GRAS NOTICE FOR 3'-SIALYLLACTOSE (3'-SL) SODIUM SALT

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

PREPARED BY:
Inbiose N.V.
Technologiepark Zwijnaarde 82 – bus 41
B-9052 Gent
Belgium

DATE:
30 June 2021

GRAS Notice for 3'-Sialyllactose (3'-SL) Sodium Salt

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Part 1. § 170.225 Signed Statements and Certification

In accordance with 21 CFR § 170 Subpart E consisting of § 170.203 through 170.285, Inbiose N.V. (Inbiose) hereby informs the United States (U.S.) Food and Drug Administration (FDA) that the intended uses of 3'-sialyllactose (3'-SL) sodium salt, as manufactured by Inbiose, in non-exempt term infant formula and various conventional food and beverage products as described in Section 1.3 below, are not subject to the premarket approval requirements of the *Federal Food, Drug, and Cosmetic Act* based on Inbiose's view that these notified uses of 3'-SL sodium salt are Generally Recognized as Safe (GRAS). To the best of our knowledge, the data and information presented in this Notice represents a complete and balanced submission that is representative of the generally available literature. Inbiose considered all unfavorable as well as favorable information that is publicly available and/or known to Inbiose and that is pertinent to the evaluation of the safety and GRAS status of 3'-SL sodium salt as a food ingredient for addition to non-exempt term infant formula and various conventional food and beverage products, as described herein.

Signed,

30 June 2021

Joeri Beauprez, PhD
Chief Scientific Officer (CSO)

Date

1.1 Name and Address of Notifier

Inbiose N.V.
Technologiepark Zwijnaarde 82 – bus 41
B-9052 Gent
Belgium

1.2 Common Name of Notified Substance

3'-Sialyllactose sodium salt; 3'-SL sodium salt

1.3 Conditions of Use

Inbiose's 3'-SL sodium salt is proposed for use as an ingredient in non-exempt term infant formula products and a variety of other food and beverage products, as described in previous GRAS Notices (U.S. FDA, 2018a, 2020a,b). Previous GRAS determinations have been made on the use of 3'-SL in non-exempt term infant formulas at levels ranging from 0.2 to 0.28 g 3'-SL sodium salt/L. For food and beverage products intended for use within the general population (*e.g.*, dairy product analogs, milk products, or beverage and beverage bases), 3'-SL sodium salt is considered GRAS at levels up to 12.9 g/L in herbal tea presweetened with low calorie sweetener or sugar, or 25.9 g/kg in meal replacement bars for weight reduction (U.S. FDA, 2018a). As this ingredient would serve as an alternative source of 3'-SL, additive increases in 3'-SL consumption are not expected to occur.

A summary of the proposed food categories and use levels, as described in GRN 766, 880, and 921, is provided in Table 1.3-1 below. The intended conditions of use for Inbiose's 3'-SL sodium salt will be the same as described in GRN 921 for non-exempt term infant formula products, and the same as those described in GRN 880 for all other listed food categories.

Table 1.3-1 Summary of the Individual Uses and Maximum Use Levels for 3'-SL Previously Determined to be GRAS in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2020c)	Proposed Food Use	Maximum Use Levels Described in Previous GRNs (g/kg or g/L)[a]		
		GRN 766[b]	GRN 880	GRN 921
Beverages and Beverage Bases	Meal Replacement Drinks, for Weight Reduction[c]	-	0.5	-
	Sports, Isotonic, and Energy Drinks, Soft Drinks, Enhanced or Fortified Waters, Fruit-based Ades	0.12	0.25	-
	Herbal Tea, Presweetened with Low Calorie Sweetener or Sugar	12.5	-	-
	Cappuccino, Non Fat, With Dairy Milk, Sweetened	0.50	-	-
Infant and Toddler Foods	Non-exempt Term Infant Formulas	0.23	0.2	0.28[d]
	Toddler Formulas	0.24	0.15	-
	Other Baby Foods for Infants and Young Children	0.22 to 1.6	1.25	-
	Other Drinks for Young Children	-	0.15	-
Grain Products and Pastas	Meal Replacement Bars, for Weight Reduction	25.9	5.0	-
	Cereal and Granola Bars	-	2.5	-
Milk, Whole and Skim	Unflavored Pasteurized and Sterilized Milk*	0.12	0.25	-
Milk Products	Buttermilk*	-	0.25	-
	Flavored Milk	0.11	0.25	-
	Milk-Based Meal Replacement Drinks, for Weight Reduction[c]	-	0.5	-
	Yogurt, Frozen	1.0 to 1.6	-	-
	Yogurt*	0.53	2.5	-
Dairy Product Analogs	Imitation Milks	0.12	-	-
	Non-dairy Yogurt	0.53	-	-

Table 1.3-1 Summary of the Individual Uses and Maximum Use Levels for 3'-SL Previously Determined to be GRAS in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2020c)	Proposed Food Use	Maximum Use Levels Described in Previous GRNs (g/kg or g/L)[a]		
		GRN 766[b]	GRN 880	GRN 921
Sugar Substitute	Sugar Substitute, Herbal Extract Powder or Liquid	10% mg/RACC[e]	-	-

3'-SL = 3'-sialyllactose; CFR = *Code of Federal Regulations*; GRAS = Generally Recognized as Safe; GRN = GRAS Notice; RACC = Reference Amounts Customarily Consumed per Eating Occasion; U.S. = United States.
[a] Proposed maximum use levels are presented as g/kg for solids and as g/L for liquids.
[b] Proposed maximum use levels were provided on both a 3'-SL and a 3'-SL sodium salt basis within GRN 766. For consistency, Inbiose has only reported the 3'-SL values in this Notice.
[c] Includes ready-to-drink and powder forms.
[d] It is assumed for the purpose of this Notice that the value reported is provided on a sodium salt basis.
[e] RACC based on values established in 21 CFR § 101.12 (U.S. FDA, 2020d). When a range of values is reported for a proposed food use, particular foods within that food use may differ with respect to their RACC.
* Inbiose's 3'-SL sodium salt is only intended for use in unstandardized products and not in foods where standards of identity exist that preclude its addition.

1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a)(b) of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2020e), Inbiose has concluded that the intended uses of 3'-SL as described herein are GRAS on the basis of scientific procedures.

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notice will be sent to the U.S. FDA upon request, or will be available for review and copying at reasonable times at the offices of:

Inbiose N.V.
Technologiepark Zwijnaarde 82 – bus 41
B-9052 Gent
Belgium

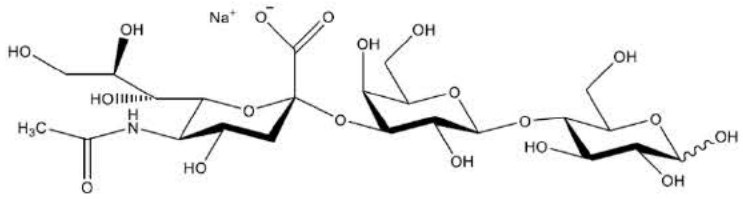
Should the FDA have any questions or additional information requests regarding this Notice, Inbiose will supply these data and information upon request.

1.6 Freedom of Information Act, 5 U.S.C. 552

It is Inbiose's view that all data and information presented in Parts 2 through 7 of this Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore, all data and information presented herein are not exempted from the *Freedom of Information Act*, 5 U.S.C. 552.

Part 2. § 170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

Common Name:	3'-Sialyllactose (3'-SL) sodium salt
Abbreviation:	3'-SL (3'SL, 3-SL, 3SL) sodium salt
International Union of Pure and Applied Chemistry (IUPAC) Name:	*N*-Acetyl-α-D-neuraminyl-(2→3)-β-D-galactopyranosyl-(1→4)-D-glucose, sodium salt
Chemical Abstracts Service (CAS) Number:	128596-80-5
Chemical Formula:	$C_{23}H_{38}NO_{19}Na$
Molecular Weight:	655.53 g/mol
Chemical Structure:	
Schematic Representation:	

2.1.1 Chemical and Physical Characteristics

3'-SL is an abundant human milk oligosaccharide (HMO), comprised of galactose, glucose, and sialic acid.

Inbiose's 3'-SL sodium salt is produced by fermentation with a genetically modified strain of *Escherichia coli* K-12 MG1655. The final product is a purified white powder containing ≥88% 3'-SL sodium salt, and small quantities of lactose, sialic acid, and other related carbohydrates.

The identity of Inbiose's 3'-SL sodium salt has been confirmed by nuclear magnetic resonance (NMR), by comparison with a 3'-SL sodium salt reference standard (Batch ID: 35/01, IsoSep AB, Sweden) derived from human milk. Based on NMR, the Inbiose 3'-SL sodium salt is structurally identical to the IsoSep reference. All peaks seen in the reference material are present in the Inbiose products with the same intensity. The typical shifts of the anomeric protons/carbons and those of the methyl group of the acetyl group further confirm the 3'-SL sodium salt structure.

2.2 Manufacturing

2.2.1 Production Microorganism

2.2.1.1 Host Organism

The host organism is *Escherichia coli* K-12 strain MG1655, which is the same host organism as described in GRN 749, 897, and 951. The taxonomy of the species is as follows:

Bacteria
Proteobacteria
Gammaproteobacteria
Enterobacteriales
Enterobacteriaceae
Escherichia
Escherichia coli
Escherichia coli K-12

The host strain, *E. coli* K-12 strain MG1655, is available from both American Type Culture Collection (ATCC) as 700926 and the Coli Genetic Stock Center as CGSC#7740. *E. coli* strains proliferate *via* asexual reproduction. This strain is nonrecombinant, stable, and can easily be maintained as a homogeneous population under the usual laboratory and production conditions. This strain does not produce spores.

E. coli K-12 strain MG1655 is derived from the well-known *E. coli* K-12 strain *via* classical, nonrecombinant genetics and cured of the temperate bacteriophage lambda and F plasmid by means of ultraviolet light and acridine orange, respectively. The genotype of the recipient microorganism is F-lambda-ilvG-rfb-50 rph-1, and the serotype is IRLH48:K- (Blattner *et al*., 1997). Later additional mutations in commonly used stocks of *E. coli* K-12 strain MG1655 were identified and determined to cause loss of function of the *glpR* and *crl* genes, which are involved in glycerol 3-phosphate and RNA polymerase formation, respectively (Freddolino *et al*., 2012). The complete genome of this strain has been sequenced (GenBank U00096[1]).

The United States Environmental Protection Agency conducted a risk assessment of *E. coli* K-12 under the *Toxic Substances Control Act* (U.S. EPA, 1997). This review concluded that "*the use of E. coli K-12 under contained conditions in fermentation facilities"* will present a low risk of release of this microorganism to the environment and would not pose any significant ecological hazards, based on the following evidence:

1. Wild-type *E. coli* is an inhabitant of the human colon.

2. Studies have demonstrated that *E. coli* K-12 is a debilitated strain, defective in at least 3 cell wall characteristics that are important for colonization. As a result, *E. coli* K-12 is unable to colonize the human intestinal tract under normal conditions. Even in germ free mice, *E. coli* K-12 is a poor colonizer.

3. Evidence indicates indigenous intestinal microorganisms have a large competitive advantage over *E. coli* K-12 strains.

[1] https://www.ncbi.nlm.nih.gov/nuccore/545778205/.

4. *E. coli* K-12 lacks the ability to produce significant quantities of toxins that affect humans. There is no record in the literature of *E. coli* K-12 enterotoxin-induced disease in fermentation workers.

5. *E. coli* K-12 has a history of safe commercial use. Its derivative strains are currently used in a large number of industrial applications, including the production of specialty substances L-aspartic, inosinic, and adenylic acids, which the human body produces, and U.S. FDA-approved human drugs such as insulin and somatostatin.

Because *E. coli* K-12 is not considered a human or animal pathogen and is not toxicogenic it falls into Biosafety Level 1 classification and meets the Organisation for Economic Co-operation and Development (OECD) Good Industrial Large-Scale Practice (GILSP) criteria (OECD, 1992). *E. coli* K-12 strain MG1655 has been classified Biosafety Level 1 by the ATCC[2].

2.2.1.2 Production Strain

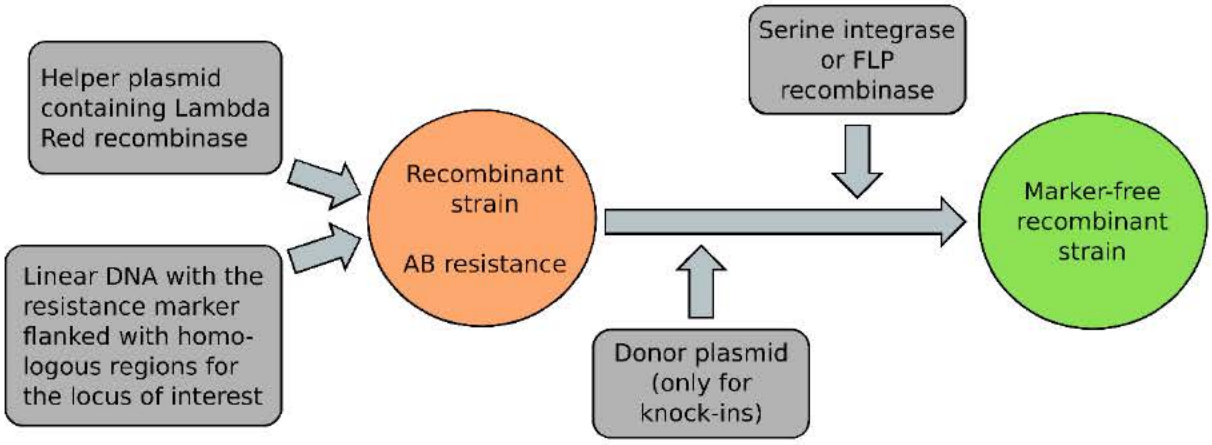
Several modifications, like gene knock-outs, gene insertions, and the addition of a production plasmid, were performed in *E. coli* K-12 strain MG1655 to create the 3'-SL production strain. A production strain, INB-3SL_01, has been developed, through which the safety was assessed.

The general method to introduce genetic modifications like gene deletions and gene knock-ins into the production strain genome is based on the methods described in detail by Datsenko and Wanner (2000) and Snoeck *et al*. (2019). The method is briefly described below in Figure 2.2.1.2-1. In all cases, gene deletions and gene insertions were verified by polymerase chain reaction (PCR), Sanger sequencing and whole genome sequencing (WGS). As validated through WGS, the final strain does not contain any trace of (i) helper plasmids; (ii) antibiotic markers present on the helper plasmids; (iii) or antibiotic markers inserted into the genome. The removal of the helper plasmid is also validated by (i) PCR and (ii) replica plating on a plate containing the antibiotic for which the marker is present on the helper plasmid. In the case of the PCR test, no amplification was observed when the plasmid was not present; in the case of the replica plate, no growth was observed for the strains that did not contain the helper plasmid.

In most cases, DNA scars (att or FRT sites) are left behind, although very small and far apart in the chromosome. Inbiose's host requires an external recombinase to recombine DNA fragments efficiently. The endogenous system requires very large stretches of homology, which are not present in the production host, and is very inefficient. After each modification, each of the previous modifications were checked by PCR and Sanger sequencing to ensure no other modifications occurred during the engineering process. No additional modifications or chromosome re-arrangements were observed, which was validated with WGS.

[2] https://www.atcc.org/~/ps/47076.ashx.

Figure 2.2.1.2-1 General Scheme of the Strain Construction Process*



* At the end after plasmid curing, a complete marker-free recombinant strain is obtained. Helper plasmids used contain a lambda Red recombinase for homologous recombination or a serine integrase recognizing att sites or a FLP recombinase recognizing FRT sites. For genomic knock-ins, an extra donor plasmid containing (heterologous) genes, flanked by att sites, needs to be added.

All heterologous genes introduced into INB-3SL_01 were produced by DNA synthesis and were based on well-known annotated genomes from the respective donor organism. As such, no PCR techniques were used, indicating that there is no risk of undesirable or unintended genes from the donor organism being introduced to the production host. If needed, the heterologous genes were codon-optimized using bio-informatic tools. Finally, before and after the introduction of these heterologous genes into the genome of the production host organism, a full Sanger sequencing of the transcription units was performed to ensure their identity.

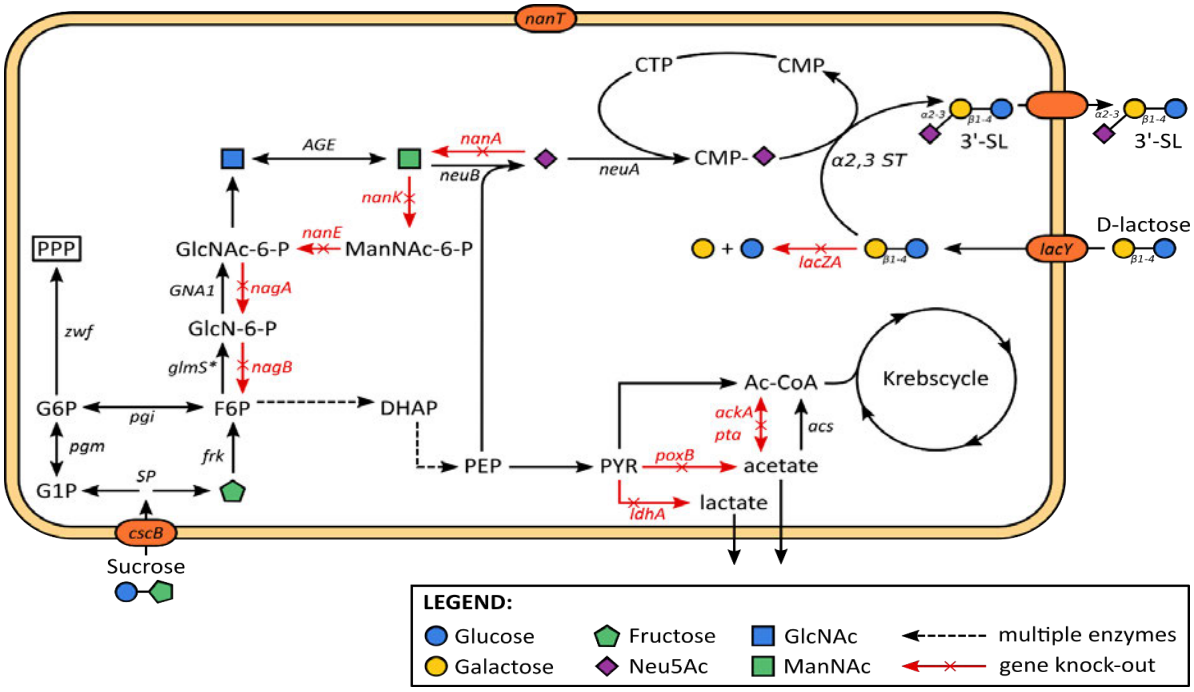
The host organism *E. coli* K-12 strain MG1655 was modified by genomic knock-outs and knock-ins by using the methods as described above to obtain efficient biosynthesis of 3'-SL (see Figure 2.2.1.2-1 above and Table 2.2.1.2-1 below).

Table 2.2.1.2-1 Genetic Modification of the Production Organisms (Gene Knock-ins)

Origin	Function
Escherichia coli	Lactose permease
Escherichia coli	Glutamine-fructose-6-phosphate aminotransferase
Saccharomyces cerevisiae	Glucosamine 6-phosphate *N*-acetyltransferase
Neisseria meningitidis	Sialic acid synthase
Bacteroides ovatus	*N*-acylglucosamine 2-epimerase
Pasteurella multocida	*N*-acylneuraminate cytidylyltransferase
Pasteurella multocida	Sialyltransferase
Escherichia coli	*N*-acetylneuraminate transporter
Escherichia coli	Sucrose permease
Bifidobacterium adolescentis	Sucrose phosphorylase
Zymomonas mobilis	Fructokinase

Knock-outs were performed to avoid breakdown of lactose and sialic acid and to prevent the by-product formation of acetate and lactate (see Figure 2.2.1.2-2). This strain was further modified to biosynthesize 3'-SL by the introduction and overexpression of genes throughout the genome, including genes to make the strain able to grow on sucrose (see Table 2.2.1.2-1 and Figure 2.2.1.2-2). In addition to the chromosomal modifications, a plasmid was also introduced in production host INB-3SL_01 for the overexpression of a *Campylobacter jejuni N*-acylneuraminate cytidylyltransferase and a *Pasteurella multocida* sialyltransferase gene. No antibiotic resistance genes were present on the plasmid. The whole vector was synthesized *de novo* and is named pINB-3SL_01. After strain construction, WGS and colony PCR checks were performed to verify all genetic modifications introduced in the 3'-SL production strain. Production strain INB-3SL_01 does not contain any antibiotic resistance marker on the plasmid or introduced inside its genome.

Figure 2.2.1.2-2 Schematic Overview of the 3'-SL Biosynthetic Pathway in INB-3SL_01 using Sucrose as Carbon Source



3'-SL = 3'-sialyllactose; Ac-CoA = acetyl-coenzyme A; CMP = cytidine monophosphate; CTP = cytidine triphosphate; DHAP = dihydroxyacetone phosphate; F6P = fructose-6-phosphate; G1P = glucose-1-phosphate; G6P = glucose-6-phosphate; GlcN 6 P = glucosamine-6-phosphate; GlcNAc-6-P = N-acetylglucosamine-6-phosphate; ManNAc-6-P = N-acetylmannosamine-6-phosphate; PEP = phosphoenolpyruvate; PPP = pentose phosphate pathway; PYR = pyruvate.

Taxonomical verification was performed with FastANI[3]. Assembled contigs of the production strain were compared to the *E. coli* K-12 MG1655 (U00096.3) reference genome. A whole-genome average nucleotide identity (ANI) of >99.95 was obtained confirming that the production strain is *E. coli* K12 MG1655.

[3] https://github.com/ParBLiSS/FastANI.

Production strain INB-3SL_01 proved to be 100% stable within the production environment after analysis by next generation sequencing of samples at the end of fermentation at pilot scale. The genes integrated into the genome cannot be mobilized or transferred by vector-mediated processes such as conjugation. There are no known lytic phages or conjugation plasmids in these host strains; therefore, transfer can only occur by natural transformation. The integrated genes can be transferred at a frequency normal for chromosomal genes.

The final INB-3SL_01 strain does not contain any trace of helper plasmids used for strain construction, or from the antibiotic marker used in the construction of the helper plasmids. Some DNA scars are left in the genome after constructing gene knock-outs or gene insertions. The removal of helper plasmids is validated by PCR and replica plating on a plate containing the antibiotic for which the marker is present on the helper plasmid.

No specific toxic or allergenic effects are expected from the proteins expressed by the introduced genes (see Section 6.5). These proteins are not secreted, and the cell mass is separated from the product during manufacturing. The absence of these substances has been confirmed in the product specification and batch analyses.

The production process of 3'-SL with INB-3SL_01 does not require the addition of any antibiotics or inducer molecules. During the fermentation process, the production strain remains intact and convert its carbon source sucrose into 3'-SL, which is partly secreted into the medium. Afterwards, the remaining intracellular 3'-SL is released after pasteurization. Finally, all remaining biomass of the production hosts is removed *via* a series of downstream processing steps. As such, the production host is solely used as a processing aid for 3'-SL biosynthesis and cannot be found in the final product.

Production strain INB-3SL_01 was deposited to an internationally recognized culture collection having acquired the status of International Depository Authority under the Budapest Treaty in Belgium.

More specifically, the strain INB-3SL_01 with deposition number LMBP 12731 was deposited at:

Belgian Co-ordinated Collections of Micro-organisms (BCCM)
GeneCorner Plasmid Collection
Ghent University - Department of Biomedical Molecular Biology
Technologiepark-Zwijnaarde 71
9052 Gent
BELGIUM

2.2.2 Raw Materials, Processing Aids, and Equipment Specifications

3'-SL sodium salt is manufactured by Inbiose in compliance with current Good Manufacturing Practice (cGMP), principles of Hazard Analysis and Critical Control Points (HACCP) and Food Safety System Certification (FSSC) 22000. The manufacture of 3'-SL is largely comparable to the production processes previously evaluated for other HMOs with GRAS status (*e.g.*, GRN 749, GRN 880, GRN 881, GRN 897, GRN 921, GRN 922, and GRN 951– U.S. FDA, 2018b, 2020a,b,f,g,h,2021). All of these processes are fermentative based processes for HMOs, similar or the same as 3'-SL sodium salt. All additives, processing aids, and food contact articles used during manufacturing are permitted by federal regulation, have been previously concluded to be GRAS for their respective uses, or have been the subject of an effective food contact notification.

2.2.3 3'-SL Manufacturing Process

In summary, the manufacturing method for 3'-SL entails a fermentation process with a K-12 based production host (see Section 2.2.1) that produces 3'-SL sodium salt. This host produces 3'-SL sodium salt through the utilization of a carbon source (glucose or sucrose) in combination with lactose in a minimal medium. The product is secreted into the medium, where it then undergoes purification before drying to produce the final dry powder product.

In the first step, the biomass is removed together with cell components and large molecules (DNA, protein, and lipopolysaccharides). After the removal of larger particles, the salts present in medium are largely removed, which are cations (*e.g.*, magnesium, calcium, ammonium) and anions (*e.g.*, phosphate and sulfate, which are minerals used for growth of the microorganism), followed by removal of color components. The product is also fully converted into the sodium form in these steps. Water is mainly removed from the product through evaporation. Before drying, the product is filtered again to ensure compliance with the microbial specifications. Figures 2.2.3-1 and 2.2.3-2 below depict the fermentation and purification processes, respectively.

Figure 2.2.3-1 Fermentation Process

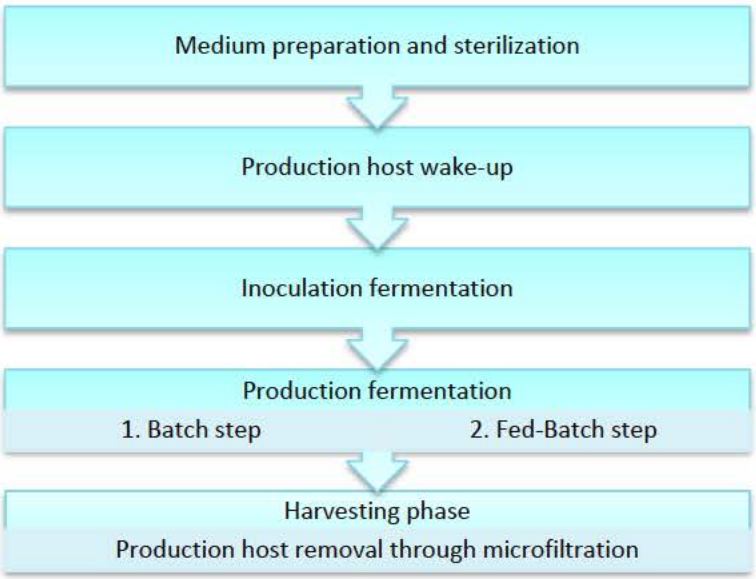
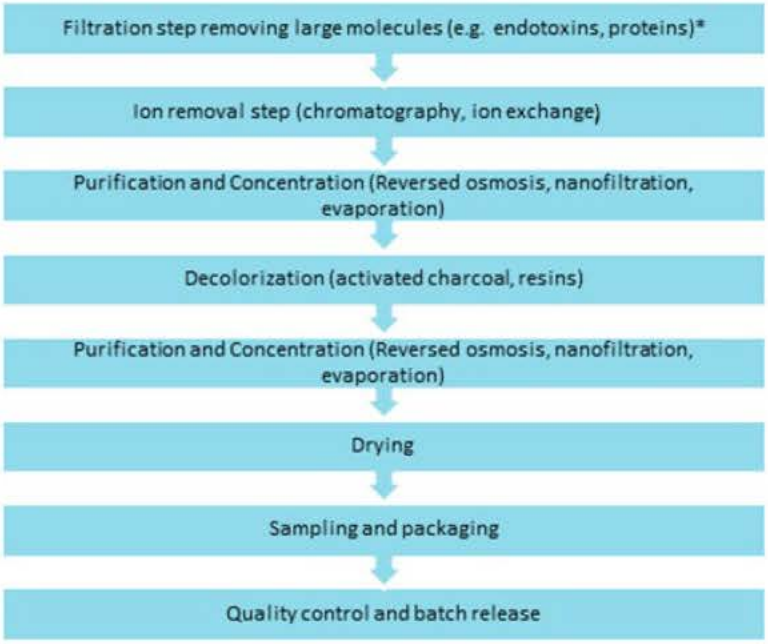


Figure 2.2.3-2 Purification Process



*The filtrations steps are done with cut-offs of 0.1 µm to 5 µm and 1 to 30 kDa.

2.3 Product Specifications and Batch Analyses

2.3.1 Specifications

To ensure consistent product quality, Inbiose has established a set of specifications for 3'-SL sodium salt, which includes the amount of 3'-SL and other main carbohydrates, chemical parameters, heavy metals, microbial contaminants, and absence of the genetically modified production strain and endotoxins. The specifications proposed for 3'-SL sodium salt are presented in Table 2.3.1-1. The specifications of GeneChem Inc's (GeneChem's), Glycom A/S's (Glycom's) and Jennewein Biotechnologie GmbH's (Jennewein's) 3'-SL sodium salt preparations (GRN 766, 880, and 921, respectively) are included in the table for comparison (Glycom A/S, 2019; Jennewein Biotechnology GmbH, 2020; U.S. FDA, 2018a, 2020a,b). All parameters were determined using compendial or validated methods.

Table 2.3.1-1 Product Specifications for Inbiose's 3'-SL Sodium Salt in Comparison to those of the 3'-SL Ingredients in GRN 766, 880, and 921

Parameter	Specification for Inbiose's 3'-SL	Method of Analysis Employed by Inbiose	Specification Reported for Other 3'-SL Products		
			GeneChem's 3'-SL Sodium Salt Siallac3® (GRN 766) (U.S. FDA, 2018a)	Glycom's 3'-SL Sodium Salt (GRN 880) (U.S. FDA, 2020a)	Jennewein's 3'-SL Sodium Salt (GRN 921) (U.S. FDA, 2020b)
Identification					
Appearance (form)	Powder	Visual	Powder	Powder or agglomerates	Spray-dried powder
Appearance (color)	White	Visual	White	White to off-white	White to ivory-colored

Table 2.3.1-1 Product Specifications for Inbiose's 3'-SL Sodium Salt in Comparison to those of the 3'-SL Ingredients in GRN 766, 880, and 921

Parameter	Specification for Inbiose's 3'-SL	Method of Analysis Employed by Inbiose	Specification Reported for Other 3'-SL Products		
			GeneChem's 3'-SL Sodium Salt Siallac3® (GRN 766) (U.S. FDA, 2018a)	Glycom's 3'-SL Sodium Salt (GRN 880) (U.S. FDA, 2020a)	Jennewein's 3'-SL Sodium Salt (GRN 921) (U.S. FDA, 2020b)
Appearance in solution	Clear, colorless to slightly yellow	Visual	Clear colorless solution at 20 mg/mL in water	NS	NS
Identity (3'-SL sodium salt)	Conform to reference standard, 3'-SL sodium salt derived from human milk	UPLC-RI	RT of standard ±3%	NS	NS
pH	4.0 to 7.0 (20°C, 10% solution)	Eurofins' internal method, potentiometry	NS	4.5 to 6.0 (20°C, 5% solution)	NS
Carbohydrates, water free (%DM)					
3'-SL	NLT 85%	UPLC-RI			
3'-SL sodium salt	NLT 88%	UPLC-RI	NLT 98%[a] (% carbohydrates)	NLT 88.0%	NLT 88%
D-Lactose	NMT 5%	UPLC-RI	NS	NMT 5.0%	NMT 5%[b]
Sialic acid	NMT 5%	UPLC-RI	NS	NMT 1.5%	NMT 10%[b]
3'-Sialyllactulose	NS	-	NS	NMT 5.0%	NS
N-acetylglucosamine	NS	-	NS	NS	NMT 5%[b]
Sum of human identical milk saccharides[c]	NLT 90%	UPLC-RI	NS	NLT 90.0%	NS
Other carbohydrates	NMT 10%[b]	UPLC-RI	NS	NMT 3.0%	NMT 12%[b]
Sodium, Na	NMT 4.5%	ICP-AES	NMT 3.5%	2.5 to 4.5%	NMT 4.2%
Chemical Analysis					
Water content, volumetric	NMT 9.0%	Karl-Fischer, volumetric	NMT 6%	NMT 8.0%	NMT 9.0%
Protein content	NMT 100 µg/g	Roti®Nanoquant	NMT 0.1 g/100g	NMT 0.01%	NMT 1%
Ash content[d]	NMT 8.5%	NEN 6810 (500–550°C)	NMT 8.5%	NS	NMT 8.5%
Fat	NS		NMT 0.5 g/100 g	NS	
Endotoxins	NMT 300 IU/g	Ph. Eur. 2.6.14	NMT 300 EU/g	NMT 10 EU/mg	NMT 10 EU/mg
Aflatoxin M1	NS		NS	NS	NMT 0.25 µg/kg
Heavy Metals					
Arsenic	NMT 0.2 mg/kg	ICP-MS	NMT 0.2 ppm	NS	NMT 0.2 ppm
Cadmium	NMT 0.01 mg/kg	ICP-MS	NMT 0.1 ppm	NS	NMT 0.1 ppm
Lead	NMT 0.05 mg/kg	ICP-MS	NMT 0.1 ppm	NMT 0.1 mg/kg	NMT 0.02 ppm
Mercury	NMT 0.1 mg/kg	ICP-MS	NMT 0.5 ppm	NS	NMT 0.5 ppm
Microbiological Contaminants					
Total plate count	NMT 5,000 CFU/g	ISO 4833	NMT 200 CFU/g	NMT 1,000 CFU/g	NMT 10,000 CFU/g

Table 2.3.1-1 Product Specifications for Inbiose's 3'-SL Sodium Salt in Comparison to those of the 3'-SL Ingredients in GRN 766, 880, and 921

Parameter	Specification for Inbiose's 3'-SL	Method of Analysis Employed by Inbiose	Specification Reported for Other 3'-SL Products		
			GeneChem's 3'-SL Sodium Salt Siallac3® (GRN 766) (U.S. FDA, 2018a)	Glycom's 3'-SL Sodium Salt (GRN 880) (U.S. FDA, 2020a)	Jennewein's 3'-SL Sodium Salt (GRN 921) (U.S. FDA, 2020b)
Yeasts	NMT 100 CFU/g	ISO 7954	NMT 200 CFU/g[e]	NMT 100 CFU/g	NMT 100 CFU/g[e]
Molds	NMT 100 CFU/g	ISO 7954	NMT 200 CFU/g[e]	NMT 100 CFU/g	NMT 100 CFU/g[e]
Coliform	NMT 10 CFU/g	ISO 4832	Negative	NS	NS
Enterobacteriaceae	Absent in 10 g	ISO 21528-1	NS	NMT 10 CFU/g	NMT 10 CFU/g
Salmonella spp.	Absent in 25 g	ISO 6579-1	Negative	Absent in 25 g	Absent in 25 g
Cronobacter (Enterobacter) sakazakii	Absent in 25 g	ISO/TS 22964	Negative	NS	Absent in 10 g
Listeria monocytogenes	Absent in 25 g	AFNOR EGS 38/05-03/17	Negative	NS	NS
Bacillus cereus	NMT 50 CFU/g	ISO 7932	NS	NS	NS
Gene residue	Negative	Negative	Negative	NS	Negative
Additional					
Chloride by IC	NS			NMT 1.0 w/w %	

3'-SL = 3'-sialyllactose; CFU = colony forming units; DM = dry matter; EU = endotoxin units; GeneChem = GeneChem Inc.; Glycom = Glycom A/S; GRN = GRAS Notice; HiMS = human-identical milk saccharides; IC = ion chromatography; ICP-AES = inductively coupled plasma atomic emission; ICP-MS = inductively coupled plasma mass spectrometry; ISO = International Organization for Standardization; IU = international units; Jennewein = Jennewein Biotechnologie GmbH; NLT = not less than; NMT = not more than; NS = not specified; Ph. Eur. = European Pharmacopoeia; ppm = parts per million; RT = retention time; UPLC-RI = ultra-performance liquid chromatography refractive index.

[a] Refers to the % of carbohydrate, not represented by % dry matter or %w/w.

[b] Expressed in area %.

[c] Human identical milk saccharides is defined as the sum of 3'-SL sodium salt, lactose, and sialic acid.

[d] Major constituents of the ash are sodium and its (oxidized) derivatives, as well as sulfates and phosphates.

[e] Specification for yeast and mold is combined.

2.3.2 Batch Analysis

Results for the analyses of 5 non-consecutive batches of 3'-SL sodium salt are summarized in Table 2.3.2-1. The data demonstrate that the production process as described in Section 2.2 results in a consistent product that meets the established product specifications.

Table 2.3.2-1 Analytical Data Obtained from 5 Batches of 3'-SL

Parameter	Specification	Lot Nos.				
		ilex13F02	ilex13F03	ilex13F04	ilex013F07	ilex13F08
Identification						
Appearance (color)	White	White	White	White	White	White
Appearance (form)	Dry powder	Dry powder	Dry powder	Dry powder	Dry powder	Dry powder
Appearance in solution	Clear, colorless to slightly yellow	Clear, colorless to slightly yellow	Clear, colorless to slightly yellow	Clear, colorless to slightly yellow	Clear, colorless to slightly yellow	Clear, colorless to slightly yellow
pH (20°C, 10% solution)	4.0 to 7.0	5.28	4.74	4.54	4.73	5.64
Carbohydrates, water free (%area)						
3'-SL	≥85	90.53	88.56	87.93	91.10	89.39
3'-SL sodium salt	≥88	93.67	91.64	90.98	94.26	92.49
D-Lactose	≤5%	0.54	1.05	1.05	0.78	1.05
Sialic acid	≤5%	1.12	0.89	2.42	0.64	1.24
Sum of human identical milk saccharides[a]	≥90%	95.33	93.57	94.44	95.68	94.78
Other carbohydrates[b]	≤5%	0.20	2.19	1.21	0.53	0.29
Chemical Analysis						
Water content, volumetric (% w/w)	≤9.0%	6.7	6.3	5.0	5.4	4.46
Protein content (µg/g)	≤100	27	<25	<25	<25	<25
Total ash (%)	≤8.5%	7.62	7.36	7.44	6.95	8.04
Sodium, Na %	≤4.5%	3.33	3.27	3.28	3.38	3.44
Endotoxin (IU/g)	≤300	2.40	1.65	7.70	27	19.5
Heavy Metals						
Arsenic (mg/kg)	≤0.2	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium (mg/kg)	≤0.01	<0.005	<0.002	<0.002	<0.005	<0.005
Lead (mg/kg)	≤0.05	<0.01	<0.004	<0.004	<0.01	0.016
Mercury (mg/kg)	≤0.1	<0.01	<0.002	<0.002	<0.01	<0.01
Microbiological Contaminants						
Standard plate count (CFU/g)	≤5000	<100	<100	<100	<100	<100
Yeast (CFU/g)	≤100	<10	<10	<10	<10	<10
Mold (CFU/g)	≤100	<10	10[c]	<10	<10	<10
Coliform (CFU/g)	≤10	<10	<10	<10	<10	<10

Table 2.3.2-1 Analytical Data Obtained from 5 Batches of 3'-SL

Parameter	Specification	Lot Nos.				
		ilex13F02	ilex13F03	ilex13F04	ilex013F07	ilex13F08
Enterobacteriaceae	Absent in 10 g	Absent	Absent	Absent	Absent	Absent
Salmonella	Absent in 25 g	Absent	Absent	Absent	Absent	Absent
Cronobacter (Enterobacter) sakazakii	Absent in 25 g	Absent	Absent	Absent	Absent	Absent
Listeria monocytogenes	Absent in 25 g	Absent	Absent	Absent	Absent	Absent
Bacillus cereus (CFU/g)	≤50	<10	<10	<10	<10	<10

3'-SL = 3'-sialyllactose; CFU = colony forming units; IU = international units.
[a] Human identical milk saccharides is defined as the sum of 3´-SL sodium salt, lactose, and sialic acid.
[b] Expressed in area %.
[c] Estimated values

2.3.3 Microbiological Endotoxins and Residual Protein Analysis

The content of endotoxins and residual proteins in the 3'-SL product is determined by methods with high sensitivity [Protein content: Roti®Nanoquant method, based on the Bradford assay; and Endotoxins: kinetic-chromogenic test (Method D) described in the European Pharmacopoeia] to ensure the consistency and quality of the 3'-SL product.

The regulatory batches contain only a small quantity of endotoxin and residual proteins, remaining below the proposed specification limits and, therefore, are not considered a safety concern (see Table 2.3.2-1).

2.3.4 Residual DNA Analysis

To ensure the absence of residual DNA of the production organism, PCRs were performed on the 3'-SL product of 5 regulatory batches. The protocol followed the European Food Safety Authority (EFSA) guidelines for the presence of recombinant DNA. A short subsequence of the inserted *N*-acylglucosamine 2-epimerase gene of *Bacteroides ovatus* on the genome and a subsequence of the *N*-acylneuraminate cytidylyltransferase gene of *Campylobacter jejuni* on the plasmid were targeted to check for residual DNA in the product. For every batch, the analysis was performed in triplicate together with 3 types of positive controls and 1 negative control. The analysis of all regulatory batches of 3'-SL showed no detectable levels of residual DNA in the final product. The limit of detection for the *N*-acylglucosamine 2-epimerase gene subsequence and the *N*-acylneuraminate cytidylyltransferase gene subsequence were both below the threshold limit of detection of 10 ng DNA per gram 3'-SL as it is stated in the EFSA guidelines (EFSA, 2018).

2.4 Stability

The stability of Inbiose's 3'-SL sodium salt is supported by the real-time and accelerated stability studies summarized in GRN 766, 880, and 921. The compositional similarities between Inbiose's 3'-SL sodium salt and the 3'-SL sodium salt ingredients summarized in GRN 766, 880, and 921 (see Section 2.3.1), indicate that the stability of the ingredients will be similar. A summary of the real-time and accelerated stability studies, as described in GRN 766, 880, and 921 is provided below (U.S. FDA, 2018a, 2020a,b).

As described in Section 2.C.5 of GRN 766, the shelf life of GeneChem's 3'-SL sodium salt was assessed *via* a 1-year stability study [25±2°C, 25±6% relative humidity (RH)]. No significant changes in purity, appearance, odor, and stability were observed. The lack of significant change in the evaluated parameters was further substantiated in a 3-month study in which the 3'-SL sodium salt was sealed and stored in a climatic chamber (40±2°C, 24±8% RH).

As described in Section 2.4 of GRN 880, the chemical, physical, microbiological, and sensory stability testing of Glycom's 3'-SL sodium salt (produced from fermentation) was assessed in an ongoing 5-year study under real-time conditions (25°C, 60% RH). The 12-month interim results of 2 representative batches confirmed that 3'-SL sodium salt is stable when stored at ambient room temperatures. The results of an accelerated stability study (40°C, 75% RH) also indicated no changes in the evaluated chemical (3'-SL sodium salt, lactose, sialic acid, unspecified impurities and water content), physical (appearance and color), and microbiological parameters [aerobic mesophilic total plate count, Enterobacteriaceae, *Salmonella spp*., *Cronobacter (Enterobacter) sakazakii*, *Listeria monocytogenes*, *Bacillus cereus*, yeasts, and molds] in 2 representative batches following storage for up to 12 months. Further to this, Glycom's 3'-SL sodium salt was subject to stress/forced stability conditions. In the solid-state testing, 3'-SL sodium salt in powdered form was stored for 28 days at 80°C at ambient and high humidity conditions. A "*negligible concurrent increase of lactose and sialic acid*" and "*slight isomerization of 3'-SL sodium salt to 3'-sialyl-lactulose*" were

observed, increasing with humidity conditions. In the aqueous solution testing, 3'-SL sodium salt was exposed to a pH range (3.0 to 9.0) for 28 days at 35°C or an acid (0.1 N HCl) and base (0.01 N NaOH) for 24 hours at 35°C. 3'-SL sodium salt was stable under neutral pH (6.9) and a minor hydrolysis of 3'-SL to sialic acid and lactose was observed under slightly acidic pH (5.5). 3'-SL sodium salt was completely hydrolyzed to sialic acid and lactose under acidic conditions (pH 3.0, 35°C for 1 month or at 0.1 N HCl, 35°C for 24 hours). A significant (15 to 50%) isomerization of 3'-SL sodium salt to 3'-sialyl-lactulose was observed under the basic conditions (pH 9.0, 35°C for 1 month or at 0.01 N NaOH 35°C for 24 hours).

The stability of Jennewein's 3'-SL sodium salt was assessed in a HMO mixture containing approximately 5% 3'-SL by dry weight, stored in high-density polyethylene bottles under ambient (25°C, 60% RH) and accelerated (40°C, 75% RH) conditions for 52 and 26 weeks, respectively (Part II, Section H; GRN 921). Under ambient conditions, 3'-SL content remained relatively unchanged and moisture increased from 5.7 to 7.8%. Under accelerated conditions, the 3'-SL content decreased and moisture content increased. These data supported the 1-year shelf life of 3'-SL sodium salt when stored under ambient conditions.

The stability of 3'-SL was also assessed under the intended conditions of use. The 3'-SL sodium salt ingredients produced by GeneChem and Glycom were assessed in powdered infant formula, as described in Section 2.C.5.2 of GRN 766 and Section 2.4.2 of GRN 880, respectively. GeneChem's 3'-SL was found to be stable at room temperature (25±2°C, 25±6% RH) for up to 24 months and up to 18 months at accelerated conditions (40±2°C, 24±8% RH). Glycom's 3'-SL added to a powdered infant formula supplemented with other human-identical milk oligosaccharides (HiMOs), long chain polyunsaturated fatty acids (LC-PUFA), vitamins, and minerals was also found to be stable at various temperatures (4°C, 20°C, 30°C, and 37°C) for up to 12 months. The stability testing of GeneChem's 3'-SL in milk and yogurt indicated that 3'-SL sodium salt was stable in milk (content, appearance, and odor) for 45 days at 4±2°C and 25±2°C and within the targeted stability range for 45 days in low temperature yogurt (4±2°C, 26±3% RH). The 3'-SL content did not comply with target stability ranges after 15 days in room temperature yogurt (25±2°C, 25±6% RH).

These results show that 3'-SL is anticipated to be stable in most food matrices.

Part 3. § 170.235 Dietary Exposure

3.1 Estimated Intake of 3'-SL

3.1.1 Methods

In line with GRN 921, Inbiose's 3'-SL sodium salt is intended for use as a food ingredient in term infant formula (0 to 12 months) and toddler formula at concentrations up to 0.28 g/L. In line with GRN 880, Inbiose's 3'-SL sodium salt is also intended for products other than beverages (*e.g*., baby foods) up to 1.25 g/kg. Inbiose's 3'-SL will also be targeted to the general U.S. population in food and beverage products including foods for special dietary use (*e.g*., meal replacement bars) up to 0.5 g/L or 5 g/kg, as described in GRN 880. In line with GRN 766, Inbiose's 3'-SL is intended for use in dairy product analogs, milk (whole and skim), milk products, grain products, beverage and beverage bases and sugar substitutes from 28 to 3,000 mg/Reference Amounts Customarily Consumed per Eating Occasion (RACC), equivalent to 121 to 12,934 mg 3'-SL sodium salt per kg (for solids) or liter (for liquids). As food uses of 3'-SL sodium salt are fully substitutional to current GRAS uses previously determined to be GRAS in GRN 766 and 880, no change in dietary intake of 3'-SL sodium salt are expected from the introduction of Inbiose's 3'-SL sodium salt ingredient to the U.S. marketplace. A summary of the estimated dietary intake of 3'-SL sodium salt from food uses described in GRN 766 and 880 are presented below and are considered applicable to GRAS uses of 3'-SL sodium salt described herein.

3.1.2 Intake Estimates for 3'-SL Sodium Salt

As described in Section 3.A of GRN 766 and Section 3.2 of GRN 880, the estimated intake of 3'-SL sodium salt as an ingredient in term infant formula (0 to 12 months), toddler formula, and other food and beverage products has been estimated from dietary survey data. The intake of 3'-SL sodium salt described in GRN 880 was estimated using food categories representative of each proposed food use chosen from the National Center for Health Statistics' 2013-2014 National Health and Nutrition Examination Survey (NHANES) (CDC, 2015, 2016; USDA, 2016). Based on the proposed uses, more than 80.1% of the evaluated population groups consisted of eligible 3'-SL sodium salt consumers, with infants (7 to <12 months) at 99.9% representing the greatest proportion of potential consumers. They were also established to represent the highest mean and 90th percentile consumer-only intakes of 3'-SL sodium salt on an absolute basis, at 0.44 and 0.82 g/person/day. The summary of the estimated dietary intake of 3'-SL sodium salt in the U.S. population, as described in GRN 880, is provided in Table 3.1.2-1 (U.S. FDA, 2020a).

Table 3.1.2-1 Summary of the Estimated Daily Intake of 3'-SL[a] from Proposed Food Uses in the U.S. by Population Group (2013-2014 NHANES Data)[b]

Population Group	Age Group (Years Unless Otherwise Specified)	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to 6 months	0.24	0.51	80.1	165	0.30	0.55
Infants	7 to <12 months	0.44	0.82	99.9	127	0.44	0.82
Toddlers	1 to 3	0.22	0.47	98.5	465	0.23	0.47
Children	4 to 10	0.18	0.36	99.0	986	0.18	0.37
Female Teenagers	11 to 18	0.16	0.34	94.5	572	0.17	0.34
Male Teenagers	11 to 18	0.23	0.41	98.2	570	0.23	0.41
Female Adults of Childbearing Age	19 to 40	0.18	0.39	92.9	826	0.19	0.39
Female Adults	19 to 64	0.18	0.42	92.9	1,764	0.20	0.43
Male Adults	19 to 64	0.22	0.49	92.7	1,522	0.23	0.50
Elderly	65 and up	0.15	0.37	92.2	917	0.17	0.38
Total Population	All ages	0.19	0.43	93.8	7,088	0.21	0.44

3'-SL = 3'-sialyllactose; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

[a] Intake data expressed as wet weight of ingredient under the proposed conditions of intended use. Wet weight is assumed in this document to be expressed on a 3'-SL sodium salt basis.

[b] Table adapted from GRN 880 (U.S. FDA, 2020a), full intake assessment reported in GRN 880 GRAS determination.

Part 4. § 170.240 Self-Limiting Levels of Use

No known self-limiting levels of use are associated with 3'-SL.

Part 5. § 170.245 Experience Based on Common Use in Food Before 1958

Not applicable.

Part 6. § 170.250 Narrative and Safety Information

6.1 Introduction

The first GRAS conclusion notified to the U.S. FDA for 3'-SL sodium salt was submitted by GeneChem in 2018 (GRN 766; U.S. FDA, 2018a). A critical and comprehensive review of the publicly available data and information pertaining to the safety of 3'-SL sodium salt for use as an ingredient in non-exempt infant formula, and various food and beverage products across multiple categories was presented in the Notice, and the published information pertinent to the safety of 3'-SL sodium salt presented by GeneChem has served as the basis for subsequent GRAS determinations for similar 3'-SL sodium salt preparations (U.S. FDA, 2020a,b). All of these 3'-SL sodium salt preparations are produced from either enzymatic synthesis using *N*-acetyl-D-glucosamine as a precursor or *via* microbial fermentation using genetically modified strains of *E. coli* K-12 DH1 or *E. coli* BL21 DE3. Despite differences in manufacturing process, these 3'-SL sodium salt ingredients are all compositionally highly similar (see Table 2.3.1-1) and therefore safety data conducted with any of these ingredients are generally applicable to all ingredients. Within the previous GRAS Notices, data and information supporting the GRAS use of 3'-SL sodium salt as an ingredient in infant formula and other foods have been critically reviewed by a number of qualified scientific experts, including the FDA, and are publicly available. Additionally, EFSA recently issued an opinion supporting the safe use of 3'-SL sodium salt as an ingredient in a variety of foods, including infant and follow-on formula (EFSA, 2020).

As reported in Part III, Section B of GRN 921, reported concentrations of 3'-SL in human milk can range from 0.08 to 0.41 g/L and unlike other HMOs, concentrations of 3'-SL were not found to differ between Secretor status of the mothers and remain steady throughout lactation time (Austin *et al*., 2016; Kunz *et al*., 2017; Sprenger *et al*., 2017; Ma *et al*., 2018). As such, the use of 3'-SL sodium salt as an ingredient in non-exempt term infant formula at levels up to 0.28 g/L is within the range that infants are exposed to following the ingestion of human milk.

Based on the equivalence of Inbiose's 3'-SL sodium salt to other 3'-SL sodium salt preparations with GRAS status, publicly available data and information establishing the GRAS status of 3'-SL sodium salt are therefore incorporated by reference to previous GRAS determinations in the sections below (U.S. FDA, 2018a, 2020a,b). Since the most recent GRAS determination notified to the U.S. FDA was in 2020, an updated comprehensive search of the publicly available scientific literature was conducted to identify new information relevant to the safety of 3'-SL sodium salt published through 24 March 2021. The following databases were accessed: AdisInsight: Trials, AGRICOLA, AGRIS, Allied & Complementary Medicine, BIOSIS Toxicology, BIOSIS Previews, CAB ABSTRACTS, Embase, Foodline: SCIENCE, FSTA, MEDLINE, NTIS: National Technical Information Service, Toxicology Abstracts, and ToxFile. A summary of the historical basis for the GRAS determination of 3'-SL sodium salt and any newly identified studies relevant to the safety of Inbiose's 3'-SL sodium salt are provided below.

6.2 Absorption, Distribution, Metabolism and Excretion

As discussed previously, 3'-SL produced by microbial fermentation is structurally identical to the 3'-SL found in human milk and will be physiologically equivalent, in terms of absorption, distribution, metabolism, and excretion. Therefore, the metabolism of this HiMO, when added to infant formula, is expected to be identical to those of other HMOs in human breast milk.

The metabolism of HMOs, including 3'-SL, has been previously described in detail (U.S. FDA, 2018a, 2020a,b). Briefly, HMOs are resistant to enzymatic hydrolysis and are therefore not significantly digested in the upper gastrointestinal tract (Brand-Miller *et al*., 1998; Engfer *et al*., 2000; Rudloff and Kunz, 2012; EFSA, 2020). As a result, 3'-SL will reach the large intestine, where HMOs have been observed to be used as nutritive factors by microbiota and may influence their activities (Rudloff *et al.*, 2019). The effects of HMOs on gastrointestinal bacterial growth are bacterial strain- and HMO structure-dependent. Different growth patterns were observed for different bacteria strains when exposed to the same HMOs *in vitro* (Cheng *et al.*, 2021).

Using Caco-2 cells, Gnoth *et al.* (2000) demonstrated that both neutral and acidic HMOs can cross the intestinal epithelial cell monolayers and that a translocation of acidic HMOs exclusively represents a paracellular flux. In an *in vitro* study of various sialyllactoses treated with artificial gastric fluid, Gnoth *et al.* (2000) observed only minor structural changes in the HMOs and concluded that <5% of those ingested would be digested and subsequently absorbed. In breastfed infants, only minimal levels of ingested HMOs have been detected unchanged in the urine (*i.e.*, 1 to 2% of the total HMO fraction).

6.3 Toxicological Studies

6.3.1 Pre-clinical Studies Conducted with Inbiose's 3'-SL sodium salt

Toxicology studies characterizing the genotoxicity and subchronic toxicity of 3'-SL sodium salt in neonatal rats is presented as conducted information on the safety of the ingredient. Findings from these studies are consistent with observations previously reported in the published literature and described in GRN 766, 880, and 921: 3'-SL sodium salt is not genotoxic and does not pose a toxicological safety concern.

6.3.1.1 Genotoxicity

Bacterial Reverse Mutation (Ames) Test (OECD Test Guideline 471)

The potential mutagenicity of Inbiose's 3'-SL sodium salt was evaluated in a bacterial reverse mutation assay conducted in accordance with OECD Test Guideline 471 (OECD, 1997) [Tóth-Gönczöl, 2020 (unpublished)]. The study included a Preliminary Compatibility Test, a Preliminary Range Finding Test (Plate Incorporation Method), an Assay 1 (Plate Incorporation Method) and an Assay 2 (Plate incorporation method without metabolic activation and Pre-Incubation Method with metabolic activation). Formulations were analyzed for concentration.

In the Preliminary Compatibility Test, the solubility of 3'-SL sodium salt was examined using distilled water. 3'-SL sodium salt was soluble at 100 mg/mL concentration using distilled water (clear solutions).

For the range finding test, *Salmonella* Typhimurium (*S.* Typhimurium) TA98 and TA100 tester strains were exposed to 3'-SL sodium salt at 10, 31.6, 100, 316, 1,000, 2,500 or 5,000 µg/plate in the absence and presence of metabolic activation. For the plate incorporation and pre-incubation method *S.* Typhimurium strains TA98 (pKM101), TA100 (pKM101), TA1535, and TA1537 and *E. coli* strain WP2 *uvrA* were exposed to 3'-SL at concentrations of 15.81, 50, 158.1, 500, 1,581, or 5,000 µg/plate (the OECD Test Guideline 471 maximum recommended concentration) in the absence and presence of external metabolic activation (S9 mix prepared from the livers of phenobarbital/β-naphthoflavone-induced rats). Distilled water served as the vehicle for 3'-SL sodium salt and as the negative control. Strain-specific positive controls were also included in the presence [2-aminoanthracene (2AA) 2 µg for all *Salmonella* strains, and 2AA 50 µg for *E. coli* strain WP2 *uvrA*] and absence [4-nitro-1,2-phenylene-diamine (NPD) for *S.* Typhimurium strain TA98,

sodium azide (NaN_3) for *S.* Typhimurium strains TA100 and TA1535, 9-aminoacridine (9AA) for *S.* Typhimurium strain TA1537, methyl-methanesulfonate (MMS) for *E. coli* strain WP2 *uvrA*] of metabolic activation. A list of controls used in the study is provided below in Table 6.3.1.1-1.

There was no evidence of mutagenicity in either test, in the absence or presence of metabolic activation. The mean number of revertant colonies did not show any biologically relevant increase compared to the solvent controls. There were no reproducible dose-related trends and there was no indication of any treatment-related effects. No precipitation nor growth inhibition and no cytotoxic effects of 3'-SL sodium salt were observed in the main assays in all examined bacterial strains.

The mean values of revertant colonies of the negative (vehicle/solvent) control plates were within the historical control range. The positive controls showed a distinct increase of induced revertant colonies in each strain with and without metabolic activation. The viability of the bacterial cells was checked by a plating experiment in each test. The study was considered to be valid. Under the experimental conditions applied in this study, 3'-SL sodium salt did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. The results of this study are provided below in Table 6.3.1.1-2.

Based on the results of the study, it was concluded that 3'-SL sodium salt is non-mutagenic at concentrations up to 5,000 µg/plate (the OECD Test Guideline 471 maximum recommended concentration).

Table 6.3.1.1-1 List of Controls Used in Inbiose's Bacterial Reverse Mutation Assay

Plate Incorporation Method – Assay I					
Test Strains	**TA 98**	**TA 100**	**TA 1535**	**TA 1357**	**WP2 uvrA**
Positive control (+/-S9 mix)	NPD (4 µg/plate)/ 2AA (2 µg/plate)	NaN_3 (2 µg/plate)/ 2AA 2 µg/plate	NaN_3 (2 µg/plate)/ 2AA 2 µg/plate	9AA (50 µg/plate)/ 2AA 2 µg/plate	MMS (2 µL/plate)/ 2AA 50 µg/plate
Plate Incorporation Method (-S9- mix), Pre-Incubation Method (+S9-mix) – Assay II					
Test Strains	**TA 98**	**TA 100**	**TA 1535**	**TA 1357**	**WP2 uvrA**
Positive control (+/-S9 mix)	NPD (4 µg/plate)/ 2AA (2 µg/plate)	NaN_3 (2 µg/plate)/ 2AA 2 µg/plate	NaN_3 (2 µg/plate)/ 2AA 2 µg/plate	9AA (50 µg/plate)/ 2AA 2 µg/plate	MMS (2 µL/plate)/ 2AA 50 µg/plate

2AA = 2-aminoanthracene; 9AA = 9-aminoacridine; MMS = methyl-methanesulfonate; NaN_3 = sodium azide; NPD = 4-nitro-1,2-phenylene-diamine; S9 = metabolic activation.

Table 6.3.1.1-2 Results of Inbiose's Bacterial Reverse Mutation Assay

Concentration (µg/plate)	Revertant Colonies per Plate (Mean ± SD)									
	Without Metabolic Activation (-S9 mix)					With Metabolic Activation (+S9 mix)				
	Salmonella Typhimurium				*Escherichia coli*	*Salmonella* Typhimurium				*Escherichia coli*
	TA98	TA100	TA1535	TA1537	WP2unA	TA98	TA100	TA1535	TA1537	WP2unA
Plate Incorporation Method – Assay I										
Distilled Water	17.7±0.58	85.3±1.15	14.7±1.15	15.7±3.21	42.7±0.58	19.0±1.00	88.0±2.00	16.0±0.00	17.0±1.00	47.7±1.53
5,000	16.3±5.51	68.7±2.89	14.7±1.53	12.7±1.53	42.7±1.15	22.0±2.65	86.0±4.00	13.0±1.00	15.3±1.15	45.0±1.00
1,581	12.0±5.00	77.0±2.65	14.3±0.58	13.0±1.73	43.0±1.00	22.3±0.58	79.7±5.51	12.0±1.00	16.0±0.00	45.7±2.52
500	17.3±1.53	75.0±2.00	13.3±2.08	11.7±1.53	42.7±0.58	24.3±2.08	98.0±10.15	14.7±0.58	15.3±1.15	47.3±1.15
158.1	19.7±1.15	79.0±3.46	14.0±2.65	13.0±0.00	42.7±1.15	25.3±0.58	90.3±7.09	13.3±1.53	15.3±1.15	47.7±0.58
50	19.7±0.58	81.7±3.21	13.7±1.53	10.7±2.08	43.3±0.58	27.0±1.76	96.0±4.00	12.7±2.31	12.7±0.58	45.7±3.21
15.81	18.0±1.00	83.0±7.00	14.0±2.00	8.3±1.15	44.0±2.00	25.7±2.52	87.0±5.57	14.0±1.00	13.7±0.58	46.3±1.53
Positive control[a]	410.7±19.73	1090.7±38.85	1192.0±40.60	402.7±22.03	1109.3±36.07	2390.7±67.69	2416.0±32.00	203.7±4.51	204.0±7.55	250.0±13.11
Plate Incorporation Method (-S9- mix), Pre-Incubation Method (+S9-mix) – Assay II										
Distilled Water	20.0±1.73	92.7±8.96	12.3±1.53	11.0±1.00	42.3±0.58	23.7±4.04	116.7±12.66	12.0±2.00	12.3±0.58	50.7±2.08
5,000	20.3±1.53	84.0±6.56	11.7±0.58	10.7±4.16	42.3±0.58	23.3±2.08	103.0±18.19	13.0±1.00	14.0±2.00	50.7±4.16
1,581	17.7±1.15	80.7±8.02	13.0±1.00	12.7±1.15	40.0±2.65	22.7±4.62	108.7±8.39	13.0±1.73	16.0±0.00	51.3±2.08
500	18.7±4.16	73.7±2.08	13.3±0.58	11.7±1.53	42.0±2.65	25.3±4.62	103.0±2.65	13.0±1.00	16.0±1.00	53.7±0.58
158.1	19.3±2.31	74.3±1.53	13.7±0.58	12.7±1.53	40.7±2.08	23.3±2.31	108.7±10.12	13.0±1.00	15.0±1.73	48.3±0.58
50	19.3±2.31	83.0±2.65	13.0±1.00	12.3±1.53	42.7±0.58	23.7±2.08	115.0±11.53	12.3±0.58	14.7±1.15	49.7±3.06
15.81	21.0±3.00	73.0±3.00	13.3±0.58	15.0±1.73	37.0±3.61	23.3±3.06	96.3±6.35	11.7±0.58	14.7±2.31	49.3±2.31
Positive control[a]	402.7±10.07	1225.3±18.90	1192.0±31.75	409.3±12.22	1064.0±36.66	2442.7±24.44	2466.7±53.27	204.0±6.24	202.0±5.29	231.3±7.02

S9 = metabolic activation; SD = standard deviation.

[a] List of positive controls is included in Table 6.3.1.1-1

***In vitro* Mammalian Cell Micronucleus Test (OECD Test Guideline 487)**

The potential clastogenicity and aneugenicity of 3'-SL sodium salt was evaluated in an *in vitro* micronucleus test with human peripheral blood lymphocytes. This study was conducted in accordance with OECD Test Guideline 487 (OECD, 2016) [Jong, 2020 (unpublished)].

For the pulse exposure, the human lymphocytes were exposed to 3'-SL sodium salt at concentrations of 0 (water for injection and vehicle), 63, 125, 250, 500, 1,000, or 2,000 3´-SL sodium salt µg/mL in the presence [Cyclophosphamide (CP) at a final concentration of 15 and 17.5 µg/mL] and absence [Mitomycin C (MMC-C) at a final concentration of 0.25 and 0.38 µg/mL and Colchicine (Colch) at a final concentration of 0.1 µg/mL] of external metabolic activation (S9 mix) for 3 hours followed by a 24-hour recovery. For the continuous exposure, the human lymphocytes were exposed to 3'-SL sodium salt at concentrations of 0 (water for injection and vehicle), 63, 125, 250, 500, 1,000, or 2,000 6'-SL sodium salt µg/mL in the absence (MMC-C at a final concentration of 0.15 and 0.23 µg/mL and Colch at a final concentration of 0.05 µg/mL) of external metabolic activation (S9 mix) for 24 hours with no recovery.

No precipitation of the test item was observed at the end of treatment. When compared to the vehicle control group, neither a statistically significant nor a biologically relevant increase in the number of micronucleated cells was observed in either independent experiment after treatment with the test item. In the assay with a 24-hour continuous exposure time, additional cells were scored for the vehicle control and intermediate dose level (1,000 µg/mL) of 3´-SL sodium salt to clarify results, which were at the border of 95% range of the historical negative control data (14 micronucleated cells per 2,000 binucleated cells, *versus* 2 micronucleated cells in the negative control). Scoring of additional cells confirmed the negative response (24 micronucleated cells per 4,000 binucleated cells, *versus* 13 in the negative control). The positive control cultures showed statistically significant increases in the frequency of micronucleated binucleated cells (MNBC). It was concluded that the metabolic activation system functioned properly and the study was valid. The results of this study are provided below in Table 6.3.1.1-3.

Based on the results of this study, 3'-SL sodium salt was concluded to have no potential for clastogenicity or aneugenicity in human lymphocytes at doses up to 2,000 µg/mL (the OECD Test Guideline 487 maximum recommended concentration).

Table 6.3.1.1-3 Results of Inbiose's *In Vitro* Mammalian Cell Micronucleus Test

Concentration (µg/mL)	Cytostasis (%)	Culture	Number of Analyzed Micronucleated Binucleated Cells	Number of Binucleated Cells with Micronuclei	
				Per Culture	Per Dose
3-h Treatment, 27-h Harvest Time: Without Metabolic Activation (-S9 mix)					
Vehicle	0	C1	1,000	0	1
		C2	1,000	1	
500	2	C1	1,000	0	2
		C2	1,000	2	
1,000	2	C1	1,000	1	1
		C2	1,000	0	
2,000	-2	C1	1,000	0	0
		C2	1,000	0	
Mitomycin C (MMC-C): 0.25 µg/mL	29	C1	1,000	18	42****
		C2	1,000	24	
Colchicine: 0.1 µg/mL	58	C1	758	10	21****
		C2	806	11	
3-h Treatment, 27-h Harvest Time: With Metabolic Activation (+S9 mix)					
Vehicle	0	C1	1,000	1	2
		C2	1,000	1	
500	-2	C1	1,000	1	2
		C2	1,000	1	
1,000	-1	C1	1,000	1	1
		C2	1,000	0	
2,000	-1	C1	1,000	0	0
		C2	1,000	0	
Cyclophosphamide (CPA): 15 µg/mL	51	C1	1,000	8	12**
		C2	1,000	4	
Cyclophosphamide (CPA): 17.5 µg/mL	57	C1	1,000	14	25****
		C2	1,000	11	

Table 6.3.1.1-3 Results of Inbiose's *In Vitro* Mammalian Cell Micronucleus Test

Concentration (µg/mL)	Cytostasis (%)	Culture	Number of Analyzed Micronucleated Binucleated Cells	Number of Binucleated Cells with Micronuclei	
				Per Culture	Per Dose
24-h Treatment, 24-h Harvest Time: Without Metabolic Activation (–S9 mix)					
Vehicle	0	C1	1,000	1	2
		C2	1,000	1	
Vehicle #Additional scoring	0	C1	1,000	5	11
		C2	1,000	6	
500	10	C2	1,000	6	10
		C1	1,000	4	
1,000	17	C2	1,000	6	14
		C1	1,000	8	
1,000 #Additional scoring	17	C2	1,000	4	10
		C1	1,000	6	
2,000	9	C2	1,000	1	8
		C1	1,000	7	
Mitomycin C (MMC): 0.15 µg/mL	41	C2	1,000	21	39****
		C2	1,000	18	
Colchicine: 0.05 µg/mL	94	C1	1,000	9	21****
		C2	1,000	12	

C1 = culture 1; C2 = culture 2; h = hour(s).
Vehicle: water for injection.
*Significantly different from control group (Chi-square test), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ or **** $P < 0.0001$.
Additional scoring was performed to clarify results. 1,000 additional binucleated cells were assessed for the presence of micronuclei in the vehicle control and intermediate dose level (1,000 µg/mL).

6.3.1.2 Acute Oral Toxicity Test (OECD Test Guideline 425)

The median lethal dose (LD_{50}) of 3'-SL sodium salt was assessed in a single dose acute toxicity study in accordance with OECD Test Guideline 425 (OECD, 2008; Orosz, 2020 [unpublished]). Three female Crl:WI Wistar rats were administered a single dose of 5,000 mg 3'-SL salt/kg body weight dissolved in distilled water *via* gavage, followed by a 14-day observation period. No mortality nor test item-related effects were observed during the study; hence, the LD_{50} was concluded to be greater than 5,000 mg 3'-SL sodium salt/kg body weight (the OECD Test Guideline 425 maximum recommended concentration).

6.3.1.3 Repeat Dose Toxicity Study (Dose Range Finding Study)

A non-Good Laboratory Practice (GLP) 21-day repeat dose toxicity study was conducted in juvenile Sprague-Dawley (SD) rats to evaluate the short-term toxicity of 3'-SL sodium salt and select a maximum dose for the subsequent 90-day subchronic toxicity study (Chalmey, 2020 [unpublished]). Groups of 8 male and female juvenile SD rats were administered 0 (sterile water for injection, vehicle) 3,000, 4,000, or 5,000 mg 3'-SL/kg/day by gavage from Post-natal Day (PND) 7 to 27 at 10 mL/kg/day. A satellite group of SD rats (4/sex) was also allocated to the 5,000 mg/kg body weight (bw)/day group, for the development and further validation of bioanalytical methods of detection of 3'-SL in plasma and urine. Satellite animals received 3'-SL sodium salt daily by gavage until PND 22. All animals were observed daily for mortality and clinical signs. Body weight and food consumption (after weaning) were recorded at designated intervals. Hematology and blood chemistry parameters were measured in principal animals at the end of the treatment period.

On PND 28 (after at least 14 hours fasting), the principal animals were euthanized, and a complete macroscopic post-mortem examination of the principal thoracic and abdominal organs was performed. Selected organs were weighed. Blood samples were collected from the satellite animals on PND 22 at designated time-points, *i.e.,* 0, 1 and 4 hours after gavage. Urine samples were collected at least 24 hours after oral exposure. Satellite animals were euthanized after urine collection and discarded without necropsy.

Two deaths were recorded on PND 28 and 27 in a male and female receiving 3,000 mg/kg bw/day. The cause of deaths could not be clearly established but the intestinal macroscopic/microscopic changes (translucent liquid was observed in the thoracic cavity of both animals, and red color of the lungs in the male) suggested procedure-related deaths. On PND 19, a male receiving 5,000 mg/kg bw/day was found dead. The necropsy revealed a torsion of the ileum around colon, the ileum was distended with gas and contained liquid, the cecum was red and contained red liquid, and the pancreas was tan. The intestinal changes may explain the death of this animal. In the absence of similar macroscopic changes in other animals treated at the same or lower dose levels, any relationship with the test item administration was considered to be unlikely. A female in the 5,000 mg/kg bw/day group was found dead and autolytic on PND 10; thus, a cause of death was not evident at necropsy. Overall, there were no unscheduled deaths that can be undoubtedly attributed to the test item.

A slight decrease (approximately 11%) in food consumption was detected only in males receiving 5,000 mg/kg bw/day, correlating with a minimally lower mean body weight (approximately 11%). However, no organ weight changes were observed that were considered to be related to 3'-SL sodium salt administration. The statistically significant changes in organ weights for brain, liver, spleen, and testis in males at 5,000 mg/kg bw/day were considered to be a reflection of reductions in final body weight compared to controls and not due to test-item-related organ toxicity. In addition, no 3'-SL sodium salt-related macroscopic findings were observed at the end of the treatment period.

There were no test item-related changes on clinical signs, hematology and blood biochemistry parameters. Despite a few statistically significant differences when compared with controls, there were no relevant findings in treated groups. These changes were of low magnitude, lacked dose-response relationship, were not observed at the highest dose level and/or not consistent between both sexes. The statistically significant observations from this study are provided below in Table 6.3.1.3-1.

Therefore, 5,000 mg/kg bw/day, the highest tested dose of 3'-SL sodium salt in this study was considered to be the acceptable high dose level for the 90-day subchronic study in juvenile rats.

Table 6.3.1.3-1 Summary of the Statistically Significant Observations in the 21-day Dose Range Finding Study using Inbiose's 3'-SL Sodium Salt Ingredient

Parameters	Exposure	Sex	Dose group (3'-SL sodium salt mg/kg bw/day)			
			0	3,000	4,000	5,000
Body Weight/Body Weight Change (Mean values ± SD)						
Body weight (g) (1)	Day 4	M	25±1.2	25±0.8	24±1.1	**22**±1.3**
Body weight (g) (1)	Day 8	M	37±1.5	38±1.2	**35**±1.6**	**34**±1.1**
Body weight (g) (1)	Day 8	F	35±1.8	37±0.9	34±2.0	**33*±1.2**
Body weight (g) (1)	Day 11	M	45±0.9	47±1.6	44±2.0	**43**±1.2**
Body weight (g) (1)	Day 15	M	61±3.1	64±2.1	60±3.1	**57*±2.1**
Body weight (g) (1)	Day 15	F	58±2.9	**62*±2.1**	57±3.7	56±1.5
Body weight (g) (1)	Day 18	F	70±3.3	**77**±3.2**	72±4.7	70±3.4
Body weight (g) (1)	Day 21	F	86±4.1	**95**±3.9**	89±6.7	87±5.0
Body weight change (g) (1)	Day 4/8	F	12±1.2	12±1.2	**11*±1.1**	11±0.7
Body weight change (g) (1)	Days 15/18	F	12±1.1	**15**±1.4**	**15**±1.4**	14±2.7
Body weight change (g) (1)	Days 15/18	M	14±1.8	**17*±2.2**	**17**±1.6**	13±2.6
Body weight change (g) (1)	Days 1/21	F	70±1.9	**78**±4.2**	73±5.8	72±4.6
Final Body Weights/Organ Weights (Mean values ± SD)						
Body weight (g) (1)	Day 28	M	89.01±7.42	94.21±4.47	90.23±5.43	**79.59*±5.57**
Body weight (g) (1)	Day 28	F	80.48±5.66	**87.17*±3.42**	82.89±5.28	80.99±4.17
Adrenal glands (g) (1) Mean % body	Day 28	M	0.03747±0.005	0.03494±0.004	0.03124*±0.004	0.04146±0.005
Adrenal Glands (g) (1) Mean % brain	Day 28	M	2.14±0.270	2.06±0.199	**1.71**±0.264**	2.08±0.218
Brain Mean weight (g) (1)	Day 28	M	1.56±0.056	1.60±0.036	**1.65**±0.032**	1.58±0.044
Brain Mean % body (3)	Day 28	M	1.75±0.113	1.70±0.050	1.84±0.093	**2.00#±0.152**
Brain Mean weight (g) (1)	Day 28	F	1.48±0.078	1.55±0.039	**1.58**±0.041**	1.54±0.054
Kidneys Mean weight (g) (1)	Day 28	M	0.96300±0.094	**1.10*±0.128**	0.95975±0.112	0.90943±0.051
Kidneys Mean weight (g) (1)	Day 28	F	0.84600±0.056	**0.98343**±0.080**	0.89788±0.096	0.89329±0.054

Table 6.3.1.3-1 Summary of the Statistically Significant Observations in the 21-day Dose Range Finding Study using Inbiose's 3'-SL Sodium Salt Ingredient

Parameters	Exposure	Sex	Dose group (3'-SL sodium salt mg/kg bw/day)			
			0	3,000	4,000	5,000
Kidneys Mean % brain (1)	Day 28	F	57.12±3.46	**63.62*±5.13**	56.86±5.49	58.17±3.30
Liver Mean % brain (3)	Day 28	M	205.4±13.35	203.3±10.16	191.3±11.29	**176.4#±32.64**
Liver Mean weight (g) (3)	Day 28	F	2.72±0.059	**3.18##±0.308**	2.98±0.238	2.87±0.375
Liver Mean % brain (1)	Day 28	F	183.6±9.54	**205.4*±16.92**	189.0±13.65	186.3±18.05
Spleen Mean % brain (1)	Day 28	M	20.78±3.06	21.52±2.95	17.90±3.94	**16.59*±2.33**
Spleen Mean weight (g) (1)	Day 28	F	0.25038±0.030	**0.30371*±0.042**	0.25813±0.032	0.28800±0.055
Testes Mean weight (g) (3)	Day 28	M	0.74213±0.063	0.73543±0.040	0.70950±0.098	**0.57943#±0.171**
Testes Mean % brain (3)	Day 28	M	47.70±3.40	46.08±2.31	42.84±5.53	**36.62##±10.98**
Thymus Mean % body (1)	Day 28	M	0.45387±0.065	**0.36319*±0.034**	0.40987±0.089	0.40931±0.058
Thyroid glands Mean weight (g) (1)	Day 28	F	0.00888±0.002	**0.01114*±0.001**	0.00988±0.001	0.00943±0.002
Hematology (Mean values ± SD)						
RBC (T/L) (3)	Day 22	M	6.06±0.308	**5.30 #±0.135**	5.56±0.371	5.60±0.287
RTC (T/L) (3)	Day 22	F	6.57±0.383	**5.22 #±0.158**	5.85±0.427	5.85±0.247
HB (G/L) (3)	Day 22	F	13.1±0.68	**11.8 #±0.23**	12.8±0.71	12.9±0.42
PCV (L/L) (3)	Day 22	F	0.44±0.029	**0.38 #±0.006**	0.43±0.031	0.41±0.000
MCV (fL) (3)	Day 22	M	65.5±1.77	71.2±1.28	**72.9##±0.48**	71.8±4.35
MCV (fL) (3)	Day 22	F	67.1±2.29	71.9±1.42	**72.7 #±1.44**	70.2±2.47
MCH (pg) (3)	Day 22	M	20.0±0.38	**22.4#±0.45**	22.3±0.35	22.3±1.06
MCH (pg) (3)	Day 22	F	19.9±0.42	**22.5#±0.47**	21.9±0.60	22.1±0.14
MCHC (g/dL) (3)	Day 22	M	30.5±0.26	**31.4#±0.31**	30.6±0.37	31.0±0.53
MCHC (g/dL) (3)	Day 22	F	29.6±0.56	**31.3#±0.06**	30.0±0.45	31.5±0.85

Table 6.3.1.3-1 Summary of the Statistically Significant Observations in the 21-day Dose Range Finding Study using Inbiose's 3'-SL Sodium Salt Ingredient

Parameters	Exposure	Sex	Dose group (3'-SL sodium salt mg/kg bw/day)			
			0	3,000	4,000	5,000
RTC (%) (3)	Day 22	M	12.08±1.014	**14.29#±0.855**	12.70±0.438	13.58±1.669
RTC (%) (3)	Day 22	F	11.42±0.328	**13.49#±0.660**	12.36±0.808	12.52±1.457
E (G/L) (3)	Day 22	F	0.02±0.013	0.01±0.006	**0.00#±0.005**	0.03±0.007
Blood Biochemistry (Mean values ± SD)						
Ca++ (mmol/L) (3)	Day 22	F	2.31±0.061	2.62±0.079	**2.63#±0.090**	2.62±0.097
GLUC (mmol/L) (3)	Day 22	F	7.37±0.281	**9.10#±0.661**	7.67±0.778	6.78±0.849
CREAT (µmol/L) (3)	Day 22	M	16.79±1.686	21.21±1.207	**21.09#±1.578**	20.53±1.307
CREAT (µmol/L) (3)	Day 22	F	17.70±1.808	**21.65#±1.786**	21.05±1.033	20.63±2.656
A/G (3)	Day 22	M	2.63±0.070	2.41±0.101	**2.37#±0.095**	2.60±0.073
ALAT (3)	Day 22	M	58±10.0	36±3.8	43±9.0	**33#±9.9**

3'-SL = 3'-sialyllactose; A/G = albumin/globulin ratio; ALAT = alanine aminotransferase; Ca^{++} = calcium; CREAT = creatinine; E = eosinophils; F = female; GLUC = glucose; HB = hemoglobin; M = male; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; MCV = mean cell volume; PCV = packed cell volume; RBC = erythrocytes; RTC = reticulocytes; SD = standard deviation; UREA = urea.

* P<0.05, ** P<0.01 (1) DUNNETT TEST

# P<0.05, ## P<0.01 (3) DUNN TEST

Assigned control group(s) : 1.

6.3.1.4 Subchronic Toxicity Study (OECD Test Guideline 408)

The 90-day study in juvenile rats is currently in progress. The end of *in vivo* phase (last day of euthanasia) was scheduled in Week 10, 2021. This study was conducted in accordance with OECD Test Guideline 408 (OECD, 2018).

The objective of this study is to evaluate the potential toxic effects of the test item, 3'-SL sodium salt, on the development of juvenile rats, following daily oral administration from PND 7 to at least PND 97. The study is intended to cover the period of development corresponding to infancy, childhood, and adolescence.

On completion of the treatment period, designated animals (control and high-dose level groups) will be held for a 5-week treatment-free period in order to evaluate the reversibility of any findings.

In addition, 3 male and 3 female satellites per group will be dosed for toxicokinetic assessment. The results of this study will be provided as supplemental information to the notice once available.

6.3.1.5 Summary of Studies Conducted with Inbiose's 3'-SL sodium salt

Table 6.3.1.5-1 Summary of Toxicological Studies to Support the Safety of Inbiose's 3'-SL Sodium Salt

Type of Study	Species or Cell Type	Length of Study	3'-SL Dose and Route of Administration	Result	Reference
Single dose acute toxicity study up and down procedure (OECD TG 425)	Three female Crl:WI Wistar rats	Single dose followed by a 14-day observation period	5,000 mg/kg bw 3'-SL sodium salt dissolved in distilled water – the highest recommended dose	LD_{50} of 3'-SL was found to be greater than 5,000 mg/kg bw	Orosz [Unpublished], 2020 final report
Bacterial reverse mutation test (OECD TG 471)	*Salmonella* Typhimurium strains TA98 (pKM101), TA100 (pKM101), TA1535, and TA1537 and *Escherichia coli* strain WP2 uvrA	Plate incorporation assay and pre-incubation method	0 (distilled water, vehicle); up to 5,000 µg/plate (±S9 mix)	3'-SL is non-mutagenic under the conditions of this test.	Tóth-Gönczöl [Unpublished], 2021 final report
In vitro micronucleus assay (OECD TG 487)	Human peripheral blood lymphocytes	+S9 = 3 h -S9 = 3 and 24 h	0 (water for injection, vehicle) 125, 250, 500, 1,000, and 2,000 3'-SL sodium salt µg/mL (2,000 µg/mL being the highest recommended limit dose level)	3'-SL sodium salt did not induce any chromosome damage or damage to the cell division apparatus.	Jong [Unpublished], 2021 final report
Preliminary toxicity study by oral route (gavage) in Juvenile rats	Group of 8 male and 8 female juvenile SD rats	21 days from PND 7	0 (water for injection, vehicle) 3,000, 4,000, or 5,000 mg/kg bw/day by gavage	5,000 mg/kg bw/day was selected as the appropriate highest dose for the main 90-day study.	Chalmey [Unpublished], 2021 final report

Table 6.3.1.5-1 Summary of Toxicological Studies to Support the Safety of Inbiose's 3'-SL Sodium Salt

Type of Study	Species or Cell Type	Length of Study	3'-SL Dose and Route of Administration	Result	Reference
90-day toxicity study by oral route (gavage) in Juvenile rats followed by a 5-week treatment-free period (OECD TG 408)	Groups of 10 male and 10 female neonatal Crl:CD(SD) rats In addition, 5 males and 5 females in control and 5,000 mg/kg bw/day group for recovery period For toxicokinetic assessment, groups of 3 male and 3 female satellite animals	90 days	0 (water for injection), 1,500, 3,000, or 5,000 mg/kg bw/day of 3'-SL sodium salt, by oral gavage	In progress.	Spezia, 2021 [Unpublished], Draft report due July 2021

3'-SL = 3'-sialyllactose sodium salt; bw = body weight; h = hours; LD_{50} = median lethal dose; OECD = Organisation for Economic Co-operation and Development; PND = Post-natal Day; S9 = metabolic activation mix; SD = Sprague Dawley; TG = Test Guideline.

6.3.2 Pre-Clinical Studies Conducted with Other 3'-SL Sodium Salt Preparations

Pivotal safety data and information has been discussed previously and is hereby incorporated by reference to Part 6.D and 6.E of GRN 766, Section 6.4 of GRN 880, and Section VI Part C of GRN 921 (U.S. FDA, 2018a, 2020a,b). Analytical data of Inbiose's 3'-SL sodium salt product establish the ingredient as chemically identical to its 3'-SL counterpart in human breast milk (see Section 2.1.1). Based on analytical data presented demonstrating that 3'-SL produced by Inbiose is of similar purity to 3'-SL preparations that have previously been concluded to be GRAS, studies characterizing the toxicity and safety of 3'-SL in animal models are considered relevant to the safety assessment of Inbiose's ingredient.

No evidence of toxicity related to the administration of 3'-SL sodium salt, or its constitutional isomer, 6'-SL sodium salt, has been reported in any previous 3'-SL sodium salt GRAS Notice submission (U.S. FDA, 2018a, 2020a,b). Additionally, there were no new data identified evaluating the potential toxicological or genotoxic effects of the ingredient since the previous 3'-SL sodium salt GRAS determination was prepared (GRN 921).

The toxicological studies in GRN 766, 880, and 921 are briefly summarized below in Table 6.3.2-1.

Table 6.3.2-1 Summary of Toxicological Studies to Support the Safety of Inbiose's 3'-SL Sodium Salt

Type of Study	Species or Cell Type	Length of Study	3'-SL Dose and Route of Administration	Result	Reference
Studies Conducted with GeneChem's 3'-SL (GRN 766)					
Bacterial reverse mutation test	*Salmonella* Typhimurium strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 uvrA (pKM101)	Plate incorporation assay and pre-incubation assay	Up to 5,000 µg/plate (±S9)	3'-SL is non-mutagenic at concentrations up to 5,000 µg/plate.	Kim *et al.* (2018)
In vitro chromosome aberration test	CHL/IU cells	Growth inhibition study (±S9) and continuous treatment (-S9)	Up to 5,000 µg/mL	3'-SL is not clastogenic at concentrations up to 5,000 µg/mL.	
In vivo mammalian erythrocyte micronucleus test	54 male and female ICR mice	3 days Vehicle control: Saline	500, 1,000, and 2,000 mg/kg bw of 3'-SL, by gavage	Under the conditions of this study, 3'-SL sodium salt did not induce micronuclei in the bone marrow cells of mice.	
Acute toxicity study	Groups of 5 male and female SD rats	Single dose	0, 5, 10, 15, or 20 g/kg bw of 3'-SL, by gavage	The LD_{50} and the MTD of 3'-SL sodium salt were greater than 20 g/kg bw, the highest dose tested.	
Subacute toxicity study	Two male and female Beagle dogs	Single dose at 4-day intervals	0, 500, 1,000, and 2,000 mg/kg bw of 3'-SL	The MTD was greater than 2,000 mg/kg bw in male and female beagle dogs.	
28-day subacute toxicity study	Groups of 20 male and female SD rats	28 days	0, 500, 1,000, or 2,000 mg/kg bw of 3'-SL, by gavage	The NOAEL was greater than 2,000 mg/kg bw, the highest dose tested.	
Subacute toxicity study	48 neonatal piglets	3 weeks	140, 200, or 500 mg/L of 3'-SL dissolved in water and added to diet	No effect on food consumption, growth, bw, hematology parameters, electrolytes and minerals and serum enzymes. 3'-SL was well tolerated.	Unpublished [Donovan (2017)]
Subacute toxicity study	54 neonatal piglets	3 weeks	0 (control), 2 or 4 g 3'-SL, 2 or 4 g 6'-SL or 2 g polydextrose/L + 2 g galacto-oligosaccharides/L *via* the diet	No significant differences in initial bw, weight gain, feed intake, feed:gain ratios, fecal consistency, and diarrhea scores across the treatment groups. The oligosaccharide diets were well tolerated by the pigs.	Jacobi *et al.* (2016)

Table 6.3.2-1 Summary of Toxicological Studies to Support the Safety of Inbiose's 3'-SL Sodium Salt

Type of Study	Species or Cell Type	Length of Study	3'-SL Dose and Route of Administration	Result	Reference
90-day subchronic toxicity study	Groups of 10 male and female SD rats	90 days	0, 500, 1,000, and 2,000 mg/kg bw/day of 3'-SL, *via* gavage	The NOAEL for 3'-SL sodium salt was determined to be higher than 2,000 mg/kg bw/day, the highest dose tested.	Kim *et al.* (2018)
Studies Conducted with Glycom's 3'-SL (GRN 880)					
Bacterial reverse mutation test	*S.* Typhimurium strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2 uvrA (pKM101)	Plate incorporation assay and pre incubation assay	Up to 5,000 µg/plate (±S9)	3'-SL is non mutagenic at concentrations up to 5,000 µg/plate.	Phipps *et al.* (2019)
In vitro mammalian cell micronucleus test	Human lymphocytes	+S9 = 3 h -S9 = 3 and 24 h	Up to 2,000 µg/mL	3'-SL is neither clastogenic nor aneugenic at concentrations up to 2,000 µg/mL.	
14-day oral toxicity study	Groups of 8 male and 8 female neonatal rats	14 days	0 (water for irrigation), 4,000, or 5,000 mg/kg bw/day of 3'-SL, by gavage	5,000 mg/kg bw/day of 3'-SL is the highest dose selected in the subchronic study.	
90-day oral toxicity study	Groups of 10 male and 10 female neonatal Crl:CD(SD) rats	90 days	0 (water for irrigation), 1,000, 3,000, or 5,000 mg/kg bw/day 3'-SL, by gavage	NOAEL is 5,000 mg/kg bw/day of 3'-SL.	
Tolerability study in neonatal pigs	38 naturally farrowed male neonatal piglets	31 to 32 days	*via* diet • Days 3 to 7 of age: 16, 45, 122, or 222 mg/kg bw/day • Day 8 of age onwards: 18, 52, 139, or 253 mg/kg bw/day	No reported adverse effects, bw gain and no differences in intestinal length or weight between controls and sialyllactose-treated groups. No test item-related differences in clinical pathology values were observed.	Monaco *et al.* (2018)
Tolerability study in neonatal pigs	Groups of 24 male and female neonatal piglets	21 days	*via* diet • Days 1 to 5 of age: 300 mL/kg bw/day • Day 6 of age onwards: 360 mL/kg bw/day	No reported adverse effects, bw gain and no differences in intestinal length or weight between controls and sialyllactose-treated groups. No test item-related differences in clinical pathology values were observed.	Monaco *et al.* (2019)

Table 6.3.2-1 Summary of Toxicological Studies to Support the Safety of Inbiose's 3'-SL Sodium Salt

Type of Study	Species or Cell Type	Length of Study	3'-SL Dose and Route of Administration	Result	Reference
Studies Conducted with Jennewein's 3'-SL as Part of an HMO Mixture[a] (GRN 921)					
Bacterial reverse mutation assay	*S.* Typhimurium strains TA98, TA100, TA102, TA1535, and TA1537	Plate incorporation test and pre incubation test	5, 10.0, 31.6, 100, 316, or 600 mg of the HMO mixture per plate containing 0.21, 0.41, 1.3, 4.1, 13.0, and 24.6 mg 3'-SL per plate	The HMO mixture, and the 3'-SL contained therein, was not mutagenic under the conditions tested.	Parschat *et al.* (2020)
In vitro micronucleus assay	Human peripheral blood lymphocytes	4 or 24 hours (±S9)	7.5, 15, 30, and 60 mg HMO mixture/mL medium (equivalent to 0.31, 0.62, 1.23, and 2.46 mg 3'-SL/mL medium)	The HMO mixture was not genotoxic under the tested conditions at concentrations up to 60 mg/mL (2.46 mg/mL 3'-SL).	
Seven-day pilot dietary toxicity study	Groups of 5 female CD/Crl:CD rats	7 days	A control diet or the same diet containing 10% of an HMO mixture (equivalent to 0.4% 3'-SL)	No HMO-related differences in behavior, appearance and consistency of the feces, bw, bw gain, or feed consumption were observed.	
90-day feeding study	Groups of 10 male and female CD/Crl:CD rats	90 days	A control diet or the same diet containing 10% of an HMO mixture (equivalent to 0.41% 3'-SL)	NOAEL for this study was 5.67 g/kg/day for male rats and 6.97 g/kg/day for the female rats. This resulted in a mean intake of 3'-SL of 0.23 g/kg/day in males and 0.29 g/kg/day in females.	

Table 6.3.2-1 Summary of Toxicological Studies to Support the Safety of Inbiose's 3'-SL Sodium Salt

Type of Study	Species or Cell Type	Length of Study	3'-SL Dose and Route of Administration	Result	Reference
21 day-neonatal piglet study	Groups of 6 male and female LD-2 Domestic Yorkshire Crossbred Swine (farm pig)	21 days	A control diet; or Oligosaccharide blend (2.8 g 2'-FL/L, 0.6 g 3-FL/L, 1.2 g LNT/L, 0.2 g 3'-SL/L, and 0.2 g 6'-SL/L) in the diet; or Oligosaccharide blend (3.9 g 2'-FL/L, 0.8 g 3-FL/L, 1.6 g LNT/L, 0.3 g 3'-SL/L, and 0.3 g 6'-SL/L) in the diet	The Oligosaccharide blend was well tolerated and did not produce adverse effects on the growth and development of the pigs. No Oligosaccharide blend-related mortalities occurred. The clinical pathology values and macroscopic and microscopic findings at necropsy did not reveal a relationship to treatment with the Oligosaccharide Blend at the concentrations evaluated. No adverse findings in gross or histopathology were noted.	Hanlon (2020)

2'-FL = 2'-fucosyllactose; 3'-SL = 3'-sialyllactose sodium salt; 3-FL = 3-fucosyllactose; 6'-SL = 6'-sialyllactose sodium salt; bw = body weight; CHL = Chinese hamster lung cells; GeneChem = GeneChem Inc.; Glycom = Glycom A/S; GRN = GRAS Notice; h = hours; HMO = human milk oligosaccharide; Jennewein = Jennewein Biotechnologie GmbH; LD_{50} = lethal dose; LNT = lacto-*N*-tetraose; MTD = maximum tolerated dose; NOAEL = no-observed-adverse-effect level; S9 = metabolic activation mix; SD = Sprague-Dawley.
[a] HMO mixture = 47.1% dry weight 2'-FL, 16.0% dry weight 3-FL, 23.7% dry weight LNT, 4.1% dry weight 3'-SL, 4.0% dry weight 6'-SL, and 5.1% dry weight other carbohydrates manufactured by Jennewein using fermentation.

As the final results of Inbiose's subchronic toxicity study are pending, please find a detailed summary of a pivotal subchronic study with an approved 3'-SL sodium salt ingredient, incorporated by reference to Sections 6.4.1.1.1 and 6.4.1.1.2 of GRN 880 (U.S. FDA, 2018a).

Briefly, 14-day repeat dose toxicity study using neonatal Crl:CD(SD) rats was performed to select a suitable high dose for the subsequent sub-chronic toxicity study with Glycom's 3'-SL sodium salt (Phipps *et al.*, 2019). The neonatal rats (8/group/sex) received 0 (water for irrigation), 4,000, or 5,000 mg 3'-SL sodium salt/kg body weight/day, by oral gavage, once daily from the PND 7 for at least 90 days. No test item-related deaths or clinical signs were reported. Also, there were no biologically relevant differences in body weight at the end of the treatment and no test item-related macroscopic abnormalities at necropsy, between test item-treated groups and vehicle controls. It was concluded that the dose of 5,000 mg/kg bw/day was a suitable high dose for the 90-day toxicity study.

In the GLP 90-day study conducted according to the OECD Test Guideline 408 (OECD, 1998), the neonatal Crl:CD(SD) rats (10/group/sex) received 0 (water for irrigation), 1,000, 3,000, or 5,000 mg 3'-SL sodium salt/kg body weight/day, by oral gavage, once daily from PND 7 for at least 90 days (Phipps *et al*., 2019). In addition, after 90-day treatment period, 5 rats/group/sex were included in the recovery phase, during which the rats were kept un-dosed for additional 4 weeks, to assess whether any effects observed at the end of the dosing phase persist, partially or fully recover. For direct comparison against the high 3'-SL sodium salt dose group, a reference control group (10 rats/sex) received GRAS non-digestible oligosaccharide fructooligosaccharides (FOS) at a dose level of 5,000 mg/kg bw/day to assess any fiber-specific effects.

No test item-related deaths, clinical signs, or ophthalmological changes were observed at the end of the treatment period.

The mean final body weights and mean body weight gain were considered not affected by 3'-SL sodium salt treatment. The mean final body weight and overall mean body weight gain for the 3'-SL dosed males dosed at 5,000 mg/kg bw/day were statistically significantly lower when compared with the vehicle control group. The differences were minor, lacked a dose-response relationship, and were concluded as biologically irrelevant and unrelated to 3'-SL sodium salt treatment.

No test item-related differences in organ weights between all 3'-SL-treated groups and vehicle control group were noted at the end of the dosing or recovery period. The statistically significant lower mean adjusted salivary gland weight observed in 3'-SL-treated males at 5,000 mg/kg bw/day, was likely a result of the lower absolute terminal body weight of these males.

The statistically significant decrease in body weight-adjusted brain, testes, and prostate weights observed in males dosed with 3'-SL sodium salt at 5,000 mg/kg bw/day as well as the increased adjusted kidney weights in all groups of 3'-SL-treated females, compared with vehicle controls, were not dose-response associated findings.

The macroscopic and microscopic findings at the end of the treatment revealed only incidental findings in all groups and were consistent with spontaneously occurring in SD rats of this age.

There were no test item-related effects on pre-weaning development parameters or the sexual maturation (as evaluated by balano-preputial skinfold separation and vaginal opening for males and females, respectively). Similarly, no test item-related differences in measured behavior parameters were noted. The statistically significant lower forelimb grip strength and rearing counts were observed only for females at 5,000 mg/kg bw/day, and these limited to one sex findings lacked the dose response relationship.

As there were no indications of the interruption of the pituitary-thyroid axis during the study, the thyroid hormones analysis was not performed.

No test item-related differences in blood chemistry, urinalysis or hematology parameters were noted between 3'-SL-treated groups and vehicle controls at the end of the treatment or recovery phase.

The statistically significant changes in biochemistry parameters at the end of the treatment period included: low sodium levels for all 3'-SL dosed males and for the high dosed 3'-SL females; low total protein and albumin for 3'-SL dosed males at 5,000 mg/kg bw/day; increased albumin/globulin (A/G) ratio for all groups of 3'-SL-treated males and creatinine for 3'-SL dosed males at 3,000 mg/kg bw/day and 5,000 mg/kg bw/day, respectively; increased triglycerides and urea for high 3´-SL dosed males and females. All these changes were considered not test item-related. The findings lacked dose-response relationship and/or

were limited to 1 sex. All individual values were within the historical control data (HCD) ranges, and reflected normal biological variations.

Other statistically significant changes included: decrease of the urine volume, total protein in addition to increased specific gravity for the 3'-SL-treated males at 5,000 mg/kg bw/day; increased pH of urine noted for all 3'-SL-treated groups. These findings were also considered biologically irrelevant and unrelated to 3'-SL sodium salt administration as there was no evidence of any alteration in kidney function, no test item-related differences in kidney weights nor macroscopic or microscopic kidney findings. In addition, no microscopic abnormalities in urine sediment were observed.

It was concluded that 3'-SL sodium salt treatment did not elicit any signs of adverse toxicity. The no-observed-adverse-effect level (NOAEL) in this study was established at 5,000 mg/kg bw/day.

6.4 Human Studies

Many studies have been published that investigated the effects of supplementing infant formula with HiMOs, including 3'-SL sodium salt. The weight of the available evidence (published clinical data) evaluating the safety and tolerance of HiMOs in infants supports the conclusion that 3'-SL sodium salt preparations are GRAS for use in infant formula at use levels of up to 0.28 mg/L. The summaries of these studies are incorporated by reference to Section 6.F of GRN 766, Part 6.5 of GRN 880 and Part VI Section G of GRN 921 (U.S. FDA, 2018a, 2020a,b). These studies in infants regarded the addition of 3'-SL sodium salt to infant formula as well-tolerated and did not indicate the manifestation of adverse effects related to growth or development. No additional studies were identified in the literature as being published subsequent to the most recent 3'-SL sodium salt GRAS Notice.

Similarly, no new studies of 3'-SL sodium salt administration in adults have been identified in the scientific literature since the most recent 3'-SL sodium salt GRAS Notice. Summaries of previously-identified studies in adults are hereby incorporated by reference to previous GRAS determination (U.S. FDA, 2018a, 2020a,b), which support well-tolerated safe use levels of up to 20 g/day of 3'-SL sodium salt in adults.

6.5 Allergenicity

The potential allergenic activity of the recombinant proteins expressed in *E. coli K-12 MG1655* INB-3SL_01 was assessed by using the Allergen Online Tool (V20, released on 10 February 2020) of the University of Nebraska – Lincoln (FARRP, 2020). The database contained 2,171 putative allergen sequences. Potential allergenicity was evaluated by scanning each possible 80-amino acid segment of the recombinant protein (sliding window) to the database, and therefore looking for matches of at least 35% identity. No sequence alerts from potential allergens were found for the recombinant proteins in INB-3SL_01.

Since lactose is used as substrate in the 3'-SL production process and small amounts of residual lactose are present in the final product, the label “contains milk”, in accordance with the requirements of the F*ood Allergy, Labelling and Consumer Protection Act of 2004*, must be added.

6.6 General Recognition

As discussed, the use of 3'-SL sodium salt as an ingredient in non-exempt term infant formula at levels up to 0.28 g/L and in various conventional food products has been evaluated by multiple experts, qualified through scientific training and experience, in the safety evaluation of food and infant formula ingredients (GRN 766, 880, and 921). The use of 3'-SL sodium salt in infant formula at concentrations up to 0.28 g/L and various food products also has been the subject of comprehensive evaluations by multiple authoritative bodies, including EFSA (EFSA, 2020). As Inbiose has demonstrated that 3'-SL sodium salt manufactured by the company is qualitatively and quantitatively highly similar to 3'-SL sodium salt ingredients that have been the subject of previous GRAS evaluations and global novel food approvals and is intended for use in the same foods and at the same use levels as those concluded to be GRAS, conclusions on the safety of 3'-SL sodium salt for these uses issued by various experts and scientific bodies forms a basis for general recognition of Inbiose's GRAS conclusion. Convening of a GRAS Panel was therefore not considered necessary to support a GRAS conclusion on the basis that this HMO ingredient has been evaluated by multiple GRAS Panels and authoritative bodies, including the U.S. FDA and EFSA.

6.7 Conclusion

Based on the above data and information presented herein, Inbiose has concluded that 3'-SL sodium salt is GRAS, on the basis of scientific procedures, for use in non-exempt term infant formula and specified conventional food and beverage products as described in Section 1.3.

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

Part 7. § 170.255 List of Supporting Data and Information

Austin S, De Castro CA, Benet T, Hou Y, Sun H, Thakkar SK, et al. (2016). Temporal change of the content of 10 oligosaccharides in the milk of Chinese urban mothers. Nutrients 8(6):346 [22pp]. DOI:10.3390/nu8060346.

Blattner FR, Plunkett G III, Bloch CA, Perna NT, Burland V, Riley M, et al. (1997). The complete genome sequence of *Escherichia coli* K-12. Science 277(5331):1453-1462 [plus supplementary data]. DOI:10.1126/science.277.5331.1453.

Brand-Miller JC, McVeagh P, McNeil Y, Messer M (1998). Digestion of human milk oligosaccharides by healthy infants evaluated by the lactulose hydrogen breath test. J Pediatr 133(1):95-98. DOI:10.1016/S0022-3476(98)70185-4.

CDC (2015). *National Health and Nutrition Examination Survey (NHANES): 2013-2014*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/search/nhanes13_14.aspx [Page last updated: October 30, 2015].

CDC (2016). *National Health and Nutrition Examination Survey (NHANES): 2013-2014 – Dietary Data*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/Search/DataPage.aspx?Component=Dietary&CycleBeginYear=2013 [Date published: September 2016].

Chalmey C (2021) [unpublished]. *3'-Sialyllactose (3'-SL) Sodium Salt - Preliminary Toxicity Study by Oral Route (Gavage) in Juvenile Rats: Final Report*. (Study No. 48322 RSR). Charles River Laboratories Evreux.

Cheng L, Akkerman R, Kong C, Walvoort MTC, de Vos P (2021). More than sugar in the milk: human milk oligosaccharides as essential bioactive molecules in breast milk and current insight in beneficial effects. Crit Rev Food Sci Nutr 61(7):1184-120. DOI:10.1080/10408398.2020.1754756.

Datsenko KA, Wanner BL (2000). One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc Natl Acad Sci USA 97(12):6640-6645. DOI:10.1073/pnas.120163297.

Donovan SM (2017) [unpublished]. *Pre-Clinical Study for 3'sialyl-Lactose Supplementation in Neonatal Piglets*. A manuscript in preparation. Cited In: GeneChem, Inc., 2018 [GRN 766].

EFSA (2018). Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA Panel on Additives and Products or Substances Used in Animal Feed/FEEDAP) (Question no EFSA-Q-2016-00069 and EFSA-Q-2017-00211, adopted: 21 February 2018 by European Food Safety Authority). EFSA J 16(3):5206 [24pp]. DOI:10.2903/j.efsa.2018.5206. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/5206.

EFSA (2020). Safety of 3'-Sialyllactose (3'-SL) sodium salt as a novel food pursuant to Regulation (EU) 2015/2283. (EFSA Panel on Nutrition, Novel Foods and Food Allergens/NDA) (Question no: EFSA-Q-2019-00204; adopted: 25 March 2020 by European Food Safety Authority). EFSA J 18(5):6098 [22pp]. DOI:10.2903/j.efsa.2020.6098. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/6098.

Engfer MB, Stahl B, Finke B, Sawatzki G, Daniel H (2000). Human milk oligosaccharides are resistant to enzymatic hydrolysis in the upper gastrointestinal tract. Am J Clin Nutr 71(6):1589-1596. DOI:10.1093/ajcn/71.6.1589.

FARRP (2020). *AllergenOnline Version 20: Home of the FARRP Allergen Protein Database*. Lincoln (NE): University of Nebraska-Lincoln, Food Allergy Research and Resource Program (FARRP). Available at: http://www.allergenonline.org [Released: February 10, 2020].

Freddolino PL, Amini S, Tavazoie S (2012). Newly identified genetic variations in common *Escherichia coli* MG1655 stock cultures. J Bacteriol 194(2):303-6. DOI:10.1128/JB.06087-11.

GeneChem, Inc. (2018). *GRAS Notification of 3'-Sialyllactose {3'-SL) Sodium Salt*. (GRN No. 766). Prepared by Clarksville (MD): NutraSource, Inc. Submitted to Daejeon, Republic of Korea: GeneChem, Inc. Submitted to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=766 [Sep. 26, 2018 - FDA response - no questions].

Glycom A/S (2019). *GRAS Notice for 3'-Sialyllactose Sodium Salt (3'-SL)*. (GRN No. 880). Prepared by Hørsholm, Denmark: Glycom A/S. Submitted to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=880 [Feb. 21, 2020 - FDA response - no questions].

Gnoth MJ, Kunz C, Kinne-Saffran E, Rudloff S (2000). Human milk oligosaccharides are minimally digested in vitro. J Nutr 130(12):3014-3020. DOI:10.1093/jn/130.12.3014.

Hanlon PR (2020). A safety evaluation of mixed human milk oligosaccharides in neonatal farm piglets. Toxicol Res Appl 4 [8pp]. DOI:10.1177/2397847320971255.

Jacobi SK, Yatsunenko T, Li D, Dasgupta S, Yu RK, Berg BM, et al. (2016). Dietary isomers of sialyllactose increase ganglioside sialic acid concentrations in the corpus callosum and cerebellum and modulate the colonic microbiota of formula-fed piglets. J Nutr 146(2):200-208 [plus supplementary data]. DOI:10.3945/jn.115.220152.

Jennewein Biotechnologie GmbH (2020). GRAS Determination for the Use of 3'-Sialyllactose Sodium Salt in Non-Exempt Term Infant Formula. (GRN No. 921). Prepared by Rockville (MD): Spherix Consulting Group. Prepared for Rheinbreitbach, Germany: Jennewein Biotechnologie GmgH. Submitted to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=921 [Oct. 30, 2020 - FDA response - no questions].

Jong BG (2021) [unpublished]. *An in vitro Micronucleus Assay with 3´-Sialyllactose (3´-SL) Sodium Salt in Cultured Peripheral Human Lymphocytes: Final Report*. (Study No. 20258017). Charles River Laboratories Den Bosch BV.

Kim D, Gurung RB, Seo W, Lee AW, Woo J (2018). Toxicological evaluation of 3'-sialyllactose sodium salt. Regul Toxicol Pharmacol 94:83-90. DOI:10.1016/j.yrtph.2018.01.020.

Kunz C, Meyer C, Collado MC, Geiger L, García-Mantrana I, Bertua-Ríos B, et al. (2017). Influence of gestational age, secretor and Lewis blood group status on the oligosaccharide content of human milk. J Pediatr Gastroenterol Nutr 64(17):789-798. DOI:10.1097/MPG.0000000000001402.

Ma L, McJarrow P, Jan Mohamed JB, Liu X, Welman A, Fong BY (2018). Lactational changes in the human milk oligosaccharide concentration in Chinese and Malaysian mothers' milk. Int Dairy J 87:1-10. DOI:10.1016/j.idairyj.2018.07.015.

Monaco MH, Gurung RB, Donovan SM (2019). Safety evaluation of 3'-siallylactose sodium salt supplementation on growth and clinical parameters in neonatal piglets. Regul Toxicol Pharmacol 101:57-64. DOI:10.1016/j.yrtph.2018.11.008.

Monaco MH, Wang M, Pan X, Li Q, Richards JD, Chichlowski M, et al. (2018). Evaluation of sialyllactose supplementation of a prebiotic-containing formula on growth, intestinal development, and bacterial colonization in the neonatal piglet. Curr Dev Nutr 2(11):nzy067 [15pp, plus supplementary tables]. DOI:10.1093/cdn/nzy067.

OECD (1992). *Safety Considerations for Biotechnology 1992*. Paris, France: Organisation for Economic Co-Operation and Development (OECD). Available at: http://www.oecd.org/dataoecd/8/3/2375496.pdf.

OECD (1997). Bacterial reverse mutation test. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline, No. 471) [updated & adopted: 21 July 1997]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: https://www.oecd-ilibrary.org/fr/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en [Latest version updated & adopted: 26 June 2020].

OECD (1998). Repeated dose 90-day oral toxicity study in rodents. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline No. 408) [Updated & Adopted: 21 September 1998]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en;jsessionid=1m6bvwspup322.delta [New Version Updated & Adopted: 27 June 2018].

OECD (2008). Acute oral toxicity: up-and-down procedure (UDP). In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline, No. 425) [Updated & Adopted: 3 October 2008]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-425-acute-oral-toxicity-up-and-down-procedure_9789264071049-en.

OECD (2016). *In Vitro* mammalian cell micronucleus test. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline, No. 487) [Updated & Adopted: 29 July 2016]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-487-in-vitro-mammalian-cell-micronucleus-test_9789264264861-en;jsessionid=58ip5ibjcf4kc.x-oecd-live-02.

OECD (2018). Repeated dose 90-day oral toxicity study in rodents. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline, No. 408) [Updated & Adopted: 27 June 2018]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en.

Orosz I (2021) [unpublished]. *A GLP Acute Oral Toxicity Study of 3´-Sialyllactose (3´-SL) Sodium Salt in Rats (Up and Down Procedure): Final Report*. (Study No. 20/066-001P). Charles River Laboratories Hungary Kft.

Parschat K, Oehme A, Leuschner J, Jennewein S, Parkot J (2020). A safety evaluation of mixed human milk oligosaccharides in rats. Food Chem Toxicol 136:Article 111118 [12pp, plus supplementary tables]. DOI:10.1016/j.fct.2020.111118.

Phipps KR, Baldwin NJ, Lynch B, Stannard DR, Šoltésová A, Gilby B, et al. (2019). Toxicological safety assessment of the human-identical milk oligosaccharide 3'-sialyllactose sodium salt [plus supplementary data]. J Appl Toxicol 39(10):1378-1393. DOI:10.1002/jat.3824.

Rudloff S, Kunz C (2012). Milk oligosaccharides and metabolism in infants. Adv Nutr 3(3, Suppl.):398S-405S. DOI:10.3945/an.111.001594.

Rudloff S, Kuntz S, Ostenfeldt Rasmussen S, Roggenbuck M, Sprenger N, Kunz C, et al. (2019). Metabolism of milk oligosaccharides in preterm pigs sensitive to necrotizing enterocolitis. Front Nutr 6:Article 23 [11pp]. DOI:10.3389/fnut.2019.00023.

Snoeck N, De Mol ML, Van Herpe D, Goormans A, Maryns I, Coussement P, et al. (2019). Serine integrase recombinational engineering (SIRE): A versatile toolbox for genome editing. Biotechnol Bioeng 116(2):364-374. DOI:10.1002/bit.26854.

Sprenger N, Lee LY, De Castro CA, Steenhout P, Thakkar SK (2017). Longitudinal change of selected human milk oligosaccharides and association to infants' growth, an observatory, single center, longitudinal cohort study. PLoS ONE 12(2):e0171814 [15pp, plus supplementary data]. DOI:10.1371/journal.pone.0171814.

Spezia (draft report due July 2021) [unpublished]. *Toxicity Study by Oral Route (Gavage) in Juvenile Rats followed by a 5-week Treatment Free Period. Draft Report*. (Study No. 48323 RSR). Charles River Laboratories Evreux.

Tóth-Gönczöl K (2020) [unpublished]. *A GLP Bacterial Reverse Mutation Assay of 3'-Sialyllactose (3'-SL) Sodium Salt. Final Report*. (Study No. 20/066-007M). Charles River Laboratories Hungary Kft.

U.S. EPA (1997). *Escherichia Coli K-12 Final Risk Assessment: Attachment I--Final Risk Assessment of Escherichia Coli K-12 Derivatives*. Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Biotechnology Program under the Toxic Substances Control Act (TSCA). Available at: https://www.epa.gov/sites/production/files/2015-09/documents/fra004.pdf [Last updated on September 27, 2012].

U.S. FDA (2018a). *Agency Response Letter GRAS Notice No. GRN 766 [3'-sialyllactose sodium salt, Daejeon, Republic of Korea: GeneChem, Inc.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=766 [Sep. 26, 2018; Correction: May 7, 2019 - FDA response - no questions].

U.S. FDA (2018b). *Agency Response Letter GRAS Notice No. GRN 749 [2'-O-fucosyllactose, Wilmington (DE): DuPont Nutrition & Health]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=749 [Apr. 23, 2018 - FDA response - no questions].

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

U.S. FDA (2020b). *Agency Response Letter GRAS Notice No. GRN 921 [3'-sialyllactose sodium salt, Reinbreitbach, Germany: Jennewein Biotechnologie GmgH]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=921 [Oct. 30, 2020 - FDA response - no questions].

U.S. FDA (2020c). Part 170—Food additives. Section §170.3—Definitions. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs* (Food and Drug Administration). Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/ [current to 4-1-20, last updated 4-1-19].

U.S. FDA (2020d). Part 101—Food labeling. §101.12—Reference amounts customarily consumed per eating occasion. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs*. (U.S. Food and Drug Administration). Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/.

U.S. FDA (2020e). Part 170—Food additives. Section §170.30—Eligibility for classification as generally recognized as safe (GRAS). In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration)*. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/ [current to 4-1-20, last updated 4-1-19].

U.S. FDA (2020f). *Agency Response Letter GRAS Notice No. GRN 881 [6'-sialyllactose sodium salt: Hørsholm, Denmark: Glycom A/S]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=881 [Feb. 24, 2020; Correction: Apr. 13, 2020 - FDA response - no questions].

U.S. FDA (2020g). *Agency Response Letter GRAS Notice No. GRN 897 [2'-O-fucosyllactose, Wilmington (DE): DuPont Nutrition and Health]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=897 [Jun. 12, 2020 - FDA response – no questions].

U.S. FDA (2020h). *Agency Response Letter GRAS Notice No. GRN 922 [6'-sialyllactose sodium salt, Reinbreitbach, Germany: Jennewein Biotechnologie GmgH]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=922 [Pending as of: Aug. 20, 2020].

U.S. FDA (2021). *Agency Response Letter GRAS Notice No. GRN 922 [6'-sialyllactose sodium salt, Reinbreitbach, Germany: Jennewein Biotechnologie GmgH]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=922 [Apr 23, 2021 - FDA response - no questions].

USDA (2016). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2013-2014.* Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release [Last Modified: October 13, 2016].

From: Joeri Beauprez
To: Morissette, Rachel; Kamila Solak - Inbiose
Subject: [EXTERNAL] Re: additional question for GRN 001074
Date: Wednesday, March 29, 2023 8:47:55 AM
Attachments: image001.png
image002.png
image003.png
image004.png
image005.png
image006.png
Outlook-5pdws0jx.png
Response letter_FDA_3SL_2nd_Follow-up GRN 001074.pdf

Dear Rachel,

Thank you for the quick turnaround on our application regarding GRN001074 on 3'SL. Please find in attachment our response letter.

Please feel free to contact me if you would have any further questions.

Kind regards,
Joeri

Joeri Beauprez, PhD

inbiose
specialty carbohydrates

T +32 9 241 57 10
M +32 496 089 584
E joeri.beauprez@inbiose.com
www.inbiose.com

Inbiose N.V.
Technologiepark Zwijnaarde 82 – bus 41
B-9052 Gent - Belgium
RPR Gent: 0534 887 593

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: 28 March 2023 22:31
To: Joeri Beauprez <Joeri.Beauprez@inbiose.com>
Subject: additional question for GRN 001074

Dear Joeri,

Based on Inbiose's responses in the March 27, 2023, amendment, our chemist has a follow-up question regarding the limit for "other carbohydrates."

In the original text of GRN 001074, Inbiose compares the specifications (Table 2.3.1-1, p. 14) for its 3′-SL ingredient with 3′-SL described in GRNs 000880 and 000921, which are of similar purity (not less than 88% 3′-SL sodium salt) and received "No Questions" letters from FDA. While GRN 000880 set a specification of not more than 5.0% for 3′-sialyllactulose, GRN 000921 did not set a specification for this impurity. The notifier (Jennewein) for GRN 000921 specifically observed on p. 26 that 3′-sialyllactulose "is not expected in Jennewein's product due to the 3′-SL production process." Inbiose notes in GRN 001074 that 3′-sialyllactulose is a known impurity of 3′-SL, produced as described in GRN 001074, but does not provide a specification for this impurity.

In the March 27, 2023, amendment, Inbiose notes that the specification for "other carbohydrates" in GRN 001074 is ≤10%. However, this specification is much higher than the results of the batch analyses for "other carbohydrates" reported in Table 2.3.2-1 and would not limit the level of 3′-sialyllactulose to levels reported previously (i.e., ≤5.0%). We request that Inbiose either lowers the specification for "other carbohydrates" to ≤5.0% or provides a specification for 3′-sialyllactulose of ≤5.0%. If Inbiose chooses to set a specification for 3′-sialyllactulose, please provide the results of a minimum of three non-consecutive batch analyses to demonstrate that the specification can be met. In addition, please indicate the analytical method used to analyze for 3′-sialyllactulose and indicate that the method is validated for its intended use.

We request a response within 5 business days. If you choose to provide a specification and need more time to provide the batch analyses, please let me know.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

March 29, 2023

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration

Regarding: Response to FDA Questions related to GRAS Notice No. GRN 001074

Dear Dr. Morissette,

In reference to your email dated March 28, 2023, regarding the follow up question for GRAS Notice GRN 001074, I am pleased to provide you with our responses to the Agency's questions in the following document.

I trust that your question and comment is adequately addressed, below, and meet the Agency's expectations. If further clarification or any additional information is required as part of this GRAS Notice, please do not hesitate to let me know.

Kind regards,

Joeri Beauprez
Chief Scientific Officer

Question 1. In the March 27, 2023, amendment, Inbiose notes that the specification for "other carbohydrates" in GRN 001074 is ≤10%. However, this specification is much higher than the results of the batch analyses for "other carbohydrates" reported in Table 2.3.2-1 and would not limit the level of 3'-sialyllactulose to levels reported previously (i.e., ≤5.0%). We request that Inbiose either lowers the specification for "other carbohydrates" to ≤5.0% or provides a specification for 3'-sialyllactulose of ≤5.0%. If Inbiose chooses to set a specification for 3'-sialyllactulose, please provide the results of a minimum of three non-consecutive batch analyses to demonstrate that the specification can be met. In addition, please indicate the analytical method used to analyze for 3'-sialyllactulose and indicate that the method is validated for its intended use.

In the original text of GRN 001074, Inbiose compares the specifications (Table 2.3.1-1, p. 14) for its 3'-SL ingredient with 3'-SL described in GRNs 000880 and 000921, which are of similar purity (not less than 88% 3'-SL sodium salt) and received "No Questions" letters from FDA. While GRN 000880 set a specification of not more than 5.0% for 3'-sialyllactulose, GRN 000921 did not set a specification for this impurity. The notifier (Jennewein) for GRN 000921 specifically observed on p. 26 that 3'-sialyllactulose "is not expected in Jennewein's product due to the 3'-SL production process." Inbiose notes in GRN 001074 that 3'-sialyllactulose is a known impurity of 3'-SL, produced as described in GRN 001074, but does not provide a specification for this impurity.

Inbiose agrees to lower the specification for "other carbohydrates" to ≤5.0%, and to add the specification of 3´- sialyllactulose of ≤5.0% (see the updated Carbohydrates composition section in Table 2.3.1-1 below).

Table 2.3.1-1 Product Specifications for Inbiose's 3'-SL Sodium Salt in Comparison to those of the 3'-SL Ingredients in GRN 766, 880, and 921 – Carbohydrates section update

Parameter	Specification for Inbiose's 3'-SL	Method of Analysis Employed by Inbiose	Specification Reported for Other 3'-SL Products		
			GeneChem's 3'-SL Sodium Salt Siallac3® (GRN 766) (U.S. FDA, 2018a)	Glycom's 3'-SL Sodium Salt (GRN 880) (U.S. FDA, 2020a	Jennewein's 3'-SL Sodium Salt (GRN 921) (U.S. FDA, 2020b)
Carbohydrates, water free (%DM)					
3'-SL	NLT 85%	UPLC-RI			
3'-SL sodium salt	NLT 88%	UPLC-RI	NLT 98%[a] (% carbohydrates)	NLT 88.0%	NLT 88%
D-Lactose	NMT 5%	UPLC-RI	NS	NMT 5.0%	NMT 5%[b]
Sialic acid	NMT 5%	UPLC-RI	NS	NMT 1.5%	NMT 10%[b]
3'-Sialyllactulose	**NMT 5%**	-	NS	NMT 5.0%	NS
N-acetylglucosamine	NS	-	NS	NS	NMT 5%[b]
Sum of human identical milk saccharides[c]	NLT 90%	UPLC-RI	NS	NLT 90.0%	NS

Table 2.3.1-1 Product Specifications for Inbiose's 3'-SL Sodium Salt in Comparison to those of the 3'-SL Ingredients in GRN 766, 880, and 921 – Carbohydrates section update

Parameter	Specification for Inbiose's 3'-SL	Method of Analysis Employed by Inbiose	Specification Reported for Other 3'-SL Products		
			GeneChem's 3'-SL Sodium Salt Siallac3® (GRN 766) (U.S. FDA, 2018a)	Glycom's 3'-SL Sodium Salt (GRN 880) (U.S. FDA, 2020a	Jennewein's 3'-SL Sodium Salt (GRN 921) (U.S. FDA, 2020b)
Other carbohydrates	**NMT 5%[b]**	UPLC-RI	NS	NMT 3.0%	NMT 12%[b]

[b] Expressed in area %.
[c] Human identical milk saccharides is defined as the sum of 3'-SL sodium salt, lactose, and sialic acid.

Inbiose confirms, that the validated UPLC-RI method is used to analyze for 3'-sialyllactulose. The analytical data for 3´-sialyllactulose for the non-consecutive batches of 3´-Sialyllactose sodium salt are shown in the Table 1 below.

Table 1. Analytical data for 3´-sialyllactulose for the non-consecutive batches of 3´-Sialyllactose sodium salt

Parameter	Specification	Lot Nos.				
		ilex13F02	ilex13F03	ilex13F04	ilex013F07	ilex13F08
3´-sialyllactulose	≤5%	0.29	< 0.01	< 0.01	< 0.01	< 0.01

From: Joeri Beauprez
To: Morissette, Rachel
Cc: Kamila Solak - Inbiose
Subject: [EXTERNAL] Re: follow-up questions for GRN 001074
Date: Monday, March 27, 2023 7:13:38 AM
Attachments: image001.png
image002.png
image003.png
image004.png
image005.png
image006.png
image008.png
image009.png
Outlook-aigrictu.png
Response letter_FDA_3SL_Follow-up GRN 001074.pdf

Dear Dr. Morissette

Thank you for following up our application regarding GRN001074 on 3'SL. Please find in attachment our response letter.

Please feel free to contact me if you would have any further questions.

Kind regards,
Joeri

Joeri Beauprez, PhD

inbiose
specialty carbohydrates

T +32 9 241 57 10
M +32 496 089 584
E joeri.beauprez@inbiose.com
www.inbiose.com

Inbiose N.V.
Technologiepark Zwijnaarde 82 – bus 41
B-9052 Gent - Belgium
RPR Gent: 0534 887 593

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: 21 March 2023 16:54
To: Joeri Beauprez <Joeri.Beauprez@inbiose.com>
Cc: Kamila Solak - Inbiose <kamila.solak@inbiose.com>
Subject: follow-up questions for GRN 001074

Hi Joeri,

We had a few clarifying questions for GRN 001074 below from our chemist. Please provide a

response within 5 business days. If that's not possible, we can discuss.

1. The original text in the notice did not include uses in meat and poultry, nor did it include suitability information for consideration by USDA FSIS. However, we note that the February 15, 2023, amendment lists several infant and toddler foods containing meat and poultry. Please clarify if the intended uses of 3'-SL include uses in meat and poultry products under the jurisdiction of USDA.

2. Please clarify the following uses in Table 1 of the amendment:
    a. The age of the population consuming enteral tube feeding formula. We note that previous GRAS notices have included the use of 2'-FL for enteral tube feeding formulas for ages 11 years and older only.
    b. Given the substitutional uses proposed in GRN 001074 and for consistency with previous GRNs, please confirm that use of 3'-SL is 25.9 g/kg in meal replacement bars for weight reduction and the use in other meal replacement bars (general use) is 2.5-5 g/kg.

3. The specification for "other carbohydrates" is not more than 10% in Table 2.3.1-1 (p. 14 of the notice) and ≤5% in Table 2.3.2-1 (p. 16). Please confirm the correct specification for "other carbohydrates".

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

I GOT MY COVID-19 VACCINATION

From: Joeri Beauprez <Joeri.Beauprez@inbiose.com>
Sent: Wednesday, February 15, 2023 10:45 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Cc: Kamila Solak - Inbiose <kamila.solak@inbiose.com>
Subject: Re: [EXTERNAL] Re: questions for GRN 001074

Dear Dr Morissette,

Hope all is well.

Thank you for the letter regarding GRN001074 on 3'SL. Please find in attachment our response letter and the requested supplementary information, Supplementary 90-day Subchronic Study Summary for 3'SL sodium salt and Appendix A - 3'-SL Food Codes.

Please feel free to contact me if you would have any further questions.

Kind regards,
Joeri

Joeri Beauprez, PhD

inbiose
specialty carbohydrates

T +32 9 241 57 10
M +32 496 089 584
E joeri.beauprez@inbiose.com
www.inbiose.com

Inbiose N.V.
Technologiepark Zwijnaarde 82 – bus 41
B-9052 Gent - Belgium
RPR Gent: 0534 887 593

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: 27 January 2023 14:30
To: Joeri Beauprez <Joeri.Beauprez@inbiose.com>
Cc: Kamila Solak - Inbiose <kamila.solak@inbiose.com>
Subject: RE: [EXTERNAL] Re: questions for GRN 001074

Dear Joeri,

That will be fine.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

From: Joeri Beauprez <Joeri.Beauprez@inbiose.com>
Sent: Friday, January 27, 2023 6:40 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Cc: Kamila Solak - Inbiose <kamila.solak@inbiose.com>
Subject: [EXTERNAL] Re: questions for GRN 001074

Dear Dr Morissette,

Thank you for the questions on GRN 001074, 3'SL. We would like to request 15 extra working days as from January 31st because we are relying on external advisors and are not able to obtain fast enough feedback. We would hence request to move the deadline to February 21st 2023.

Thank you for your consideration.

Kind regards
Joeri

Joeri Beauprez, PhD

T +32 9 241 57 10
M +32 496 089 584
E joeri.beauprez@inbiose.com
www.inbiose.com

Inbiose N.V.
Technologiepark Zwijnaarde 82 – bus 41
B-9052 Gent - Belgium
RPR Gent: 0534 887 593

Disclaimer

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: 18 January 2023 17:21
To: Joeri Beauprez <Joeri.Beauprez@inbiose.com>
Subject: questions for GRN 001074

Dear Dr. Beauprez,

Please see below our questions for GRN 001074. We request that you provide responses within 10 business days. If you need more time, please let me know.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

March 27, 2023

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration

Regarding: Response to FDA Questions related to GRAS Notice No. GRN 001074

Dear Dr. Morissette,

In reference to your email dated March 21, 2023, regarding the follow up questions for GRAS Notice GRN 001074, I am pleased to provide you with our responses to the Agency's questions in the following document.

I trust that all of your questions and comments are adequately addressed, below, and meet the Agency's expectations. If further clarification or any additional information is required as part of this GRAS Notice, please do not hesitate to let me know.

Kind regards,

Joeri Beauprez
Chief Scientific Officer

Question 1. The original text in the notice did not include uses in meat and poultry, nor did it include suitability information for consideration by USDA FSIS. However, we note that the February 15, 2023, amendment lists several infant and toddler foods containing meat and poultry. Please clarify if the intended uses of 3'-SL include uses in meat and poultry products under the jurisdiction of USDA.

The inclusion of NHANES food codes for "Other baby foods for infants and young children", which represent foods that may be regulated by U.S. Department of Agriculture (USDA), was intended as a highly conservative measure in the estimation of dietary exposure. However, it is recognized that certain meat, poultry, and egg products are subject to regulation by the USDA and these uses are therefore excluded from the scope of this GRAS Notice.

Question 2. Please clarify the following uses in Table 1 of the amendment:

a. The age of the population consuming enteral tube feeding formula. We note that previous GRAS notices have included the use of 2'-FL for enteral tube feeding formulas for ages 11 years and older only.

b. Given the substitutional uses proposed in GRN 001074 and for consistency with previous GRNs, please confirm that use of 3'-SL is 25.9 g/kg in meal replacement bars for weight reduction and the use in other meal replacement bars (general use) is 2.5-5 g/kg.

a. While Inbiose notes that no age group was specified for the GRAS use of 3'-SL in enteral tube feeding formulas as part of GRN 001015, it is acknowledged that GRAS Notices for other HMO ingredients (*i.e.*, 2'-FL) have specified use in enteral tube feeding formulas for ages 11 years and older only. Inbiose confirms that the food use of 3'-SL for enteral tube feeding formulas is intended to align with previous GRAS Notices and is therefore intended for this use by individuals ages 11 years and older only.

b. Inbiose confirms that use of 3'-SL is 25.9 g/kg in meal replacement bars for weight reduction and the use Cereal and granola bars ("general use") is 2.5 g/kg.

Question 3. The specification for "other carbohydrates" is not more than 10% in Table 2.3.1-1 (p. 14 of the notice) and ≤5% in Table 2.3.2-1 (p. 16). Please confirm the correct specification for "other carbohydrates".

Thank you for identifying this inconsistency. Inbiose confirms that the specification for "other carbohydrates" is intended to be "not more than 10%", as indicated in Table 2.3.1-1. Please find below the updated Table 2.3.2-1 with the correct specification for "other carbohydrates" (in **bold**).

Table 2.3.2-1 Analytical Data Obtained from 5 Batches of 3'-SL

Parameter	Specification	Lot Nos.				
		ilex13F02	**ilex13F03**	**ilex13F04**	**ilex013F07**	**ilex13F08**
Identification						
Appearance (color)	White	White	White	White	White	White
Appearance (form)	Dry powder	Dry powder	Dry powder	Dry powder	Dry powder	Dry powder
Appearance in solution	Clear, colorless to slightly yellow	Clear, colorless to slightly yellow	Clear, colorless to slightly yellow	Clear, colorless to slightly yellow	Clear, colorless to slightly yellow	Clear, colorless to slightly yellow
pH (20°C, 10% solution)	4.0 to 7.0	5.28	4.74	4.54	4.73	5.64
Carbohydrates, water free (%area)						
3'-SL	≥85	90.53	88.56	87.93	91.10	89.39
3'-SL sodium salt	≥88	93.67	91.64	90.98	94.26	92.49
D-Lactose	≤5%	0.54	1.05	1.05	0.78	1.05
Sialic acid	≤5%	1.12	0.89	2.42	0.64	1.24
Sum of human identical milk saccharides[a]	≥90%	95.33	93.57	94.44	95.68	94.78
Other carbohydrates[b]	**≤10%**	0.20	2.19	1.21	0.53	0.29
Chemical Analysis						
Water content, volumetric (% w/w)	≤9.0%	6.7	6.3	5.0	5.4	4.46
Protein content (µg/g)	≤100	27	<25	<25	<25	<25
Total ash (%)	≤8.5%	7.62	7.36	7.44	6.95	8.04
Sodium, Na %	≤4.5%	3.33	3.27	3.28	3.38	3.44
Endotoxin (IU/g)	≤300	2.40	1.65	7.70	27	19.5
Heavy Metals						
Arsenic (mg/kg)	≤0.2	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium (mg/kg)	≤0.01	<0.005	<0.002	<0.002	<0.005	<0.005
Lead (mg/kg)	≤0.05	<0.01	<0.004	<0.004	<0.01	0.016
Mercury (mg/kg)	≤0.1	<0.01	<0.002	<0.002	<0.01	<0.01
Microbiological Contaminants						
Standard plate count (CFU/g)	≤5000	<100	<100	<100	<100	<100
Yeast (CFU/g)	≤100	<10	<10	<10	<10	<10
Mold (CFU/g)	≤100	<10	10[c]	<10	<10	<10
Coliform (CFU/g)	≤10	<10	<10	<10	<10	<10
Enterobacteriaceae	Absent in 10 g	Absent	Absent	Absent	Absent	Absent
Salmonella	Absent in 25 g	Absent	Absent	Absent	Absent	Absent

Table 2.3.2-1 Analytical Data Obtained from 5 Batches of 3'-SL

Parameter	Specification	Lot Nos.				
		ilex13F02	**ilex13F03**	**ilex13F04**	**ilex013F07**	**ilex13F08**
Cronobacter (Enterobacter) sakazakii	Absent in 25 g	Absent	Absent	Absent	Absent	Absent
Listeria monocytogenes	Absent in 25 g	Absent	Absent	Absent	Absent	Absent
Bacillus cereus (CFU/g)	≤50	<10	<10	<10	<10	<10

3'-SL = 3'-sialyllactose; CFU = colony forming units; IU = international units.

[a] Human identical milk saccharides is defined as the sum of 3´-SL sodium salt, lactose, and sialic acid.

[b] Expressed in area %.

[c] Estimated values

From: Joeri Beauprez
To: Morissette, Rachel
Cc: Kamila Solak - Inbiose
Subject: Re: [EXTERNAL] Re: questions for GRN 001074
Date: Wednesday, February 15, 2023 10:45:29 AM
Attachments: image008.png
image009.png
image010.png
image011.png
image012.png
image013.png
image014.png
image015.png
image016.png
image017.png
image018.png
image019.png
image020.png
Outlook-zcgm3543.png
Appendix A - 3"-SL Food Codes.pdf
Questions for notifier GRN 001074.pdf
Response letter_FDA_3SL_1502023.pdf
Supplementary 90-day Subchronic Study Summary_3SL sodium salt.pdf

Dear Dr Morissette,

Hope all is well.

Thank you for the letter regarding GRN001074 on 3'SL. Please find in attachment our response letter and the requested supplementary information, Supplementary 90-day Subchronic Study Summary for 3'SL sodium salt and Appendix A - 3'-SL Food Codes.

Please feel free to contact me if you would have any further questions.

Kind regards,
Joeri

Joeri Beauprez, PhD

inbiose
specialty carbohydrates

T +32 9 241 57 10
M +32 496 089 584
E joeri.beauprez@inbiose.com
www.inbiose.com

Inbiose N.V.
Technologiepark Zwijnaarde 82 – bus 41
B-9052 Gent - Belgium
RPR Gent: 0534 887 593

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: 27 January 2023 14:30
To: Joeri Beauprez <Joeri.Beauprez@inbiose.com>
Cc: Kamila Solak - Inbiose <kamila.solak@inbiose.com>
Subject: RE: [EXTERNAL] Re: questions for GRN 001074

Dear Joeri,

That will be fine.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

I GOT MY COVID-19 VACCINATION

From: Joeri Beauprez <Joeri.Beauprez@inbiose.com>
Sent: Friday, January 27, 2023 6:40 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Cc: Kamila Solak - Inbiose <kamila.solak@inbiose.com>
Subject: [EXTERNAL] Re: questions for GRN 001074

Dear Dr Morissette,

Thank you for the questions on GRN 001074, 3'SL. We would like to request 15 extra working days as from January 31st because we are relying on external advisors and are not able to

obtain fast enough feedback. We would hence request to move the deadline to February 21st 2023.

Thank you for your consideration.

Kind regards
Joeri

Joeri Beauprez, PhD

inbiose
specialty carbohydrates

T +32 9 241 57 10
M +32 496 089 584
E joeri.beauprez@inbiose.com
www.inbiose.com

Inbiose N.V.
Technologiepark Zwijnaarde 82 – bus 41
B-9052 Gent - Belgium
RPR Gent: 0534 887 593

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: 18 January 2023 17:21
To: Joeri Beauprez <Joeri.Beauprez@inbiose.com>
Subject: questions for GRN 001074

Dear Dr. Beauprez,

Please see below our questions for GRN 001074. We request that you provide responses within 10 business days. If you need more time, please let me know.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION


I GOT MY
COVID-19
VACCINATION

February 15, 2023

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration

Regarding: Response to FDA Questions related to GRAS Notice No. GRN 001074

Dear Dr. Morissette,

In reference to your email dated January 18, 2023, regarding Inbiose's GRAS Notice GRN 001074 for the intended uses of 3´-sialyllactose sodium salt (3´-SL), I am pleased to provide you with our responses to the Agency's questions in the following document.

I trust that all of your questions and comments are adequately addressed, below, and meet the Agency's expectations. If further clarification or any additional information is required as part of this GRAS Notice, please do not hesitate to let me know.

Kind regards,

Joeri Beauprez
Chief Scientific Officer

Question 1. Please Specify the protein base of the infant formula into which the 3'-SL would be added (e.g., cow milk, soy, etc).

Inbiose is the bulk ingredient manufacturer of this ingredient, and therefore does not control the Specific protein source that infant formula manufacturers may choose to use during the formulation of end use products. Protein sources used in the manufacture of infant formulae are defined by the infant formula manufacturer. Therefore, it is reasonable to expect that Inbiose's HMO ingredients, including 3'-SL, may be used in any of the available protein bases (*e.g.*, milk, soy, whey) that are currently used to manufacture non-exempt infant formula products.

Question 2. In Section 1.3 Conditions of Use (Table 1.3-1, p. 4), Inbiose lists the maximum use levels in Specified food categories from GRNs 000766, 000880, 000921 and states that intended uses for GRN 001074's 3'-SL "will be the same as described in GRN 000921 for non-exempt term infant formula products, and the same as those described in GRN 000880 for all other listed food categories." However, in Section 3.1 (p. 20) Estimated Intake of 3'-SL, Inbiose states that "In line with GRN 000921, Inbiose's 3'-SL sodium salt is intended for use as a food ingredient in term infant formula (0 to 12 months) and toddler formula at concentrations up to 0.28 g/L." Additionally, Inbiose states that uses in baby foods and meal replacement bars will be similar to GRN 000880, and "In line with GRN 000766, Inbiose's 3'-SL is intended for use in dairy product analogs, milk (whole and skim), milk products, grain products, beverages and beverage bases and sugar substitutes…"

a. We note that since GRN 001074 was submitted, FDA responded to GRN 001015 with a "No Questions" letter, dated July 15, 2022. GRN 001015 includes uses of 3'-SL in "toddler formula" at a level up to 0.28 g/L. While the stated use of 3'-SL in "toddler formula" is consistent with GRN 001015 (which is not cited in the current notice), it is inconsistent with the 0.24 g/L use level Specified in Table 1.3-1. Please clarify the use level in "toddler formula."

b. GRN 001015 included a use-level increase (0.45 g/L) for non-carbonated drinks (e.g., sports and energy drinks, flavored water), and expanded the uses of 3'-FL to include milk-based meal replacement drinks (general use, not just for weight loss) at levels up to 0.9 g/L for adults and children and in formulas for enteral feeding at levels up to 1.5 g/L. Please clarify if the intended uses of 3'-SL in GRN 001074 also include the uses in GRN 001015.

c. For clarity, please provide a summary of the intended uses of GRN 001074 in table form. In the table, please clarify that use levels match the information provided in the text, that the analogous uses (e.g., GRNs 000766, 000880, 000921, and, if applicable, 001015) are correctly stated, and that the basis (3'-SL or 3'-SL sodium salt) for expression of use levels is clearly indicated.

a. Thank you for the opportunity to clarify. The use of Inbiose's 3'-SL as a food ingredient in term infant formula (0 to 12 months) and "toddler formula" are both intended to be used at levels up to 0.28 g/L, which is consistent with the GRAS use levels of other 3'-SL preparations evaluated as part of GRAS Notification GRN 000921 and 001015.

b. The intended uses of 3'-SL in GRN 001074 also include the uses evaluated as part of GRN 001015, which include the use-level increase for non-carbonated drinks (*e.g.*, sports and energy drinks, flavored water), and the expanded uses of 3'-SL to include milk-based meal replacement drinks (general use, not just for weight loss) at levels up to 0.9 g/L for adults and children, and in formulas for enteral feeding at levels up to 1.5 g/L.

c. For clarity, the intended uses of GRN 001074 are provided in table form, below, which have been corrected to accurately reflect the discussion above. Notably, the uses of Inbiose's 3'-SL listed in Table 1 are fully substitutional to other forms of 3'-SL that have already been determined GRAS.

Table 1. Summary of the Individual Proposed Food Uses and Maximum Use Levels Notified as GRAS for 3'-Sialyllactose Sodium Salt in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2020a)	Food Uses[a,b]	Maximum Cumulative Use Levels Described in Previous GRNs (g/kg or g/L)
Beverages and Beverage Bases	Soft drinks (regular and diet)	0.25
	Sports, Isotonic, and Energy Drinks; Enhanced or Fortified Waters	0.45
	Non-milk-based meal replacement drinks	0.90
Coffee and Tea	Cappucino, non-fat, with dairy milk, sweetened	0.52
	Herbal tea, presweetened with low calorie sweetener or sugar	12.9
Dairy Product Analogs	Imitation milk	0.12
	Non-dairy yogurt	0.55
Frozen Dairy Desserts	Frozen yogurts	1.7
Grain Products and Pastas	Cereal and granola bars	2.5
	Meal replacement bars	25.9
Infant and Toddler Foods	Non-exempt term infant formula	0.28 (as consumed)
	Toddler formula	0.28 (as consumed)
	Milk-based meal replacement beverages for children (Pediasure)	0.9
	Instant cereals for babies and toddlers	1.66
	Other baby foods for infants and young children[b]	1.25
	Other drinks for young children	0.15
Milk, Whole, and Skim	Unflavored pasteurized and sterilized milk (whole milk, reduced-fat milk, low-fat milk, non-fat milk; including powdered milks, reconstituted)	0.25
Milk Products	Buttermilk	0.25
	Flavored milk	0.25
	Milk-based meal replacement beverages	0.90
	Yogurt	2.5
	Formula intended for pregnant women ("mum" formulas, -9 to 0 months)[c]	0.9
Processed Fruits and Fruit Juices	Fruit flavored drinks and ades	0.25
Sugar Substitutes	Sugar substitute, herbal extract powder or liquid[d]	100
Foods For Special Dietary Use	Enteral tube feeding[e]	1.5

- = not applicable; 3'-SL = 3'-sialyllactose; CFR = *Code of Federal Regulations*; FDA = Food and Drug Administration; GRAS = Generally Recognized as Safe; incl. = including; NHANES = National Health and Nutrition Examination Survey; RTE = ready-to-eat; U.S. = United States.

[a] Inbiose's 3'-SL sodium salt is intended for use in unstandardized products when standards of identity do not permit its addition, as established under 21 CFR §130 to 169, do not permit its addition in standardized products.

[b] The use of 3'-SL sodium salt was previously concluded to be GRAS in GRN 000880 at a use level of 1.25 g/kg in baby foods other than non-exempt term infant formulas, toddler formulas, and drinks for young children. Therefore, since this food category is representative of the various proposed infant and toddler food uses in GRN 000766 (*e.g.*, "cereals for babies, jarred", "cereal bar with fruit fillings", etc.) at a higher use level, the infant and toddler food uses in GRN 000766 were not

Table 1. Summary of the Individual Proposed Food Uses and Maximum Use Levels Notified as GRAS for 3'-Sialyllactose Sodium Salt in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2020a)	Food Uses[a,b]	Maximum Cumulative Use Levels Described in Previous GRNs (g/kg or g/L)

summarized (with the exception of "instant cereals for babies and toddlers" which has been separated out) as they are considered represented by this use.

[c] Food codes for "mum formulas" were not available in the 2017-2018 NHANES. However, representative food code(s) for nutritional drinks (previously categorized in "meal replacement drink" categories) were utilized as surrogates for "mum formulas" in the dietary exposure assessment.

[d] The use of 3'-SL sodium salt was previously concluded to be GRAS in herbal extract sugar substitutes at a use level of 10% (equivalent to 100 g/kg).

[e] Foods for special dietary use were assessed separately from the intended food uses of 3'-SL sodium salt in conventional foods, as they are intended for supplying a particular dietary need and/or supplementing the intake of a dietary component. Intake of 3'-SL sodium salt from foods for special dietary use is, therefore, not expected to be cumulative to other dietary sources.

Question 3. In Footnote d of Table 2.3.1-1 (p. 15), Inbiose notes that sodium and its oxidized derivatives are major components of the ash and that sulfates and phosphates are also present. Based on the Specifications, ash may comprise up to 8.5% of the ingredient and sodium may comprise up to 4.5% of the ingredient. Please provide additional information to characterize the ash component.
a. Sodium has a Specification of NMT 4.5% of the total ingredient. Does the 4.5% include the oxidized forms of sodium and/or its derivatives?
b. Please indicate other minerals present in the ash fraction and their approximate levels as a portion of the 3'-SL.

a. The sodium specification of NMT 4.5% includes only elemental sodium and not its oxidized forms or derivatives.

b. To further characterize the composition of the ash fraction, Inbiose has conducted an ash composition analysis of the 3'-SL ash fraction using inductively coupled plasma atomic emission spectroscopy (ICP-AES); a method widely used for elemental composition characterization (*i.e.*, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Phosphate, Sulfate, and Zn). Ash fractions obtained from two commercially representative batches of 3'-SL (Batch IDs: ilex13F04 and ilex13F08), which were included in the GRAS Notification, were analyzed. Results from this analysis indicate that the most abundant minerals detected in the ash of 3'-SL are, in order from most to least abundant; sodium, phosphate (calculated from phosphorus), sulfate (calculated from sulfur), and potassium. Trace levels of minor constituents such as calcium, magnesium, and iron were also detected. Notably, the composition of the 3'-SL ash fraction, is not only composed of metals, but also contains oxygen and carbon, which cannot be measured by ICP-AES. During the ashing process, oxygen is introduced, therefore the measured elements are expected to be present in their oxidized forms and derivatives. Since the carbon and oxygen cannot be measured, the mass balance of the analyzed ash based on the ICP-AES will not be achieved.

   Furthermore, elemental analysis was conducted on five batches the final 3'-SL ingredient (Batch IDs: ilex13F02, ilex13F03, ilex13F04, ilex13F07 and ilex13F08), using ICP-AES or

inductively coupled plasma mass spectroscopy (except for sulfur, which was measured as sulfate using ion chromatography coupled with conductivity detector), to confirm the elemental composition obtained from the ash analysis. The maximum detected levels of each mineral in Inbiose's 3'-SL product are summarized in Table 2, below. Overall, the elements detected in the powder are consistent with those found in the ash, consisting primarily of sodium, phosphorus, sulfur, and potassium.

Table 2. Overview of the Maximum Mineral Levels Detected in Inbiose's 3'-SL

Mineral	Method	LOQ (mg/kg)	Maximum Detected Level* (mg/kg)	Maximum Detected Level* (w/w %)
Ca	ICP-AES	40	< 40 (LOQ)	< 0.004
Cu	ICP-MS	0.1	0.34	0.000034
Fe	ICP-AES	0.4	1.3	0.00013
K	ICP-AES	40	58	0.0058
Mg	ICP-AES	0.01	< 0.01 (LOQ)	< 0.000001
Mn	ICP-AES	0.14	5.2	0.00052
Mo	ICP-MS	0.1	< 0.1 (LOQ)	< 0.00001
Na	ICP-AES	100	34,400	3.44
P	ICP-AES	5.0	≤ 2,800	0.28
(as phosphate)	conversion	-	≤ 8,585	0.86
S (as sulfate)	IC-CD	5.0	120	0.012
Zn	ICP-AES	0.2	3.6	0.00036

*Maximum level from five commercially representative 3´-SL powder batches

Question 4. The notifier identifies ultra-high performance liquid chromatography coupled with a refractive index detector (UPLC-RI) as the method of analysis for 3'-SL and other carbohydrates present in Inbiose's 3'-SL ingredient. By Specification, other carbohydrates may be present at up to 5% of total carbohydrates.

a. Please confirm that the method includes resolution and identification of "other carbohydrates" present in the 3'-SL ingredient.

b. Please describe the minor components present in this fraction, including levels of 3'-siallylactulose and N-acetylglucosamine, if present.

a. Inbiose confirms that the method has the required resolution to ensure separation and identification of the "other carbohydrates" from 3´-SL, lactose and sialic acid.

b. The minor components present in the ''other carbohydrates'' fraction are carbohydrates, such as glucose, sucrose, 3-sialylgalactose, 3'-sialyllactulose and N-acetylglucosamine. The level of 3'-siallylactulose determined in Inbiose´s 3´-SL product is up to 0.29% w/w. N-acetylglucosamine has not been quantified by UPLC-RI (LOQ: 0.01 % dry matter), but trace amounts are present.

Question 5. The Specification for arsenic (≤0.2 mg/kg) is well above the results of the batch analyses (<0.01 mg/kg), as provided in Table 2.3.2-1 (p. 16). Please consider reducing this Specification to reflect the results of the batch analyses and to ensure that dietary exposure to arsenic is as low as possible. Based on the results of batch analyses, we request that you consider lowering your arsenic Specification from ≤0.2 mg/kg to reflect more closely the results of batch analyses (<0.01 mg/kg).

Inbiose highlights that the arsenic specification proposed for this GRAS notice (i.e. ≤0.2 mg/kg) is in line with other HMOs that have been notified to the U.S. FDA without objection from the agency (e.g. GRNs: 001016, 001015, 001014, 000951, 000929, 000925, 000923, 000922). The specifications reported in these GRN notices are specified in Table 3.

Other recent application (GRN 001059) does not provide specification limits for arsenic, but only provides a specification for lead (see Table 3).

Inbiose would like to highlight that the HMOs indicated in Table 3., likewise Inbiose's 3´-SL product, were produced from fermentation processes using *Escherichia coli* strains, and using similar production techniques.

Table 3. Arsenic specifications reported in previous GRN notices

GRN no.	Date of filing	Date of closure	FDA's Letter	Notifier	As specifications (mg/kg)	Reference
001074	Sep 30, 2022	On-going	N/A	INBIOSE	≤0.2	Current GRN notice
001059	Jun 8, 2022	Dec 2, 2022	"No questions"	Glycom	NS	Specs: Table 2.3.1-4, p. 10
001016	Sep 24, 2021	Jul 15, 2022	"No questions"	Hansen	≤0.2	Specs: Table 2, p. 8
001015	Sep 24, 2021	Jul 15, 2022	"No questions"	Hansen	≤0.2	Specs: Table 2, pp. 8-9
001014	Oct 18, 2021	Jul 15, 2022	"No questions"	Hansen	≤0.2	Specs: Table 2, pp. 10-11
000951	Oct 02, 2021	Aug 12, 2021	"No questions"	Danisco	≤0.2	Specs: Table 3, p. 17
000929	Jun 19, 2020	Feb 26, 2021	"No questions"	Jennewein	≤0.2	Specs: Table 4, p. 7
000925	May 15, 2020	Feb 08, 2021	"No questions"	Jennewein	≤0.2	Specs: Table 3, p. 11
000923	May 14, 2020	Feb 02, 2021	"No questions"	Jennewein	≤0.2	Specs: Table 3, p. 11
000922	June 3, 2020.	Apr 23, 2021	"No questions"	Jennewein	≤0.2	Specs: Table 3, p. 7
000921	May 14, 2020	Oct 30, 2020	"No questions"	Jennewein	≤0.2	Specs: Table 3, p. 12
000919	May 12, 2020	Oct 30, 2020	"No questions"	Jennewein	≤0.2	Specs: Table 3, p. 14-15

NS: Not Specified. N/A – to available

Nonetheless, despite the specifications (or their absence) for arsenic in other GRAS notices, Inbiose would like to reduce the proposed specifications of Arsenic (from initially proposed ≤0.2 mg/kg to ≤0.1 mg/kg).

Question 6. Based on the results of batch analyses (Table 2.3.2-1, p. 16), at least one of the results for protein is above the apparent limit of quantitation (25 µg/g). Additionally, the Specification limit for protein (≤100 µg/g) is well above the limit of quantitation.
a. Please clarify the likely source of residual protein, including possible fermentation media components.
b. Please clarify if the same method is used for batch analyses provided in the notice and routine measures to confirm Specifications are met.

The residual protein level measured in one batch (Lot No. ilex13F02) was indeed slightly above the LOQ (*i.e.*, 27 µg/g, LOQ: 25 µg/g); however, due to the presence of protein at a very low level that remained within the defined specification parameter (which is aligned with numerous GRNs filed without objection; see Table 4 below) the exact nature of this protein was not further characterized.

a. To identify the likely source of residual protein, Inbiose has assessed the manufacturing process and process checks, as well as the conditions under which the analyzed batches of 3'-SL were manufactured. First, production of 3'-SL sodium salt is conducted *via* the utilization of the production organism, a glucose or sucrose carbon source, and lactose (sourced from bovine milk) in a minimal fermentation medium. At this stage, the bovine-derived lactose and production organism itself would be the only potential sources of protein. However, the multiple post-fermentation downstream processing steps (*i.e.*, microfiltration and ultrafiltration steps with low molecular weight cut-offs) are then conducted to remove any protein residue that may originate from the raw materials or production organism.

   Therefore, consideration must also be made as to the conditions under which the analyzed batches were produced. The pilot plant facility (HACCP and FSSC 22000 certified) used in the production of the analyzed batches of 3'-SL ingredient also processes other food materials that contain milk protein while utilizing the same drying equipment. Although rigorous cleaning procedures are used in between processes, residual traces of milk protein may still be present as a cross contaminant in any batches of 3'-SL produced in this facility. Because Inbiose's 3'-SL is manufactured using lactose sourced from cow's milk as a raw material and it may therefore contain traces of milk protein, it will be labeled as "contains milk" to remain in accordance with the requirements of the Food Allergy, Labelling and Consumer Protection Act of 2004.

   Therefore, the likely source of residual protein in the analyzed batch of Inbiose's 3'-SL is milk protein. The level of residual protein in Inbiose's 3'-SL is controlled and monitored to ensure the level of protein in the final ingredient remains within the specification parameter (*i.e.*, ≤100 µg/g), which is aligned with numerous recent HMO preparations that have been notified and received "no questions" letters from the FDA. Hence, production of Inbiose's 3'-SL ingredient in a facility where other milk products are also produced is not anticipated to be a safety concern.

b. The Roti®-Nanoquant method that was used for batch analysis in this GRN is the same method used for routine batch inspection to confirm the product specifications are met. Notably, the LOQ for the method used to determine protein content in Inbiose's 3'-SL sodium salt is aligned with

those described in a wide range of GRNs for other HMO ingredients produced using similar manufacturing techniques (*i.e.*, fermentation) (see Table 4, below).

Table 4 Specifications and LOD/LOQs reported in previous GRN notices #

GRN	Date of filing	Date of closure	FDA's Letter	Method	Specification	LOD/LOQ	Reference
001074	Sep 30, 2022	Ongoing	N/A	Roti®Nanoquant	≤ 100 µg/g	25 µg/g	Current GRAS Notice
001016	Sep 24, 2021	Jul 15, 2022	"No questions"	Nanoquant (modified Bradford)	≤ 100 µg/g	10 µg/g	Spec and LOQ: Table 2, page 8
001015	Sep 24, 2021	Jul 15, 2022	"No questions"	Nanoquant (modified Bradford)	≤ 100 µg/g	10 µg/g	Spec and LOQ: Table 2, page 8-9
001014	Oct 18, 2021	Jul 15, 2022	"No questions"	Nanoquant (modified Bradford)	≤ 100 µg/g	10 µg/g	Spec and LOQ: Table 2, page 10-11
000951	Oct 02, 2021	Aug 12, 2021	"No questions"	Nanoquant (modified Bradford)	≤ 100 µg/g	25 µg/g	Spec and LOQ: Table 3, page 17
000929	Jun 19, 2020	Feb 26, 2021	"No questions"	Nanoquant (modified Bradford)	≤ 100 µg/g	10 µg/g	Spec: GRN 929, Table 4, page 7 LOQ: GRN 929 amendments, Attachment 1, Table A-11, page 26
000925	May 15, 2020	Feb 08, 2021	"No questions"	Nanoquant (modified Bradford)	≤ 100 µg/g	10 µg/g	Spec and LOQ: Table 3, page 11
000923	May 14, 2020	Feb 02, 2021	"No questions"	Nanoquant (modified Bradford)	≤ 100 µg/g	10 µg/g	Spec and LOQ: Table 3, page 11
000922	June 3, 2020.	Apr 23, 2021	"No questions"	Nanoquant (modified Bradford)	≤ 100 µg/g	10 µg/g	Spec and LOQ: Table 3, page 7
000921	May 14, 2020	Oct 30, 2020	"No questions"	Nanoquant (modified Bradford)	≤ 100 µg/g	10 µg/g	Spec and LOQ: Table 3, page 12

HMOs indicated in Table 4., likewise Inbiose's 3´-SL product, were produced from fermentation processes using *Escherichia coli* strains, and using similar production techniques.
N/A – to available

Question 7. The exposure estimates presented in section 3.1.2 (p. 21) for infants, toddlers, children, teens, and adults, consuming food containing 3'-SL, appear to be based on GRN 000880 uses only, as stated in Footnote b to Table 3.1.2-1. The estimates do not appear to be cumulative estimates of dietary exposure that include the increased use in "toddler formula" (0.28 g/L) or the uses provided in GRN 000766 (e.g., herbal teas, cappuccino drinks, imitation and flavored milks, sugar substitutes) that are not included in the intended uses in GRN 000880. We request that you address estimated dietary

exposure to 3'-SL from intended uses in GRN 001074 and the cumulative estimated dietary exposure to 3'-SL from existing uses.

a. Using the combined intended uses (i.e., infant formula, infant and "toddler" foods, other food categories) in GRN 001074, please provide an updated dietary exposure estimate to 3'-SL for infants, toddlers (1-3 y), children (4-10 y) and the total consuming population ages 2 years and older on a consumers- only basis.

b. Please clearly discuss if your dietary exposure estimates are cumulative estimates to 3'-FL from existing and intended uses or reflect only partial substitution of existing 3'-FL uses.

a. A summary of the cumulative food uses and maximum use levels of 3'-SL utilized in the determination of the cumulative estimated dietary exposure is provided in Table 1 (see response to FDA Question 2c, above). The uses of 3'-SL and maximum use levels were amalgamated as those that have previously been determined GRAS and notified to the U.S. FDA in GRNs 000766, 000880, 000921, and 001015. As discussed above, the proposed uses of Inbiose's 3'-SL are fully substitutional to other forms of 3'-SL that have already been determined GRAS. All food codes included in the cumulative intake assessment are provided in Appendix A.

Table 5 summarizes the estimated total intake of 3'-SL (g/person/day) from maximum conditions of use previously determined to be GRAS in the U.S. for each of the requested population groups. Table 6 presents these data on a per kilogram body weight basis (mg/kg body weight/day). The percentage of consumers was high among all age groups evaluated in the current intake assessment; more than 68.9% of the population groups consisted of consumers of these food products (see Table 5). The consumer-only estimates are more relevant to risk assessments, as they represent exposures in the target population; consequently, only the consumer-only intake results are discussed in detail herein.

Among the total population (2 years and older), the mean and 90th percentile consumer-only intakes of 3'-SL were determined to be 0.39 and 0.71 g/person/day, respectively. Of the individual population groups, the infants 7 to 12 months of age were determined to have the greatest mean and 90th percentile consumer-only intakes of 3'-SL on an absolute basis, at 0.48 and 0.89 g/person/day (see Table 5).

Table 5. Summary of the Estimated Cumulative Daily Intake of 3'-SL Sodium Salt Based on Maximum Conditions of Use Previously Determined to be GRAS in the U.S. by Population Group (2017-2018 NHANES Data)

Population Group	Age Group	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to 6 m	0.19	0.43	68.9	128	0.28	0.53
Infants	7 to <12 m	0.46	0.89	97.7	123	0.48	0.89
Toddlers	1 to 3 y	0.22	0.42	98.4	407	0.23	0.42
Children	4 to 10 y	0.23	0.50	98.3	761	0.23	0.51
Total population	≥2 y	0.35	0.67	91.2	5,622	0.39	0.71

3'-SL = 3'-sialyllactose; GRAS = Generally Recognized as Safe; m = months; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States; y = years.

On a body weight basis, the total population (2 years and older) mean and 90th percentile consumer-only intakes of 3'-SL were determined to be 6 and 12 mg/kg body weight/day, respectively. Among the individual population groups, infants 7 to <12 months of age were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 52 and 96 mg/kg body weight/day, respectively (see Table 6).

Table 6. Summary of the Estimated Cumulative Daily Per Kilogram Body Weight Intake of 3'-SL Sodium Salt Based on Maximum Conditions of Use Previously Determined to be GRAS in the U.S. by Population Group (2017-2018 NHANES Data)

Population Group	Age Group	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to 6 m	29	65	68.9	128	41	66
Infants	7 to <12 m	51	96	97.7	123	52	96
Toddlers	1 to 3 y	17	32	98.4	397	17	33
Children	4 to 10 y	8	17	98.3	759	8	17
Total population	≥2 y	6	11	91.2	5,574	6	12

3'-SL = 3'-sialyllactose; bw = body weight; GRAS = Generally Recognized as Safe; m = months; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States; y = years.

b. The dietary exposure estimates of 3'-SL presented in Tables 5 and 6, above, are cumulative estimates of all 3'-SL uses previously determined to be GRAS in the U.S. (*i.e.*, GRNs 000766, 000880, 000921, and 001015) at each respective maximum use level. The intended use of Inbiose's 3'-SL is entirely substitutional for other forms of 3'-SL that are currently considered GRAS for use in infant formula and conventional foods. Importantly, the intended food uses and use levels of 3'-SL described above have not been expanded in this response in any manner from those that have already been concluded to be GRAS.

Microbiology:

Question 8. Please confirm whether *E. coli* "K-12 MG1655 INB-3SL_01" is non-pathogenic and non-toxigenic. Also, briefly discuss (with relevant references, as appropriate) any phenotypic characteristics of *E. coli* K-12 "K-12 MG1655 INB-3SL_01" (e.g., production of antimicrobials, production of secondary metabolites, antimicrobial resistance) and whether these pose a safety concern.

As indicated in Subsection 2.2.1.1 of the GRAS Notice, the host organism, *E. coli* K-12 MG1655, is not considered a human or animal pathogen and is non-toxigenic. As this organism is classified as Biosafety Level 1 classification by the American Type Culture Collection (ATCC), and meets the Organisation for Economic Co-operation and Development (OECD)'s Good Industrial Large-Scale Practice (GILSP) criteria for working with genetically modified microorganisms (OECD, 1992).

Inbiose hereby confirms that the 3´-SL sodium salt ingredient subject to this GRAS notification is produced using a non-pathogenic and non-toxigenic production organism.

Phenotypic characteristics of the E. coli K-12 and its derivative MG1655 strain are described in the 'Attachment I of the Final Risk Assessment of Escherichia coli K-12 derivatives' (EPA, 1997). Some

phenotypic characteristics are highlighted below and were already discussed in GRNs: 000749, 000897and 000951, which used Inbiose´s technology for the host strain development.

- E. coli K-12 lacks virulence factors and has a deficient O-specific side chain on its lipopolysaccharide
- E. coli K-12 cannot colonize the human colon
- E. coli K-12 appears to lack the ability to produce significant amounts of toxins that affect humans
- There is no evidence that E. coli K-12 may lead to hazardous effects to other microorganisms in the environment, or plants or animals. As such, there is no evidence at all that E. coli K-12 is producing antimicrobials or other harmful secondary metabolites
- E. coli K-12 has no known survival mechanisms in the environment, e.g. it is not able to produce spores

In addition, no extra genetic modifications were introduced into production host E. coli "K-12 MG1655 INB-3SL_01" that would lead to harmful antimicrobial or secondary metabolites. Also, no antibiotic markers, and thus antimicrobial resistance, is present in the production host as described in the Section 2.2.1.2 of the GRAS notice. As such, there is no evidence that safety concerns arise with the usage of production host E. coli "K-12 MG1655 INB-3SL_01" to produce 3'-SL.

Reference

EPA (1997). *Escherichia Coli K-12 Final Risk Assessment: Attachment I--Final Risk Assessment of Escherichia Coli K-12 Derivatives*. Washington (DC): U.S. Environmental Protection Agency (U.S. EPA), Biotechnology Program under the Toxic Substances Control Act (TSCA). Available at: https://www.epa.gov/sites/production/files/2015-09/documents/fra004.pdf [Last updated on September 27, 2012].

Question 9. On p. 8 of the notice, Inbiose states that "*E. coli* K-12 strain MG1655 has been classified Biosafety Level 1 by the ATCC" and provides a weblink under Footnote 2. We note that the weblink results in a "Page Not Found (404 Error)." Please provide an updated weblink for Footnote 2.

Thank you for alerting us to this issue. Indeed, the previous weblink was incorrect. The correct weblink is, as follows: https://www.atcc.org/products/700926 (accessed: February 8, 2023).

Question 10. Please provide a description of the fermentation process and details on the in-process controls Inbiose has in place. Additionally, please state whether the fermentation process is conducted in a contained, sterile environment.

The strain purity is ensured as described in the response to the FDA Question 12.

The overall fermentation process used in the production of Inbiose's 3´-SL is conducted within a contained, sterile, environment, and the use of strict process controls (like monitoring the temperature, pH, dissolved oxygen (pO2) ensures that the production strain grows at its most ideal

and optimal conditions and further ensures the purity and genetic makeup of the host culture remains stable throughout fermentation. Using the same conditions for each fermentation ensures that the same time profiles of the fermentation parameters are obtained (e.g. pO2, pH, CO_2 production, concentration of the different sugars). When contamination occurs, those profiles immediately change. Additionally, the microbial contaminants during the fermentation are controlled via strain specific plating method. Hence, any contamination is easily detected.

Question 11. Please identify any materials used in the production and formulation of 3'-SL that are derived from major allergens (other than cow-milk allergens). Please state whether any of these materials will be present in the final product. If no allergens are present, please provide a statement confirming this. Please note that sesame is now considered a major food allergen as of January 1, 2023 (https://www.fda.gov/food/cfsan- constituent-updates/faster-act-video-food-industry-and-other-stakeholders).

Inbiose's 3´-SL sodium salt is produced using milk-derived lactose. As such, any products that include this ingredient would be required to include "contains milk" on the label in accordance with the requirements of the Food Allergy, Labelling and Consumer Protection Act (FALCPA) of 2004. None of the other raw materials used in the fermentation are themselves considered, or are derived from, major allergens as defined by FALCPA (*i.e*., milk, egg, fish, Crustacea shellfish, tree nuts, wheat, peanuts, and soybeans or sesame).

Question 12. Please briefly describe how the purity of *E. coli* "K-12 MG1655 INB-3SL_01" is ensured.

As indicated on page 12 of the GRAS notice, the production strain INB-3SL_01 proved to be 100% stable within the production environment, which was verified by next-generation sequencing of microbial samples taken at the end of the fermentation at the pilot scale. This confirmed no contamination of the host strain and thus the fermentations were considered pure.

The overall fermentation process used in the production of Inbiose's 3´-SL is conducted within a contained, sterile, environment, and the use of strict process controls ensures that the purity and genetic makeup of the host culture remains stable throughout fermentation.

Once the identity of the INB-3SL_01 production strain was originally established, a batch of cryovials was collected from the strain for 3´-SL production. The following quality checks are performed on randomly selected cryovial to ensure purity and genetic uniformity of the production strain prior to use in the fermentation process:

- Inoculation and growth on Lysogeny broth (LB) and Minimal medium, followed by the extraction of genomic DNA for Whole Genome Sequencing (WGS) *via* the Illumina platform (*i.e.*, 150 bp paired-end sequencing), to monitor genetic uniformity and purity of the collected cryovials
- Polymerase chain reaction (PCR) checks to confirm the presence of all integrated genes in the collected samples
- Growth in a shake flask with minimal medium, followed by gram-staining of the production organism, measurement of sugar production and the optical density at 600 nm.
- Measurement of colony forming unit (CFU) counts and colony morphology checks. Random colonies are selected from all plated samples and integrated genes are checked *via* PCR. A

growth experiment is also performed with random selected colonies from all plated samples to evaluate the growth speed (μ_{max}) and 3´-SL production after 72 hours of growth.

After the cryovial quality is verified, fermentations with INB-3SL_01 are performed using cryovials from the batch as an inoculum.

As indicated in the GRAS Notice Section 2.2.1.2, the "*production strain INB-3SL_01 proved to be 100% stable within the production environment after analysis by next generation sequencing of samples at the end of fermentation at pilot scale.*" This purity and genetic stability analysis of the INB-3SL_01 strain host culture was conducted post-fermentation, following five non-consecutive fed-batch fermentations, using WGS of genomic DNA obtained from the residual biomass at the end of each fermentation. Each of the tested samples of residual biomass correspond to the five batches of 3´-SL as described in Section 2.3.4 of the GRAS Notice. Results from the WGS of post-production biomass were compared with results from an overnight LB culture of the production strain, INB-3SL_01. No evidence of significant mutation was observed in any of the five samples collected post-fermentation relative to the production strain INB-3SL_01. All mutations identified in post-fermentation samples were comparable to those of the INB-3SL_01 strain reference from the initial cryovials. Overall, these analyses support that the genetic stability and purity of the production strain is maintained throughout fermentation.

Question 13. On p. 18, Inbiose states that it performs microbiological endotoxin, residual protein, and residual DNA analyses on "the regulatory batches." Please define what "regulatory batches" means. Additionally, please explain the relevance of these tests to Inbiose's conclusion that 3'-SL is safe for its intended use.

Thank you for providing us with the opportunity to bring clarity regarding our use of the term "regulatory batches". In this context, the term was used to describe the commercially representative batches that were analyzed to establish compliance with the proposed specifications (or "regulatory compliance") with the defined product specifications. A more appropriate term for these batches in this context would be "commercially representative batches". The additional batch analyses discussed on page 18 of the GRN (*i.e.*, microbial endotoxins, residual protein, and residual DNA) were all conducted on the same 5 non-consecutive commercial batches of Inbiose's 3'-SL sodium salt that underwent the standard product batch analyses summarized in Table 2.3.2-1 (page 16) of the GRN. The 5 tested batches of Inbiose's 3'-SL (Batch IDs:. ilex13F02, ilex13F03, ilex13F04, ilex013F07, ilex13F08) are representative of the final 3'-SL ingredient that is to be commercialized. Each of the tested batches were measured to contain minimal levels of endotoxin and residual protein that are well below the proposed specification limits for each parameter and, therefore, are not considered a safety concern. Additionally, no detectable levels of residual DNA were measured in these 5 commercially representative batches of 3'-SL, which indicates an absence of residual production organism DNA in the final product. These additional analyses were conducted to ensure that Inbiose's manufacturing process for 3'-SL results in a final product that does not contain any of these potential contaminants at levels that could be of concern. The results from these analyses indicate that commercially representative batches of 3'-SL, produced *via* the manufacturing process described in the GRN, contain levels of these analytes that are safe for the intended uses of 3'-SL.

Question 14. On p. 15 of the notice, the Specification for *Cronobacter* (*Enterobacter*) *sakazakii* is listed as "Absent in 25 g" and the analytical method is ISO/TS 22964.

a. We note that the current version of this method is ISO 22964:2017. The method states that it has been validated for test portions of 10 g. We recommend that *C. sakazakii* testing be performed on sample sizes no larger than 10 g to prevent the possibility of false negatives.

b. ISO/TS 22964:2017 corresponds to "Microbiology of the Food Chain - Horizontal Method for the Detection of *Cronobacter* spp." Please clarify whether Inbiose tests for the presence of *Cronobacter* spp. or *C. sakazakii*, Specifically. If it is the former, please state whether presumptive positives are further analyzed to determine if the isolate is *C. sakazakii*.

a. Inbiose confirms that the analysis for *Cronobacter* spp. is now performed in compliance with the current version of this test method, ISO 22964:2017. While Inbiose acknowledges that ISO 22964:2017 is validated for test portions of 10 g or smaller, larger test portions can be used when "*validation/verification study has shown that there are no negative effects on the detection of Cronobacter spp.*" (ISO, 2017). Use of this test method for the analysis of *Cronobacter* spp. in 25 g test portions has been validated by the external lab contracted by Inbiose to conduct this analysis; however, Inbiose appreciates the recommendation provided by FDA concerning the sample size and will take it into consideration in future GRAS notices.

b. Inbiose clarifies that the test method utilized to evaluate the presence of *Cronobacter* spp. in the final 3'-SL ingredient is ISO 22964:2017, which has been validated by the testing facility for use in test portions of 25 g. Indeed, this version of the ISO method, "Microbiology of the Food Chain – Horizontal Method for the Detection of *Cronobacter* spp." tests for the presence of *Cronobacter* spp., of which *C. sakazakii* is included. The description for the *Cronobacter* specification parameter included in this GRN should therefore be described as "*Cronobacter* spp." instead of "*Cronobacter (Enterobacter) sakazakii*".

According to the ISO 22964:2017 method, the method is used to determine the presence or absence of *Cronobacter* spp. (including *C. sakazakii*). Since *Cronobacter* spp. was not detected in any 3'-SL batches analyzed and included in this GRN, no further identification for *C.* sakazakii were performed. However, if the presence of *Cronobacter* spp. is confirmed in any future production batch of Inbiose's 3'-SL, the batch would subsequently be rejected and an additional step would be performed to determine if the isolate contains *C. sakazakii.*

Question 15. *C. sakazakii* has been isolated from foods intended for very young children and can cause infection in infants and young children. Inbiose lists the intended use of 3'-SL as an ingredient in formula and drinks intended for young children (>12 months of age) and in foods intended for infants and young children. We note there remains a potential risk to these vulnerable populations if *C. sakazakii* is not controlled for during the production of 3'-SL or if foods formulated with this ingredient are not treated with an inactivation step (e.g., retort) before consumption by infants or young children. The following publications discuss the prevalence and potential concerns of *C. sakazakii* presence in foods intended for infants and young children:

- Chen, Q., Zhu, Y., Qin, Z., Qiu, Y., & Zhao, L. (2018). *Cronobacter spp.*, foodborne pathogens threatening neonates and infants. Frontiers of Agricultural Science and Engineering, 5(3), 330-339.
- Forsythe, S. J. (2015). New insights into the emergent bacterial pathogen *Cronobacter*. In Food Safety (pp. 265-308). Academic Press.

Given that the intended uses include in foods intended for consumption by infants and very young children, please provide a discussion on how Inbiose plans to control for the presence of *C. sakazakii* in formula and drinks intended for young children (>12 months of age) and in foods intended for infants and young children. Additionally, please describe why a *C. sakazakii* Specification is not provided for these products.

Inbiose acknowledges the FDA's concerns related to *C. sakazakii* as described in the Question above. For this reason, *Cronobacter* spp. analysis, conducted in compliance with ISO 22964:2017 (see Response 14), is proposed as a specification parameter for Inbiose's 3'-SL. The presence of *C. sakazakii* is therefore a routinely measured parameter in the production of this ingredient *via* a more generalized test for *Cronobacter* spp. (*i.e.*, ISO 22964:2017). Moreover, the absence (or presence) of *C. sakazakii* is monitored and controlled throughout the production of this 3'-SL ingredient with the use of critical control points in the production process (*e.g.*, sterile filtration before drying step). Any batch of Inbiose's 3'-SL that is detected to contain measurable levels of *Cronobacter* spp. would be rejected during quality control checks as it would not satisfy the established specification parameters described in this GRN. Additional testing would be conducted on rejected batches of 3'-SL to further analyze presumptive positives and to determine if any detected *Cronobacter* isolates are *C. sakazakii* (see Response 14).

Furthermore, batch data from five non-consecutive batches of Inbiose's 3'-SL (Batch IDs: ilex13F02, ilex13F03, ilex13F04, ilex013F07, ilex13F08) confirm the absence of *Cronobacter* spp. (which includes *C. sakazakii*).

Nonetheless, Inbiose is not a manufacturer of formula and drinks intended for young children (>12 months of age) or foods intended for infants and young children and is therefore not in a position to create additional specifications for such end-use products.

Toxicology:

Question 16 In Table 2.3.1-1 on p. 14, Inbiose indicates that 3'-sialyllactulose is "not Specified." However, as noted in recent scientific opinions on 3'-SL by the European Food Safety Authority, lactulose can have a laxative effect.[1,2] Please provide a justification for why a Specification for 3'-sialyllactulose is not required to ensure the safety of Inbiose's 3'-SL, especially considering the use in a sensitive subpopulation, such as infants, who consume formula as a sole source of nutrition.

[1] EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Turck D, Bohn T, et al. Safety of 3'- sialyllactose (3'-SL) sodium salt produced by derivative strains of *Escherichia coli* BL21 (DE3) as a Novel Food pursuant to Regulation (EU) 2015/2283. *EFSA J*. 2022;20(5):07331. doi:10.2903/j.efsa.2022.7331

[2] EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Turck D, Castenmiller J, et al. Safety of 3'-sialyllactose (3'-SL) sodium salt as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA J*. 2020;18(5):6098. doi: 10.2903/j.efsa.2020.6098

As indicated in the response to FDA Question 4, 3'-sialyllactulose was measured in 5 batches of Inbiose's 3'-SL ingredient at levels up to 0.29% w/w. The level of 3'-sialyllactulose in Inbiose's is therefore at least 17-fold lower than the maximum specified level of 3'-sialyllactulose in 3'-SL (*i.e.*, 5.0% w/w) that has previously been affirmed as GRAS in GRN 880 and concluded to be safe by the European Food Safety Authority (EFSA) in its safety opinion on this ingredient (EFSA, 2020). Moreover, the highest anticipated consumer-only intakes of Inbiose's 3'-SL in infants are expected to be 0.28 and 0.53 g/day (ages 0 to 6 months) and 0.48 to 0.89 g/day (ages 7 to <12 months) at the means and 90th percentiles, respectively (see Response 7, above). On a body weight basis, these 90th percentile intakes correspond to intakes of 66 and 96 mg 3'-SL/kg body weight/day for infants ages 0 to 6 and 7 to <12 months, respectively. Using the 90th percentile intakes for these sensitive subpopulations, and the highest measured quantity of 3'-sialyllactulose measured in Inbiose's 3'-SL (*i.e.*, 0.29% w/w), the 90th percentile consumer-only intakes of Inbiose's 3'-SL in infants would only result in 3'-sialyllactulose intakes of 0.0015 and 0.0026 g/day (or 0.19 and 0.28 mg/kg body weight/day) in infants ages 0 to 6 and 7 to <12 months, respectively. According to EFSA Panel 3'-sialyllactulose levels up to 3.6 mg/kg body weight are considered not having any laxative effect (EFSA NDA Panel, 2020). Therefore, the intake of 3'-sialyllactulose from the proposed uses and use levels of Inbiose's 3'-SL will result in a much smaller intake of 3'-sialyllactulose than the 3.6 mg/kg body weight/day evaluated in the EFSA Opinion (*i.e.*, estimated daily intake of 71 mg 3'-SL/kg body weight in infants up to 11 months and up to 5% w/w 3'-sialyllactulose in the assessed ingredient). Additionally, no laxative effects were identified in the 90-day subchronic study of Inbiose's 3'-SL conducted in rats at dose levels up to 5,000 mg/kg body weight/day (Spezia, 2022 [unpublished]).

Reference

EFSA NDA Panel, 2020. EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Turck D, Castenmiller J, et al. Safety of 3'-sialyllactose (3'-SL) sodium salt as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA J*. 2020;18(5):6098. doi: 10.2903/j.efsa.2020.6098

Question 17. On p. 20, Inbiose states, "In line with GRN 921, Inbiose's 3'-SL sodium salt is intended for use as a food ingredient in term infant formula (0-12 months) and toddler formula at concentrations up to 0.28 g/L." We note that GRN 000921 did not include uses in formula for young children (>12 months; i.e., "toddler" formula). However, we note that in GRN 001015, a use level of 0.28 g/L was concluded to be GRAS in formulas for young children. Please discuss the data supporting a use level of 0.28 g/L in formula for young children either by incorporating into the notice information from GRN 001015 and/or by providing an updated safety narrative for young children consuming 3'-SL.

In recent years, numerous GRAS conclusions have been notified to the U.S. FDA for 3'-SL sodium salt (*i.e.*, GRN 000766, GRN 000880, GRN 000921, and GRN 001015). Within these GRNs (and GRN 001074), critical and comprehensive reviews of the publicly available data and information

pertaining to the safety of 3'-SL sodium salt for use as an ingredient in non-exempt infant formula, and various food and beverage products from multiple categories have been presented. In each, data and information supporting the GRAS use of 3'-SL sodium salt as an ingredient in such products have been critically reviewed by a number of qualified scientific experts, including the U.S. FDA. Following review of the data presented in these previous GRNs, 3'-SL is currently considered GRAS for all uses and use levels presented in Table 1 above (see response to FDA Question 2c), which includes the use of 3'-SL in formulae for young children at levels up to 0.28 g/L.

As reported in GRN 000921 (Part III, Section B) and GRN 001074 (Part 6.1), measured concentrations of 3'-SL in human milk range from 0.08 to 0.41 g/L; levels that remain steady throughout lactation (Austin *et al*., 2016; Kunz *et al*., 2017; Sprenger *et al*., 2017; Ma *et al*., 2018). The proposed use levels of 3'-SL in infant and toddler formulae described within GRN 001015 and this response document for GRN 001074 (*i.e.*, up to 0.28 g/L) are consistent with, and therefore supported by, the measured levels of 3'-SL in human milk. Since current GRAS uses of 3'-SL sodium salt as an ingredient in non-exempt term infant formula and toddler formula at levels up to 0.28 g/L are within the established range that infants are exposed to following the ingestion of human milk, there is no safety concern for excessive exposure from infant formula manufacturers using 3'-SL at these defined use levels.

Moreover, toxicological data used to support the use level of 0.28 g 3'-SL/L in formula for young children were previously discussed in detail in Section C of GRN 000921 (Pages 25 to 38) and Section 6.4.1 of GRN 000880 (Pages 37 to 42), which were subsequently incorporated by reference in GRN 001015 (Section C; Pages 20 to 21). Within these 3'-SL GRNs, a study conducted to assess the toxicological safety of 3'-SL published by Phipps *et al.* (2019) is frequently referenced as the pivotal toxicological study and was therefore incorporated by reference as such in the safety narrative of GRN 001015. A brief discussion of these data is included below.

A battery of toxicological tests was conducted in the risk assessment of 3'-SL published by Phipps *et al.* (2019) that includes a bacterial reverse mutation test, an *in vitro* mammalian cell micronucleus test, and 14- and 90-day *in vivo* studies conducted in neonatal rats. Briefly, in the bacterial reverse mutation test and the *in vitro* mammalian cell micronucleus test, 3'-SL was reported to be non-mutagenic at concentrations up to 5,000 µg/plate and neither clastogenic nor aneugenic at concentrations up to 2,000 µg/mL, respectively. Both *in vitro* studies were conducted in accordance with the respective OECD test guidelines.

In the subchronic 90-day toxicity study conducted according to the OECD Test Guideline 408 (OECD, 1998), neonatal rats (8/group/sex) received 0 (water for irrigation), 1,000, 3,000, or 5,000 mg 3'-SL sodium salt/kg body weight/day, by oral gavage, once daily from the PND 7 for at least 90 days. Tested dose levels were selected based on the results of the 14-day study (also conducted in neonatal rats). Following the 90-day treatment period, an additional 5 rats/group/sex were included in a recovery phase, during which the rats were kept un-dosed for additional 4 weeks, to assess whether any effects observed at the end of the dosing phase persist, partially or fully recover. For direct comparison against the high 3'-SL sodium salt dose group, a reference control group (10 rats/sex) received GRAS non-digestible oligosaccharide fructooligosaccharides (FOS) at a dose level of 5,000 mg/kg body weight/day to assess any fiber-specific effects. No test item-related deaths,

clinical signs, or ophthalmological changes were observed at the end of the treatment period and the no-observed-adverse-effect level (NOAEL) in this study was established at 5,000 mg/kg body weight/day. In conjunction with Inbiose's product-specific toxicological data included in GRN 001074 (and updated in Response to FDA question 18, below), which indicate that 3'-SL is non-genotoxic, non-mutagenic, and safe in juvenile Sprague Dawley rats at levels up to 5,000 mg/kg body weight/day, these data support the safe use of 3'-SL in formulae for young children at levels up to 0.28 g/L.

Additionally, two studies conducted to evaluate the safety and tolerance of 3'-SL in neonatal piglets (Donovan *et al.*, 2017; Hanlon, 2020) were discussed briefly in Section D of GRN 001015 (Pages 21 to 23), which were incorporated by reference to Section 6.E.4 of GRN 000766 (Pages 53 to 56) and Section D of GRN 000921 (Pages 38 to 70) where the studies were discussed in greater detail. In the study conducted by Donovan *et al.* (2017), neonatal piglets were administered up to 500 mg 3'-SL sodium salt/L during the first 3 weeks of postpartum, which was observed to be well tolerated and supportive of normal growth patterns. In the Hanlon (2020) study, neonatal piglets were provided an "oligosaccharide blend" containing several HMOs including up to 0.3 g 3'-SL/L, over a 3-week test period. The "oligosaccharide blend" was observed to be well tolerated at all tested use levels and did not elicit any adverse effects on the safety parameters measured. These studies are therefore considered corroborative of the safety narrative discussed herein.

With regards to the clinical data presented in GRN 001015 to support a use level of 0.28 g/L in formula for young children (Section F.1; Pages 25 to 38), it was concluded HMOs are generally well tolerated in infants up to 1 g/day. Additionally, it was identified that non-digestible carbohydrates are well tolerated in enteral tube feeding formulas up to 63 g/day (GRN 000897; response to FDA Question 8; Pages 40-48 GRN 001015; Section F.2; Pages 39 to 48). Furthermore, in Section 6.F of GRN000766 (Pages 62 to 64), four intervention studies were discussed in which infant diets were supplemented with infant formulae containing up to 10 g/L bovine milk oligosaccharides (BMOs), the concentration of 3'-SL was not specified (Meli *et al.*, 2014; Simeoni *et al.*, 2016; Cooper *et al.*, 2017; Radke *et al.*, 2017). In each of these studies, the infant formulae supplemented with bovine 3'-SL were described as well tolerated and supportive of normal healthy growth.

Additionally, 3 newly identified clinical studies are incorporated by reference and briefly summarized in tabular format in Annex II: Summaries of Newly Identified Clinical Trials Conducted with 3'-SL sodium salt (see the response to the FDA question 20 and Annex II below). Overall, the results from the recently published studies, in conjunction with those evaluated in previous GRNs, support the conclusion that 3'-SL is safe and well tolerated in infants when provided at levels consistent with the proposed uses of Inbiose's 3'-SL described in the GRAS Notification.

The results of the cumulative intake assessment of all current GRAS uses of 3'-SL, presented in Response to FDA Question 7a (above), were estimated to be 0.48 and 0.89 g/person/day in infants 7 to 12 months of age at the mean and 90th percentile, respectively, on a consumer-only basis. These values remain below the levels of 3'-SL that are well tolerated in infants who have participated in clinical trials published in the scientific literature. Additionally, while the intake estimates for infant subpopulations presented herein are higher than what was presented in GRN 001015, the basis for

those numbers is unclear and we therefore believe the data presented herein are a more accurate estimation of intakes in infant subpopulations, which aligns with the intake estimates for these subpopulations presented in GRN 000880.

References

Amyris, Inc. (2022). GRAS Notification of 2'-fucosyllactose (2'-FL). (GRN No. 1015). Prepared by Emeryville (CA): Amyris, Inc. Submitted to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.fda.gov/media/154998/download

Austin S, De Castro CA, Benet T, Hou Y, Sun H, Thakkar SK, et al. (2016). Temporal change of the content of 10 oligosaccharides in the milk of Chinese urban mothers. Nutrients 8(6):346 [22pp]. DOI:10.3390/nu8060346.

Chr. Hansen A/S (2022). GRAS Notification of 3'-Sialyllactose {3'-SL) Sodium Salt. (GRN No. 1015). Prepared by Rockville (MD): Spherix Consulting Group. Submitted to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.fda.gov/media/155917/download

Cooper, P., Bolton, K. D., Velaphi, S., de Groot, N., Emady-Azar, S., Pecquet, S., & Steenhout, P. (2017). Early Benefits of a Starter Formula Enriched in Prebiotics and Probiotics on the Gut Microbiota of Healthy Infants Born to HIV+ Mothers: A Randomized Double-Blind Controlled Trial. Clinical medicine insights. Pediatrics, 10, 119–130. https://doi.org/10.4137/CMPed.S40134

Donovan SM (2017) [unpublished]. Pre-Clinical Study for 3'sialyl-Lactose Supplementation in Neonatal Piglets. A manuscript in preparation. Cited In: GeneChem, Inc., 2018 [GRN 766].

GeneChem, Inc. (2018). GRAS Notification of 3'-Sialyllactose {3'-SL) Sodium Salt. (GRN No. 766). Prepared by Clarksville (MD): NutraSource, Inc. Submitted to Daejeon, Republic of Korea: GeneChem, Inc. Submitted to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=766 [Sep. 26, 2018 - FDA response - no questions].

Glycom A/S (2019). GRAS Notice for 3'-Sialyllactose Sodium Salt (3'-SL). (GRN No. 880). Prepared by Hørsholm, Denmark: Glycom A/S. Submitted to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=880 [Feb. 21, 2020 - FDA response - no questions].

Hanlon PR (2020). A safety evaluation of mixed human milk oligosaccharides in neonatal farm piglets. Toxicol Res Appl 4 [8pp]. DOI:10.1177/2397847320971255.

Jennewein Biotechnologie GmbH (2020). GRAS Determination for the Use of 3'-Sialyllactose Sodium Salt in Non-Exempt Term Infant Formula. (GRN No. 921). Prepared by Rockville (MD): Spherix Consulting Group. Prepared for Rheinbreitbach, Germany: Jennewein Biotechnologie GmgH. Submitted to College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=921 [Oct. 30, 2020 - FDA response - no questions].

Kunz C, Meyer C, Collado MC, Geiger L, García-Mantrana I, Bertua-Ríos B, et al. (2017). Influence of gestational age, secretor and Lewis blood group status on the oligosaccharide content of human milk. J Pediatr Gastroenterol Nutr 64(17):789-798. DOI:10.1097/MPG.0000000000001402.

Ma L, McJarrow P, Jan Mohamed JB, Liu X, Welman A, Fong BY (2018). Lactational changes in the human milk oligosaccharide concentration in Chinese and Malaysian mothers' milk. Int Dairy J 87:1-10. DOI:10.1016/j.idairyj.2018.07.015.

Meli, F., Puccio, G., Cajozzo, C., Ricottone, G. L., Pecquet, S., Sprenger, N., & Steenhout, P. (2014). Growth and safety evaluation of infant formulae containing oligosaccharides derived from bovine milk: a randomized, double-blind, noninferiority trial. BMC pediatrics, 14, 306. https://doi.org/10.1186/s12887-014-0306-3

OECD (2018), Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264070707-en (adopted on the 25 June 2018)

Phipps KR, Baldwin NJ, Lynch B, Stannard DR, Šoltésová A, Gilby B, et al. (2019). Toxicological safety assessment of the human-identical milk oligosaccharide 3'-sialyllactose sodium salt [plus supplementary data]. J Appl Toxicol 39(10):1378-1393. DOI:10.1002/jat.3824.

Radke, M., Picaud, J. C., Loui, A., Cambonie, G., Faas, D., Lafeber, H. N., de Groot, N., Pecquet, S. S., Steenhout, P. G., & Hascoet, J. M. (2017). Starter formula enriched in prebiotics and probiotics ensures normal growth of infants and promotes gut health: A randomized clinical trial. Pediatric Research, 81(4), 622–631. https://doi.org/10.1038/pr.2016.270

Simeoni, U., Berger, B., Junick, J., Blaut, M., Pecquet, S., Rezzonico, E., Grathwohl, D., Sprenger, N., Brüssow, H., Szajewska, H., Bartoli, J. M., Brevaut-Malaty, V., Borszewska-Kornacka, M., Feleszko, W., François, P., Gire, C., Leclaire, M., Maurin, J. M., Schmidt, S., … Verdot, J. J. (2016). Gut microbiota analysis reveals a marked shift to bifidobacteria by a starter infant formula containing a synbiotic of bovine milk-derived oligosaccharides and Bifidobacterium animalis subsp. lactis CNCM I-3446. Environmental Microbiology, 18(7), 2185–2195. https://doi.org/10.1111/1462-2920.13144

Sprenger N, Lee LY, De Castro CA, Steenhout P, Thakkar SK (2017). Longitudinal change of selected human milk oligosaccharides and association to infants' growth, an observatory, single center, longitudinal cohort study. PLoS ONE 12(2):e0171814 [15pp, plus supplementary data]. DOI:10.1371/journal.pone.0171814.

Question 18. On p. 36, Inbiose states "The 90-day study in juvenile rats is currently in progress. The end of the *in vivo* phase (last day of euthanasia) was scheduled in Week 10, 2021." Thus, Inbiose's GRAS conclusion was made prior to knowing the results of this study. Please provide a summary of the results from this 90-day study and discuss whether these results support Inbiose's GRAS conclusion for the proposed uses of 3'-SL. We note that since Inbiose acknowledged the existence of this study in the current notice, we will not be able to complete our evaluation until Inbiose provides information regarding the outcome of the study and Inbiose's safety conclusions.

Following the filing of this GRAS Notice, the 90-day study was completed. Inbiose conducted this 90-day subchronic study in juvenile rats to corroborate the safety of 3´-Sialyllactose (3´-SL) sodium salt and to support premarket approvals in global jurisdictions where such studies may be necessary (Spezia, 2022 [final report, unpublished]). This study was conducted in accordance with the OECD Test Guideline 408 (2018).

In the main study, groups of 20 (10/sex/group) juvenile Sprague-Dawley rats were administered Inbiose´s 3´-SL sodium salt at dose levels of 0, 1,500, 3,000, or 5,000 mg/kg body weight/day *via* oral gavage, from post-natal day (PND) 7 to at least PND 98. An additional group was included for

reference, in which a group of 20 (10/sex) juvenile Sprague-Dawley rats were administered 5,000 mg fructooligosaccharides (FOS)/kg body weight/day. The control and high-dose groups also included 10 additional rats (5/sex/group) in the recovery phase, during which the rats were kept un-dosed for additional 5-week treatment-free period, to assess whether any effects observed at the end of the dosing phase persist, partially or fully recover. Finally, another 6 animals (3/sex/group) were included for toxicokinetic evaluation in each of the 0, 1,500, 3,000, or 5,000 mg 3´-SL sodium salt /kg body weight/day treatments.

The following parameters and end points were evaluated in this study during the dosing period: clinical observations, body weights, food consumption, growth (tibia length), ophthalmology, developmental pre-weaning end points, sexual maturation, estrous cycles, and neuro-behavioral development (behavioral functional observational battery, learning and memory retention, and locomotor activity), clinical pathology (hematology, coagulation, clinical chemistry, and urinalysis), thyroid hormone levels, gross necropsy findings, organ weights, sperm analysis data and histopathologic findings.

No 3´-SL sodium salt related deaths, clinical signs, adverse effects, or macroscopic and microscopic changes were observed following oral administration. The study researchers reported that Inbiose's 3´-SL sodium salt was "…*clinically well tolerated*…" *"No effects were noted on the hematology, blood biochemistry or urinalysis parameters, coagulation factors or thyroid hormone levels…".*

The no-observed-adverse-effect level (NOAEL) in this study was therefore established by the study director as 5,000 mg/kg body weight/day for both males and females, which supports Inbiose's GRAS conclusion that 3´-SL sodium salt is safe for use in non-exempt term infant formula and specified conventional food and beverage products as described in Section 1.3 of the GRAS Notice.

For the sake of completeness, Inbiose is also providing the U.S. FDA with the summary of this study, which was produced by the contract research organization, and is included in the attached pdf document entitled: **"Supplementary 90-day Subchronic Study Summary_3SL sodium salt".**

Question 19. Since each GRAS notice must independently support the safety of an ingredient for its intended use, please provide brief summaries of the clinical studies that Inbiose is incorporating into the notice in Section 6.4 on p. 43. As part of this discussion, we suggest including the infant population, study treatments including concentrations of the 3'-SL used in the test formulas, and outcome measures of the study as they relate to Inbiose's conditions of use.

The brief summaries of clinical studies incorporated by Inbiose in Section 6.4 are provided in Annex I (Summary of clinical studies incorporated in Section 6.4 of the GRAS notice). In four studies conducted in healthy infants fed with formula containing bovine milk-derived oligosaccharides (BMOs), the concentration of 3'-SL was not specified. In conclusion, the supplement of BMOs in infant formula is well tolerated and support the normal growth.

Question 20. Please provide an updated literature search for data and information related to the safety of 3'-SL, since Inbiose's literature search concluded in March 2021. As part of this search, please indicate if any clinical studies have been recently published in which 3'-SL was included in a test formula consumed by an infant population.

To address this question, Inbiose has conducted an updated literature search through February 15 2023 to identify any new publicly available data pertaining to the safety of 3'-SL that have been published since the original literature search was conducted in March 2021. No new data were identified in the updated search of the published literature that could be perceived as counter to Inbiose's 3'-SL GRAS conclusion; however, several new clinical studies were identified in support of the GRAS conclusion. While the results from these studies are not counter to the GRAS conclusion, these studies are summarized in Annex II (Summaries of Newly Identified Clinical Trials Conducted with 3´-SL sodium salt), for completeness. Briefly, 3'-SL (in combination with another HMOs) was not observed to elicit adverse effects in humans. Results from these recently published studies support that 3'-SL is safe and well tolerated in infants when provided at levels consistent with the proposed uses of Inbiose's 3'-SL described in the GRAS Notification. Inbiose therefore maintains that this 3'-SL ingredient is GRAS, on the basis of scientific procedures, for use in non-exempt term infant formula and Specified conventional food and beverage products, as described in the GRAS Notification.

Parschat et al. (2021) evaluated the effects of infant formula supplemented with 5 HMOs mixture (5.75 g/L total, comprising 52% 2'-FL, 13% 3'-FL, 26% LNT, 4% 3'-SL, and 5% 6'-SL) in healthy term infants. The increase of mean daily body weight and changes in anthropometric parameters, such as weight, length, and head circumference, were recorded over a 4-month period. The safety was measured via occurrence of adverse events, while the tolerability and behavioral parameters were measured via stool frequency and consistency, gurgitation, vomiting, flatulence, fussiness, crying, and awakening at night. The infants were allocated to test (N=86) and control (N=91) group and received infant formula with 5HMO-Mix and infant formula without 5HMO-Mix, respectively. In addition, a reference breast-fed infant group was included (N=88). No differences in weight, length, or head circumference gain were observed between the two formula groups. The frequency of AEs in the two formula groups were similar, which was slightly but not significantly higher than that for the breast-fed group. The authors showed that the mixture of 5 HMOs had positive effect on normal infant growth and was safe and well tolerated for use in healthy term infants.

Lasekan et al. (2022) conducted a randomized, controlled, multicenter, double-blind, parallel feeding growth and tolerance study to investigate the growth and gastrointestinal tolerance of milk-based infant formula supplemented with 5 HMOs in healthy term babies. The 5 HMO blend content used in this study was exactly the same as used by Parschat et al., 2021, i.e. 3.0 g/L of 2'-FL, 0.8 g/L of 3'-FL, 1.5 g/L of LNT, 0.2 g/L of 3'-SL and 0.3 g/L of 6'-SL. Infants were randomized to receive either a control (N = 129) or an experimental formula with blend of 5 HMOs (N = 130) through approximately 4 months of age. The breastfed infants (N = 101) were included as a reference group, as well. Weight, length, head circumference (HC), mean rank stool consistency (MRSC) number of stools per day and a percentage of feedings with spit-up/vomit associated with feeding were measured from day (D) 14 to D119. No differences were observed among the three groups for weight gain per day from 14 to 119 days ($p \geq 0.337$). Infants fed with experimental formula had more soft, frequent and yellow stools and were similar to the reference group. There were no differences between serious and non-serious adverse events among three groups. The blend of 5 HMOs was concluded to be safe and well-tolerated as well as supportive of normal growth. These results were in line with data published by Parschat et al. (2021).

In a randomized, controlled, double-blind trial, Bosheva et al. (2022) investigated the gut maturation effects (microbiota, metabolites, and selected maturation markers) of an infant formula containing a Specific blend of five HMOs. Healthy full-term infants were assigned to control group (CG) fed a standard IF without HMOs, test group 1 (TG1) and test group 2 (TG2) fed with the same standard IF

containing the five-HMO blend at a concentration of 1.5 g/L and 2.5 g/L, respectively. A non-randomized human milk-fed infants (HMG) served as reference group. Fecal samples collected at baseline, age 3 and 6 months, were analyzed for microbiome (shotgun metagenomics), pH and organic acids, as well as the biomarkers (immunoglobulin A (sIgA), calprotectin and alpha-1-antitrypsin). Higher bifidobacterial and lower toxigenic C. difficile abundance were observed in the TGs vs. CG. Early life intestinal immune response was improved as indicated by the higher fecal sIgA concentration in the TGs vs. CG. The authors concluded that the infant formula contained Specific HMO blend was able to support the development of the intestinal immune system, and shaped the gut microbiota directionally toward that of breastfed infants.

Question 21. On p. 24, Inbiose cites GRN 000921 and discusses the concentrations of 3'-SL in human milk to support the use level of 0.28 g/L in infant formula. Since the proposed use level of 0.28 g/L of 3'-SL in infant formula has previously been concluded to be GRAS, we note the following for informational purposes and do not require a response. Since the closure of GRN 000921, additional information on 3'-SL concentrations in human milk have been published in the literature, including systematic reviews, and we suggest including this information in future GRAS notices for HMO ingredients.

Inbiose appreciates the suggestion provided by FDA and will take it into consideration in future GRAS notices for HMO ingredients.

Additional corrections:

Inbiose took this opportunity to revise the content of the GRAS Notice 001074.

A. Heavy metal values in Table 2.3.2-1

Inbiose would like to inform that the Heavy metals values of Table 2.3.2-1 have been wrongly reported by the external lab performing the Heavy Metal analysis on two batches: ilex13F03 and ilex13F04. The external lab stated that:

> ''*Previously reported LOQs were incorrect due to a technical problem with Primoris' automatic reporting of the LOQs for heavy metals.*
> *Therefore, this report is regenerated with the correct and current LOQs.*''

Therefore, Inbiose would like to update the section of Heavy metals indicated in Table 2.3.2.1 of this GRN 001074, with the correct values (in blue) shown in Table 7 below..

Table 7. Analytical Data Obtained from 5 Batches of 3'-SL (only heavy metals specification is indicated).

Parameter	Specification	Batch IDs				
		ilex13F02	ilex13F03	ilex13F04	ilex13F07	ilex13F08
Heavy Metals						
Arsenic (mg/kg)	≤0. 1**	<0. 01	<0. 01	<0. 01	<0. 01	<0. 01

Parameter	Specification	Batch IDs				
		ilex13F02	ilex13F03	ilex13F04	ilex13F07	ilex13F08
Cadmium (mg/kg)	≤0. 1	<0. 005	<0. 005*	<0. 005*	<0. 005	<0. 005
Lead (mg/kg)	≤0. 02	<0. 01	<0. 01*	<0. 01*	<0. 01	<0. 01
Mercury (mg/kg)	≤0. 5	<0. 01	<0. 01*	<0. 01*	<0. 01	<0. 01

LOQs: As = 0.01 mg/kg; Cd = 0.005 mg/kg; Pb = 0.01 mg/kg; Hg = 0.01 mg/kg;
* Corrected heavy metal values
** Proposed specification limit of Arsenic, see the response to Question 5

B. Residual endotoxins specification

The specification for residual endotoxin level was erroneously expressed as 300 EU/g. The correct specification is 10 EU/mg, as indicated in Table 8 below with the corrected value and unit (in blue).

Table 8. Corrected Residual endotoxins specification for Inbiose's 3'-SL in Comparison to those of the 3'-SL Ingredients in GRN 833 and 923

Parameter	Specification for Inbiose's 3'-SL	Method of Analysis Employed by Inbiose	Specification Reported for Other 3'-SL Products		
			GeneChem's 3'-SL Sodium Salt Siallac3® (GRN 766) (U.S. FDA, 2018a)	Glycom's 3'-SL Sodium Salt (GRN 880) (U.S. FDA, 2020a	Jennewein's 3'-SL Sodium Salt (GRN 921) (U.S. FDA, 2020b)
Chemical Analysis					
Endotoxins	NMT 10 EU/mg	Ph. Eur. 2.6.14	NMT 300 EU/g	NMT 10 EU/mg	NMT 10 EU/mg

Annex I. Summary of clinical studies incorporated in Section 6.4 of the GRAS notice.

Type of Study	Population	Length of Study	Dose	Result	Reference
Randomized, double-blind, single-center study	Healthy term infants ≤14 days old	From enrolment until 4 months of age	Standard formula (control; n = 84) Standard formula with BMOS at a total oligosaccharide concentration of 7.3 ± 1.0 g/100 g of powder formula (IF-BMOS; n = 99) Standard formula with BMOS and probiotics (contained BMOS (7.3 ± 1.0 g/100 g of powder formula) as well as the probiotics *Bifidobacterium longum* ATCC BAA-999 (Bl999) and *Lactobacillus rhamnosus* CGMCC 1.3724 (LPR) each at 2 × 107 colony forming units (CFUs) per gram) (IF-BMOS + Pro; n = 98). Breastfed reference group (n = 30)	Compared with control, infants in the BMOS groups had similar Mean daily gains in length and head circumference during the first 4 months, more frequent ($p < 0.0001$) and less hard ($p = 0.0003$) stools. No significant differences of flatulence, vomiting, spitting up, crying, fussing, and colic were observed between the control and BMOS groups. Compared with control group, IF-BMOS + Pro group had higher stool bifidobacteria and lactobacilli counts ($p < 0.05$), whereas Clostridia counts were lower ($p < 0.05$) in both BMOS groups.	Meli et al., 2014

Type of Study	Population	Length of Study	Dose	Result	Reference
Randomized, placebo controlled, double-blind study	Healthy term newborns	From birth until 4 months of age	Control formula (N = 37) Formula supplemented with a prebiotic (bovine milk-derived oligosaccharides, BMOS) at a total oligosaccharide concentration of 5.7 ± 1.0 g/100 g of powder formula (N = 39) Breast milk (N = 39)	Compared with a non-supplemented control formula, Similar tolerability and a similar growth in healthy newborns (followed for 12 weeks) was observed for the test formula groups. Higher faecal pH and a significantly higher diversity of the faecal microbiota was observed in infants fed with control formula compared to the breast-fed reference group. In stool samples of the test group, the probiotic *B. lactis* increased by 100-fold and was detected in all supplemented infants.	Simeoni et al., 2016
Multicenter, randomized, double-blind, and controlled study	Healthy full-term infants aged 0–14 d old	12 months	Control formula (N = 207) Same formula supplemented with BMOS (milk oligosaccharides such as 3'-SL and 6'-SL) at concentration of 5.8 ± 1.0 g/100 g and a probiotic *B. lactis* (CNCM I-3446, 1×10^7 cfu/g) (N = 206) Breast milk (N = 63)	No significant differences were observed for diarrhea and febrile infections incidence between groups at 6 and 12 months. The test group showed improvements of the gut microbiota pattern, fecal IgA and stool pH. All these parameters were closely comparable with those of breastfed infants.	Radke et al., 2017

Type of Study	Population	Length of Study	Dose	Result	Reference
A Randomized Double-Blind Controlled study	Healthy, full-term, newborn infants ≤3 days of age born to HIV positive mothers	4 months	Cesarean-delivered infants fed with the test formula (n = 92) (a starter infant formula [IF] containing BMOS at a total oligosaccharide concentration of 5.8 ± 1.0 g/100 g of powder formula [8 g/L in the reconstituted formula] plus B. lactis [1×10^7 cfu/g]) Cesarean-delivered infants fed with the Control IF (n = 101) Vaginally delivered infants fed with the same test formula (n = 115) Vaginally delivered infants fed with the control formulas (n = 113)	Compared with the control formula group, the infants from test-group with cesarean birth or vaginal birth had significant higher Bifidobacteria counts up to 4 weeks and significant lower fecal pH at 10 days and 4 weeks. For both cesarean-delivered infants and vaginally delivered infants the number of serious adverse events was comparable in the control and test formula group.	Cooper et al., 2017

References

Cooper, P., Bolton, K. D., Velaphi, S., de Groot, N., Emady-Azar, S., Pecquet, S., & Steenhout, P. (2017). Early Benefits of a Starter Formula Enriched in Prebiotics and Probiotics on the Gut Microbiota of Healthy Infants Born to HIV+ Mothers: A Randomized Double-Blind Controlled Trial. Clinical medicine insights. Pediatrics, 10, 119–130. https://doi.org/10.4137/CMPed.S40134

Meli, F., Puccio, G., Cajozzo, C., Ricottone, G. L., Pecquet, S., Sprenger, N., & Steenhout, P. (2014). Growth and safety evaluation of infant formulae containing oligosaccharides derived from bovine milk: a randomized, double-blind, noninferiority trial. BMC pediatrics, 14, 306. https://doi.org/10.1186/s12887-014-0306-3

Radke, M., Picaud, J. C., Loui, A., Cambonie, G., Faas, D., Lafeber, H. N., de Groot, N., Pecquet, S. S., Steenhout, P. G., & Hascoet, J. M. (2017). Starter formula enriched in prebiotics and probiotics ensures normal growth of infants and promotes gut health: A randomized clinical trial. Pediatric Research, 81(4), 622–631. https://doi.org/10.1038/pr.2016.270

Simeoni, U., Berger, B., Junick, J., Blaut, M., Pecquet, S., Rezzonico, E., Grathwohl, D., Sprenger, N., Brüssow, H., Szajewska, H., Bartoli, J. M., Brevaut-Malaty, V., Borszewska-Kornacka, M., Feleszko, W., François, P., Gire, C., Leclaire, M., Maurin, J. M., Schmidt, S., … Verdot, J. J. (2016). Gut microbiota analysis reveals a marked shift to bifidobacteria by a starter infant formula containing a synbiotic of bovine milk-derived oligosaccharides and Bifidobacterium animalis subsp. lactis CNCM I-3446. Environmental Microbiology, 18(7), 2185–2195. https://doi.org/10.1111/1462-2920.13144

Annex II. Summaries of Newly Identified Clinical Trials Conducted with 3´-SL sodium salt.

Type of Study	Population	Length of Study	Dose	Outcome	Reference
Randomized, controlled, parallel-group clinical study	Term, healthy infants ≤14 days of age	112 ± 3 days	Infant formula mixed with 5HMO-Mix (2.99 g/L 2'-FL, 0.75 g/L 3-FL, 1.5 g/L LNT, 0.23 g/L 3'-SL, and 0.28 g/L 6'-SL) (N = 113) Control formula without HMOs (N = 112) Placebo: Breast milk (N = 116)	The results demonstrated that 5HMO-Mix at 5.75 g/L in infant formula was safe and well tolerated by healthy term infants during the first months of life.	Parschat et al., 2021
Randomized, double-blind, controlled parallel feeding growth trial	Healthy term infants (gestational age 37–42 weeks) between 0 and 14 days of age with a birth weight ≥ 2490 g.	Time of enrolment at ≤14 Days (D) of age until D 119 or up to D 183	Control milk-based formula (CF; n = 129); experimental formula (EF; N = 130) containing five HMOs (5.75 g/L; 2'-FL (3.0 g/L), 3-FL (0.8 g/L), LNT (1.5 g/L), 3'-SL (0.2 g/L) and 6'-SL (0.3 g/L)); reference group: human milk (HM; N = 104)	No significant differences among the three groups for weight gain per day and gains in weight and length (p ≥ 0.05) from D 14 to D 119. Color of stool, its consistency and frequencycy per day were more similar between EF and HM groups. Serious and non-serious adverse events were not different among groups. The results indicated that EF containing five HMOs was safe and well-tolerated and supported age-appropriate growth.	Lasekan et al., 2022

Type of Study	Population	Length of Study	Dose	Outcome	Reference
Randomized, controlled, double-blind trial	Healthy full-term infants (7–21 days old)	Time of enrolment of age until up to 6 months	Test group 1 (TG1) fed standard infants formula with a concentration of 1.5 g/L of the five-HMO blend: 0.87, 0.10, 0.29, 0.11 and 0.14 g/L for 2'-FL, DFL, LNT, 3'-SL and 6'-SL, respectively. (N = 230) Test group 2 (TG2) fed standard IF with a concentration of 2.5 g/L of the five-HMO blend: 1.45, 0.14, 0.48, 0.18 and 0.24 g/L for 2'-FL, DFL, LNT, 3'-SL and 6'-SL, respectively (N = 230). Control group (CG): standard cow's milk-based infant formula (N = 233) Placebo: standard IF without HMOs (HMG) (N = 96)	Relative abundance of *Bifidobacterium longum* subsp. *infantis (B. infantis)* was higher in TGs vs. CG. At both post-baseline visits, toxigenic *Clostridioides difficile* abundance was 75–85% lower in TGs vs. CG ($P < 0.05$) and comparable with HMG. At 3 months, TGs (vs. CG) had higher secretory immunoglobulin A (sIgA) and lower alpha-1-antitrypsin ($P < 0.05$).	Bosheva et al., 2022

References:

Bosheva, M., Tokodi, I., Krasnow, A., Pedersen, H. K., Lukjancenko, O., Eklund, A. C., Grathwohl, D., Sprenger, N., Berger, B., & Cercamondi, C. I. (2022). Infant Formula With a Specific Blend of Five Human Milk Oligosaccharides Drives the Gut Microbiota Development and Improves Gut Maturation Markers: A Randomized Controlled Trial. Frontiers in Nutrition, 9. https://doi.org/10.3389/fnut.2022.920362

Lasekan, J., Choe, Y., Dvoretskiy, S., Devitt, A., Zhang, S., Mackey, A., Wulf, K., Buck, R., Steele, C., Johnson, M., & Baggs, G. (2022). Growth and Gastrointestinal Tolerance in Healthy Term Infants Fed Milk-Based Infant Formula Supplemented with Five Human Milk Oligosaccharides (HMOs): A Randomized Multicenter Trial. Nutrients, 14(13), 2625. https://www.mdpi.com/2072-6643/14/13/2625

Parschat, K., Melsaether, C., Jäpelt, K. R., & Jennewein, S. (2021). Clinical Evaluation of 16-Week Supplementation with Tolerability , Safety , and Effect on Growth. Nutrients, 13, 287

U.S. FOOD & DRUG ADMINISTRATION
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January 18, 2023

Joeri Beauprez, Ph.D.
Inbiose N.V.
Technologiepark Zwijnaarde 82 – bus 41
B-9052 Gent
BELGIUM

Re: GRAS Notice No. GRN 001074

Dear Dr. Beauprez:

After reviewing Inbiose N.V. (Inbiose)'s GRAS Notice GRN 001074 for the intended use of 3′-sialyllactose sodium salt (3′-SL), we note the following questions to be addressed. We respectfully request a response to these questions within 10 business days. If you are unable to complete the response within that time frame or have questions, please contact me to discuss at 240-402-1212 or via email.

Chemistry:

1. Please specify the protein base of the infant formula into which the 3′-SL would be added (e.g., cow milk, soy, etc).

2. In Section 1.3 Conditions of Use (Table 1.3-1, p. 4), Inbiose lists the maximum use levels in specified food categories from GRNs 000766, 000880, 000921 and states that intended uses for GRN 001074's 3′-SL "will be the same as described in GRN 000921 for non-exempt term infant formula products, and the same as those described in GRN 000880 for all other listed food categories." However, in Section 3.1 (p. 20) Estimated Intake of 3′-SL, Inbiose states that "In line with GRN 000921, Inbiose's 3′-SL sodium salt is intended for use as a food ingredient in term infant formula (0 to 12 months) and toddler formula at concentrations up to 0.28 g/L." Additionally, Inbiose states that uses in baby foods and meal replacement bars will be similar to GRN 000880, and "In line with GRN 000766, Inbiose's 3′-SL is intended for use in dairy product analogs, milk (whole and skim), milk products, grain products, beverages and beverage bases and sugar substitutes…"

    a. We note that since GRN 001074 was submitted, FDA responded to GRN 001015 with a "No Questions" letter, dated July 15, 2022. GRN 001015 includes uses of 3′-SL in "toddler formula" at a level up to 0.28 g/L. While the stated use of 3′-SL in "toddler formula" is consistent with GRN 001015 (which is not cited in the current notice), it is inconsistent with the 0.24 g/L use level specified in Table 1.3-1. Please clarify the use level in "toddler formula."

    b. GRN 001015 included a use-level increase (0.45 g/L) for non-carbonated drinks (e.g., sports and energy drinks, flavored water), and expanded the uses

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of 3′-FL to include milk-based meal replacement drinks (general use, not just for weight loss) at levels up to 0.9 g/L for adults and children and in formulas for enteral feeding at levels up to 1.5 g/L. Please clarify if the intended uses of 3′-SL in GRN 001074 also include the uses in GRN 001015.

c. For clarity, please provide a summary of the intended uses of GRN 001074 in table form. In the table, please clarify that use levels match the information provided in the text, that the analogous uses (e.g., GRNs 000766, 000880, 000921, and, if applicable, 001015) are correctly stated, and that the basis (3′-SL or 3′-SL sodium salt) for expression of use levels is clearly indicated.

3. In Footnote d of Table 2.3.1-1 (p. 15), Inbiose notes that sodium and its oxidized derivatives are major components of the ash and that sulfates and phosphates are also present. Based on the specifications, ash may comprise up to 8.5% of the ingredient and sodium may comprise up to 4.5% of the ingredient. Please provide additional information to characterize the ash component.

   a. Sodium has a specification of NMT 4.5% of the total ingredient. Does the 4.5% include the oxidized forms of sodium and/or its derivatives?

   b. Please indicate other minerals present in the ash fraction and their approximate levels as a portion of the 3′-SL.

4. The notifier identifies ultra-high performance liquid chromatography coupled with a refractive index detector (UPLC-RI) as the method of analysis for 3′-SL and other carbohydrates present in Inbiose's 3′-SL ingredient. By specification, other carbohydrates may be present at up to 5% of total carbohydrates.

   a. Please confirm that the method includes resolution and identification of "other carbohydrates" present in the 3′-SL ingredient.

   b. Please describe the minor components present in this fraction, including levels of 3′-siallylactulose and N-acetylglucosamine, if present.

5. The specification for arsenic (≤0.2 mg/kg) is well above the results of the batch analyses (<0.01 mg/kg), as provided in Table 2.3.2-1 (p. 16). Please consider reducing this specification to reflect the results of the batch analyses and to ensure that dietary exposure to arsenic is as low as possible. Based on the results of batch analyses, we request that you consider lowering your arsenic specification from ≤0.2 mg/kg to reflect more closely the results of batch analyses (<0.01 mg/kg).

6. Based on the results of batch analyses (Table 2.3.2-1, p. 16), at least one of the results for protein is above the apparent limit of quantitation (25 µg/g). Additionally, the specification limit for protein (≤100 µg/g) is well above the limit of quantitation.

   a. Please clarify the likely source of residual protein, including possible fermentation media components.

   b. Please clarify if the same method is used for batch analyses provided in the notice and routine measures to confirm specifications are met.

7. The exposure estimates presented in section 3.1.2 (p. 21) for infants, toddlers,

children, teens, and adults, consuming food containing 3′-SL, appear to be based on GRN 000880 uses only, as stated in Footnote b to Table 3.1.2-1. The estimates do not appear to be cumulative estimates of dietary exposure that include the increased use in "toddler formula" (0.28 g/L) or the uses provided in GRN 000766 (e.g., herbal teas, cappuccino drinks, imitation and flavored milks, sugar substitutes) that are not included in the intended uses in GRN 000880. We request that you address estimated dietary exposure to 3′-SL from intended uses in GRN 001074 and the cumulative estimated dietary exposure to 3′-SL from existing uses.

a. Using the combined intended uses (i.e., infant formula, infant and "toddler" foods, other food categories) in GRN 001074, please provide an updated dietary exposure estimate to 3′-SL for infants, toddlers (1-3 y), children (4-10 y) and the total consuming population ages 2 years and older on a consumers-only basis.

b. Please clearly discuss if your dietary exposure estimates are cumulative estimates to 3′-FL from existing and intended uses or reflect only partial substitution of existing 3′-FL uses.

Microbiology:

8. Please confirm whether *E. coli* "K-12 MG1655 INB-3SL_01" is non-pathogenic and non-toxigenic. Also, briefly discuss (with relevant references, as appropriate) any phenotypic characteristics of *E. coli* K-12 "K-12 MG1655 INB-3SL_01" (e.g., production of antimicrobials, production of secondary metabolites, antimicrobial resistance) and whether these pose a safety concern.

9. On p. 8 of the notice, Inbiose states that "*E. coli* K-12 strain MG1655 has been classified Biosafety Level 1 by the ATCC" and provides a weblink under Footnote 2. We note that the weblink results in a "Page Not Found (404 Error)." Please provide an updated weblink for Footnote 2.

10. Please provide a description of the fermentation process and details on the in-process controls Inbiose has in place. Additionally, please state whether the fermentation process is conducted in a contained, sterile environment.

11. Please identify any materials used in the production and formulation of 3′-SL that are derived from major allergens (other than cow-milk allergens). Please state whether any of these materials will be present in the final product. If no allergens are present, please provide a statement confirming this. Please note that sesame is now considered a major food allergen as of January 1, 2023 (https://www.fda.gov/food/cfsan-constituent-updates/faster-act-video-food-industry-and-other-stakeholders).

12. Please briefly describe how the purity of *E. coli* "K-12 MG1655 INB-3SL_01" is ensured.

13. On p. 18, Inbiose states that it performs microbiological endotoxin, residual protein, and residual DNA analyses on "the regulatory batches." Please define what "regulatory batches" means. Additionally, please explain the relevance of these tests to Inbiose's conclusion that 3′-SL is safe for its intended use.

14. On p. 15 of the notice, the specification for *Cronobacter* (*Enterobacter*) *sakazakii* is listed as "Absent in 25 g" and the analytical method is ISO/TS 22964.

    a. We note that the current version of this method is ISO 22964:2017. The method states that it has been validated for test portions of 10 g. We recommend that *C. sakazakii* testing be performed on sample sizes no larger than 10 g to prevent the possibility of false negatives.

    b. ISO/TS 22964:2017 corresponds to "Microbiology of the Food Chain - Horizontal Method for the Detection of *Cronobacter* spp." Please clarify whether Inbiose tests for the presence of *Cronobacter* spp. or *C. sakazakii*, specifically. If it is the former, please state whether presumptive positives are further analyzed to determine if the isolate is *C. sakazakii*.

15. *C. sakazakii* has been isolated from foods intended for very young children and can cause infection in infants and young children. Inbiose lists the intended use of 3′-SL as an ingredient in formula and drinks intended for young children (>12 months of age) and in foods intended for infants and young children. We note there remains a potential risk to these vulnerable populations if *C. sakazakii* is not controlled for during the production of 3′-SL or if foods formulated with this ingredient are not treated with an inactivation step (e.g., retort) before consumption by infants or young children. The following publications discuss the prevalence and potential concerns of *C. sakazakii* presence in foods intended for infants and young children:

    • Chen, Q., Zhu, Y., Qin, Z., Qiu, Y., & Zhao, L. (2018). Cronobacter spp., foodborne pathogens threatening neonates and infants. Frontiers of Agricultural Science and Engineering, 5(3), 330-339.

    • Forsythe, S. J. (2015). New insights into the emergent bacterial pathogen Cronobacter. In Food Safety (pp. 265-308). Academic Press.

    Given that the intended uses include in foods intended for consumption by infants and very young children, please provide a discussion on how Inbiose plans to control for the presence of *C. sakazakii* in formula and drinks intended for young children (>12 months of age) and in foods intended for infants and young children. Additionally, please describe why a *C. sakazakii* specification is not provided for these products.

Toxicology:

16. In Table 2.3.1-1 on p. 14, Inbiose indicates that 3′-sialyllactulose is "not specified." However, as noted in recent scientific opinions on 3′-SL by the European Food Safety Authority, lactulose can have a laxative effect.[1,2] Please provide a justification for why a specification for 3′-sialyllactulose is not required to ensure the safety of Inbiose's 3′-SL, especially considering the use in a sensitive

[1] EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Turck D, Bohn T, et al. Safety of 3′-sialyllactose (3′-SL) sodium salt produced by derivative strains of *Escherichia coli* BL21 (DE3) as a Novel Food pursuant to Regulation (EU) 2015/2283. *EFSA J*. 2022;20(5):07331. doi:10.2903/j.efsa.2022.7331

[2] EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Turck D, Castenmiller J, et al. Safety of 3′-sialyllactose (3′-SL) sodium salt as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA J*. 2020;18(5):6098. doi: 10.2903/j.efsa.2020.6098

subpopulation, such as infants, who consume formula as a sole source of nutrition.

17. On p. 20, Inbiose states, "In line with GRN 921, Inbiose's 3′-SL sodium salt is intended for use as a food ingredient in term infant formula (0-12 months) and toddler formula at concentrations up to 0.28 g/L." We note that GRN 000921 did not include uses in formula for young children (>12 months; i.e., "toddler" formula). However, we note that in GRN 001015, a use level of 0.28 g/L was concluded to be GRAS in formulas for young children. Please discuss the data supporting a use level of 0.28 g/L in formula for young children either by incorporating into the notice information from GRN 001015 and/or by providing an updated safety narrative for young children consuming 3′-SL.

18. On p. 36, Inbiose states "The 90-day study in juvenile rats is currently in progress. The end of the *in vivo* phase (last day of euthanasia) was scheduled in Week 10, 2021." Thus, Inbiose's GRAS conclusion was made prior to knowing the results of this study. Please provide a summary of the results from this 90-day study and discuss whether these results support Inbiose's GRAS conclusion for the proposed uses of 3′-SL. We note that since Inbiose acknowledged the existence of this study in the current notice, we will not be able to complete our evaluation until Inbiose provides information regarding the outcome of the study and Inbiose's safety conclusions.

19. Since each GRAS notice must independently support the safety of an ingredient for its intended use, please provide brief summaries of the clinical studies that Inbiose is incorporating into the notice in Section 6.4 on p. 43. As part of this discussion, we suggest including the infant population, study treatments including concentrations of the 3′-SL used in the test formulas, and outcome measures of the study as they relate to Inbiose's conditions of use.

20. Please provide an updated literature search for data and information related to the safety of 3′-SL, since Inbiose's literature search concluded in March 2021. As part of this search, please indicate if any clinical studies have been recently published in which 3′-SL was included in a test formula consumed by an infant population.

21. On p. 24, Inbiose cites GRN 000921 and discusses the concentrations of 3′-SL in human milk to support the use level of 0.28 g/L in infant formula. Since the proposed use level of 0.28 g/L of 3′-SL in infant formula has previously been concluded to be GRAS, we note the following for informational purposes and do not require a response. Since the closure of GRN 000921, additional information on 3′-SL concentrations in human milk have been published in the literature, including systematic reviews, and we suggest including this information in future GRAS notices for HMO ingredients.

Sincerely,

Rachel Morissette, Ph.D.
Division of Food Ingredients
Center for Food Safety
and Applied Nutrition

3. SUMMARY

The objective of this study was to evaluate the potential toxic effects of the test item, 3'-Sialyllactose (3'-SL) sodium salt, on the development of juvenile rats, following daily oral administration from post-natal Day (PND) 7 to at least PND 98. The study was intended to cover the period of development corresponding to infancy, childhood and adolescence.

On completion of the treatment period, designated animals (control and high-dose level groups) were held for a 5-week treatment-free period in order to evaluate the reversibility of any findings.

In addition, satellite animals were dosed for toxicokinetic assessment.

Methods

Four groups of 10 male and 10 female juvenile Sprague-Dawley rats received the test item, 3'-Sialyllactose (3'-SL) sodium salt (batch No. ilex13F04), daily by the oral route (gavage), at the dose level of 0, 1500, 3000 or 5000 mg/kg/day, from PND 7 to at least PND 98. The test item was administered as a solution in sterile water for injection under a constant dosage volume of 10 mL/kg/day. For direct comparison against the highest test item dose group, another group of 10 males and 10 females received the reference item, non-digestible Fructo-Oligosaccharides [FOS (Actilight 950P; batch No. 0175092941)], under the same experimental conditions.

Five animals/sex in control- and high-dose groups were also dosed under the same conditions and then retained untreated for 5 weeks in order to assess recovery of test item-related effects.

Satellite animals were included in the control and test-item treated groups (three males and three females per group) for the determination of plasma and urine test item levels at the end of the treatment period.

The actual test or reference item concentrations were determined on four occasions during the study.

The animals were checked at least twice daily for mortality and at least once daily for clinical signs. Body weight and food consumption were recorded twice weekly from weaning until the end of the treatment period.

The length of the tibia was measured from PND 7, every 2 days during the lactation period and then twice weekly after weaning until the end of the treatment period.

All animals were assessed for pre-weaning development, including eye opening, tooth eruption, auditory canal opening, air righting test and cliff avoidance.

Functional observation battery, reflexes, and motor activity were tested at the end of the treatment period. Animals were tested for learning and memory (5-T Biel water maze) at the end of the treatment and treatment free periods.

Reproductive development [cleavage of the balanopreputial groove (preputial separation) or vaginal opening] was observed for all males every day from PND 40 until positive, and for all females every day from PND 28 until positive.

Ophthalmological examination was performed on all animals at the beginning of the post-weaning period and on the first 10 surviving animals per sex and group (groups 1 to 5) at the end of the treatment period.

In the first 10 surviving females per group, estrous cycle stage was determined for 5 consecutive days at the end of the treatment period.

Blood and urine were collected from all animals at the end of the treatment and treatment-free period for hematology, coagulation, blood biochemistry and urinary investigations as well as determination of thyroid hormone levels.

Seminology investigations (count, motility and morphology) were performed on all principal males before scheduled euthanasia at the end of the treatment period.

Animals were euthanized on completion of the treatment or treatment-free period and a complete macroscopic *post-mortem* examination was performed. Selected organs were weighed and preserved. A microscopic examination was performed on selected tissues from the control, reference and high-dose animals, and on all macroscopic lesions.

Results

Actual concentrations of the test item and reference item in the dose formulations analyzed during the treatment period remained within an acceptable variation range except on Study Day 1 (PND 7) for the test item low-dose level (+11.2% and -12.7% of nominal value).

After oral administration of 3'-Sialyllactose sodium salt from PND 7 until PND 97 in male and female Sprague-Dawley rats, all treated rats were exposed to 3'-Sialyllactose, with quantifiable concentrations measured up to 6 or 24 hours after administration. The peak of plasma concentrations of 3'-Sialyllactose was observed 1 hour after administration. Some TK profiles showed a second peak of plasma concentrations observed 6 hours after administration. Based on the AUC, the male rats showed higher systemic exposure to 3'-Sialyllactose than the female rats due to the higher endogenous baseline levels of 3'-Sialyllactose. Between 1500 and 3000 mg/kg/day, the C_{max} and AUC_{0-t} increased proportionally with doses, while between 3000 and 5000 mg/kg/day a trend to more than dose proportional increase was observed. Higher excreted amount of 3'-Sialyllactose was observed in males (up to 6-fold) due to the higher endogenous baseline levels of 3´-SL. However, the amounts of 3´-SL recovered in urine (as the percentage of the doses) during 24-hour exposure were negligible low, *i.e.* <0.5% for both males and females.

No test item or reference item related deaths occurred during the study.

In test item-treated animals, there were no test item treatment-related clinical signs.

In reference item-treated animals, yellowish feces, reddish/soiled/swollen urogenital area were observed as early as PND 9 (Study Day 3). In addition, when compared with controls, there were higher incidences of pups with increased in size abdomen/urogenital area. Taken together, these findings were considered as adverse.

No test item or reference item effects were noted on mean body weight, mean body weight change and mean food consumption. All individual values were within the historical control data (HCD) ranges and reflected normal biological variations.

No test item or reference item effects were noted on long bone growth (tibia length), developmental landmarks and neurologic development, functional observation battery parameters, motor activity, learning/memory functions, reproductive development or ophthalmology.

Estrous cycle and seminology parameters were not impacted by the test item or reference treatment.

No effects were noted on the hematology, blood biochemistry or urinalysis parameters, coagulation factors or thyroid hormone levels.

At pathology, there were no organ weight differences, macroscopic or microscopic findings related to the administration of 3'-Sialyllactose (3'-SL) sodium salt. The reference item induced non-adverse accumulation of hyaline droplets consisted with α2u globulin in the kidneys of males that correlated with gross irregular or granular surface, and non-adverse increased extra-medullary hematopoiesis in the spleen correlated with increased weight.

Conclusion

The test item, 3'-Sialyllactose (3'-SL) sodium salt, was administered once daily from post-natal day (PND) 7 to at least PND 98, by oral gavage, to juvenile Sprague Dawley rats at the dose level of 1500, 3000 or 5000 mg/kg/day. The reference item for comparison with the highest 3'-Sialyllactose (3'-SL) sodium salt group, Actilight 950P, was administered at the dose level of 5000 mg/kg/day. The study was intended to cover the period of development corresponding to infancy, childhood through adolescence. On completion of the treatment period, designated animals (control and high-dose level groups) were held for a 5-week treatment-free period in order to evaluate the reversibility of any findings.

The test item was clinically well tolerated.

The No Observed Adverse Effect Level (NOAEL) in this study was established at 5000 mg/kg/day for juvenile males and females, based on the absence of adverse effects at this dose level.

The reference item induced yellowish feces, reddish/soiled/swollen urogenital area along with higher incidence of pups with increased in size abdomen/urogenital area that were considered as adverse.

Appendix A
Representative Food Codes for Cumulative Dietary Exposure Assessment of 3'-Sialyllactose Sodium Salt in the U.S. (2017-2018 NHANES Data)

Representative Food Codes for Cumulative Dietary Exposure Assessment of 3'-Sialyllactose Sodium Salt in the U.S. (2017-2018 NHANES Data)

Beverages and Beverage Bases

Soft drinks (regular and diet)
[3'-SL Sodium Salt] = 0.025 g/100 g

92400000	Soft drink, NFS
92400100	Soft drink, NFS, diet
92410310	Soft drink, cola
92410315	Soft drink, cola, reduced sugar
92410320	Soft drink, cola, diet
92410340	Soft drink, cola, decaffeinated
92410350	Soft drink, cola, decaffeinated, diet
92410360	Soft drink, pepper type
92410370	Soft drink, pepper type, diet
92410390	Soft drink, pepper type, decaffeinated
92410400	Soft drink, pepper type, decaffeinated, diet
92410410	Soft drink, cream soda
92410420	Soft drink, cream soda, diet
92410510	Soft drink, fruit flavored, caffeine free
92410520	Soft drink, fruit flavored, diet, caffeine free
92410550	Soft drink, fruit flavored, caffeine containing
92410560	Soft drink, fruit flavored, caffeine containing, diet
92410610	Soft drink, ginger ale
92410620	Soft drink, ginger ale, diet
92410710	Soft drink, root beer
92410720	Soft drink, root beer, diet
92410810	Soft drink, chocolate flavored
92410820	Soft drink, chocolate flavored, diet
92411510	Soft drink, cola, fruit or vanilla flavored
92411520	Soft drink, cola, chocolate flavored
92411610	Soft drink, cola, fruit or vanilla flavored, diet
92411620	Soft drink, cola, chocolate flavored, diet

Sports, Isotonic, and Energy Drinks; Enhanced or Fortified Waters
[3'-SL Sodium Salt] = 0.045 g/100 g

92410110	Carbonated water, sweetened
92410250	Carbonated water, sweetened, with low-calorie or no-calorie sweetener
94100200	Water, bottled, sweetened, with low calorie sweetener

94100300	Water, bottled, flavored (Capri Sun Roarin' Waters)
94210100	Water, bottled, flavored (Propel Water)
94210200	Water, bottled, flavored (Glaceau Vitamin Water)
94210300	Water, bottled, flavored (SoBe Life Water)
94220215	Water, bottled, flavored, sugar free (Glaceau Vitamin Water)
94220310	Water, bottled, flavored, sugar free (SoBe)
95310200	Energy drink (Full Throttle)
95310400	Energy drink (Monster)
95310500	Energy drink (Mountain Dew AMP)
95310550	Energy drink (No Fear)
95310555	Energy drink (No Fear Motherload)
95310560	Energy drink (NOS)
95310600	Energy drink (Red Bull)
95310700	Energy drink (Rockstar)
95310750	Energy drink (SoBe Energize Energy Juice Drink)
95310800	Energy drink (Vault)
95311000	Energy Drink
95312400	Energy drink, low calorie (Monster)
95312410	Energy drink, sugar free (Monster)
95312500	Energy drink, sugar free (Mountain Dew AMP)
95312550	Energy drink, sugar free (No Fear)
95312555	Energy drink, sugar-free (NOS)
95312560	Energy drink (Ocean Spray Cran-Energy Juice Drink)
95312600	Energy drink, sugar-free (Red Bull)
95312700	Energy drink, sugar free (Rockstar)
95312800	Energy drink, sugar free (Vault)
95312900	Energy drink (XS)
95312905	Energy drink (XS Gold Plus)
95313200	Energy drink, sugar free
95320200	Sports drink (Gatorade G)
95320500	Sports drink (Powerade)
95321000	Sports drink, NFS
95322200	Sports drink, low calorie (Gatorade G2)
95322500	Sports drink, low calorie (Powerade Zero)
95323000	Sports drink, low calorie
95330100	Fluid replacement, electrolyte solution
95330500	Fluid replacement, 5% glucose in water

<u>Foods adjusted for being present in dried form</u>
Reconstitution factor of 16.625
[3'-SL Sodium Salt] = 0.75 g/100 g

92900300	Sports drink, dry concentrate, not reconstituted

Non-milk-based meal replacement drinks
[3'-SL Sodium Salt] = 0.09 g/100 g

95104000	Nutritional drink or shake, ready-to-drink, sugar free (Glucerna)
95120050	Nutritional drink or shake, liquid, soy-based

Foods adjusted for being present in dried form
Reconstitution factor of 6 to 10
[3'-SL Sodium Salt] = 0.54 to 0.90 g/100 g

95201300	Nutritional powder mix (EAS Soy Protein Powder)
95201600	Nutritional powder mix (Isopure)
95201700	Nutritional powder mix (Kellogg's Special K20 Protein Water)
95230010	Nutritional powder mix, protein, soy based, NFS

Coffee and Tea

Cappucino, non-fat, with dairy milk, sweetened
[3'-SL Sodium Salt] = 0.52 g/100 g

92161000	Coffee, Cappuccino
92161001	Coffee, Cappuccino, nonfat
92161002	Coffee, Cappuccino, with non-dairy milk
92162000	Coffee, Cappuccino, decaffeinated
92162001	Coffee, Cappuccino, decaffeinated, nonfat
92162002	Coffee, Cappuccino, decaffeinated, with non-dairy milk

Tea
[3'-SL Sodium Salt] = 1.29 g/100 g

92306000	Tea, hot, herbal

Dairy Product Analogs

Milk substitutes such as soy milk and imitation milks
[3'-SL Sodium Salt] = 0.012 g/100 g

11300100	Non-dairy milk, NFS
11320000	Soy milk
11320100	Soy milk, light
11320200	Soy milk, nonfat
11321000	Soy milk, chocolate
11321100	Soy milk, light, chocolate
11321200	Soy milk, nonfat, chocolate

11350000	Almond milk, sweetened
11350010	Almond milk, sweetened, chocolate
11350020	Almond milk, unsweetened
11350030	Almond milk, unsweetened, chocolate
11360000	Rice milk
11370000	Coconut milk
11512030	Hot chocolate / Cocoa, ready to drink, made with non-dairy milk
11512120	Hot chocolate / Cocoa, ready to drink, made with non-dairy milk and whipped cream
11513310	Chocolate milk, made from dry mix with non-dairy milk
11513375	Chocolate milk, made from reduced sugar mix with non-dairy milk
11513385	Chocolate milk, made from dry mix with non-dairy milk (Nesquik)
11513395	Chocolate milk, made from no sugar added dry mix with non-dairy milk (Nesquik)
11513750	Chocolate milk, made from syrup with non-dairy milk
11513805	Chocolate milk, made from light syrup with non-dairy milk
11513855	Chocolate milk, made from sugar free syrup with non-dairy milk
11514150	Hot chocolate / Cocoa, made with dry mix and non-dairy milk
11514360	Hot chocolate / Cocoa, made with no sugar added dry mix and non-dairy milk
11519215	Strawberry milk, non-dairy
42401010	Coconut milk, used in cooking

Mixed foods containing milk substitutes

Adjusted for milk substitute content of 42.2 to 83.6%

[3'-SL Sodium Salt] = <0.015 to 0.010 g/100 g

92101906	Coffee, Latte, with non-dairy milk, flavored
92101913	Coffee, Latte, decaffeinated, with non-dairy milk
92101919	Coffee, Latte, decaffeinated, with non-dairy milk, flavored
92101923	Frozen coffee drink, with non-dairy milk
92101928	Frozen coffee drink, with non-dairy milk and whipped cream
92101933	Frozen coffee drink, decaffeinated, with non-dairy milk
92101938	Frozen coffee drink, decaffeinated, with non-dairy milk and whipped cream
92101960	Coffee, Cafe Mocha, with non-dairy milk
92101975	Coffee, Cafe Mocha, decaffeinated, with non-dairy milk
92102020	Frozen mocha coffee drink, with non-dairy milk
92102050	Frozen mocha coffee drink, with non-dairy milk and whipped cream
92102080	Frozen mocha coffee drink, decaffeinated, with non-dairy milk
92102110	Frozen mocha coffee drink, decaffeinated, with non-dairy milk and whipped cream
92102502	Coffee, Iced Latte, with non-dairy milk
92102505	Coffee, Iced Latte, with non-dairy milk, flavored
92102512	Coffee, Iced Latte, decaffeinated, with non-dairy milk
92102515	Coffee, Iced Latte, decaffeinated, with non-dairy milk, flavored
92102602	Coffee, Iced Cafe Mocha, with non-dairy milk
92102612	Coffee, Iced Cafe Mocha, decaffeinated, with non-dairy milk

Non-dairy yogurt
[3'-SL Sodium Salt] = 0.055 g/100 g

41420380	Yogurt, soy
42401100	Yogurt, coconut milk

Frozen Dairy Desserts and Mixes

Frozen yogurts
[3'-SL Sodium Salt] = 0.17 g/100 g

11459990	Frozen yogurt, NFS
11460000	Frozen yogurt, vanilla
11460100	Frozen yogurt, chocolate
11460500	Frozen yogurt, soft serve, vanilla
11460510	Frozen yogurt, soft serve, chocolate
11461200	Frozen yogurt sandwich
11461210	Frozen yogurt bar, vanilla
11461220	Frozen yogurt bar, chocolate
11461250	Frozen yogurt cone, chocolate
11461260	Frozen yogurt cone, vanilla
11461300	Frozen yogurt cone, vanilla, waffle cone
11461320	Frozen yogurt cone, chocolate, waffle cone

Grain Products and Pastas

Cereal and granola bars
[3'-SL Sodium Salt] = 0.25 g/100 g

53710400	Cereal or granola bar (General Mills Fiber One Chewy Bar)
53710500	Cereal or granola bar (Kellogg's Nutri-Grain Cereal Bar)
53710502	Cereal or granola bar (Kellogg's Nutri-Grain Yogurt Bar)
53710504	Cereal or granola bar (Kellogg's Nutri-Grain Fruit and Nut Bar)
53710600	Milk 'n Cereal bar
53710700	Cereal or granola bar (Kellogg's Special K bar)
53710800	Cereal or granola bar (Kashi Chewy)
53710802	Cereal or granola bar (Kashi Crunchy)
53710810	Cereal or granola bar (KIND Fruit and Nut Bar)
53710900	Cereal or granola bar (General Mills Nature Valley Chewy Trail Mix)
53710902	Cereal or granola bar, with yogurt coating (General Mills Nature Valley Chewy Granola Bar)
53710904	Cereal or granola bar (General Mills Nature Valley Sweet and Salty Granola Bar)
53710906	Cereal or granola bar (General Mills Nature Valley Crunchy Granola Bar)
53711000	Cereal or granola bar (Quaker Chewy Granola Bar)

53711002 Cereal or granola bar (Quaker Chewy 90 Calorie Granola Bar)
53711004 Cereal or granola bar (Quaker Chewy 25% Less Sugar Granola Bar)
53711006 Cereal or granola bar (Quaker Chewy Dipps Granola Bar)
53711100 Cereal or granola bar (Quaker Granola Bites)
53712000 Snack bar, oatmeal
53712100 Cereal or Granola bar, NFS
53712200 Cereal or granola bar, lowfat, NFS
53712210 Cereal or granola bar, nonfat
53713000 Cereal or granola bar, reduced sugar, NFS
53713010 Cereal or granola bar, fruit and nut
53713100 Cereal or granola bar, peanuts , oats, sugar, wheat germ
53714200 Cereal or granola bar, chocolate coated, NFS
53714210 Cereal or granola bar, with coconut, chocolate coated
53714220 Cereal or granola bar with nuts, chocolate coated
53714230 Cereal or granola bar, oats, nuts, coated with non-chocolate coating
53714250 Cereal or granola bar, coated with non-chocolate coating
53714300 Cereal or granola bar, high fiber, coated with non-chocolate yogurt coating
53714400 Cereal or granola bar, with rice cereal

Meal replacement bars
[3'-SL Sodium Salt] = 2.59 g/100 g

53714500 Breakfast bar, NFS
53714510 Breakfast bar, date, with yogurt coating
53714520 Breakfast bar, cereal crust with fruit filling, lowfat
53720100 Nutrition bar (Balance Original Bar)
53720200 Nutrition bar (Clif Bar)
53720210 Nutrition bar (Clif Kids Organic Zbar)
53720300 Nutrition bar (PowerBar)
53720400 Nutrition bar (Slim Fast Original Meal Bar)
53720500 Nutrition bar (Snickers Marathon Protein Bar)
53720600 Nutrition bar (South Beach Living Meal Bar)
53720610 Nutrition bar (South Beach Living High Protein Bar)
53720700 Nutrition bar (Tiger's Milk)
53720800 Nutrition bar (Zone Perfect Classic Crunch)
53729000 Nutrition bar or meal replacement bar, NFS

Infant and Toddler Foods

Term infant formula
[3'-SL Sodium Salt] = 0.028 g/100 g

11710000 Infant formula, NFS

11710350 Infant formula, NS as to form (Similac Advance)
11710351 Infant formula, ready-to-feed (Similac Advance)
11710352 Infant formula, liquid concentrate, made with water, NFS (Similac Advance)
11710353 Infant formula, powder, made with water, NFS (Similac Advance)
11710354 Infant formula, liquid concentrate, made with tap water (Similac Advance)
11710355 Infant formula, liquid concentrate, made with plain bottled water (Similac Advance)
11710356 Infant formula, liquid concentrate, made with baby water (Similac Advance)
11710357 Infant formula, powder, made with tap water (Similac Advance)
11710358 Infant formula, powder, made with plain bottled water (Similac Advance)
11710359 Infant formula, powder, made with baby water (Similac Advance)
11710360 Infant formula, NS as to form (Similac Advance Organic)
11710361 Infant formula, ready-to-feed (Similac Advance Organic)
11710363 Infant formula, powder, made with water, NFS (Similac Advance Organic)
11710367 Infant formula, powder, made with tap water (Similac Advance Organic)
11710368 Infant formula, powder, made with plain bottled water (Similac Advance Organic)
11710369 Infant formula, powder, made with baby water (Similac Advance Organic)
11710370 Infant formula, NS as to form (Similac Sensitive)
11710371 Infant formula, ready-to-feed (Similac Sensitive)
11710372 Infant formula, liquid concentrate, made with water, NFS (Similac Sensitive)
11710373 Infant formula, powder, made with water, NFS (Similac Sensitive)
11710374 Infant formula, liquid concentrate, made with tap water (Similac Sensitive)
11710375 Infant formula, liquid concentrate, made with plain bottled water (Similac Sensitive)
11710376 Infant formula, liquid concentrate, made with baby water (Similac Sensitive)
11710377 Infant formula, powder, made with tap water (Similac Sensitive)
11710378 Infant formula, powder, made with plain bottled water (Similac Sensitive)
11710379 Infant formula, powder, made with baby water (Similac Sensitive)
11710380 Infant formula, NS as to form (Similac for Spit-Up)
11710381 Infant formula, ready-to-feed (Similac for Spit-Up)
11710383 Infant formula, powder, made with water, NFS (Similac for Spit-Up)
11710620 Infant formula, NS as to form (Enfamil Newborn)
11710621 Infant formula, ready-to-feed (Enfamil Newborn)
11710626 Infant formula, powder, made with water, NFS (Enfamil Newborn)
11710627 Infant formula, powder, made with tap water (Enfamil Newborn)
11710628 Infant formula, powder, made with plain bottled water (Enfamil Newborn)
11710629 Infant formula, powder, made with baby water (Enfamil Newborn)
11710630 Infant formula, NS as to form (Enfamil Infant)
11710631 Infant formula, ready-to-feed (Enfamil Infant)
11710632 Infant formula, liquid concentrate, made with water, NFS (Enfamil Infant)
11710633 Infant formula, liquid concentrate, made with tap water (Enfamil Infant)
11710634 Infant formula, liquid concentrate, made with plain bottled water (Enfamil Infant)
11710635 Infant formula, liquid concentrate, made with baby water (Enfamil Infant)
11710636 Infant formula, powder, made with water, NFS (Enfamil Infant)

11710637 Infant formula, powder, made with tap water (Enfamil Infant)
11710638 Infant formula, powder, made with plain bottled water (Enfamil Infant)
11710639 Infant formula, powder, made with baby water (Enfamil Infant)
11710660 Infant formula, NS as to form (Enfamil A.R.)
11710661 Infant formula, ready-to-feed (Enfamil A.R.)
11710663 Infant formula, powder, made with water, NFS (Enfamil A.R.)
11710664 Infant formula, powder, made with tap water (Enfamil A.R.)
11710668 Infant formula, powder, made with plain bottled water (Enfamil A.R.)
11710669 Infant formula, powder, made with baby water (Enfamil A.R.)
11710670 Infant formula, NS as to form (Enfamil Gentlease)
11710671 Infant formula, ready-to-feed (Enfamil Gentlease)
11710673 Infant formula, powder, made with water, NFS (Enfamil Gentlease)
11710677 Infant formula, powder, made with tap water (Enfamil Gentlease)
11710678 Infant formula, powder, made with plain bottled water (Enfamil Gentlease)
11710679 Infant formula, powder, made with baby water (Enfamil Gentlease)
11710910 Infant formula, NS as to form (Gerber Good Start Gentle)
11710911 Infant formula, ready-to-feed (Gerber Good Start Gentle)
11710912 Infant formula, liquid concentrate, made with water, NFS (Gerber Good Start Gentle)
11710913 Infant formula, powder, made with water, NFS (Gerber Good Start Gentle)
11710914 Infant formula, liquid concentrate, made with tap water (Gerber Good Start Gentle)
11710915 Infant formula, liquid concentrate, made with plain bottled water (Gerber Good Start Gentle)
11710916 Infant formula, liquid concentrate, made with baby water (Gerber Good Start Gentle)
11710917 Infant formula, powder, made with tap water (Gerber Good Start Gentle)
11710918 Infant formula, powder, made with plain bottled water (Gerber Good Start Gentle)
11710919 Infant formula, powder, made with baby water (Gerber Good Start Gentle)
11710920 Infant formula, NS as to form (Gerber Good Start Protect)
11710923 Infant formula, powder, made with water, NFS (Gerber Good Start Protect)
11710927 Infant formula, powder, made with tap water (Gerber Good Start Protect)
11710928 Infant formula, powder, made with plain bottled water (Gerber Good Start Protect)
11710929 Infant formula, powder, made with baby water (Gerber Good Start Protect)
11710960 Infant formula, NS as to form (Store Brand)
11710961 Infant formula, liquid concentrate, made with water, NFS (Store Brand)
11710962 Infant formula, powder, made with water, NFS (Store Brand)
11710963 Infant formula, ready-to-feed (Store Brand)
11710964 Infant formula, liquid concentrate, made with tap water (Store Brand)
11710965 Infant formula, liquid concentrate, made with plain bottled water (Store Brand)
11710966 Infant formula, liquid concentrate, made with baby water (Store Brand)
11710967 Infant formula, powder, made with tap water (Store Brand)
11710968 Infant formula, powder, made with plain bottled water (Store Brand)
11710969 Infant formula, powder, made with baby water (Store Brand)
11720310 Infant formula, NS as to form (Enfamil ProSobee)

11720311 Infant formula, ready-to-feed (Enfamil ProSobee)
11720312 Infant formula, liquid concentrate, made with water, NFS (Enfamil ProSobee)
11720313 Infant formula, powder, made with water, NFS (Enfamil ProSobee)
11720314 Infant formula, liquid concentrate, made with tap water (Enfamil ProSobee)
11720315 Infant formula, liquid concentrate, made with plain bottled water (Enfamil ProSobee)
11720316 Infant formula, liquid concentrate, made with baby water (Enfamil ProSobee)
11720317 Infant formula, powder, made with tap water (Enfamil ProSobee)
11720318 Infant formula, powder, made with plain bottled water (Enfamil ProSobee)
11720319 Infant formula, powder, made with baby water (Enfamil ProSobee)
11720410 Infant formula, NS as to form (Similac Isomil Soy)
11720411 Infant formula, ready-to-feed (Similac Isomil Soy)
11720412 Infant formula, liquid concentrate, made with water, NFS (Similac Isomil Soy)
11720413 Infant formula, powder, made with water, NFS (Similac Isomil Soy)
11720414 Infant formula, liquid concentrate, made with tap water (Similac Isomil Soy)
11720415 Infant formula, liquid concentrate, made with plain bottled water (Similac Isomil Soy)
11720416 Infant formula, liquid concentrate, made with baby water (Similac Isomil Soy)
11720417 Infant formula, powder, made with tap water (Similac Isomil Soy)
11720418 Infant formula, powder, made with plain bottled water (Similac Isomil Soy)
11720419 Infant formula, powder, made with baby water (Similac Isomil Soy)
11720610 Infant formula, NS as to form (Gerber Good Start Soy)
11720611 Infant formula, ready-to-feed (Gerber Good Start Soy)
11720612 Infant formula, liquid concentrate, made with water, NFS (Gerber Good Start Soy)
11720613 Infant formula, powder, made with water, NFS (Gerber Good Start Soy)
11720614 Infant formula, liquid concentrate, made with tap water (Gerber Good Start Soy)
11720615 Infant formula, liquid concentrate, made with plain bottled water (Gerber Good Start Soy)
11720616 Infant formula, liquid concentrate, made with baby water (Gerber Good Start Soy)
11720617 Infant formula, powder, made with tap water (Gerber Good Start Soy)
11720618 Infant formula, powder, made with plain bottled water (Gerber Good Start Soy)
11720619 Infant formula, powder, made with baby water (Gerber Good Start Soy)
11720800 Infant formula, NS as to form (Store Brand Soy)
11720801 Infant formula, ready-to-feed (Store brand Soy)
11720802 Infant formula, liquid concentrate, made with water, NFS (Store Brand Soy)
11720803 Infant formula, powder, made with water, NFS (Store Brand Soy)
11720807 Infant formula, powder, made with tap water (Store Brand Soy)
11720808 Infant formula, powder, made with plain bottled water (Store Brand Soy)
11720809 Infant formula, powder, made with baby water (Store Brand Soy)

Toddler formula

[3'-SL Sodium Salt] = 0.028 g/100 g

11720430 Infant formula, NS as to form (Similac Expert Care for Diarrhea)
11720431 Infant formula, ready-to-feed (Similac Expert Care for Diarrhea)
11710480 Infant formula, NS as to form (Similac Go and Grow)

11710481	Infant formula, powder, made with water, NFS (Similac Go and Grow)
11710680	Infant formula, NS as to form (Enfamil Enfagrow Toddler Transitions)
11710681	Infant formula, ready-to-feed (Enfamil Enfragrow Toddler Transitions)
11710683	Infant formula, powder, made with water, NFS (Enfamil Enfragrow Toddler Transitions)
11710687	Infant formula, powder, made with tap water (Enfamil Enfagrow Toddler Transitions)
11710688	Infant formula, powder, made with plain bottled water (Enfamil Enfagrow Toddler Transitions)
11710689	Infant formula, powder, made with baby water (Enfamil Enfagrow Toddler Transitions)
11710690	Infant formula, NS as to form (Enfamil Enfagrow Toddler Transitions Gentlease)
11710693	Infant formula, powder, made with water, NFS (Enfamil Enfagrow Toddler Transitions Gentlease)
11710697	Infant formula, powder, made with tap water (Enfamil Enfagrow Toddler Transitions Gentlease)
11710698	Infant formula, powder, made with plain bottled water (Enfamil Enfagrow Toddler Transitions Gentlease)
11710699	Infant formula, powder, made with baby water (Enfamil Enfagrow Toddler Transitions Gentlease)
11710930	Infant formula, NS as to form (Gerber Graduates Gentle)
11710940	Infant formula, NS as to form (Gerber Graduates Protect)
11720320	Infant formula, NS as to form (Enfamil Enfagrow Toddler Transitions Soy)
11720323	Infant formula, powder, made with water, NFS (Enfamil Enfagrow Toddler Transitions Soy)
11720620	Infant formula, NS as to form (Gerber Graduates Soy)

Milk-based meal replacement beverages for children (Pediasure)
[3'-SL Sodium Salt] = 0.09 g/100 g

11710800	Infant formula, NS as to form (PediaSure)
11710801	Infant formula, ready-to-feed (PediaSure)
11710805	Infant formula, with fiber, NS as to form (PediaSure Fiber)
11710806	Infant formula, with fiber, ready-to-feed (PediaSure Fiber)

Instant cereals for babies and toddlers
[3'-SL Sodium Salt] = 0.166 g/100 g

Foods adjusted for being present in dried form
Reconstitution factor of 8.33
[3'-SL Sodium Salt] = 1.38 g/100 g

57801000	Barley cereal, baby food, dry, instant
57803000	Mixed cereal, baby food, dry, instant
57804000	Oatmeal cereal, baby food, dry, instant
57805000	Rice cereal, baby food, dry, instant
57805080	Rice cereal with apples, baby food, dry, instant
57805090	Rice cereal with mixed fruits, baby food, dry, instant

57805100 Rice cereal with bananas, baby food, dry, instant
57805500 Brown rice cereal, baby food, dry, instant
57806000 Mixed cereal with bananas, baby food, dry, instant
57806050 Multigrain, whole grain cereal, baby food, dry, instant
57806100 Oatmeal cereal with bananas, baby food, dry, instant
57806200 Oatmeal cereal with fruit, baby food, dry, instant, toddler
57807010 Whole wheat cereal with apples, baby food, dry, instant

Other baby foods for infants and young children

[3'-SL Sodium Salt] = 0.125 g/100 g

11480010 Yogurt, whole milk, baby food
11480020 Yogurt, whole milk, baby food, with fruit and multigrain cereal puree, NFS
11480030 Yogurt, whole milk, baby food, with fruit and multigrain cereal puree, plus iron
11480040 Yogurt, whole milk, baby food, with fruit and multigrain cereal puree, plus DHA
20000070 Meat, baby food, NS as to type, NS as to strained or junior
20000090 Meat sticks, baby food, NS as to type of meat
21701000 Beef, baby food, NS as to strained or junior
21701010 Beef, baby food, strained
21701020 Beef, baby food, junior
22810010 Ham, baby food, strained
22820000 Meat stick, baby food
23410010 Lamb, baby food, strained
23420010 Veal, baby food, strained
24701000 Chicken, baby food, NS as to strained or junior
24701010 Chicken, baby food, strained
24701020 Chicken, baby food, junior
24703000 Turkey, baby food, NS as to strained or junior
24703010 Turkey, baby food, strained
24703020 Turkey, baby food, junior
24705010 Chicken stick, baby food
24706010 Turkey stick, baby food
27601000 Beef stew, baby food, toddler
27610100 Beef and egg noodles, baby food, NS as to strained or junior
27610110 Beef and egg noodles, baby food, strained
27610120 Beef and egg noodles, baby food, junior
27610710 Beef with vegetables, baby food, strained
27610730 Beef with vegetables, baby food, toddler
27640050 Chicken and rice dinner, baby food, strained
27640100 Chicken noodle dinner, baby food, NS as to strained or junior
27640110 Chicken noodle dinner, baby food, strained
27640120 Chicken noodle dinner, baby food, junior
27640810 Chicken, noodles, and vegetables, baby food, toddler
27641000 Chicken stew, baby food, toddler

27642100 Turkey, rice and vegetables, baby food, NS as to strained or junior
27642110 Turkey, rice and vegetables, baby food, strained
27642120 Turkey, rice and vegetables, baby food, junior
27642130 Turkey, rice, and vegetables, baby food, toddler
27644110 Chicken soup, baby food
58503000 Macaroni, tomatoes, and beef, baby food, NS as to strained or junior
58503010 Macaroni, tomatoes, and beef, baby food, strained
58503020 Macaroni, tomatoes, and beef, baby food, junior
58503050 Macaroni with beef and tomato sauce, baby food, toddler
58508000 Macaroni and cheese, baby food, strained
58508300 Macaroni and cheese, baby food, toddler
58509020 Spaghetti, tomato sauce, and beef, baby food, junior
58509100 Ravioli, cheese-filled, with tomato sauce, baby food, toddler
58509200 Macaroni with vegetables, baby food, strained
67100100 Fruit, baby food, NFS
67100200 Tropical fruit medley, baby food, strained
67100300 Apples, baby food, toddler
67101000 Apple-raspberry, baby food, NS as to strained or junior
67101010 Apple-raspberry, baby food, strained
67101020 Apple-raspberry, baby food, junior
67102000 Applesauce, baby food, NS as to strained or junior
67102010 Applesauce, baby food, strained
67102020 Applesauce, baby food, junior
67104000 Applesauce and apricots, baby food, NS as to strained or junior
67104010 Applesauce and apricots, baby food, strained
67104020 Applesauce and apricots, baby food, junior
67104030 Applesauce with bananas, baby food, NS as to strained or junior
67104040 Applesauce with bananas, baby food, strained
67104060 Applesauce with bananas, baby food, junior
67104070 Applesauce with cherries, baby food, strained
67104080 Applesauce with cherries, baby food, junior
67104090 Applesauce with cherries, baby food, NS as to strained or junior
67105030 Bananas, baby food, strained
67106010 Bananas with apples and pears, baby food, strained
67106030 Bananas with orange, baby food, strained
67106050 Banana with mixed berries, baby food, strained
67108000 Peaches, baby food, NS as to strained or junior
67108010 Peaches, baby food, strained
67108020 Peaches, baby food, junior
67108030 Peaches, baby food, toddler
67109000 Pears, baby food, NS as to strained or junior
67109010 Pears, baby food, strained
67109020 Pears, baby food, junior

67109030 Pears, baby food, toddler
67110000 Prunes, baby food, strained
67113000 Apples and pears, baby food, NS as to strained or junior
67113010 Apples and pears, baby food, strained
67113020 Apples and pears, baby food, junior
67114000 Pears and pineapple, baby food, NS as to strained or junior
67114010 Pears and pineapple, baby food, strained
67114020 Pears and pineapple, baby food, junior
67304000 Plums, baby food, NS as to strained or junior
67304010 Plums, baby food, strained
67304020 Plums, baby food, junior
67304030 Plums, bananas, and rice, baby food strained
67304500 Prunes with oatmeal, baby food, strained
67307000 Apricots, baby food, NS as to strained or junior
67307010 Apricots, baby food, strained
67307020 Apricots, baby food, junior
67308000 Bananas, baby food, NS as to strained or junior
67308020 Bananas, baby food, junior
67309000 Bananas and pineapple, baby food, NS as to strained or junior
67309010 Bananas and pineapple, baby food, strained
67309020 Bananas and pineapple, baby food, junior
67309030 Bananas and strawberry, baby food, junior
67501000 Apples and chicken, baby food, strained
67501100 Apples with ham, baby food, strained
67600100 Apples and sweet potatoes, baby food, strained
76102010 Spinach, creamed, baby food, strained
76102030 Broccoli, carrots and cheese, baby food, junior
76201000 Carrots, baby food, NS as to strained or junior
76201010 Carrots, baby food, strained
76201020 Carrots, baby food, junior
76201030 Carrots, baby food, toddler
76202000 Carrots and peas, baby food, strained
76205000 Squash, baby food, NS as to strained or junior
76205010 Squash, baby food, strained
76205020 Squash, baby food, junior
76205030 Squash and corn, baby food, strained
76205060 Corn and sweet potatoes, baby food, strained
76209000 Sweet potatoes, baby food, NS as to strained or junior
76209010 Sweet potatoes, baby food, strained
76209020 Sweet potatoes, baby food, junior
76401000 Beans, green string, baby food, NS as to strained or junior
76401010 Beans, green string, baby food, strained
76401020 Beans, green string, baby food, junior

76401060 Beans, green string, baby food, toddler
76402000 Green beans and potatoes, baby food, strained
76403010 Beets, baby food, strained
76405000 Corn, creamed, baby food, NS as to strained or junior
76405010 Corn, creamed, baby food, strained
76405020 Corn, creamed, baby food, junior
76407000 Mixed vegetables, garden vegetables, baby food, NS as to strained or junior
76407010 Mixed vegetables, garden vegetables, baby food, strained
76407020 Mixed vegetables, garden vegetables, baby food, junior
76409000 Peas, baby food, NS as to strained or junior
76409010 Peas, baby food, strained
76409020 Peas, baby food, junior
76409030 Peas, baby food, toddler
76420000 Potatoes, baby food, toddler
76501000 Vegetables and rice, baby food, strained
76502000 Peas and brown rice, baby food
76602000 Carrots and beef, baby food, strained
76603000 Vegetable and beef, baby food, NS as to strained or junior
76603010 Vegetable and beef, baby food, strained
76603020 Vegetable and beef, baby food, junior
76604000 Broccoli and chicken, baby food, strained
76604500 Sweet potatoes and chicken, baby food, strained
76605000 Vegetable and chicken, baby food, NS as to strained or junior
76605010 Vegetable and chicken, baby food, strained
76605020 Vegetable and chicken, baby food, junior
76607100 Potatoes with cheese and broccoli, baby food, toddler
76611000 Vegetable and turkey, baby food, NS as to strained or junior
76611010 Vegetable and turkey, baby food, strained
76611020 Vegetable and turkey, baby food, junior
56210000 Cereal, nestum
57820000 Cereal, baby food, jarred, NFS
57820100 Rice cereal, baby food, jarred, NFS
57822000 Mixed cereal with applesauce and bananas, baby food, jarred
57823000 Oatmeal with applesauce and bananas, baby food, jarred
57824000 Rice cereal with applesauce and bananas, baby food, jarred
57824500 Rice cereal with mixed fruit, baby food, jarred
13310000 Custard pudding, flavor other than chocolate, baby food, NS as to strained or junior
13311000 Custard pudding, baby food, flavor other than chocolate, strained
13312000 Custard pudding, baby food, flavor other than chocolate, junior
67404000 Fruit dessert, baby food, NS as to strained or junior
67404010 Fruit dessert, baby food, strained
67404020 Fruit dessert, baby food, junior
67404050 Fruit Supreme dessert, baby food

67404070 Apple yogurt dessert, baby food, strained
67404110 Banana apple dessert, baby food, strained
67404300 Blueberry yogurt dessert, baby food, strained
67404500 Mixed fruit yogurt dessert, baby food, strained
67404550 Cherry cobbler, baby food, junior
67405000 Peach cobbler, baby food, NS as to strained or junior
67405010 Peach cobbler, baby food, strained
67405020 Peach cobbler, baby food, junior
67408010 Banana pudding, baby food, strained
67408500 Banana yogurt dessert, baby food, strained
67410000 Cherry vanilla pudding, baby food, strained
67412000 Dutch apple dessert, baby food, NS as to strained or junior
67412010 Dutch apple dessert, baby food, strained
67412020 Dutch apple dessert, baby food, junior
67413700 Peach yogurt dessert, baby food, strained
67414010 Pineapple dessert, baby food, strained
67414100 Mango dessert, baby food
67415000 Tutti-fruitti pudding, baby food, NS as to strained or junior
67415010 Tutti-fruitti pudding, baby food, strained
67415020 Tutti-fruitti pudding, baby food, junior
67430500 Yogurt and fruit snack, baby food
53801000 Cereal bar with fruit filling, baby food
53803050 Cookie, fruit, baby food
53803100 Cookie, baby food
53803250 Cookie, teething, baby
53803300 Cookie, rice, baby
54350000 Crackers, baby food
54350010 Gerber Finger Foods, Puffs, baby food
54350020 Finger Foods, Puffs, baby food
54360000 Crunchy snacks, corn based, baby food
54408100 Pretzel, baby food
57830100 Gerber Graduates Finger Snacks Cereal, baby food
67100110 Fruit bar, with added vitamin C, baby food, toddler
67430000 Fruit flavored snack, baby food

Other drinks for young children, including yogurt and juice beverages identified as "baby drinks"
[3'-SL Sodium Salt] = 0.015 g/100 g

67202000 Apple juice, baby food
67202010 Apple juice, with added calcium, baby food
67203000 Apple-fruit juice blend, baby food
67203200 Apple-banana juice, baby food
67203400 Apple-cherry juice, baby food

67203500	Apple-grape juice, baby food
67203600	Apple-peach juice, baby food
67203700	Apple-prune juice, baby food
67203800	Grape juice, baby food
67204000	Mixed fruit juice, not citrus, baby food
67204100	Mixed fruit juice, not citrus, with added calcium, baby food
67205000	Orange juice, baby food
67211000	Orange-apple-banana juice, baby food
67212000	Pear juice, baby food
67230000	Apple-sweet potato juice, baby food
67230500	Orange-carrot juice, baby food
67250100	Banana juice with lowfat yogurt, baby food
67250150	Mixed fruit juice with lowfat yogurt, baby food
67260000	Fruit juice and water drink, with high vitamin C and added calcium, baby food

Milk, Whole, and Skim

Unflavored pasteurized and sterilized milk
[3'-SL Sodium Salt] = 0.025 g/100 g

11100000	Milk, NFS
11111000	Milk, whole
11111100	Milk, low sodium, whole
11111150	Milk, calcium fortified, whole
11111160	Milk, calcium fortified, low fat (1%)
11111170	Milk, calcium fortified, fat free (skim)
11112110	Milk, reduced fat (2%)
11112120	Milk, acidophilus, low fat (1%)
11112130	Milk, acidophilus, reduced fat (2%)
11112210	Milk, low fat (1%)
11113000	Milk, fat free (skim)
11114300	Milk, lactose free, low fat (1%)
11114320	Milk, lactose free, fat free (skim)
11114330	Milk, lactose free, reduced fat (2%)
11114350	Milk, lactose free, whole
11116000	Goat's milk, whole
11120000	Milk, dry, reconstituted, NS as to fat content
11121100	Milk, dry, reconstituted, whole
11121210	Milk, dry, reconstituted, low fat (1%)
11121300	Milk, dry, reconstituted, fat free (skim)

Mixed foods containing milk
Adjusted for milk content of 42.1 to 83.6%
[3'-SL Sodium Salt] = 0.011 to 0.021 g/100 g

92101900 Coffee, Latte
92101901 Coffee, Latte, nonfat
92101903 Coffee, Latte, with non-dairy milk
92101904 Coffee, Latte, flavored
92101905 Coffee, Latte, nonfat, flavored
92101910 Coffee, Latte, decaffeinated
92101911 Coffee, Latte, decaffeinated, nonfat
92101917 Coffee, Latte, decaffeinated, flavored
92101918 Coffee, Latte, decaffeinated, nonfat, flavored
92101920 Frozen coffee drink
92101921 Frozen coffee drink, nonfat
92101925 Frozen coffee drink, with whipped cream
92101926 Frozen coffee drink, nonfat, with whipped cream
92101930 Frozen coffee drink, decaffeinated
92101931 Frozen coffee drink, decaffeinated, nonfat
92101935 Frozen coffee drink, decaffeinated, with whipped cream
92101936 Frozen coffee drink, decaffeinated, nonfat, with whipped cream
92101950 Coffee, Cafe Mocha
92101955 Coffee, Cafe Mocha, nonfat
92101965 Coffee, Cafe Mocha, decaffeinated
92101970 Coffee, Cafe Mocha, decaffeinated, nonfat
92102000 Frozen mocha coffee drink
92102010 Frozen mocha coffee drink, nonfat
92102030 Frozen mocha coffee drink, with whipped cream
92102040 Frozen mocha coffee drink, nonfat, with whipped cream
92102060 Frozen mocha coffee drink, decaffeinated
92102070 Frozen mocha coffee drink, decaffeinated, nonfat
92102090 Frozen mocha coffee drink, decaffeinated, with whipped cream
92102100 Frozen mocha coffee drink, decaffeinated, nonfat, with whipped cream
92102500 Coffee, Iced Latte
92102501 Coffee, Iced Latte, nonfat
92102503 Coffee, Iced Latte, flavored
92102504 Coffee, Iced Latte, nonfat, flavored
92102510 Coffee, Iced Latte, decaffeinated
92102511 Coffee, Iced Latte, decaffeinated, nonfat
92102513 Coffee, Iced Latte, decaffeinated, flavored
92102514 Coffee, Iced Latte, decaffeinated, nonfat, flavored
92102600 Coffee, Iced Cafe Mocha
92102601 Coffee, Iced Cafe Mocha, nonfat
92102610 Coffee, Iced Cafe Mocha, decaffeinated
92102611 Coffee, Iced Cafe Mocha, decaffeinated, nonfat

Foods adjusted for being present in dried form
Reconstitution factor of 11
[3'-SL Sodium Salt] = 0.275 g/100 g

11810000	Milk, dry, not reconstituted, NS as to fat content
11811000	Milk, dry, not reconstituted, whole
11812000	Milk, dry, not reconstituted, low fat (1%)
11813000	Milk, dry, not reconstituted, fat free (skim)

Milk Products

Buttermilk
[3'-SL Sodium Salt] = 0.025 g/100 g

11115000	Buttermilk, fat free (skim)
11115100	Buttermilk, low fat (1%)
11115200	Buttermilk, reduced fat (2%)
11115300	Buttermilk, whole

Flavored milk
[3'-SL Sodium Salt] = 0.025 g/100 g

11115400	Kefir, NS as to fat content
11511000	Chocolate milk, NFS
11511100	Chocolate milk, ready to drink, whole
11511200	Chocolate milk, ready to drink, reduced fat
11511300	Chocolate milk, ready to drink, fat free
11511400	Chocolate milk, ready to drink, low fat
11511550	Chocolate milk, ready to drink, reduced sugar, NS as to milk
11511600	Chocolate milk, ready to drink, low fat (Nesquik)
11511610	Chocolate milk, ready to drink, fat free (Nesquik)
11511700	Chocolate milk, ready to drink, low fat, no sugar added (Nesquik)
11512010	Hot chocolate / Cocoa, ready to drink
11512020	Hot chocolate / Cocoa, ready to drink, made with nonfat milk
11512100	Hot chocolate / Cocoa, ready to drink, with whipped cream
11512110	Hot chocolate / Cocoa, ready to drink, made with nonfat milk and whipped cream
11513000	Chocolate milk, made from dry mix, NS as to type of milk
11513100	Chocolate milk, made from dry mix with whole milk
11513150	Chocolate milk, made from dry mix with reduced fat milk
11513200	Chocolate milk, made from dry mix with low fat milk
11513300	Chocolate milk, made from dry mix with fat free milk
11513350	Chocolate milk, made from reduced sugar mix, NS as to type of milk
11513355	Chocolate milk, made from reduced sugar mix with whole milk

11513360 Chocolate milk, made from reduced sugar mix with reduced fat milk
11513365 Chocolate milk, made from reduced sugar mix with low fat milk
11513370 Chocolate milk, made from reduced sugar mix with fat free milk
11513380 Chocolate milk, made from dry mix, NS as to type of milk (Nesquik)
11513381 Chocolate milk, made from dry mix with whole milk (Nesquik)
11513382 Chocolate milk, made from dry mix with reduced fat milk (Nesquik)
11513383 Chocolate milk, made from dry mix with low fat milk (Nesquik)
11513384 Chocolate milk, made from dry mix with fat free milk (Nesquik)
11513390 Chocolate milk, made from no sugar added dry mix, NS as to type of milk (Nesquik)
11513391 Chocolate milk, made from no sugar added dry mix with whole milk (Nesquik)
11513392 Chocolate milk, made from no sugar added dry mix with reduced fat milk (Nesquik)
11513393 Chocolate milk, made from no sugar added dry mix with low fat milk (Nesquik)
11513394 Chocolate milk, made from no sugar added dry mix with fat free milk (Nesquik)
11513400 Chocolate milk, made from syrup, NS as to type of milk
11513500 Chocolate milk, made from syrup with whole milk
11513550 Chocolate milk, made from syrup with reduced fat milk
11513600 Chocolate milk, made from syrup with low fat milk
11513700 Chocolate milk, made from syrup with fat free milk
11513800 Chocolate milk, made from light syrup, NS as to type of milk
11513801 Chocolate milk, made from light syrup with whole milk
11513802 Chocolate milk, made from light syrup with reduced fat milk
11513803 Chocolate milk, made from light syrup with low fat milk
11513804 Chocolate milk, made from light syrup with fat free milk
11513850 Chocolate milk, made from sugar free syrup, NS as to type of milk
11513851 Chocolate milk, made from sugar free syrup with whole milk
11513852 Chocolate milk, made from sugar free syrup with reduced fat milk
11513853 Chocolate milk, made from sugar free syrup with low fat milk
11513854 Chocolate milk, made from sugar free syrup with fat free milk
11514100 Hot chocolate / Cocoa, made with dry mix and water
11514110 Hot chocolate / Cocoa, made with dry mix and whole milk
11514120 Hot chocolate / Cocoa, made with dry mix and reduced fat milk
11514130 Hot chocolate / Cocoa, made with dry mix and low fat milk
11514140 Hot chocolate / Cocoa, made with dry mix and fat free milk
11514310 Hot chocolate / Cocoa, made with no sugar added dry mix and water
11514320 Hot chocolate / Cocoa, made with no sugar added dry mix and whole milk
11514330 Hot chocolate / Cocoa, made with no sugar added dry mix and reduced fat milk
11514340 Hot chocolate / Cocoa, made with no sugar added dry mix and low fat milk
11514350 Hot chocolate / Cocoa, made with no sugar added dry mix and fat free milk
11519040 Strawberry milk, NFS
11519050 Strawberry milk, whole
11519105 Strawberry milk, reduced fat
11519200 Strawberry milk, low fat

11519205 Strawberry milk, fat free
11519210 Strawberry milk, reduced sugar
11526000 Milk, malted
11531000 Eggnog
11541400 Milk shake with malt
11542100 Milk shake, fast food, chocolate
11542200 Milk shake, fast food, flavors other than chocolate
11543000 Milk shake, bottled, chocolate
11543010 Milk shake, bottled, flavors other than chocolate
11551050 Licuado or Batido
11553100 Fruit smoothie, NFS
11553110 Fruit smoothie, with whole fruit and dairy
11553120 Fruit smoothie, with whole fruit and dairy, added protein
11553130 Fruit smoothie juice drink, with dairy
11560000 Chocolate milk drink

Foods adjusted for being present in dried form
Reconstitution factor of 10.6
[3'-SL Sodium Salt] = 0.265 g/100 g

11830150 Cocoa powder, not reconstituted
11830160 Chocolate beverage powder, dry mix, not reconstituted
11830165 Chocolate beverage powder, light, dry mix, not reconstituted
11830260 Milk, malted, dry mix, not reconstituted
11830400 Strawberry beverage powder, dry mix, not reconstituted

Milk-based meal replacement beverages for weight reduction
[3'-SL Sodium Salt] = 0.09 g/100 g

95101000 Nutritional drink or shake, ready-to-drink (Boost)
95101010 Nutritional drink or shake, ready-to-drink (Boost Plus)
95102000 Nutritional drink or shake, ready-to-drink (Carnation Instant Breakfast)
95103000 Nutritional drink or shake, ready-to-drink (Ensure)
95103010 Nutritional drink or shake, ready-to-drink (Ensure Plus)
95105000 Nutritional drink or shake, ready-to-drink (Kellogg's Special K Protein)
95106000 Nutritional drink or shake, ready-to-drink (Muscle Milk)
95106010 Nutritional drink or shake, ready-to-drink, light (Muscle Milk)
95110000 Nutritional drink or shake, ready-to-drink (Slim Fast)
95110010 Nutritional drink or shake, ready-to-drink, sugar free (Slim Fast)
95110020 Nutritional drink or shake, high protein, ready-to-drink (Slim Fast)
95120010 Nutritional drink or shake, high protein, ready-to-drink, NFS
95120020 Nutritional drink or shake, high protein, light, ready-to-drink, NFS

Foods adjusted for being present in dried form
Reconstitution factor of 6 to 10
[3'-SL Sodium Salt] = 0.54 to 0.9 g/100 g

95201000	Nutritional powder mix (Carnation Instant Breakfast)
95201010	Nutritional powder mix, sugar free (Carnation Instant Breakfast)
95201200	Nutritional powder mix (EAS Whey Protein Powder)
95201500	Nutritional powder mix, high protein (Herbalife)
95202000	Nutritional powder mix (Muscle Milk)
95202010	Nutritional powder mix, light (Muscle Milk)
95210000	Nutritional powder mix (Slim Fast)
95210010	Nutritional powder mix, sugar free (Slim Fast)
95210020	Nutritional powder mix, high protein (Slim Fast)
95220010	Nutritional powder mix, high protein, NFS
95230000	Nutritional powder mix, whey based, NFS
95230020	Nutritional powder mix, protein, light, NFS
95230030	Nutritional powder mix, protein, NFS

Yogurt
[3'-SL Sodium Salt] = 0.25 g/100 g

11400000	Yogurt, NFS
11400010	Yogurt, Greek, NS as to type of milk or flavor
11410000	Yogurt, NS as to type of milk or flavor
11411010	Yogurt, NS as to type of milk, plain
11411100	Yogurt, whole milk, plain
11411200	Yogurt, low fat milk, plain
11411300	Yogurt, nonfat milk, plain
11411390	Yogurt, Greek, NS as to type of milk, plain
11411400	Yogurt, Greek, whole milk, plain
11411410	Yogurt, Greek, low fat milk, plain
11411420	Yogurt, Greek, nonfat milk, plain
11430000	Yogurt, NS as to type of milk, fruit
11431000	Yogurt, whole milk, fruit
11432000	Yogurt, low fat milk, fruit
11433000	Yogurt, nonfat milk, fruit
11433990	Yogurt, Greek, NS as to type of milk, fruit
11434000	Yogurt, Greek, whole milk, fruit
11434010	Yogurt, Greek, low fat milk, fruit
11434020	Yogurt, Greek, nonfat milk, fruit
11434090	Yogurt, NS as to type of milk, flavors other than fruit
11434100	Yogurt, whole milk, flavors other than fruit
11434200	Yogurt, low fat milk, flavors other than fruit

11434300	Yogurt, nonfat milk, flavors other than fruit
11435000	Yogurt, Greek, NS as to type of milk, flavors other than fruit
11435010	Yogurt, Greek, whole milk, flavors other than fruit
11435020	Yogurt, Greek, low fat milk, flavors other than fruit
11435030	Yogurt, Greek, nonfat milk, flavors other than fruit
11435100	Yogurt, Greek, with oats
11436000	Yogurt, liquid
11446000	Yogurt parfait, low fat, with fruit

Formula intended for pregnant women ("mum" formulas, -9 to 0 months)
[3'-SL Sodium Salt] = 0.09 g/100 g

95120000	Nutritional drink or shake, ready-to-drink, NFS

Foods adjusted for being present in dried form
Reconstitution factor of 6
[3'-SL Sodium Salt] = 0.54 g/100 g

95220000	Nutritional powder mix, NFS

Processed Fruits and Fruit Juices

Fruit flavored drinks and ades
[3'-SL Sodium Salt] = 0.025 g/100 g

42403010	Coconut water, unsweetened
42404010	Coconut water, sweetened
92432000	Fruit juice drink, citrus, carbonated
92433000	Fruit juice drink, noncitrus, carbonated
92510610	Fruit juice drink
92510650	Tamarind drink
92510720	Fruit punch, made with fruit juice and soda
92510730	Fruit punch, made with soda, fruit juice, and sherbet or ice cream
92510955	Lemonade, fruit juice drink
92510960	Lemonade, fruit flavored drink
92511015	Fruit flavored drink
92511250	Fruit juice beverage, 40-50% juice, citrus
92513000	Slush frozen drink
92513010	Slush frozen drink, no sugar added
92530410	Fruit flavored drink, with high vitamin C
92530510	Cranberry juice drink, with high vitamin C
92530610	Fruit juice drink, with high vitamin C
92530950	Vegetable and fruit juice drink, with high vitamin C
92531030	Fruit juice drink (Sunny D)

92541010 Fruit flavored drink, powdered, reconstituted
92542000 Fruit flavored drink, with high vitamin C, powdered, reconstituted
92550030 Fruit juice drink, with high vitamin C, light
92550035 Fruit juice drink, light
92550040 Fruit juice drink, diet
92550110 Cranberry juice drink, with high vitamin C, light
92550200 Grape juice drink, light
92550350 Orange juice beverage, 40-50% juice, light
92550360 Apple juice beverage, 40-50% juice, light
92550370 Lemonade, fruit juice drink, light
92550380 Pomegranate juice beverage, 40-50% juice, light
92550400 Vegetable and fruit juice drink, with high vitamin C, diet
92550405 Vegetable and fruit juice drink, with high vitamin C, light
92550610 Fruit flavored drink, with high vitamin C, diet
92550620 Fruit flavored drink, diet
92552000 Fruit flavored drink, with high vitamin C, powdered, reconstituted, diet
92552010 Fruit flavored drink, powdered, reconstituted, diet
92552020 Fruit juice drink, reduced sugar (Sunny D)
92552030 Fruit juice drink (Capri Sun)
92582100 Fruit juice drink, with high vitamin C, plus added calcium
92582110 Fruit juice drink, added calcium (Sunny D)
92610030 Horchata beverage, made with milk
92611100 Oatmeal beverage with milk
92612010 Sugar cane beverage
92613510 Cornmeal beverage with chocolate milk
92804000 Shirley Temple

Foods adjusted for being present in dried form
Reconstitution factor of 4 to 10.23
[3'-SL Sodium Salt] = 0.048 to 0.123 g/100 g

92511000 Lemonade, frozen concentrate, not reconstituted
92900100 Fruit flavored drink, with high vitamin C, powdered, not reconstituted
92900110 Fruit flavored drink, powdered, not reconstituted
92900200 Fruit flavored drink, powdered, not reconstituted, diet

Sugar Substitutes

Sugar substitute, herbal extract powder or liquid
[3'-SL Sodium Salt] = 10 g/100 g

91106010 Sugar substitute and sugar blend
91107000 Sugar substitute, sucralose, powder
91108000 Sugar substitute, stevia, powder

91108010	Sugar substitute, stevia, liquid
91108020	Sugar substitute, monk fruit, powder
91200000	Sugar substitute, powder, NFS
91200005	Sugar substitute, liquid, NFS
91200040	Sugar substitute, saccharin, powder
91200110	Sugar substitute, saccharin, liquid
91201010	Sugar substitute, aspartame, powder
91302020	Agave liquid sweetener