMPA)

It is generally recognized that a majority of infants with IgE- and non-IgE-mediated allergy to cow's milk protein can tolerate EHF, analytical data demonstrating the absence of milk protein in 2'-FL strongly supports the view that the majority of infants with IgE- and non-IgE-mediated allergy will tolerate infant formula containing this HiMO. This conclusion is supported by findings reported by Nowak-Wegrzyn *et al*. (2019a) who evaluated the safety and tolerance of adding 2'-FL to an extensively hydrolyzed hypoallergenic infant formula. The study was conducted in accordance with the AAP statement guidance on hypoallergenic infant formulas, which requires that for a formula to be:

> "[…] *labeled hypoallergenic, these formulas, after appropriate preclinical testing, must demonstrate in clinical studies that they do not provoke reactions in 90% of infants or children with confirmed cow's milk allergy with 95% confidence when given in prospective randomized, double-blind, placebo-controlled trials*" (AAP, 2000).

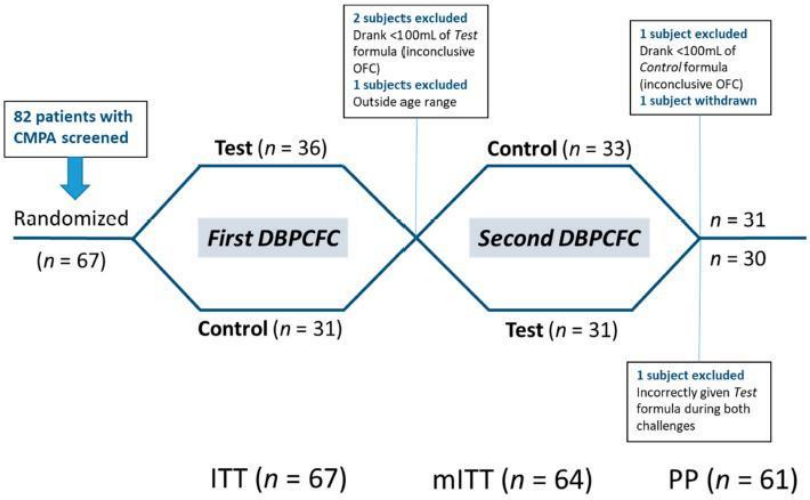
These criteria for hypoallergenic infant formula are also endorsed by other relevant scientific bodies such as the World Allergy Organization (Fiocchi *et al*., 2010), the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (Koletzko *et al*., 2012), and the European Academy of Allergy and Clinical Immunology (Muraro *et al*., 2014).

The test formula used in the study was a 100% whey-based EHF supplemented with 2'-FL (produced by Glycom as described in GRN 650 – Glycom A/S, 2016a) and LNnT (produced by Glycom as described in GRN 659 – Glycom A/S, 2016b). Infants and children between 2 months and 4 years of age [modified intention-to-treat (mITT) cohort: mean age at enrollment of 24.1 ± 13.2 months] with clinically diagnosed CMPA were recruited for the study. The infants were randomized into one of two groups provided a commercially available control infant formula (Althéra®, Nestlé, Vevey, Switzerland) without HMOs, or a test infant formula [Althéra® supplemented with 2'-FL (1.0 g/L) and LNnT (0.5 g/L)] in cross-over fashion resulting in two double-blind placebo-controlled food challenges (DBPCFCs) (see Figure 6.5.1-1). The control formula was previously qualified as hypoallergenic in accordance with AAP guidelines (Nowak-Wegrzyn *et al*., 2019b)[5]. The control and test formula were demonstrated to be free of residual milk proteins, as confirmed by gel electrophoresis (SDS-PAGE, Pharmacia PhastSystem™ with silver staining) and high-sensitivity ELISA testing (Euroclone Spa, Pero, Italy; limits of quantification 10 ppb for β-lactoglobulin and 20 ppb for casein). The first DBPCFC occurred within 3 to 28 days after enrolment, and the second DBPCFC within 2 to 7 days of the first DBPCFC. For subjects < 1 year of age, the initial dose was a lip smear with the assigned infant formula, followed by oral doses of 5, 10, 20, 30, 30, 35, and 50 mL at 10- to 15-minute intervals (total volume of 180 mL). For subjects > 1 year of age, the initial dose was a lip smear, followed by oral doses of 5 , 10, 25, 45, 45, 45, and 65 mL at 10- to 15-minute intervals (total volume of 240 mL). A DBPCFC was considered evaluable if subjects had consumed a minimum of 100 mL of formula. The subjects were observed for a minimum period of 1 hour after the second DBPCFC for any allergic signs or symptoms

[5] The production of hypoallergenic formula involves enzymatic hydrolysis, heat-treatment and ultrafiltration steps that are specific to each manufacturer. Accordingly, both the control formula and the test formula must be qualified as hypoallergenic in accordance with AAP guidelines. Extrapolation of findings between studies is therefore not possible.

(cutaneous, gastrointestinal, respiratory, or cardiovascular) attributable to the challenge formula. If both DBPCFCs were negative, subjects participated in a one-week (7-9 days), open food challenge (OFC) with the test infant formula (instructed to drink a minimum of 240 mL daily). During this time, daily formula intake as well as several clinical parameters, including allergenic or adverse events were recorded: (1) Daily stool frequency, color, consistency, and odor; (2) frequency of flatulence; (3) frequency of spitting-up and/or vomiting; (4) any potential allergic symptoms; and (5) any other adverse or serious adverse events.

Figure 6.5.1-1 Double-Blind Placebo-Controlled Food Challenge Study Flow Chart (Nowak-Wegrzyn *et al.*, 2019a)



ITT = intention-to-treat; mITT = modified intention-to-treat; OFC = open food challenge; PP = per protocol analysis cohorts. Patients were allocated to perform two double-blind, placebo-controlled food challenges (DBPCFC) with the Test and Control formula in randomized order.

The results of the DBPCFCs reported one positive allergic reaction to the test infant formula during the first DBPCFC, and one positive allergic reaction to the control infant formula in the second food DBPCFC (same 12-month-old girl). Based on the mITT analysis, 63 out of 64 (98.4%; 95% CI lower bound of 92.8%) participants tolerated the test infant formula. Based on the per protocol (PP) analysis, 60 out of 61 (98.4%; 95% CI lower bound of 92.5%) participants tolerated the test infant formula (see Table 6.5.1-1). Therefore, under both analyses the test infant formula with two HMO (2'-FL and LNnT) met the defined criteria for hypoallergenic formulas (AAP, 2000). Additionally, no serious adverse events occurred during the OFC.

Table 6.5.1-1 Outcome of Double-Blind Placebo-Controlled Food Challenge to the Test and Control Formula by Group Allocation in the Modified Intention-to-Treat Cohort (Nowak-Wegrzyn *et al*., 2019a)

	Challenge Outcome	DBPCFC 1 n (%)	DBPCFC 2 n (%)	Total n (%)	Exact 96.5% Lower Bound
Test Formula (EHF + 2'-FL/LNnT)	Positive	1 (3.0%)	0 (0.0%)	**1 (1.6%)**	92.8%
	Negative	32 (97.0%)	31 (100%)	63 (98.4%)	
Control Formula	Positive	0 (0.0%)	1 (6.1%)	**1 (2.9%)**	92.6%
	Negative	31 (100%)	30 (93.9%)	61 (97.1%)	

2'-FL = 2'-fucosyllactose; DBPCFC = double-blind placebo-controlled food challenge; EHF = extensively hydrolyzed formula; LNnT = lacto-*N*-neotetraose.
The 95% confidence interval lower bound was greater than 90% for both formulas.

The authors concluded that "*the hypoallergenicity of this novel EHF supplemented with two HMOs* [2'-FL and LNnT] *was confirmed by DBPCFC in children with CMPA, in line with the established guidelines for hypoallergenic formulas*" (Nowak-Wegrzyn *et al*., 2019a); therefore, this conclusion supports the safety and tolerance of 2'-FL and LNnT as ingredients for use in exempt hypoallergenic infant formula.

Ramirez-Farias and colleagues (2021) reported findings from a study evaluating the growth, tolerance and compliance of infants fed an EHF with added 2'-FL. Forty-eight infants < 60 days of age with a history of food allergy requiring the use of EHF were enrolled in a prospective, single group, non-randomized, multicenter, in-home study. The test formula used for the study was an extensively hydrolyzed casein based infant formula (Similac Alimentum, Abbott Nutrition) containing 2'-FL at a concentration of 0.2 g/L. Infants were provided the test formula for *ad libitum* home use and the study duration was 60 ± 5 days. Study endpoints were evaluated on Days 1, 30, and 60, and the primary endpoints monitored in the study were maintenance of weight for age z-score from Day 1 to Day 60. Secondary endpoints related to weight, length, head circumference, formula intake, tolerance, stool consistency and questionnaires related to tolerance and clinical symptoms were obtained. The authors reported that the 2'-FL test formula was well tolerated, and the infants maintained their weight for age z-scores at Days 30 and 60. No apparent worsening of clinical symptoms related to diarrhea, constipation, blood in stool, vomiting, spit-up/gagging/reflux, fussiness, or rash/eczema were observed at Day 30 or Day 60. Due to several limitations in the study design (*e.g*., no control group, lack of characterization of the test formula and 2'-FL, small sample size, age of infants) conclusions on the hypoallergenicity of 2'-FL used in the infant formula are not possible; additionally, the level of 2'-FL investigated in the clinical study (0.2 g/L) was markedly lower than the proposed level herein (2.4 g/L). However, trends towards improvements of the aforementioned symptoms were reported across the various categories of clinical symptoms suggesting the possibility the 2'-FL may have beneficial effects on food intolerance symptoms in infants with confirmed milk allergy.

6.5.2 Other Infant Studies

The results of the updated literature search identified seven new interventional clinical trials in which endpoints related to the safety of 2'-FL were identified. These studies are summarized in the subsections below and tabulated in Section 6.5.2.6. Studies exclusively examining benefits of 2'-FL supplementation were not included herein.

6.5.2.1 Randomized, Double-Blind, Controlled Clinical Study Examining Safety of 2'-FL and LNnT in a Liquid Supplement for Premature Infants (Hascoët et al., 2021 [abstract])

The effect of a supplement containing 2'-FL and LNnT on growth, safety, and feeding tolerance was examined in a multicenter, randomized, double-blind, controlled clinical study conducted in France [Hascoët *et al.*, 2021 (abstract); National Clinical Trial (NCT) 03607942]. In this study, preterm infants (27 to 33 weeks gestation, birth weight < 1,700 g) were randomly allocated to receive either a supplement containing 2'-FL and LNnT in a 10:1 ratio (administered as a total of 0.374 g/kg body weight/day, dissolved in water buffered with a pH adjusting agent) or an isocaloric placebo supplement consisting of only glucose (0.140 g/kg body weight/day) from randomization (as early as possible) to discharge from the neonatal unit. The primary outcome was feeding tolerance, measured by non-inferiority in days to reach full enteral feeding from birth in the 2'-FL+LNnT group compared to the placebo group (non-inferiority margin of +4 days). Anthropometric z scores were calculated using Fenton growth standards. Other secondary outcomes include fecal markers of gut health/maturation and microbiota.

A total of 43 infants were allocated to the 2'-FL+LNnT supplement group and 43 to the placebo control group. The mean chronological age at the initiation of supplementation were 6.3 days in the 2'-FL+LNnT group and 6.2 days in the placebo group. The mean total duration of intervention was 41 (range: 2 to 80) days in the 2'-FL+LNnT group and 34.5 (range: 2 to 125) days in placebo group. Non-inferiority in time to reach full enteral feeding in the 2'-FL+LNnT group *versus* the placebo was achieved in the full analysis set (least squares mean difference = 2.16 days; 95% confidence level -5.33, 1.00; upper bound of 95% CI < non-inferiority margin). Similar results were observed in the per protocol set. The adjusted mean time to reach full enteral feeding from birth was 2 days shorter in the 2'-FL+LNnT group compared to placebo (12.2 days *versus* 14.3 days) but this finding did not reach statistical significance (p = 0.177). There was no difference in weight-for-age z-scores between the groups. Length-for-age z-scores were statistically significantly higher in the 2'-FL+LNnT supplement group *versus* the control group at full enteral feeding days 14 (least squares mean difference of 0.29; p = 0.037) and 21 (least squares mean difference of 0.31; p = 0.037). Head circumference-for-age z score was significantly higher in the group receiving 2'-FL+LNnT *versus* the control at discharge (least squares mean difference of 0.42; p = 0.007). Gastrointestinal tolerance measures, incidence of gastrointestinal adverse events, incidence of necrotizing colitis, and incidence of other illnesses and infections were similar between groups. No cases of illnesses and infections were deemed related to the intervention.

6.5.2.2 2'-FL in a 100% Whey, Partially Hydrolyzed Infant Formula (Storm et al., 2019)

A randomized, controlled, double-blind, multicenter study was conducted to evaluate the feeding tolerance of 2'-FL in healthy infants enrolled at 2 weeks of age (± 5 days) [Storm *et al*., 2019; NCT03307122]. The infants were randomly assigned to receive one of two infant formulas for 6 weeks. Both formulas contained a 100% whey, partially hydrolyzed protein base, and both were supplemented with the probiotic *Bifidobacterium lactis* strain Bb12. Both formulas provided 0.67 kcal/mL and 2.2 g protein/L. The only difference between the two formulas was the addition of 0.25 g/L 2'-FL to the test formula. At the enrollment visit (V0), the Infant Gastrointestinal Symptom Questionnaire (IGSQ) was administered, and anthropometric measurements were taken by trained study staff. Caregivers began to feed the infants with either the test or control formula *ad libitum* after V0. Following 42 days of feeding, subjects returned for a second visit (V1). For 2 days prior to V1, caregivers completed a diary recording the amount of formula intake, stool parameters (frequency, consistency, and whether the infant had difficulties passing bowel movements), the frequency of spit-up and vomit, and duration of crying and fussing. At V1, the IGSQ and anthropometric measurements were repeated.

Seventy-nine infants were enrolled and 63 completed the study per protocol (n = 30 in the Test group, n = 33 in the Control group). Body weights and lengths of the infants were similar between the Test and Control groups at both baseline (V0) and at the end of the 6-week intervention period (V1). No serious adverse events were reported in the study, the incidence of adverse events was comparable between the Test and Control groups. No significant differences were observed in the IGSQ scores between the Test and Control groups. There were also no significant differences in the stool frequency or consistency, and the duration of crying and fussing and vomiting frequency were similar between groups. The study authors reported that no safety concerns were noted with either of the study formulas. 100% whey protein, partially hydrolyzed infant formula containing 2'-FL and *B. lactis* is well tolerated, though it was also recognized that the level of 2'-FL tested in this study is in the lower ranges of what has been reported in human milk.

6.5.2.3 Use of a 100% Whey Protein, Partially Hydrolyzed Infant Formula with Lactobacillus reuteri in Infants with Caregiver-Perceived Intolerance (Czerkies et al., 2019)

A non-randomized, single arm, multicenter study was conducted in healthy, exclusively formula-fed, full-term infants (14 to 16 days of age) with caregiver-perceived fussiness (Czerkies *et al*., 2019; clinical trial registry number NCT03679234). Infants whose caregivers identified them as "very" or "extremely" fussy were eligible for enrollment. All subjects were switched to a commercially available 100% whey protein, partially hydrolyzed infant formula (2.2 g protein/100 kcal) with reduced lactose (30% of carbohydrate source), 2-fucosyllactose (0.25 g/L reconstituted formula), and *L. reuteri*[6] (1×10^6 CFU/g powder) for 3 weeks. The primary outcome of this study was formula tolerance based on the IGSQ. The IGSQ was performed at baseline and then repeated after 3 weeks of study formula use. A total of 50 subjects (54% male; mean age of 28.9 ± 14.5 days at enrollment) were enrolled and 41 completed the study per protocol.

In the intention-to-treat population, the baseline IGSQ score was 34.9 ± 10.0 and decreased to 22.1 ± 7.5 ($p < 0.001$) after 3 weeks of study formula use. In the per protocol population, the IGSQ score was 34.1 ± 10.0 and decreased to 21.4 ± 7.0 ($p < 0.001$) after 3 weeks of study formula use. All caregivers stated their infants liked the study formula. Twelve subjects reported 17 adverse events throughout the study with one adverse effect (hard stools) considered study formula related. The authors reported no serious adverse events.

6.5.2.4 Real-World Study in Infants Fed 2'-FL and LNnT (Román Riechmann et al., 2020)

A non-randomized, open-label, prospective study was conducted in healthy, term infants (Román Riechmann *et al.*, 2020; clinical trial registry number NCT04055363). In this real-world study, infants were enrolled at age 7 days to 2 months and fell into one of three groups: an exclusively formula-fed group, a mixture of formula and human milk fed, or exclusively breastfed infants (serving as a reference population). Formula-fed infants received a partially hydrolyzed, 100% whey, term infant formula (67 kcal/100 mL, 1.9 g protein/199 kcal, 11.5 g carbohydrates/100 kcal, 5.1 g lipids/100 kcal, 1.0 g 2'-FL/L, and 0.5 g LNnT/L) that contained *L. reuteri* (dose not reported), vitamins, and minerals, *ad libitum* for 8 weeks.

[6] Published as *Lactobaccllus reuteri* but current day is referred to as *Limosilactobacillus reuteri.*

Anthropometry measures (weight, length, head circumference) were measured at baseline and at Week 8. Z-scores for weight-for-age, length-for-age, head circumference-for-age, and body mass index-for-age were calculated. Gastrointestinal symptoms were evaluated *via* the IGSQ. Adverse events were recorded from the time of enrolment through the end of study.

A total of 66 exclusively formula fed, 48 mixed fed, and 45 exclusively breastfed infants were included in the analyses. When comparing baseline characteristics of the enrolled infants, the exclusively formula fed group was slightly younger at enrolment ($p < 0.01$) and had a higher proportion of male infants ($p > 0.05$) compared to the mixed-fed and breastfed group. Consistent with the slightly younger age group, baseline weight and length were slightly lower in the exclusively formula-fed group. Other baseline anthropometric characteristics were comparable across groups.

Through the study, age-appropriate growth was reported in all groups. Differences in baseline weight and length did not persist by Week 8; there were no significant differences between any groups for any of the anthropometric measures. The composite IGSQ scores showed low gastrointestinal distress in all groups at all time points. No significant differences were reported in four of the subdomains of gassiness, fussiness, crying, and spitting-up/vomiting. In the last subdomain of stooling, the formula-fed and mixed feeding group exhibited a statistically significant different score at baseline compared to exclusively breastfed infants. This was significantly improved at Week 8 in exclusively formula-fed infants, with scores moving closer to the stooling profile of the exclusively breastfed group. Stooling scores in mixed fed infants remained significantly different at Week 8.

Three patients experienced potentially product-related adverse events, including two instances of cow's milk intolerance (one in exclusively formula fed group, one in the mixed-feeding group and one instance of irritability in the exclusively formula fed group. No serious adverse events were attributed to the study feeding. The authors noted that the incidence of adverse events was low overall and was not significantly different between the groups.

6.5.2.5 2'-FL in Combination with Short-Chain Galacto-Oligosaccharides, Long-Chain Fructo-Oligosaccharides, 3'-Galactosyllactose, and Milk Fat in Healthy Term Infants (Vandenplas et al., 2020)

A double-blind, randomized, controlled, parallel group growth equivalence study of a partly fermented infant formula product containing 2'-FL, 3'-galactosyllactose (3'-GL), short-chain galacto-oligosaccharides (scGOS), long-chain fructo-oligosaccharides (lcFOS), and milk fat was conducted in healthy term infants from multiple locations in Belgium, Hungary, Poland, Spain, and Ukraine (Vandenplas *et al.*, 2020; clinical trial registry number NCT03476889). Groups of infants (≤ 2 weeks of age at study initiation) were exclusively fed either a commercially available complete cow's milk based infant formula (n = 86 at test completion) or the same infant formula supplemented with 1 g/L 2'-FL, 0.15 g/L 3'-GL, and anhydrous milk fat (n = 90 at test completion), until 17 weeks of age. Both test formulas also contained 8 g/L scGOS/lcFOS (9:1 ratio). A control group of entirely breastfed infants (n = 56 at test completion) also was included for reference.

The primary outcome measurement was weight gain, which was recorded at baseline (≤ 2 weeks of age), 4, 8, 12, and 17 weeks of age. Other growth parameters, including length and head circumference, were also measured at the same time intervals. Parameters of gastrointestinal tolerance (*i.e.*, regurgitation, vomiting, stool characteristics) were reported by the parents throughout the test period, and any additional safety outcomes (*i.e.*, adverse events) were reported by the investigators. Dietary intake of the test product also was recorded throughout the test period.

Formula consumption was consistent across the formula test groups, both with respect to intake/day and intake/kg body weight/day. The mean daily weight gain of infants receiving either formula was consistent throughout the test period; however, weight gain was significantly greater in these test groups compared to the breastfed infants. Regarding gastrointestinal tolerance, occurrence of regurgitation was reported to be slightly greater in breastfed infants; 23.4% of infants receiving the 2'-FL formula, 25.8% in the commercially available formula, and 45.8% of infants in the breastfed group at 8 weeks of age experienced frequent regurgitation, the time point where the highest incidence of regurgitation was reported. No significant difference was reported in symptoms of vomiting in any groups. Stool frequency was significantly reduced in both formula groups compared with breastfed infants.

At least one adverse event was reported in 39.3% of infants receiving the 2'-FL test formula, 31.7% in infants receiving the control formula, and 24.6% in breastfed infants. No statistical significance was reported in regurgitation, vomiting, frequent watery stools, or infrequent hard stools between either group receiving infant formula. 11 total serious adverse events were noted in formula-fed infants; 7 in infants who received the 2'-FL test formula and 4 in infants who received the control formula; however, this difference was not statistically significant and was determined to be unrelated to the study product by the investigators. No serious adverse events were reported in breastfed infants.

The study authors concluded that the infant formula containing 2'-FL, 3'-GL, scGOS/lcFOS, and milk fat was safe and well tolerated in healthy term infants, and supportive of adequate infant growth.

6.5.2.6 Randomized, Double-Blind, Multicenter, Controlled, Parallel-Designed 4-Month Growth, Safety, and Tolerability Study of Formula for Infants Containing 5HMO-Mix (Parschat et al., 2021)

The growth, safety, and tolerability of an infant formula supplemented with a mixture of five HMOs (5HMO-Mix) during the first 4 months of life has been evaluated in a randomized, double-blind, multicenter[7], controlled, non-inferiority trial (Parschat *et al*., 2021; Clinical Trial Registry NCT03513744). The 4-month intervention period was followed by a 2-month voluntary follow-up period during which parents could choose to continue intervention up to 6 months. Healthy term infants 14 days of age or younger were eligible to participate in the study. Infants whose mother independently and voluntarily chose not to breastfeed were randomized to receive infant formula with or without the addition of the 5HMO-Mix. In parallel, a group of exclusively breastfed infants were enrolled as a reference group.

The basic infant formula providing the macro- and micro-nutrients required for infant nutrition was manufactured in compliance with regulations of the European Union for infant formulae. The Test formula was identical to the basic infant formula apart from the partial replacement of maltodextrin with the 5HMO-Mix. Specifically, the 5-HMO-Mix was added at a concentration providing 5.75 g/L of the reconstituted infant formula. The concentration of individual HMOs from the 5HMO-Mix manufactured by Chr. Hansen HMO GmBH (Rheinbreitbach, Germany) added to the Test formula is presented in Table 6.5.2.6-1 below. The reconstituted Control and Test formulas contained similar energy within natural and tolerable ranges (68 and 67 kcal/100 mL, respectively), and identical amounts of protein (1.4 g/100 mL), fat (3.6 g/100 mL), carbohydrates (7.2 g/100 mL), lactose (5.2 g/100 mL), vitamins, and other nutrients.

[7] Subjects were recruited from 12 sites across Germany (2 sites), Italy (5 sites), and Spain (5 sites) from December 2018 to November 2020.

Table 6.5.2.6-1 Concentrations of Individual HMOs from the 5HMO-Mix in the Powdered and Reconstituted Test Infant Formula (Parschat *et al.*, 2021)

HMO	Proportion of 5HMO-Mix (%)	Powdered Test Infant Formula (g/100 g)	Reconstituted Test Infant Formula (g/L)
5HMO-Mix	**100**	**4.35**	**5.75**
2'-FL	52	2.26	2.99
3-FL	13	0.57	0.75
LNT	26	1.13	1.5
3'-SL	4	0.17	0.23
6'-SL	5	0.22	0.28

2'-FL = 2'-fucosyllactose; 3-FL = 3-fucosyllactoes; 3'-SL = 3'-sialyllactose; 5HMO-Mix = mixture of 5 HMOs; 6'-SL = 6'-sialyllactose; HMO = human milk oligosaccharide; LNT = lacto-*N*-tetraose.

All infants were fed *ad libitum* according to their assigned feeding group. The total daily intake was recorded by parents in 3-day diaries. In the formula groups, compliance was determined based on the weight of delivered *versus* returned packages of the study product and was defined as the consumption of at least 80% of the anticipated quantity as calculated from the average intake by infants 0 to 4 months of age.

The primary objective of the trial was to demonstrate that infant formula supplemented with the 5HMO-Mix supports normal noninferior growth during the first 4 months of age by comparing the mean daily body weight gain after 4 months intervention between the formula-fed groups. Secondary outcomes included other anthropometric measures (absolute data, changes, increments, and World Health Organization (WHO) growth standard z-scores for weight, length, and head circumference), tolerability (stool frequency and consistency assessed using the Amsterdam Stool Chart), digestive tolerance (regurgitation, vomiting, and flatulence), and behavior (fussiness, crying, and awakening at night). Tolerability, digestive tolerance, and behavioral endpoints were evaluated based on parent ratings for pre-determined scales in 3-day diaries, recorded either after (Day 0) or before (Days 14, 28, 56, 84, or 112) each visit. The primary endpoint was evaluated in the full-analysis dataset (FAS)[8] and the per-protocol dataset (PPS)[9]. Growth parameters were evaluated in the FAS, while all other secondary outcomes were evaluated in the safety dataset (SS)[10].

Overall, 341 infants were enrolled in the study, 225 of which were formula-fed and randomized to the formula groups (113 to the 5HMO-Mix Test Group and 112 to the Control Group); the remaining 116 breastfed infants were allocated to the Reference Group. The study was completed by 265 infants (77.7%), while 76 infants discontinued the study (Control Group: n = 21; Test Group: n = 27; Reference Group: n = 28).

The mean daily intake of infant formula on a volume (mL/day) and energy (kcal/day) basis were similar between the Test and Control Groups. The average daily intake of the 5HMO-Mix steadily increased throughout intervention, ranging from 2.6 ± 0.8 g/day at enrollment to 5.2 ± 1.0 g/day at 4 months[11].

[8] All subjects enrolled in the study who received at least one feeding, had any tolerability data available up to 4 months, and had at least 1 body weight value at baseline and after baseline.
[9] All subjects from the FAS without any major deviations.
[10] All subjects enrolled in the study who received at least one feeding and had any tolerability data available up to 4 months.
[11] Calculated from mean infant formula consumption volumes ranging from 459.7 ± 137.7 mL/day at enrolment to 902.5 ± 170.0 mL/day at 4 months.

The mean daily body weight gain after 4 months of intervention was within the non-inferiority margin of -3 g/day in the Test Group compared to the Control Group for both the FAS and PPS (non-inferiority $p < 0.001$). Furthermore, there were no significant differences in any anthropometric measures evaluated between the formula-fed groups throughout intervention.

Stool frequency was similar between the Test Group and breastfed Reference Group from 2 to 4 months; at 4 months, infants from the Control Group passed fewer stools on a daily basis compared to infants from the Test Group ($p = 0.0428$) and breastfed infants ($p = 0.0136$). A significantly higher frequency of soft stools was observed in the Test Group compared to the Control Group during the first 2 months of intervention ($p < 0.05$), while breastfed infants generally had a higher frequency of soft stools compared to both formula-fed groups at most timepoints. There was no difference in flatulence, vomiting, or fussiness without crying between the formula-fed groups. Regurgitation was higher in the Test Group compared to Control from 1 to 4 months ($p < 0.05$) but comparable to breastfed infants. Crying was less frequent in the Test Group compared to breastfed infants at most timepoints ($p < 0.05$), though no significant difference between the formula-fed groups was observed. Throughout intervention, infants from the formula-fed groups woke less frequently at night compared to breastfed infants ($p < 0.05$).

The number and intensity of reported adverse events were similar between all 3 groups, and there was no significant difference in adverse events categorized according to the Medical Dictionary for Regulatory Activities (MedDRA) by primary system, organ, and class (SOC) between the formula-fed groups. Among specific adverse events, a higher incidence of genital fungal infection was reported in the Test Group ($n = 5$) compared to Control ($n = 0$; $p = 0.0290$), and hematochezia and plagiocephaly were more frequent in the Test Group compared to the breastfed Reference Group. For hematochezia, the study authors noted that the overall frequency was low (Test Group: $n = 5$; Control Group: $n = 2$; Reference Group: $n = 2$) and could be caused by factors unrelated to the intervention. The majority of serious adverse events were reported in the Control Group (Control Group: $n = 9$; Test Group: $n = 3$; Reference Group: $n = 4$). In each of the formula-fed groups, 2 of the reported serious adverse effects were determined to be related to the investigational product. In the Test Group, one subject was hospitalized due to choking and gastroesophageal reflux who later recovered and continued the study, and another subject experienced severe diarrhea who was treated with hydrolysed milk and removed from the study. Both serious adverse effects reported in the Control Group resulted in the diagnosis of bovine milk protein allergy.

Overall, the study authors concluded that infant formula supplemented with a mixture of 5 HMOs (2'-FL, 3-FL, LNT, 3'-SL, and 6'-SL) at concentrations similar to those naturally occurring in human milk supported normal infant growth and was safe and well-tolerated.

6.5.2.7 Randomized, Double-Blind, Controlled Study in Infants Provided Formula with 2'-FL and Lactobacillus reuteri (Corsello et al., 2020 [abstract])

A randomized, double-blind, controlled trial was conducted in healthy infants receiving cow's milk-based infant formula (Corsello *et al.*, 2020 [abstract]; clinical trial registry number NCT03090360). In the study, 289 infants aged less than 14 days old residing in Italy and Belgium were provided a standard bovine milk-based whey-predominant term infant formula containing *L. reuteri*[12] at 1 x 10^7 CFU/g (control) or the same formula supplemented with 1.0 g/L of 2'-FL (test formula) until Day 180. Subjects were allowed progressive introduction of complementary foods or liquids after Study Day 120 (approximately 4 months of age). A non-randomized group comprising of infants exclusively breastfed to 4 months served as a reference (n = 60). The primary outcome was weight gain (in g/day) through 4 months of age. Secondary safety endpoints included additional anthropometric measures, stool pattern, gastrointestinal tolerance (including spitting-up, flatulence), associated behaviors (including crying, fussiness, sleep) *via* a 3-day parent diary, and adverse events. Secondary efficacy endpoints included an analysis of the fecal bacterial species at 1, 2, and 3 months of age, and enterotoxin targets *via* quantitative polymerase chain reaction (qPCR).

Among the 352 infants screened, 289 were randomized to receive either the control or test formula. Fifty-six infants receiving control formula, 44 infants receiving test formula, and 25 breastfed infants did not complete the study. The main reason for non-completion was withdrawal without explanation (n = 62), followed by withdrawal by adverse event (n = 29), withdrawal with explanation (n = 24), lost to follow-up (n = 4), and other (n = 6). The mean duration of formula intake and the volume of formula consumed per day were comparable between the two formula-fed groups.

The results of the safety measures indicated that weight gain was similar between formula-fed groups and was above the non-inferiority margin (defined as -3 g/day). Anthropometric z-scores for weight-for-age, length-for-age, weight-for-length, and head circumference-for-age were comparable between all groups including the reference group and/or statistical differences were not clinically relevant. Growth measures including weight-for-age, length-for-age, head circumference-for-age, and weight-for-length for all infants were plotted individually against WHO reference standards. Both formulas were well tolerated and no differences in stool characteristics were reported. Parent-reported gastrointestinal symptoms and behavioral patterns, and physician-confirmed gastrointestinal adverse events were low and similar between formula groups.

The study authors concluded that *L. reuteri*-containing infant formula supplemented with 2'-FL at 1.0 g/L supported age-appropriate growth and was well tolerated.

6.5.2.8 Summary of Interventional Clinical Infant Studies Identified

Glycom performed a search of the scientific literature for new interventional infant studies relevant to the safety of 2'-FL. Overall, the new clinical studies examining the effect of the administration of 2'-FL to infants have not identified any safety concerns (see Table 6.5.2.8-1).

[12] Published as *Lactobaccllus reuteri* but current day is referred to as *Limosilactobacillus reuteri.*

Table 6.5.2.8-1 Summary of the Interventional Clinical Infant Studies Conducted on 2'-FL

Study Population	Duration of Intervention	Study Groups and Test Articles	References
86 preterm infants (27 to 33 weeks gestation, birth weight < 1,700 g) 43 per group Average 6 days of age at intervention initiation	Enrolment to discharge from neonatal unit	***Control Supplement:*** Glucose (0.140 g/kg bw/day) ***Test Supplement:*** 2'-FL and LNnT in 10:1 ratio (0.374 g/kg bw/day)	Hascoët *et al.* (2021) ***Clinical trial number*** NCT03607942
79 healthy full-term singleton **infants** 39–40 per group 14 ± 5 days of age at enrolment	42 days	***Control Formula:*** 100% whey partially hydrolyzed formula containing *Bifidobacterium lactis* ***Test Formula:*** Same As control, but with 0.25 g/L **2'-FL**	Storm *et al.* (2019) ***Clinical trial number*** NCT03307122
50 healthy full-term **infants** 14–60 days of age at enrolment	3 weeks	***Test Formula***: 100% whey partially hydrolyzed formula containing *Lactobacillus reuteri* and 0.25 g/L **2'-FL** (single-arm study)	Czerkies *et al.* (2019) ***Clinical trial number*** NCT03679234
159 healthy full-term **infants** 45–66 per group 7 days–2 months old at enrolment	8 weeks	***Exclusively Formula Fed Group:*** *Ad libitum* formula containing 1.0 g 2'-FL/L and 0.5 g **LNnT**/L ***Mixed Formula Fed and Breastfed Group***: *Ad libitum* formula containing 1.0 g 2'-FL/L and 0.5 g **LNnT**/L ***Exclusively Breastfed Group (Reference Group):*** Breastfed enrolled at the same time as formula fed infants	Román Riechmann *et al.* (2020) ***Clinical trial number*** NCT04055363
176 healthy formula-fed infants 56 healthy breastfed infants (reference group) 14 days of age or less at enrolment Double-blind, randomized, controlled study	15 weeks	***Control Formula:*** Cow's milk-based infant formula containing 8 g/L scGOS/lcFOS (9:1 ratio) ***Test Formula:*** Same as control, plus 1.0 g/L 2'-FL, 0.15 g/L 3'-GL, and anhydrous milk fat	Vandenplas *et al*. (2020) ***Clinical trial number*** NCT03476889

Table 6.5.2.8-1 Summary of the Interventional Clinical Infant Studies Conducted on 2'-FL

Study Population	Duration of Intervention	Study Groups and Test Articles	References
225 healthy term infants and 116 healthy breastfed infants (Reference Group) 112–113 per formula group ≤ 14 days of age at enrolment	4 months	***Control Formula:*** Basic infant formula ***Test Formula:*** Same as control, plus a target HMO content of 5.75 g/L (2.99 g/L of 2'-FL, 1.5 g/L of LNT, 0.75 g/L of 3-FL, 0.28 g/L of 6'-SL, and 0.23 g/L of 3'-SL) ***Reference Group:*** Breastfed infants	Parschat *et al*. (2021) ***Clinical trial number*** NCT03513744
289 healthy formula-fed infants 60 healthy breastfed infants (reference group) Less than 14 days of age at enrolment Double-blind, randomized, controlled study	6 months	***Control Formula:*** Cow's milk-based infant formula containing *L. reuteri* (1 x 10^7 CFU/g) ***Test Formula:*** Same as control, plus 1.0 g/L 2'-FL	Corsello *et al.* (2020) [abstract] ***Clinical trial number*** NCT03090360

2'-FL = 2'-fucosyllactose; 3-FL = 3-fucosyllactose; 3'-GL = 3'-galactosyllactose; 3'-SL = 3'-sialyllactose; 6'-SL = 6'-sialyllactose; bw = body weight; CFU = colony forming units; HMO = human milk oligosaccharide; lcFOS = long-chain fructo-oligosaccharides; LNnT = lacto-*N*-neotetraose; LNT = lacto-*N*-tetraose; scGOS = short-chain galacto-oligosaccharides.

6.6 Animal Studies

The updated literature search identified two new animal toxicology studies conducted using test articles containing 2'-FL (Parschat *et al*., 2020; Phipps *et al*., 2021). Parschat conducted a bacterial reverse mutation assay, an *in vitro* micronucleus assay, and a 90-day repeated dose oral toxicity study in rats using a mixture HMOs (2'-FL, 3-FL, LNT, 3'-SL, and 6'-SL). The authors reported that the HMO mixture was not mutagenic or genotoxic. A NOAEL value for the repeat-dose toxicity study was 10% of the HMO mixture in the diet, corresponding to 5.67 g HMO mixture/kg body weight for males and 6.97 mg/kg body weight per day for females. Phipps and colleagues reported findings from safety studies of a mixture of LNFP-I and 2'-FL. The assessment included a bacterial reverse mutation assay, an in vitro mammalian cell micronucleus test, and a 90-day repeat-dose oral toxicity study in neonatal Sprague-Dawley rats. The HMO mixture was demonstrated to be non-mutagenic and non-genotoxic. In the oral toxicity study, separate groups of control and high-dose rats were included in a 4-week recovery period as well as a reference control group administered oligofructose. The study was conducted in accordance with OECD 408 guidelines modified to include neonatal pups. No evidence of test article related toxicity was reported and a NOAEL of 5,000 mg/kg body weight was derived. The studies by Parschat *et al*. (2020) and Phipps *et al*. (2021) provide further corroborating evidence that 2'-FL is of low oral toxicity and there have been no findings in toxicity studies of 2'-FL published to date to suggest that 2'-FL would be unsafe for use in infant formula or exempt infant formula.

Several additional studies evaluating various biological endpoints were identified in the literature. Azagra-Boronat *et al*. (2019) evaluated the immunomodulatory and prebiotic effects of 2'-FL in suckling rats and reported that supplementation of 2'-FL in early life has a pre-biotic and intestinal trophic effects and promotes maturation of the immune system. The same group also reported that 2'-FL modulated rotavirus-associated dysbiosis and toll-like receptor (TLR) gene-expression in neonatal rats (Azagra-Boronat *et al*., 2019). Wu and colleagues reported that 2'-FL reduces neurodegeneration in a rat brain stroke model and stated that administration of 2'-FL after stroke damage facilitated repair in the rats (Wu *et al*., 2020). Noye Tuplin *et al.* (2021) reported that 2'-FL + 3'-SL supplementation of weanling Sprague-Dawley rats produced various changes in the mesolimbic dopamine system related to obesity. Sex-specific effects were observed; however, the implications of the authors findings were not clear and required further research. Chleilat *et al.* (2020) also reported sex specific effects of 2'-FL and 3'-SL in 3-week-old Sprague-Dawley rats and stated that HMOs found in breast milk have complex sex-dependent risk/benefit profiles and require further long-term investigation of gut microbial profiles and supplementation with other HMOs during early development. Two studies in piglets were identified. Fleming *et al.* (2020a) reported that dietary oligofructose alone or in combination with 2'-FL differentially improves recognition memory and hippocampal mRNA expression and the same group also reported that 2'-FL and LNnT improved recognition memory concurrent with alterations in regional brain volumes and hippocampal mRNA expression (Fleming *et al.*, 2020b). None of these investigative findings suggest that 2'-FL is unsafe or unsuitable for use in exempt infant formula.

6.7 Other Considerations – Additive Dietary Intakes of 2'-FL with Other HiMOs

While Glycom is not a manufacturer of infant formula, the company anticipates that their portfolio of HiMOs will be used in combination to produce infant formula products that are as compositionally representative of human breast milk as possible, taking into account their natural variation. Glycom recognizes that there are known gastrointestinal tolerance issues that can develop if consumed levels of indigestible carbohydrates, such as HiMOs, are too high in sensitive populations including infants. As discussed in detail previously, in Glycom's view, GRAS uses of individual HiMOs in infant formula should be representative of levels that have been reported for human milk samples obtained from lactating women across all lactational stages considering natural variation. Consequently, the maximum level of HiMOs used in combination (*i.e.*, an additive manner) in infant formula should not exceed mean quantities of total HMOs that have been measured in pooled samples of human breast milk (Kunz *et al*., 1999, 2000).

Glycom also recognized the possibility that the company's HiMOs may be used in combination with other non-digestible carbohydrate sources such as GOS and fructo-oligosaccharides (FOS), which have GRAS status for use in infant formula. Although Glycom is not a manufacturer of infant formula, and is therefore not in a position to comment on the levels of resistant oligosaccharides such as GOS or FOS that could be used with a HiMO, or even the likelihood that such combinations would be introduced to the market, Glycom notes that any new infant formula containing a new HiMO or new HiMO combination will be subject to the laws and implementing regulations governing infant formula under Section 412 of the *Federal Food, Drug, and Cosmetic Act* [21 USC §350(a)]. Specifically, under Section 412(d)(1) of the *Federal Food, Drug, and Cosmetic Act*, a manufacture of a new infant formula must notify the U.S. FDA at least 90 days before marketing their infant formula, and this must include, among other things, a description of any reformulation of the formula or change in processing of the infant formula. Accordingly, the manufacturer will need to provide the Agency with information supporting that a particular oligosaccharide combination (*e.g*., use of 2'-FL with an indigestible oligosaccharide such as GOS in exempt infant formula) would be well tolerated as part of the Agency's 90-day notification procedure. Under 21 CFR §107.50, a manufacturer of a

new exempt infant formula must notify the U.S. FDA at least 90 days before the first processing of the infant formula for commercial or charitable distribution, and include the infant formula label, a complete quantitative formulation, and a detailed description of targeted medical conditions (U.S. FDA, 2020b).

Similar to 2'-FL where a petition for exemption of 2'-FL from the allergy labeling requirements of FALCPA is necessary for use in hypoallergenic exempt infant formula, a petition exempting LNnT has been submitted to the Agency. A notification for the use of LNnT also will follow to allow such uses.

6.8 General Recognition

Glycom has concluded that crystallized 2'-FL is GRAS for use in non-exempt term infant formula, as described in Section 1.3, on the basis of scientific procedures. This GRAS conclusion is based on general principles of risk-assessment of food allergens proposed by the Threshold Working Group of the U.S. FDA in 2008 (Buchanan *et al*., 2008). Clinical thresholds for milk allergenicity have now been validated by qualified scientific experts as a part of the VITAL program of the ABA using publicly available data and approaches that are generally accepted in the scientific community (Allen *et al*., 2014; Taylor *et al*., 2014; Remington *et al*., 2020). Using this risk-based approach Glycom has concluded that accepted milk allergy thresholds relative to the validated detection limits for milk protein in 2'-FL manufactured by Glycom are sufficient to protect the U.S. population of infants with CMPA. The safety of adding 2'-FL to infant formula is being evaluated by the FDA under the Agency's petition procedure for exemption of 2'-FL from the allergen labeling requirements of FALCPA further supporting the general recognition standard (U.S. FDA, 2020a). The risk-based approach was further supported using published findings from a clinical safety study of 2'-FL in infants with CMPA conducted in accordance with AAP guidelines for the evaluation of hypoallergenic infant formula (AAP, 2000).

6.9 Conclusion

Based on the above data and information presented herein, Glycom has concluded that the intended uses of crystallized 2'-FL in exempt hypoallergenic infant formula, as described in Section 1.3, is GRAS based on scientific procedures. General recognition of Glycom's GRAS conclusion is supported by previously established and now widely accepted risk-based procedures for assessment of milk allergy thresholds ensuring the protection of 99% of the population of infants with CMPA.

2'-FL therefore may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the *Code of Federal Regulations*.

Part 7. List of Supporting Data and Information

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Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	Section §	Last Amended	Section Title
101—Food labeling	101.12	4-1-20	Reference amounts customarily consumed per eating occasion
107—Infant formula	107	4-1-20	[Full part]
	107.3	4-1-20	Definitions
	107.50	4-1-20	Terms and conditions
170—Food additives	170.3	4-1-19	Definitions
	170.30	4-1-19	Eligibility for classification as generally recognized as safe (GRAS)
	Subpart E (170.203 through 170.285)	4-1-19	Generally Recognized as Safe (GRAS) Notice

U.S. FDA (2020c). *Agency Response Letter GRAS Notice No. GRN 880 [3'-Sialyllactose sodium salt: Hørsholm, Denmark: Glycom A/S]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=880 [Feb. 21, 2020; Correction: Apr. 13, 2020 - FDA response - no questions].

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Glycom A/S, Kogle Allé 4, 2970 Hørsholm, Denmark

07 July 2022

Ellen Anderson
Regulatory Review Scientist
Division of Food Ingredients
Center for Food Safety & Applied Nutrition
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5001 Campus Drive
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Re: GRAS Notice No. GRN 001034

Dear Ms. Anderson,

Please see the below responses to the United States (U.S.) Food and Drug Administration (FDA)'s letter dated 02 June 2022 pertaining to information provided within Glycom A/S (Glycom)'s Generally Recognized as Safe (GRAS) Notice for the intended use 2'-fucosyllactose (2'-FL) in exempt hypoallergenic infant formula for term infants filed by the Agency under GRN 001034.

FDA.1. *On page 3, the notice states that Glycom intends "to expand the current GRAS uses of 2'-FL as described in GRN 650 to also include use in hypoallergenic exempt infant formula for infants with cow's milk protein allergy (CMPA) or multiple food allergies," and that "these formulas may also be appropriate for infants with non-allergenic gut impairment and malabsorptive conditions." Moreover, on page 5, Glycom states that, in addition to infants with CMPA, the target populations include infants with other medical conditions such as multiple food allergies, fat malabsorption, food protein-induced enterocolitis syndrome (FPIES), multiple food protein allergy of infancy [non-immunoglobulin E (IgE)-mediated], short bowel syndrome, or eosinophilic gastrointestinal disorders.*

We find that the narrative in GRN 001034 does not provide sufficient publicly available safety data and information that could support the intended use of 2'-FL in exempt hypoallergenic infant formula for term infants other than those with CMPA. Thus, we suggest that Glycom narrows the targeted infant population specified in GRN 001034 to only term infants with CMPA to ensure the completion of this review in a timely manner. If you have any questions about this recommendation, please request a teleconference to discuss this further.

Glycom agrees to narrow the targeted infant population specified in GRN 1034 to only term infants with cow's milk protein allergy (CMPA).

FDA.2. *In Part 2.2.2 on page 7, Glycom states that the manufacture of 2'-FL includes upstream (fermentation) and downstream (purification) stages as described in GRN 000650.*

a) For the record, please identify where the description of the upstream and downstream processes can be found in GRN 000650.

b) *Please confirm that raw materials and processing aids used in the manufacture of 2'-FL are the same as those specified in GRN 000650 (Table II.B.2-1)*

Part A)

The description of the upstream and downstream processes is described in Section II.B.3 of GRN 650. Specifically, the upstream process is described in Section II.B.3.1 (page 13), while the downstream processing is described in Section in Section II.B.3.1 (pages 13-14).

Part B)

Glycom confirms that raw materials and processing aids used in the manufacture of 2'-FL are the same as those specified in Table II.B.2-1 of GRN 650 (pages 11-12).

Although the production process for 2'-FL as described in GRN 650 does not result in the transfer of allergenic milk protein to 2'-FL, extended quality control criteria for 2'-FL intended to be added to exempt infant formula for term infants with CMPA are described in Section 2.2.3 of the GRAS notice.

FDA.3. *Based on the information provided in Table 2.2.2-1 on page 8, 2'-FL is crystallized from water with acetic acid. However, the first paragraph on the same page states that the production of 2'-FL includes a crystallization step with methanol. Please clarify what solvent(s) is/are used for crystallization of 2'-FL.*

We thank the FDA for catching this error. The only solvent used in the crystallization of 2'-FL is acetic acid. Methanol is not used in the production of 2'-FL; rather, it was a "copy-and-paste" error from LNnT.

FDA.4. *In Part 2.2.3 on page 9, Glycom refers to data and information described in Petition No. FDA-2020-FL-1865. We note that a significant portion of the petition is designated as confidential, including parts describing the manufacturing method for 2'-FL, ingredient protein characterization, methods used to test for proteins, and the results of analyses.*

a. *Please explain how there could be a basis for a conclusion of GRAS status of the intended use of 2'-FL if qualified experts do not have access to the safety-related data and information designated as confidential in Petition No. FDA-2020-FL-1865.*

b. *In addition, please provide a brief non-confidential description of the steps incorporated during the purification of 2'-FL to ensure removal of milk protein.*

Part A)

The exemption of 2'-FL from allergen labelling requirements according to the Food Allergen Labeling and Consumer Protection Act (FALCPA) of 2004 would demonstrate that the scientific evidence submitted by Glycom supports that 2'-FL (manufactured by Glycom) does not cause an allergic response that poses a risk to human health. However, the evaluation of Glycom's petition (submitted July 6th, 2020) remains pending (FDA-2020-FL-1865).

In the meantime, a non-confidential summary of the scientific evidence submitted by Glycom in the food allergen labeling exemption petition for 2'-FL is provided below. Some if this information has been previously summarized in Section 2.4.1.1 of the GRAS notice.

Briefly, Glycom has demonstrated the absence of detectable milk protein from the lactose raw material produced from whey in the final 2'-FL ingredient using three different highly sensitive analytical methods for protein detection and quantification:

- **Modified Bradford Assay**
  Confirmed the removal of total protein during various unit operation of the manufacturing process (described in Part b). This method has been validated to quantify total residual protein down to a level of 0.0017%.

- **Enzyme-Linked Immunosorbent Assay (ELISA)**
  There were no detectable levels of β-lactoglobulin and casein (two major whey proteins) nor total milk allergen from nonfat dried milk (casein and whey proteins from cows's milk) in the final 2'-FL ingredient. These assays have been validated for the detection of milk allergens from food matrices at limits of detection ranging from 1.5 ppb to 1.0 ppm.

- **Proteomic Analyses**
  No major milk proteins in the lactose raw material (including β-lactoglobulin and casein) were detectable in 2'-FL samples using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Similarly, no proteins from high-purity bovine serum albumin (BSA) were detectable in 2'-FL samples. The limit of quantitation of this method is 20 ppb.

The results of these analyses demonstrate that Glycom's manufacturing process efficiently removes residual milk protein potentially introduced from the lactose raw material as the final 2'-FL ingredient is devoid of detectable levels of milk antigens.

Part B)

The lactose used during fermentation is the only source of allergenic protein introduced to the manufacturing processes of 2'-FL. The steps incorporated during the purification of 2'-FL to ensure removal of all types of protein (including milk protein) include ultrafiltration, ion-exchange/adsorption resin, activated charcoal filtration, and crystallization. The ultrafiltration step removes large biomolecules such as protein. The ion-exchange/adsorption resin and activated charcoal filtration steps remove compounds with chemo-physical properties different from 2'-FL, including proteins composed of charged amino acids and containing hydrophobic moieties. Finally, the crystallization step further isolates and concentrates 2'-FL.

FDA.5. *In Part 2.3.1 on page 10, Glycom states that some of the analytical methods used to test for the specification parameters were "developed by Glycom A/S (Glycom) using validated in-house methods." Please confirm that all developed in-house methods are validated for the stated purpose.*

All methods developed by Glycom have been internally validated for the evaluation of the conformance of 2'-FL batches with specifications for 2'-FL.

FDA.6. *On page 11, Table 2.4-1 lists the results of analyses from three nonconsecutive batches of 2'-FL.*

a) According to Table 2.4-1, two tested batches were manufactured using Escherichia coli K-12 DH1 MDO strain SCR6 and one batch was manufactured using E. coli K-12 DH1 MDO strain MAP1001d. We note that in Part 2.2.1 (page 7), it states that Glycom is currently using strain MAP1001d to manufacture 2'-FL. Please clarify whether the 2'-FL that is the subject of GRN 001034 is manufactured using only strain MAP1001d or does Glycom intend to use strain SCR6 as an alternative production strain. If the 2'-FL that is intended for use in exempt infant formulas is manufactured using strain MAP1001d only, please provide the results of analysis from a minimum one additional non-consecutive batch manufactured using this strain.

b) Please clarify whether all results reported in Table 2.4-1 as ranges (e.g., <0.05 mg/kg for lead) represent limits of quantification (LOQ) for the corresponding analytes.

c) We note that the specification limit for lead is ≤0.1 mg/kg while the levels of lead in each of the three 2'-FL batches are reported to be <0.05 mg/kg. We request Glycom to consider lowering the specification limit for lead to reflect the results of batch analyses and to be as low as possible.

Part A)

MAP1001d is the improved strain that is currently used in the manufacture of 2'-FL that is the subject of GRN 1034.

Table 2.4-1 of the GRAS notice has been updated below to include the results of analysis of an additional non-consecutive batch of 2'-FL manufactured using the MAP1001d production strain (Batch No. (b) (4) [redacted]. The updated table also specifies results that are below LOQs (see response to Part B) and the lower specification limit for lead (see response to Part C).

Table 2.4-1 Batch Analysis of 2'-FL Produced by Fermentation (UPDATED)

Specification Parameter	Specification Limit	2'-FL from SCR6		2'-FL from MAP1001d	
		(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]
Appearance	Powder, agglomerates, or powder with agglomerates	Powder	Powder	Powder with agglomerates	Powder with agglomerates
Color	White, white to off white, off white	White	White	White	White
Identification	RT of standard ± 3%	Complies	Complies	Complies	Complies
Assay (water free) – HiMS[a] (w/w %)	≥ 96.0	99.2	96.9	98.3	97.7
Assay (water free) – 2'-FL (w/w %)	≥ 94.0	98.9	97.5	97.8	97.4
D-Lactose (w/w %)	≤ 1.0	0.28	0.29	0.20	0.24
L-Fucose (w/w %)	≤ 1.0	< 0.03[a]	< 0.03[a]	< 0.03[a]	< 0.03[a]
Difucosyllactose (w/w %)	≤ 1.0	0.07	0.34	0.29	0.07

Table 2.4-1 Batch Analysis of 2'-FL Produced by Fermentation (UPDATED)

Specification Parameter	Specification Limit	2'-FL from SCR6		2'-FL from MAP1001d	
		(b) (4)	(b) (4)	(b) (4)	(b) (4)
2'-Fucosyl- D-lactulose (w/w %)	≤ 1.0	0.18	0.48	0.29	0.15
pH (20°C, 5% solution)	3.2 to 5.0	3.7	3.7	3.6	3.7
Water (w/w %)	≤ 5.0	0.12	0.11	0.06	0.21
Ash, sulfated (w/w %)	≤ 1.5	0.10	< 0.10[a]	< 0.10[a]	< 0.10[a]
Acetic acid (w/w %)	≤ 1.0	0.23	0.27	0.34	0.3
Residual proteins (w/w %)	≤ 0.002	< 0.0017[a]	< 0.0017[a]	< 0.0017[a]	< 0.0017[a]
β-Lactoglobulin (mg/kg)	≤ 0.05	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]
Casein (mg/kg)	≤ 0.5	< 0.2[a]	< 0.2[a]	< 0.2[a]	< 0.2[a]
Lead (mg/kg)	≤ 0.05	< 0.05[a]	< 0.05[a]	< 0.05[a]	< 0.05[a]
Salmonella (in 25 g)	Absent	Absent	Absent	Absent	Absent
Total plate count (CFU/g)	≤ 500	< 10[a]	< 10[a]	< 10[a]	< 10[a]
Enterobacteriaceae (in 10 g)	Absent	Absent	Absent	Absent	Absent
Cronobacter (Enterobacter) *sakazakii* (in 10 g)	Absent	Absent	Absent	Absent	Absent
Listeria monocytogenes (in 25 g)	Absent	Absent	Absent	Absent	Absent
Bacillus cereus (CFU/g)	≤ 50	< 10[a]	< 10[a]	< 10[a]	< 10[a]
Yeasts (CFU/g)	≤ 10	< 10[a]	< 10[a]	< 10[a]	< 10[a]
Molds (CFU/g)	≤ 10	< 10[a]	< 10[a]	< 10[a]	< 10[a]
Residual endotoxins (E.U./mg)	≤ 10	< 0.00025[a]	< 0.00024[a]	< 0.00025[a]	<0.00025[a]

2'-FL = 2'-fucosyllactose; CFU = colony forming units; E.U. = endotoxin units; HiMS = human-identical milk saccharides; LOQ = limit of quantitation; RT = retention time.

[a] Result is below the LOQ: 0.03 w/w % for L-Fucose; 0.0017 w/w % for residual proteins; 0.01 mg/kg for β-Lactoglobulin; 0.2 mg/kg for casein; 0.05 mg/kg for lead; 10 CFU/g for total plate count, *Bacillus cereus*, yeasts, and molds; and 0.050 E.U./mL for endotoxins (converted to approximately 0.00024 to 0.00025 E.U./mg depending on the sample weight).

Part B)

Glycom confirms that all results reported as ranges in Table 2.4-1 of the GRAS notice represent LOQs for the corresponding analytes at the time of their measurement.

Residual endotoxins are analyzed by a third-party laboratory from which results are reported as endotoxin units (E.U.) per mL of sample. The LOQ for residual endotoxins is 0.050 E.U./mL, which is converted to approximately 0.00024 to 0.00025 E.U./mg depending on the sample weight.

Part C)

Glycom is agreeable to lower the specification limit for lead to ≤ 0.05 mg/kg. The lower specification limit is equivalent to the LOQ for lead at the time of measurement from representative batches of 2'-FL. However, the sensitivity of the inductively coupled plasma mass spectrometry (ICP-MS) method according to EPA 6020A has since been improved, resulting in the reduction of the LOQ for lead from 0.05 to 0.01 mg/kg for future batches.

FDA.7. *In Part 3.3.1 on page 16, Glycom discusses dietary exposure to 2'-FL resulting from the intended use in exempt infant formula proposed in GRN 001034 and existing uses specified in GRN 000650. We note that 2'-FL is the subject of multiple GRAS notices received after our evaluation of GRN 000650 was completed. We also note that the cumulative dietary exposure assessment to 2'-FL should take into consideration food uses proposed in GRN 001034 and all current uses in foods that may be consumed by infants.*

a. Please clarify whether there are any additional food uses or higher use levels of 2'-FL that were not included in either GRN 000650 or GRN 001034 but were included in other GRAS notices.

b. Please discuss whether these food uses, or higher use levels not considered in the dietary exposure assessment in GRN 001034 would meaningfully affect the estimates presented in Tables 3.3.1-1 and 3.3.1-2 and whether this will affect Glycom's safety conclusion.

Part A)

A summary of all individual food uses and use levels of 2'-FL previously notified as GRAS to the U.S. FDA and receiving a "no questions" letter from the Agency is provided in Table 1 below. For transparency, maximum use levels of 2'-FL notified in GRN 650 are included for comparison.

The maximum use level of 2'-FL proposed in the current GRAS notice (GRN 1034) for use in exempt term infant formula for infants with CMPA is the same as the maximum use level of 2'-FL (2.4 g/L of formula as consumed) that has previously been notified as GRAS for use in non-exempt term infant formula (GRNs 546, 650, 735, 749, 852, 932). Infants with CMPA requiring exempt term infant formula (*i.e.*, extensively hydrolyzed protein formula or amino acid-based formula) are anticipated to be mutually exclusive consumers from infants fed non-exempt term infant formula due to milk protein sensitivity. 2'-FL has previously been notified as GRAS for use as an ingredient in exempt hypoallergenic infant formula for term infants (including both extensively hydrolyzed cow milk protein and amino acid-based formula) but at a slightly lower use level (2 g/L of formula as consumed; GRN 929).

Table 1 Summary of Individual Food Uses and Maximum Use levels Notified as GRAS for 2'-FL in the U.S.[a]

Food Category (21 CFR §170.3) (U.S. FDA, 2021a)	Proposed Food Use[b]	Maximum Use Level (g/kg or g/L)[c]	
		Notified in GRN 650 or GRN 1034[d]	Other GRAS Notices with a "No Questions" letter
Beverages and Beverage Bases	**Soft Drinks**	-	**1.5 (GRN 815[e])**
	Non-Milk Meal Replacement and Nutritional Beverages[f]	5.0	5.0
	Sports, Isotonic, and Energy Drinks; **Enhanced or Fortified Waters**	1.2	**1.5 (GRN 815[e])**
Breakfast Cereals	**Hot Cereals**	-	**31.0 (GRN 897)**
	RTE Cereals	-	**80.0 (puffed) 30.0 (high fiber) 20 (biscuit type) (GRN 735)**
	Milk Substitutes	1.2	1.2

Table 1 Summary of Individual Food Uses and Maximum Use levels Notified as GRAS for 2'-FL in the U.S.[a]

Food Category (21 CFR §170.3) (U.S. FDA, 2021a)	Proposed Food Use[b]	Maximum Use Level (g/kg or g/L)[c]	
		Notified in GRN 650 or GRN 1034[d]	Other GRAS Notices with a "No Questions" letter
Dairy Product Analogs	Non-Dairy Yogurts	5.3	**12.0 (GRN 897)**
Frozen Dairy Desserts and Mixes	**Frozen desserts including ice creams and frozen yogurts, frozen novelties**	-	**17.0 (GRN 735)**
Gelatins, Puddings, and Fillings	**Dairy-based puddings, custards, and mousses**	-	**17.0 (GRN 735)**
	Fruit pie filling	-	**14.1 (GRN 735)**
	Fruit filling in bars, cookies, yogurt, and cakes	-	**30.0 (GRN 735)**
Grain Products and Pastas	Meal Replacement Bars, for Weight Reduction	40.0	40.0
	Cereal and Nutrition Bars	-	**30.0 (GRN 897)**
Infant and Toddler Foods	Non-Exempt Term Infant Formulas	2.4	2.4
	Exempt Term Infant Formulas for food allergies (*i.e.*, extensively hydrolyzed formula and amino acid-based formula)	2.4	2.0
	Toddler Formulas[g]	2.4	2.4
	Other Baby Foods for Infants and Young Children	12.0	12.0
	Other Drinks for Young Children	1.2	**10.0 (GRN 735)**
	Baby crackers, pretzels, cookies, and snack items	-	**57.0 (GRN 735)**
Jams and Jellies	**Jellies and jams, fruit preserves, and fruit butters**	-	**60.0 (GRN 735)**
Milk, Whole and Skim	Unflavored Pasteurized and Sterilized Milk (whole milk, reduced-fat milk, low-fat milk, non-fat milk; including powdered milks, reconstituted)	1.2	**1.5 (GRN 815[e])**
Milk Products	Buttermilk	1.2	**1.5 (GRN 815[e])**
	Flavored Milk	1.2	**1.5 (GRN 815[e])**
	Milk-Based Meal Replacement and Nutritional Beverages[f]	5.0	5.0
	Smoothies (Dairy and Non-Dairy)	-	**5.0 (GRN 897)**
	Yogurt	5.3	**15.0 (GRN 815[e])**
	Formula intended for pregnant women (-9 to 0 months)[h]	-	**6.0 (GRN 735)**
Processed Fruits and Fruit Juices	**Fruit Drinks and Ades**	-	**1.5 (GRN 815[e])**
	Fruit Juices and Nectars	1.2	1.2
Processed Vegetables and Vegetable Juices	**Vegetable Juice**	-	**1.2 (GRN 897)**

Table 1 Summary of Individual Food Uses and Maximum Use levels Notified as GRAS for 2'-FL in the U.S.[a]

Food Category (21 CFR §170.3) (U.S. FDA, 2021a)	Proposed Food Use[b]	Maximum Use Level (g/kg or g/L)[c]	
		Notified in GRN 650 or GRN 1034[d]	Other GRAS Notices with a "No Questions" letter
Sweet Sauces, Toppings, and Syrups	**Syrups used to flavor milk beverages**	-	**7.0 (GRN 735)**
Foods for Special Dietary Use	**Oral Nutritional Supplements and Enteral Tube Feeding (> 11 years)[i]**	-	**20.0 (GRN 735)**

2'-FL = 2'-fucosyllactose; '-' = not applicable; CFR = Code of Federal Regulations; GRAS = Generally Recognized as Safe; GRN = GRAS notice; RTE = Ready-to-Eat; U.S. = United States.

[a] Food uses that have been previously notified as GRAS to the U.S. FDA (which were not included in GRN 650 or GRN 1034), and received a "no questions" letter, are **bolded**. This includes GRNs 546, 571, 735, 749, 815, 852, 897, 929, and 932.

[b] 2'-FL is intended for use in unstandardized products where standards of identity, as established under 21 CFR §130 to 169, do not permit its addition in standardized products.

[c] Use level expressed on a 2'-FL basis in the final food, as consumed.

[d] The intended condition of use requested in the current GRAS notice (GRN 1034) is shaded in blue.

[e] Assuming the 2'-FL/DFL mixture contains a minimum of 75 w/w % 2'-FL content (as per the ingredient specification).

[f] Includes ready-to-drink and powder forms.

[g] Formula products targeted toward young children (> 12 months of age).

[h] Food codes for formula intended for pregnant women were not available in the 2017-2018 NHANES. This intended use is excluded from the calculation of estimated daily intakes due to absence of consumption data.

[i] Foods for special dietary use are assessed separately from the intended food uses of 2'-FL in conventional foods, as they are intended for supplying a particular dietary need and/or supplementing the intake of a dietary component. Intake of 2'-FL from foods for special dietary use is, therefore, not expected to be cumulative to other dietary sources.

Part B)

Cumulative dietary exposure assessments of 2'-FL have been recently conducted as part of GRAS notices for 2'-FL intended for use in exempt hypoallergenic infant formula for term infants at a use level of 2 g/L (GRN 929 amendment) and in non-exempt infant formula for term infants at a use level of 2.4 g/L (GRN 932 amendment). Notably, the cumulative dietary exposure assessment presented in the GRN 929 amendment excluded milk and dairy products given the cow's milk protein sensitivity of the target population. Results of these assessments for infant population groups are summarized in Table 2 below.

Table 2 Summary of the Cumulative Estimated Daily Intake of 2'-FL by Infants Calculated in Recent GRAS Notices

GRN (Dataset)	Population Group	Age Group (months)	Consumer-Only Intake			
			Absolute Basis (g/day)		Body Weight Basis (mg/kg bw/day)	
			Mean	90th Percentile	Mean	90th Percentile
GRN 929 Amendment (2015-16 NHANES)	Infants	0 to 5	1.9	2.6	310	403
	Infants	6 to 11	2.0	2.9	230	320
GRN 932 Amendment (2017-18 NHANES)	Infants	0 to 5	2.06	3.17	329	529
	Infants	6 to 11	2.60	4.95	290	532

2'-FL = 2'-fucosyllactose; bw = body weight; n = sample size; GRAS = generally recognized as safe; NHANES = National Health and Nutrition Examination Survey.

As Glycom is proposing a slightly higher use level of 2'-FL in exempt hypoallergenic term infant formula in the current GRAS notice (2.4 g/L in GRN 1034 *versus* 2.0 g/L in GRN 929), and given the sensitivity of the target population (infants with CMPA in GRN 1034 versus the general infant population in GRN 932), Glycom conducted its own cumulative dietary exposure assessment.

Table 3 summarizes the estimated cumulative intake of 2'-FL on an absolute basis (g/person/day) and on a per kilogram body weight basis (mg/kg body weight/day) from all individual food uses and highest use levels of 2'-FL previously notified as GRAS to the U.S. FDA and receiving a "no questions" letter from the Agency (See Table 1 above). A use level of 2.4 g/L of 2'-FL was applied to all infant formula. Furthermore, dairy-based foods other than infant formula (*i.e.,* frozen dairy desserts; dairy-based puddings, custards, and mousses; milk and milk beverages; and yogurt) were excluded from the cumulative dietary intake assessment as these products would not be anticipated to be consumed by infants with CMPA.

The percentage of consumers was high among all age groups evaluated in the current intake assessment; more than 72.1% of the infant population groups consisted of consumers of food products in which 2'-FL may be used (see Table 3). Infants aged 7 to <12 months had the greatest proportion of consumers at 97.4%. The consumer-only estimates are more relevant to risk assessments as they represent exposures in the target population; consequently, only the consumer-only intake results are discussed in detail herein.

Among infants aged 0 to 6 months, the mean and 90th percentile consumer-only intakes of 2'-FL on an absolute basis were determined to be 2.15 and 3.73 g/person/day, respectively. Among infants aged 7 to <12 months, the mean and 90th percentile consumer-only intakes of 2'-FL on an absolute basis were determined to be 3.31 and 6.02 g/person/day, respectively. On a body weight basis, the mean and 90th percentile consumer-only intakes of 2'-FL for infants aged 0 to 6 months were determined to be 326 and 499 mg/kg body weight/day, respectively. Among infants aged 7 to <12 months, the mean and 90th percentile consumer-only intakes were determined to be 365 and 664 mg/kg body weight/day, respectively.

Table 3 Summary of the Cumulative Estimated Daily Intake of 2'-FL from All Current Uses in Foods by Infants in the U.S. (2017-2018 NHANES Data)

Population Group	Age Group (months)	Consumer-Only Intake					
		Percentage of Population (%)	n	Absolute Basis (g/day)		Body Weight Basis (mg/kg bw/day)	
				Mean	90th Percentile	Mean	90th Percentile
Infants	0 to 6	72.1	133	2.15	3.73	326	499
Infants	7 to <12	97.4	121	3.31	6.02	365	664

2'-FL = 2'-fucosyllactose; bw = body weight; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

Cumulative estimated daily intakes of 2'-FL in infant population groups are lower compared to those from GRN 650 presented in Tables 3.3.1-1 and 3.3.1-2 of the GRAS notice. The decreases observed can likely be attributed to both the removal of dairy-based foods and the introduction of additional food uses that are proportionally smaller contributors to the overall intake of 2'-FL in infant population groups. Similar estimated daily intakes of 2'-FL in infant population groups were reported in the other recent cumulative dietary exposure assessments of 2'-FL from the GRN 929 and 932 amendments (see Table 2), which were also notably lower compared to those from GRN 650.

As estimates from the cumulative dietary exposure assessment are lower than the estimates from GRN 650 presented in Tables 3.3.1-1 and 3.3.1-2 of the GRAS notice, there is no change to Glycom's safety conclusion.

FDA.8. *In Part 3.3.2 on page 16, Glycom discusses dietary exposure to 2'-FL resulting from the intended use in exempt infant formula only and provides the 99th percentile estimates for infants (eaters-only) on a per day basis. We agree that the 99th percentile estimates represent consumption for heavy consumers; however, we typically use the 90th percentile estimates in our safety assessment.*

a. Please provide the 90th percentile estimates of dietary exposure for infants (eaters-only) aged 0-6 months and 7 to <12 months on both a per day basis and a body weight basis.

b. In addition, please provide the mean estimates of dietary exposure for the populations listed above on a body weight basis.

Table 4 summarizes the mean and 90th percentile estimated daily intakes of 2'-FL from infant formula only on an absolute basis (g/person/day) and on a per kilogram body weight basis (mg/kg body weight/day) using the 2011-2012 NHANES data.

On an absolute basis, infants aged 0 to 6 months were determined to have the greatest mean and 90th percentile consumer-only intakes of 2'-FL, at 2.1 and 3.2 g/person/day, respectively. On a body weight basis, infants aged 0 to 6 months were also identified as having the highest mean and 90th percentile consumer-only intakes, of 330 and 566 mg/kg body weight/day, respectively.

Table 4 Summary of the Estimated Daily Intake of 2'-FL from Infant Formula Only in U.S. Infant Population Groups (2011-2012 NHANES Data)

Population Group	Age Group (months)	Consumer-Only Intake					
		Percentage of Population (%)	n	Absolute Basis (g/day)		Body Weight Basis (mg/kg bw/day)	
				Mean	90th Percentile	Mean	90th Percentile
Infants	0 to 6	76.0	161	2.1	3.2	330	566
Infants	7 to <12	75.8	94	1.8	2.4	193	264

2'-FL = 2'-fucosyllactose; bw = body weight; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

FDA.9. *Glycom discusses two clinical studies which assessed the safety and tolerability of extensively hydrolyzed formulas (EHF) containing 2'-FL in populations of infants and young children. The Nowak-Wegrzyn et al., 2019 study used a test formula containing 2'-FL at a concentration of 1.0 g/L in combination with another human milk oligosaccharide, lacto-N-neotetraose (LNnT), at 0.5 g/L in a population of infants and young children with confirmed CMPA. The study by Ramirez-Farias et al., 2021 used an extensively hydrolyzed study formula containing 2'-FL at 0.2 g/L for infants with clinical conditions that required consumption of EHF. Thus, neither of these clinical studies examined the safety and tolerability of 2'-FL in infants and toddlers with medical conditions requiring feeding with EHF at the proposed use level of 2.4 g/L, a level much higher than was used in either of the clinical studies. Please provide a thorough discussion and scientific rationale that addresses why 2'-FL at the proposed use level of 2.4 g/L (and especially when used without LNnT) is not a safety concern for infants and toddlers which require feeding with EHF.*

The proposed use level of 2'-FL in the GRAS notice of 2.4 g/L was chosen based on the range of observed concentrations of 2'-FL naturally occuring in human milk that have an established safe history of consumption by breastfed infants. This is the same use level that was notified as GRAS for Glycom's 2'-FL ingredient manufactured using the same production strain intended for use in non-exempt term infant formula (GRN 650). In the current GRAS notice, Glycom has demonstrated that the production process for 2'-FL as described in GRN 650 does not result in the transfer of allergenic milk protein to the final 2'-FL ingredient. Furthermore, Glycom has extended quality control criteria for 2'-FL intended for use in exempt term infant formula for infants with CMPA.

Infants with CMPA have an allergy against cow's milk protein. Numerous published guidelines for the management of CMPA in breastfed infants recommend that breastfeeding should continue while mothers avoid the consumption of cow's milk and milk products from cow's milk in their own diet (Vandenplas *et al*., 2007; Koletzko *et al*., 2012; Caffarelli *et al*., 2010). Thus, breastfed infants with CMPA would continue to be exposed to carbohydrates, such as HMOs (including 2'-FL) produced in the mammary gland, from human milk. On a body weight basis, the intake of 2'-FL under the proposed conditions of use at the mean (193 to 330 mg/kg body weight/day, respectively – see Table 4) and high-level (264 to 566 mg/kg body weight/day, respectively – see Table 4) is within the naturally occurring range of 2'-FL in mature human milk (ranging from 170 to 660 mg/kg body weight/day on average, but may be up to 1,150 mg/kg boy weight/day – see Section IV.B.1 of GRN 650, pgs. 24-25). The intake of 2'-

FL by breastfed infants from human milk is also expected to be safe for other population groups including toddlers.

2'-FL has previously been notified as GRAS for use as an ingredient in exempt hypoallergenic infant formula for term infants (GRN 929) at the same use level notified as GRAS by the same manufacturer for use in non-exempt infant formula (GRN 571), at 2 g/L of infant formula as consumed. This use level is also higher than the concentration of 2'-FL evaluated in the clinical study by Nowak-Wegrzyn *et al*. (2019) conducted in infants with CMPA available at the time of notification, but resulted in a similar dietary exposure of 2'-FL to that of breastfed infants from human milk.

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We hope this information adequately addresses the Agency's questions on GRN 001034, and if there is any additional information or further clarification that is required, Glycom will be happy to provide such information upon request.

Sincerely,

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Maryse Darch
Sr. Regulatory Affairs Specialist
Glycom A/S

ADDITIONAL REFERENCES NOT CITED IN THE NOTICE

Caffarelli C, Baldi F, Bendandi B, Calzone L, Marani M, Pasquinelli P; EWGPAG (2010). Cow's milk protein allergy in children: a practical guide. Ital J Pediatr 36:5. DOI:10.1186/1824-7288-36-5.

Koletzko S, Niggemann B, Arato A, Dias JA, Heuschkel R, Husby S, et al.; European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (2012). Diagnostic approach and management of cow's-milk protein allergy in infants and children: ESPGHAN GI Committee practical guidelines. J Pediatr Gastroenterol Nutr 55(2):221-9. DOI:10.1097/MPG.0b013e31825c9482.

Vandenplas Y, Koletzko S, Isolauri E, Hill D, Oranje AP, Brueton M, et al. (2007). Guidelines for the diagnosis and management of cow's milk protein allergy in infants. Arch Dis Child 92(10):902-8. Erratum in: Arch Dis Child 92(10):following 908. Erratum in: Arch Dis Child 93(1):93. DOI:10.1136/adc.2006.110999.

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26 September 2022

Ellen Anderson
Regulatory Review Scientist
Division of Food Ingredients
Center for Food Safety & Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive
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Re: GRAS Notice No. GRN 001034

Dear Ms. Anderson,

Please see the below responses to the United States (U.S.) Food and Drug Administration (FDA)'s email correspondences dated 26 August 2022 and 02 September 2022 pertaining to information provided within Glycom A/S (Glycom)'s Generally Recognized as Safe (GRAS) Notice for the intended use 2'-fucosyllactose (2'-FL) in exempt hypoallergenic infant formula for term infants filed by the Agency under GRN 001034.

FDA.1. *In response to Question #6a in the amendment dated July 7, 2022 (attached), Glycom provided the results of analysis from one additional nonconsecutive batch of 2'-FL manufactured using E. coli K-12 MDO strain MAP1001d (i.e., DSM 32775) and stated that only this strain is currently used in the manufacture of 2'-FL that is the subject of GRN 001034. We typically request that notifiers provide results from a minimum three nonconsecutive batch analyses to demonstrate that a substance can be manufactured to meet the established specifications. Therefore, we request that Glycom provide analytical results from one additional non-consecutive batch of 2'-FL manufactured using strain MAP1001d to satisfy the minimum number of batch analyses typically requested. We realize that the wording of Question #6a was not clear, in that we originally asked for "the results of analysis from a minimum of one additional non-consecutive batch manufactured using" strain MAP1001d. At that time, we should have requested the results of analysis from at least two additional non-consecutive batches. We apologize for this oversight.*

Table 2.4-1 of the GRAS notice has been updated below to include the results of analysis for three non-consecutive batches of 2'-FL intended for use in exempt hypoallergenic infant formula for term infants manufactured using the MAP1001d production strain. The updated table also specifies results that are below LOQs and the lower specification limit for lead (as per the July amendment).

Table 2.4-1 Batch Analysis of 2'-FL Produced by Fermentation (UPDATED)

Specification Parameter	Specification Limit	2'-FL from SCR6		2'-FL from MAP1001d		
		(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Appearance	Powder, agglomerates, or powder with agglomerates	Powder	Powder	Powder with agglomerates	Powder with agglomerates	Powder
Color	White, white to off white, off white	White	White	White	White	White

Table 2.4-1 Batch Analysis of 2'-FL Produced by Fermentation (UPDATED)

Specification Parameter	Specification Limit	2'-FL from SCR6		2'-FL from MAP1001d		
		(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Identification	RT of standard ± 3%	Complies	Complies	Complies	Complies	Complies
Assay (water free) – HiMS[a] (w/w %)	≥ 96.0	99.2	96.9	98.3	97.7	99.2
Assay (water free) – 2'-FL (w/w %)	≥ 94.0	98.9	97.5	97.8	97.4	98.9
D-Lactose (w/w %)	≤ 1.0	0.28	0.29	0.20	0.24	0.13
L-Fucose (w/w %)	≤ 1.0	< 0.03[a]	< 0.03[a]	< 0.03[a]	< 0.03[a]	< 0.03[a]
Difucosyllactose (w/w %)	≤ 1.0	0.07	0.34	0.29	0.07	0.15
2'-Fucosyl- D-lactulose (w/w %)	≤ 1.0	0.18	0.48	0.29	0.15	0.08
pH (20°C, 5% solution)	3.2 to 5.0	3.7	3.7	3.6	3.7	3.7
Water (w/w %)	≤ 5.0	0.12	0.11	0.06	0.21	0.10
Ash, sulfated (w/w %)	≤ 1.5	0.10	< 0.10[a]	< 0.10[a]	< 0.10[a]	<0.05[a]
Acetic acid (w/w %)	≤ 1.0	0.23	0.27	0.34	0.3	0.2
Residual proteins (w/w %)	≤ 0.002	< 0.0017[a]	< 0.0017[a]	< 0.0017[a]	< 0.0017[a]	< 0.0017[a]
β-Lactoglobulin (mg/kg)	≤ 0.05	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]	< 0.01[a]
Casein (mg/kg)	≤ 0.5	< 0.2[a]	< 0.2[a]	< 0.2[a]	< 0.2[a]	< 0.2[a]
Lead (mg/kg)	≤ 0.05	< 0.05[a]	< 0.05[a]	< 0.05[a]	< 0.05[a]	0.003[b]
Salmonella (in 25 g)	Absent	Absent	Absent	Absent	Absent	Absent
Total plate count (CFU/g)	≤ 500	< 10[a]	< 10[a]	< 10[a]	< 10[a]	< 10[a]
Enterobacteriaceae (in 10 g)	Absent	Absent	Absent	Absent	Absent	Absent
Cronobacter (Enterobacter) *sakazakii* (in 10 g)	Absent	Absent	Absent	Absent	Absent	Absent
Listeria monocytogenes (in 25 g)	Absent	Absent	Absent	Absent	Absent	Absent
Bacillus cereus (CFU/g)	≤ 50	< 10[a]	< 10[a]	< 10[a]	< 10[a]	< 10[a]
Yeasts (CFU/g)	≤ 10	< 10[a]	< 10[a]	< 10[a]	< 10[a]	< 10[a]
Molds (CFU/g)	≤ 10	< 10[a]	< 10[a]	< 10[a]	< 10[a]	< 10[a]
Residual endotoxins (E.U./mg)	≤ 10	< 0.00025[a]	< 0.00024[a]	< 0.00025[a]	<0.00025[a]	<0.00025[a]

2'-FL = 2'-fucosyllactose; CFU = colony forming units; E.U. = endotoxin units; HiMS = human-identical milk saccharides; LOQ = limit of quantitation; RT = retention time.

[a] Result is below the LOQ: 0.03 w/w % for L-Fucose; 0.10 or 0.05 w/w % for ash depending on the testing laboratory; 0.0017 w/w % for residual proteins; 0.01 mg/kg for β-Lactoglobulin; 0.2 mg/kg for casein; 0.05 mg/kg for lead depending on the testing laboratory; 10 CFU/g for total plate count, *Bacillus cereus*, yeasts, and molds; and 0.050 E.U./mL for endotoxins (converted to approximately 0.00024 to 0.00025 E.U./mg depending on the sample weight).

[b] As per the July amendment, the sensitivity of the inductively coupled plasma mass spectrometry (ICP-MS) method according to EPA 6020A has been improved, resulting in the reduction of the LOQ for lead from 0.05 to 0.01 or 0.001 mg/kg (depending on the testing laboratory) for more recent batches.

FDA.2. *We would like to clarify a mis-statement in Table 1 on page 8 of the amendment dated July 7, 2022. Table 1 indicates that GRN 000735 included the use of 2'-FL in oral nutritional supplements and enteral tube feeding within the food category "Foods for special dietary use". This is incorrect, as per our request, the notifier removed these uses from the scope of GRN 000735. We realize that including this use in Table 1 has no impact on your dietary exposure calculation; we only wanted to clarify the GRN 000735 intended uses for the administrative record. No other action is required on your part regarding this issue.*

This was an oversight, and we thank the FDA for the correction.

FDA.3. *The notice states that 2'-FL is intended to be used in exempt hypoallergenic extensively hydrolyzed infant formula and amino acid-based infant formula for term infants. For the administrative record, could you please identify the protein source(s) of the extensively hydrolyzed infant formula to which 2'-FL is intended to be added?*

The protein sources of the extensively hydrolyzed infant formula to which 2'-FL is intended to be added is from mammalian milk such as from cow's milk or goat's milk.

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We hope this information adequately addresses the Agency's questions on GRN 001034, and if there is any additional information or further clarification that is required, Glycom will be happy to provide such information upon request.

Sincerely,

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