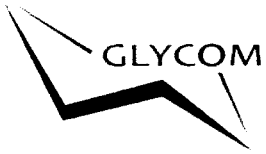
ORIGINAL SUBMISSION

GRN 000546



Glycom A/S, Diplomvej 373, DK - 2800 Kongens Lyngby, Denmark

September 23, 2014

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Dr. Gaynor:

Re: GRAS Exemption Claim for 2'-*O*-Fucosyllactose

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized as Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)], I am submitting one hard copy and one electronic copy (on CD), as the notifier [Glycom A/S, Diplomvej 373, DK-2800 Kgs. Lyngby, Denmark], a Notice of the determination, on the basis of scientific procedures, that 2'-*O*-fucosyllactose, produced by Glycom A/S, as defined in the enclosed documents, is GRAS under specific conditions of use in term infant formula and in food, and therefore, is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act*. Information setting forth the basis for the GRAS determination, which includes detailed information on the notified substance and a summary of the basis for the GRAS determination, as well as a consensus opinion of an independent panel of experts in support of the safety of 2'-*O*-fucosyllactose under the intended conditions of use, also 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,

(b) (6)

Christoph H. Röhrig, Ph.D.
Project Manager Regulatory Affairs & Glycobiology
Glycom A/S

RECEIVED
SEP 25 2014
OFFICE OF
FOOD ADDITIVE SAFETY

Glycom A/S, Diplomvej 373, DK - 2800 Kongens Lyngby, Denmark

September 23, 2014

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Christoph H. Röhrig, Ph.D.
Project Manager Regulatory Affairs & Glycobiology
Glycom A/S

Glycom A/S, Diplomvej 373, DK - 2800 Kongens Lyngby, Denmark

September 23, 2014

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Dr. Gaynor:

Re: GRAS Exemption Claim for 2'-*O*-Fucosyllactose

I hereby certify that the enclosed electronic files for the Notification entitled, "GRAS Exemption Claim for 2'-*O*-Fucosyllactose (2'-FL)" were scanned for viruses prior to submission and are thus certified as being virus-free using McAfee VirusScan 8.8.

Sincerely,

(b) (6)

Christoph H. Röhrig, Ph.D.
Project Manager Regulatory Affairs & Glycobiology
Glycom A/S

GRN 000546

GRAS Exemption Claim for 2'-*O*-Fucosyllactose (2'-FL)

Submitted to:

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835
USA

Submitted by:

Glycom A/S
Diplomvej 373
DK-2800 Kgs. Lyngby
Denmark

September 23, 2014

RECEIVED
SEP 25 2014
OFFICE OF
FOOD ADDITIVE SAFETY

GRAS Exemption Claim for 2'-*O*-Fucosyllactose (2’-FL)

Submitted to:

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
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USA

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Diplomvej 373
DK-2800 Kgs. Lyngby
Denmark

September 23, 2014

GRAS Exemption Claim for 2'-*O*-Fucosyllactose (2'-FL)

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Appendix B Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of the Proposed Infant Formula and Food Uses of Human-identical Milk Oligosaccharide 2'-*O*-Fucosyllactose (2'-FL)

I. GRAS EXEMPTION CLAIM

I.A Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)]

Glycom A/S hereby claims that the use of 2'-*O*-fucosyllactose (2'-FL) in term infant formula and in foods, as described in Section I.D below, is exempt from the requirement of premarket approval of the *Federal Food, Drug, and Cosmetic Act* because we have determined that such uses are Generally Recognized as Safe (GRAS).

Signed,

(b) (6)

23.09.2014

Christoph H. Röhrig, Ph.D.
Project Manager Regulatory Affairs & Glycobiology
Glycom A/S
christoph.roehrig@glycom.com

Date

I.B Name and Address of Notifier

Glycom A/S
Diplomvej 373
DK-2800 Kgs. Lyngby
Denmark
Tel: +45 4525 2247
Fax: +45 3841 1720

I.C Common Name of the Notified Substance

2'-*O*-Fucosyllactose

I.D Conditions of Intended Use

I.D.1 Conditions of Intended Use in Infant Formula

2'-*O*-fucosyllactose (2'-FL) is intended for use in term infant formulas at a maximum use level of 2,400 mg/L (reconstituted formula). This maximum use level of 2'-FL in term infant formulas is based on providing a similar level of 2'-FL as that which occurs in mature human breast milk, which ranges from 1,100 to 4,260 mg/L on average, but may be up to 7,000 mg/L (see Section IV.B.1).

I.D.2 Conditions of Intended Use in Food

2'-FL also is intended for use in foods and beverages of multiple categories as described in Table I.D.2-1 at use levels up to the maximum use levels specified.

Table I.D.2-1 Summary of the Individual Proposed Food Uses and Use Levels for 2'-FL in the U.S.

Food Category	Proposed Food-Uses	RACC[a]	Proposed Use Level (g/RACC)	Proposed Maximum Use Level (g/kg or g/L)[b]
Baked Goods and Baking Mixes	Breads and Baked Goods, Gluten-free	50 g	1.2 to 2.4	24 to 48
	Cereal Bars	40 g	0.48	12
Beverages and Beverage Bases	Carbonated Beverages	240 mL	0.28	1.2
	Flavored and Enhanced Waters	240 mL	0.28	1.2
	Meal Replacement Drinks, for Weight Reduction	240 mL	1.2	5
	Sports, Isotonic, and Energy Drinks	240 mL	0.28	1.2
Coffee and Tea	Coffee	240 mL	1.2 to 2.4	5.0 to 10.0
	Tea	240 mL	1.2 to 2.4	5.0 to 10.0
Dairy Product Analogs	Beverage Whiteners	2 g	1.2	600
	Imitation Milks	240 mL	0.28	1.2
	Non-Dairy Yogurt	225 g	1.2 to 2.4	5.4 to 10.6
Infant and Toddler Foods	Toddler Formulas	100 mL[c]	0.24	2.4
	Other Baby Foods for Infants and Young Children	7 to 170 g	0.084 to 2.04	12
	Other Drinks for Young Children	120 mL	0.14	1.2
Grain Products and Pastas	Meal Replacement Bars, for Weight Reduction	30 g	1.2	40
Milk, Whole and Skim	Unflavored Pasteurized and Sterilized milk[d]	240 mL	0.28	1.2
Milk Products	Buttermilk	240 mL	0.28	1.2
	Flavored Milk	240 mL	0.28	1.2
	Yogurt	225 g	1.2 to 2.4	5.4 to 10.6
Processed Fruits and Fruit Juices	Fruit Flavored Drinks and Ades	240 mL	0.28	1.2
	Fruit Juices and Nectars	240 mL	0.28	1.2
Processed Vegetables and Vegetable Juices	Vegetable Juices and Nectars	240 mL	0.28	1.2
Sugar Substitutes	Table Top Sweeteners	4 g	0.12	300

RACC: Reference Amounts Customarily Consumed
[a] Serving sizes were based on Reference Amounts Customarily Consumed (RACC) per Eating Occasion in the U.S. CFR (21 CFR §101.12 - U.S. FDA, 2013a).
[b] The proposed maximum use level is presented on a g/kg basis for solids and on a g/L basis for liquids.
[c] RACC not available, 100 mL employed as an approximation.
[d] When the milk is fortified with 2'-FL, it will then be classified as a milk product. The intake of the category 'Unflavored pasteurized and sterilized milks' was used here as a conservative proxy for the dietary pattern of the fortified milk drink product.

I.E Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30 of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2013b), 2'-*O*-fucosyllactose has been determined by Glycom A/S to be GRAS through scientific procedures.

I.F Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the United States (U.S.) Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

Glycom A/S
Diplomvej 373
DK-2800 Kgs. Lyngby
Denmark

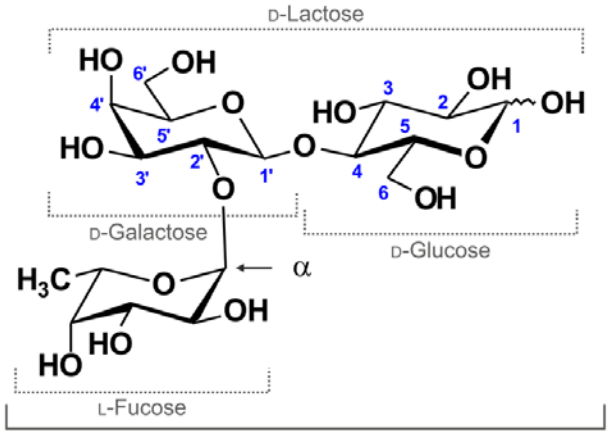
Should the FDA have any questions or additional information requests regarding this notification, Glycom will supply these data and information.

II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE

II.A Identity

II.A.1 Chemical Identity

Common Name:	2'-*O*-Fucosyllactose
Common Abbreviation:	2'-FL (2'FL, 2-FL, 2FL)
IUPAC Name:	α-D-Fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucopyranose
Alternative Denotations:	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:	



α-L-Fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-D-glucopyranose

= 2'-*O*-Fucosyllactose

II.A.2 Chemical and Physical Characteristics

2'-FL is a trisaccharide consisting of L-fucose, D-galactose, and D-glucose. Alternatively, 2'-FL 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. The molecular structure of 2'-FL was first elucidated by Richard Kuhn in 1955 (using classical chemical techniques) (Kuhn *et al.*, 1955) and shortly thereafter by Jean Montreuil (Montreuil, 1956). Since then the structure of 2'-FL was confirmed independently by others using a range of modern structure characterization techniques, including spectroscopic techniques [*e.g.*, ^{1}H-, ^{13}C, and 2D-nuclear magnetic resonance (NMR)] (Jenkins *et al.*, 1984; Ishizuka *et al.*, 1999; Rundlöf and Widmalm,

2001; Urashima *et al.*, 2002, 2004, 2005; Almond *et al.*, 2004; Wada *et al.*, 2008), mass spectrometric (MS) techniques (Fura and Leary, 1993; Asres and Perreault, 1996; Perreault and Costello, 1999), and x-ray crystallography (Kuhn *et al*., 1956; Svensson *et al*., 2002).

2'-FL is a naturally occurring trisaccharide found in mammalian milk with the highest concentrations occurring in *human* milk, and is therefore typically referred to as a *human* milk oligosaccharide (HMO). An oligosaccharide is a generic term for a saccharide polymer with a typical degree of polymerization below 20 (~3-20). Whilst human milk contains a combination of oligosaccharides (HMOs) it is important to emphasize that 2'-FL is a clearly defined trisaccharide that occurs only as one specific constitutional isomer. In simple terms, it is as clearly defined, for example, as sucrose is as a disaccharide consisting of glucose and fructose.

Glycom has confirmed, based on ^{1}H-, ^{13}C-NMR-spectroscopy, mass spectrometry and x-ray crystallography data, that the 2'-FL manufactured by Glycom is chemically and structurally fully identical to the 2'-FL that is present in human breast milk. Glycom's 2'-FL is therefore referred to as a human identical milk oligosaccharide (HiMO).

II.B Method of Manufacture

II.B.1 Raw Materials and Processing Aids

The raw material from which 2'-FL is derived is benzyl-2'-FL, which is produced by Glycom as the material is not currently commercially available. The benzyl-2'-FL raw material is produced from L-fucose and D-lactose in the first stage of the manufacturing process as described below in Section II.B.2.1. The L-fucose source raw material also is produced by Glycom and is of high purity and quality. The D-lactose source raw material is European Phamacopeia-grade, the monograph for which is harmonized with that of the National Formulary. The benzyl-2'-FL raw material is produced through a tightly controlled process, and all batches are controlled for conformity to established strict specifications. The benzyl-2'-FL produced is of high quality and purity. In addition, all processing aids used in the manufacture of 2'-FL from benzyl-2'-FL are food-grade[1], and all filters and filter aids are those commonly used by the food industry in the purification of food ingredients. All such processing aids and filters/filter aids are permitted by U.S. regulation, or previously determined to be GRAS, for their respective uses during manufacturing. The materials used in the manufacture of 2'-FL from benzyl-2'-FL are listed in Table II.B1-1.

Table II.B.1-1 Raw Material and Processing Aids Used in the Manufacture of 2'-FL

Material	Function
Raw Materials	

[1] Compliant with the specifications set forth in the Food Chemicals Codex or equivalent international food or pharmacopeia standard.

L-Fucose	Source raw material
D-Lactose	Source raw material
Benzyl-2'-FL	Raw material
Processing Aids	
Citric acid	pH Adjustment
Nitrogen	To produce an inert atmosphere in hydrogenation reactor
Palladium/Carbon catalyst	To catalyze hydrogenation reaction
Hydrogen (H_2)	Hydrogenation
Filter and Filter Aids	
Circulation filter	Filtration
Cartridge filter (2 µm)	Filtration
Activated charcoal	To remove coloration and impurities
Microfilter (0.2 µm)	Bacterial microfiltration

II.B.2 Manufacturing Process

2'-FL is manufactured in compliance with current Good Manufacturing Practice (cGMP) and the principles of Hazard Analysis and Critical Control Points (HACCP). The raw material from which 2'-FL is derived is benzyl-2'-FL, which is produced by Glycom as the material is not currently commercially available. The manufacturing process can be broadly divided into 2 stages, which are described in brief in Section II.B.2.1 and II.B.2.2, respectively.

II.B.2.1 Manufacturing Stage 1: Precursor Formation

Stage 1 of the manufacturing process consists of manufacturing the raw material, benzyl-2'-FL, used to produce the final 2'-FL ingredient. Benzyl-2'-FL is produced from the source raw materials, L-fucose and D-lactose, through a series of reactions, involving the production of several intermediates. The final intermediate is recrystallized to produce benzyl-2'-FL. As mentioned, the benzyl-2'-FL primary raw material produced is a high quality and high purity material, manufactured through a tightly controlled process with critical control points in place at key steps. All precursors and intermediates are single, well-characterized, and pure compounds and isolated intermediates are analyzed at critical control points to ensure conformance to strict specifications. Benzyl-2'-FL also is a single, well-characterized, and crystalline compound, and all batches of benzyl-2'-FL are controlled for conformity to established strict specifications by certificate of analysis, ensuring that only batches of high purity primary raw material enter Manufacturing Stage 2.

II.B.2.2 Manufacturing Stage 2: Production of 2'-FL

Stage 2 of the manufacturing process consists of manufacturing 2'-FL from benzyl-2'-FL. To this end, benzyl-2'-FL is subjected to a hydrogenation reaction in the presence of citric acid and a palladium/charcoal chemical catalyst to generate 2'-FL. The purification process consists of

filtration (circulation filter and a 2 µm cartridge filter) to remove the catalyst and treatment with activated charcoal and additional filtration to remove coloration and any potential impurities. The resultant 2'-FL solution is then subjected to microfiltration (0.2 µm filter) to ensure absence of microbiological contamination. The solution then undergoes vacuum distillation resulting in the production of a syrup, evaporation, a final microfiltration step (0.2 µm filter), and spray drying to generate the final 2'-FL powdered product. The manufacturing process for 2'-FL is tightly controlled with critical control points in place at key steps where in-process controls are applied to minimize the amount of potential inherent impurities as technically possible.

II.B.3 Quality Control

As mentioned, a HACCP plan and quality control procedures are in place in order to ensure that 2'-FL complies with the product specifications. Manufacturing Stage 1 and Manufacturing Stage 2 are each controlled by a HACCP plan in which critical control points have been identified and in-process controls are applied. Additionally, specifications for key materials are set according to HACCP principles, and conformity is monitored by certificate of analysis at each key-stage of the production process. Batch release procedures are then followed. Thus, appropriate control measures and analytical testing are implemented throughout the manufacturing process to ensure production of a high quality ingredient.

II.C Specifications for Food Grade Material and Product Analysis

II.C.1 Specifications for Food Grade Material

Appropriate food-grade product specifications have been established for 2'-FL and are presented in Table II.C.1-1 below. Specifications established for potential impurities and contaminants and microbial endotoxins are further discussed in Sections II.C.3 and II.C.4, respectively. All methods of analysis are nationally or internationally recognized or have been validated by Glycom or a third party laboratory.

Table II.C.1-1 Proposed Product Specifications for 2'-FL

Parameter	Specification	Method
Appearance	Powder	Visual
Color	White to off white	Visual
Identification	RT of main component corresponds to RT of standard ± 3%	HPLC (Glycom method)
Assay by HPLC (water free)	Min. 95.0%	HPLC (Glycom method)
pH (20°C, 5% solution)	3.0 to 7.5	WBSE-77:2012
Water	Max. 9.0%	Karl Fischer (EP 2.5.32)
Ash, sulphated	Max. 0.2%	EP 2.4.14
Acetic acid (as free acid and/or sodium acetate)	Max. 0.3%	MSZ EN ISO 10304-1:2009

Table II.C.1-1 Proposed Product Specifications for 2'-FL		
Parameter	**Specification**	**Method**
Residual solvents	Max. 50 mg/kg singly Max. 200 mg/kg in combination	EP GC 2.4.24
Residual proteins	0.1%[a]	External laboratory in-house method
Lead	Max. 0.8 mg/kg	EPA 6020A:2007
Microbiological Specifications		
Salmonella	Absent in 25 g	MSZ-EN-ISO 6579:2006
Aerobic mesophilic total count	Max. 500 CFU/g	MSZ-EN-ISO 4833:2003
Enterobacteriaceae	Absent in 10 g	MSZ-ISO 21528-2:2007
Cronobacter (Enterobacter) sakazakii	Absent in 10 g	ISO-TS 22964:2006
Listeria monocytogenes	Absent in 25 g	MSZ-EN-ISO 11290-1:1996, 1998/A1:2005
Bacillus cereus	Max. 50 CFU/g	MSZ-EN-ISO 7932:2005
Yeasts	Max. 10 CFU/g	MSZ-ISO 7954:1999
Moulds	Max. 10 CFU/g	MSZ-ISO 7954:1999
Residual endotoxins	Max. 50 EU/mg[a]	LAL Kinetic chromogenic assay (EP 2.6.14)

CFU = colony forming units; EP = European Pharmacopeia; EPA = (United States) Environmental Protection Agency; EU = endotoxin units; GC = gas chromatography; HPLC = high performance liquid chromatography; ISO = International Organization for Standardization; LAL = *Limulus* amebocyte lysate; RT = retention time

[a] Specification may be amended (*i.e.*, lowered) should additional data become available in the future.

II.C.2 Product Analysis

Batch analyses for 5 separate commercial batches supporting the product specifications laid out in Section II.C.1 are presented in Table II.C.2-1.

Table II.C.2-1 Batch Analyses for 2'-FL

Parameter	Specification	Batch Results				
		L06112K	**PSD42013 1210/166**	**PSD42013 1210/167**	**PSD42013 1210/168**	**PSD42013 1210/169**
Appearance	Powder	Powder	Powder	Powder	Powder	Powder
Color	White to off-white	White	White	White	White	White
Identification	RT of main component corresponds to RT of standard ± 3%	Complies	Complies	Complies	Complies	Complies
Assay by HPLC (water free) (%)	Min. 95	96.9	97.0	98.2	97.0	97.8
pH (20°C, 5% solution)	3.5 to 7.5	6.2	3.9	3.9	3.7	3.6
Water (%)	Max. 9.0	0.3	2.8	3.2	3.2	3.1
Ash, sulphated (%)	Max. 0.2	0.15	< 0.01	0.07	0.05	0.05
Acetic acid (as free acid and/or sodium acetate) (%)	Max. 0.3	0.003	0.05	0.05	0.03	0.03
Residual solvents Singly (mg/kg) Combination (mg/kg)	 Max. 50 Max. 200	 n/a[a] n/a[a]	 <10 <70	 <10 <70	 <10 <70	 <10 <70
Residual proteins (%)	Max. 0.1	<LOQ[b]	<LOQ[b]	<LOQ[b]	<LOQ[b]	<LOQ[b]
Lead (mg/kg)	Max. 0.8	<0.1	<0.1	<0.1	<0.1	<0.1
Microbiological Specifications						
Salmonella	Absent in 25 g	Complies	Complies	Complies	Complies	Complies
Aerobic mesophilic total count (CFU/g)	Max. 500	<10	<0	<10	<10	<10
Enterobacteriaceae	Absent in 10 g	Complies	Complies	Complies	Complies	Complies
Cronobacter (Enterobacter) sakazakii	Absent in 10 g	Complies	Complies	Complies	Complies	Complies
Listeria monocytogenes	Absent in 25 g	n/a[c]	Complies	Complies	Complies	Complies
Bacillus cereus (CFU/g)	Max. 50	n/a[c]	<10	<10	<10	<10
Yeasts (CFU/g)	Max. 10	Complies	Complies	Complies	Complies	Complies
Moulds (CFU/g)	Max. 10	Complies	Complies	Complies	Complies	Complies
Residual endotoxins (EU/mg)	Max. 50	<LOR[d]	<LOR[d]	<LOR[d]	<LOR[d]	<LOR[d]

CFU = colony forming units; EU = endotoxin units; HPLC = high-performance liquid chromatography; LOR = level of reporting; LOQ = limit of quantification; n/a = not applicable

[a] Not applicable to specifications; batch L06112K was not spray-dried.

[b] LOQ = 0.1 %.

[c] Parameter was not measured for batch L06112K.

[d] LOR = 0.01 EU/mg.

II.C.3 Potential Impurities and Contaminants

Potential impurities and contaminants present in the final 2'-FL product are listed in the product specifications presented in Section II.C.1 and analytical results provided in Section II.C.2. Potential contaminants fall into 2 categories:

1. External contaminants; and,
2. Potential contaminants from the production process.

Impurities which may potentially remain in the 2'-FL product at the end of the manufacturing process are covered at top-level by the final product specifications and in more detail by additional internal specifications. These specifications include those for the raw materials and processing aids used in the production process and a low number of other carbohydrate-type impurities which stem from the primary raw material benzyl-2'-FL that may be carried over into the final 2'-FL product. Importantly, an internal specification of 1 mg/kg is set for the palladium catalyst used in the production of 2'-FL.

While no solvents, other than water, are used in the manufacture of 2'-FL from the primary raw material benzyl-2'-FL, residual solvents may be produced from the raw material during manufacturing. The potential for residual solvents produced from the raw material is controlled by specifications. Residual solvents are covered by a single and combined residual solvent specification of 50 mg/kg and 200 mg/kg, respectively, in the final product specifications.

As described in Section II.B.3, a detailed HACCP plan is in place to clearly identify and insert control specifications to limit or eliminate carryover of potential contaminants. The specifications on the final product are, in the end, confirmatory final controls in the long chain of control points for the process.

II.C.4 Specification for Microbial Endotoxins

While Glycom's 2'-FL ingredient is currently produced through chemical synthesis, many oligosaccharides are known to be manufactured using full cell fermentation technologies. Additionally, the source raw materials, L-fucose and D-lactose, also may be potentially produced using full cell fermentation technologies. These technologies can present a potential risk in the carry-over of microbial metabolites, particularly endotoxins, and it is therefore of interest to provide reassurance that Glycom's 2'-FL ingredient is controlled for residual endotoxins. While regulatory threshold levels for food regarding endotoxin contamination currently do not exist, typical ranges of endotoxin load have been reported for drinking water (Anderson *et al.*, 2002; O'Toole *et al.*, 2008), cow's milk (Gehring *et al.*, 2008), and infant formula powder (Townsend *et al.*, 2007). Because of the sensitivity of infants to microbial toxins, in addition to monitoring for microorganisms, Glycom has established a specification for endotoxins to provide reassurance that 2'-FL produced through Glycom's method of manufacture is controlled for residual levels of endotoxins. The endotoxin specification for 2'-FL is set to not contribute additional exposure to

endotoxins that would result in exposures above the range of levels reported for infant formula powder currently on the market (Townsend *et al.*, 2007). The endotoxin specification established for 2'-FL is set at a maximum of 50 endotoxin units/mg as specified in Section II.C.1 (Table II.C.1-1).

II.D Stability

II.D.1 Bulk Stability

II.D.1.1 Real-Time Stability Study Data

The current shelf-life of crystalline 2'-FL is established as 36 months when stored at ambient temperature and protected from humidity. The current shelf-life of crystalline 2'-FL is supported by the data available to date from a 5-year long-term stability study (25°C, 60% relative humidity) on crystalline 2'-FL (Batch L06112K), which is ongoing. In this study, eight 3 g samples were collected for chemical analysis and were packed into polyethylene bags as the primary packaging material, which was then packed into polyethylene/aluminum/polyester triple layer foil bags as the secondary packaging material. Five 40 g samples for microbiological testing were similarly packaged. Samples were stored at 25°C and 60% relative humidity in a climatic chamber for periods of up to 36 months. Chemical analysis and microbiological testing were performed for one sample per time point. 2'-FL was analyzed by high performance liquid chromatography (HPLC) and water content was analyzed by Karl Fischer titration at each time point. The analytical data are presented in Table II.D.1.1-1, including the initial analytical data for the batch.

Table II.D.1.1-1 Real-Time Stability Study on 2'-FL (25°C and 60% relative humidity) (Batch L06112K)

Parameter Tested	Analytical Data[a]							
	Initial	**3 Months**	**6 Months**	**9 Months**	**12 Months**	**18 Months**	**24 Months**	**36 Months**
Chemical Analysis								
Description	White crystalline powder	Not reported	Not reported	White crystalline powder	White crystalline powder	White crystalline powder	White crystalline powder	White crystalline powder
Assay (w/w %) (calculated for water-free substance)	99.9	98.5	97.7	98.9	99.0	100	99.9	97.0
Water (w/w %)	0.1	0.1	0.2	0.1	0.3	0.5	0.5	0.4
Lactose (w/w %)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	Not reported	Not reported	0.07
Microbiological Analysis								
Salmonella (CFU/25 g)	0	n/a	Negative	n/a	Negative	n/a	Negative	Negative
Aerobic mesophilic total count (CFU/g)	<10	n/a	<10	n/a	<10	n/a	<10	<10

Table II.D.1.1-1 Real-Time Stability Study on 2'-FL (25°C and 60% relative humidity) (Batch L06112K)								
Parameter Tested	**Analytical Data[a]**							
	Initial	**3 Months**	**6 Months**	**9 Months**	**12 Months**	**18 Months**	**24 Months**	**36 Months**
Enterobacteriaceae (CFU/10 g)	0	n/a	Negative	n/a	Negative	n/a	Not reported	Negative
Cronobacter (*Enterobacter*) *sakazakii* (CFU/10 g)	0	n/a	Negative	n/a	Negative	n/a	Not reported	Negative

CFU = colony forming units; n/a = not applicable
[a] Chemical analysis was conducted on samples separate from those subjected to microbiological testing.

No significant change was observed in the assay value for 2'-FL for up to 36 months of storage. A very slight increase in water content was observed by 18 months of storage; however, the water content remained low throughout the 36 months of storage, indicating that the packaging is appropriate in protecting 2'-FL from water absorption. Lactose, the potential degradation product of 2'-FL, was below 0.1% at all time points up to 36 months of storage. No unknown degradation products were observed in the HPLC chromatogram at any time point during the 36 months of storage. These results indicate that 2'-FL is stable and does not degrade when stored at a temperature of 25°C for periods of up to 36 months. Furthermore, microbiological purity was maintained throughout the duration of the 36 month storage period. The current shelf-life of crystalline 2'-FL has thus been established as 36 months when stored at ambient temperature, protected from humidity until longer term data is available following completion of the 5-year stability study.

II.D.1.2 Accelerated Study Data

A 6-month accelerated stability study (40°C, 75% relative humidity) has been performed on crystalline 2'-FL (Batch L06112K). Two 3 g samples for chemical analysis were packed into polyethylene bags (primary packaging material), which was then packed into polyethylene/aluminum/polyester triple layer foil bags (secondary packaging material). A separate 40 g sample for microbiological testing was similarly packaged. Samples were stored at 40°C and 75% relative humidity in a climatic chamber. One 3 g sample was analyzed for 2'-FL, water, and impurities at 3 months of storage and the other at 6 months of storage. Microbiological testing on the 40 g sample was performed after 6 months of storage. 2'-FL was analyzed by HPLC and water content was analyzed by Karl Fischer titration. The analytical data are presented in Table II.D.1.2-1, including the initial analytical data for the batch.

Table II.D.1.2-1 Accelerated Stability Study on 2'-FL (40°C and 75% relative humidity) (Batch L06112K)			
Parameter Tested	**Analytical Data[a]**		
	Time 0	**3 Months**	**6 Months**

Chemical Analysis			
Assay (w/w %) (calculated for water-free substance)	99.9	97.9	98.2
Water (w/w %)	0.1	0.1	0.1
Lactose (w/w %)	< 0.1	< 0.1	< 0.1
Microbiological Analysis			
Salmonella (CFU/25 g)	0	n/a	Negative
Aerobic mesophilic total count (CFU/g)	<10	n/a	< 10
Enterobacteriaceae (CFU/10 g)	0	n/a	Negative
Cronobacter (*Enterobacter*) *sakazakii* (CFU/10 g)	0	n/a	Negative

CFU = colony forming units; LOD = limit of detection; n/a = not applicable
[a] Chemical analysis was conducted on samples separate from those subjected to microbiological testing.

No significant change was observed in the assay value for 2'-FL for up to 6 months of storage under accelerated conditions. In addition, the low water content after 6 months of storage indicates that the packaging is appropriate in protecting 2'-FL from water absorption. Lactose, the potential degradation product of 2'-FL, was below 0.1% after 3 and 6 months of storage. No unknown degradation products were observed in the HPLC chromatogram. These results indicate that 2'-FL is stable and does not degrade when stored under accelerated conditions for periods of up to 6 months. Furthermore, microbiological purity was maintained throughout the duration of the 6 month study.

II.D.2 Stability Under the Intended Conditions of Use

II.D.2.1 Stability in Powdered Infant Formula

The stability of 2'-FL in a representative infant formula was assessed in an ongoing 3 year study in which 2'-FL was added to infant formula powder in combination with another HiMO, lacto-*N*-neotetraose (LNnT) which mimics the intended conditions of use of 2'-FL in infant formula. The infant formula powder used in the study is a whey-based starter formula containing long chain polyunsaturated fatty acids (LC-PUFA) and vitamins and minerals at concentrations intended for full nutritional support of infants from birth to 6 months of age. The composition of the infant formula powder is representative of commercial infant formulas on the market and is presented in Table II.D.2.1-1. The caloric density of the infant formula when reconstituted is 66.9 kcal/100 mL.

Table II.D.2.1-1 Composition of Infant Formula Powder Used in the 3-Year Stability Study on 2'-FL	
Parameter	**Content**
Protein (Whey proteins/caseins)	1.889 g/100 kcal (71.6%/28.4%)
Carbohydrate	11.182 g/100 kcal
Fat	5.302 g/100 kcal
Dry matter	97.51%
Moisture	2.49%

The infant formula powder samples were produced following a commercial infant formula production process in which 2'-FL from a single batch (Batch L06112K) and LNnT were added in the wet mixture together with other ingredients (salts, carbohydrates, proteins). 2'-FL was added at a target concentration of 0.90 g/100 g (dry matter) of infant formula [LNnT added at a target concentration of 0.45 g/100 g (dry matter)]. Following dissolution of the ingredients, the mixtures were heat-treated at a temperature of 105°C for 5 seconds. Subsequent steps consisted of evaporation, homogenization, and spray drying to produce a powdered product. Samples were stored in gassed (N_2/CO_2) tin cans (1 can per time and temperature point) at a temperature of 4, 20, 30, or 37°C. While the study is currently ongoing, data is available for time points of up to 540 days (approximately 18 months) of storage. Specifically, 2'-FL content was measured prior to and following 90, 180, 360, and 540 days of storage. 2'-FL was analyzed in duplicate for each sample by ultra HPLC with fluorescent detection. The analytical data are presented in Table II.D.2.1-2.

Table II.D.2.1-2 Stability of 2'-FL (Batch L06112K) in Powdered Infant Formula

Days of Storage	Replicate 1 (g/100 g)	Replicate 2 (g/100 g)	Mean (g/100 g)	% CV	g/100 g Dry Matter	% Recovery	% Losses
Initial Concentration							
0	n/a	n/a	0.836	n/a	0.86	n/a	n/a
Temperature = 4°C							
180[a]	0.858	0.843	0.851	1.2	0.87	101.71	-1.71
540[b]	0.870	0.900	0.885	2.4	0.91	105.84	-5.84
Temperature = 20°C							
90[c]	0.859	0.871	0.865	1.0	0.89	103.47	-3.47
180[a]	0.833	0.836	0.835	0.3	0.86	99.80	0.20
360[d]	0.821	0.836	0.829	1.3	0.85	99.08	0.92
540[b]	0.869	0.878	0.874	0.7	0.90	104.46	-4.46
Temperature = 30°C							
90[a]	0.858	0.856	0.857	0.2	0.88	102.49	-2.49
180[b]	0.844	0.836	0.840	0.7	0.86	100.46	-0.46
360[d]	0.820	0.824	0.822	0.3	0.84	98.31	1.69

Table II.D.2.1-2 Stability of 2'-FL (Batch L06112K) in Powdered Infant Formula							
Days of Storage	**Replicate 1 (g/100 g)**	**Replicate 2 (g/100 g)**	**Mean (g/100 g)**	**% CV**	**g/100 g Dry Matter**	**% Recovery**	**% Losses**
Initial Concentration							
0	n/a	n/a	0.836	n/a	0.86	n/a	n/a
540[b]	0.851	0.881	0.866	2.4	0.89	103.57	-3.57
Temperature = 37°C							
180[a]	0.845	0.832	0.839	1.1	0.86	100.28	-0.28
540[b]	0.856	0.869	0.863	1.1	0.88	103.15	-3.15

CV = coefficient of variation; n/a = not applicable
[a] Date of analyses: 27/11/2012
[b] Date of analyses: 05/09/2013
[c] Date of analyses: 07/06/2012
[d] Date of analyses: 04/03/2013

The analytical data demonstrate no significant loss of 2'-FL when added to an infant formula powder prior to processing and stored at temperatures of up to 37°C over a period of 540 days when compared to the initial 2'-FL concentration. 2'-FL is therefore stable under the intended conditions of use in powdered infant formula under the recommended storage conditions and when subjected to higher storage temperatures (of up to 37°C) for prolonged periods.

II.D.2.2 Stability in Other Food Applications

The stability of 2'-FL in other food applications was assessed using representative food products, such as yogurts, ready-to-drink flavored milk, and citrus fruit beverages, in stability studies as described below. The 2'-FL content in all food matrices is measured by HPLC with fluorescent detection consistent with the method applied in the analysis of 2'-FL in infant formula as indicated in Section II.D.2.1. All stability studies were conducted using formulations representative of commercial food products on the market and under typical processing (*i.e.*, pasteurization and/or ultra high-temperature heating) and typical storage conditions (*e.g.*, temperature and shelf-life) for such products. All test samples were analyzed for 2'-FL content in duplicate and the results provided in Tables II.D.2.2-1, II.D.2.2-2, and II.D.2.2-3.

Table II.D.2.2-1 Stability of 2'-FL (Batch L06112K) in Yogurt Following Processing				
Sample Number	**Time Point (days)**	**2'-FL Concentration (g/100 g)**		**Average 2'-FL Concentration (g/100 g)**
		Sample Replicate 1	**Sample Replicate 2**	
2'-FL Added to Yogurt Base				
P14-00712-23	0	1.28	1.28	1.28
P14-00712-34	10	1.26	1.19	1.23
P14-00712-41	21	1.30	1.36	1.33
2'-FL Added to Fruit Preparation				
P14-00712-25	0	1.23	1.13	1.18
P14-00712-35	10	1.21	1.21	1.21
P14-00712-42	21	1.33	1.31	1.32

Table II.D.2.2-2 Stability of 2'-FL (Batch L06112K) in a Citrus Fruit Drink Following Pasteurization				
Sample	**Time Point (days)**	**2'-FL Concentration (g/100 mL)**		**Average 2'-FL Concentration (g/100 mL)**
		Sample Replicate 1	**Sample Replicate 2**	
P14-00712-6	Pre-processing	0.78	0.80	0.79
P14-00712-2	0	0.82	0.82	0.82
P14-00712-27	14	0.80	0.81	0.81
P14-00712-37	28	0.85	0.82	0.84

Table II.D2.2-3 Stability of 2'-FL (Batch L06112K) in Ready-to-Drink Chocolate-Flavored Milk Following Processing				
Sample Number	**Time Point (days)**	**2'-FL Concentration (g/100 g)**		**Average 2'-FL Concentration (g/100 g)**
		Sample Replicate 1	**Sample Replicate 2**	
Samples Subjected to Pasteurization				
P14-00712-8	Pre-processing	0.75	0.72	**0.74**
P14-00712-4	0	0.76	0.75	**0.76**
P14-00712-17	7	0.75	0.76	**0.76**
P14-00712-29	14	0.73	0.78	**0.76**
Samples Subjected to Ultra-High Temperature Processing				
P14-00712-19	Pre-processing	0.74	0.74	**0.74**
P14-00712-21	0	0.73	0.73	**0.73**
P14-00712-39	14	0.73	0.73	**0.73**
P14-00712-50	28	0.73	0.72	**0.73**

The analytical data demonstrate that there was no loss of 2'-FL in yogurt, a citrus fruit drink, or ready-to drink chocolate-flavored milk at each time point tested when compared to the initial 2'-FL concentration. Testing in a citrus fruit drink and ready-to drink chocolate-flavored milk

also demonstrated no loss of 2'-FL between pre-processing and the start of the stability study; pasteurization and/or ultra-high temperature heating did not affect the stability of 2'-FL in these foods. The analytical data therefore demonstrate that 2'-FL is stable when added to yoghurt, citrus fruit drinks, and ready-to-drink chocolate-flavored milk following typical processing conditions and when stored at 4°C over the shelf-life of these foods.

III. SELF-LIMITING LEVELS OF USE

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

IV. BASIS FOR GRAS DETERMINATION

The determination that 2'-FL, as manufactured by Glycom, is GRAS under the intended conditions of use in infant formula and in food as described in Sections I.D.1 and I.D.2, respectively, is on the basis of scientific procedures. The data and information supporting this GRAS determination is summarized below. A discussion of the estimated exposure of the U.S. population to 2'-FL under the intended conditions of use is presented in Section IV.A. Information supporting the safety of 2'-FL for use in infant formula alone and its combined use in infant formula and in food in consumers of infant formula (*i.e.*, infants and toddlers) is summarized in Section IV.B. The basis for the safety of 2'-FL for use in food in all other population groups is summarized in Section IV.C.

As mentioned, Glycom's 2'-FL is chemically and structurally identical to the 2'-FL present in human breast milk, and therefore, the safety of Glycom's 2'-FL for all intended uses and population groups was found to be supported by the known consumption of 2'-FL from human breast milk in infants. A summary of the levels of 2'-FL in human breast milk and the corresponding intakes of 2'-FL from human breast milk are provided in Section IV.B.1. A comparison of these intakes of 2'FL from human breast milk to those of Glycom's 2'-FL is summarized in Sections IV.B.2 and IV.B.3 for infants and toddlers and in Section IV.C.1 for all other population groups. The safety of Glycom's 2'-FL is further supported by the results of published toxicological studies on Glycom's manufactured 2'-FL, which are summarized in Section IV.B.5, and which comprise the pivotal studies for the safety assessment of Glycom's 2'-FL. To identify other data and information relevant to the safety of infant formula and food uses of 2'-FL, a comprehensive search of the published scientific literature was conducted through June 2014. Published studies identified during the literature search consisted of studies relating to the metabolic fate of 2'-FL and which are summarized in Section IV.B.4 below. No other toxicological or safety studies on 2'-FL were identified.

The data and information summarized below were reviewed by a Panel of Experts, qualified by scientific training and experience to evaluate the safety of 2'-FL, who concluded that 2'-FL is GRAS under the aforementioned conditions of intended use in infant formula and in food based on scientific procedures (as indicated in Section IV.D).

IV.A Probable Consumption

IV.A.1 Estimated Daily Intake of 2'-FL from Use in Infant Formula (Infants and Toddlers)

Estimates for the daily intake of 2'-FL from its use in term infant formulas were calculated based on a maximum use level of 2,400 mg/L as indicated in Section I.D.1 in conjunction with infant formula consumption data included in the U.S. National Center for Health Statistics' (NCHS) 2009-2010 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2011; USDA, 2012). The population groups consuming infant formulas consisted of infants (0 to 6 and 7 to 12 month age groups) and toddlers (1 to 3 years), with infants ages 0 to 6 months identified as the largest consumer group as expected. For toddlers, only 1.1% of respondents were identified as potential consumers of 2'-FL.

Estimates for the daily intake of 2'-FL from its use in term infant formulas are summarized in Table IV.A.1-1 on a per person basis by population group. Table IV.A.1-2 presents these data on a per kilogram body weight basis.

From the use of 2'-FL in infant formulas only, the all-user estimated mean and 90th percentile intakes of 2'-FL were greatest in infants 0 to 6 months of age at 2.02 and 2.91 g/person/day, respectively. On a body weight basis, these intakes were determined to be 332.8 and 535.6 mg/kg body weight/day, respectively. In infants 7 to 12 months of age, the estimated mean and 90th percentile intakes of 2'-FL from infant formulas were determined to be at 1.70 and 2.63 g/person/day, respectively. On a body weight basis, these intakes were determined to be 188.9 and 295.8 mg/kg body weight/day, respectively. In toddlers 1 to 3 years of age, the estimated mean and 90th percentile all-user intakes of 2'-FL from infant formulas were determined to be 1.08 and 1.41 g/person/day, respectively. On a body weight basis, these intakes were determined to be 89.3 and 117.1 mg/kg body weight/day, respectively.

Table IV.A.1-1 Summary of the Estimated Daily Intake of 2'-FL from Infant Formulas in the U.S. Infant and Toddler Population Groups (2009-2010 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (g/day)		All-Users Consumption (g/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Infants	0 to 6 months	1.51	2.74	74.8	161	2.02	2.91
Infants	7 to 12 months	1.25	2.49	73.6	128	1.70	2.63
Toddlers	1 to 3	0.01[a]	na	1.1	7	1.08[a]	1.41[a]

na = not available
[a] Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table IV.A.1-2	Summary of the Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Infant Formulas in the U.S. Infant and Toddler Population Groups (2009-2010 NHANES Data)						
Population Group	**Age Group (Years)**	**All-Person Consumption (mg/kg bw/day)**		**All-Users Consumption (mg/kg bw/day)**			
		Mean	**90th Percentile**	**%**	**n**	**Mean**	**90th Percentile**
Infants	0 to 6 months	249.0	477.2	74.8	161	332.8	535.6
Infants	7 to 12 months	138.9	266.5	73.6	128	188.9	295.8
Toddlers	1 to 3	1.0[a]	na	1.1	7	89.3[a]	117.1[a]

na = not available
[a] Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

IV.A.2 Estimated Daily Intake of 2'-FL from Use in Infant Formula and in Food Combined (Infants and Toddlers)

2'-FL is intended for use in term infant formulas and in foods, including baby foods. Considering that older infants begin consuming baby food and other foods while still consuming infant formula, the estimated daily intakes of 2'-FL from its use in term infant formulas combined with its use in foods were calculated in order to determine the total daily intake of the ingredient from all sources in the population groups identified as consumers of infant formula (*i.e.*, infants and toddlers). The estimated daily intakes were calculated based on a maximum use level of 2,400 mg/L in term infant formulas and on the individual food uses and maximum use levels presented in Table I.D.2-1 in conjunction with infant formula and food consumption data included in NHANES (CDC, 2011; USDA, 2012). Food codes were grouped in food use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (U.S. FDA, 2013b). Product-specific adjustment factors were developed based on data provided in the standard recipe file for the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996, 1998 survey (USDA, 2000). Estimates for the total daily intake of 2'-FL from its use in term infant formulas combined with all other intended food uses are summarized in Table IV.A.2-1 on a per person basis by population group. Table IV.A.2-2 presents these data on a per kilogram body weight basis.

For the infant population group aged 0 to 6 months, 80.5% of respondents were identified as potential consumers of 2'-FL, while in the other infant and toddler population groups, the percentage of consumers exceeded 99%. As a result of the high percentage of users identified within these population groups, the intake estimates for the all-person and all-user categories were similar; therefore, only the all-user results are discussed in detail.

Among the individual population groups, infants aged 7 to 12 months were determined to have the greatest estimated mean and 90th percentile intakes of 2'-FL on an absolute and body

weight basis. In this age group, the estimated mean and 90th percentile intakes of 2'-FL on an absolute basis were 4.63 and 8.36 g/person/day, respectively, and on a body weight basis, 520.2 and 987.1 mg/kg body weight/day, respectively. The second greatest estimated intakes of 2'-FL on an absolute and body weight basis were determined to be in infants aged 0 to 6 months. The estimated mean and 90th percentile daily intakes in infants aged 0 to 6 months were 4.63 and 8.36 g/person/day, respectively, and on a body weight basis, 520.2 and 987.1 mg/kg body weight/day, respectively. In toddlers 1 to 3 years of age, the estimated mean and 90th percentile intakes of 2'-FL were determined to be 1.39 and 2.59 g/person/day, respectively, and on a body weight basis, 107.1 and 200.0 mg/kg body weight/day, respectively.

Of note, the greatest contributors to the estimated daily intakes of 2'-FL in the infant population groups were use in infant formulas and use in baby foods for infants and young children. In infants aged 0 to 6 months, use in infant formulas contributed 68.8% to the estimated intakes of 2'-FL, while use in baby foods for infants and young children contributed 30.8%. In infants aged 7 to 12 months, use in infant formulas contributed 26.7% to the estimated intakes of 2'-FL, while use in baby foods for infants and young children contributed 65.1%.

Table IV.A.2-1 Summary of the Estimated Daily Intake of 2'-FL from Infant Formulas and from all Proposed Food Uses in the U.S. Infant and Toddler Population Groups (2009-2010 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (g/day)		All-Users Consumption (g/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Infants	0 to 6 months	2.36	5.11	80.5	168	2.93	5.29
Infants	7 to 12 months	4.63	8.36	100	161	4.63	8.36
Toddlers	1 to 3	1.39	2.58	99.7	644	1.39	2.59

Table IV.A.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Infant Formulas and from all Proposed Food Uses in the U.S. Infant and Toddler Population Groups (2009-2010 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to 6 months	362.1	681.7	80.5	168	449.7	712.4
Infants	7 to 12 months	520.2	987.1	100	161	520.2	987.1
Toddlers	1 to 3	106.8	199.9	99.7	644	107.1	200.0

IV.A.3 Estimated Daily Intake of 2'-FL from Use in Foods (Ages ≥4 Years)

For population groups ≥4 years of age, estimates for the intake of 2'-FL were calculated based on the individual food uses and maximum use levels presented in Table I.D.2-1 in conjunction with food consumption data included in NHANES (CDC, 2011; USDA, 2012). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (U.S. FDA, 2013b). Product-specific adjustment factors were developed based on data provided in the standard recipe file for the CSFII 1994-1996, 1998 survey (USDA, 2000). Estimates for the total daily intake of 2'-FL from all intended food uses are summarized in Table IV.A.3-1 on a per person basis by population group. Table IV.A.3-2 presents these data on a per kilogram body weight basis.

Approximately 98.1% of the total U.S. population was identified as potential consumers of foods containing 2'-FL (8,236 actual users identified). As a result of the high percentage of users identified within all population groups, the intake estimates for the all-person and all-user categories were similar; therefore, only the all-user results are discussed in detail.

The mean and 90th percentile intake of 2'-FL by the total U.S. population from all intended food uses was estimated to be 1.61 and 3.23 mg/person/day, respectively. The total population estimated mean and 90th percentile intakes on a body weight basis were determined to be 34.1 and 65.5 mg/kg body weight/day, respectively.

Among the individual population groups, female adults were determined to have the greatest estimated mean and 90th percentile intakes of 2'-FL on an absolute basis at 1.73 and 3.63 g/ person/day, respectively. Similar absolute estimated daily intakes of 2'-FL were calculated for all other adult population groups, while children were determined to have the lowest estimated mean and 90th percentile intakes of 2'-FL at 1.16 and 1.96 g/person/day, respectively.

On a body weight basis, children were determined to have the greatest estimated mean and 90th percentile intakes of 2'-FL at 44.2 and 77.2 mg/kg body weight/day, respectively. The elderly and male adults were identified as having the lowest estimated mean intakes of 2'-FL with similar values of 18.9 and 19.5 mg/kg body weight/day, respectively. Male adults were determined to have the lowest 90th percentile intake of 2'-FL at 39.3 mg/kg body weight/day.

Table IV.A.3-1	Summary of the Estimated Daily Intake of 2'-FL from Proposed Food-Uses in the U.S. by Population Group (2009-2010 NHANES Data)						
Population Group	**Age Group (Years)**	**All-Person Consumption (g/day)**		**All-Users Consumption (g/day)**			
		Mean	**90th Percentile**	**% Users**	**n**	**Mean**	**90th Percentile**
Children	4 to10	1.15	1.96	99.7	1092	1.16	1.96
Female Teenagers	11 to 18	1.27	2.32	99.7	551	1.27	2.32
Male Teenagers	11 to 18	1.48	2.76	99.4	571	1.50	2.76
Female Adults of child bearing age	19 to 40	1.55	3.11	97.7	977	1.58	3.19
Female Adults	19 to 64	1.70	3.59	98.2	2042	1.73	3.63
Male Adults	19 to 64	1.66	3.31	97.6	1822	1.70	3.35
Elderly	65 and up	1.41	3.11	97.0	1185	1.45	3.28
Total Population	All Ages	1.58	3.21	98.1	8236	1.61	3.23

Table IV.A.3-2	Summary of the Estimated Daily Per Kilogram Body Weight Intake of 2'-FL from Proposed Food-Uses in the U.S. by Population Group (2009-2010 NHANES Data)						
Population Group	**Age Group (Years)**	**All-Person Consumption (mg/kg bw/day)**		**All-Users Consumption (mg/kg bw/day)**			
		Mean	**90th Percentile**	**%**	**n**	**Mean**	**90th Percentile**
Children	4 to 10	44.1	77.2	99.7	1,092	44.2	77.2
Female Teenagers	11 to 18	22.5	41.4	99.7	551	22.6	41.4
Male Teenagers	11 to 18	23.5	47.5	99.4	571	23.7	48.1
Female Adults of child bearing age	19 to 40	21.6	44.2	97.7	977	22.1	44.6
Female Adults	19 to 64	23.4	50.4	98.2	2,042	23.8	50.5
Male Adults	19 to 64	19.0	38.8	97.6	1,822	19.5	39.3
Elderly	65 and up	18.3	42.4	97.0	1,185	18.9	44.0
Total Population	All Ages	33.4	64.4	98.1	8,236	34.1	65.5

IV.B Information to Support the Safety of 2'-FL for Use in Infant Formula Alone and in Infant Formula and in Food Combined (Infants and Toddlers)

IV.B.1 Background Dietary Intake of 2'-FL from Human Breast Milk

2'-FL is one of the naturally occurring fucosylated milk oligosaccharides present in a range of mammalian milks (Urashima *et al.*, 2001; Castanys-Muñoz *et al*., 2013), with the highest concentrations found in human breast milk (Kuhn *et al.*, 1955). 2'-FL is an important member of a complex family of structurally-related milk oligosaccharides which make up the third largest

solid component of human breast milk (Kuhn, 1952; Kunz and Rudloff, 1993; Bode, 2012; Newburg, 2013). These oligosaccharides are known as HMOs as they were first discovered in human breast milk (Malpress and Hytten, 1958) and also since they occur in human milk at much higher concentrations than in any other mammalian milk (Urashima *et al.*, 2001). More than 100 members of this family have been fully described on a structural basis (Urashima *et al.*, 2011), and an even greater number of members have been detected by sensitive mass spectrometry techniques (Finke *et al.*, 1999; Wu *et al.*, 2010, 2011). The highest concentrations of HMOs occur in human colostrum (20 to 25 g/L), and concentrations between 5 to 20 g/L occur in mature human milk (Bode, 2012). In comparison, bovine milk contains approximately 20 times lower concentrations of a far less complex oligosaccharide mixture (Tao *et al.*, 2009; Aldredge *et al.*, 2013; Urashima *et al.*, 2013), and which does not contain fucosylated oligosaccharides at any appreciable level (Gopal and Gill, 2000; Aldredge *et al.*, 2013) (see Section 6.2).

It is well-established that 2'-FL is formed from lactose by the action of α-1,2-fucosyltransferase, which is responsible for fucosylating lactose at the 2'-*O*-position and which is excreted by the myoepithelial cells of the mammary gland into the lactiferous ducts (Wiederschain and Newburg, 2001). Lactose itself is synthesized in the Golgi of mammary gland cells during a process called lactogenesis in which a complex of α-lactalbumin with the enzyme uridine diphosphate (UDP)-Gal:N-acetylglucosamine β1-4-galactosyltransferase (Gal-T) catalyses the utilization of UDP-D-galactose to enable selective addition of D-galactose to D-glucose in a β(1-3)-bond (Urashima *et al*., 2012; Castanys-Muñoz *et al*., 2013). Further details on the biosynthesis of HMOs in the mammary gland are not yet well understood due to lack of suitable cell models (Bode, 2012; Urashima *et al.*, 2012). 2'-FL, however, is not produced in women who do not express α-1,2-fucosyltransferase in their mammary glands (approximately 20% of women). 2'-FL is therefore not detected in the breast milk of these women. This phenotype is referred to as the *non-secretor* (Se^-) phenotype and forms, together with the phenotype for another enzyme (α1,4-fucosyltransferase, which is required to synthesize the so-called *Lewis* antigens a and b), the basis for categorization of human milk into 4 different phenotypes (Thurl *et al.*, 1997, 2010; Coppa *et al.*, 2011). Descriptions of these 4 phenotypes are summarized in Table IV.B.1-1.

Table IV.B.1-1 Categorization of Human Milk According to Enzyme Phenotype[a]

Milk Group	**1**	**2**	**3**	**4**
Phenotype	**Se+/Le(a– b+)**	**Se–/Le(a+ b–)**	**Se+/Le(a– b–)**	**Se–/Le(a– b–)**
Fuc (α-1,2)	+	–	+	–
Fuc (α-1,3)	+	+	+	+
Fuc (α-1,4)	+	+	–	–
Frequency (Caucasian)	~ 70%	~ 20%	~ 9%	~ 1%

+ = present; - = absent; Fuc = Fucose
[a] Based on information from Thurl *et al.* (1997, 2010) and Coppa *et al.* (2011).

2'-FL is by far the most abundant HMO in human breast milk on average (*i.e*., based on pooled[2] milk data) (Erney *et al.*, 2000; Chaturvedi *et al.*, 2001a; Musumeci *et al.*, 2006; Asakuma *et al.*, 2008; Castanys-Muñoz *et al*., 2013). The 2'-FL content of human breast milk has been reported in several publications from independent research groups (see Appendix A for further details), whereby extensive data has been provided according to Secretor and Lewis-blood group status (Thurl *et al.*, 1996, 2010; Coppa *et al.*, 2011; Galeotti *et al.*, 2012, 2014), ethnicity (Erney *et al.*, 2000; Musumeci *et al.*, 2006), lactation period (Coppa *et al.*, 1999; Erney *et al.*, 2000; Sumiyoshi *et al.*, 2003; Asakuma *et al.*, 2008; Leo *et al.*, 2010; Gabrielli *et al.*, 2011; Bao *et al.*, 2013), term/preterm birth (Nakhla *et al.*, 1999; Gabrielli *et al.*, 2011), and the average content of mature milk (Chaturvedi *et al.*, 1997, 2001a; Nakhla *et al.*, 1999; Erney *et al.*, 2000, 2001; Sumiyoshi *et al.*, 2003; Morrow *et al*., 2004; Leo *et al.*, 2010; Thurl *et al.*, 2010; Asakuma *et al*., 2011; Coppa *et al.*, 2011; Galeotti *et al.*, 2012; Smilowitz *et al*., 2013; Hong *et al*., 2014). As mentioned, the presence of 2'-FL in human breast milk is dependent on the Secretor phenotype, and therefore, may manifest as a dependence on ethnicity (reviewed in Urashima *et al*., 2012). The concentration of 2'-FL in human breast milk also appears to be dependent on the lactation period within a given population group, with the highest levels detected in colostrum and decreasing levels detected thereafter (Erney *et al.*, 2000). Although the levels of 2'-FL decrease as lactation progresses, the levels of 2'-FL remain relatively high at ≥1,500 mg/L even at 31 to 452 days post-partum (Coppa *et al.*, 1999; Erney *et al.*, 2000; Thurl *et al.*, 2010). Additionally, a wide variation in 2'-FL concentrations in human breast milk exists between individual mothers.

The range of average concentrations of 2'-FL in pooled mature human breast milk (from full-term birth mothers at approximately lactation days 5 to 100 and considering milk types in accordance to their frequency) is reported as being between 1,100 and 4,260 mg/L, based on all available data (see Table IV.B.1-2). The values reported by Sumiyoshi *et al*. (2003) and Leo *et al*. (2010) fall out of the typical observed range, with reported values of 2'-FL at 220 and 330 mg/L. However, when the data is viewed according to individual milk type (*i.e.*, non-pooled data), it is not uncommon to observe concentrations of 2'-FL that span a much wider range from 0 to approximately 7,000 mg/L, with the greatest levels occurring in those with the Secretor phenotype (Gabrielli *et al.*, 2011; Galeotti *et al.*, 2012). In the mature breast milk of mothers with the Secretor phenotype, 2'-FL concentrations range from approximately 1,000 to 7,000 mg/L. Therefore, based on these data, the level of 2'-FL present in mature human breast milk ranges from 1,100 to 4,260 mg/L on average, but may be up to approximately 7,000 mg/L.

Based on the levels of 2'-FL present in mature human breast milk as detailed above and on a 6.5 kg infant consuming approximately 1 L of breast milk per day (Davies *et al.*, 1994; Hester *et al.*, 2012), the average intake of 2'-FL from mature human breast milk is calculated to be approximately 170 to 660 mg/kg body weight/day. However, in infants from Secretor mothers, the intake of 2'-FL from mature human breast milk may be up to approximately 1,150 mg/kg

[2] Data from "pooled milk" refers to data obtained from actual pooled milk samples from different women with varying milk types or to an average value calculated from individual values obtained from different women with varying milk types.

body weight/day. Therefore, the background dietary range of 2'-FL intakes in infants is 170 to 660 mg/kg body weight/day on average, but may be up to 1,150 mg/kg body weight/day.

Considering that Glycom's 2'-FL is chemically and structurally identical to the 2'-FL present in human breast milk, the background dietary range of 2'-FL intakes from mature human breast milk in infants serves as the safe range of Glycom's 2'-FL intakes for infants. In addition, considering that infants are a susceptible population group from a safety perspective (SCF, 1998), it may be concluded that this background dietary range of 2'-FL intakes in infants also would be safe for older population groups. This background exposure to 2'-FL therefore serves as the safe reference range of Glycom's 2'-FL intakes for all population groups.

Table IV.B.1-2 Average 2'-FL Concentrations in Pooled, Mature (Lactation Days 5 to 100), Full-Term, Human Breast Milk in Various Populations[a]

Publication	2'-FL Concentrations (mg/L)				
	Europe	**US**	**Latin America**	**Asia**	**Overall**
Thurl *et al.* (1997)	1,840				1,840
Chaturvedi *et al.* (1997)			1210		1,210
Coppa *et al.* (1999)	2,810				2,810
Nakhla *et al.* (1999)	1,270				1,270
Erney *et al.* (2000)	2,710	2,340	2,440	2,100	2,710, 2,340, 2,100, 2,440
Erney *et al.* (2001)		2,380[b]			2,380
Chaturvedi *et al.* (2001a)		2,430			2,430
Sumiyoshi *et al.* (2003)				~~220~~[c]	~~220~~[c]
Morrow *et al*. (2004)			3,850		3,850
Thurl *et al.* (2010)	2,650				2,650
Leo *et al.* (2010)				~~330~~[c]	~~330~~[c]
Asakuma *et al.* (2011)				1,480	1,480
Coppa *et al.* (2011)	2,060				2,060
Galeotti *et al.* (2012)	3,190				3,190
	3,520				3,520
Bao *et al.* (2013)		1,100			1,100
Smilowitz *et al.* (2013)		1,220			1,220
Galeotti *et al.* (2014)	4,260				4,260
Hong *et al*. (2014)	2,080				2,080
Average	**2,559**	**1,773**	**2,500**	**1,790**	**2,350**

[a] Further details are provided in Appendix A.
[b] Samples from mothers from the United States, Mexico, and Europe were combined, and therefore, the results represent a mixed population. This value was not used to calculate the average value within the individual population groups.
[c] Values with a strikethrough are considered to be outliers and were not included as part of the calculation of the average value.

IV.B.2 Comparison of Estimated Intake of 2'-FL from Use in Infant Formula to Background Dietary Intake of 2'-FL from Human Breast Milk (Infants and Toddlers)

As mentioned, the maximum use level of 2'-FL of 2,400 mg/L in term infant formulas is based on providing a similar level of 2'-FL as that which occurs in mature human breast milk, which ranges from 1,100 and 4,260 mg/L on average, but may be up to approximately 7,000 mg/L. As summarized in Section IV.A.1, the resulting heavy consumer (90th percentile) intake of 2'-FL from its use in term infant formulas was estimated to be 535.6 mg/kg body weight/day in infants aged 0 to 6 months, 295.8 mg/kg body weight/day in infants aged 7 to 12 months, and 117.1 mg/kg body weight/day in toddlers aged 1 to 3 years. In comparison, as summarized in Section IV.B.1, the intake of 2'-FL from mature human breast milk ranges from 170 to 660 mg/kg body weight/day on average, but may be up to 1,150 mg/kg body weight/day, in infants. The intake of 2'-FL from its use in term infant formulas in the population groups identified as consumers is therefore within the background dietary range of 2'-FL intake from mature human breast milk in infants, which is considered to be the safe reference range of 2'-FL intakes for all population groups as previously noted.

The safety of the use of 2'-FL in term infant formulas is therefore supported by the fact that 2'-FL manufactured by Glycom is chemically and structurally identical to 2'-FL present in human breast milk and that the estimated intakes of 2'-FL from its use in term infant formulas is within the safe reference range of 2'-FL intakes for infants and older population groups.

Furthermore, considering that term infant formulas are consumed in substitution for breast milk, the addition of 2'-FL to term infant formulas would not add to the background dietary intake of 2'-FL from breast milk.

IV.B.3 Comparison of Estimated Intake of 2'-FL from Use in Infant Formula and in Food Combined to Background Dietary Intake of 2'-FL from Human Breast Milk (Infants and Toddlers)

As summarized in Section IV.A.2, for the population groups identified as consuming infant formulas (*i.e.*, infants and toddlers), the estimated daily intakes of 2'-FL from its use in term infant formulas combined with its use in foods were calculated in order to determine the total daily intake of the ingredient from all sources in these population groups. The resulting heavy consumer (90th percentile) intake of 2'-FL from its use in term infant formulas combined with its use in foods was estimated to be 712.4 mg/kg body weight/day in infants aged 0 to 6 months, 987.1 mg/kg body weight/day in infants aged 7 to 12 months, and 200.0 in toddlers aged 1 to 3 years. The use of 2'-FL in foods therefore increases the 2'-FL intakes in these population groups from the intakes from use in infant formula alone. These combined 2'-FL intakes, however, remain within the background exposure to 2'-FL intakes from mature human breast milk in infants (up to 1,150 mg/kg body weight/day), supporting the safety of the use of the ingredient in term infant formulas combined with its use in foods.

IV.B.4 Absorption, Distribution, Metabolism, and Excretion

HMOs, including 2'-FL, do not undergo any significant digestion in the upper gastrointestinal tract and only small amounts are absorbed intact. *In vitro* data from Engfer *et al*. (2000) and Gnoth *et al*. (2000) demonstrate that HMOs, including 2'-FL, are highly resistant to digestion by digestive enzymes under conditions simulating the gastrointestinal tract. This was shown by Engfer *et al*. (2000) using digestive enzymes from sources consisting of human pancreatic juice (duodenal aspirates) together with human or porcine intestinal brush border membranes (hydrolases) (human samples were prepared from human adult duodenum samples). In this study, HMOs were subjected to digestion both as a total fraction of neutral or acidic HMOs and as purified HMOs. The total fraction of neutral HMOs contained 2'-FL as a component, and purified 2'-FL also was tested. There was no change observed in matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) spectra and high-pH anion exchange chromatography (HPAEC) elution profiles between samples of the HMO fractions containing control heat-inactivated enzymes and those containing active enzymes following 20 hours of incubation. These results were observed for both the neutral and acidic HMO fractions. Moreover, when purified 2'-FL was subjected to digestion, the HMO was recovered intact with no hydrolysis products detected by MALDI-MS. Similar results were obtained by Gnoth *et al*. (2000) in which incubation of a neutral HMO fraction, containing 2'-FL as a component, with human salivary amylase, porcine pancreatic amylase, or porcine intestinal brush border membranes for 2 hours failed to cleave the neutral HMOs as assessed by HPAEC analysis; no change was observed in the elution profiles between control and enzyme-treated fractions. Similar results were obtained with an acidic HMO fraction. While incubation with porcine intestinal brush border membranes for periods up to 24 hours resulted in the hydrolysis of small amounts of neutral non-fucosylated HMOs to lacto-*N*-triose, lactose, and galactose, the majority of the neutral fraction remained undigested [as assessed by HPAEC and silica-high performance thin layer chromatography (HPTLC)]. In contrast, incubation of neutral fucosylated HMOs with porcine intestinal brush border membranes demonstrated that the enzymes within the brush border membranes were unable to cleave these types of oligosaccharides. Acidic HMOs also were shown to be largely resistant to digestion. Gnoth *et al*. (2000) concluded that the results suggest that <5% of the HMO amount would be digested in the intestinal tract. The results of these studies therefore indicate that a large proportion of an ingested amount of HMOs, including 2'-FL, goes undigested in the intestinal tract of humans in general, and therefore, only small amounts of monosaccharides derived from HMOs would be available for absorption.

Consistent with the above data, studies in human infants and rats demonstrate that HMOs, including 2'-FL are orally absorbed intact to a small extent (Goehring *et al*., 2014; Ruhaak *et al*., 2014; Santos-Fandila *et al*., 2014). 2'-FL specifically has been detected at measurable levels in the plasma of exclusively and partially breast-fed infants in recent investigations (Goehring *et al*., 2014; Ruhaak *et al*., 2014). 2'-FL levels in the plasma of exclusively formula-fed infants were minimal (and statistically significantly less) when compared to those in exclusively breast-

fed infants (Goehring *et al*., 2014). In addition, the concentration of 2'-FL in the plasma of exclusively breast-fed infants was shown to correlate with the concentration of 2'-FL ingested *via* breast milk (Goehring *et al*., 2014). While the precise proportion of ingested 2'-FL (or other HMOs) that is absorbed has not been quantified, the finding that the concentration of 2'-FL in the plasma of breast-fed infants is only 0.1% of that detected in breast milk suggests that the proportion of ingested 2'-FL (and other HMOs) that is absorbed is small (Ruhaak *et al*., 2014). Studies in human infants in which HMOs were detected unchanged in the urine of breast-fed infants further demonstrate that HMOs are orally absorbed intact. The results of such studies indicate that a small portion of the total amount of ingested HMO is excreted unchanged in urine; it has been estimated that approximately 1 to 2% of the total amount of ingested HMO is excreted *via* the urinary route (Rudloff *et al*., 1996, 2006, 2012; Obermeier *et al.*, 1999; Chaturvedi *et al*., 2001b; Rudloff and Kunz, 2012; Dotz *et al*., 2014; Goehring *et al*., 2014; Ruhaak *et al*., 2014). 2'-FL and/or 3-FL have been specifically detected unchanged in the urine of breast-fed infants; however, the level of 2'-FL excreted in the urine relative to the amount ingested has not been quantified (Rudloff *et al*., 1996, 2012; Obermeier *et al.*, 1999; Chaturvedi *et al.*, 2001b; Dotz *et al*., 2014). Given that the biosynthesis of HMOs occurs exclusively in the lactating mammary gland (Messer and Urashima, 2002; Jantscher-Krenn and Bode, 2012; Urashima *et al*., 2012), the presence of intact HMOs in the urine of infants in the above studies is attributed to oral absorption from the ingestion of breast milk. The mechanism by which HMOs are absorbed intact has not yet been fully elucidated; however, data from *in vitro* studies using the Caco-2 human intestinal epithelial cell model suggest that neutral HMOs, such as 2'-FL, are transported transcellularly across the intestinal epithelium by receptor-mediated transcytosis, as well as by paracellular means (Gnoth *et al*., 2001). Acidic HMOs were determined to be absorbed by paracellular transport (Gnoth *et al*., 2001). It is expected that only small amounts of intact 2'-FL would be absorbed in toddlers as well considering that intestinal permeability decreases with maturation of the intestinal tract.

Data from human infant studies analyzing HMO digestion by intestinal bacterial microflora and HMO fecal excretion further support that the proportion of ingested HMO that is absorbed is relatively small. The data indicate that a large proportion of an ingested physiological amount of HMOs by infants undergoes fermentation by the intestinal bacterial microflora as assessed by the lactulose hydrogen breath test (Brand-Miller *et al.*, 1995, 1998). Specifically, the proportion subjected to fermentation was estimated to be on average 100% following a loading test in infants with the purified HMO fraction isolated from their respective mother's milk (Brand-Miller *et al.*, 1998). The analysis was performed by the lactulose hydrogen breath test, and therefore, the amount of HMO reaching the large intestine was not specifically measured. In other studies, HMOs, including 2'-FL, were detected unchanged (by HPLC) in fecal samples of infants at total levels amounting to 40 to 50% of the ingested amount following consumption of breast milk (Chaturvedi *et al*., 2001b; Coppa *et al.*, 2001). While some discrepancy exists among these studies regarding the proportion of ingested HMOs that is excreted in the feces *versus* the proportion that is fermented by the intestinal microflora, the available data indicate that a large

proportion of an ingested amount of an HMO, including 2'-FL, reaches the large intestine and is either fermented by the intestinal microflora or is excreted unchanged in the feces in infants. The same fate of 2'-FL would occur in toddlers considering that only small amounts of intact 2'-FL would be absorbed in this age group.

It should be noted that while Glycom's HiMO 2'-FL may be absorbed to some extent as suggested by the above data, any level of 2'-FL that is absorbed in infants would be considered normal on the basis that consumption levels of the ingredient (from use in infant formula and in food) would be similar to or less than those from human breast milk in infants. Therefore, the potential absorption of Glycom's 2'-FL from its consumption is not a safety concern for infants. Since infants comprise a susceptible age group, it may be concluded that the potential absorption of Glycom's 2'-FL would not pose a safety concern for older age groups, including toddlers, in which the consumption of 2'-FL would be less than in infants.

IV.B.5 Toxicological Studies

As mentioned, product-specific studies on Glycom's 2'FL have been conducted, consisting of a 90-day oral toxicity study in juvenile Wistar [Crl:WI(Han)] rats, preceded by a 14-day dose-range finding study, and 2 *in vitro* mutagenicity studies. These studies comprise the pivotal studies for the safety assessment of Glycom's 2'-FL for use in infant formula and in food and are summarized below in Sections IV.B.5.1 and IV.B.5.2. No other toxicological studies on 2'-FL were identified in the publicly available scientific literature.

IV.B.5.1 Repeated Oral Exposure Studies on Glycom's 2'-FL

Glycom's 2'-FL ingredient was investigated in an adapted subchronic (90-day) oral toxicity study using 7-day-old Wistar [Crl:WI(Han)] rats as described in detail in the publication by Coulet *et al*. (2014). The 90-day study was preceded by a 14-day dose-range finding study also performed in juvenile rats (Coulet *et al*., 2014). Juvenile rats were selected for the 90-day study to supplement the standard Organisation for Economic Co-operation and Development (OECD) 408 protocol to better reflect use of the ingredient for consumption by infants, in addition to use in foods for consumption by the general population (OECD, 1998a; Barrow, 2007). Thus, the studies were conducted in rats starting at post-natal day (PND) 7 to adequately include coverage of the developmental window during which infants would be exposed. In both studies, oligofructose (OF) (also known as short-chain fructo-oligosaccharide) was used as the reference control on the basis that it is currently used in infant formula. The doses selected for these studies (*i.e.,* 2,000, 5,000, and 6,000 or 7,500 mg/kg body weight/day) and overall experimental design were based on a published subchronic 90-day toxicity study conducted in rats investigating the safety of other oligosaccharides (*i.e.*, galatooligosaccharides and fructooligosaccharides) for use in infant formulas (Anthony *et al.*, 2006). It should be noted that the highest dose tested in the 90-day study of 6,000 mg/kg body weight/day was the highest dose able to be tested as administration of macronutrients at higher levels may result in

nutritional imbalances, which would complicate a proper safety assessment (Borzelleca, 1996; Munro *et al*., 1996). The results of these studies are summarized below.

In the dose-range finding study, Wistar [Crl:WI(Han)] pups (5/sex/group) were administered by gavage 0 (vehicle control), 2,000 (low-dose), 5,000 (mid-dose), or 7,500 (high-dose) mg/kg body weight/day of Glycom's 2'-FL (purity of 99%) for 14 days starting from PND 7 to weaning on PND 20 (Coulet *et al*., 2014). A reference control group was orally administered 6,000 mg OF/kg body weight/day for the same period of time. Individual dams with litters of at least 5 male and 5 female pups were housed in plastic cages. One pup of each sex in each litter was randomly allocated to 1 of the 5 groups. Animals were observed twice daily for mortality and morbidity, and clinical observations were performed daily. Detailed clinical examination was performed weekly, and body weights were measured on PND 1, 4, 7, 10, 14, 17, and 20. Feed intake was not measured. All animals were euthanized at the end of the administration period on PND 21 and macroscopic examinations were performed.

One female in the high-dose group was found partially cannibalized on day 6 of the 14-day study, prior to which the animal lost body weight between days 0 and 3 and presented with clinical signs. A second female of the high-dose group died on day 12, for which there were no significant findings noted at necropsy and the cause of death could not be determined. Liquid feces and/or yellowish liquid feces were observed in some animals of the mid- and high-dose groups and the OF reference control group from days 1 to 3 up to days 9 to 11. These findings were occasionally accompanied by erythema in the urogenital region. Lower body weight gains were observed in all animals of the high-dose and OF groups between days 0 to 3 relative to the control group. This effect also was observed, to a lesser extent, in animals of the mid-dose group. Terminal body weights, however, were similar among groups, with the exception of slight reductions observed in females of the high-dose and OF groups (7.7 and 6.3% lower than the control group, respectively). There were no compound-related macroscopic findings observed at necropsy. Based on the results of this study, the authors determined that the highest dose of 2'-FL considered suitable for the 90-day study in juvenile rats should be lower than 7,500 mg/kg body weight/day.

The subchronic 90-day toxicity study was conducted in compliance with the OECD Principles of Good Laboratory Practice (GLP) and according to OECD Test No. 408 (OECD, 1998a,b), with adaptation to include the use of juvenile rats. A 28-day recovery period was included in the study. Wistar [Crl:WI(Han)] pups were orally administered by gavage 0 (water vehicle control), 2,000 (low-dose), 5,000 (mid-dose), or 6,000 (high-dose) mg/kg body weight/day of 2'-FL (purity of 99%) from PND 7 to up to 13 weeks of age. The control and high-dose groups each consisted of 15 males and 15 females, while the low- and mid-dose groups each consisted of 10 males and 10 females. A reference control group (15 rats/sex/group) administered 6,000 mg/kg body weight/day of OF was included in the study. Individual dams with litters of at least 5 male and 5 female pups were housed in plastic cages until weaning on PND 21. One pup of each sex in each litter was randomly allocated to one of the 5 groups. On PND 21, pups were

weaned and placed in plastic cages according to sex and dose group such that a total of 5 pups of the same sex and dose group were housed per cage. A standard diet (A04C-10) and water were provided *ad libitum*. Animals were observed twice daily for mortality and morbidity, and clinical observations were performed daily. A detailed clinical examination was performed weekly. Body weights were assessed at time of randomization, prior to dosing, twice weekly during the first 8 weeks of the administration period, and then once weekly thereafter. Food intake also was measured twice weekly after weaning on PND 21 up until the 8th week of the administration period (when animals were 9 weeks of age), and then once weekly thereafter. Ophthalmology was performed on all animals from the control, high-dose 2'-FL, and OF groups during the last week of administration. Fasting blood and urine samples were collected from all animals of all groups for clinical pathology analysis (*i.e.*, hematology, coagulation, clinical chemistry, and urinalysis) at the end of the administration period. Clinical pathology also was performed for all animals from all groups at the end of the recovery period. Twenty (20) animals from each group (10/sex) were euthanized and necropsied on the day after the last day of dosing. The remaining animals in the control, high-dose, and OF groups (5/sex/group) were monitored over a 4 week recovery period to evaluate the regression of any observed effects and then euthanized and necropsied. Complete necropsy was performed and selected organs were removed and weighed for all animals. Histopathological examinations of all organs and tissues were performed for all animals in the control, high-dose 2'-FL, and OF groups at the end of the administration period. Kidneys from all females in the low- and mid-dose groups and in all recovery groups also were microscopically examined.

One male and 1 female in the high-dose group and 2 males in the OF group died on Days 2, 2, 13, and 12 of the administration period, respectively. One female in the high-dose group died on Study Day 108 during the recovery period. The cause of death of these animals could not be determined by histopathological examination. As in the dose-range finding study, colored and/or liquid feces were observed in some animals of the low-dose group, in all animals of the mid- and high-dose groups, and in all animals of the OF group from Day 0 to 13 of the administration period (*i.e.*, up to the time of weaning). These observations are attributed to the non-digestible, fermentable nature of these oligosaccharides and were associated with erythema in the urogenital area in the high-dose and OF groups. Other clinical signs included marked hypersalivation 1 hour after dosing on Days 57 and 71 in some animals of the mid-dose group and in the majority of animals in the high-dose and OF groups. No compound-related ophthalmological findings were reported.

At the beginning of the study (Days 0 to 3), transient, yet statistically significant, lower body weight gains were observed in the mid- and high-dose groups, as well as in the OF group, when compared to the control group. This finding was associated with transient, yet statistically significant, lower body weights in high-dose males, mid- and high-dose females, and in both males and females of the OF group. The lower body weights observed in males of the high-dose group persisted throughout the remainder of the administration period, but this effect did not attain statistical significance at any other time point. Moreover, body weights at the end of

the administration period were not significantly different between any test group and the control group. No significant differences in food consumption were observed between the test groups and the control group.

Some statistically significant changes were noted in the clinical pathology data of the 2'-FL administered groups when compared to the control data; however, all findings were incidental and slight in nature, not dose-dependent, occurred in only one sex, and/or were within the range of historical data, and therefore, were considered to be toxicologically insignificant.

A few statistically significant changes in organ weights were noted at the end of the administration period in the 2'-FL groups compared to the control group. These consisted of the following changes: decreased absolute adrenal weights in mid- and high-dose males; decreased relative adrenal weights in high-dose males; increased epididymide and heart weights in mid-dose males; and decreased relative brain and relative kidney weights in high-dose females. Apart from the decrease in kidney weights noted in females, the organ weight changes observed were not accompanied by histopathological changes, and therefore, were considered to be toxicologically insignificant. Histopathological examination of the kidneys revealed a higher incidence of minimal cortical tubular epithelial cytoplasmic vacuolation in mid- and high-dose females and in females of the OF group at the end of the administration period. However, considering that this finding was not accompanied by histological evidence of renal degeneration and that similar findings were noted in females of the control group at the end of the recovery period, the investigators concluded that the finding was toxicologically insignificant. No other compound-related histopathological findings were reported.

Based on the results of the 90-day study, the investigators concluded that the oral administration of up to 5,000 mg/kg body weight/day of 2'-FL for 13 weeks was well-tolerated by juvenile Wistar [Crl:WI(Han)] rats, with the only notable observations being transient lower body weight gain and colored/liquid feces during the first few days of the administration period. The authors determined the no-observed-adverse-effect level (NOAEL) for 2'-FL to be 5,000 mg/kg body weight/day in Wistar [Crl:WI(Han)] rats. The NOAEL was conservatively placed at the mid-dose of 5,000 mg/kg body weight/day based on the 2 deaths observed at the higher dose of 6,000 mg/kg body weight/day, since the deaths could not be definitely determined to be non-compound-related although there was no evidence of a direct toxic effect.

IV.B.5.2 Mutagenicity Studies on Glycom's 2'-FL

The potential mutagenicity of Glycom's 2'-FL (purity of 99%) was evaluated in the bacterial reverse mutation assay (Ames test) using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102 in the presence or absence of metabolic activation (S9), using the plate-incorporation and pre-incubation methods (Coulet *et al*., 2014). This study was conducted in compliance with the OECD principles of GLP and according to OECD Test No. 471 (OECD, 1997a, 1998b). The water vehicle served as a negative control for all strains. One of the

following compounds was employed as a positive control, depending on the strain, in assays conducted in the absence of S9: 2-nitrofluorene (TA98), sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), or t-butyl hydroperoxide (TA102). For assays conducted in the presence of S9, 2-aminoathracene was employed as the positive control. In the first experiment using the plate incorporation method, bacterial strains were treated with 2'-FL at concentrations of 52, 164, 512, 1,600, or 5,000 µg/plate. In the second experiment using the pre-incubation method, bacterial strains were incubated with 2'-FL at concentrations of 492, 878, 1,568, 2,800, or 5,000 µg/plate. No cytotoxicity or precipitation was observed in any strain treated with 2'-FL in the presence or absence of S9. In both experiments, treatment with 2'-FL did not result in a biologically significant increase in the number of revertant colonies compared with the negative control at any concentration either in the presence of absence of S9. In contrast, positive control agents substantially induced the number of revertant colonies compared to the negative control. Thus, 2'-FL was determined to be non-mutagenic in the Ames test at concentrations up to 5,000 µg/plate.

The mutagenic potential of 2'-FL (purity of 99%) was further investigated in an *in vitro* mammalian cell gene mutation test in L5178Y tk+/- mouse lymphoma cells (Coulet *et al*., 2014). This study also was conducted in compliance with the OECD principles of GLP and according to OECD Test No. 476 (OECD, 1997b, 1998b). Methymethanesulfonate (MMS) and cyclophosphamide were used as the positive controls in the absence and presence of S9, respectively, while water was used as the negative control. In the first experiment, cells were incubated with 2'-FL at concentrations ranging from 1.7 to 5,000 µg/mL for 24 hours without S9. In the second experiment, cells were treated with 2'-FL at concentrations ranging from 492 to 5,000 µg/mL for 4 hours with or without S9. In both experiments, no precipitation or cytotoxicity was observed in cells treated with 2'-FL at any concentration. Also in both experiments, no statistically or biologically significant increases in the frequency of mutations were observed in cells treated with 2'-FL, with or without S9. Therefore, 2-FL was determined to be non-mutagenic in L5178Y tk +/- mouse lymphoma cells.

IV.B.7 Conclusions on the Safety of 2'-FL for Use in Infant Formula Alone and in Infant Formula and in Food Combined (Infants and Toddlers)

The weight of the available evidence supports the safety of the use of 2'-FL in term infant formulas. The safety of the intended uses of 2'-FL in infant formula is supported by Glycom's 2'-FL being chemically identical to the 2'-FL present in human breast milk with resultant intakes being within the range of 2'-FL intakes from mature human breast milk in infants.

The safety of the intended use of 2'-FL in term infant formulas in combination with its use in foods also is supported by Glycom's 2'-FL being chemically identical to the 2'-FL present in human breast milk with resultant combined estimated intakes from its use in infant formula and in food (*i.e.,* in infants and toddlers) being within the established safe background dietary range of 2'-FL intakes from mature human breast milk in infants.

The safety of the intended use of 2'-FL in term infant formulas alone and in term infant formulas in combination with its use in foods also is supported by the available pivotal toxicological data. In the 90-day study conducted in juvenile Wistar [Crl:WI(Han)] rats, the NOAEL for 2'-FL was determined to be 5,000 mg/kg bodyweight/day. It should be noted that while the NOAEL for 2'-FL was determined to be the mid-dose tested as a result of the 2 deaths observed at the highest dose tested of 6,000 mg/kg body weight/day (which could not be definitely determined to be non-compound-related), the NOAEL is determined to be a conservative NOAEL as there was no evidence of a direct toxic effect at the highest dose tested. It should also be noted that the mid and highest doses tested represent the highest doses able to be tested as administration of macronutrients at even greater levels may result in nutritional imbalances, which would complicate a proper safety assessment (Borzelleca, 1996; Munro *et al*., 1996). Thus, the established NOAEL for 2'-FL together with the fact that the greatest 90th percentile daily intakes for the infant and toddler population groups fall within the established safe background dietary range of 2'-FL intakes from mature human breast milk in infants support the safety of the intended use of 2'-FL in term infant formulas and in foods. No safety concerns are therefore raised with respect to the conditions of use of 2'-FL in term infant formulas combined with its use in foods.

IV.C Information to Support the Safety of 2'-FL for Use in Foods (Ages ≥4 Years)

IV.C.1 Comparison of Estimated Intake of 2'-FL from Use in Food to Background Dietary Intake of 2'-FL from Human Breast Milk

As summarized in Section IV.A.3, the greatest estimated heavy consumer intake of 2'-FL among population groups ≥4 years of age was determined to be 77.2 mg/kg body weight/day, which occurred in children. In comparison, as summarized in Section IV.B.1, the background exposure to 2'-FL from mature human breast milk in infants ranges from 170 to 660 mg/kg body weight/day on average, but may be up to 1,150 mg/kg body weight/day. The intake of 2'-FL in population groups ≥4 years of age from its use in food is therefore within the background dietary range of 2'-FL intake from mature human breast milk in infants on a mg per kg body weight basis, which is considered to be the safe reference range of 2'-FL intakes for all population groups.

The safety of the use of 2'-FL in food is therefore supported by the fact that 2'-FL manufactured by Glycom is chemically and structurally identical to 2'-FL present in human breast milk and that the estimated intakes of 2'-FL in population groups ≥4 years of age from its use in food is within the safe reference range of 2'-FL intakes from mature human breast milk in infants.

IV.C.2 Absorption, Distribution, Metabolism, and Excretion

The available data pertaining to the absorption, distribution, metabolism, and excretion of 2'-FL is summarized in Section IV.B.4. As mentioned, HMOs, including 2'-FL, do not undergo any

significant digestion in the upper gastrointestinal tract. While this conclusion is drawn from the results of infant studies, the data also applies to older age groups. Consistent with this, the *in vitro* study performed by Engfer *et al*. (2000) in which HMO fractions and purified HMOs, including 2'-FL, were subjected to *in vitro* digestion was performed using duodenal aspirates and brush border membranes prepared from human *adult* duodenum samples. The results of this study demonstrated that digestive enzymes from human adult samples failed to digest HMOs, including 2'-FL (Engfer *et al*., 2000). Also as previously mentioned, the available data indicate that a large proportion of an ingested amount of an HMO, including 2'-FL, reaches the large intestine and is either fermented by the intestinal microflora or is excreted unchanged in the feces.

IV.C.3 Toxicological Studies

As mentioned the pivotal studies for the safety assessment of Glycom's 2'-FL for use in food are the published product-specific studies, consisting of the 90 day oral toxicity study in juvenile Wistar [Crl:WI(Han)] rats, preceded by a 14 day dose-range finding study, and the *in vitro* mutagenicity studies. These studies are summarized in Sections IV.B.5.1 and IV.B.5.2 above. The rat studies were performed in a juvenile rat model to reflect use of the ingredient for consumption by both infants and by the general population. Based on the results of the 90-day study, the NOAEL for 2'-FL was determined to be 5,000 mg/kg body weight/day in Wistar [Crl:WI(Han)] rats (Coulet *et al*., 2014). As previously noted, while the NOAEL for 2'-FL was determined to be the mid-dose tested, as a result of the 2 deaths observed at the highest dose tested of 6,000 mg/kg body weight/day (which could not be definitely determined to be non-compound-related), the NOAEL is determined to be conservative as there was no evidence of a direct toxic effect at the highest dose tested. Also as previously noted, the mid and highest doses tested represent the highest doses able to be tested as administration of macronutrients at even greater levels may result in nutritional imbalances, which would complicate a proper safety assessment (Borzelleca, 1996; Munro *et al*., 1996). In addition, 2'-FL was shown to be non-mutagenic in the bacterial reverse mutation test and the *in vitro* mammalian cell gene mutation test (Coulet *et al*., 2014).

IV.C.4 Conclusions on the Safety of 2'-FL for Use in Foods

The weight of the available evidence supports the safety of the use of 2'-FL in foods. The safety of the intended uses of 2'-FL in food is supported by Glycom's 2'-FL being chemically identical to the 2'-FL present in human breast milk with resultant estimated intakes in all population groups ≥4 years of age being within the established safe background dietary range of 2'-FL intakes from mature human breast milk in infants on a mg per kg body weight basis and on the available pivotal toxicological data.

The safety of the intended use of 2'-FL in foods also is supported by the available pivotal toxicological data. The NOAEL for 2'-FL was determined to be 5,000 mg/kg bodyweight/day

based on the results of a 90 day study conducted in juvenile Wistar [Crl:WI(Han)] rats. In comparison to the NOAEL for 2'-FL of 5,000 mg/kg body weight/day, the greatest estimated heavy consumer intake of 2'-FL among population groups ≥4 years of age was determined to be 77.2 mg/kg body weight/day, which occurred in children. Thus, a large margin of safety exists between the NOAEL for 2'-FL and the estimated 90th percentile daily intakes in all population groups ≥4 years of age.

The established NOAEL for 2'-FL together with the fact that the greatest 90th percentile daily intakes in all population groups ≥4 years of age fall within the established safe background dietary range of 2'-FL intakes from mature human breast milk in infants support the safety of the intended use of 2'-FL in foods. No safety concerns are therefore raised with respect to the conditions of use of 2'-FL in foods.

IV.D Expert Panel Evaluation

Glycom A/S has determined that 2'-FL is GRAS for use in infant formula and in food as described in Section I.D on the basis of scientific procedures. This GRAS determination is based on data generally available in the public domain pertaining to the safety of 2'-FL, as discussed herein, and on consensus among a panel of experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of infant formula ingredients and food ingredients. The Expert Panel consisted of the following qualified scientific experts: Dr. Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Dr. Ronald Kleinman (Mass General Hospital for Children), Dr. Robert J. Nicolosi (University of Massachusetts Lowell), and Dr. John A. Thomas (Indiana University School of Medicine).

The Expert Panel, convened by Glycom A/S, independently and critically evaluated all data and information presented herein, and also concluded that 2'-FL was GRAS for use in infant formula and in food as described in Section I.D based on scientific procedures. A summary of data and information reviewed by the Expert Panel, and evaluation of such data as it pertains to the proposed GRAS uses of 2'-FL is presented in Appendix B.

IV.E Conclusion

Based on the above data and information presented herein, Glycom A/S has concluded that the intended uses of 2'-FL in infant formula and in food, as described in Section I.D, is GRAS based on scientific procedures. General recognition of Glycom A/S's GRAS determination is supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training, to evaluate the use of 2'-FL in infant formula and in food, who similarly concluded that the intended use of 2'-FL in infant formula and in food as described herein is GRAS.

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.

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APPENDIX A

QUANTITATIVE DATA ON THE CONTENT OF 2’-FL IN HUMAN BREAST MILK

A.1 Content of 2'-*O*-Fucosyllactose (2'-FL) in Human Breast Milk

Individual quantitative data has been reported for ~ **35** different human milk oligosaccharides (HMOs) in at least 30 publications as presented in Figure A.1-1. All available quantitative data on the 2'-FL content of human breast milk derived from diverse samples is presented in Figure A.1-2, while a "reference frame" *average* value for *pooled* mature full-term breast-milk is provided in Figure A.1-3.

Figure A.1-1 Publications reporting quantitative data for individual HMOs[1-30]

#	Reference	Milk-type	Neutral	Acidic	Samples	Population	Term/ Preterm	Lactation day	Method
1	Kunz et al. *Acta Pædiatr.* **1993**	pool	+	+	n/a	Europe	full-term	mature milk	1
2	Thurl & Sawatzki, *Anal. Biochem*. **1996**	**1**	+	+	1	Europe	full-term	mature milk	1
3	Chaturvedi et al. *Anal. Biochem.* **1997**	pool	+		50	Latin America	full-term	30-60	2
4	Coppa et al. *Acta Paediatr. Suppl*. **1999**	**1**	+	+	18	Europe	full-term	4, 10, 30, 60, 90	1
5	Nakhla et al. *Br. J. Nutr*. **1999**	pool	+		15	USA	*preterm*	0-33	1
6	Shen et al. *Anal. Biochem.* **2000**	pool		+	n.d.	USA	full-term	n/a	3
7	Erney et al. *JPGN* **2000**	pool	+		80 Asia 68 Europe 197 Lat. Amer. 36 USA		full-term	0-2, 3-10, 11-30, >31	1
8	Kunz et al. *Ann. Rev. Nutr*. **2000**	pool	+	+	4	Europe	full-term	2-19	n.d.
9	Erney et al, *Bioactive Comp. Hum. Milk* **2001**	pool	+		368	America & Europe	full-term	1-100	1
10	Chaturvedi et al. *Glycobiol.* **2001**	pool	+		12	USA	full-term	mature milk	2
11	Sumiyoshi et al. *Br. J. Nutr.* **2003**	pool	+		16	Japan	full-term	4, 10, 30, 100	4
12	Martin-Sosa et al. *J. Dairy Sci.* **2003**	pool		+	12	Europe	full-term	1-4, 12-17, 28-32	5
13	Morrow et al, *J. Pediatr.* **2004**	pool	+		93	Latin America	full-term	1-100	2
14	Musumeci et al. *JPGN* **2006**	pool	+		53 Africa, 50 Europe		full-term	1, 2, 3	1
15	Bao et al. *Anal. Biochem.* **2007**	pool		+	13	USA	full-term	2-4, 12-67	3
16	Asakuma et al. *Biosci. Biotech. Biochem.* **2007**	pool		+	20	Japan	full-term	1-3	6

#	Reference	Milk-type	Neutral	Acidic	Samples	Population	Term/ Preterm	Lactation day	Method
17	Asakuma et al. *Eur. J. Clin. Nutr.* **2008**	pool	+		12	Japan	full-term	1-3	4, 6
18	Thurl et al. *Br. J. Nutr.* **2010**	**1-3**	+	+	30	Europe	full-term	3-90	1
19	Leo et al. *J. Chromatogr.* **2009**	pool	+	+	16	Samoa	full-term	5-10, 21-155	7
20	Leo et al. *Biosci. Biotech. Biochem.* **2010**	pool	+	+	16	Samoa	full-term	5-10, 21-155	7
21	Asakuma et al. *J. Biol. Chem.* **2011**	pool	+		57	Japan	full-term	30-120	8
22	Coppa et al. *JPGN* **2011**	**1-4**	+		40	Europe	full-term	25-35	1
23	Gabrielli et al. *Pediatr*. **2011**	**1-4**	+	+	63	Europe	*preterm*	4-30	1
24	Galeotti et al. *Anal. Biochem*. **2012**	**1-4**	+	+	n/a	Europe	full-term	4-30	10 & 11
25	De Leoz et al. *J. Proteome Res.* **2012**	**1-4**	+	+	15	USA	*preterm*	mature milk	12
26	Totten et al. *J. Proteome Res.* **2012**	**1-4**	+	+	60	Africa	full-term	1-180	12
27	Bao et al. *Anal. Biochem*. **2013**	**1**	+		4	USA	full-term	3, 14-29	9
28	Smilowitz et al. *J. Nutr.* **2013**	pool	+	+	52	USA	full-term	90	13
29	Galeotti et al. *Electrophoresis* **2014**	**1-4**	+	+	9	Europe	full-term	4-30	14
30	Hong et al. *Anal. Chem.* **2014**	pool	+	+	20	USA	full-term	35	15

Method 01: Gel permeation chromatography, then high-pH anion-exchange chromatography and pulsed amperometric detection HPAEC-PED.
Method 02: Reduced OS, perbenzoylated, reversed-phase HPLC, UV-detection at 229 nm.
Method 03: High-Performance Capillary Electrophoresis of underivatized HMO with UV detection at 205 nm (MEKC micellar electrokinetic chromatography).
Method 04: Gel permeation chromatography, Anion exchange chromatography DEAE sephadex, 2-Aminopyridine derivatization, electrodialysis, RP-HPLC
Method 05: Gel filtration (sephadex), then HPLC using an NH_2-bound silica column and UV detection at 206 nm
Method 06: PMP-derivatization (1-phenyl-3-methyl-5-pyrazolone), RP-HPLC and UV-detection at 245 nm.
Method 07: Anthranilic acid derivatization, RP-HPLC
Method 08: 2-Anthranilic acid fluorescence-labeling, pre-purification (Discovery DPA-6S column), filtration (PTFE membrane), HPLC
Method 09: LC-MS, HMO fraction reduced with sodium borohydrate to alditols, porous graphitic carbon HPLC
Method 10: 2-Aminoacridone derivatization, online HPLC-ESI-MS
Method 11: 2-Aminoacridone derivatization, HPLC with fluorescence detection
Method 12: MALDI FTICR MS and nano-HPLC Chip/TOF with calibration to in-house library foir m/z and retention time values.
Method 13: 600 MHz 1H-NMR, quantitation by peak annotation & integration, and calibration to internal standard
Method 14: 2-Aminoacridone derivatization, Capillary Electrophoresis

Method 15: LC-ESI-MS with multiple reaction monitoring (MRM) on triple quadropole instrument (QqQ). Hypercarb column. Underivatized HMO, calibration to standards.

Figure A.1-2 Quantitative data on the content of 2'-*O*-fucosyllactose (2'-FL) in human breast milk

#	Reference	Samples	Population	Term/ Preterm	Lactation phase (days)	Milk-type	2'-FL (g/L)
2	Thurl et al. *Anal. Biochem.* **1996**	1	Europe	term	mature milk	**1**	**1.84**
3	Chaturvedi et al. *Anal. Biochem*. **1997**	50	Latin America	term	30-60	pool	**1.21**
4	Coppa et al. *Acta Paediatr. Suppl.* **1999**	18	Europe	term	**4**	**1**	**3.93**
					10		**3.02**
					30		**2.78**
					60		**1.84**
					90		**2.46**
5	Nahkla et al. *Br. J. Nutr*. **1999**	13	US	***preterm***	0-33	pool	**1.13**
		2		term	4-128		**1.27**
7	Erney et al. *JPGN* **2000**	11	Asia	term	**0-2**	pool	**2.29**
		25			3-10		**2.26**
		20			11-30		**2.36**
		24			>31		**1.50**
		8	Europe		**0-2**		**3.40**
		14			3-10		**2.69**
		21			11-30		**2.38**
		25			>31		**2.36**
		19	Latin America		3-10		**2.79**
		129			11-30		**2.61**
		49			>31		**1.91**
		4	US		3-10		**2.78**
		8			11-30		**2.56**
		24			>31		**1.69**
9	Erney et, *Bioactive Comp. Hum. Milk* **2001**	268	America & Europe[a]	term	1-100	pool	**2.38**
10	Chaturvedi et al. *Glycobiol.* **2001**	12	US	term	mature milk	pool	**2.43**
11	Sumiyoshi et al. *Br. J. Nutr*. **2003**	16	Japan	term	**4**	pool	**0.20**
					10		**0.34**
					30		**0.29**
					100		**0.05**
13	Morrow et al, *J. Pediatr.* **2004**	93	Latin America	term	1-100	pool	**3.95**
14	Musumeci et al. *JPGN* **2006**	53	***Africa***	term	**1**	pool	**1.80**
					2		**4.50**
					3		**8.40**
		50	Europe		**1**		**1.00**
					2		**2.10**
					3		**4.20**
17	Asakuma et al. *Eur. J. Clin.*	12	Japan	term	**1-3**	pool	**2.03**

#	Reference	Samples	Population	Term/ Preterm	Lactation phase (days)	Milk-type	2'-FL (g/L)
	Nutr. **2008**						
18	Thurl et al. *Br. J. Nutr*. **2010**	30	Germany	term	3-90	**1**	**3.13**
						2	**0.00**
						3	**4.57**
20	Leo et al. *Biosci. Biotech. Biochem.* **2010**[b]	16	***Samoa***	term	5-10	pool	**0.22**
					22-155		**0.69**
21	Asakuma et al. *J. Biol. Chem.* **2011**	57	Japan	term	30-120	pool	**1.48**
22	Coppa et al. *JPGN* **2011**	10	Europe	term	25-35	**1**	**2.56**
		19				**2**	**0.00**
		6				**3**	**2.66**
		4				**4**	**0.00**
23	Gabrielli et al. *Pediatr.* **2011**	35	Europe	***preterm***	**4**	**1**	**7.23**
					10		**5.36**
					20		**4.72**
					30		**4.41**
		18			**4**	**2**	**0.00**
					10		**0.00**
					20		**0.00**
					30		**0.00**
		7			**4**	**3**	**7.36**
					10		**6.69**
					20		**5.83**
					30		**5.85**
		3			**4**	**4**	**0.00**
					10		**0.00**
					20		**0.00**
					30		**0.00**
24	Galeotti et al. *Anal. Biochem*. **2012**	n/a	Europe	term	4-30	**1**	**3.71**
						1	**4.11**
						2	**0.00**
						2	**0.00**
						3	**6.57**
						3	**7.15**
						4	**0.00**
						4	**0.00**
27	Bao et al. *Anal. Biochem.* **2013**	4	US	term	3	**1**	**1.12**
					14-29		**1.08**
28	Smilowitz et al. J. Nutr. **2013**	52	US	term	90	**pool**	**1.22**
29	Galeotti et al. *Electrophoresis* **2014**	3	Europe	term	4-30	**1**	**5.00**
		3				**2**	**0.00**
		2				**3**	**7.80**

#	Reference	Samples	Population	Term/ Preterm	Lactation phase (days)	Milk-type	2'-FL (g/L)
		1				**4**	**0.00**
30	Hong et al. *Anal. Chem.* **2014**	20	USA	term	35	**1+3**	**2.50**
						2+4	**0.48**
RANGE							**0.00 – 8.40**

[a] Samples from mothers from the United States, Mexico, and Europe were combined, and therefore, the results represent a mixed population.
[b] The data in Leo et al. (2010) are duplicates of those presented in Leo et al. (2009).

Figure A.1-3 *Averaged* quantitative data on the content of 2'-*O*-fucosyllactose (2'-FL) in *pooled* full-term mature (lactation days ~ 5-100) human breast milk

#	Reference	Samples	Population	Lactation phase (days)	2'-FL (g/L)
2	Thurl et al. *Anal. Biochem.* **1996**	1	Europe	"mature" milk	**1.84**
3	Chaturvedi et al. *Anal. Biochem*. **1997**	50	Latin America	30-60	**1.21**
4	Coppa et al. *Acta Paediatr. Suppl.* **1999**	18	Europe	< 90	**2.81**
5	Nahkla et al. *Br. J. Nutr*. **1999**	2	US	4-128	**1.27**
7	Erney et al. *JPGN* **2000**	80	Asia	< 90	**2.10**
		68	Europe	< 90	**2.71**
		197	Latin America	< 90	**2.44**
		36	US	< 90	**2.34**
9	Erney et al., *Bioactive Comp. Hum. Milk* **2001**	268	America & Europe[a]	1-100	**2.38**
10	Chaturvedi et al. *Glycobiol.* **2001**	12	US	"mature" milk	**2.43**
11	Sumiyoshi et al. *Br. J. Nutr*. **2003**	16	Japan	< 100	~~0.22~~[b]
13	Morrow et al, *J. Pediatr.* **2004**	93	Latin America	1-100	**3.85**
18	Thurl et al. *Br. J. Nutr*. **2010**	30	Germany	3-90	**2.65**
20	Leo et al. *Biosci. Biotech. Biochem.* **2010**	16	*Samoa*	5-10	~~0.33~~[b]
21	Asakuma et al. *J. Biol. Chem.* **2011**	57	Japan	30-120	**1.48**
22	Coppa et al. *JPGN* **2011**	39	Europe	25-35	**2.06**
24	Galeotti et al. *Anal. Biochem*. **2012**	n/a	Europe	4-30	**3.19**
					3.52
27	Bao et al. *Anal. Biochem.* **2013**	4	US	< 30	**1.10**
28	Smilowitz et al. J. Nutr. **2013**	52	US	90	**1.22**
29	Galeotti et al. *Electrophoresis* **2014**	9	Europe	4-30	**4.26**
30	Hong et al. *Anal. Chem.* **2014**	20	USA	35	**2.08**
AVERAGE					**2.35**

[a] Samples from mothers from the United States, Mexico, and Europe were combined, and therefore, the results represent a mixed population.
[b] Values with a strikethrough are considered outliers and were not included as part of the calculation of the average value.

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APPENDIX B

2’-FL Expert Panel Consensus Statement

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of the Proposed Infant Formula and Food Uses of Human-identical Milk Oligosaccharide 2'-*O*-Fucosyllactose (2'-FL)

August 7, 2014

INTRODUCTION

Glycom A/S (Glycom) convened a panel of independent scientists (the "Expert Panel"), qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients, to conduct a critical and comprehensive evaluation of the available pertinent data and information on the human-identical milk oligosaccharide 2'-*O*-fucosyllactose (2'-FL), and to determine whether the proposed uses of 2'-FL in food production would be Generally Recognized as Safe (GRAS) based on scientific procedures. The Expert Panel consisted of the below-signed qualified scientific experts: Dr. Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Dr. Ronald Kleinman (Mass General Hospital for Children), Dr. Robert J. Nicolosi (University of Massachusetts Lowell), and Dr. John A. Thomas (Indiana University School of Medicine).

The Expert Panel, independently and collectively, critically evaluated a comprehensive package of scientific information and data compiled from the literature. This information was presented in a dossier provided by Glycom [Documentation Supporting the Evaluation of the Human-identical Milk Oligosaccharide 2'-*O*-Fucosyllactose (2'-FL) as Generally Recognized as Safe (GRAS) for Use in Infant Formula and in Food (June 2014)], which included an evaluation of all available scientific data and information, both favorable and unfavorable, relevant to the safety of the intended food uses of Glycom's 2'-FL ingredient. This information was prepared in part from a comprehensive search of the scientific literature performed by Glycom through June 2014 and also included information characterizing the identity and purity of the ingredient, manufacture of the ingredient, product specifications, supporting analytical data, intended conditions of use, estimated exposure under the intended uses, information on the history of consumption from human breast milk, and information on the safety of Glycom's 2'-FL ingredient. In addition, the Expert Panel evaluated other information deemed appropriate or necessary.

Following its independent critical evaluation, the Expert Panel unanimously concluded that the intended uses described herein of Glycom's 2'-FL ingredient, meeting appropriate food-grade specifications, and manufactured consistent with current Good Manufacturing Practice (cGMP), are GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion appears below.

SUMMARY AND BASIS FOR GRAS

The human-identical milk oligosaccharide 2'-*O*-fucosyllactose (2'-FL) is intended to be used as an ingredient in term infant formulas (0 to 12 months) and in a variety of other foods. 2'-FL is a trisaccharide consisting of L-fucose, D-galactose, and D-glucose. Alternatively, 2'-FL can be described as consisting of the monosaccharide L-fucose and the disaccharide D-lactose, which are linked by an alpha(1→2) bond of L-fucose to the galactose moiety to form the trisaccharide. The compound occurs naturally in mammalian milk with the highest concentrations present in *human* breast milk (reviewed in Urashima *et al.*, 2001 and Castanys-Muñoz *et al.*, 2013), and is therefore typically referred to as a *human* milk oligosaccharide (HMO). The molecular structure of 2'-FL has been characterized and is documented in the literature (Kuhn *et al.*, 1956; Jenkins *et al.*, 1984; Fura and Leary, 1993; Asres and Perreault, 1996; Perreault and Costello, 1999; Ishizuka *et al.*, 1999; Rundlof and Widmalm, 2001; Svensson *et al.*, 2002; Urashima *et al.*, 2002, 2004, 2005; Almond *et al.*, 2004; Wada *et al.*, 2008). Glycom's 2'-FL has been analytically determined by ^{1}H-, ^{13}C, and 2D-nuclear magnetic resonance (NMR), mass spectrometry (MS), and x-ray crystallography to be chemically and structurally fully identical to the 2'-FL that is present in human breast milk and is, therefore, referred to as a human-identical milk oligosaccharide (HiMO). 2'-FL, therefore, has an established long history of safe consumption as a normal nutritive component of human breast milk in infants on the basis that the 2'-FL manufactured by Glycom is chemically identical to 2'-FL present in human breast milk.

The Expert Panel critically reviewed details of the manufacturing process for Glycom's 2'-FL that is produced in a process that is highly controlled and compliant with cGMP and the principles of Hazard Analysis and Critical Control Points (HACCP). Appropriate control measures and analytical testing are implemented throughout manufacture to ensure production of a high quality ingredient. The manufacturing process can be broadly divided into two stages. Stage 1 consists of manufacturing the raw material, benzyl-2'-FL, used to produce the final ingredient 2'-FL. In Stage 1, benzyl-2'-FL is produced from highly pure D-lactose, meeting specifications of the National Formulary and the European Pharmacopeia, and highly pure L-fucose through a series of reactions, involving the production of several intermediates. The final intermediate is recrystallized to produce the primary raw material benzyl-2'-FL. The production process for benzyl-2'-FL is tightly controlled under a HACCP plan with critical control points in place at key steps. All precursors and intermediates are single, well-characterized, and pure compounds and isolated intermediates are analyzed at critical control points to ensure conformance to strict specifications. Certificates of analysis are then issued and batch release procedures are followed. The primary raw material, recrystallized benzyl-2'-FL, is a single, well-characterized, and crystalline compound, and all batches of benzyl-2'-FL are controlled for conformity to established strict specifications by certificate of analysis, which ensures production of a high quality and high purity material that is acceptable for use in the production of food ingredients/food.

In Stage 2 of the manufacturing process, the benzyl-2'-FL raw material is subjected to a hydrogenation reaction in the presence of catalytic amounts of citric acid and a palladium/charcoal chemical catalyst to generate 2'-FL. The purification process consists of filtration using a circulation filter and a cartridge filter (2 µm) to remove the catalyst and treatment with activated charcoal and additional filtration to remove coloration and any potential impurities. Microfiltration using a 0.2 µm filter is then applied to ensure absence of microbiological contamination in the 2'-FL solution. The solution then undergoes a series of steps, involving vacuum distillation resulting in the production of a syrup, evaporation, a final microfiltration step (using a 0.2 µm filter), and spray drying to generate the final 2'-FL powdered product. The manufacturing process for 2'-FL is tightly controlled under a HACCP plan with critical control points in place at key steps where in-process controls are applied to minimize the amount of potential inherent impurities to the level technically possible. All processing aids and all filters and filter aids used in the manufacture of 2'-FL from benzyl-2'-FL are food-grade and approved by the United States Food and Drug Administration (U.S. FDA).

Glycom have established appropriate food-grade specifications for 2'-FL. The specifications for 2'-FL include parameters relating to physical properties, purity, water, ash content, lead, and microbiological contaminants. The assay minimum for 2'-FL is set at 95%. Specifications also have been established for acetic acid, carbohydrate-type compounds, and residual solvents to control for their use/production during manufacturing and carryover from raw materials. While Glycom's 2'-FL ingredient is currently manufactured using chemical processes, specifications for residual proteins and microbial endotoxins have been established to provide a control for impurities that might stem from the use of raw materials produced from alternative production processes (*i.e.,* fermentation) and to provide further reassurance that Glycom's 2'-FL ingredient does not contain residual proteins or microbial endotoxins. All analytical methods are nationally or internationally recognized or have been validated. The Expert Panel reviewed the results from 5 batches of 2'-FL and confirm that the data demonstrate that the manufacturing process produces a consistent material in conformance with the product specifications.

The current shelf-life of crystalline 2'-FL has been established by Glycom as 36 months under ambient storage conditions based on the results of an ongoing 5-year real-time stability study. The Expert Panel reviewed the analytical results of the real-time stability study available to date, which confirm that 2'-FL is stable for 36 months, with no significant change in the assay value, when stored at a temperature of 25°C and 60% relative humidity. While a very slight increase in water content (increase of 0.4 w/w %) was observed by 18 months of storage, the water content remained low throughout the 36 months of storage. A 6-month accelerated stability study also was conducted, the results of which demonstrate that 2'-FL is stable for up to 6 months under accelerated conditions (40°C, 75% relative humidity). The Expert Panel also reviewed the results of the stability studies conducted in food matrices representative of the conditions of use of 2'-FL in infant formula and other food and beverage products. 2'-FL has been demonstrated to be stable in powdered infant formula for up to 540 days (approximately 18 months) when

stored at temperatures of up to 37°C. 2'-FL was further demonstrated to be stable in yogurt and in a citrus fruit drink following pasteurization and storage over a period of 21 and 28 days, respectively, when stored under refrigeration (4°C). Therefore, 2'-FL has been sufficiently demonstrated to be stable in infant formula and representative food and beverages under the conditions of these studies.

2'-FL is intended for use in term infant formulas and in a variety of other foods and beverages from selected food categories, including infant and toddler foods (toddler formulas, other baby foods for infants and young children, and other drinks for infants and young children), baked goods and baking mixes, beverages and beverage bases, coffee and tea, dairy product analogs, grain products and pastas, milk (whole and skim), milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and sugar substitutes. The maximum level of use of 2'-FL in term infant formulas is 2,400 mg/L (2.4 g/kg) and is based on providing the same level of 2'-FL that occurs in mature human breast milk, which typically range between 1,100 and 4,260 mg/L (average of approximately 2,350 mg/L) based on pooled data (Chaturvedi *et al.*, 1997, 2001a; Thurl *et al.*, 1996; Coppa *et al.*, 1999, 2011; Nakhla *et al.*, 1999; Erney *et al.*, 2000, 2001; Sumiyoshi *et al.*, 2003; Morrow *et al.*, 2004; Leo *et al.*, 2010; Thurl *et al.*, 2010; Asakuma *et al.*, 2011; Galeotti *et al.*, 2012, 2014; Smilowitz *et al.*, 2013; Hong *et al.*, 2014). Considering that the presence of 2'-FL in human breast milk depends on the "Secretor" phenotype[1], the highest levels of 2'-FL occur in breast milk from the "Secretor" sub-population in which the range is up to 7,000 mg/L in mature human breast milk when the data is viewed according to individual milk type (*i.e.*, non-pooled data) (Gabrielli *et al.*, 2011, Galeotti *et al.*, 2012). The maximum use level in toddler formulas is also 2,400 mg/L. The maximum use level in other drinks for young children is 1,200 mg/L and that in other baby foods for infants and young children is 12 g/kg. The maximum intended use level in most other foods range from 1.2 to 12 g/kg, while the maximum intended use level in breads and baked goods, meal replacement bars, beverage whiteners, and table top sweeteners are 24 to 48, 40, 600, and 300 g/kg, respectively.

An estimate of the dietary exposure to 2'-FL in the U.S. population was performed using individual proposed food uses and use levels in conjunction with survey data obtained from the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) 2009-2010 (CDC, 2011; USDA, 2012). The estimated daily intake of 2'-FL in infants from the use of 2'-FL in infant formulas only was calculated as part of the dietary exposure assessment and reviewed to determine the estimated daily intake of 2'-FL from this use alone in the target population group (*i.e.*, infants). In infants aged 0 to 6 months, the use of 2'-FL in infant formulas only resulted in an all-user estimated 90th percentile intake of 2.91 g 2'-FL/person/day on an absolute basis and 535.6 mg/kg body weight/day on a body weight basis. In infants aged 7 to 12 months, the use of 2'-FL in infant formulas only resulted in an all-user

[1] The "Secretor" phenotype refers to the ability to express the enzyme, α1,2-fucosyltransferase, in the mammary gland, which is responsible for fucosylating lactose at the 2'-*O*-position (approximately 80% of women).

estimated 90th percentile intake of 2.63 g 2'-FL/person/day on an absolute basis and 295.8 mg/kg body weight/day on a body weight basis.

The estimated daily intake of 2'-FL in all other population groups from all food uses of 2'-FL, including infant formula, also was calculated as part of the above dietary exposure assessment. From this assessment, the estimated 90th percentile all-user daily intake of 2'-FL from all uses including infant formula in the total U.S. population was calculated to be 3.21 g/person/day and, on a body weight basis, 65.3 mg/kg body weight/day. The greatest all-user estimated daily intakes of 2'-FL calculated among all population groups on an absolute and body weight basis were determined to be in infants aged 7 to 12 months. The estimated 90th percentile daily intake in infants aged 7 to 12 months was calculated to be 8.36 g/person/day and, on a body weight basis, 987.1 mg/kg body weight/day. The greatest contributor to the estimated daily intake of 2'-FL in this infant population group was the use in baby foods for infants and young children at a contribution level of 65.1%, while use in infant formulas contributed 26.7%. The second greatest all-user estimated daily intakes of 2'-FL calculated among all population groups on an absolute and body weight basis were determined to be in infants aged 0 to 6 months. The estimated 90th percentile daily intake in infants aged 0 to 6 months was calculated to be 5.29 g/person/day and, on a body weight basis, 712.4 mg/kg body weight/day. The greatest contributor to the estimated daily intake of 2'-FL in this infant population group was the use in infant formula at a contribution level of 68.8%, while the use in baby foods for infants and young children contributed 30.8%.

The above estimated daily intakes of 2'-FL were compared to the intake of 2'-FL from mature human breast milk in infants. As mentioned, the level of 2'-FL present in mature human breast milk typically ranges between 1,100 and 4,260 mg/L based on pooled data, but may be up to 7,000 mg/L in milk from mother's with the Secretor phenotype. Based on these levels and on a 6.5-kg infant drinking approximately 1 L of mature human breast milk per day (Davies *et al.*, 1994; Hester *et al.*, 2012), the intake of 2'-FL from mature human breast milk was calculated to be approximately 170 to 660 mg/kg body weight/day and potentially up to 1,150 mg/kg body weight/day. 2'-FL also is present in high concentrations in colostrum, with levels typically ranging from 1,120 to 4,900 mg/L (average of approximately 3,025 mg/L) based on pooled data. However, concentrations as high as 8,400 mg/L have been reported to occur in colostrum from mothers with the Secretor phenotype. Based on these levels and on a 3.4-kg newborn infant drinking an average of 250 mL of colostrum per day during the first 5 days, the intake of 2'-FL from colostrum was calculated to be approximately 80 to 360 mg/kg body weight/day, and may be up to 620 mg/kg body weight/day, for the newborn infant.

In comparison to the intakes of 2'-FL from mature human breast milk, the greatest estimated 90th percentile daily intake of Glycom's 2'-FL in infants from its use in infant formula alone was calculated to be 535.6 mg/kg body weight/day, which was determined to occur in infants ages 0 to 6 months. The estimated intake of 2'-FL from infant formula only is therefore well within the

range of 2'-FL intake from mature human breast milk in infants of up to 1,150 mg/kg body weight/day. When all food uses, including infant formula, were considered, the greatest estimated 90th percentile daily intake of 2'-FL in infants was determined to be 987.1 mg/kg body weight/day, which was determined to occur in infants ages 7 to 12 months. The estimated intake of 2'-FL from all intended food uses, including infant formula, is therefore also within the range of 2'-FL intake from mature human breast milk in the infants. Therefore, the estimated intake of 2'-FL in infants from the consumption of infant formula and all other foods and beverages lies within the safe intake levels of 2'-FL from mature human breast milk.

The estimated intakes of 2'-FL from all food uses in all other population groups also lie within the safe intake levels of 2'-FL from mature human breast milk in infants, which serve as the safe reference intakes.

The Expert Panel critically evaluated published data and information characterizing the safety of 2'-FL. The data included information pertaining to the metabolic fate of HMOs, which indicate that HMOs, including 2'-FL, do not undergo any significant digestion in the upper gastrointestinal tract (Brand-Miller *et al.*, 1995; Brand-Miller *et al.*, 1998; Engfer *et al.*, 2000; Gnoth *et al.*, 2000; Chaturvedi *et al.*, 2001b; Coppa *et al.*, 2001). A small proportion of the total amount of ingested HMO is absorbed as intact HMO; HMOs, including 2'-FL, have been detected unchanged in the urine of breast-fed infants (Rudloff *et al*., 1996, 2006, 2012; Obermeier *et al.*, 1999; Chaturvedi *et al*., 2001b; Rudloff and Kunz, 2012; Dotz *et al*., 2014). From these data, it has been estimated that approximately 1 to 2% of the total amount of ingested HMO is excreted unchanged in urine (Rudloff *et al*., 1996, 2006, 2012; Obermeier *et al.*, 1999; Chaturvedi *et al*., 2001b; Rudloff and Kunz, 2012; Dotz *et al*., 2014). *In vitro* data suggest that the mechanism of absorption of intact HMOs may involve both transcellular receptor-mediated transcytosis and paracellular transport for neutral HMOs and paracellular transport for acidic HMOs (Gnoth *et al.*, 2001). Most of an ingested HMO, however, including 2'-FL, reaches the large intestine where the HMO serves as a substrate for metabolism by the intestinal bacterial microflora or is excreted unchanged in the feces. The proportion of ingested HMO that is available for metabolism by intestinal bacterial microflora has been reported to be near 100% of the ingested amount (Brand-Miller *et al.*, 1998) and the proportion that is excreted in the feces has been reported to be 40 to 50% (Chaturvedi *et al.*, 2001b; Coppa *et al.*, 2001). The data suggest that absorption of Glycom's HiMO 2'-FL is limited and any level of 2'-FL that is absorbed following ingestion would be considered normal on the basis that consumption levels of the ingredient in infants, under the intended conditions of use, would be similar to the intake of 2'-FL from human breast milk in infants, and therefore, the absorption levels would be similar. Therefore, the potential absorption of Glycom's 2'-FL from its consumption is not a safety concern for infants. Since infants are the most sensitive age group, it may be concluded that the absorption of Glycom's 2'-FL does not pose a safety concern for other age groups.

The Expert Panel critically evaluated the toxicological data on Glycom's 2'-FL, including a 14-day oral dose-range finding study and a sub-chronic (90-day) oral toxicity study, in 7-day-old Han Wistar rats (Coulet *et al*., 2014). The 90-day study was conducted in compliance with the Organisation for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practices (GLP) and according to OECD Testing Guidelines No. 408 (OECD, 1998a,b). Juvenile 7-day-old rats were selected for these studies to supplement the standard OECD protocol to better reflect the intended use in the infant target population (Barrow, 2007) and to adequately cover the developmental window during which infants will be exposed. In both studies, oligofructose (OF) (also known as short-chain fructo-oligosaccharide), was used as an oligosaccharide reference control to determine any physiological effects that may be attributed to the administration of oligosaccharides. OF was selected as an appropriate reference control on the basis that it is an oligosaccharide food ingredient with a long and well-established history of safe use in foods and is specifically currently used in infant formula. The doses selected for these studies and overall experimental design were based on a published sub-chronic 90-day rat oral toxicity study on galacto-oligosaccharides and fructo-oligosaccharides (Anthony *et al.*, 2006). A control group administered the vehicle alone (water) was included in both studies. The 14-day dose-range finding study was conducted with 2'-FL doses of 2,000, 5,000, and 7,500 mg/kg body weight/day and an OF dose of 7,500 mg/kg body weight/day; both 2'-FL and OF were administered by gavage. Observations at the highest dose tested included yellow liquid feces and reddening of the urogenital region and 2 unexplained deaths. Based on these results, the 90-day study was conducted with 2'-FL doses of 2,000 (low-dose), 5,000 (mid-dose), and 6,000 (high-dose) mg/kg body weight/day administered by gavage and an OF dose of 6,000 mg/kg body weight/day also administered by gavage. The results of the 90-day sub-chronic study demonstrated no compound-related adverse effects of 2'-FL at doses up to 5,000 mg/kg body weight/day. The no-observed-adverse-effect level (NOAEL) for the oral toxicity of 2'-FL was determined to be 5,000 mg/kg body weight/day based on the results of the 90-day study conducted in juvenile Wistar rats. The NOAEL was conservatively placed at the mid-dose based on 2 unexplained deaths reported at the highest dose, since the deaths could not be definitely determined to be non-compound-related although there was no evidence of a direct toxic effect. It should be noted that while yellow liquid feces and reddening of the urogenital region were observed prior to weaning in animals administered 2'-FL at the mid- and high-doses, the effect also was observed in animals administered the OF reference control at a dose of 6,000 mg/kg body weight/day. The gastrointestinal effects were therefore attributed to the non-digestible, fermentable nature of these oligosaccharides; 2'-FL was as well tolerated as OF. It is further noted that testing at doses greater than those tested in this study was not performed as administration of macronutrients at higher levels may result in nutritional imbalances, complicating a proper safety assessment. Based on outcomes from the 90-day oral toxicity study, the Expert Panel concluded that the oral toxicity of 2'-FL is low and without significant adverse effects.

The toxicological data on Glycom's 2'-FL also included a bacterial reverse mutation assay and an *in vitro* mammalian cell gene mutation test in L5178Y tk+/- mouse lymphoma cells) (Coulet *et al*., 2014). Both tests were conducted in compliance with the OECD principles of GLP and according to OECD Testing Guidelines No. 471 and 476, respectively. All test results reported indicate that 2'-FL is non-mutagenic (OECD, 1997a,b).

The Expert Panel noted that the safety of Glycom's 2'-FL for use in term infant formulas and in foods is supported by the substance being chemically identical to 2'-FL present in human breast milk and by the safe intake of 2'-FL from mature human breast milk by infants at levels greater than those estimated from its addition to infant formula and to food, as well as by the available scientific literature.

CONCLUSION

We, the members of the Expert Panel, have independently and collectively critically evaluated the information summarized above and conclude that the intended use as an ingredient in term infant formula and in food of Glycom A/S's 2'-*O*-fucosyllactose (2'-FL) ingredient, meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice, is safe and suitable.

We, the members of the Expert Panel, have independently and collectively critically evaluated the information summarized above and conclude that the intended use as an ingredient in term infant formula and in food of Glycom A/S's 2'-*O*-fucosyllactose (2'-FL), meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice, is Generally Recognized as Safe (GRAS), based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

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08 August 2014

Professor Joseph F. Borzelleca, Ph.D.
Virginia Commonwealth University School of Medicine

Date

(b) (6)

08/12/2014

Ronald E. Kleinman, M.D.
Mass General Hospital for Children

Date

(b) (6)

14 August 2014

Professor Robert J. Nicolosi, Ph.D.
University of Massachusetts Lowell

Date

(b) (6)

18 August 2014

Professor John A. Thomas, Ph.D.
Indiana University School of Medicine

Date

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