KYOWA HAKKO BIO CO., LTD.

1-9-2, Otemachi, Chiyoda-ku, Tokyo, 100-0004, Japan
Tel: +81-3-5205-7303 Fax: +81-3-5205-7149
http://www.kyowahakko-bio.co.jp/

January 14th, 2022

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

Dear Dr. Gaynor:

Re: GRAS Notice for 6'-Sialyllactose sodium salt

In accordance with 21 CFR §170 Subpart E consisting of §§ 170.203 through 170.285, Kyowa Hakko Bio Co., Ltd. (Kyowa; 1-9-2, Otemachi, Chiyoda-ku, Tokyo, 100-0004, Japan), as the notifier, is submitting one hard copy and one electronic copy (on CD) of all data and information supporting the company's conclusion that 6'-sialyllactose (6'-SL) sodium salt is GRAS on the basis of scientific procedures, for use in non-exempt term infant formula and various conventional food and beverage products across multiple food categories; these food uses of 6'-SL sodium salt are therefore not subject to the premarket approval requirements of the *Federal Food, Drug and Cosmetic Act*. Information setting forth the basis for Kyowa's GRAS conclusion, as well as a consensus opinion of an independent panel of experts, also are enclosed for review by the agency.

I certify that the enclosed electronic files were scanned for viruses prior to submission and are thus certified as being virus-free using Symantec Endpoint Protection 12.1.5.

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

Sincerely,

[redacted]

Yoko Kawada, Pharmacist
External Relations Department Manager
Kyowa Hakko Bio Co., Ltd.

GRAS NOTICE FOR 6'-SIALYLLACTOSE SODIUM SALT FOR USE IN NON-EXEMPT INFANT FORMULA AND SPECIFIED CONVENTIONAL FOOD PRODUCTS

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

SUBMITTED BY:
Kyowa Hakko Bio Co., Ltd.
1-9-2, Otemachi, Chiyoda-ku
Tokyo, 100-0004,
Japan

DATE:
14 January 2022

GRAS Notice for 6'-Sialyllactose Sodium Salt for Use in Non-Exempt Infant Formula and Specified Conventional Food Products

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GRAS Notice for 6'-Sialyllactose Sodium Salt for Use in Non-Exempt Infant Formula and Specified Conventional Food Products

Part 1. §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §§170.203 through 170.285, Kyowa Hakko Bio Co., Ltd. (Kyowa) hereby informs the United States (U.S.) Food and Drug Administration (FDA) that the intended uses of 6'-sialyllactose (6'-SL) sodium salt, as manufactured by Kyowa in non-exempt infant formula, specified conventional food products, and foods for special dietary uses 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 Kyowa's view that these notified uses of 6'-SL sodium salt are Generally Recognized as Safe (GRAS). In addition, as a responsible official of Kyowa, the undersigned hereby certifies that all data and information presented in this notice represents a complete and balanced submission that is representative of the generally available literature. Kyowa considered all unfavorable as well as favorable information that is publicly available and/or known to Kyowa and that is pertinent to the evaluation of the safety and GRAS status of 6'-SL sodium salt as a food ingredient for addition to non-exempt infant formula, specified conventional food products, and foods for special dietary uses, as described herein.

Signed,

14 January, 2022
Date

Yoko Kawada, Pharmacist
External Relations Department Manager
Kyowa Hakko Bio Co., Ltd.
yoko.kawada@kyowa-kirin.co.jp

1.1 Name and Address of Notifier

Kyowa Hakko Bio Co., Ltd.
1-9-2, Otemachi, Chiyoda-ku
Tokyo, 100-0004,
Japan

1.2 Common Name of Notified Substance

6'-sialyllactose sodium salt (6'-SL sodium salt)

1.3 Conditions of Use

Kyowa's proposed food uses and use levels for 6'-SL sodium salt in the U.S. are presented in Table 1.3-1, whereby food uses are organized according to 21 CFR §170.3 (U.S. FDA, 2020a). As discussed below in Section 3.1, 6'-SL sodium salt has previously been concluded to be GRAS for use in term (non-exempt) infant formula, infant and toddler foods (including toddler formula intended for ages 1 to 3 years), and specified conventional foods [GRAS Notice (GRN) 881; GRN 922]. Kyowa's 6'-SL ingredient is intended as an alternative to other sources of 6'-SL currently on the U.S. market.

Kyowa notes that human milk is a complex fluid containing over 150 human milk oligosaccharides (HMOs) and is proposing the addition of 6'-SL sodium salt to non-exempt term infant formula to provide a source of 6'-SL for formula-fed infants. Kyowa's proposed use level in non-exempt term infant formula (0.50 g/L) is within the range of average levels of 6'-SL calculated from studies in which levels of 6'-SL were assessed in the milk of healthy human mothers following the birth of healthy infants (see Section 3.2.2.2 below).

Compared to the conditions of use previously notified as GRAS for 6'-SL sodium salt, Kyowa's proposed food uses include all food uses previously concluded to be GRAS; however, Kyowa intends to use 6'-SL sodium salt at different use levels in several food uses (see Table 1.3-1). The use levels proposed by Kyowa for 6'-SL sodium salt are representative of the proportion of 6'-SL to 2'-fucosyllactose (2'-FL) in human milk and were calculated from Kyowa's proposed use levels for 2'-FL using the ratio of 6'-SL to 2'-FL in human milk [*i.e.*, 6'-SL is present at approximately one fifth the level of 2'-FL and thus, Kyowa's proposed use levels for 6'-SL are one fifth those of 2'-FL (see GRAS Notice for 2'-FL submitted by Kyowa)]. Kyowa's proposed use levels for 2'-FL (see GRAS Notice for 2'-FL submitted by Kyowa) are the same as those previously concluded to be GRAS and notified to the Agency without objection in GRNs 546, 571, 650, 735, 749, 852, 897, and 932 (U.S. FDA, 2015a,b, 2016a, 2018a,b, 2019a, 2020b, 2021a). In addition to the uses previously concluded as GRAS for 6'-SL sodium salt, Kyowa also proposes the use of 6'-SL sodium salt in the following uses: breads and baked goods (including gluten-free varieties), protein drinks, hot breakfast cereals, ready-to-eat breakfast cereals, chewing gum, coffee, tea, milk imitates, beverage whiteners, non-dairy cream, non-dairy yogurt, frozen dairy desserts (including ice cream and frozen yogurt), edible ices, sherbet and sorbet, dairy-based puddings, custards, and mousses, fruit pie filling, "fruit prep" fillings, energy and protein bars, jellies and jams, fruit preserves, and fruit butters, evaporated and condensed milk, formula intended for pregnant women, fruit juices and nectars, canned fruit, fruit-based desserts, vegetable juices and nectars, table-top sweeteners, syrups for flavoring milk beverages, and foods for special dietary use (oral nutritional supplements and enteral tube feeding) (**bolded** in Table 1.3-1). The use of 6'-SL sodium salt in foods for special dietary uses for oral nutritional supplements is intended for the general population (ages 2 and up). The recommended conditions of use are 0.42 g 6'-SL sodium salt/45 g powdered serving or 250 mL ready to consume product, consumed twice per day for a total daily intake of 0.84 g 6'-SL sodium salt/day. The use of 6'-SL sodium salt in enteral tube feeding formula is intended for ages 11 and up and is proposed at a use level of 4.1 g/L in the final, ready-to-consume product. The recommended conditions of use for enteral tube feeding formula are 1.0 g 6'-SL sodium salt per 250 mL, consumed twice per day, for a total intake of 2.0 g/day.

Table 1.3-1 Summary of the Individual Proposed Food Uses and Use Levels for 6'-Sialyllactose Sodium Salt in the U.S.

Food Category (21 CFR §170.3)	Food Uses[a,b]	Use Levels (g/L or g/kg)
Baked Goods and Baking Mixes	**Breads and baked goods, incl. gluten-free**	10
Beverages and Beverage Bases	Soft drinks (regular and diet)[c]	0.25
	Enhanced, fortified, and flavored waters (incl. carbonated waters)[c]	0.25
	Non-milk-based meal replacement drinks	1.0
	Sports, isotonic, and energy drinks	0.5
	Protein drinks	1.0
Breakfast Cereals	**Hot breakfast cereals (*e.g.*, oatmeal, grits), instant and RTE**	1.0
	RTE breakfast cereals	
	Puffed cereals	17
	High-fiber cereals	6.2
	Biscuit-type cereals	4.2
Chewing Gum	**Chewing gum**	62
Coffee and Tea	**Coffee**	2.1
	Tea	2.1
Dairy Product Analogs	**Milk substitutes such as soy milk and imitation milks**	0.25
	Beverage whiteners	125
	Non-dairy cream	125
	Non-dairy yogurt	2.2
Frozen Dairy Desserts	**Frozen desserts incl. ice creams and frozen yogurts, frozen novelties**	3.5
Fruit and Water Ices	**Edible ices, sherbet, and sorbet**	3.5
Gelatins, Puddings, and Fillings	**Dairy-based puddings, custards, and mousses[d]**	3.5
	Fruit pie filling	2.9
	"Fruit Prep" such as fruit filling in bars, cookies, yogurt, and cakes	6.25
Grain Products and Pastas	Cereal and granola bars incl. **energy, protein,** and meal replacement bars[e]	10
Infant and Toddler Foods	Term infant formula[f]	0.50 (as consumed)
	Toddler formula[f] (intended for age 1 to 3 years)	0.50 (as consumed)
	Other baby foods for infants and young children	2.5
	Hot cereals (dry and RTE)[g]	2.3
	Other drinks for young children, incl. yogurt and juice beverages identified as "baby drinks"[h]	0.25 to 2.1
	Desserts incl. fruit desserts, cobblers, yogurt/fruit combinations ("junior type desserts")[g]	2.3
	Baby crackers, pretzels, cookies, and snack items[g]	12
Jams and Jellies	**Jellies and jams, fruit preserves, and fruit butters**	12
Milk, Whole, and Skim	Unflavored pasteurized and sterilized milk (whole milk, reduced-fat milk, low-fat milk, non-fat milk; including powdered milks, reconstituted)	0.5
Milk Products	Buttermilk[i]	0.25
	Flavored milk[i]	0.25

Table 1.3-1 Summary of the Individual Proposed Food Uses and Use Levels for 6'-Sialyllactose Sodium Salt in the U.S.

Food Category (21 CFR §170.3)	Food Uses[a,b]	Use Levels (g/L or g/kg)
	Evaporated and condensed milk	0.25
	Milk-based meal replacement beverages for weight reduction	1.0
	Yogurt	5.0
	Formula intended for pregnant women ("mum" formulas, -9 to 0 months)[j]	12.5
Processed Fruits and Fruit Juices	Fruit flavored drinks and ades[k]	0.25
	Fruit juices	0.25
	Fruit nectars	0.25
	Canned fruit	3.5
	Fruit-based desserts	3.5
Processed Vegetables and Vegetable Juices	**Vegetable juices and nectars**	0.25
Sugar Substitutes	**Table-top sweeteners**	62
Sweet Sauces, Toppings, and Syrups	**Syrups used to flavor milk beverages**	1.5
Foods For Special Dietary Use	**Oral nutritional supplements and enteral tube feeding (11 years and older)[l]**	4.1[m]

6'-SL = 6'-sialyllactose; CFR = *Code of Federal Regulations*; GRAS = Generally Recognized as Safe; incl. = including; NHANES = National Health and Nutrition Examination Survey; RTE = ready-to-eat; U.S. = United States.

[a] 6'-SL are 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] Additional food uses proposed by Kyowa that have not been previously concluded as GRAS and notified to the U.S. FDA are **bolded.**

[c] The use of 6'-SL sodium salt in soft drinks and enhanced, fortified, and flavored waters was previously concluded to be GRAS at a use level of 0.50 g/L.

[d] Includes gelatin desserts.

[e] The use of 6'-SL sodium salt was previously concluded to be GRAS in cereal and granola bars at a use level of 5 g/kg and in meal replacement bars at a use level of 10 g/kg. Kyowa now proposes to also use 6'-SL sodium salt in energy and protein bars and at a use level of 10 g/kg for all bar types.

[f] The use of 6'-SL sodium salt was previously concluded to be GRAS in term infant formula at a use level of 0.4 g/L and toddler formula at a use level of 0.3 g/L.

[g] The use of 6'-SL sodium salt was previously concluded to be GRAS in baby foods at a use level of 2.5 g/kg.

[h] The use of 6'-SL sodium salt was previously concluded to be GRAS in drinks for young children at a use level of 0.3 g/L.

[i] The use of 6'-SL sodium salt was previously concluded to be GRAS in buttermilk and flavored milk at a use level of 0.5 g/L.

[j] Food codes for "mum formulas" were not available in the 2017-2018 NHANES. This intended use is excluded from the calculation of estimated daily intakes due to absence of consumption data.

[k] The use of 6'-SL sodium salt was previously concluded to be GRAS in fruit flavored drinks and ades at a use level of 0.5 g/L.

[l] Foods for special dietary use were assessed separately from the intended food uses of 6'-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 6'-SL sodium salt from foods for special dietary use is, therefore, not expected to be cumulative to other dietary sources.

[m] Use level of 4.1 g/L represents the level of 6'-SL sodium salt in the final, ready-to-consume product.

1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a)(b) of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2020c), Kyowa has concluded that the intended uses of 6'-SL sodium salt 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 Notification 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:

Yoko Kawada, Pharmacist
External Relations Department
Manager
1-9-2, Otemachi
Chiyoda-ku
Tokyo, 100-0004
Japan

Email: yoko.kawada@kyowa-kirin.co.jp
Phone: +81 70 3145 4956

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

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

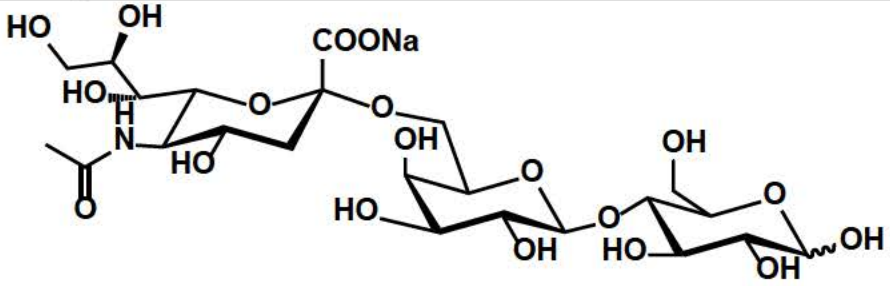
It is Kyowa's view that all data and information presented in Parts 2 through 7 of this Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore, all data and information presented herein are not exempted from the Freedom of Information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

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

2.1 Identity

6'-SL is a sialylated oligosaccharide that is composed of lactose at the reducing terminus and a sialic acid residue at the nonreducing end that is connected to the galactose unit of lactose at the 6 position *via* an α-2,6 linkage (ten Bruggencate *et al.*, 2014; Jacobi *et al.*, 2016). Kyowa's 6'-SL sodium salt manufactured by microbial fermentation using a genetically modified strain of *Escherichia coli* W contains by specification ≥82% 6'-SL, with lesser amounts of *N*-Acetyl D-neuraminic acid (NeuAc) (≤9%), D-glucose and D-lactose (≤3 % each), 6'-sialyllactulose (≤5%), 3'-sialyllactose (3'-SL) sodium salt (≤1%), and sodium (≤5%). Information regarding the chemical identity of Kyowa's 6'-SL sodium salt ingredient is provided in Table 2.1-1 below.

Table 2.1-1 Chemical Identity of 6'-Sialyllactose Sodium Salt

Common Name	6'-Sialyllactose sodium salt; 6'-O-sialyllactose sodium salt
Trade Name	6'-Sialyllactose sodium salt; 6'-O-sialyllactose sodium salt
Common Abbreviations	6'-SL; 6-SL; 6SL
International Union of Pure and Applied Chemistry (IUPAC) Name	sodium;(2S,4S,5R,6R)-5-acetamido-2-[(2R,3S,4S,5R,6S)-3,5-dihydroxy-2-(hydroxymethyl)-6-[(2R,3S,4R,5R)-4,5,6-trihydroxy-2-(hydroxymethyl)oxan-3-yl]oxyoxan-4-yl]oxy-4-hydroxy-6-[(1R,2R)-1,2,3-trihydroxypropyl]oxane-2-carboxylate
Synonyms	Neu5Ac-a-2-6-Gal-b1-4-Glc sodium salt; 6'-*N*-Acetylneuraminyl-D-lactose sodium salt
Chemical Abstract Service (CAS) Number	157574-76-0
Chemical Formula	$C_{23}H_{38}NO_{19}Na$
Molecular Weight	655.53 g/mol
Structural Formula	

Sialyllactose, the predominant sialylated oligosaccharide in human and bovine milk (Goedhart and Bindels, 1994), is composed of a sialic acid moiety conjugated to a lactose molecule. The predominant forms of sialyllactose are 6'-SL and 3'-SL, in which sialic acid is connected to the galactose unit of lactose at the 6 and 3 positions, respectively (Jacobi *et al.*, 2016). Sialyllactoses, including 6'-SL and 3'-SL, are present in the milk from various species, including mice, pigs, dogs, cows, elephants, and humans (Grollman *et al.*, 1965; Prieto *et al.*, 1995; Kunz *et al.*, 1999; Shen *et al.*, 2000; Nakamura *et al.*, 2003; Leo *et al.*, 2010; Smilowitz *et al.*, 2013; Salcedo *et al.*, 2016).

The chemical and structural identity of Kyowa's 6'-SL produced by fermentation with a genetically engineered strain of *E. coli* W (Lot C) was confirmed against 6'-SL isolated from bovine milk or colostrum (SIGMA-ALDRICH, Lot No. SLCD8825) using proton nuclear magnetic resonance spectroscopy (^{1}H NMR), carbon-13 nuclear magnetic resonance spectroscopy (^{13}C NMR), and liquid chromatography–mass spectrometry (LC-MS). A representative ^{1}H NMR, an enlarged portion of the ^{13}C NMR, and a representative LC-MS spectrum of Kyowa's 6'-SL sodium salt (Lot C) compared against the bovine milk or colostrum standard (SIGMA-ALDRICH, Lot No. SLCD8825) are presented in Figures 2.1-1 through 2.1-3 below.

Batch analyses of 5 lots of 6'-SL sodium salt produced by fermentation with a genetically modified strain of *E. coli* W demonstrate that it is a high-purity product (~87 to 92% 6'-SL) with low levels of other structurally related saccharides detected (see Section 2.3.3).

Figure 2.1-1 **^{1}H NMR Spectrums of 6'-Sialyllactose Sodium Salt (Lot C) and 6'-Sialyllactose Standard (SIGMA-ALDRICH, Lot No. SLCD8825)**

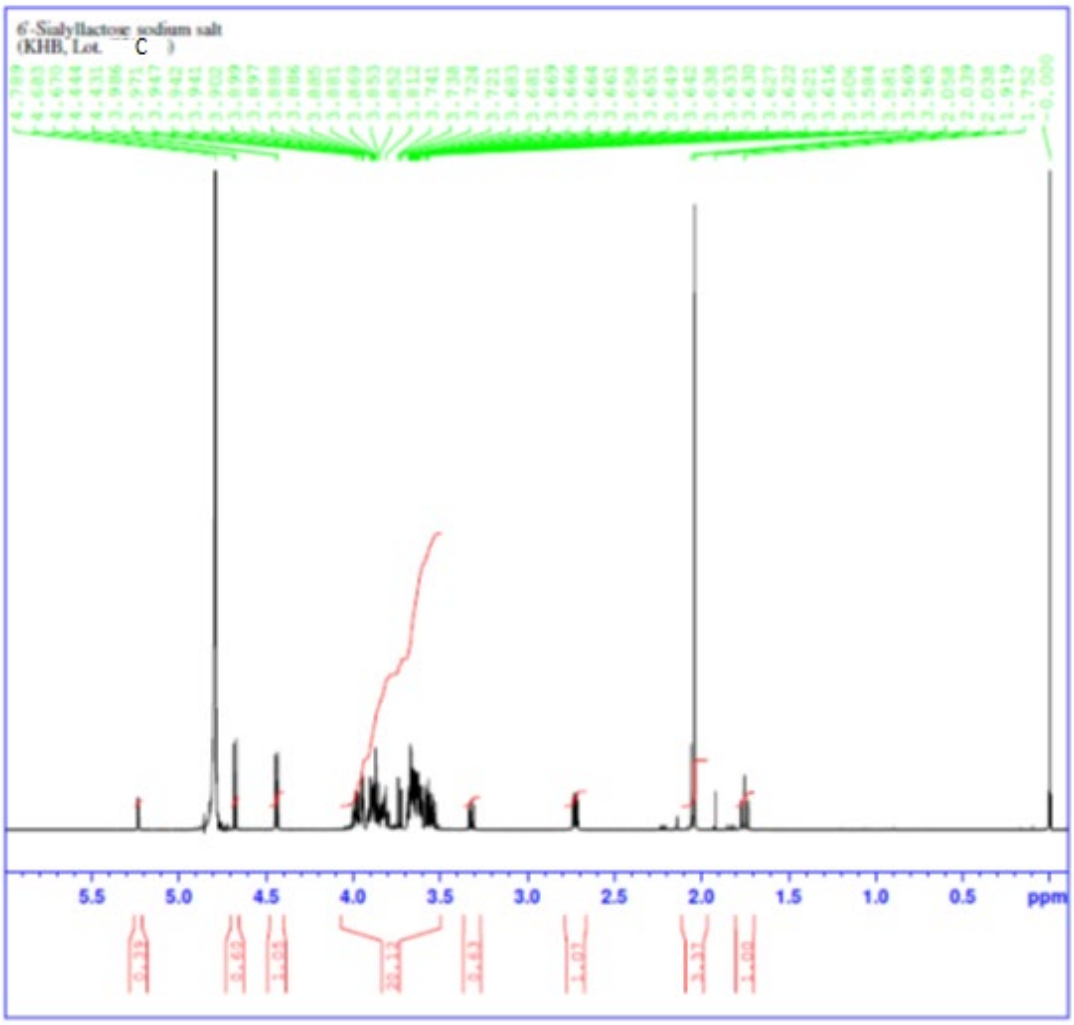
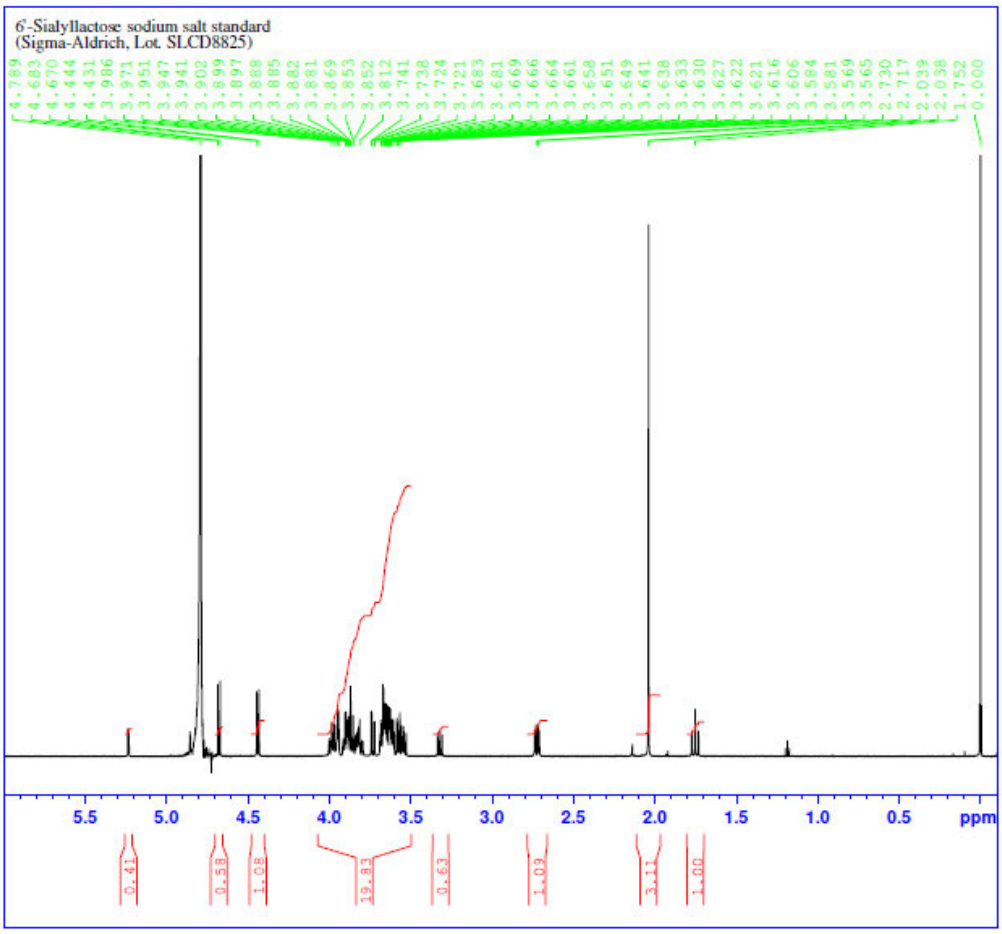
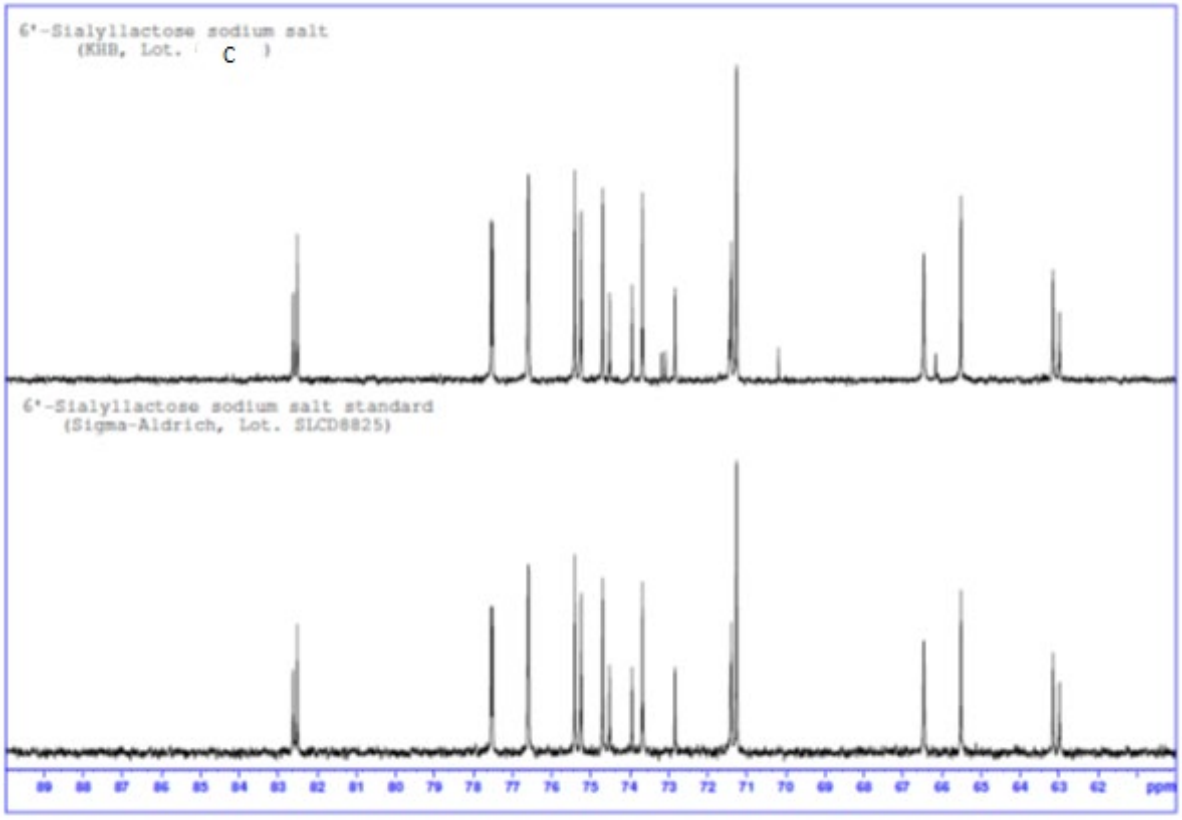


Figure 2.1-1 **^{1}H NMR Spectrums of 6'-Sialyllactose Sodium Salt (Lot C) and 6'-Sialyllactose Standard (SIGMA-ALDRICH, Lot No. SLCD8825)**



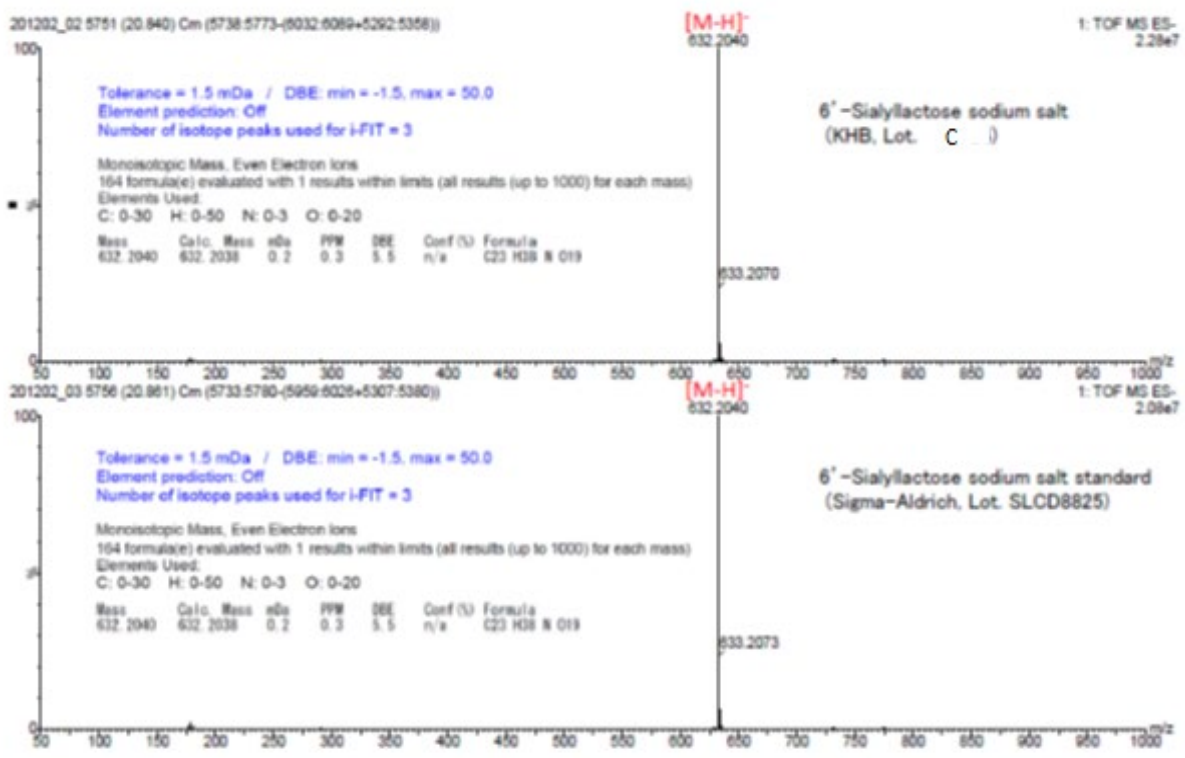
^{1}H NMR = proton nuclear magnetic resonance spectroscopy.

Figure 2.1-2 **Enlarged ^{13}C NMR Spectrums of 6'-Sialyllactose Sodium Salt (Lot C) and 6'-Sialyllactose Standard (SIGMA-ALDRICH, Lot No. SLCD8825)**



^{13}C NMR = carbon-13 nuclear magnetic resonance spectroscopy.

Figure 2.1-3 LC-MS Spectra and Estimated Composition Formula of 6'-Sialyllactose Sodium Salt (Lot C) and 6'-Sialyllactose Standard (SIGMA-ALDRICH, Lot No. SLCD8825)



LC-MS = liquid chromatography–mass spectrometry.

2.2 Method of Manufacture

2.2.1 Production Microorganism

2.2.1.1 Host Organism (E. coli W)

The host organism used in the production of 6'-SL sodium salt is *E. coli* W. The current taxonomic classification of *E. coli* W is summarized in Table 2.2.1.1-1.

Table 2.2.1.1-1 Taxonomic Classification of *Escherichia coli* strain W

Superkingdom	*Bacteria*
Phyllum	*Protobacteria*
Class	*Gammaproteobacteria*
Order	*Enterobacteriales*
Family	*Enterobacteriaceae*
Genus	*Escherichia*
Species	*Escherichia coli*
Strain	W
Culture Collection	American Type Culture Collection (ATCC)
Deposit Number[a]	ATCC 9637

[a] https://www.atcc.org/products/all/9637.aspx.

The *E. coli* W strain is a Gram-negative, rod-shaped, facultative anaerobe that has been used in the industrial production of amino acids for foods, feeds, medicines, and various other applications for nearly 80 years (Archer *et al.*, 2011; UniProt, 2021). *E. coli* W was first isolated from the soil of a cemetery near Rutgers University by Selman A. Waksman, who observed the strain's high sensitivity to streptomycin compared to other isolated *E. coli* strains in his collection, and is thus commonly referred to as "Waksman's strain" or "W strain" (Archer *et al.*, 2011). Early reported uses of *E. coli* W are related to the strain's susceptibility to streptomycin and other antibiotics (Archer *et al.*, 2011).

E. coli W is 1 of 4 strains designated safe for laboratory use (K-12, B, C, and W). These 4 strains and their derivatives are designated as Risk Group 1 or Biosafety Level 1 organisms in biological safety guidelines (Archer *et al.*, 2011; ATCC, 2021a), as they are well-characterized and do not cause disease in healthy adult humans (NIH, 2019), and do not colonize the human gut (Bauer *et al.*, 2008). The *E. coli* W strain has been deposited in the American Type Culture Collection (ATCC 9637 – ATCC, 2021a), and its genome has been sequenced, annotated, and compared to other safe *E. coli* strains and group B1 commensal/pathogenic *E. coli* strains (Archer *et al.*, 2011). Although *E. coli* W has genes that encode pathogenicity determinants, these have been mutationally inactivated or are missing key components required for pathogenicity, similar to other safe strains (Archer *et al.*, 2011). Genomic analyses also confirmed the lack of genes encoding toxins that can be secreted. As such, *E. coli* W is non-pathogenic and non-toxigenic.

Compared to other Risk Group 1 *E. coli* strains (K-12, B, and C), *E. coli* W has a larger genome (the chromosome is 4,900,968 bp and encodes 4,764 open reading frames), belongs to phylogroup B1 rather than A (both of which are classified as non-pathogenic commensal strains), grows faster, and utilizes a wider range of carbon sources including, unlike the other 3 Risk Group 1 strains, sucrose (Archer *et al.*, 2011; UniProt, 2021). *E. coli* W contains 2 cryptic plasmids, namely pRK1 and pRK2. The pRK1 plasmid (102,536 bp) encodes 118 genes (114 proteins coding genes, 1 pseudogene, and 3 non-coding RNAs). This plasmid was demonstrated to belong to Incompatibility Group 1 (IncI1) *via* Basic Local Alignment Search Tool (BLAST) analysis, and although genes for antibiotic resistance are typically found on most IncI1 plasmids, the pRK1 plasmid does not encode any antibiotic resistance genes (Archer *et al.*, 2011). The pRK1 plasmid is removed from the host strain and is not present in the production strain. The pRK2 plasmid (5,360 bp; previously sequenced by Štěpánek *et al.*, 2005) encodes 16 genes (15 protein coding genes and 1 non-coding RNA) and is a cryptic ColE1-type plasmid (Archer *et al.*, 2011). The pRK2 plasmid remains in the production strain and no genetic modification was made by Kyowa to the pRK2 plasmid.

2.2.1.2 Host Modifications

The host strain *E. coli* W was genetically modified to produce the 6'-SL recombinant production strain. The production strain was optimized to produce 6'-SL *via* the fermentation of glucose and lactose.

Method of Modification

Target genes are cloned by polymerase chain reaction (PCR) from chromosomal DNA of defined donor organisms and fused to a constitutive promoter originating from *E. coli* W and expressed at the insertion loci. Desired host modifications are introduced to the *E. coli* W strains in a step-wise manner for the construction of the production strain.

In all instances, genetic modifications were achieved using a modified lambda Red recombination system (Datsenko and Wanner, 2000), a common technique used to make targeted genetic modifications in *E. coli* at loci specified by flanking homology regions including insertions, deletions, and point mutations (Murphy, 1998; Yu *et al.*, 2000; Sharan *et al.*, 2009). Lambda Red recombination genes are expressed from the Red recombinase pKD46 plasmid under the inducible arabinose promoter (P_{araB}) containing a temperature-sensitive replicon (Datsenko and Wanner, 2000; GenBank Accession No. AY048746 – NCBI, 2021). Following expression of the recombinase enzymes, linear DNA substrates are introduced by electroporation, and recombination is catalyzed by the Lambda-derived proteins (Sharan *et al.*, 2009).

Genes of Interest

The production strain contains 5 heterologous gene sequences (encoding glucosamine 6-phosphate *N*-acetyltransferase, *N*-acylglucosamine 2-epimerase, CMP-*N*-acetylneuraminic acid synthetase, α-2,6-sialyltransferase, and *N*-acetylneuraminic acid synthetase) originating from defined donor organisms that are inserted into the chromosomal DNA of the host organism, *E. coli* W. The gene encoding glucosamine 6-phosphate *N*-acetyltransferase originates from *Saccharomyces cerevisiae* S288C (ATCC 204508 – ATCC, 2021b). The gene encoding *N*-acylglucosamine 2-epimerase originates from *Synechocystis* sp. PCC 6803 (ATCC 27184 – ATCC, 2021c). The gene encoding *N*-acetylneuraminic acid synthetase originates from *Rhodobacter capsulatus* NBRC16581 (NBRC16581 – NBRC, 2001). The gene encoding CMP-*N*-acetylneuraminic acid synthetase originates from *Pasteurella multocida* subsp. *multocida* str. Pm70 (ATCC BAA-1909 – ATCC, 2021d). The gene encoding an α-2,6-sialyltransferase (Yamamoto *et al.*, 1998) originates from *Photobacterium damselae* NBRC 15633 (NBRC15633 – NBRC, 1994).

In all cases, the target genes were cloned by PCR and fused to a constitutive promoter originating from *E. coli* W and expressed at the insertion loci. In some cases, site-directed mutagenesis, according to Kamada and Koizumi (2007), was used to produce a protein with the desired activity but whose amino acid sequence has at least 1 amino acid that was deleted, substituted, or added. No unspecified DNA is expected to be associated with the transfer of the genes, as the DNA inserts are well-characterized and confirmed to consist of the desired sequences only. Furthermore, the expression products have well-defined functions in the biosynthesis of 6'-SL and are not associated with any potential toxicity or pathogenic traits of the donor organism.

Host modifications also include the deletion of 8 gene sequences, which serve as insertion loci for the inserted gene products described above.

2.2.1.3 Selection of Final Strain

Selection of the final *E. coli* W production strain is achieved *via* negative selection using the *Bacillus subtilis sacB* gene coding for levansucrase as a counter-selectable marker (Mizoguchi *et al*., 2007). The enzyme catalyzes the hydrolysis of sucrose and synthesis of high-molecular weight fructose polymers called levans (Gay *et al.*, 1983). When the *sacB* gene is expressed in *E. coli*, the strain cannot grow in the presence of sucrose.

A marker cassette containing the *sacB* gene and the *cat* gene, an antibiotic resistance gene that encodes chloramphenicol acetyl transferase and confers chloramphenicol resistance, is inserted into the *E. coli* W strains for the construction of the production strain by homologous recombination following the deletion of the target region using the lamba Red recombinase system. Desired host modifications are then introduced to the *E. coli* W strain in a step-wise manner for the construction of the production strain. Cells expressing the desired genetic traits are selected using the antibiotic resistance marker. The marker cassette is then removed using the lamba Red recombinase system, and cells are plated with sucrose. Cells able to grow in the presence of sucrose are selected as the final strains (as cells containing the *sacB* gene cannot survive in the presence of sucrose). In this manner, the strains containing the desired genetic modifications but lacking the antibiotic resistance gene (which is present in the marker cassette with the *sacB* gene) are selected, as the antibiotic resistance gene cannot be present in order for the cell to survive in the presence of sucrose. After the strains have been selected, PCR at the recombination point is used to verify that all desired genetic modifications have been incorporated.

2.2.1.4 Final Production Strain

The final 6'-SL production strain is non-pathogenic and non-toxigenic and has the same virulence profile as the host organism, as all genetic modifications are well-characterized, confirmed to consist of the desired sequences only, have a well-defined function in the biosynthesis of 6'-SL, and are not associated with any potential toxicity or pathogenic traits of the donor organism. The final 6'-SL production strain is not capable of DNA transfer to other organisms. Therefore, the use of the 6'-SL production strain in the manufacture of Kyowa's 6'-SL sodium salt is not expected to result in any safety concerns.

2.2.2 Fermentation Media Components, Processing Aids, and Raw Materials

The fermentation media used for culturing the genetically modified strain of *E. coli* W contains nutrient sources and ingredients that are commonly used in microbial growth media. Fermentation media components include ammonia-based salts as a nitrogen source, and vitamins, amino acids, essential mineral mix, trace elements, and yeast extract as sources of nutrients to promote growth.

All additives, processing aids, and food contact materials used in the manufacturing process are food-grade quality or of a higher standard and are used in accordance with an applicable federal regulation, previous conclusion of GRAS status, or have been the subject of an effective food contact notification and are used consistent with current Good Manufacturing Practice (cGMP) requirements. Glucose and lactose are the only carbon sources added to the fermentation medium during the fermentation process. Lactose monohydrate used as a carbon source for the production of 6'-SL by fermentation is derived from cow's milk, which is a major food allergen; however, Kyowa's purification processes (see Section 2.2.3) are effective in the removal of residual proteins and no milk proteins were detected in Kyowa's final 6'-SL ingredient, as described in Section 6.6.

2.2.3 Manufacturing Process

The manufacturing process for Kyowa's 6'-SL sodium salt is controlled by a Hazard Analysis Critical Control Points (HACCP) plan and is conducted in accordance with cGMP as established by 21 CFR §117 (U.S. FDA, 2020d). The production of 6'-SL by fermentation with a genetically modified strain of *E. coli* W involves 2 main steps: fermentation and purification. Each of the 2 steps is briefly described below, along with a schematic overview of the fermentation and purification processes (see Figure 2.2.3.2-1).

2.2.3.1 Fermentation Process

The fermentation processes for the production of 6'-SL are conducted in chemically defined nutrient media under sterile conditions. A master frozen cell bank is prepared for the production strain. Cells from the master cell bank are inoculated to produce the working frozen cell bank. The genetic stability from a minimum of 3 cell passages from the master and working cell bank is verified based on 6'-SL production, cell growth, oxygen consumption, and other functional parameters indicating a change in cell culture behavior.

Cells from the working cell bank are then inoculated to produce the flask seed culture. Cells are cultured in the flask seed medium and then transferred to the factory seed medium and cultured. The process conditions are tightly controlled (*e.g.*, time, temperature, pH, and feeding rate). The seed culture step is complete when a specific optical density is reached.

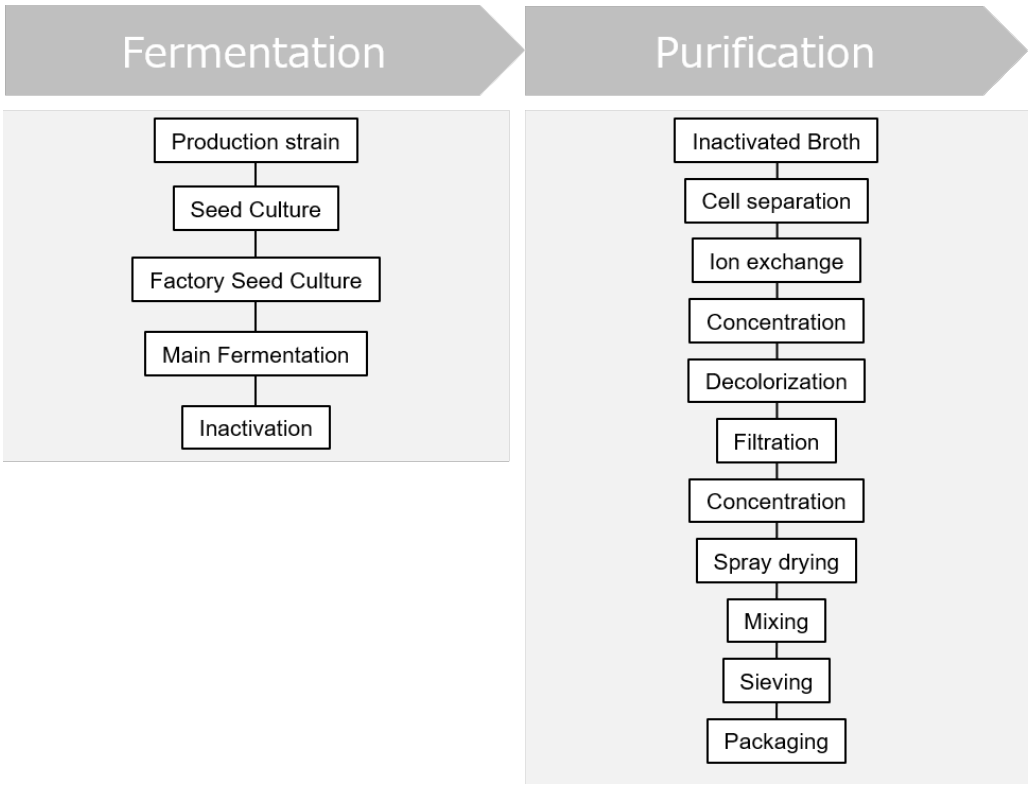
In the main fermentation, the medium is first inoculated with factory seed cultures and fermented in the presence of glucose. Following the depletion of glucose in the culture medium, lactose and glucose are fed to the culture medium. The main fermentation is maintained at a constant temperature until the completion of feeding. During the feeding step, the production strain takes up the lactose and glucose for the synthesis of 6'-SL, which is excreted into the media. As with the initial fermentations, the process conditions of the main fermentation are tightly controlled (*e.g.*, time, temperature, pH, and feeding rate). The production of 6'-SL is terminated *via* heat treatment (sterilization), after which the broth is cooled and acidified.

2.2.3.2 Purification Processes

The intact cells are removed *via* microfiltration. The obtained solution is then passed through a series of cationic resin and anionic resin ion exchangers to remove cations, anions, minerals, and organic impurities. The pH of the effluent is adjusted, and the concentrated solution is decolorized with activated carbon and the pH is adjusted again. The solution is then filtered using an ultra-filtration membrane to remove endotoxins, as well as any residual protein, organic impurities, and production organisms not removed by the cationic/anionic exchange resins. The obtained solution is concentrated, filtered, spray-dried, homogenized, and then passed through a sieve to remove foreign materials to obtain the final 6'-SL sodium salt product.

A schematic overview of the fermentation and purification steps is provided in Figure 2.2.3.2-1.

Figure 2.2.3.2-1 Schematic Overview of the Fermentation and Purification Processes of 6'-Sialyllactose Sodium Salt Produced Using a Genetically Modified Strain of *Escherichia coli* W



2.3 Product Specifications

2.3.1 Chemical Specifications

Food-grade chemical specifications have been established for the 6'-SL sodium salt produced with a genetically modified strain of *E. coli* W and are presented in Table 2.3.1-1.

Kyowa has established qualitative and quantitative limits for the 6'-SL sodium salt to confirm identity and purity. Kyowa's final product is a white to off-white powder with a purity of at least 82% 6'-SL as determined by an in-house validated method [high-performance liquid chromatography with charged aerosol detection (HPLC-CAD)]. Kyowa also has established limits for potential impurities of the production process, including NeuAc (*N*-Acetyl D-neuraminic acid) (≤9%), 6'-sialyllactulose (≤5%), and 3'-SL sodium salt (≤1%) determined by HPLC-CAD, and D-glucose (≤3%) and D-lactose (≤3%) determined an in-house validated by high-performance liquid chromatography with pulsed aerosol detection (HPLC-PAD) method. *N*-acetyl D-neuraminic acid, lactose, and 3'-SL are naturally-occurring components of human milk, while glucose is a naturally-occurring breakdown product of lactose, a common dietary component, and serves as a starting material for the biosynthesis of 6'-SL. 6'-sialyllactulose is an isomerization product of 6'-SL formed when the

terminal glucose moiety isomerizes into fructose (EFSA, 2020a). In addition, residual proteins are specified to be ≤10 mg/kg (determined using a dot-blot method).

The specified limit for sodium in the final 6'-SL sodium salt is ≤5.0% on a dry weight basis (dwb) (as determined by the compendial method specified in the United States Pharmacopeia, section 233) and water content is ≤10.5 w/w% as determined by Karl-Fischer titration (as specified in the Japanese Pharmacopoeia, 17th Edition, Section 2.48). The ash component of the final product is expected to be fully accounted for by the sodium content, and as such, a specification for ash was not established. The final product is specified to have a pH between 4.0 and 9.0 when analyzed in 5% solution at 20°C.

The specification limits for lead, arsenic, cadmium, and mercury of ≤0.2 mg/kg (individually) and the specification limit for iron of ≤10 mg/kg in the final product are in accordance with the requirements for a food-grade quality ingredient and are similar to the limits for heavy metals in other HMO ingredients that have been concluded to be GRAS (see Section 6.1).

Methods of analysis used by Kyowa were obtained from the United States or Japanese Pharmacopeia or were developed in-house. Methods obtained from the United States and Japanese Pharmacopeia are validated for their intended uses. Kyowa uses validated internal HPLC-CAD and HPLC-PAD methods for the identification and quantification of the carbohydrate components. Residual protein is assessed using an internal dot-blot method that has been developed and concluded to be suitable for its intended use by Kyowa.

Table 2.3.1-1 Chemical Specifications for 6'-Sialyllactose Sodium Salt Produced with a Genetically Modified Strain of *Escherichia coli* W

Specification Parameter	Specification	Method
Organoleptic		
Appearance	Powder	Visual observation
Color	White to off-white	General Notice, JP 17[a]
Physicochemical		
Identification	RT of standard ± 3%	HPLC-CAD (internal method)
Purity (6'-SL)	≥82% dry basis	HPLC-CAD (internal method)
Water	≤10.5 w/w%	JP 2.48[a]
Sodium (Assay)	≤5.0% dry basis	USP 233[b]
Residual protein	≤10 mg/kg	Dot-blot (internal method)
pH (20°C, 5% solution)	4.0 to 9.0	JP 2.54[a]
Other Carbohydrates		
N-Acetyl D-neuraminic acid	≤9 w/w%	HPLC-CAD (internal method)
D-glucose	≤3 w/w%	HPLC-PAD (internal method)
D-lactose	≤3 w/w%	HPLC-PAD (internal method)
6'-Sialyllactulose	≤5 w/w%	HPLC-CAD (internal method)
3'-Sialyllactose sodium salt	≤1 w/w%	HPLC-CAD (internal method)
Heavy Metals		
Arsenic	≤0.2 mg/kg	USP 233[b]
Cadmium	≤0.2 mg/kg	USP 233[b]
Lead	≤0.2 mg/kg	USP 233[b]
Mercury	≤0.2 mg/kg	USP 233[b]

Table 2.3.1-1 Chemical Specifications for 6'-Sialyllactose Sodium Salt Produced with a Genetically Modified Strain of *Escherichia coli* W

Specification Parameter	Specification	Method
Iron	≤10 mg/kg	USP 233[b]

6'-SL = 6'-sialyllactose; HPLC-CAD = high-performance liquid chromatography coupled with charged aerosol detection; HPLC-PAD = high-performance liquid chromatography coupled with pulsed amperometric detection; JP = Japanese Pharmacopeia; RT = retention time; USP = United States Pharmacopeia.

[a] Method is consistent with the compendial method specified in 17th edition of the Japanese Pharmacopeia (2016).

[b] Method is consistent with the compendial method specified in the United States Pharmacopeia 35th revision (2011).

2.3.2 Microbiological Specifications

Kyowa has established food-grade limits for standard microbial parameters, such as aerobic plate count, molds, and yeasts, as well as limits for a comprehensive list of potential pathogenic organisms, including *Salmonella* spp., *Enterobacteriaceae*, *Cronobacter* spp., *Listeria monocytogenes,* and *Bacillus cereus*. Microbial parameters are analyzed using standards from the International Organization for Standardization (ISO). Kyowa also has established a limit of ≤10 EU/mg (determined with Section 4.01, kinetic-turbidimetric method, of the Japanese Pharmacopoeia, 17th Edition) for residual endotoxins at to ensure that there is no potential contamination from the production organism. All methods are validated for their intended uses. The microbiological specifications for 6'-SL sodium salt are presented in Table 2.3.2-1.

Table 2.3.2-1 Microbiological Specifications for 6'-Sialyllactose Sodium Salt Produced with a Genetically Modified Strain of *Escherichia coli* W

Specification Parameter	Specification	Method
Aerobic plate count	≤1,000 CFU/g	ISO 4833-1:2013
Molds	≤100 CFU/g	ISO 21527-2:2008
Yeasts	≤100 CFU/g	ISO 21527-2:2008
Salmonella[a]	Negative in 100 g	ISO 6579-1:2017
Enterobacteriaceae	Negative in 10 g	ISO 21528-1:2017
Cronobacter spp.[b] *(Enterobacter sakazakii)*	Negative in 100 g	ISO 22964:2017
Listeria monocytogenes	Negative in 25 g	ISO 11290-1:2017
Bacillus cereus	≤50 CFU/g	ISO 7932:2004
Residual endotoxins	≤10 EU/mg	JP 4.01 (kinetic-turbidimetric method)[c]

CFU = colony-forming units; EU = endotoxin units; ISO = International Organization for Standardization; JP = Japanese Pharmacopeia.

[a] Four individual samples of 25 g are analyzed as per the validated method. All 4 samples must be negative to meet the specification limit.

[b] Ten individual samples of 10 g are analyzed as per the validated method. All 10 samples must be negative to meet the specification limit.

[c] Method is consistent with the compendial method specified in 17th edition of the Japanese Pharmacopeia (2016).

2.3.3 Product Analysis

2.3.3.1 Chemical Analysis of 6'-SL Sodium Salt

Analysis of 5 representative lots of 6'-SL sodium salt (3 of which were non-consecutive) manufactured by fermentation using a genetically modified strain of *E. coli* W (Lots A, B, C, D, and E) demonstrates that the manufacturing process as described in Section 2.2.3 produces a consistent product that meets specifications. Across all 5 lots, the purity ranged between 87 to 92% dwb, with low levels (≤5.4% w/w) of NeuAc, trace levels of D-Lactose and 6'-sialyllactulose (≤0.5% w/w), and no 3'-SL sodium salt or residual D-glucose detected. A summary of the chemical analysis for the 5 lots of 6'-SL sodium salt is presented in Table 2.3.3.1-1.

Table 2.3.3.1-1 Summary of Batch Analyses for the Final 6'-Sialyllactose Sodium Salt Powdered Ingredient Produced with a Genetically Modified Strain of *Escherichia coli* W

Specification Parameter	Specification	Methods of Analysis	Manufacturing Lot				
			A	B	C	D	E
Properties							
Appearance	Powder	Visual observation	Complies	Complies	Complies	Complies	Complies
Color	White to off-white	JP 17; General Notice[a]	Complies	Complies	Complies	Complies	Complies
Identification	RT of standard ± 3%	HPLC-CAD (internal method)	Complies	Complies	Complies	Complies	Complies
Purity	≥82% dry basis	HPLC-CAD (internal method)	87	92	90	92	92
Purity as free acid	Not established[b]	By calculation[c]	84.08	88.92	86.98	88.92	88.92
Water	≤10.5 w/w%	JP 2.48[a]	5.3	5.0	5.4	5.6	5.0
Sodium	≤5.0% dry basis	USP 233[d]	3.8	3.8	3.8	3.7	3.8
pH (20˚C, 5% solution)	4.0 to 9.0	JP 2.54[a]	6.4	6.5	6.5	6.5	6.5
Residual proteins	≤10 mg/kg	Dot-blot (internal method)	≤1	≤1	≤1	≤1	≤1
Other Carbohydrates							
NeuAc	≤9% w/w	HPLC-CAD (internal method)[e]	5.1	3.5	4.9	4.3	5.4
D-Glucose	≤3% w/w	HPLC-PAD (internal method)[f]	ND	ND	ND	ND	ND
D-Lactose	≤3% w/w	HPLC-PAD (internal method)[f]	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05
6'-Sialyllactulose	≤5% w/w	HPLC-CAD (internal method)[e]	0.4	0.4	0.5	0.5	0.4
3'-Sialyllactose sodium salt	≤1% w/w	HPLC-CAD (internal method)[e]	ND	ND	ND	ND	ND
Mass balance	NA	By calculation[g]	93.5	96.7	96.3	97.5	98.6

Table 2.3.3.1-1 Summary of Batch Analyses for the Final 6'-Sialyllactose Sodium Salt Powdered Ingredient Produced with a Genetically Modified Strain of *Escherichia coli* W

Specification Parameter	Specification	Methods of Analysis	Manufacturing Lot				
			A	B	C	D	E
Heavy Metals							
Arsenic	≤0.2 mg/kg	USP 233[d,h]	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05
Cadmium	≤0.2 mg/kg	USP 233[d,h]	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05
Lead	≤0.2 mg/kg	USP 233[d,h]	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05
Mercury	≤0.2 mg/kg	USP 233[d,h]	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05
Iron	≤10 mg/kg	USP 233[d,h]	0.3	0.2	0.3	0.3	0.6

HPLC-CAD = high-performance liquid chromatography coupled with charged aerosol detection; HPLC-PAD = high-performance liquid chromatography coupled with pulsed amperometric detection; JP = Japanese Pharmacopeia; LOD = limit of detection; LOQ = limit of quantification; NA = not applicable; ND = not detected; RT = retention time; USP = United States Pharmacopeia.

[a] Method is consistent with the compendial method specified in 17th edition of the Japanese Pharmacopeia (2016).

[b] No specification limit established as purity as free acid was calculated for the purposes of calculating mass balance.

[c] Purity as free acid was calculated as Purity * Mw 6'-SL (633.55)/Mw 6'-SL Na (655.53).

[d] Method is consistent with the compendial method specified in the United States Pharmacopeia 35th revision (2011).

[e] LOD for NeuAc, 6'-Sialyllactulose, and 3'-sialyllactose sodium salt is 0.01 w/w% and LOQ for NeuAc, 6'-Sialyllactulose, and 3'-sialyllactose sodium salt is 0.2 w/w% as 6'-Sialyllactose sodium salt.

[f] LOD for D-glucose and D-lactose is 0.02 w/w% and LOQ for D-glucose and D-lactose is 0.05 w/w% as D-lactose.

[g] Mass balance = sum of purity as free acid, sodium, NeuAc, D-glucose, D-lactose, 6'-sialyllactulose, 3'-sialyllactose sodium salt. Results that were ND were replaced with the respective LOD values. Results that were ≤ LOQ were replaced with the LOQ values.

[h] LOQ for heavy metals (*i.e.*, arsenic, cadmium, lead, and mercury) is 0.05 mg/kg.

2.3.3.2 Microbiological Analysis

Analysis of the same 5 representative lots of 6'-SL sodium salt (Lots A, B, C, D, and E) demonstrates that the product meets the microbiological specifications outlined in Section 2.3.2. A summary of the results of the microbiological analyses for the 5 lots of 6'-SL sodium salt is presented in Table 2.3.3.2-1.

Table 2.3.3.2-1 Summary of the Microbiological Product Analysis for 5 Lots of 6'-Sialyllactose Sodium Salt

Parameter	Specification	Methods of Analysis	Manufacturing Lot				
			A	B	C	D	E
Aerobic plate count	≤1,000 CFU/g	ISO 4833-1:2013[a]	<10	<10	<10	<10	<10
Molds	≤100 CFU/g	ISO 21527-2:2008[b]	<100	<100	<100	<100	<100
Yeasts	≤100 CFU/g	ISO 21527-2:2008[b]	<100	<100	<100	<100	<100
Salmonella[c]	Negative in 100 g	ISO 6579-1:2017[d]	Negative	Negative	Negative	Negative	Negative
Enterobacteriaceae	Negative in 10 g	ISO 21528-1:2017[e]	Negative	Negative	Negative	Negative	Negative
Cronobacter spp.[f] *(Enterobacter sakazakii)*	Negative in 100 g	ISO 22964:2017[d]	Negative	Negative	Negative	Negative	Negative
Listeria monocytogenes	Negative in 25 g	ISO 11290-1:2017[g]	Negative	Negative	Negative	Negative	Negative
Bacillus cereus	≤50 CFU/g	ISO 7932:2004[h]	<10	<10	<10	<10	<10
Residual endotoxins	≤10 EU/mg	JP17; JP 4.01 (kinetic-turbidimetric method)[i]	0.006	0.011	0.020	0.034	0.026

CFU = colony-forming units; EU = endotoxin units; ISO = International Organization for Standardization; JP = Japanese Pharmacopeia; LOD = limit of detection.
[a] LOD = 10 CFU/g
[b] LOD = 100 CFU/g
[c] Four individual samples of 25 g are analyzed as per the validated method. All 4 samples must be negative to meet the specification limit.
[d] Qualitative test to confirm "absent in 100 g".
[e] Qualitative test to confirm "absent in 10 g".
[f] Ten individual samples of 10 g are analyzed as per the validated method. All 10 samples must be negative to meet the specification limit.
[g] Qualitative test to confirm "absent in 25 g".
[h] LOD = 10 CFU/g.
[i] Method is consistent with the compendial method specified in 17th edition of the Japanese Pharmacopeia (2016).

2.3.3.3 Additional Chemical Characterization

2.3.3.3.1 Absence of Production Organism and DNA

As indicated in Section 2.2.3.2, the production organism is removed during the purification processes of the manufacturing process by a combination of microfiltration, filtration through cationic and anionic exchange resins, and ultra-filtration. The absence of the production organism in the final 6'-SL sodium salt ingredient is further demonstrated by microbial testing for *Enterobacteriaceae* in microbiological batch analyses according to internationally recognized methods (ISO 21528-1:2017) (see Table 2.3.3.2-1).

In addition, Kyowa's final 6'-SL sodium salt ingredient was assessed for residual production organism using a culture method conducted in accordance with European Food Safety Authority's (EFSA's) *Guidance on the characterization of microorganisms used as feed additives or as production organisms* (EFSA, 2018). Briefly, 3 lots of 6'-SL sodium salt produced with a genetically modified strain of *E. coli* W (Lots A, C, and E) were cultured in triplicate in Luria-Bertani (LB) medium at 30°C for 44 hours. A polymerase chain reaction (PCR) analysis was then conducted using primers specific to the production organism. The production organism cultured in LB medium at 30°C overnight was obtained and diluted, inoculated with sample solution, and subsequently cultured at 30°C for 44 hours and used as a positive control. The results of this test demonstrated that the primers used were appropriate for the detection of the production organism, and that the production organism was absent from the final 6'-SL sodium salt ingredient.

To confirm the absence of residual production organism-derived DNA in the final product, Kyowa conducted a quantitative PCR analysis using 3 lots of 6'-SL sodium salt produced using a genetically modified strain of *E. coli* W (Lots A, C, and E; assayed in triplicate). The analysis was conducted in accordance with EFSA's *Guidance on the characterization of microorganisms used as feed additives or as production organisms* (EFSA, 2018). The quantitative PCR assay was conducted using primers specific to the production organism, with DNA extracted from the production organism used as a positive control. No residual DNA was detected (limit of quantification of 4 µg/kg or 4 ppb) in the final 6'-SL sodium salt ingredient.

2.3.3.3.2 Solubility

Kyowa has conducted a solubility test on the final 6'-SL sodium salt powdered product (Lot A) in accordance with Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 105 (Flask Method) (OECD, 1995). The results of this study demonstrate that Kyowa's final 6'-SL sodium salt ingredient has a water solubility of 888 g/L. Given its high solubility in water, no safety concerns related to the particle size of the 6'-SL sodium salt ingredient are expected.

2.4 Stability of 6'-SL Sodium Salt

Kyowa has investigated the stability of the 6'-SL sodium salt ingredient under accelerated storage conditions (see Section 2.4.1) and normal storage conditions (see Section 2.4.2) to assess the physicochemical and biochemical stability of the ingredient and also to investigate the potential degradation products. Microbiological stability of the 6'-SL sodium salt final ingredient has been addressed through the investigation of water activity (see Section 2.4.3), and stability in final food matrices has been assessed using publicly available information on other 6'-SL sodium salt final ingredients, the structural isomer 3'-SL, and other HMO preparations (see Section 2.4.4).

2.4.1 Accelerated Storage Conditions

Kyowa conducted a study to assess the physicochemical and biochemical bulk stability of 3 independently produced representative lots of 6'-SL sodium salt produced using a genetically modified strain of *E. coli* W (Lots A, C, and E) under accelerated conditions (temperature of 40 ± 2°C; 75 ± 5% relative humidity) over a 6-month period. Kyowa's 6'-SL sodium salt ingredient was stored in polyethylene bags within an aluminum chuck bag, which are similar packaging materials to those intended for storage and distribution of the commercial product. The results are shown in Table 2.4.1-1. 6'-SL sodium salt was stable and remained within specification limits throughout the 6-month storage period with no significant change in physicochemical parameters (appearance, color, pH, water activity) or biochemical parameters (purity, carbohydrate profile, and water content). A slight increase in the isomerization product 6'-sialyllactulose was observed across all lots, with a maximum observed level of 1.2% of the 6'-SL sodium salt ingredient, which is well below the specification limit of 5%. Assuming a worst-case level of 5% 6'-sialyllactulose in the 6'-SL sodium salt ingredient (based on the specification limit) would result in a worst-case daily intake of 9.5 mg/kg body weight/day in infants 7 to <12 months of age (highest 90th percentile intake of 6'-SL sodium salt of 190 mg/kg body weight/day; see Section 3.4). This level of intake is substantially lower than the level of lactulose that is reported to be recommended to treat constipation in infants and assumed to have laxative effects (*i.e.*, 1.65 g/day) (EFSA, 2020a). The content of lactulose in commercially available infant formulas has been reported to be 1 to 7% of the lactose content (Beach and Menzies, 1983), while heat-treated human milk also has been reported to contain lactulose at a significant proportion of the content of lactose (Gomez de Segura *et al.*, 2012). Since the formation of 6'-sialyllactulose and lactulose result from a similar isomerization process (*i.e.*, pH- and temperature-dependent isomerization of the terminal glucose to fructose), it is expected that 6'-sialyllactulose would be present at a similar ratio to 6'-SL as the contents of lactulose to lactose in heat-treated human milk (Beach and Menzies, 1983; Schuster-Wolff-Bühring *et al.*, 2010; Gómez de Segura *et al.*, 2012). It is therefore expected that 6'-sialyllactulose has a history of safe consumption as a component of heat-treated human milk, and as such, there are no safety concerns with the low levels of 6'-sialyllactulose in the 6'-SL sodium salt ingredient. The results of the accelerated stability study support a shelf-life of 3 years.

Table 2.4.1-1 Summary of Accelerated Stability Testing (40 ± 2°C; 75 ± 5% Relative Humidity) for 6'-Sialyllactose Sodium Salt Produced with a Genetically Modified Strain of *Escherichia coli* W

Parameter	Specification	Storage Time (months)			
		0	2	4	6
Lot A					
Appearance	Powder	Complies	Complies	Complies	Complies
Color	White to off-white	Complies	Complies	Complies	Complies
Purity	≥82% dry basis	87	89	90	90
Water	≤10.5 w/w%	5.3	5.3	5.1	5.1
Water Activity (Aw)	NA	0.17	-	-	0.16
Sodium	≤5.0% dry basis	3.8	3.7	3.8	3.8
pH (20°C; 5% solution)	4.0 to 9.0	6.4	6.2	6.1	6.2
NeuAc	≤9 w/w%	5.1	5.4	5.2	4.9
D-Glucose	≤3 w/w%	ND[a]	ND[a]	ND[a]	ND[a]
D-Lactose	≤3 w/w%	≤0.05[b]	≤0.05[b]	≤0.05[b]	≤0.05[b]
6'-Sialyllactulose	≤5 w/w%	0.4	0.7	0.8	1.0

Table 2.4.1-1 Summary of Accelerated Stability Testing (40 ± 2°C; 75 ± 5% Relative Humidity) for 6'-Sialyllactose Sodium Salt Produced with a Genetically Modified Strain of *Escherichia coli* W

Parameter	Specification	Storage Time (months)			
		0	2	4	6
3'-Sialyllactose sodium salt	≤1 w/w%	ND[c]	ND[c]	ND[c]	ND[c]
Lot C					
Appearance	Powder	Complies	Complies	Complies	Complies
Color	White to off-white	Complies	Complies	Complies	Complies
Purity	≥82% dry basis	90	92	90	91
Water	≤10.5 w/w%	5.4	5.2	5.1	5.1
Water Activity (Aw)	NA	0.14	-	-	0.14
Sodium	≤5.0% dry basis	3.8	3.6	3.7	3.7
pH (20°C; 5% solution)	4.0 to 9.0	6.5	6.4	6.2	6.1
NeuAc	≤9 w/w%	4.9	5.2	5.4	5.0
D-Glucose	≤3 w/w%	ND[a]	ND[a]	ND[a]	ND[a]
D-Lactose	≤3 w/w%	≤0.05[b]	≤0.05[b]	≤0.05[b]	≤0.05[b]
6'-Sialyllactulose	≤5 w/w%	0.5	0.7	1.0	1.2
3'-Sialyllactose sodium salt	≤1 w/w%	ND[c]	ND[c]	ND[c]	ND[c]
Lot E					
Appearance	Powder	Complies	Complies	Complies	Complies
Color	White to off-white	Complies	Complies	Complies	Complies
Purity	≥82% dry basis	92	90	91	91
Water	≤10.5 w/w%	5.0	5.0	4.8	4.9
Water Activity (Aw)	NA	0.14	-	-	0.12
Sodium	≤5.0% dry basis	3.8	3.6	3.8	3.8
pH (20°C; 5% solution)	4.0 to 9.0	6.5	6.4	6.2	6.2
NeuAc	≤9 w/w%	5.4	5.6	5.2	5.0
D-Glucose	≤3 w/w%	ND[a]	ND[a]	ND[a]	ND[a]
D-Lactose	≤3 w/w%	≤0.05[b]	≤0.05[b]	≤0.05[b]	≤0.05[b]
6'-Sialyllactulose	≤5 w/w%	0.4	0.7	0.9	1.2
3'-Sialyllactose sodium salt	≤1 w/w%	ND[c]	ND[c]	ND[c]	ND[c]

- = not analyzed or planned for analysis; 6'-SL = 6'-sialyllactose; LOD = limit of detection; LOQ = limit of quantification; NA = not applicable; ND = not detected; NeuAc = *N*-Acetyl D-neuraminic acid.

[a] LOD for D-glucose is 0.02 w/w% as D-lactose.

[b] LOQ for D-glucose and D-lactose is 0.05 w/w% as D-lactose.

[c] LOD for 3'-sialyllactose sodium salt is 0.03 w/w% as 6'-sialyllactose sodium salt.

2.4.2 Normal Storage Conditions

The recommended storage conditions for 6'-SL sodium salt are at room temperature. A real-time study to assess the physicochemical and biochemical stability of a representative lot of 6'-SL sodium salt produced using a genetically modified strain of *E. coli* W (Lot C) under standard room temperature conditions (25 ± 2°C; 60 ± 5% relative humidity) is ongoing. The 6'-SL sodium salt ingredient was stored in polyethylene bags within an aluminum chuck bag, which are similar packaging materials to those intended for storage and distribution of the commercial product. The duration of the study is planned to be 36 months (*i.e.*, the proposed shelf-life for the 6'-SL sodium salt), with analyses planned at 0, 2, 4, 6, 9, 12, 18, 24, 30, and 36 months. Results are available up to 12 months (see Table 2.4.2-1). The interim results demonstrate that 6'-SL sodium salt was stable throughout the first 12 months of storage and remained within specification limits, with no significant change in physicochemical (appearance, color, pH, water activity) or biochemical (purity, carbohydrate profile, and water content) parameters.

Table 2.4.2-1 Summary of Stability Testing of 1 Lot of 6'-Sialyllactose Sodium Salt (Lot C) Produced Using a Genetically Modified Strain of *Escherichia coli* W Under Standard Conditions (25 ± 2°C; 60 ± 5% Relative Humidity)

Specification Parameter	Specification	Storage Time (months)					
		0	2	4	6	9	12
Appearance	Powder	Complies	Complies	Complies	Complies	Complies	Complies
Color	White to off-white	Complies	Complies	Complies	Complies	Complies	Complies
Purity	≥82% dry basis	90	92	93	93	90	92
Water	≤10.5 w/w%	5.4	5.2	5.0	5.2	5.1	5.4
Water Activity (Aw)	NA	0.14	-	-	0.14	-	-
Sodium	≤5.0% dry basis	3.8	3.8	3.7	3.7	3.7	3.7
pH (20°C; 5% solution)	4.0 to 9.0	6.5	6.5	6.3	6.4	6.3	6.3
N-Acetyl D-neuraminic acid	≤9 w/w%	4.9	5.3	4.9	4.9	4.8	4.7
D-Glucose	≤3 w/w%	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
D-Lactose	≤3 w/w%	≤0.05[b]	≤0.05[b]	≤0.05[b]	≤0.05[b]	≤0.05[b]	≤0.05[b]
6'-Sialyllactulose	≤5 w/w%	0.5	0.4	0.5	0.6	0.6	0.7
3'-Sialyllactose sodium salt	≤1 w/w%	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]

- = not analyzed or planned for analysis; LOD = limit of detection; LOQ = limit of quantification; NA = not applicable; ND = not detected.

[a] LOD for D-glucose is 0.02 w/w% as D-lactose.

[b] LOQ for D-lactose is 0.05 w/w%.

[c] LOD for 3'-sialyllactose sodium salt is 0.03 w/w% as 6'-sialyllactose sodium salt.

2.4.3 Microbiological Stability

It has been noted that microbial survival and growth in composite products and foods in general is affected by factors including low water activity, whereby, in general, foods with measured water activity of <0.88 prevent the growth and formation of toxins by food-borne pathogenic bacteria (EFSA, 2012). Kyowa therefore measured the water activity of 6'-SL sodium salt after 0 and 6 months of storage under accelerated conditions (40 ± 2°C; 75 ± 5% relative humidity), and after 0 and 6 months of storage under standard conditions (25 ± 2°C; 60 ± 5% relative humidity). Additional analyses are planned at 18, 24, 30, and 36 months of storage under standard conditions. As shown above in Tables 2.4.1-1 and 2.4.2-1, the water activity of 6'-SL sodium salt was considerably lower than 0.88 at all time-points of evaluation and conditions of storage, with values not exceeding 0.17. The low water content of the analyzed lots of 6'-SL sodium salt indicate also that the storage packaging prevents water absorption by the 6'-SL sodium salt ingredient. Based on the low water content and water activity values, microbial growth or toxin formation in Kyowa's 6'-SL sodium salt ingredient is unlikely.

2.4.4 Stability in Intended Food Uses

Kyowa's 6'-SL has been demonstrated to be chemically and structurally equivalent to 6'-SL from bovine milk or colostrum (see Section 2.1), which has been demonstrated to be structurally and chemically identical to 6'-SL in human milk (Aldredge *et al.*, 2013). On this basis, stability data on other 6'-SL ingredients that have been demonstrated to be structurally and chemically identical to 6'-SL in human or bovine milk or colostrum are relevant to the stability of Kyowa's 6'-SL ingredient. Furthermore, 6'-SL is a structural isomer to 3'-SL, with the only difference being the position of the connection of sialic acid to the galactose unit, with sialic acid attached at the 6 position in 6'-SL and attached at the 3 position in 3'-SL (Jacobi *et al*., 2016). On the basis that 6'-SL and 3'-SL are structural isomers with no differences expected in degradation based on the position of the sialic acid attachment, stability data on other 3'-SL ingredients that have been demonstrated to be structurally and chemically identical to 3'-SL in human or bovine milk or colostrum are relevant to the stability of Kyowa's 6'-SL ingredient.

6'-SL sodium salt manufactured by Glycom A/S (Glycom) has been demonstrated to be chemically and structurally identical to 6'-SL that is naturally present in human breast milk (Glycom A/S, 2019a – GRN 881). Data supporting the stability of Glycom's 6'-SL sodium salt ingredient was reported in GRN 881 and is incorporated herein by reference (Glycom A/S, 2019a – GRN 881, Section 2.4.2.1, pages 26 to 27). Glycom's 6'-SL sodium salt ingredient was stable in a commercially representative whey-based infant formula powder for up to 12 months when stored at temperatures of 4, 20, 30, and 37°C. On the basis that Kyowa's 6'-SL is structurally and chemically identical to Glycom's 6'-SL, these results support the stability of Kyowa's 6'-SL sodium salt in powdered infant formula when stored under the same conditions.

GeneChem Inc.'s (GeneChem's) 3'-SL was demonstrated to be structurally and chemically identical to 3'-SL in bovine milk or colostrum (Sigma standard A8681) (GeneChem, Inc., 2018 – GRN 766). The stability of GeneChem's 3'-SL sodium salt ingredient stored in infant formula (powder) as well as other food matrices including milk and yoghurt has been reported in GRN 766 and is incorporated herein by reference (GeneChem, Inc., 2018 – GRN 766, Section 2.C.5.2, pages 35 to 37). The stability of the 3'-SL sodium salt ingredient in the relevant matrices was supported based on parameters including 3'-SL content (mg/L), appearance, and odor. When stored under room temperature conditions (25°C and 25% humidity), 3'-SL sodium salt incorporated into powdered infant formula was concluded to be stable for the full 24-month duration of the study on the basis of no changes in 3'-SL content, appearance, or odor. When incorporated into powdered infant formula and stored under accelerated conditions (40°C and 24% humidity), the 3'-SL

content, appearance, and odor were concluded to be stable for 18 months, with a change in color and decreased 3'-SL content reported at 24 months. When 3'-SL sodium salt was stored in commercial ready-to-drink milk at 4°C and 26% humidity or 25°C and 25% humidity, the 3'-SL content remained stable and the appearance and odor did not change over a period of 45 days. In a commercial yoghurt, following storage at 4°C and 26% humidity, the color and odor did not change, and the 3'-SL content decreased slowly over the 45-day storage period but remained within the target range of 80 to 120%. In contrast, when stored in yoghurt at 25°C and 26% humidity, the 3'-SL content decreased substantially, with the levels being out of target after 15 days. It was concluded that 3'-SL was less stable in yoghurt than in water or milk and it was proposed that microorganisms present in yoghurt digest the 3'-SL sodium salt (Yu *et al.*, 2013; GeneChem, 2018 – GRN 766). The results of the stability studies on GeneChem's 3'-SL sodium salt demonstrate that 3'-SL is stable in powdered infant formula stored at room temperature for 24 months, milk stored at 4 and 25°C for 45 days, and yoghurt stored at 4°C for 45 days. On the basis that Kyowa's 6'-SL is a structural isomer to 3'-SL and no differences in stability are expected between the isomers, these results support the stability of Kyowa's 6'-SL sodium salt in powdered infant formula, milk, and yoghurt when stored under the same conditions.

3'-SL sodium salt manufactured by Glycom A/S (Glycom) has been demonstrated to be chemically and structurally identical to 3'-SL that is naturally present in human breast milk (Glycom A/S, 2019b – GRN 880). Data supporting the stability of Glycom's 3'-SL sodium salt ingredient was reported in GRN 880 and is incorporated herein by reference (Glycom A/S, 2019b – GRN 880, Section 2.4.2.1, page 26). Glycom's 3'-SL sodium salt ingredient was stable in a commercially representative whey-based infant formula powder for up to 12 months when stored at temperatures of 4, 20, 30, and 37°C. On the basis that Kyowa's 6'-SL is a structural isomer to 3'-SL and no differences in stability are expected between the isomers, these results support the stability of Kyowa's 6'-SL sodium salt in powdered infant formula when stored under the same conditions.

Stability studies on other structurally and chemically related HMOs also are relevant to the stability of Kyowa's 6'-SL sodium salt ingredient on the basis of their related structures.

Data on the stability of 2'-fucosyllactose (2'-FL), a 2'-fucosyllactose/difucosyllactose (2'-FL/DFL) mixture, lacto-*N*-neotetraose (LNnT), and sialic acid in infant formula and follow-on formula, as well as other food matrices, have previously been evaluated by EFSA (EFSA, 2015a,b, 2017, 2019) and reviewed during previous GRAS evaluations (GRNs 546, 547, 602, 650, 815 – Glycom A/S, 2014a,b, 2015, 2016b, 2018). When the HMOs were assessed in infant formula and follow-on formula, 2'-FL and LNnT were demonstrated to be stable when stored at temperatures of 4, 20, 30, and 37°C over a period of 3 years, a mixture of 2'-FL/DFL was stable when stored at temperatures of 4, 20, 30, and 37°C for up to 6 months, and sialic acid was stable when stored at temperatures of 5, 25, 30, and 40°C for up to 360 days. When assessed in yoghurts, 2'-FL and LNnT were stable when stored at 4°C for 21 days. When assessed in ready-to-drink flavored milk, 2'-FL and LNnT were stable when stored at 4°C for 14 days (pasteurized) and 28 days [ultra-high temperature treated (UHT)]. When assessed in citrus fruit drinks, 2'-FL and LNnT were stable when stored at 4°C for 28 days. Sialic acid was also shown to be stable when stored in yogurts, ready-to-drink flavored milk, and citrus fruit drinks at 4°C over the shelf-life of the foods (duration not reported) and in cereal bars at ambient conditions for 3 months.

Following their review of the stability data on 2'-FL, 2'-FL/DFL mixture, LNnT, and sialic acid, in each case, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (previously the EFSA Panel on Dietetic Products, Nutrition and Allergies) (NDA Panel) concluded that "*the data provide sufficient information with respect to the stability of the NFI* [novel food ingredient]".

The results of the stability studies on other related HMOs such as 2'-FL, 2'-FL/DFL, LNnT, and sialic acid support the stability of 6'-SL in the evaluated food matrices [infant formula, follow-on formula, yogurts, ready-to-drink flavored milk (pasteurized or UHT), citrus fruit drinks, and cereal bars] when stored under the same conditions. The totality of stability data in food matrices on other 6'-SL sodium salt ingredients, 3'-SL sodium salt ingredients, and other related HMOs support the general stability of Kyowa's 6'-SL sodium salt ingredient under the proposed conditions of use.

Part 3. §170.235 Dietary Exposure

3.1 Current Regulatory Status and Safety Assessments for Other 6'-SL Preparations and Structurally Related HMOs

3.1.1 6'-SL

In the U.S., 6'-SL sodium salt has been the subject of 2 GRAS Notices to which the U.S. Food and Drug Administration (FDA) responded with no questions (GRNs 881 and 922 – Glycom A/S, 2019a; Jennewein Biotechnologie GmbH, 2020a; U.S. FDA, 2020e, 2021b). In GRN 881, the notifier describes the use of 6'-SL sodium salt (produced by fermentation using a genetically modified strain of *E. coli* K-12 DH1, ≥90% 6'-SL) in infant formulas (at levels up to 0.4 g/L), follow-on formula and other beverages for young children (0.3 g/L), in foods for young children (2.5 g/kg), foods and beverages for the general population, including yoghurt, buttermilk and fluid milk, cereal and granola bars, soft drinks, fruit-based drinks, sports drinks, "energy drinks," and enhanced waters (0.5 g/L or 5 g/kg), foods for special dietary use such as meal replacement drinks and bars (1 g/L or 10 g/kg) (GRN 881 – Glycom A/S, 2019a). These uses were estimated to result in intakes of up to 1,640 mg/person/day or 176 mg/kg body weight/day. In this GRAS Notice, the levels of 6'-SL sodium salt added to infant formulas and other foods and beverages were intended to result in intakes of 6'-SL comparable to those obtained from human milk.

In GRN 922, the notifier describes the use of 6'-SL sodium salt [produced by fermentation using a genetically modified strain of *E. coli* BL21 (DE3), ≥90% 6'-SL] as a substitute for other forms of 6'-SL in cow's milk-based, non-exempt infant formula for term infants at a level of 0.4 g/L. The notifier reported that since 6'-SL was intended as a substitute for other currently marketed 6'-SL ingredients for use at the same concentration as what was concluded to be GRAS in GRN 881, intakes would be the same as those in GRN 881 and those intakes were incorporated by reference.

The GRAS Notices and intended uses of 6'-SL sodium salt are summarized in Table 3.1.1-1.

In the EU, the EFSA NDA Panel concluded that the addition of 6'-SL sodium salt (produced by fermentation by a genetically modified strain of *E. coli* K-12 DH1, ≥90% 6'-SL) to a variety of foods (including infant and follow-on formula, foods for infants and toddlers, foods for special medical purposes, and food supplements) is safe under the proposed conditions of use for the proposed target populations (EFSA, 2020a). Intake of 6'-SL sodium salt resulting from the intended use in infant formula was estimated to be up to 104 mg/kg body weight/day, while intakes resulting from intended uses in other foods and food supplements were estimated to be 192 and 60 mg/kg body weight/day, respectively, for the general population (including infants). The EFSA NDA Panel noted that the estimated intakes of 6'-SL sodium salt are comparable to the high estimate of 6'-SL from human milk in infants. The use of 6'-SL sodium salt produced with a genetically modified strain of *E. coli* K-12 DH1 in the EU was authorized by the European Commission under Commission Implementing Regulation (EU) 2021/82 of 27 January 2021 (EU, 2021a).

Table 3.1.1-1 GRAS Notices for 6'-Sialyllactose Sodium Salt Submitted to the U.S. FDA

GRN Number	Applicant	Ingredient	Source	Purity	Intended Food Uses and Use Levels (g/kg or g/L)
881	Glycom A/S	6'-SL sodium salt	Fermentation (*Escherichia coli* K-12 "MAP425")	≥90 % on a dry matter basis	Intended for use as an ingredient at levels up to 0.4 g/L in non-exempt infant formula for term infants; 1 0.3 g/L in beverages and formula for young children (>12 months of age); 2.5 g/kg in foods for infants and young children; 5 g/kg in yogurt; 0.5 g/L in buttermilk and fluid milk (flavored and unflavored); 1 g/L in meal replacement drinks; 10 g/kg in meal replacement bars; 5 g/kg in cereal and granola bars; and 0.5 g/L in soft drinks, fruit-based drinks, sports drinks, energy drinks, and enhanced waters.
922	Jennewein Biotechnologie GmbH	6'-SL sodium salt	Fermentation [*E. coli* BL21 (DE3) strain DSM 33492]	≥90 % on a dry matter basis	Intended for use as an ingredient in cow milk-based, non-exempt infant formula for term infants at a level of 0.4 g/L.

6'-SL = 6'-sialyllactose; FDA = Food and Drug Administration; GRAS = Generally Recognized as Safe; GRN = GRAS Notice; U.S. = United States.

3.1.2 3'-SL

In the U.S., 3'-SL sodium salt has been the subject of 3 GRAS Notices to which the U.S. FDA responded with no questions (GRNs 766, 880, 921 – GeneChem, Inc., 2018; Glycom A/S, 2019b; Jennewein Biotechnologie GmbH, 2020b; U.S. FDA, 2018c, 2020f,g). In GRN 766, the notifier described the use of 3'-SL sodium salt (produced by enzymatic synthesis, ≥98% 3'-SL) in infant formulas (at levels up to 238 mg/L, providing 230 mg/L 3'-SL), resulting in all-user estimated intakes from infant formula of 266 mg 3'-SL/person/day, or 41 mg 3'-SL/kg body weight/day, in infants aged 0 to 11.9 months old (GeneChem, 2018 – GRN 766). The notifier also described the use of 3'-SL sodium salt as an ingredient in dairy product analogues, infant and toddler foods, milk (whole and skim), milk products, grain products, beverages and beverages bases, and sugar substitutes intended for the general population (at levels up to 3,104 mg/serving), resulting in estimated intakes of up to 326 mg/person/day, or 43.9 mg/kg body weight/day (in infant formula consumers aged 0 to 11.9 months).

In GRN 880, the notifier described the use of 3'-SL sodium salt (produced by fermentation using a genetically modified strain of *E. coli* K-12 DH1, ≥88% 3'-SL) in infant formulas (at levels up to 200 mg/L), other beverages and foods for young children (at levels up to 0.15 g/L or 1.25 g/kg), other foods and beverages intended for the general population, including yoghurt, buttermilk and fluid milk, cereal and granola bars, soft drinks, fruit-based drinks, sports drinks, "energy drinks," and enhanced waters (at levels up to 0.25 g/L or 2.5 g/kg), and foods for special dietary uses such as meal replacement drinks and bars (at levels 0.5 g/L or 5 g/kg) (Glycom A/S, 2019b – GRN 880). These use levels were estimated to result in intakes of up to 820 mg/person/day, or 87.9 mg/kg body weight/day, which occurred in infants aged 7 to 12 months.

In GRN 921, the notifier described the use of 3'-SL sodium salt [produced by fermentation using a genetically modified strain of *E. coli* BL21 (DE3), ≥88% 3'-SL] in infant formulas at levels up to 0.28 g/L (Jennewein Biotechnologie GmbH, 2020b – GRN 921). This use level was estimated to result in intake of up to 0.325 g/day (50.4 mg/kg body weight/day) in infants 0 to 12 months of age.

In all 3 of these GRAS Notices, the levels of 3'-SL sodium salt added to infant formulas and other foods and beverages were intended to result in intakes of 3'-SL comparable to those obtained from human milk.

In the European Union (EU), the EFSA NDA Panel concluded that the addition of 3'-SL sodium salt (produced by fermentation by a genetically modified strain of *E. coli* K-12 DH1, ≥88% 3'-SL) to a variety of foods (including infant and follow-on formula, foods for infants and toddlers, foods for special medical purposes, and food supplements) is safe under the proposed conditions of use for the proposed target populations (EFSA, 2020b). Intake of 3'-SL sodium salt resulting from the intended use in infant formula was estimated to be up to 52 mg/kg body weight/day, while intakes resulting from intended uses in other foods and food supplements were estimated to be 71 and 30 mg/kg body weight/day, respectively, for the general population (including infants). The EFSA NDA Panel noted that although the maximum daily intake of 3'-SL sodium salt resulting from the intended uses in foods is slightly above the high estimate for consumption of 3'-SL from human milk, it was concluded that this intake level is considered to be safe. The use of 3'-SL sodium salt produced with a genetically modified strain of *E. coli* K-12 DH1 in the EU was authorized by the European Commission under Commission Implementing Regulation (EU) 2021/96 of 28 January 2021 (EU, 2021b).

3.1.3 *N*-acetyl-D-neuraminic acid

NeuAc has been the subject of 1 GRAS Notice to which the U.S. FDA responded with no questions (GRN 602 – Glycom A/S, 2015; U.S. FDA, 2016b). In GRN 602, the notifier described the use of sialic acid (produced by enzymatic synthesis, ≥97% NeuAc*$2H_2O$) in infant formulas (at levels up to 50 mg/L), resulting in all-user estimated intakes of up to 64.1 mg/person/day, or 11.6 mg/kg body weight/day (GRN 602 – Glycom A/S, 2015). The notifier also described the use of sialic acid as an ingredient in a variety of other foods and beverages intended for the general population (at levels up to 1,000 mg/serving), resulting in estimated intakes of up to 154.3 mg/person/day, or 3.2 mg/kg body weight/day. In this GRAS Notice, the levels of sialic acid added to infant formulas and other foods and beverages were intended to result in intakes of sialic acid comparable to those obtained from human milk.

In the EU, the EFSA NDA Panel concluded that the addition of NeuAc (produced by enzymatic synthesis, ≥97% NeuAc*$2H_2O$) to a variety of foods (including infant and follow-on formula, foods for infants and toddlers, foods for special medical purposes, and foods for the general population) is safe under the proposed conditions of use for the proposed target populations (EFSA, 2017). Intake of NeuAc resulting from the intended use in infant formula was estimated to be up to 8.7 mg/kg body weight/day, while intakes resulting from intended uses in other foods and food supplements were estimated to be up to 7.1 and 60 mg/kg body weight/day, respectively, for the general population (including infants). The Panel noted that the estimated intakes of NeuAc from the intended uses and the background diet is comparable to the daily intake of NeuAc from human milk for teenagers and adults. For individuals below 10 years of age, the anticipated intake of NeuAc from food supplements alone would exceed the range of intake from human milk, while the anticipated intake from the intended food uses (excluding food supplements) in addition to the background diet would be within the range of intake from human milk. The EFSA NDA Panel therefore concluded that NeuAc is safe for use in foods other than food supplements at the proposed levels for the general population and for use in food supplements alone and in fortified foods plus food supplements for individuals over 10 years of age, while the safety of NeuAc was not established in food supplements alone for individuals under 10 years of age. NeuAc is an authorized novel food on the Union list (EU, 2017).

3.2 History of Use

3.2.1 Background on HMOs

HMOs consist of neutral and acidic oligosaccharides (ten Bruggencate *et al.*, 2014), which are classified as non-digestible (non-glycemic) carbohydrates (EFSA, 2014). Neutral and acidic HMOs are characterized primarily by the presence of fucose or sialic acid, respectively, conjugated to an oligosaccharide chain (ten Bruggencate *et al.*, 2014). It has been reported that 10 to 30% of the HMOs identified in human milk are sialic acid conjugates (EFSA, 2014; ten Bruggencate *et al.*, 2014). HMOs have been reported to be the third most abundant component by mass of human milk (behind lactose and lipids), with 100 different HMOs identified in human milk (ten Bruggencate *et al.*, 2014). Concentrations of oligosaccharides in general are much lower in bovine milk than in human milk (ten Bruggencate *et al.*, 2014). The oligosaccharide composition of human milk varies with infant gestation time, maternal genetics and blood type, duration of lactation, and time of day (ten Bruggencate *et al.*, 2014). Total HMOs, as well as the fucosylated HMOs, sialylated HMOs, undecorated HMOs, and fucosylated and sialylated HMOs, as classes are reported to significantly decrease over time in the mother's milk (Davis *et al.*, 2017). When examined individually, however, concentrations of 6'-SL tend to be higher during early lactation, while concentrations of 3'-SL remain fairly stable, with no indication of significant changes in concentration, over the duration of lactation (ten Bruggencate *et al.*, 2014).

Sialic acids are N- and O- substituted derivatives of neuraminic acid (a 9-carbon acidic sugar), and are present in the tissues, fluids, and secretions of mammals (Nakano, 1999; ten Bruggencate *et al.*, 2014). Mammalian milk contains sialic acid primarily in the form of conjugates of oligosaccharides, glycolipids, and glycoproteins, with very little sialic acid (approximately 3%) present in unbound form (Nakano, 1999; ten Bruggencate *et al.*, 2014). Human milk contains more overall sialic acid than bovine milk, the latter of which is commonly used in the production of infant formula (Nakano, 1999).

The trisaccharide sialyllactose, the predominant sialylated oligosaccharide in human and bovine milk (Goedhart and Bindels, 1994; Nakano, 1999), is composed of a lactose at the reducing terminus and sialic acid residue at the nonreducing end (ten Bruggencate *et al.*, 2014). The predominant forms of sialyllactose are 3'-SL and 6'-SL, which are constitutional isomers wherein the sialic acid moiety is connected to the galactose unit of lactose at the 3 or 6 position *via* an α-2,3 linkage or α-2,6 linkage, respectively (ten Bruggencate *et al.*, 2014; Jacobi *et al*., 2016). Although 6'-SL predominates in human milk, 3'-SL predominates in bovine milk (ten Bruggencate *et al.*, 2014).

HMOs are considered to be one of the primary growth factors of the infant gut microbiota and are therefore considered to be responsible for the composition of the infant gut microbiota of breastfed infants (EFSA, 2014). Sialic acid conjugates have been reported to have several physiological effects on neonatal development, primarily related to the development of the gastrointestinal and immune systems *via* prebiotic effects on the developing intestinal microbiota (Nakano, 1999; German *et al.*, 2008; ten Bruggencate *et al.*, 2014; Vasquez *et al.*, 2017). The results of studies in animals indicate that neonatal mammals have limited ability to synthesize sialic acids, and that endogenous sialic acid production is insufficient to meet the needs of rapid neonatal development; thus, sialic acid may be considered conditionally essential in neonates (Nakano, 1999; ten Bruggencate *et al.*, 2014).

3.2.2 Natural Occurrence of 3'-SL and 6'-SL

Sialyllactoses, including 3'-SL and 6'-SL, are present in the colostrum and milk from various species, including mice, pigs, dogs, goats, camels, sheep, cows, elephants, and humans (Grollman *et al.*, 1965; Prieto *et al.*, 1995; Nakamura *et al.*, 1998; Kunz *et al.*, 1999; Shen *et al.*, 2000; Nakamura *et al.*, 2003; McJarrow *et al.*, 2004; Barile *et al.*, 2010; Fukuda *et al.*, 2010; Goto *et al.*, 2010; Leo *et al.*, 2010; Sundekilde *et al.*, 2012; Alhaj *et al.*, 2013; Kelly *et al.*, 2013; Smilowitz *et al.*, 2013; Claps *et al.*, 2014, 2016; Kim *et al.*, 2015; Lee *et al.*, 2015; Salcedo *et al.*, 2016; Vicaretti *et al.*, 2018).

HMOs, including 3'-SL and 6'-SL, have been detected in the plasma of human infants (concentrations not reported in abstract) (Ruhaak *et al.*, 2014), while non-specified HMOs have been detected in fecal samples from breastfed infants (Chow *et al.*, 2014). At birth, human amniotic fluid has been reported to contain HMOs, including 6'-SL, indicating that infants are exposed to these compounds *in utero* (Wise *et al.*, 2018). 3'-SL also has been detected in cord blood serum, and was significantly higher in the cord blood of infants born to mothers with gestational diabetes (Jantscher-Krenn *et al.*, 2016 [abstract only]). Increased 3'-SL in the cord blood of mothers with gestational diabetes was also reported by Hoch *et al.* (2021). 3'-SL levels were reported to be 0.63 and 0.17 nmol/mL in mothers with gestational diabetes and mothers with a normal glucose tolerance, respectively. Sialyllactose is excreted in the urine of rats (Maury, 1972), healthy human subjects (Maury and Wegelius, 1981; Maury *et al.*, 1981), and both breastfed and formula-fed infants (Kunz and Rudloff, 1993), indicating its endogenous presence in the human body.

3.2.2.1 Levels in Animal Milk

In porcine, camel, goat, sheep, and cow milk, 3'-SL and 6'-SL are among the most abundant oligosaccharides (Nakamura *et al*., 1998; Fukuda *et al*., 2010; Alhaj *et al*., 2013; Claps *et al*., 2014, 2016; Salcedo *et al.*, 2016). In goats, concentrations of 3'-SL and 6'-SL decreased from immediately following parturition throughout the lactation period (Claps *et al*., 2014, 2016). Concentrations of 3'-SL and 6'-SL in goat milk were reported to be 125 to 254 and 20 to 175 mg/L, respectively, immediately following parturition and decreased to 71 to 111 and 0 to 78 mg/L, respectively, on Post-partum Day 90 (Claps *et al*., 2014, 2016).

In 3 samples of elephant milk, 3'-SL and 6'-SL comprised between 6 and 14% of the total oligosaccharides and were present at levels ranging from 0.86 to 2.79 g/L for 3'-SL and 0.13 to 0.34 g/L for 6'-SL (Kunz *et al.*, 1999).

In bovine milk, the concentrations of 3'-SL and 6'-SL were reported to vary considerably over the first 7 days postpartum, with the highest concentrations of 3'-SL and 6'-SL reported immediately following parturition (Nakamura *et al.*, 2003; McJarrow *et al*., 2004; Barile *et al*., 2010; Fischer *et al.*, 2018; Vicaretti *et al*., 2018). The results of analyses of bovine milk samples taken from several days before parturition through several months postpartum demonstrate that 3'-SL is more abundant than 6'-SL (Nakamura *et al*., 2003; McJarrow *et al*., 2004; Barile *et al*., 2010; Goto *et al*., 2010; Sundekilde *et al*., 2012; Lee *et al*., 2015; Fischer *et al.*, 2018; Vicaretti *et al*., 2018).

The concentration of 3'-SL ranged from 94 to 1,245 mg/L and the concentration of 6'-SL ranged from 29 to 243 mg/L in bovine colostrum, and both were reported to decrease over milkings (Nakamura *et al*., 2003; McJarrow *et al*., 2004; Fong *et al.*, 2011; Lee *et al*., 2015). Concentrations of 3'-SL and 6'-SL also were lower in mature milk compared to colostrum, and ranged from 30 to 325 and 14 to 88 mg/L, respectively. In samples of commercially-available skim and homogenized cows' milk the concentrations of 3'-SL and 6'-SL

ranged from 48 to 55 and 6.3 to 9.6 mg/L (McJarrow *et al*., 2004; Goto *et al*., 2010; Fong *et al.*, 2011; Lee *et al*., 2015).

3.2.2.2 Levels in Human Milk

The levels of 6'-SL in human milk have been quantified by many investigators, with highly variable concentrations reported within and between studies. The concentration of 6'-SL has been reported by most authors to decrease as lactation progresses, but to be unaffected by maternal diet, age, parity, ethnicity, obesity, smoking, mode of delivery, gestational age, or birth weight (Asakuma *et al.*, 2007; Eckhardt *et al.*, 2016; Azad *et al*., 2018; Neville *et al.*, 2021).

Studies identified in searches of the published literature (see Section 6.2) in which levels of 6'-SL were measured in the milk of healthy human mothers following the birth of healthy, full-term infants are summarized in Table 3.2.2.2-1. Literature searches were initially conducted through 19 April 2021 for the identification of studies in which levels of 6'-SL were measured in the milk of healthy human mothers following the birth of healthy, full-term infants. Studies identified in these searches were used for the derivation of use levels for 6'-SL sodium salt in infant formula. In these studies, mean concentrations of 6'-SL ranged from 39 to 1,770 mg/L, with ranges of 276 to 520, 209 to 1,770, and 39 to 1,310 mg/L reported in colostrum (1 to 2 days postpartum), transitional milk (3 to 30 days postpartum), and mature milk (>30 days postpartum), respectively. The average level of 6'-SL in transitional and mature milk from mothers who had given birth to full-term infants from the studies in Table 3.2.2.2-1 was calculated to be 428 and 376 mg/L, respectively, while the overall average level in both transitional and mature milk was calculated to be 345 mg/L.

Thurl *et al.* (2017) conducted a systematic review of levels of individual HMOs in human breast milk from healthy mothers with documented duration of pregnancy, lactation days of the sample, and defined Secretor status for neutral HMOs and calculated the mean concentration and 95% confidence intervals (CI) for each individual HMO examined. For 6'-SL, the results from 10 studies conducted with term infants were included[1]. The mean concentration of 6'-SL in milk from Secretor mothers who gave birth to term infants was 0.64 g/L (95% CI: 0.38-0.91 g/L), and the mean concentration in milk from mothers regardless of Secretor status who gave birth to term infants was 0.35 g/L (95% CI: 0.29-0.42). Despite a lower calculated mean when including milk regardless of Secretor status, there was no significant difference between the level of 6'-SL in milk from Secretor and non-Secretor mothers of term infants.

Taking into consideration the individual studies reviewed in Table 3.2.2.2-1 and the systematic review conducted by Thurl *et al.* (2017), overall average levels of 6'-SL in human milk range from 0.345 to 0.64 g/L in milk of mothers giving birth to term infants. Kyowa selected a use level of 0.50 g 6'-SL sodium salt/L in non-exempt term infant formula, as this use level was mid-range of the calculated average from all identified studies and mean levels determined in the review publication by Thurl *et al.* (2017).

[1] Coppa *et al.* (1999); Kunz *et al.* (1999); Martin-Sosa *et al.* (2003); Sumiyoshi *et al.* (2003); Asakuma *et al.* (2007); Bao *et al.* (2007); Leo *et al.* (2010); Smilowitz *et al.* (2013); Hong *et al.* (2014); Spevacek *et al.* (2015).

An updated search of the published literature was conducted in December 2021, and 5 additional studies were identified[2]. Of these 5 reports, 3 were research studies (of which individual levels of 6'-SL in human milk were reported in 2), 1 was a literature review, and 1 was a systemic review and meta-analysis. The 6'-SL content of colostrum (Lactation Days 0 to 7) was 409 mg/L in the individual research study (Liu *et al.*, 2021) and ranged from 400 to 433 mg/L in the review publications (Soyyilmaz *et al.*, 2021; Zhou *et al.*, 2021). Levels of 6'-SL in transitional milk ranged from 602 to 782 mg/L in the individual research studies (Liu *et al.*, 2021; Plows *et al.*, 2021) and from 584 to 710 mg/L in the review publications. Levels of 6'-SL in mature milk ranged from 23 to 300 mg/L in the individual research studies and from 34.6 to 403 mg/L in the review publications. Overall, mean levels of 6'-SL in transitional and mature human milk in the recently published studies ranged from 23 to 782 mg/L. In the recent publications, the concentration of 6'-SL in breast milk was increased during the transitional milk phase compared to colostrum, and it then decreased over time in mature milk.

[2] Liu *et al.,* 2021; Neville *et al.,* 2021; Plows *et al.,* 2021; Soyyilmaz *et al.,* 2021; Zhou *et al.,* 2021.

Table 3.2.2.2-1 Levels of 6'-Sialyllactose in the Milk of Healthy Human Mothers Following the Birth of Healthy, Full-Term Infants

Reference	Population (Health Status, Sample Size, Age, Location, Genotype [if reported])	Duration of Lactation at Time of Sample	Level in Milk (mg/L)[a]			
			Colostrum 1 to 2 d	Transitional 3 to 30 d	Mature >30 d	Average Level in Transitional and Mature 3 to >30 d
Asakuma *et al.* (2007)	Healthy women 19 to 37 years of age n=20 (10 primiparous and 10 multiparous) Japan	Milk samples were collected during the first 3 d of lactation. Values reported as mean ± SD	Overall mean 369.86 ± 108.19	NR	NR	NA
Austin *et al.* (2016)	Healthy women (no gestational diabetes, hypertension, cardiac diseases, acute communicable diseases, or postpartum depression) who gave birth to a healthy full-term infant Women exclusively breastfeeding for at least 4 m were included in the study. n=446 (5 to 11 d and 12 to 30 d postpartum: n=88; 1 to 2 m, 2 to 4 m, and 4 to 8 m postpartum: n=90) Age (mean ± SD): 27 ± 4 years China (Beijing, Suzhou, and Guangzhou)	Milk samples were collected during the following lactation stages: 5 to 11 d, 12 to 30 d, 1 to 2 m, 2 to 4 m, or 4 to 8 m postpartum. Values reported as mean ± SD (range)	NR	250 to 330	39 to 140	167.4

Table 3.2.2.2-1 Levels of 6'-Sialyllactose in the Milk of Healthy Human Mothers Following the Birth of Healthy, Full-Term Infants

Reference	Population (Health Status, Sample Size, Age, Location, Genotype [if reported])	Duration of Lactation at Time of Sample	Level in Milk (mg/L)[a]			
			Colostrum 1 to 2 d	Transitional 3 to 30 d	Mature >30 d	Average Level in Transitional and Mature 3 to >30 d
Austin *et al.* (2019) Garcia-Rodenas *et al.* (2018)	Healthy women (no diabetes, drug or alcohol consumption, or insufficient ability to follow study procedures) who intended to breastfeed for ≥4 m n=28 individuals; 28 infants (Group 1: n=21 samples, Group 2: n=5 samples, Group 3: n=1 sample, Group 4: n=1 sample) Age 31.2 ± 4.2 years Lausanne, Switzerland	Milk samples were collected once per week at 7 ± 1-d intervals, from Day 7 until Day 56 postpartum. Group 1: Secretors with active FUT2 and FUT3 enzymes Group 2: Secretors with only FUT3 activity Group 3: Secretors with only FUT2 activity Group 4: non-Secretors with no activity for FUT3 or FUT2 Values reported as a range of means over the sample period	NR	**Group 1:** 493.4 to 658.6 **Group 2:** 417.7 to 561.1 **Group 3:** 419.6 to 648.7 **Group 4:** 436.4 to 888.0	**Group 1:** 238.3 to 363.1 **Group 2:** 161.8 to 336.2 **Group 3:** 192.3 to 355.0 **Group 4:** 150.4 to 357.8	403.75
Azad *et al.* (2018)	Women who gave birth to healthy infants born ≥35 wk of gestation n=427 (Secretors: n=307; non-Secretors: n=120) Age (mean ± SD): 33.0 ± 4.2 years Canada (Vancouver, Edmonton, Manitoba, and Toronto)	Milk samples were collected 3 to 4 m postpartum. Lactation stage (mean ± SD): 17.1 ± 5.0 wk postpartum Secretor status was defined by the presence or near absence of 2'-FL. Values reported as mean ± SD (range)[b]	NR	NR	All subjects: 162 ± 127 (22 to 1,713) Secretors only: 157 ± 105 Non-Secretors: 173 ± 171	162

Table 3.2.2.2-1 Levels of 6'-Sialyllactose in the Milk of Healthy Human Mothers Following the Birth of Healthy, Full-Term Infants

Reference	Population (Health Status, Sample Size, Age, Location, Genotype [if reported])	Duration of Lactation at Time of Sample	Level in Milk (mg/L)[a]			
			Colostrum 1 to 2 d	Transitional 3 to 30 d	Mature >30 d	Average Level in Transitional and Mature 3 to >30 d
Bao *et al.* (2007)	Healthy donors n=13 (2 to 4 d postpartum: n=5; 12 to 67 d postpartum: n=1/time point - 5 total) Age NR United States	Milk samples were collected 2 to 4 d postpartum or 12 to 67 d postpartum.	276 ± 99	383 to 426	43 to 306	306
	Women for sequential sampling (*i.e.*, "matched samples"); n=3/collection period	Matched milk samples were collected from 3 donors (Donor 1 Days 4 and 21 postpartum; Donor 2: Days 3 and 15 postpartum; Donor 3: Days 5 and 9 postpartum).	NR	335 to 396	NR	NA
Coppa *et al.* (1999)	Mothers who had delivered at term, with phenotype Secretor A, B, H, and Lewis n=18 Age NR Italy[c]	Milk samples were collected 4, 10, 30, 60, and 90 d postpartum. Values reported as mean ± SD[b]	NR	440 to 590	240 to 300	382.5

Table 3.2.2.2-1 Levels of 6'-Sialyllactose in the Milk of Healthy Human Mothers Following the Birth of Healthy, Full-Term Infants

Reference	Population (Health Status, Sample Size, Age, Location, Genotype [if reported])	Duration of Lactation at Time of Sample	Level in Milk (mg/L)[a]			
			Colostrum 1 to 2 d	Transitional 3 to 30 d	Mature >30 d	Average Level in Transitional and Mature 3 to >30 d
Kunz *et al.* (1999)	Healthy women who were exclusively breastfeeding n=10 Age NR Germany	Milk samples were collected 2 to 28 d postpartum. Values reported as mean ± SD	NR	380 ± 50	NR	380
Kunz *et al.* (1999); Kunz *et al.* (2000)	Healthy women who were exclusively breastfeeding n=4 Age NR Germany	Milk samples were collected 2 to 19 d postpartum. Absolute values were reported in the publication. Mean values by lactation period were calculated by Kyowa.	330 (n=1)	382 to 572 (n= 2 to 3)	NR	479.7
Leo *et al.* (2010)	Health status NR n=16 (transitional milk: n=8; mature milk: n=8) Age NR Samoa	Milk samples were collected 5 to 10 d postpartum (transitional milk) or 21 to 155 d postpartum (mature milk). Samples were collected in the morning prior to breastfeeding. Values reported as mean ± SD (range)	NR	343 ± 235 (109 to 781)	189 ± 265 (42 to 659)	266

Table 3.2.2.2-1 Levels of 6'-Sialyllactose in the Milk of Healthy Human Mothers Following the Birth of Healthy, Full-Term Infants

Reference	Population (Health Status, Sample Size, Age, Location, Genotype [if reported])	Duration of Lactation at Time of Sample	Level in Milk (mg/L)[a]			
			Colostrum 1 to 2 d	Transitional 3 to 30 d	Mature >30 d	Average Level in Transitional and Mature 3 to >30 d
McGuire *et al.* (2017)	Mothers who were breastfeeding or pumping ≥5 times/day, with healthy infants N=413 (Ethiopia rural, n= 41; Ethiopia urban, n=40, Gambia rural, n=40; Gambia urban, n=40; Ghana, n=40; Kenya, n=42; Peru, n=43; Spain, n=41; Sweden, n=24; Washington United States, n=41; California United States, n= 19) Age 21.7 ± 0.5 to 34.3 ± 0.6 years Multi-center	Milk samples collected between 49 ± 4 and 73 ± 4 d postpartum Values reported as mean ± SEM	NR	NR	Average from all Sites 306.8	306.8
McJarrow *et al.* (2019)	Women with a singleton pregnancy in the third trimester, free of chronic diseases, autoimmune disorders, HIV, or hepatitis n=80 (transitional milk: n=41; mature milk: n=40) 19 to 40 years of age United Arab Emirates (Emirati or Arab expatriates in the Emirates of Sharjah, Dubai, and Ajman)	Milk samples were collected 5 to 15 d postpartum (transitional milk) or 6 m postpartum (mature milk). Secretor status was defined by the presence or near absence of 2'-FL and lacto-*N*-fucopentaose I. Values reported as mean ± SD	NR	<u>All subjects</u> 621 ± 212 <u>Secretors only</u> 643 ± 204 <u>Non-Secretors</u> 562 ± 232	<u>All subjects</u> 91 ± 108 <u>Secretors only</u> 95 ± 124 <u>Non-Secretors</u> 81 ± 52	356
Monti *et al.* (2015)	Health status NR n=2 Age NR Country NR	NR Values reported as <u>mean.</u>	NR	NR	*72.2*	*72*

Table 3.2.2.2-1 Levels of 6'-Sialyllactose in the Milk of Healthy Human Mothers Following the Birth of Healthy, Full-Term Infants

Reference	Population (Health Status, Sample Size, Age, Location, Genotype [if reported])	Duration of Lactation at Time of Sample	Level in Milk (mg/L)[a]			
			Colostrum 1 to 2 d	Transitional 3 to 30 d	Mature >30 d	Average Level in Transitional and Mature 3 to >30 d
Sakaguchi *et al.* (2014)	Primiparous woman (no further details provided) n=1 Age: 21 years Japan[c]	Milk samples were collected 10 d postpartum and 3 m postpartum. Absolute value reported[b]	NR	209	95	152
Seppo *et al.* (2019)	Women (n=1,223) carrying fetuses at hereditary risk for allergy (*i.e.*, the offspring had 1 or both parents with physician-diagnosed allergic rhinitis, eczema, and/or asthma). n=81 (placebo group: n=30; probiotic group: n=51) Age NR Helsinki, Finland	Details NR; described as colostrum Probiotic Group: Administered probiotic supplementation (*Lactobacillus Rhamnosus* GG, *Lactobacillus rhamnosus* LC705, *Bifidobacterium breve* b99, and *Propionibacterium freudenreichii* subspecies *shermanii* JS) twice daily from gestation wk 36 to birth of infant Values reported as mean ± SD[d]	Control Group 359 ± 113 Probiotic Group 299 ± 101	NR	NR	NA
Smilowitz *et al.* (2013)	Healthy women who delivered full-term infants n=52 Age NR United States	Milk samples were collected in the morning on Day 90 postpartum. Values reported as mean ± SD (range)[d]	NR	NR	75 ± 34.8 (27.4 to 183)	75

Table 3.2.2.2-1 Levels of 6'-Sialyllactose in the Milk of Healthy Human Mothers Following the Birth of Healthy, Full-Term Infants

Reference	Population (Health Status, Sample Size, Age, Location, Genotype [if reported])	Duration of Lactation at Time of Sample	Level in Milk (mg/L)[a]			
			Colostrum 1 to 2 d	Transitional 3 to 30 d	Mature >30 d	Average Level in Transitional and Mature 3 to >30 d
Spevacek *et al.* (2015)	Health status NR 0 to 5 d postpartum: n=15 term and n=10 preterm; 14 d postpartum: n=14 term and n=10 preterm; 28 d postpartum: n=15 term and n=6 preterm Age NR United States	Milk samples were collected 0 to 5 d postpartum, 14 d postpartum, and 28 d postpartum. In mothers of term infants, milk samples were collected between 2 and 4 hours after feeding. Values reported as mean ± SD[e]	520 ± 152 (term only)	367 to 558 (term only)	NR	462.5
Sprenger *et al.* (2017)	Healthy women (no pre-eclampsia, gestational diabetes, arterial hypertension above 140/90 mm Hg) who gave birth at gestational age 37 to 42 weeks n=49 to 50 (low 2'-FL: n=16; high 2'-FL: n=33 at 4 m; n=34 at 1 and 2 m) Age: 18 to 40 years Singapore	Milk samples were collected 0, 60, and 120 d postpartum. Samples were collected in the morning after full expression during feeding. 2'-FL concentrations measured in 30 d postpartum milk samples were used to group the mother infant pairs into those with low (considered Secretor negative) and high 2'-FL concentrations. Values reported as mean ± SD	NR	NR	Low 2'-FL (Non-Secretors): 150 to 496 High 2'-FL (Secretors): 120 to 561	312
Sumiyoshi *et al.* (2003)	Health status of the mother NR n=23 at 100 d; 24 at 4, 10, and 30 d Age NR Japan	Milk samples were collected 4, 10, 30, and 100 d postpartum. Values reported as mean ± SD (range)	NR	409.8 to 412.2	95.7 to 275.5	261.3

Table 3.2.2.2-1 Levels of 6'-Sialyllactose in the Milk of Healthy Human Mothers Following the Birth of Healthy, Full-Term Infants

Reference	Population (Health Status, Sample Size, Age, Location, Genotype [if reported])	Duration of Lactation at Time of Sample	Level in Milk (mg/L)[a]			
			Colostrum 1 to 2 d	Transitional 3 to 30 d	Mature >30 d	Average Level in Transitional and Mature 3 to >30 d
Thurl *et al.* (2010)	Women who had given birth to healthy infants who were exclusively breastfed during the study period n=30 individuals (3 d postpartum: n=21 samples; 8 d postpartum: n=19 samples; 15 d postpartum: n=17 samples; 22 d postpartum: n=16 samples; 30 d postpartum: n=14 samples; 60 d postpartum: n=12 samples; 90 d postpartum: n=10 samples; Group 1: n=109 samples; Group 2: n=28 samples; Group 3: n=17 samples). 20 to 35 years of age Germany	Samples were collected in the morning, mid-feed, 3 to 90 d postpartum. Group 1 (n=22): Secretors with Lewis blood group Le(a – b +), who produced all 20 HMOs. Group 2 (n=5): non-Secretors with Lewis blood group Le(a + b –), who produced all HMOs except α1,2-fucosylated compounds. Group 3 (n=3): Secretors with Lewis blood group Le(a – b –), who lacked α1,4-fucosyloligosaccharides Mean values reported	NR	Group 1: 1,310 to 1,770	Group 1, 2, 3: 490 to 1,310	1,223.3
Tonon *et al.* (2019)	Health status NR n=10 Age NR Brazil	Milk samples were collected between 17 and 45 d postpartum Values reported as mean ± SD (range)	NR	NR	433 ± 101 (326 to 670)	433

Table 3.2.2.2-1 Levels of 6'-Sialyllactose in the Milk of Healthy Human Mothers Following the Birth of Healthy, Full-Term Infants

Reference	Population (Health Status, Sample Size, Age, Location, Genotype [if reported])	Duration of Lactation at Time of Sample	Level in Milk (mg/L)[a]			
			Colostrum 1 to 2 d	Transitional 3 to 30 d	Mature >30 d	Average Level in Transitional and Mature 3 to >30 d
Studies from Updated Literature Search						
Liu *et al.* (2021)	Healthy women (n=335), who had lived in the area for more than 2 years, had singleton pregnancies, intention to breastfeed for more than 3 months, and had a gestational age of 37 to 42 weeks. 20 to 35 years of age China (Guangzhou City)	Milk samples were taken at 5 different time points post-partum: 0 to 5 days (n=96), 10 to 15 days (n=96), 40 to 45 days (n=104), 200 to 240 days (n=100), and 300 to 400 days (n=92). Mean values reported.	Day 0 to 5: 409	Days 10 to 15: 602	Days 40 to 45: 300 Days 200 to 240: 39 Days 300 to 400: 23	241
Plows *et al.* (2021)	Health status NR; Hispanic women who had singleton pregnancies, intention to breastfeed more than 3 months, and enrollment within 1 month of infant's birth. n varied by timepoint (1 month: n=207; 6 months: n=119; 12 months: n=83; 18 months: n=59; 24 months: n=28). Age NR United States of America (California)	Milk samples were taken at 5 different time points post-partum: 1, 6, 12, 18, and 24 months. Breast milk was collected at least 1.5 hours after the previous feeding and after the mother had fasted at least 1 hour. Median values reported.	NR	Secretors Day 30: 621 Non-Secretors Day 30: 782	Secretors Day 180: 156 Day 365: 67.9 Day 548: 50.4 Day 730: 51.9 Non-Secretors Day 180: 182 Day 365: 107 Day 548: 44.8 Day 730: 39.6	Secretors: 190 Non-Secretors: 231

Table 3.2.2.2-1 Levels of 6'-Sialyllactose in the Milk of Healthy Human Mothers Following the Birth of Healthy, Full-Term Infants

Reference	Population (Health Status, Sample Size, Age, Location, Genotype [if reported])	Duration of Lactation at Time of Sample	Level in Milk (mg/L)[a]			
			Colostrum 1 to 2 d	Transitional 3 to 30 d	Mature >30 d	Average Level in Transitional and Mature 3 to >30 d
Zhou *et al.* (2021)	Systemic review and meta-analysis of studies (n=8 studies) investigating concentrations of HMOs in a Chinese population; health status NR; 6 of 8 studies conducted in mothers of full-term infants and in 2 of 8 studies, birth status was NR. China	Results of heterogeneity analysis were summarized across all included studies. Values reported as <u>mean ± SD</u>	Day 1 to 7: 433.8 ± 81.3	Day 8 to 14: 584 ± 25.2	Day 15 to 60: 197.6 ± 43.2 Day 61 to 120: 293 ± 178.2 Day >121: 34.6 ± 5.8	277
Soyyilmaz *et al.* (2021)	Literature review of studies including healthy mothers of term infants and HMO concentration in breast milk at defined lactation periods on a global scale (n=69 studies). Secretors and non-secretors were pooled. 31 countries	Results of HMO quantification by lactation stage (4 groups) were pooled. Mean of all studies reported.	Day 0 to 5: 400	Day 6 to 14: 710	Day 15 to 90: 403 Day >90: 300	471

2'-FL = 2'-fucosyllactose; 6'-SL = 6'-sialyllactose; d = days; FUT = fucosyltransferase; HIV = human immunodeficiency virus; HMO = human milk oligosaccharide; m = months; NICU = neonatal intensive care unit; NR = not reported; SD = standard deviation; SEM = standard error of the mean; wk = weeks.

[a] Levels inferred from a figure by Kyowa are <u>underlined</u>; levels calculated by Kyowa are *italicized*.
[b] Values were reported in nmol/L and converted using the molecular weight of 3'-SL (633.553 g/mol): (nmol/L * 633.533 g/mol)/1,000.
[c] Assumed based on location of the study authors.
[d] Values were reported in umol/L and converted using the molecular weight of 3'-SL (633.553 g/mol): (umol/L * 633.533 g/mol)/1,000.
[e] Values were reported in mmol/L and converted using the molecular weight of 3'-SL (633.553 g/mol): (mmol/L * 633.533 g/mol).

3.2.3 Background Exposure to 6'-SL

As discussed above, 6'-SL is naturally present in bovine and human milk. In addition, 6'-SL was detected in two commercial whey-protein derived infant formulas at levels of 3.8 to 4.6 mg/L in the reconstituted infant formula (Fong *et al.*, 2011). In a separate study of dry powdered infant formula, the mean concentration of 6'-SL in infant formula powder, follow-on milk powder, and growing-up milk powder was reported to be 91, 119, and 95 µg 6'-SL/g dry powder in products purchased in Malaysia (n=20) and 100, 94, and 93 µg 6'-SL/g dry powder in products purchased in China (n=36), respectively (Ma *et al.*, 2019). The authors reported that these concentrations in powder were equivalent to 12.1 to 15.5 mg 6'-SL/L when the powder products were reconstituted at 130 g/L, which was the average of the manufacturers recommendations.

Using the range of mean concentrations reported in individual studies for 6'-SL in human breast milk (see Section 3.2.2.2 above), as well as the overall average level of 6'-SL from Secretor mothers who gave birth to term infants reported by Thurl *et al.* (2017) of 0.64 g/L (*i.e.*, the highest calculated average from the results of multiple studies for term infants) the background exposure was calculated to be 23 to 2,124 mg/day (mean = 512 mg/day), equivalent to 3 to 317 mg/kg body weight/day (mean = 76 mg/kg body weight/day). Background intakes of 6'-SL from infant formula were calculated to be 3 to 13 mg/day, equivalent to 0.4 to 2 mg/kg body weight/day, while background intakes from commercial cow's milk were calculated to be 35 to 40 mg/day, equivalent to 3 mg/kg body weight/day.

As such, humans that consume either breast milk or whey protein infant formulas during infancy or cow's milk at later stages of life have background dietary exposure to 6'-SL. A summary of the reported concentrations and background exposure to 6'-SL is provided in Table 3.2.3-1 below.

Table 3.2.3-1 Summary of Background Dietary Sources and Estimated Intake of 6'-Sialyllactose in Infants and Toddlers

Food Source	Intake of Milk or Formula (mL/day)	Infant Body Weight (kg; 50th Percentile, 4 months)[a]	6'-SL Concentration in Transitional and Mature Milk		6'-SL Intake			
			Concentration (mg/L) (mean)[b]	Concentration (mg/L) (range)[b]	Intake (mg/day) (mean)	Intake (mg/day) (range)	Intake (mg/kg bw/day) (mean)	Intake (mg/kg bw/day) (range)
Human Milk (mean)	800[c]	6.7	640	39 to 1,770	512	31 to 1,416	76	5 to 211
Human Milk (range)	510 to 1,200[c]	6.7	640	39 to 1,770	384 to 768	23 to 2,124	57 to 115	3 to 317
Infant Formula (mean)	761.8[d]	6.7	NA	3.8 to 15.5	NC	3 to 12	NC	0.4 to 2
Infant Formula (range)	696.8 to 856.[d]	6.7	NA	3.8 to 15.5	NC	3 to 13	NC	0.4 to 2
Commercial Cow's Milk	720[e]	11.8[f]	NA	6.3 to 9.6	NC	35 to 40	NC	3

6'-SL = 6'-sialyllactose; NA = not available; NC= not calculated; NR = not relevant.
[a] Source: WHO Growth Chart (https://www.cdc.gov/growthcharts/who_charts.htm); average of 50th percentile for boys and girls.
[b] Data from studies summarized in Table 3.2.2.2-1 above, Fong *et al.* (2011), and Ma *et al.* (2019).
[c] Source: Butte *et al.* (2002); da Costa *et al.* (2010); Nielsen *et al.* (2011); EFSA (2013).
[d] Source: Hester *et al.* (2012).
[e] Recommended daily dairy intake of 2 to 3, 240-mL servings per day; source: https://www.cnpp.usda.gov/2015-2020-dietary-guidelines-americans.

Table 3.2.3-1 Summary of Background Dietary Sources and Estimated Intake of 6'-Sialyllactose in Infants and Toddlers

Food Source	Intake of Milk or Formula (mL/day)	Infant Body Weight (kg; 50th Percentile, 4 months)[a]	6'-SL Concentration in Transitional and Mature Milk		6'-SL Intake			
			Concentration (mg/L) (mean)[b]	Concentration (mg/L) (range)[b]	Intake (mg/day) (mean)	Intake (mg/day) (range)	Intake (mg/kg bw/day) (mean)	Intake (mg/kg bw/day) (range)

[f] Source: WHO Growth Chart (https://www.cdc.gov/growthcharts/who_charts.htm); average of 50th percentile for boys and girls at age 24 months.

3.3 Nutritional Purpose for Use in Non-Exempt Term Infant Formula

Kyowa intends to market 6'-SL sodium salt as a nutritional ingredient for use in non-exempt term infant formula, as well as specified foods and beverages as defined under 21 CFR §170.3(n) (U.S. FDA, 2020a). The proposed uses and maximum use levels are summarized in Table 1.3-1. Kyowa's 6'-SL sodium salt is intended as an alternative source of 6'-SL to other 6'-SL ingredients on the market in the U.S.

As indicated in Section 3.2, 6'-SL is a naturally-occurring oligosaccharide in human milk (ten Bruggencate *et al.*, 2014; Jacobi *et al.*, 2016). The group of HMOs, which comprise both neutral and acidic oligosaccharides, is reported to be the third most abundant component by mass of human milk and 10 to 30% of the HMOs identified in human milk are sialic acid conjugates (EFSA, 2014; ten Bruggencate *et al.*, 2014). Human milk offers all essential nutrients for infant growth and development. For this reason, infant formulae are formulated to match the nutrient composition of human milk as closely as possible. Kyowa notes that human milk is a complex fluid containing over 150 HMOs and is proposing the addition of 6'-SL sodium salt to term infant formula to provide a source of 6'-SL for formula-fed infants.

3.4 Estimated Intake of 6'-SL Sodium Salt Based Upon Intended Food Uses

3.4.1 Methodology

An assessment of the estimated intake of 6'-SL sodium salt as an ingredient under the intended conditions of use (see Table 1.3-1) was conducted using data available in the 2017-2018 cycle of the U.S. National Center for Health Statistics' National Health and Nutrition Examination Survey (NHANES) (CDC, 2021a,b; USDA, 2021). The assessment included all uses previously concluded to be GRAS for 3'-SL sodium salt in order to provide cumulative estimates of intake.

The NHANES data are collected and released in 2-year cycles with the most recent cycle containing data collected in 2017-2018. Information on food consumption was collected from individuals *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2). Sample weights were incorporated with NHANES data to compensate for the potential under-representation of intakes from specific populations and allow the data to be considered nationally representative (CDC, 2021a,b; USDA, 2021). The NHANES data were employed to assess the mean and 90th percentile intake of 6'-SL sodium salt for each of the following population groups:

- Infants, ages 0 to 6 months;
- Infants, ages 7 to <12 months;
- Toddlers, ages 1 to 3 years;
- Children, ages 4 to 11 years;
- Female teenagers, ages 12 to 19 years;

- Male teenagers, ages 12 to 19 years;
- Female adults of childbearing age, ages 14 to 50 years;
- Female adults, ages 20 to 64 years;
- Male adults, ages 20 to 64 years;
- Elderly, ages ≥ 65 years; and
- Total population (≥2 years, gender groups combined[3]).

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of 6'-SL sodium salt by the U.S. population[4]. Estimates for the daily intake of 6'-SL sodium salt represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2017-2018; these average amounts comprised the distribution from which mean and percentile intake estimates were determined. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of 6'-SL sodium salt averaged over all individuals surveyed, regardless of whether they consumed food products in which 6'-SL sodium salt is proposed for use, and therefore includes individuals with "zero" intakes (*i.e*., those who reported no intake of food products containing 6'-SL sodium salt during the 2 survey days). "Consumer-only" intake refers to the estimated intake of 6'-SL sodium salt by those individuals who reported consuming food products in which the use of 6'-SL sodium salt is currently under consideration. Individuals were considered "consumers" if they reported consumption of 1 or more food products in which 6'-SL sodium salt is proposed for use on either Day 1 or Day 2 of the survey.

The estimates for the intake of 6'-SL sodium salt were generated using the maximum use level indicated for each intended food use, as presented in Table 1.3-1, together with food consumption data available from the 2017-2018 NHANES datasets. The results for these assessments are presented in Section 3.4.2.

3.4.2 Results of Intake Estimates for 6'-SL Sodium Salt

3.4.2.1 Estimated Daily Intake of 6'-SL Sodium Salt from All Proposed Conditions of Use

A summary of the estimated daily intake of 6'-SL sodium salt from all proposed food uses is provided in Table 3.4.2.1-1 on an absolute basis (g/person/day), and in Table 3.4.2.1-2 on a body weight basis (mg/kg body weight/day).

The percentage of consumers was high among all age groups evaluated in the current intake assessment; greater than 72.1% of the population groups consisted of consumers of food products in which 6'-SL sodium salt is currently proposed for use (see Table 3.4.2.1-1). With the exception of infants 0 to 6 months of age, the proportion of consumers was close to or equal to 100.0% in all population groups. 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 6'-SL sodium salt were determined to be 1.95 and 3.60 g/person/day, respectively. Of the individual population groups, the elderly were determined to have the greatest mean consumer-only intakes of

[3] Although there are 2 female adult population groups, female adults were not double counted within the total population intake results.

[4] Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2018). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

6'-SL sodium salt on an absolute basis, at 2.29 g/person/day, while female adults had the greatest 90th percentile consumer-only intakes, at 4.26 g/person/day. Infants 0 to 6 months of age had the lowest mean and 90th percentile consumer-only intakes on an absolute basis, at 0.49 and 0.90 g/person/day, respectively (see Table 3.4.2.1-1).

Table 3.4.2.1-1 Summary of the Estimated Daily Intake of 6'-Sialyllactose Sodium Salt from All Proposed Food Uses in the U.S. by Population Group (2017-2018 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants[a]	0 to 6 m	0.35	0.76	72.1	133	0.49	0.90
Infants[a]	7 to <12 m	1.00	1.74	100	124	1.00	1.74
Toddlers	1 to 3 y	1.05	1.69	99.9	414	1.06	1.69
Children	4 to 11 y	1.41	2.32	99.9	889	1.41	2.32
Female Teenagers	12 to 19 y	1.42	2.64	99.3	446	1.43	2.64
Male Teenagers	12 to 19 y	1.57	3.07	99.7	440	1.57	3.07
Females of childbearing age	14 to 50 y	2.12	3.62	99.7	1,354	2.13	3.64
Female Adults	20 to 64 y	2.18	4.26	99.7	1,626	2.18	4.26
Male Adults	20 to 64 y	1.95	4.12	99.3	1,424	1.96	4.17
Elderly	≥65 y	2.28	4.11	99.6	1,057	2.29	4.11
Total Population	≥2 y	1.94	3.59	99.6	6,143	1.95	3.60

m = months; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States; y = years.
[a] Consumption in infants also includes intakes from hypoallergenic infant formula, which is not currently being considered as an intended use.

On a body weight basis, the total population (2 years and older) mean and 90th percentile consumer-only intakes of 6'-SL sodium salt were determined to be 30 and 64 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 110 and 190 mg/kg body weight/day, respectively. Male adults had the lowest mean consumer-only intakes of 22 mg/kg body weight/day, while both female teenagers and male adults had the lowest 90th percentile consumer-only intakes of 46 mg/kg body weight/day (see Table 3.4.2.1-2).

Table 3.4.2.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of 6'-Sialyllactose Sodium Salt from All Proposed Food Uses in the U.S. by Population Group (2017-2018 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants[a]	0 to 6 m	53	116	72.1	133	74	119
Infants[a]	7 to <12 m	110	190	100	124	110	190
Toddlers	1 to 3 y	77	134	99.9	404	77	134
Children	4 to 11 y	50	88	99.9	887	50	88
Female Teenagers	12 to 19 y	24	46	99.3	439	24	46
Male Teenagers	12 to 19 y	25	51	99.7	437	25	51
Females of childbearing age	14 to 50 y	29	51	99.7	1,342	29	52
Female Adults	20 to 64 y	30	58	99.7	1,619	30	58
Male Adults	20 to 64 y	22	46	99.3	1,416	22	46
Elderly	≥65 y	28	52	99.6	1,038	28	52

Table 3.4.2.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of 6'-Sialyllactose Sodium Salt from All Proposed Food Uses in the U.S. by Population Group (2017-2018 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Total Population	≥2 y	30	64	99.6	6,089	30	64

bw = body weight; m = months; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States; y = years.

[a] Consumption in infants also includes intakes from hypoallergenic infant formula, which is not currently being considered as an intended use.

The total U.S. population (except infants 0 to <12 months of age) was identified as being significant consumers of "Breads and baked goods, including gluten-free" (80 to 93% consumers), "Unflavored pasteurized and sterilized milk" (42 to 80% consumers), "Fruit juices and nectars" (23 to 59% consumers), "Ready-to-eat breakfast cereals" (23 to 57% consumers), and "Soft drinks (regular and diet)" (21 to 54% consumers). Infants 0 to 6 months of age were identified as being significant consumers of "Term infant formula" (58% consumers), whereas infants 7 to <12 months were identified as being significant consumers of "Other baby foods for infants and young children" (64% consumers) and "Term infant formula" (60% consumers).

In terms of contribution to total mean intake of 6'-SL sodium salt, "Breads and baked goods, including gluten-free" (which contributed 28 to 54% to total mean intakes) and "Beverage whiteners" (which contributed 1 to 47% to total mean intakes) were the main sources of intake across the total U.S. population (except in infants 0 to <12 months of age); all other food uses contributed less than 14% to total mean 6'-SL sodium salt intakes. In infants 0 to 6 months of age, "Term infant formula" was the main source of intake (contributed 65% to total mean intakes), whereas in infants 7 to <12 months of age "Other baby foods for infants and young children" (contributed 25% to total mean intakes) and "term infant formula" (contributed 20% to total mean intakes) were the main sources of intake.

3.4.2.2 Estimated Daily Intake of 6'-SL Sodium Salt from Infant Formula and Toddler Formula

A summary of the estimated daily intake of 6'-SL sodium salt in younger population groups from the maximum proposed use levels in non-exempt term infant formula and toddler formula (intended for age 1 to 3 years), as well as from use in hypoallergenic infant formula (which is not a proposed food use), is provided in Table 3.4.2.2-1 on an absolute basis (g/person/day), and in Table 3.4.2.2-2 on a body weight basis (mg/kg body weight/day).

The proportion of consumers ranged between 64.7 and 69.6% in infants, whereas only 4.1 to 4.2% of toddlers were determined to be consumers of infant and toddler formulas (see Table 3.4.2.2-1). It should be noted that intake estimates derived for toddlers may not be statistically reliable as only 18 toddlers from the NHANES 2017-2018 cycle were identified as consuming term infant formula, , hypoallergenic infant formula, and/or toddler formula. As a result, estimates for this population group are presented in Tables 3.4.2.2-1 and 3.4.2.2-2, but not further discussed.

The mean and 90th percentile consumer-only intakes of 6'-SL sodium salt from use in non-exempt term infant formula, hypoallergenic infant formula, and toddler formula were highest in infants 0 to 6 months of age, at 0.39 and 0.62 g/person/day, respectively (see Table 3.4.2.2-1). Intake estimates were also highest in this population group on a body weight basis, at up to 61 and 103 mg/kg body weight/day at the mean and 90th percentile, respectively (see Table 3.4.2.2-2).

Table 3.4.2.2-1 Summary of the Estimated Daily Intake of 6'-Sialyllactose Sodium Salt from Infant Formula and Toddler Formula in the U.S. by Population Group (2017-2018 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (g/day)[a]		Consumer-Only Intake (g/day)[a]			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to 6 m	0.25	0.56	64.7	118	0.39	0.62
Infants	7 to <12 m	0.23	0.52	69.6	84	0.33	0.56
Toddlers	1 to 3 y	<0.01*	NA	4.1	18	0.12*	0.24*

m = months; n = sample size; NA = not applicable; NHANES = National Health and Nutrition Examination Survey; U.S. = United States; y = years.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).
[a] Consumption estimates also include intakes from hypoallergenic infant formula, which is not currently being considered as an intended use.

Table 3.4.2.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of 6'-Sialyllactose Sodium Salt from Infant Formula and Toddler Formula in the U.S. by Population Group (2017-2018 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)[a]		Consumer-Only Intake (mg/kg bw/day)[a]			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to 6 m	40	95	64.7	118	61	103
Infants	7 to <12 m	25	55	69.6	84	37	61
Toddlers	1 to 3 y	<1*	NA	4.2	18	11*	24*

bw = body weight; m = months; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States; y = years.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).
[a] Consumption estimates also include intakes from hypoallergenic infant formula, which is not currently being considered as an intended use.

3.4.2.3 Comparison of the Estimated Daily Intake of 6'-SL Sodium Salt from Proposed Conditions of Use (Infants) versus Human Milk

The estimated daily intake of 6'-SL sodium salt in infants from all proposed conditions of use (taken from Table 3.4.2.1-2) is compared to that from breast milk (taken from in Table 3.2.3-1) in Table 3.4.2.3-1, on a body weight basis. Mean consumer-only intakes of 6'-SL sodium salt in infants from all proposed conditions of use, ranging between 74 and 110 mg/kg body weight/day, are within the average range of 6'-SL intakes resulting from the mean consumption of human milk of 5 to 211 mg/kg body weight/day, whereas 90th percentile intakes of 6'-SL sodium salt, ranging between 119 and 190 mg/kg body weight/day, are below the maximum estimated daily intake of 6'-SL from the high-level consumption of human milk of 317 mg/kg body weight/day (see Table 3.4.2.3-1).

As indicated in Section 1.3, Kyowa's proposed use level in non-exempt term infant formula (0.50 g/L) was based on the range of average levels of 6'-SL calculated from studies in which levels of 6'-SL were assessed in the milk of healthy human mothers following the birth of healthy infants (discussed in detail in Section 3.2.2.2 above). The estimated daily intake of 6'-SL sodium salt from term infant formula and toddler formula (taken from Table 3.4.2.2-2) is compared to that from breast milk (taken from in Table 3.2.3-1) in Table 3.4.2.3-1, on a body weight basis. Mean and 90th percentile consumer-only intakes of 6'-SL sodium salt from term infant formula and toddler formula, of up to 61 and 103 mg/kg body weight/day, respectively, are within the average range of 6'-SL intakes from the mean consumption of human milk of 5 to 211 mg/kg body weight/day, and below maximum 6'-SL intakes from the high-level consumption of human milk of 317 mg/kg body weight/day (see Table 3.4.2.3-1).

Table 3.4.2.3-1 Comparison of the Estimated Daily Per Kilogram Body Weight Intake of 6'-Sialyllactose Sodium Salt from All Proposed Conditions of Use, Infant Formulas and Toddler Formula Only, and Human Milk

Population Group	Age Group	Consumer-Only Intake from All Proposed Uses (mg/kg bw/day)[a]		Consumer-Only Intake from Infant Formulas and Toddler Formula (mg/kg bw/day)[a]		Intake from Human Milk (mg/kg bw/day)			
						Mean Human Milk Intake (800 mL/day)		Range of Human Milk Intake (510 to 1,200 mL/day)	
		Mean	P90	Mean	P90	Mean Concentration (640 mg/L)	Mean Concentration Range (39 to 1,770 mg/L)	Mean Concentration (640 mg/L)	Mean Concentration Range (39 to 1,770 mg/L)
Infants	0 to 6 m	74	119	61	103	76	5 to 211	57 to 115	3 to 317
Infants	7 to <12 m	110	190	37	61				

6'-SL = 6'-sialyllactose; bw = body weight; m = months; P90 = 90th percentile; y = years.
[a] Consumption estimates also include intakes from hypoallergenic infant formula, which is not currently being considered as an intended use.

The intakes presented in the above scenarios do not take into account the possibility that a breastfed infant could consume complementary foods with 6'-SL sodium salt. To assess the potential exposure, the concentration of 6'-SL in breast milk, the amount of breast milk consumed, and the intakes of 6'-SL from complementary foods must all be considered. Notably, as consumption of complementary foods increase, consumption of breast milk decreases, such that additive exposure will be occasional and transient. Therefore, it is highly unlikely that a breastfed infant would be both a high consumer of 6'-SL from breast milk and a high consumer of 6'-SL from complementary foods, and as such, no safety concerns are anticipated due to consumption of complementary foods supplemented with 6'-SL by breastfed infants.

3.4.3 Dietary Intake from Foods for Special Dietary Uses

Kyowa also intends to market 6'-SL sodium salt for use in foods for special dietary uses, specifically, oral nutritional supplements and formula for enteral tube feeding. Oral nutritional supplements are intended for the general population (ages 2 and up). The recommended conditions of use are 0.42 g 6'-SL sodium salt/45 g powdered serving or 250 mL ready-to-consume product, consumed twice per day for a total daily intake of 0.84 g 6'-SL sodium salt/day. The use of 6'-SL sodium salt in enteral tube feeding formula is intended for ages 11 and up and is proposed at a use level of 4.1 g/L in the final,

ready to consume product. The recommended conditions of use for enteral tube feeding formula are 1.0 g 6'-SL sodium salt per 250 mL, consumed twice per day, for a total intake of 2.0 g/day.

Foods for special dietary use containing 6'-SL sodium salt are not intended to be consumed in combination with any other supplemental sources of 6'-SL sodium salt and will be labeled as such. Consumption of 6'-SL sodium salt from foods for special dietary use will be substitutional and not additive to consumption of 6'-SL sodium salt from other sources.

3.4.4 Summary and Conclusions

Consumption data and information pertaining to the intended food uses of 6'-SL sodium salt were used to estimate the *per capita* and consumer-only intakes of this ingredient for specific demographic groups and for the total U.S. population. Intake from use of 6'-SL sodium salt in infant formula and toddler formula (intended for age 1 to 3 years) only was also evaluated in infants and toddlers. There were a number of assumptions included in the assessment which render exposure estimates suitably conservative. For example, it has been assumed in this exposure assessment that all food products within a food category contain 6'-SL sodium salt at the maximum specified level of use. In reality, the levels added to specific foods will vary depending on the nature of the food product and it is unlikely that 6'-SL sodium salt will have 100% market penetration in all identified food categories.

More than 72.1% of the population groups consisted of consumers of food products in which 6'-SL sodium salt is currently proposed for use. Considering all proposed food uses, the resulting consumer-only mean and 90th percentile intakes of 6'-SL sodium salt by the total U.S. population (≥2 years of age) were estimated to be 1.95 g/person/day (30 mg/kg body weight/day) and 3.60 g/person/day (64 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean intakes of 6'-SL sodium salt on an absolute basis were determined to be 2.29 g/person/day (28 mg/kg body weight/day), as identified among the elderly, while the highest 90th percentile intakes of 6'-SL sodium salt on an absolute basis were determined to be 4.26 g/person/day (58 mg/kg body weight/day), as identified among female adults. While infants 0 to 6 months of age had the lowest consumer-only intakes on an absolute basis (0.49 and 0.90 g/person/day at the mean and 90th percentile, respectively), infants 7 to <12 months of age had the highest daily mean and 90th percentile intakes on a body weight basis, of up to 110 mg/kg body weight/day (1.00 g/person/day) and 190 mg/kg body weight/day (1.74 g/person/day), respectively. Top contributors to total mean intakes were: “Term infant formula” in infants 0 to 6 months of age (contributed 65% to total mean intakes); “Term infant formula” and “Other baby foods” in infants 7 to <12 months of age (contributed 20 and 25% to total mean intakes, respectively); and “Breads and baked goods” in all remaining population groups (contributed 28 to 54% to total mean intakes). The mean and 90th percentile consumer-only intakes of 6'-SL sodium salt from use in infant formula and toddler formula only were highest in infants 0 to 6 months of age on both an absolute and body weight basis, at 61 mg/kg body weight/day (0.39 g/person/day) and 103 mg/kg body weight/day (0.62 g/person/day), respectively.

The estimated daily intake of 6'-SL sodium salt from all proposed conditions of use in infants was compared to that from human milk. 6'-SL sodium salt intakes are up to approximately 3-fold higher when additive exposure from formula and conventional foods are considered together. Mean consumer-only intakes from all proposed conditions of use (74 to 110 mg/kg body weight/day) are within the average range of 6'-SL intakes resulting from the mean consumption of human milk (5 to 211 mg/kg body weight/day), whereas 90th percentile intakes (119 to 190 mg/kg body weight/day) are below the maximum estimated daily intake of 6'-SL from the high-level consumption of human milk (317 mg/kg body weight/day). Considering exposure from infant formula and toddler formula only, mean and 90th percentile consumer-only intakes of 6'-SL sodium salt (up to 61 and 103 mg/kg body weight/day, respectively) are within the average range of 6'-SL intakes from the mean consumption of human milk (5 to 211 mg/kg body weight/day), and below maximum 6'-SL intakes from the high-level consumption of human milk (317 mg/kg body weight/day). As 6'-SL sodium salt intakes from all proposed conditions of use are within background exposure to 6'-SL from human milk in infants, a vulnerable population group, 6'-SL sodium salt is considered to be safe for all population groups.

Breastfed infants are not expected to be high consumers of both 6'-SL from breast milk and 6'-SL from complementary foods, as the consumption of breast milk would decrease as the consumption of complementary foods increases. Thus, additive exposure from high-level consumption of 6'-SL from breast milk and high-level consumption of complementary foods is unlikely. Therefore, no safety concerns are anticipated due to consumption of complementary foods supplemented with 6'-SL by breastfed infants.

Part 4. §170.240 Self-Limiting Levels of Use

No known self-limiting levels of use are associated with 6'-SL sodium salt.

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 conclusion that 6'-SL sodium salt produced by fermentation using a genetically modified strain of *E. coli* W is GRAS for use as an ingredient in non-exempt term infant formula, conventional foods, and foods for special dietary uses is based on scientific procedures.

Kyowa's 6'-SL has been demonstrated to be chemically and structurally equivalent to 6'-SL from bovine milk or colostrum by LC-MS, ^{1}H NMR, and ^{13}C NMR, which has been demonstrated to be structurally and chemically identical to 6'-SL in human milk (Aldredge *et al.*, 2013). On the basis of the chemical and structural identity to 6'-SL from human milk, the natural background dietary exposure to 6'-SL from the consumption of human milk is the primary consideration in the assessment of the safety of Kyowa's 6'-SL sodium salt ingredient. As previously noted by EFSA (EFSA, 2020a):

> "*As with other oligosaccharides, which are natural components of human milk, the safety assessment is mainly based on the comparison between the natural intake in breastfed infants and the estimated intake as NF* [novel food]*. The same considerations apply for lactose and other mono- and oligosaccharides (i.e. sialic acid) that are only present as a very small fraction in the NF and considered of no safety concern*".

Background dietary exposure to 6'-SL was discussed in Section 3.2 and the mean intake of 6'-SL from transitional and mature human milk by infants was determined to range between 5 and 211 mg/kg body weight/day, with a maximum intake of up to 317 mg/kg body weight/day from the upper range of the reported mean concentrations of 6'-SL and high-level consumption of human milk. The estimated daily intake of 6'-SL sodium salt from the proposed conditions of use was discussed in Section 3.4. Mean consumer-only intakes from all proposed conditions of use in infants 0 to <12 months (74 to 110 mg/kg body weight/day) are within the average range of 6'-SL intakes resulting from the mean consumption of breast milk (5 to 211 mg/kg body weight/day), whereas 90th percentile intakes of 6'-SL sodium salt (119 to 190 mg/kg body weight/day) are below the maximum estimated daily intake of 6'-SL from the upper range of the reported mean concentrations of 6'-SL and high-level consumption of human milk (317 mg/kg body weight/day). Infants 7 to <12 months of age were identified as having the highest mean and 90th percentile consumer-only intakes of any population group on a body weight basis, of 110 and 190 mg/kg body weight/day, respectively. Therefore, natural background dietary intakes of 6'-SL from the consumption of human milk are higher than those estimated under the proposed conditions of use of Kyowa's 6'-SL sodium salt and support the safety of Kyowa's 6'-SL sodium salt ingredient under the proposed conditions of use. As 6'-SL intakes from all proposed conditions of use are within background exposure from human milk in infants, a vulnerable population group, 6'-SL is considered to be safe for all population groups.

The composition of Kyowa's 6'-SL sodium salt is similar to other 6'-SL sodium salt ingredients previously concluded to be GRAS and notified to the U.S. FDA. Specifications for Kyowa's 6'-SL sodium salt produced by a genetically modified strain of *E. coli* W are compared to those for other 6'-SL sodium salt ingredients previously concluded to be GRAS and notified to the FDA without questions (GRNs 881 and 922 –Glycom A/S, 2019a; Jennewein Biotechnologie GmbH, 2020a; U.S. FDA, 2020e, 2021b) in Table 6.1-1 below. The proposed purity specification for Kyowa's 6'-SL sodium salt produced using a genetically modified strain of *E. coli* W is similar to but slightly lower than the purity of 6'-SL sodium salt ingredients produced using genetically modified strains of *E. coli* K-12 or *E. coli* BL21 (*i.e.*, ≥82% *vs.* ≥90.0% dwb; as specified in GRNs 881 and 922). The specification limits for other carbohydrates in Kyowa's 6'-SL sodium salt produced with a genetically modified strain of *E. coli* W are comparable to the limits for other carbohydrates in 6'-SL sodium salt ingredients notified to the FDA by Glycom A/S

(Glycom) and Jennewein Biotechnologie GmbH (Jennewein) as GRAS for their intended uses (see Table 6.1-1). Additionally, the results of analytical testing show that typical lots of Kyowa's final product contain 3.85 to 5.75% total other carbohydrates (see Section 2.3.3.1), while total other carbohydrates in Glycom's 6'-SL sodium salt were 3.52 to 4.67% in representative lots (Glycom A/S, 2019a – GRN 881). In the 6'-SL sodium salt ingredient notified to the FDA by Jennewein, the levels of all other carbohydrates (*i.e.*, other than 6'-SL) were below the limit of quantification (Jennewein Biotechnologie GmbH, 2020a – GRN 922).

Kyowa's 6'-SL sodium salt ingredient is purified using similar processes as those previously reported to the U.S. FDA for the purification of other 6'-SL and 3'-SL sodium salt ingredients. Purification processes described in other GRAS notices for 6'-SL and 3'-SL sodium salt ingredients generally involve several microfiltration, ultrafiltration, and/or nanofiltration steps to remove microbial biomass, proteins, DNA, lipopolysaccharides, minerals, and other small molecules (GRNs 571, 766, 880, 881, 921, and 922 – Jennewein Biotechnologie GmbH, 2015, 2020a,b; GeneChem, Inc., 2018; Glycom A/S, 2019a,b; U.S. FDA, 2015, 2018c, 2020e,f,g, 2021b). The manufacturing process for Kyowa's 6'-SL sodium salt similarly includes several microfiltration steps and an ultra-filtration step. In all previously described manufacturing processes for 6'-SL and 3'-SL sodium salt ingredients, the use of anionic and/or cationic resins to remove charged compounds (*e.g.,* proteins, DNA, organic acids, inorganic salts, and colored compounds) was noted, and Kyowa uses a series of cationic resin and anionic resin ion exchangers for the same purposes. Electrodialysis also has been reported as a method of removal of charged molecules (GRN 571). One or more treatments with adsorbent materials (activated carbon, activated charcoal, or unspecified) are used for the removal of colorants and other unspecified impurities (GRNs 571, 766, 880, 881, 921, and 922); Kyowa uses activated carbon for this purpose.

It was concluded that Kyowa's 6'-SL sodium salt produced with a genetically modified strain of *E. coli* W is equivalent to other 6'-SL sodium salt ingredients produced from microbial sources and notified to the FDA on the basis of the similarity in purity and specified limits for other carbohydrate impurities, and therefore safety data included in GRAS Notices for other 6'-SL sodium salt ingredients are applicable to the current assessment.

The safety of Kyowa's 6'-SL sodium salt ingredient is supported by the results of published preclinical toxicology and human studies conducted on other 6'-SL sodium salt ingredients and the conclusions of various experts qualified by scientific training and experience to evaluate the safety of food ingredients including those used in infant formula (GRNs 881, 922 – Glycom A/S, 2019a; Jennewein Biotechnologie GmbH, 2020a; U.S. FDA, 2020e, 2021b) and EFSA (EFSA, 2020a). Safety data from GRNs 881 and 922 are incorporated herein by reference and discussed briefly below in Sections 6.3 through 6.5. An updated literature search was conducted to identify any new published scientific information pertinent to the safety of 6'-SL published since the previous GRAS evaluations (see Section 6.2). No studies were identified that would contradict Kyowa's conclusion of GRAS status for 6'-SL sodium salt. The identified studies are discussed in Sections 6.3 through 6.5.

Kyowa's 6'-SL sodium salt ingredient produced with a genetically modified strain of *E. coli* W (Lot C) has been evaluated in a series of toxicology studies, including a bacterial reverse mutation assay, an *in vivo* mouse micronucleus assay, and a 90-day oral toxicity study in Crl:CD (SD) rats [Kikuchi, 2020a (unpublished); Oguma, 2020a (unpublished); Tsuboi, 2021a (unpublished)]. The toxicology studies were performed in accordance with the OECD principles of Good Laboratory Practice (GLP) and appropriate OECD test guidelines (OECD, 1998). Kyowa's 6'-SL sodium salt was non-mutagenic at concentrations up to 5,000 µg/plate in the bacterial reverse mutation assay and did not demonstrate any potential for induction of chromosomal aberrations in male ICR mice at doses up to 2,000 mg/kg body weight. In the 90-day oral repeat dose toxicity study, there were no statistically significant, toxicologically relevant, test item-related adverse effects, and the no-observed-adverse-effect level (NOAEL) was concluded by the study authors to be 2,168 mg/kg body weight/day (the highest dose tested). Detailed descriptions

of these studies are presented in Section 6.4.1 below and the results corroborate the safety of Kyowa's 6'-SL sodium salt ingredient as well as the microbial source.

As discussed in Section 3.2.1, 3'-SL and 6'-SL are constitutional isomers wherein the sialic acid moiety is connected to the galactose unit of lactose at the 3 or 6 position *via* an α-2,3 linkage or α-2,6 linkage, respectively (ten Bruggencate *et al.*, 2014; Jacobi *et al.*, 2016). Kyowa has conducted a bacterial reverse mutation test, an *in vivo* micronucleus test, and a subchronic 90-day repeat dose toxicity study with their 3'-SL sodium salt ingredient [Kikuchi, 2020b (unpublished); Oguma, 2020b (unpublished); Tsuboi, 2021b (unpublished)]. Considering that 3'-SL is structurally related to 6'-SL, the results of the studies on Kyowa's 3'-SL sodium salt are relevant to the safety of their 6'-SL sodium salt. The studies are summarized below in Section 6.4.3 and corroborate the safety of Kyowa's 6'-SL sodium salt. The results of studies conducted with other 3'-SL ingredients also corroborate the safety of Kyowa's 6'-SL sodium salt and are discussed in Section 6.4.4.

The use of Kyowa's 6'-SL sodium salt as an ingredient in enteral tube feeding formula at levels up to 4.1 g/L (for patients 11 years of age or older) is supported by the comprehensive body of safety data pertaining to 6'-SL sodium salt in pre-clinical studies and the safety of poorly-digestible carbohydrates in general in enteral feeding at levels that exceed the recommended intake of 6'-SL sodium salt from the intended use in formula for enteral tube feeding (see Section 6.5.3).

Finally, Kyowa's 6'-SL sodium salt ingredient was concluded to be of low allergenic risk due to the effective removal of the production organism, residual DNA, and proteins; the lack of residual milk proteins; and the lack of published reports of sensitization, case reports of allergic reactions, or allergenicity studies on 6'-SL (see Section 6.6).

Table 6.1-1 Comparison of Kyowa's Specifications for 6'-Sialyllactose Sodium Salt Produced with a Genetically Modified Strain of *Escherichia coli* W to Other 6'-Sialyllactose Sodium Salt Ingredients Notified to the U.S. FDA as GRAS

Specification Parameter	Specification Limits		
	Kyowa's 6'-SL Sodium Salt	Glycom's 6'-SL Sodium Salt GRN 881	Jennewein's 6'-SL Sodium Salt GRN 922
Organoleptic			
Appearance	Powder	Powder or agglomerates	Spray-dried powder
Color	White to off-white	White to off white	White to ivory
Physicochemical			
Identification	RT of standard ± 3%	RT of standard ± 3%	-
Purity (6'-SL)	≥82% dry basis	≥90.0% dwb	≥90.0% dwb
Purity (sum of HiMS)	-	≥94.0% dwb	-
Water	≤10.5 w/w%	≤6.0 w/w%	≤9.0%
Ash	-	-	≤8.5%
Sodium (Assay)	≤5.0% dry basis	2.5 to 4.5 w/w%	≤4.2%
Chloride by IC	-	≤1.0 w/w%	-
Residual protein	≤10 mg/kg	≤0.01 w/w%	≤100 µg/g
pH (20°C, 5% solution)	4.0 to 9.0	4.5 to 6.0	-
Other Carbohydrates			
N-Acetyl D-neuraminic acid	≤9 w/w%	≤2.0 w/w%	≤10%
D-glucose	≤3 w/w%	-	-
D-lactose	≤3 w/w%	≤5.0 w/w%	≤5%
6'-sialyllactulose	≤5 w/w%	≤3.0 w/w%	-
3'-sialyllactose sodium salt	≤1 w/w%	-	-

Table 6.1-1 Comparison of Kyowa's Specifications for 6'-Sialyllactose Sodium Salt Produced with a Genetically Modified Strain of *Escherichia coli* W to Other 6'-Sialyllactose Sodium Salt Ingredients Notified to the U.S. FDA as GRAS

Specification Parameter	Specification Limits		
	Kyowa's 6'-SL Sodium Salt	Glycom's 6'-SL Sodium Salt GRN 881	Jennewein's 6'-SL Sodium Salt GRN 922
N-acetylglucosaine	-	-	≤5%
Sum of "other" carbohydrates	-	≤3.0 w/w%	≤10%
Heavy Metals			
Arsenic	≤0.2 mg/kg	-	≤0.2 mg/kg
Cadmium	≤0.2 mg/kg	-	≤0.1 mg/kg
Lead	≤0.2 mg/kg	≤0.1 mg/kg	≤0.02 mg/kg
Mercury	≤0.2 mg/kg	-	≤0.5 mg/kg
Iron	≤10 mg/kg	-	-
Microbiological Parameters			
Aerobic plate count	≤1,000 CFU/g	≤1,000 CFU/g	≤10,000 CFU/g
Molds	≤100 CFU/g	≤100 CFU/g	≤100 CFU/g
Yeasts	≤100 CFU/g	≤100 CFU/g	
Salmonella	Negative in 100 g	Absent in 25 g	Negative in 25 g
Enterobacteriaceae	Negative in 10 g	≤10 CFU/g	≤10 CFU/g
Cronobacter spp. (Enterobacter sakazakii)	Negative in 100 g	-	Negative in 10 g
Listeria monocytogenes	Negative in 25 g	-	-
Bacillus cereus	≤50 CFU/g	-	-
Residual endotoxins	≤10 EU/mg	≤10 EU/mg	≤10 EU/mg
Aflatoxin M1	-	-	≤0.25 µg/kg
GMO residues	-	-	Negative

- = parameter not established; 6'-SL = 6'-sialyllactose; CFU = colony-forming units; dwb = dry weight basis; EU = endotoxin units; GeneChem = GeneChem Inc.; Glycom = Glycom A/S; GMO = genetically modified organism; GRAS = Generally Recognized as Safe; GRN = GRAS Notice; HiMS = human-identical milk saccharides; IC = ion chromatography; Jennewein = Jennewein Biotechnologie GmbH.

6.2 Literature Search

Kyowa considered the totality of publicly available data and information relevant to the safety of 6'-SL sodium salt and literature searches for studies relevant to the safety of 6'-SL sodium salt were conducted. Comprehensive and detailed searches of the published scientific literature were conducted for studies published through 08 December 2021 using the electronic search tool, ProQuest Dialog™, with several databases, including Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile®. Consistent with the requirements of the GRAS standard, conclusions on the GRAS status of 6'-SL sodium salt have considered all publicly available sources of information including favorable and potentially unfavorable information. Based on Kyowa's search of the literature, the company is not aware of published studies to suggest 6'-SL sodium salt is unsafe for use as a food ingredient.

6.3 Absorption, Distribution, Metabolism, and Elimination

Kyowa's 6'-SL is structurally and chemically identical to 6'-SL that is naturally present in bovine milk or colostrum [see comparative nuclear magnetic resonance (NMR) and LC-MS analyses in Section 2.1], which has been demonstrated to be structurally and chemically identical to 6'-SL in human milk (Aldredge *et al.*, 2013). Therefore, on the basis that Kyowa's 6'-SL is structurally and chemically identical to 6'-SL present in human milk, the absorption, distribution, metabolism, and elimination (ADME) of Kyowa's 6'-SL would be identical to 6'-SL consumed from human breast milk. The ADME of 6'-SL has been previously reviewed in GRAS Notices for 6'-SL ingredients submitted to the U.S. FDA (GRNs 881, 922 – Glycom A/S, 2019a; Jennewein Biotechnologie GmbH, 2020a; incorporated herein by reference) and by the EFSA NDA Panel (EFSA, 2020a). HMOs, including 6'-SL, are considered to be non-digestible oligosaccharides that "*do not undergo any significant digestion in the upper gastrointestinal tract*" (EFSA, 2020a). HMOs in general are fermented in the colon by the intestinal microbiota, with 40 to 97% of ingested HMOs are excreted unchanged in the feces of breastfed infants, and up to 2% excreted unchanged in the urine (EFSA, 2020a). Breastfed infants were reported to excrete up to 3 mg/day of individual oligosaccharides following consumption of 150 mg oligosaccharides/feed and it was reported that approximately 4% of the amount of 6'-SL consumed *via* breast milk was excreted in the urine (EFSA, 2020a). In their opinion on the safety of Glycom's 6'-SL sodium salt ingredient, the EFSA NDA Panel concluded that *"limited digestion of the NF* [novel food] *occurs in the upper gastrointestinal tract and that only small amounts are expected to be absorbed*" (EFSA, 2020a). As Kyowa's 6'-SL is structurally and chemically identical to 6'-SL that is naturally present in human milk, the absorption of 6'-SL from the use of Kyowa's 6'-SL ingredient would also be limited and not different from the absorption of 6'-SL from the natural background dietary exposure from human breast milk.

A search of the published literature identified no new ADME studies of 6'-SL published since the GRAS evaluations of 6'-SL sodium salt submitted to the FDA and one study of 3'-SL that was not included in previous GRAS Notices to the U.S. FDA or included in the evaluation by the EFSA NDA Panel (EFSA, 2020a). Considering that 3'-SL and 6'-SL are constitutional isomers wherein the sialic acid moiety is connected to the galactose unit of lactose at the 3 or 6 position *via* an α-2,3 linkage or α-2,6 linkage, respectively (ten Bruggencate *et al.*, 2014; Jacobi *et al*., 2016; EFSA, 2020a), the newly identified ADME study of 3'-SL is relevant as a "read-across" in understanding the ADME of 6'-SL, and as such, is summarized herein.

The study was conducted to investigate the absorption and distribution of ^{13}C-labelled 3'-SL and NeuAc (sialic acid) in 8-week-old male NMRI mice administered ^{13}C-labelled 3'-SL, ^{13}C-labelled NeuAc, or saline vehicle by gavage or intravenous injection (Galuska *et al.*, 2020). The ^{13}C label was detected in the plasma from 3 hours after oral administration until the end of the 9-hour observation period (*i.e*., time points corresponding with the compounds reaching the lower gastrointestinal tract), and at the same time points but to a lesser extent in the brain, liver, heart, spleen, and kidney. Urinary and fecal excretion peaked 5 hours after oral dosing, with levels of ^{13}C label reported to be higher than in plasma and tissues. Intact NeuAc following the oral administration of ^{13}C labelled 3'-SL or NeuAc was detected in urine at 5 and 9 hours, respectively, and in 3 and 1 plasma samples at 5 hours following administration of ^{13}C labelled 3'-SL or NeuAc. The authors interpreted these results to indicate that intact NeuAc was absorbed into systemic circulation and immediately excreted into the urine after oral administration of 3'-SL and NeuAc. The authors noted that there was no uptake of ^{13}C-3'-SL or ^{13}C-NeuAc from the blood to the brain or other tissues after intravenous administration (the compounds were instead excreted quickly in the urine). The authors hypothesized that ^{13}C uptake after oral administration is not organ-specific but occurs in parallel to increases in plasma levels, and that ^{13}C enrichment of brain tissues was not derived from the intact compounds, but from the absorption of small amounts of metabolic products of the intestinal microbiota, intestinal epithelial cells, and/or liver cells. The authors noted that both administered compounds were labelled at the C1, C2, and C3

positions, and suggested that the cleavage of pyruvate from the NeuAc moiety would yield ^{13}C-labelled pyruvate, which could have been taken up by various tissues including the brain. The results of this study support the previous conclusions that there is no significant absorption of 3'-SL, and by extension as a "read-across" substance, 6'-SL, from the upper gastrointestinal tract and that it is fermented by the intestinal microbiota.

The levels of other carbohydrates in Kyowa's 6'-SL sodium salt produced with a genetically modified strain of *E. coli* W are comparable to the levels in Glycom's 6'-SL ingredient notified to the U.S. FDA as GRAS for its intended uses (see Table 6.1-1). The results of analytical testing show that typical lots of Kyowa's final product contain 3.85 to 5.75% total other carbohydrates (see Section 2.3.3.1), while total other carbohydrates in Glycom's 6'-SL sodium salt were 3.52 to 4.67% in representative lots (Glycom A/S, 2019a – GRN 881). These other carbohydrates (*N*-acetyl D-neuraminic acid, glucose, lactose, and 3'-SL) are naturally occurring components of human milk, or in the case of glucose, a breakdown product of the naturally occurring milk sugar lactose, or in the case of 6'-sialyllactulose, an isomerization product of 6'-SL formed when the terminal glucose moiety isomerizes into fructose (EFSA, 2020a). As discussed in Section 2.4.1, it is expected that 6'-sialyllactulose would be present at a similar ratio to 6'-SL as the contents of lactulose to lactose in heat-treated human milk (Beach and Menzies, 1983; Schuster-Wolff-Bühring *et al.*, 2010; Gómez de Segura *et al.*, 2012), and as such, would have a history of safe consumption as a component of heat-treated human milk. Furthermore, the ADME profile of 6'-sialyllactulose and the other naturally-occurring carbohydrates following the consumption of Kyowa's 6'-SL sodium salt is not expected to differ from the ADME profile of these compounds from human milk.

As the absorption and metabolism of 6'-SL and other components of the 6'-SL ingredient (*i.e.*, other carbohydrates) would not differ from the absorption and metabolism of these compounds from human milk, it can be concluded that there is no concern for safety from the potential limited absorption of 6'-SL and the absorption of the naturally occurring other carbohydrates from the ingredient. Absorption of 6'-sialyllactulose also does not pose a concern for safety due to the history of safe consumption from heat-treated human milk and considering intakes resulting from the proposed uses are substantially lower than the levels of lactulose recommended for laxative purposes (EFSA, 2020a).

6.4 Toxicological Studies

6.4.1 Studies Conducted on Kyowa's 6'-SL Sodium Salt

Kyowa has conducted a battery of toxicology studies on their 6'-SL sodium salt ingredient, including a bacterial reverse mutation test, an *in vivo* micronucleus test, and a 90-day repeat dose oral toxicity study. The results from these studies are discussed below and corroborate the results of published toxicology studies on other 6'-SL sodium salt ingredients and corroborate the safety of Kyowa's 6'-SL sodium salt ingredient.

6.4.1.1 Genotoxicity

6.4.1.1.1 Bacterial Reverse Mutation Test

The potential mutagenicity of 6'-SL sodium salt (Lot C; purity of 90% dwb, equivalent to 85.1% 6'-SL sodium salt on an as-is basis) was evaluated in a bacterial reverse mutation test, which was performed in compliance with the OECD principles of GLP (OECD, 1998) and according to OECD TG 471 (OECD, 1997) [Oguma, 2020a (unpublished)].

Two main tests, conducted as pre-incubation assays, were performed using *Salmonella* Typhimurium strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2 uvrA, which were exposed to 6'-SL

sodium salt at concentrations of 313, 625, 1,250, 2,500 or 5,000 µg/plate (the OECD TG 471 maximum recommended concentration) in the absence and presence of external metabolic activation (S9 mix).

Water (for injection) served as the vehicle for 6'-SL sodium salt and as the negative control. Positive controls were also included in the presence (2-aminoanthracene and benzo[a]pyrene) and absence [(2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, sodium azide and 2-methoxy-6-chloro-9-(3-(2-chloroethyl)aminopropylamino) acridine, dihydrochloride] of metabolic activation.

The test solutions, test strain, and metabolic activation (where applicable) were incubated while shaking at 37°C for 20 minutes. Top agar kept at 45°C was then added, and the mixture shaken, and overlaid on a minimal glucose agar plate medium. After solidification of the overlaid top agar, the plates were incubated upside down at 37°C for 48 hours. After the incubation period, the plates were observed for coloration and precipitation, and the numbers of revertant colonies were counted using a Dot Counter (IEDA Trading Co.). Growth inhibition was observed using a stereoscopic microscope. A positive result for mutagenicity was defined as a dose-dependent and biologically relevant greater-than-2-fold increase in the number of revertant colonies, compared to that of the vehicle control group.

There was no evidence of mutagenicity in either test, in the absence or presence of metabolic activation. The mean number of revertant colonies was less than twice that of the negative control at all test concentrations, and there was no dose response observed in any test system with or without metabolic activation. No growth inhibition or precipitation of the test substance was observed.

Based on the results of the study, it was concluded that 6'-SL sodium salt is non-mutagenic at concentrations up to 5,000 µg/plate (the OECD TG 471 maximum recommended concentration).

6.4.1.1.2 In Vivo Micronucleus Test

The potential clastogenicity and aneugenicity of 6'-SL sodium salt (Lot C; purity of 90% dwb, equivalent to 85.1% 6'-SL sodium salt on an as-is basis) was evaluated in an *in vivo* micronucleus test with ICR mice (Inasa Branch, Japan SLC, Inc.). This study was conducted in compliance with the OECD principles of GLP (OECD, 1998) and OECD TG 474 (OECD, 2016) [Kikuchi, 2020a (unpublished)].

In a dose-range finding study conducted to determine the dose levels for the main study, ICR [Slc:ICR] mice (3/sex/group) were administered 6'-SL sodium salt by gavage at doses of 0, 500, 1,000, or 2,000 mg/kg body weight. No clinical signs or mortality were observed at any test dose, and no significant changes in body weight or bone marrow were observed. Therefore, in the main study, male ICR mice (5/group; 9 weeks old) were administered 6'-SL sodium salt by gavage twice (at a 24-hour interval) at doses of 500, 1,000, or 2,000 mg/kg body weight. Mitomycin C (2 mg/kg body weight) (administered intraperitoneally) served as the positive control and the vehicle (water for injection) was used as the negative control. General observations of the animals were performed before the initial administration, at least 1 hour after administration, and 2, 3, and 6 hours after administration of the first- (Day 0) and second doses (Day 1). Body weights were recorded on Days 0, 1, and 2.

Animals were euthanized by cervical dislocation on Day 2 and femoral bone marrow cells were harvested for analysis. Bone marrow cells were washed with fetal bovine serum, centrifuged, and re-suspended. Smear preparations were dried, fixed in methanol, stained with 3% Giemsa solution, and rinsed with tap water. Samples of immature erythrocytes (IMEs) and mature erythrocytes (MEs) were separately counted using an oil-immersed object lens magnifying 100 diameters. The proportion of IMEs among total erythrocytes was determined by counting 1,000 erythrocytes (IMEs and MEs) per animal. A total of 4,000 IMEs were scored for the incidence of micronucleated immature erythrocytes (MNIMEs). A finding was considered to be positive if the incidence of MNIMEs in at least 1 test group increased significantly and in a dose-dependent manner. The acceptability of the test was determined

by the frequencies of MNIMEs in the negative and positive control groups being within the ranges of in-house background data, and the positive control resulting in a statistically significant increase in MNIMEs compared to the negative control.

No clinical signs or abnormalities and no statistically significant changes in the body weights of any animal were observed in the test substance, negative, and positive control groups. No significant changes in MNIME frequency were observed between the test substance and negative control groups. Conversely, the frequency of MNIMEs was significantly increased in the positive control group compared to the negative control group, thus confirming the acceptability of the study. No significant difference in the proportion of IMEs among total erythrocytes was observed among the study groups.

Based on the results of this study, 6'-SL sodium salt was concluded to have no potential for induction of chromosomal aberrations in male ICR mice at doses up to 2,000 mg/kg body weight.

6.4.1.2 Subchronic Toxicity

6.4.1.2.1 90-Day Toxicity Study in Rats

A 90-day repeat dose toxicity study was conducted to evaluate the potential subchronic toxicity of 6'-SL sodium salt when administered by gavage to Crl:CD(SD) rats [Tsuboi, 2021a (unpublished)]. The study was conducted in compliance with the OECD principles of GLP (OECD, 1998) and according to OECD TG 408 (OECD, 2018).

Animals were quarantined and acclimated for 8 days following receipt. Groups of 10 male and 10 female Crl:CD(SD) rats received 0 (distilled water for injection), 542, 1,084, or 2,168 mg 6'-SL sodium salt/kg body weight/day, by gavage at a dose volume of 10 mL/kg body weight for 90 days. Lot C was used, which had a purity of 90% dwb, equivalent to 85.1% 6'-SL sodium salt on an as-is basis. Reported dose-levels were based on the reported purity[5]. Animals were observed twice daily before and after administration (Days 1 to 90) and once on Day 91, before necropsy. Body weights were recorded on Days 1, 4, 8, 12, 15, 19, 22, 26, 29, 36, 43, 50, 57, 64, 71, 78, 85, 90, and 91. Food intake was recorded on Days 1, 3, 7, 11, 14, 18, 21, 25, 28, 35, 42, 49, 56, 63, 70, 77, 84, and 89. The weight of the remaining diet was measured on the following days (*i.e.*, Days 1, 4, 8, 12, 15, 19, 22, 26, 29, 36, 43, 50, 57, 64, 71, 78, 85, and 90) to calculate the daily food consumption of each animal. Detailed observations of all animals were conducted once during the quarantine period and weekly 1 to 2 hours after administration. In Week 11 (males: Day 72; females: Day 75) the sensory reactivity (reactions to auditory, visual, proprioceptive, and pain stimuli), grip strength, and locomotor activity were examined in all animals. Ophthalmologic examinations of all animals were performed once in the quarantine period and once in Week 13 for all animals in the control and 2,168 mg/kg body weight/day groups. No examinations were performed for the 542 and 1,084 mg/kg body weight/day groups in Week 13 as the 2,168 mg/kg body weight/day group did not display any ophthalmological abnormalities. Urine samples were collected for urinalysis in Week 13. Blood samples were taken for evaluation of hematology, blood chemistry, and blood coagulation parameters on Day 91 following an overnight fast (with free access to water). The estrus cycle of all females was examined on Day 91 *via* vaginal smear.

At the end of the treatment period, all surviving animals were euthanized by exsanguination and subjected to a gross necropsy, which included macroscopic examination of the body surface, orifices, cranial cavity, thoracic cavity, abdominal cavity, and contents of each. The following organs and tissues were collected and fixed: adrenal glands, aorta, brain (cerebrum, cerebellum, and medulla oblongata),

[5] Doses were planned to be 0, 500, 1,000, and 2,000 mg/kg body weight/day, with the highest dose selected in accordance with OECD TG 420: *Acute oral toxicity - Fixed dose procedure*; however, due to a correction in the analysis of the purity of the test article, which resulted in a higher purity value than initially reported, the doses used in the study were calculated to be 0, 542, 1,084, and 2,168 mg/kg body weight/day.

cervical lymph nodes, duodenum, epididymides, eyeball (including optic nerve), femur (bone and marrow), femoral muscle, Harderian glands, heart, ileum (including Peyer's patch), jejunum, cecum, colon, kidneys, liver, lungs (with bronchi), mammary gland, mesenteric lymph nodes, esophagus, ovaries, pancreas, pituitary gland, prostate gland, rectum, salivary glands, sciatic nerve, seminal vesicles (including coagulation glands), spinal cord, spleen, skin, sternum (bone and marrow), stomach (forestomach and glandular stomach), testes, thymus, thyroid glands (with parathyroids), tongue, trachea, urinary bladder, uterus (with cervix) and vagina. Histopathological evaluation of all organs and tissues was conducted on animals in the vehicle control and 2,168 mg/kg body weight/day groups. Due to the lack of toxicologically relevant results in high-dose animals, histopathological examinations were not conducted for the 542 and 1,084 mg/kg body weight/day groups. The heart, thymus, lungs, thyroid glands, spleen, liver, kidneys, pituitary gland, adrenal glands, testes, epididymides, uterus, ovaries, and brain (cerebrum, cerebellum, and medulla oblongata) were weighed prior to fixation and organ weight relative to body weights on the day of necropsy were calculated.

There were no test item-related deaths, clinical signs, or changes in body weight or food consumption in any of the groups throughout the administration period. There were no abnormal findings in any of the groups in the detailed or functional observation, gross pathology, or ophthalmological or estrous cycle parameters.

No test item-related differences in values for urinary parameters were observed in any of the groups. Significant differences in urinary electrolytes [increased sodium (Na) concentration and Na excretion in the mid- and high-dose males and increased Na concentration in mid-dose females, decreased chloride (Cl) concentration in high-dose males and decreased Cl excretion in high-dose males and females, decreased potassium (K) excretion in high-dose males and females] were concluded to be not toxicologically relevant as no abnormal changes in blood electrolytes or associated organs were observed.

No test item-related differences in values for hematological parameters were observed in any of the groups. A significant increase in platelet count was reported in low-dose males but was considered by the study authors to be not toxicologically relevant due to a lack of dose-dependence, absence of hypercoagulative changes in any organs, as well as the small magnitude and sex-specificity of the effect.

No test item-related differences in values for blood chemical parameters were observed in any of the groups. Statistically significant increases in alanine aminotransferase and sodium were reported in high-dose males and females, respectively. However, these differences were not considered to be toxicologically relevant by the study authors due to the small magnitude of change compared to concentrations reported in control animals.

No test item-related differences in organ weights were observed in any of the groups. Several statistically significant differences were observed but were considered by the authors to be not toxicologically relevant due to the lack of dose-dependency.

No test item-related differences or test item-related histopathological findings were observed in any of the animals. Several findings were noted more frequently in high-dose animals than in controls but were considered to be spontaneous and not test item-related due to their low frequency, morphological conformity between controls and high-dose animals, and/or unilateral observation. These findings include the following:

- Heart:
    - Mononuclear cell infiltration of ventricular wall (2 males and 1 female in the 2,168 mg/kg body weight/day group);

- Pancreas:
    - Focal islet fibrosis (1 male in the 2,168 mg/kg body weight/day group);
    - Focal atrophy of acinar cells (1 male in the 2,168 mg/kg body weight/day group);

- Kidney:
    - Unilateral scarring (1 female in the 2,168 mg/kg body weight/day group);

    - Unilateral basophilic change of the tubular cortex (1 male in the 2,168 mg/kg body weight/day group);

- Stomach:
    - Dilatation of the glandular stomach lumen (1 male in the control group, 2 males in the 2,168 mg/kg body weight/day group);

    - Focal ectopic mucosal tissue in the glandular stomach (1 male in the 2,168 mg/kg body weight/day group);

- Adrenal:
    - Unilateral accessory adrenal gland (1 female in the 2,168 mg/kg body weight/day group);

- Eyeball:
    - Unilateral retinal dysplasia (1 male and 1 female in the control group, 3 males in the 2,168 mg/kg body weight/day group).

Other histopathological findings were deemed to be unrelated to the test item due to observation in high-dose animals at equal or lesser frequency than control animals.

In the absence of any statistically significant, toxicologically relevant, test item-related adverse effects, the NOAEL was concluded by the study authors to be 2,168 mg/kg body weight/day (the highest dose tested).

6.4.2 Studies Conducted with Other 6'-SL Preparations

6.4.2.1 Overview

Toxicological studies have been conducted on other 6'-SL preparations and reported in the literature. The source and purity of these ingredients are summarized and compared to Kyowa's 6'-SL sodium salt produced from a genetically modified strain of *E. coli* W in Table 6.4.2.1-1 below. As demonstrated in the table, the purity of the 6'-SL preparations are similar, and as such, toxicological data on these other 6'-SL preparations are relevant to the safety assessment of Kyowa's 6'-SL ingredient. The preclinical toxicology studies reported by Jacobi *et al.* (2016), Gurung *et al*. (2018), and Phipps *et al.* (2019a) have been reviewed during previous GRAS evaluations notified to the U.S. FDA and filed as GRNs 766, 880, 881, and 922 (GeneChem, Inc., 2018; Glycom A/S, 2019a,b; Jennewein Biotechnologie GmbH, 2020a), to which the FDA responded with no questions (U.S. FDA, 2018c, 2020e,fc, 2021b).

An additional gastrointestinal developmental toxicity study conducted by Monaco *et al.* (2020) that was not included in previous GRAS evaluations notified to the U.S. FDA was identified in the literature search and is further discussed in Section 6.4.2.5 below.

Table 6.4.2.1-1 Test Articles Used in Safety Studies Conducted with Other 6'-Sialyllactose Preparations

Parameter	6'-SL Preparations Tested		
	Kyowa's 6'-SL by Microbial Fermentation	**Glycom's 6'-SL by Microbial Fermentation (Phipps *et al.*, 2019a)**	**GeneChem's 6'-SL by Enzymatic Synthesis (Gurung *et al.*, 2018; Monaco *et al.*, 2020)**
Production Organism	GM strain of *Escherichia coli* W	GM strain of *E. coli* K-12	Enzymes
Purity (6'-SL assay)	90%	96.8%	98.8%
Toxicology/Safety Studies Conducted	• Bacterial reverse mutation test • *In vivo* mammalian cell micronucleus test • 90-day oral toxicity study	• Bacterial reverse mutation test • *In vitro* mammalian cell micronucleus test • 90-day oral toxicity study (with neonatal rats)	• Bacterial reverse mutation test • *In vitro* chromosome aberration test • *In vivo* mammalian erythrocyte micronucleus test • Acute toxicity study (with weaned rats) • 13-week oral toxicity study (with weaned rats) • 21-day oral toxicity and gastrointestinal developmental study (with neonatal piglets)

6'-SL = 6'-sialyllactose sodium salt; GeneChem = GeneChem Inc.; Glycom = Glycom A/S; GM = genetically modified.

6.4.2.2 Genotoxicity

The potential genotoxicity of other 6'-SL sodium salt preparations was evaluated *in vitro* in bacterial and mammalian test systems (Gurung *et al.*, 2018; Phipps *et al.*, 2019a) and *in vivo* in mice (Gurung *et al.*, 2018). These studies have been included in previous GRAS evaluations that have been notified to the U.S. FDA with no objections (GRNs 880, 881, and 922). The results of these studies are summarized in Table 6.4.2.2-1. The consistently negative results reported in *in vitro* and *in vivo* studies demonstrate that 6'-SL sodium salt lacks genotoxic potential.

Gurung *et al.* (2018) evaluated the genotoxic potential of GeneChem's 6'-SL sodium salt in a bacterial reverse mutation test, an *in vitro* chromosome aberration test and an *in vivo* mammalian erythrocyte micronucleus test.

A bacterial reverse mutation assay was conducted using a plate incorporation method in *S.* Typhimurium strains TA97, TA98, TA100, TA102, and TA1535 in the absence and presence of metabolic activation at concentrations of 0 (solvent control), 100, 300, 625, 1,250, 2,500, or 5,000 µg 6'-SL sodium salt/plate (98.8% purity; produced by enzymatic synthesis; GeneChem) in triplicate (Gurung *et al.*, 2018). 4-Nitro-o-phenylenediamine (NPD), daunomycin, sodium azide, and methyl methanesulfonate in the absence of metabolic activation, and 2-aminofluorene, 1,8-dihydroxyanthraquinone, and 2-aminoanthracene in the presence of metabolic activation, served as the positive controls. All plates were incubated at 37°C for 72 hours and the number of revertant colonies were counted. The number of revertant colonies in all strains treated with 6'-SL sodium salt at all concentrations in the presence and absence of metabolic activation were less than twice that of the negative control values. Growth inhibition was observed in *S.* Typhimurium strain TA98 at concentrations 2,500 and 5,000 µg 6'-SL sodium salt/plate. Based on the results of the study, the authors concluded that 6'-SL sodium salt was not mutagenic.

The clastogenicity of 6'-SL sodium salt (98.8% purity; produced by enzymatic synthesis) was assessed by Gurung *et al.* (2018) in 2 separate *in vitro* chromosome aberration tests in Chinese hamster lung (CHL/IU) cells at concentrations of 0 (solvent control), 225, 450, or 900 µg/mL in the presence and absence of metabolic activation. Mitomycin C and cyclophosphamide served as the positive controls. In the short-term assay, CHL cells were incubated for 6 hours followed by an 18-hour expression period in the presence of metabolic activation, while cells in the continuous assay were incubated for 24 hours in the absence of metabolic activation. In both assays and at all concentrations, there was no increase in the frequency of cells with structural or numerical aberrations compared to the negative control culture. Moreover, cell growth was not inhibited at any concentrations of 6'-SL sodium salt. Based on the results of the study, the authors of the study concluded that 6'-SL sodium salt was non-mutagenic and non-clastogenic in the presence and absence of metabolic activation (Gurung *et al.*, 2018).

An *in vivo* micronucleus test also was carried out by Gurung *et al.* (2018) in 4- to 5-week-old Kunming mice (SPF grade; n=5/group) administered 6'-SL sodium salt (98.8% purity; produced by enzymatic synthesis) at doses of 500, 1,000, or 2,000 mg/kg body weight/day of *via* gavage for 2 consecutive days at 18-hour intervals. Cyclophosphamide (40 mg/kg) and purified water served as the positive and negative controls, respectively. Clinical signs were observed regularly until sacrifice. Animals were sacrificed 24 or 48 hours after final dosing and femurs were removed, cleaned, and bone marrow was collected. The proportion of immature erythrocytes (PCEs) to total erythrocytes [immature and mature erythrocytes (normochromatic erythrocytes, NCEs)] and incidence of micronucleated polychromatic erythrocytes (MNPCEs) were assessed. No clinical signs of toxicity were observed, and no statistically significant changes in mean body weights were reported in any group compared to controls. No significant changes in the incidence of MNPCE or PCE/NCE were observed in animals administered 6'-SL sodium salt compared to the control group. Based on the results of the study, the authors determined that 6'-SL sodium salt was not clastogenic (Gurung *et al.*, 2018).

Phipps *et al.* (2019a) evaluated the genotoxic potential of Glycom's 6'-SL sodium salt in a bacterial reverse mutation assay and an *in vitro* chromosome aberration test.

Phipps *et al.* (2019a) conducted a bacterial reverse mutation assay using *S.* Typhimurium strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2*uvr*A (pKM101) with 6'-SL sodium salt (96.8% purity) at concentrations of 5, 15, 50, 150, 500, 1,500, or 5,000 µg/mL in the absence and presence of metabolic activation. Sodium azide, 2-nitrofluorene, 9-aminoacridine, and 4-nitroquinoline-1-oxide served as positive controls in the absence of metabolic activation, whereas 2-aminoanthracene and benzo[a]pyrene served as the positive controls in the presence of metabolic activation. Water served as the negative/vehicle control. In both the presence and absence of metabolic activation, no biologically relevant differences in revertant colonies were observed relative to the negative control, and the authors concluded that 6'-SL sodium salt was not genotoxic.

An *in vitro* micronucleus test was conducted by Phipps *et al.* (2019a) using human peripheral blood lymphocytes from healthy non-smoking adults, which were exposed to 500, 1,000, or 2,000 µg 6'-SL sodium salt/mL for 3 hours with and without metabolic activation, or for 20 hours without metabolic activation. Mitomycin C and colchicine, or cyclophosphamide, served as positive controls in the absence and presence of metabolic activation, respectively. Water was used as the vehicle control. No biologically relevant differences were observed in the percentage of micronucleated cells between the cells incubated with 6'-SL sodium salt and the vehicle controls, and the authors concluded that 6'-SL sodium salt was not genotoxic.

Table 6.4.2.2-1 Genotoxicity Studies of Other 6'-Sialyllactose Preparations

Test	Test System/Animal Species	Test Article Concentration/Dose	Results	Reference
***In Vitro* Studies**				
Bacterial reverse mutation test	*Salmonella* Typhimurium TA98, TA100, TA102, TA1535, and TA1537	6'-SL sodium salt 0, 100, 300, 625, 1,250, 2,500, or 5,000 µg/plate +/- S9	Negative	Gurung *et al.* (2018)
Bacterial reverse mutation test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, and *Escherichia coli* WP2*uvr*A (pKM101)	6'-SL sodium salt 0, 5, 15, 50, 150, 500, 1,500, or 5,000 µg/mL +/- S9	Negative	Phipps *et al.* (2019a)
Chromosomal aberration	Chinese hamster lung cells	6'-SL sodium salt 0, 225, 450, or 900 µg/mL +/- S9	Negative	Gurung *et al.* (2018)
Micronucleus test	Human peripheral lymphocytes	6'-SL sodium salt 0, 500, 1,000, or 2,000 µg/mL 3 hours: +/- S9 20 hours: - S9	Negative	Phipps *et al.* (2019a)
***In Vivo* Studies**				
Micronucleus test	Kunming mice, SPF grade (4- to 5-week-old; 5/group)	6'-SL sodium salt 0, 500, 1,000, or 2,000 mg/kg bw/day Oral (gavage), 2 consecutive days	Negative	Gurung *et al.* (2018)

+ S9 = with metabolic activation; - S9 = without metabolic activation; 6'-SL = 6'-sialyllactose sodium salt; bw = body weight.

6.4.2.3 Subchronic Toxicity

Two 90-day repeat-dose studies of 6'-SL sodium salt were identified in the literature (Gurung *et al.*, 2018; Phipps *et al.*, 2019a). These studies have been included in previous GRAS evaluations that have been notified to the U.S. FDA with no objections (GRNs 880, 881, and 922). These studies are summarized below and in Table 6.4.2.3-1. Overall, no compound-related adverse effects were reported in rats administered doses up to 5,000 mg 6'-SL sodium salt/kg body weight/day for 90 days.

In a 90-day toxicity study, 6- to 7-week old Sprague-Dawley rats (11/sex/group) were administered 6'-SL sodium salt (98.8% purity; produced by enzymatic synthesis; GeneChem) by gavage at doses of 0 (purified water), 1,000, 2,500, or 5,000 mg/kg body weight/day (Gurung *et al.*, 2018). The animals were observed for clinical signs of toxicity twice daily. Body weights were measured pre-test, once weekly during the treatment period, and prior to sacrifice. Ophthalmic examinations were conducted during the pre-dose phase and at termination. At the end of the study period, animals were fasted, and blood collected. Clinical chemistry parameters included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bile acids, total protein, albumin, total bilirubin, gamma-glutamyl transferase, glucose, cholesterol, creatinine, urea nitrogen, triglycerides, phosphorus, sodium, potassium, calcium, chloride, and globulin. Hematological parameters included hemoglobin, hematocrit, red blood cells, total leukocyte count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, and differential leukocyte counts (*i.e.*, neutrophils, lymphocytes, and monocytes). External and internal gross pathological examination was performed on sacrificed animals following organ weight measurements.

Histopathological examination included the adrenal glands, femur, eyes, vagina, aorta, bone marrow (sternum), brain, cecum, colon, uterus, duodenum, epididymis, esophagus, heart, ileum, jejunum, kidneys, liver, lung, mandibular lymph nodes, mesenteric lymph nodes, mammary glands, nasal turbinates, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, and urinary bladder. No clinical signs of toxicity or mortality were observed at any dose. Body weights and food consumption were comparable among treatment and control groups. No statistically significant, dose-dependent, or compound-related effects were noted with respect to ophthalmoscopy, hematology, or urinalysis parameters.

Statistically significant differences were reported with respect to several blood biochemical parameters; however, these results were sex-specific, not dose-related, and/or were considered by the authors to be incidental changes or biological variations and not adverse or compound-related. These differences included: increased total serum protein in mid- and high-dose females; increased serum urea in low-dose females; increased cholesterol in high-dose females; increased serum sodium in low-dose males; decreased total serum protein in mid- and high-dose males; decreased serum globulin in all treated animals; decreased cholesterol in low-dose males; decreased serum chloride in mid-dose females; decreased serum creatinine in low- and mid-dose females; decreased ALP in all treated males; and decreased absolute and relative adrenal gland weight in mid-dose males and females. Significant differences in organ weights (absolute and/or relative to body weight) were considered to be of no toxicological relevance, as they were limited to one sex only, did not demonstrate dose -dependency, and were within the laboratory's range for historical controls. Observations on macroscopic examination were concluded to be incidental and unrelated to the administration of 6'-SL sodium salt. Histopathological findings were not reported. Based on the lack of compound-related adverse effects, the authors determined a NOAEL of >5,000 mg/kg body weight/day, the highest dose tested, for 6'-SL sodium salt in male and female rats.

In another 90-day study, 7-day old neonatal Sprague-Dawley rats (10/sex/group) were administered 6'-SL sodium salt (96.8% purity; Glycom) at doses of 0 (vehicle control), 0 (5,000 mg fructooligosaccharides/kg body weight/day reference control), 1,000, 3,000, or 5,000 mg/kg body weight/day *via* gavage (Phipps *et al.*, 2019a). Physical observations, body weights, and food consumption were recorded throughout the exposure period, and ophthalmic examinations were conducted in the final week of dosing. Developmental indices consisting of pre-weaning auditory and visual function, age of first eye opening, age when air righting reflex became apparent, ulna length, and age to achieve sexual maturity. During Week 11 of the dosing period, animals were assessed using a functional observational battery test, followed by a spatial learning and memory assessment using the Morris water maze during Week 12. During Week 13, blood samples were collected for hematology (red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count, platelet count, reticulocyte count, red cell distribution width, neutrophils, lymphocytes, monocytes, eosinophils, basophils, large unstained cells), coagulation (prothrombin time and activated partial thromboplastin time), and blood chemistry (sodium, potassium, chloride, calcium, phosphorus, total bilirubin, ALP, AST, ALT, urea, creatinine, total protein, albumin, albumin:globulin ratio, triglyceride, total cholesterol, and glucose) parameters. Urinalysis parameters analyzed prior to necropsy included clarity, color, volume, pH, specific gravity, ketones, bilirubin, blood pigments, protein, creatinine, and glucose. The following organs and tissues were weighed and subject to gross and microscopic examination: adrenal glands, aorta, brain, cecum, colon, duodenum, epididymides, femur, Harderian glands, head, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric and left axillary), esophagus, ovaries, pancreas, pituitary gland, prostate, salivary glands (submandibular, parotid, sublingual), sciatic nerves, seminal vesicles, skeletal muscle, skin (with mammary glands), spinal cord, spleen, sternum, stomach, thymus, thyroid glands (with parathyroids), trachea, urinary bladder, uterus (with cervix), and vagina.

No test article-related changes were reported with respect to mortality, clinical signs, ocular observations, time to sexual maturity, food consumption, or mean body weight. Minor differences were observed in time to completion of balano-preputial separation, completion of vaginal opening, and body weight at vaginal opening; however, these findings were not dose-dependent. Pre-weaning development, animal behavior, and Morris maze performance were comparable across groups. Statistically significant increases in overall mean ulna growth in all male 6'-SL sodium salt groups compared to controls were considered to be unrelated to the test article due to the lack of a dose response relationship, the small magnitude of the differences, and the lack of any differences observed in the female groups. No compound-related effects on organ weights, gross pathology, or histopathology were noted following 6'-SL sodium salt administration. Statistically significant differences in hematology, clinical chemistry, and urinalysis parameters were not considered by the study authors to be compound-related due to sex-specificity, lack of a dose-response relationship, and lack of deviation from historical control ranges. These differences include: increased eosinophils and activated partial thromboplastin time in high-dose females; increased AST in all treated males; increased albumin:globulin ratio in mid- and high-dose males; decreased prothrombin time in mid- and high-dose males and high-dose females; decreased hemoglobin in all treated males and high-dose females; decreased platelets in all treated females; decreased hematocrit and red blood cell count in high-dose females; decreased serum chloride in mid- and high-dose males and females; decreased serum bilirubin in low- and mid-dose males; decreased total serum protein in all treated animals; decreased serum albumin in all treated females; decreased serum cholesterol in high-dose males; decreased urinary protein in high-dose males and females; and increased urinary pH in all treated females. The only notable macroscopic and histopathological findings were reported for the testis and epididymis of males in the highest dose group; however, there was no dose-response, the findings were unilateral, and there was no gradation in severity and these observations were therefore considered unrelated to the test item. Based on the lack of compound-related adverse effects, the authors determined a NOAEL of 5,000 mg/kg body weight/day, the highest dose tested, for 6'-SL sodium salt in male and female rats.

Table 6.4.2.3-1 Summary of Subchronic Studies Conducted with Other 6'-Sialyllactose Preparations

Species	Duration	Test Article [Method]	Dose (mg/kg bw/d)	Outcome Parameters	Results Relevant to Safety	Reference
Rat						
Sprague-Dawley (11/sex/group; 6 to 7 weeks old)	90 days	6'-SL sodium salt (produced by enzymatic synthesis, 98.8% purity) [gavage]	0, 1,000, 2,500, or 5,000	Bw, clinical observations, food consumption, ophthalmology, clinical chemistry, hematology, urinalysis, organ weights, gross and histopathological examination	No compound-related adverse effects on measured parameters. Authors concluded that 6'-SL showed no evidence of toxicity. NOAEL = >5,000 mg/kg bw/d for males and females	Gurung *et al.* (2018)
Sprague-Dawley (Crl:CD[SD]; 10/sex/group; 7 days old)	90 days	6'-SL sodium salt (produced by microbial fermentation, 96.8% purity) [gavage]	0, 1,000, 3,000, or 5,000	Bw, clinical observations, food and water consumption, ophthalmology, clinical chemistry, hematology, urinalysis, organ weights, developmental indices (pre-weaning auditory and visual function, time to sexual maturity)	No compound-related adverse effects on measured parameters. Authors concluded that 6'-SL is safe for use in infant formula and other foods for the general population. NOAEL = 5,000 mg/kg bw/d for males and females	Phipps *et al.* (2019a)

6'-SL = 6'-sialyllactose sodium salt; bw = body weight; d = day; NOAEL = no-observed-adverse-effect level.

6.4.2.4 Reproductive and Developmental Toxicity

Two gastrointestinal developmental toxicity studies of 6'-SL in piglets were identified in the literature (Jacobi *et al.*, 2016; Monaco *et al.*, 2020). The study by Jacobi *et al.* (2016) was included in a previous GRAS evaluation that was notified to the U.S. FDA with no objections (GRN 766), while the study by Monaco *et al.* (2020) was not included in previous GRAS evaluations notified to the U.S. FDA. The 2 identified studies are summarized below and included in Table 6.4.2.4-1. Overall, no compound-related adverse effects were reported in piglets administered up to 1,200 mg 6'-SL/kg body weight/day for up to 21 days.

The safety of orally administered 6'-SL sodium salt (>98% purity; produced by enzymatic synthesis; GeneChem), delivered *via* a non-medicated sow-milk replacer formula for 21 days, was evaluated in 2-day-old piglets (6/sex/group; strain NR) (Monaco *et al.*, 2020). Diets were formulated to contain 0, 300, 600, or 1,200 mg 6'-SL sodium salt/L, and were administered to the piglets 10 times daily *via* a peristaltic pump at 300 or 330 mL diet/kg body weight on Study Days 1 to 5 or 6 to 21, respectively. Body weights of piglets were measured daily in the mornings prior to feeding and formula intake was recorded. On Days 8 and 22 of feeding, blood samples were collected to measure clinical chemistry (calcium, phosphorus, magnesium, sodium, potassium, chloride, glucose, total cholesterol, triglycerides, total protein, albumin, globulin, albumin/globulin ratio, alkaline phosphatase, aspartate transaminase, creatine phosphokinase, glutamate dehydrogenase, gamma glutamyltransferase, blood urea nitrogen, creatinine, urea, total bilirubin, bicarbonate, and anion gap) and coagulation parameters. Urine samples were collected immediately prior to necropsy for urinalysis (pH, protein, glucose, ketones, bilirubin, blood, and urine sediments). Upon necropsy, organs (spleen, stomach, kidneys, heart, lungs, and liver) were weighed and fixed, and the small intestine was excised to measure total intestine length. The length of the large intestine was measured and the cecal and colonic contents were collected to measure pH. Histological analyses were conducted on tissues (stomach, spleen, liver, gallbladder, kidney, cecum, colon, mesenteric lymph nodes, heart, duodenum, jejunum, ileum, and brain) from the control and high-dose groups. There were no significant differences among groups in total body weight gain, food consumption, intestinal length, organ weights, colonic pH, coagulation parameters, blood chemistry, hematology, and urinalysis parameters. The histological effects reported in the high-dose 6'-SL sodium salt group (lymphocyte infiltration in the stomach, and small and large intestines, as well as hepatic glycogen accumulation, and colonic lymphoid nodules) were comparable to control piglets and not considered to be toxicologically relevant by the study authors. The authors concluded that there were no dose-dependent adverse effects in the study, and that 6'-SL sodium salt was well tolerated and supported normal growth and development at concentrations up to 1,200 mg/L in reconstituted formula.

An additional study of 6'-SL was identified in the literature in which gastrointestinal parameters were evaluated in piglets. In this study, 1-day-old piglets (9/group, sex, and strain not reported) were provided with 0, 600, or 1,200 mg 3'-SL or 6'-SL/kg body weight/day in formula for 21 days [from Postnatal Day (PND) 2 to 22] and brain sialic acid content and the colonic microbiota were investigated (Jacobi *et al.*, 2016). The source of the 3'-SL and 6'-SL test articles was not reported. In this study, there was no effect of 3'-SL or 6'-SL on feed intake, growth, intestinal pH, or diarrhea scores, with authors reporting that both oligosaccharide diets were well tolerated by the pigs across all treatment groups.

Table 6.4.2.4-1 Summary of Gastrointestinal Developmental Studies of Other 6'-Sialyllactose Preparations

Species	Duration	Test Article [Method]	Dose (mg/kg bw/d)	Outcome Parameters	Results Relevant to Safety	Reference
Piglet (6/sex/group; 2 days old; strain NR)	21 days	6'-SL sodium salt (>98% purity; produced by enzymatic synthesis) [non-medicated sow-milk replacer formula, Advance Liqui-Wean]	Dose in mg/kg bw/d NR [0, 300, 600, or 1,200 mg/L]	Growth, bw gain, feed intake, organ weights, intestinal length, histopathology, clinical chemistry, hematology, urinalysis	No compound-related, toxicologically relevant adverse effects on measured parameters. 6'-SL was well tolerated, and the authors concluded it supported normal growth and development.	Monaco *et al.* (2020)
Piglet (crossbred; 9/group; full-term; 1 day old; strain and sex NR)	21 days (PND 2 to 22)	6'-SL (purity and source NR) [formula]	0, 600, or 1,200 [0, 2, or 4 g/L]	Sialic acid content of the brain, microbial composition of digesta, intestinal pH, feed intake, growth, fecal consistency	No compound-related adverse effects on measured parameters. The 6'-SL diet was reported to be well tolerated.	Jacobi *et al.* (2016)

6'-SL = 6-sialyllactose sodium salt; bw = body weight; d = day; NR = not reported; PND = Postnatal Day.

6.4.3 Studies Conducted with Kyowa's Structurally-Related 3'-SL Sodium Salt

6.4.3.1 Overview

As discussed in Section 3.2.1, 3'-SL and 6'-SL are constitutional isomers wherein the sialic acid moiety is connected to the galactose unit of lactose at the 3 or 6 position *via* an α-2,3 linkage or α-2,6 linkage, respectively (ten Bruggencate *et al.*, 2014; Jacobi *et al*., 2016). Kyowa has conducted a bacterial reverse mutation test, an *in vivo* micronucleus test, and a subchronic 90-day repeat dose toxicity study with their 3'-SL sodium salt ingredient. Considering that 3'-SL is structurally related to 6'-SL, the results of the studies on Kyowa's 3'-SL sodium salt are relevant to the safety of their 6'-SL sodium salt and are summarized below. The results of the unpublished studies on Kyowa's 3'-SL sodium salt ingredient corroborate the safety of Kyowa's 6'-SL sodium salt ingredient.

6.4.3.2 Genotoxicity

6.4.3.2.1 Bacterial Reverse Mutation Test

The potential mutagenicity of 3'-SL sodium salt (Lot G; 92.8% assay) was evaluated in a bacterial reverse mutation test that was performed in compliance with the OECD principles of GLP (OECD, 1998) and according to OECD TG 471 (OECD, 1997) [Oguma, 2020b (unpublished)].

Two main tests, conducted as pre-incubation assays, were performed using *S.* Typhimurium strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2 uvrA, which were exposed to 3'-SL sodium salt at concentrations of 313, 625, 1,250, 2,500, or 5,000 µg/plate (the OECD TG 471 maximum recommended concentration) in the absence and presence of external metabolic activation (S9 mix).

Water (for injection) served as the vehicle for 3'-SL sodium salt and as the negative control. Positive controls were also included in the presence (2-aminoanthracene and benzo[a]pyrene) and absence [(2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, sodium azide, and 2-methoxy-6-chloro-9-(3-(2-chloroethyl)aminopropylamino) acridine, dihydrochloride] of metabolic activation.

The test solutions, test strain, and metabolic activation (where applicable) were incubated while shaking at 37°C for 20 minutes. Top agar kept at 45°C was then added, and the mixture shaken, and overlaid on a minimal glucose agar plate medium. After solidification of the overlaid top agar, the plates were incubated upside down at 37°C for 48 hours. After the incubation period, the plates were observed for coloration and precipitation, and the numbers of revertant colonies were counted using a Dot Counter (IEDA Trading Co.). Growth inhibition was observed using a stereoscopic microscope. A positive result for mutagenicity was defined as a dose-dependent and biologically relevant >2-fold increase in the number of revertant colonies, compared to that of the vehicle control group.

There was no evidence of mutagenicity in either test, in the absence or presence of metabolic activation. The mean number of revertant colonies was less than twice that of the negative control at all test concentrations, and there was no dose response observed in any test system with or without metabolic activation. No growth inhibition or precipitation of the test substance was observed.

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 TG 471 maximum recommended concentration).

6.4.3.2.2 In Vivo Micronucleus Test

The potential clastogenicity and aneugenicity of 3'-SL sodium salt (Lot G; 92.8% assay) was evaluated in an *in vivo* micronucleus test with ICR mice (Inasa Branch, Japan SLC, Inc.). This study was conducted in in compliance with the OECD principles of GLP (OECD, 1998) and OECD TG 474 (OECD, 2016) [Kikuchi, 2020b (unpublished)].

In a dose-range finding study conducted to determine the dose levels for the main study, ICR [Slc:ICR] mice (3/sex/group) were administered 3'-SL sodium salt by gavage at doses of 0, 500, 1,000, or 2,000 mg/kg body weight. No clinical signs or mortality were observed at any test dose, and no significant changes in body weight or bone marrow were observed. Therefore, in the main study, male ICR mice (5/group) were administered 3'-SL sodium salt by gavage twice (at a 24-hour interval) at doses of 500, 1,000, or 2,000 mg/kg body weight. Mitomycin C (2 mg/kg body weight) served as the positive control and the vehicle (water for injection) was used as the negative control. General observations of the animals were performed before the initial administration, at least 1 hour after administration, and 2, 3, and 6 hours after administration of the first (Day 0) and second doses (Day 1). Body weights were recorded on Days 0, 1, and 2.

Animals were euthanized on Day 2 by cervical dislocation and femoral bone marrow cells were harvested for analysis. Bone marrow cells were washed with fetal bovine serum, centrifuged, and re-suspended. Smear preparations were dried, fixed in methanol, stained with 3% Giemsa solution, and rinsed with tap water. Samples of IMEs and MEs were separately counted using an oil-immersed object lens magnifying 100 diameters. The proportion of IMEs among total erythrocytes was determined by counting 1,000 erythrocytes (IMEs and MEs) per animal. A total of 4,000 IMEs were scored for the incidence of MNIMEs. A finding was considered to be positive if the incidence of MNIMEs in at least 1 test group increased significantly and in a dose-dependent manner. The acceptability of the test was determined by the MNIME frequencies in the negative- and positive-control groups being within the ranges of in-house background data, and the positive control resulting in a statistically significant increase in MNIMEs compared to the negative control.

No clinical signs or abnormalities and no statistically significant changes in the body weights of any animal were observed in the test substance, negative-, and positive-control groups. No significant changes in MNIME frequency were observed between the test substance and negative control groups. Conversely, the frequency of MNIMEs was significantly increased in the positive control group compared to the negative control group, thus confirming the acceptability of the study. No significant difference in the proportion of IMEs among total erythrocytes was observed among the study groups.

Based on the results of this study, 3'-SL sodium salt was concluded to have no potential for induction of chromosomal aberrations in male ICR mice at doses up to 2,000 mg/kg body weight.

6.4.3.3 Subchronic Toxicity

6.4.3.3.1 90-Day Toxicity Study in Rats

A 90-day repeat dose toxicity study was conducted to evaluate the potential subchronic toxicity of 3'-SL sodium salt when administered by gavage to Crl:CD(SD) rats [Tsuboi, 2021b (unpublished)]. The study was conducted in compliance with the OECD principles of GLP (OECD, 1998) and according to OECD TG 408 (OECD, 2018).

Animals were quarantined and acclimated for 8 days following receipt. Groups of 10 male and 10 female Crl:CD(SD) rats received 0 (distilled water for injection), 502, 1,003, or 2,007 mg 3'-SL sodium salt/kg body weight/day at a dose volume of 10 mL/kg body weight for 90 days. Lot G was used, which

had a purity value of 93% on a dwb, equivalent to 88.4% 3'-SL Na on an as-is basis, based on the purity results reported after the study[6]. Animals were observed twice daily before and after administration (Days 1 to 90) and once on Day 91, before necropsy. Body weights were recorded on Days 1, 4, 8, 12, 15, 19, 22, 26, 29, 36, 43, 50, 57, 64, 71, 78, 85, 90, and 91. Food intake was recorded on Days 1, 3, 7, 11, 14, 18, 21, 25, 28, 35, 42, 49, 56, 63, 70, 77, 84, and 89. The weight of the remaining diet was measured on the following days (*i.e.*, Days 1, 4, 8, 12, 15, 19, 22, 26, 29, 36, 43, 50, 57, 64, 71, 78, 85, and 90) to calculate the daily food consumption of each animal. Detailed observations of all animals were conducted once during the quarantine period and weekly 1 to 2 hours after administration. In Week 11 (males: Day 76; females: Day 77) the sensory reactivity (reactions to auditory, visual, proprioceptive, and pain stimuli), grip strength, and locomotor activity were examined in all animals. Ophthalmologic examinations of all animals were performed once in the quarantine period and once in Week 13 for all animals in the control and 2,007 mg/kg body weight/day groups. No examinations were performed for the 502 and 1,003 mg/kg body weight/day groups in Week 13 as the 2,007 mg/kg body weight/day group did not display any ophthalmological abnormalities. Urine samples were collected for urinalysis in Week 13. Blood samples were taken for evaluation of hematology, blood chemistry, and blood coagulation parameters on Day 91 following an overnight fast (with free access to water). The estrus cycle of all females was examined on Day 91 *via* vaginal smear.

At the end of the treatment period, all surviving animals were euthanized by exsanguination and subjected to a gross necropsy, which included macroscopic examination of the body surface, orifices, cranial cavity, thoracic cavity, abdominal cavity, and contents of each. The following organs and tissues were collected and fixed: adrenal glands, aorta, brain (cerebrum, cerebellum, and medulla oblongata), cervical lymph nodes, duodenum, epididymides, eyeball (including optic nerve), femur (bone and marrow), femoral muscle, Harderian glands, heart, ileum (including Peyer's patch), jejunum, cecum, colon, kidneys, liver, lungs (with bronchi), mammary gland, mesenteric lymph nodes, esophagus, ovaries, pancreas, pituitary gland, prostate gland, rectum, salivary glands, sciatic nerve, seminal vesicles (including coagulation glands), spinal cord, spleen, skin, sternum (bone and marrow), stomach (forestomach and glandular stomach), testes, thymus, thyroid glands (with parathyroids), tongue, trachea, urinary bladder, uterus (with cervix) and vagina. Histopathological evaluation of all organs and tissues was conducted on animals in the vehicle control and 2,007 mg/kg body weight/day groups. Due to the lack of toxicologically relevant results in high-dose animals, histopathological examinations were not conducted for the 502 and 1,003 mg/kg body weight/day groups. The heart, thymus, lungs, thyroid glands, spleen, liver, kidneys, pituitary gland, adrenal glands, testes, epididymides, uterus, ovaries, and brain (cerebrum, cerebellum, and medulla oblongata) were weighed prior to fixation and organ weight relative to body weights on the day of necropsy were calculated.

There were no test item-related deaths, clinical signs, or changes in body weight or food-consumption in any of the groups throughout the administration period. There were no abnormal findings in any of the groups in the detailed or functional observation or ophthalmological examination.

No compound-related differences in values for urinary parameters were observed in any of the groups. Significant differences in urinary electrolytes (increased Na excretion in mid-dose females and Na excretion and Na concentration in high-dose males and females, decreased K concentration in mid-and high-dose males, decreased K excretion in low- and high-dose females, decreased Cl concentration in mid- and high-dose females, decreased Cl excretion in low- and high-dose females) were concluded to

[6] Doses were planned to be 0, 500, 1,000, and 2,000 mg/kg body weight/day, with the highest dose selected in accordance with OECD TG 420. Doses were initially calculated using a preliminary Certificate of Analysis with one significant digit; however, upon re-calculation using the rounded assay value reported on the final Certificate of Analysis, the doses were calculated to be 0, 502, 1,003, and 2,007 mg/kg body weight/day.

be not toxicologically relevant as no abnormal changes in blood electrolytes or associated organs were observed.

No compound-related differences in values for hematological parameters were observed in any of the groups. The statistically significant decreases in prothrombin time and activated partial thromboplastin time in high-dose males were deemed by the authors to be not toxicologically relevant due to the absence of hypercoagulative changes in any organs, as well as the small magnitude and sex-specificity of the effects. Basophil count was significantly increased in mid-dose males; however, this was not considered toxicologically relevant as the change was not dose-dependent.

A few inconsistent, statistically significant differences were reported in blood chemical parameters and organ weights; however, these changes were concluded to not be toxicologically relevant due to a lack of dose-dependency in the results. There was no bias towards any estrous stage in any of the groups, suggesting proper progression of the estrous cycle.

No toxicologically relevant abnormal gross pathological findings were noted in any of the animals. No test item-related differences or abnormal histopathological findings were observed in any of the animals. Several findings were noted more frequently in high-dose animals than in controls, but were considered to be spontaneous and not test item-related due to their low frequency, morphological conformity between controls and high-dose animals, and/or unilateral observation. These findings include the following:

- Heart:
    - Mononuclear cell infiltration of the ventricular wall, epicardium, or endocardium (1 male in the 2,007 mg/kg body weight/day group for each tissue);

- Pancreas:
    - Focal fibrosis in the islet (4 males in the control group and 7 males in the 2,007 mg/kg body weight/day group);

    - Focal atrophy of acinar cells (1 male in the control group and 2 males in the 2,007 mg/kg body weight/day group);

- Kidney:
    - Unilateral scarring (1 male in the 2,007 mg/kg body weight/day group);

    - Unilateral medullar cyst (1 male and 1 female of the 2,007 mg/kg body weight/day group);

- Pituitary:
    - Pseudocyst, anterior lobe (1 female in the control group, and 1 male and 1 female in the 2,007 mg/kg body weight/day group);

    - Dilatation of Rathke's pouch (3 females in the control group and 4 females in the 2,007 mg/kg body weight/day group);

- Harderian gland:
    - Focal interstitial mononuclear cell infiltration (1 male in the 2,007 mg/kg body weight/day group);

- Prostate gland:

- Focal interstitial mononuclear cell infiltration (1 male in the control group and 2 males in the 2,007 mg/kg body weight/day group);

- Femur:
  - Focal epiphyseal fibrosis (1 male in the 2,007 mg/kg/day group).

Other histopathological findings were deemed to be unrelated to the test item due to observation in high-dose animals at equal or lesser frequency than control animals.

In the absence of any statistically significant, toxicologically relevant, test item-related adverse effects, the NOAEL was concluded by the study authors to be 2,007 mg/kg body weight/day (the highest dose tested).

6.4.4 Studies Conducted on Other Preparations of the Structurally-Related 3'-SL

6.4.4.1 Overview

Toxicological studies have been conducted on other 3'-SL preparations and reported in the literature. As discussed in Section 3.2.1, 3'-SL and 6'-SL are constitutional isomers wherein the sialic acid moiety is connected to the galactose unit of lactose at the 3 or 6 position *via* an α-2,3 linkage or α-2,6 linkage, respectively (ten Bruggencate *et al.*, 2014; Jacobi *et al*., 2016). Considering that 6'-SL is structurally related to 3'-SL, the results of the studies on other 3'-SL preparations are relevant to the safety assessment of Kyowa's 6'-SL ingredient. The preclinical toxicology studies reported by Jacobi *et al.* (2016), Kim *et al*. (2018), Phipps *et al.* (2019b), and Monaco *et al.* (2019) have been reviewed during previous GRAS evaluations (GRNs 766, 880, 881, and 921 – GeneChem, Inc., 2018; Glycom A/S, 2019a,b; Jennewein Biotechnologie GmbH, 2020b; U.S. FDA, 2018c, 2020e,f,g). The human studies reported by Opekun *et al.* (1999) and Parente *et al.* (2003) discussed below in Section 6.5 have also been reviewed during GeneChem's GRAS evaluation of 3'-SL sodium salt (GeneChem, Inc., 2018 – GRN 766). Two additional subchronic toxicity studies conducted by Mysore *et al.* (1999) and Chleilat *et al.* (2020) were identified in the literature search and are further discussed in Section 6.4.4.3 below.

6.4.4.2 Genotoxicity

The potential genotoxicity of other 3'-SL sodium salt preparations was evaluated *in vitro* in bacterial and mammalian test systems (Kim *et al*., 2018; Phipps *et al.*, 2019b) and *in vivo* in mice (Kim *et al*., 2018). These studies have been included in previous GRAS evaluations that have been notified to the U.S. FDA with no objections (GRNs 766, 880, 881, and 921). The results of these studies are summarized in Table 6.4.4.2-1. The consistently negative results reported in *in vitro* and *in vivo* studies demonstrate that 3'-SL sodium salt lacks genotoxic potential.

Kim *et al.* (2018) evaluated the genotoxic potential of GeneChem's 3'-SL sodium salt in a bacterial reverse mutation test, an *in vitro* chromosome aberration test, and an *in vivo* mammalian erythrocyte micronucleus test (Kim *et al*., 2018).

A bacterial reverse mutation assay was performed in *S.* Typhimurium strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2*uvrA* (pKM101) in the absence and presence of metabolic activation using 3'-SL sodium salt (98.8% purity; produced by enzymatic synthesis) at concentrations of 0 (unspecified vehicle control), 5, 10, 50, 100, 250, 500, 1,000, 2,500, or 5,000 µg/mL. Sodium azide, 2-nitrofluorene, 2-aminoanthracene, 9-aminoacridine, and 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide served as the positive controls. The mean numbers of revertant colonies observed in strains treated with 3'-SL at all concentrations in the presence and absence of metabolic activation were less than twice those of the negative control values, and growth inhibition and precipitation of the test substance were not observed. Based on the results of the study, the authors concluded that 3'-SL sodium salt was not mutagenic.

The clastogenicity of 3'-SL sodium salt (98.8% purity; produced by enzymatic synthesis) was assessed in a chromosomal aberration test in Chinese Hamster lung (CHL/IU) cells, in the presence and absence of metabolic activation, at concentrations of 5, 10, 50, 100, 250, 500, 1,000, 2,500, or 5,000 µg 3'-SL sodium salt/mL (Kim *et al*., 2018). Mitomycin C and benzo[a]pyrene served as positive controls and the vehicle of the test substance (not specified) served as the negative control. In the short-term assay, CHL cells were incubated for 6 hours followed by an 18-hour expression period in the presence and absence of metabolic activation, while cells in the continuous assay were incubated for 24 hours in the absence of metabolic activation. In both assays and at all concentrations, the frequencies of cells with structural and numerical chromosome aberrations were less than 5%, and no precipitation was observed. Based on the results of this study, the authors concluded that 3'-SL sodium salt does not induce chromosomal aberrations and is non-clastogenic in the presence or absence of metabolic activation.

An *in vivo* micronucleus test was conducted in which 8-week-old ICR mice (9/sex/group administered 3'-SL) were administered 3'-SL sodium salt (98.8% purity; produced by enzymatic synthesis) at doses of 0, 500, 1,000, or 2,000 mg/kg body weight/day, dissolved in saline, *via* gavage for 3 consecutive days (Kim *et al*., 2018). Mitomycin C (2 mg/kg body weight) and saline served as the positive and negative controls, respectively. Animals were monitored for clinical signs and mortality immediately after administration, at 2 hours, and at Days 1, 2, and 3 post-dosing. Bone marrow cells were collected at 24, 48, and 72 hours after dosing. No clinical signs were observed, and the test substance was well tolerated. Incidence of MNPCE in PCE was not statistically significant at any dose of 3'-SL sodium salt compared to the negative control. No statistically significant differences in the ratio of PCE to total erythrocytes was observed among the 3'-SL sodium salt dose groups compared to the negative control value. Based on the results of this study, the authors concluded that 3'-SL sodium salt does not induce micronuclei in the bone marrow cells of mice.

Phipps *et al.* (2019b) evaluated the genotoxic potential of Glycom's 3'-SL sodium salt in a bacterial reverse mutation assay and an *in vitro* chromosome aberration test.

The mutagenic potential of 3'-SL sodium salt was investigated in a bacterial reverse mutation assay reported by Phipps *et al.* (2019b). In this study, *S.* Typhimurium strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2*uvrA* (pKM101) were exposed to 3'-SL sodium salt (90.3% purity; produced by microbial fermentation) at concentrations of 0, 5, 15, 50, 150, 500, 1,500, or 5,000 µg/mL in the absence and presence of metabolic activation. Sodium azide, 2-nitrofluorene, 9-aminoacridine, and 4-nitroquinoline-1-oxide served as positive controls in the absence of metabolic activation, while 2-aminoanthracene and benzo[a]pyrene served as the positive controls in the presence of metabolic activation. Water served as the negative control. No biologically relevant differences in the numbers of revertant colonies were observed in the presence or absence of metabolic activation relative to the negative control, and the authors concluded that 3'-SL sodium salt was not mutagenic based on the results of the study.

An *in vitro* micronucleus test was reported by Phipps *et al.* (2019b) using human peripheral blood lymphocytes from healthy non-smoking adults, which were exposed to 3'-SL sodium salt (90.3% purity; produced by microbial fermentation) at concentrations of 0, 500, 1,000, or 2,000 µg/mL for 3 hours with and without metabolic activation, or for 20 hours without metabolic activation. Mitomycin C and colchicine served as positive controls in the absence of metabolic activation, and cyclophosphamide served as the positive control in the presence of metabolic activation. Water was used as the vehicle control. No biologically relevant differences were reported in the percentage of micronucleated cells between the 3'-SL sodium salt groups and the vehicle controls, and the authors concluded that 3'-SL sodium salt did not have aneugenic or clastogenic potential *in vitro*.

Table 6.4.4.2-1 Genotoxicity Studies of Other 3'-Sialyllactose Preparations

Test	Test System/Animal Species	Test Article Concentration/Dose	Results	Reference
***In Vitro* Studies**				
Bacterial reverse mutation test	*Salmonella* Typhimurium TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2*uvr*A (pKM101)	3'-SL sodium salt 0, 5, 10, 50, 100, 250, 500, 1,000, 2,500, or 5,000 µg/mL +/- S9	Negative	Kim *et al.* (2018)
Bacterial reverse mutation test	*S.* Typhimurium TA98, TA100, TA1535, and TA1537 and *E. coli* WP2*uvr*A (pKM101)	3'-SL sodium salt 0, 5, 15, 50, 150, 500, 1,500, or 5,000 µg/mL +/- S9	Negative	Phipps *et al.* (2019b)
Chromosome aberration test	Chinese hamster lung cells	3'-SL sodium salt 0, 5, 10, 50, 100, 250, 500, 1,000, 2,500, or 5,000 µg/mL +/- S9	Negative	Kim *et al.* (2018)
Micronucleus test	Human peripheral blood lymphocytes	3'-SL sodium salt 0, 500, 1,000, or 2,000 µg/mL 3 hours: +/- S9 20 hours: - S9	Negative	Phipps *et al.* (2019b)
***In Vivo* Studies**				
Micronucleus test	ICR mice (8-week old; 9/sex/group)	3'-SL sodium salt 0, 500, 1,000, or 2,000 mg/kg bw/day Oral (gavage); 3 consecutive days	Negative	Kim *et al.* (2018)

+ S9 = with metabolic activation; - S9 = without metabolic activation; 3'-SL = 3'-sialyllactose sodium salt; bw = body weight.

6.4.4.3 Subchronic Toxicity

Five publications including six repeat-dose studies of other 3'-SL preparations in rats and monkeys were identified in the literature; these studies are described below and summarized in Table 6.4.4.3-1. The test articles included GeneChem's 3'-SL sodium salt manufactured by enzymatic synthesis (98.8% purity; Kim *et al.*, 2018), Glycom's 3'-SL sodium salt manufactured by microbial synthesis (90.3% purity; Phipps *et al.*, 2019b), Glycom's 3'-SL sodium salt manufactured by an unspecified method (97.5% purity; Chleilat *et al.*, 2020), and Neose Technologies' 3'-SL sodium salt manufactured by an unspecified method (purity not reported; Mysore *et al.*, 1999). The studies reported by Kim *et al.* (2018) and Phipps *et al.* (2019b) have been included in previous GRAS evaluations for 3'-SL sodium salt ingredients that have been notified to the U.S. FDA with no objections (GRNs 766, 880, and 921). Overall, no compound-related adverse effects were reported in these studies following administration of up to 7,500 mg 3'-SL/kg body weight/day to rats and monkeys for test durations of up to 90 days.

In a 28-day toxicity study, 6-week-old Sprague-Dawley (Crl:CD[SD]) rats (10/sex/group) were administered 3'-SL sodium salt (98.8% purity; produced by enzymatic synthesis; GeneChem) at doses of 0, 500, 1,000, or 2,000 mg/kg body weight/day *via* gavage (Kim *et al*., 2018). Clinical signs, body weight, food consumption, absolute and relative organ weights (individual organs not reported), urinalysis, hematology, and clinical chemistry (individual parameters not reported) were measured, and ophthalmology and gross pathology examinations were conducted. Histopathological investigations were conducted only on specific tissues (further details not reported). The animals did not show any signs of compound-related abnormalities with respect to body weight gain, feed consumption, clinical chemistry, hematology, absolute or relative organ weights, or histopathology.

In a 56-day toxicity study that was not included in previous GRAS evaluations for 3'-SL sodium salt notified to the U.S. FDA, weanling Sprague-Dawley rats (10/sex/group) were administered diets providing 3'-SL (97.5% purity; method of manufacture not reported) at doses of 0 or 625 mg/kg body weight/day, or 625 mg 3'-SL/kg body weight/day in combination with 625 mg 2'-fucosyllactose (96.1% purity; method of manufacture not reported)/kg body weight/day (Chleilat *et al.*, 2020). Body weight and food intake were measured weekly, and fecal samples were collected for microbial profiling at 3, 7, and 11 weeks of age. Animals were administered an insulin tolerance test and an oral glucose tolerance test 8 days prior to the end of the dosing period and during the final week of dosing, respectively. At the end of the dosing period, lean mass, fat mass, body fat percent, bone mineral content, bone mineral density, intestinal permeability, serum cytokines [tumor necrosis factor-α (TNFα), interleukin (IL)-1α, IL-1β, IL-5, IL-10, IL-18, leptin], and gastrointestinal organ weights (cecum, colon, and jejunum) were measured. A significant decrease in body weight was measured in males who were administered 3'-SL in the diet relative to the controls, but this finding was only significant on test completion and not throughout the exposure. Females administered 3'-SL consumed significantly more food than the controls at the beginning of dosing; however, they consumed significantly less food than the controls by test completion. Serum leptin levels were significantly lower in rats that consumed the 3'-SL diet. The weight of the cecum from females administered the 3'-SL + 2'-FL mixture diet was significantly higher than controls. Conversely, female colon weight was significantly lower in the 3'-SL + 2'-FL group compared to the controls. Gut barrier permeability of females administered HMO diets was reduced relative to control animals. No statistically significant adverse effects were reported, and the authors reported that the changes observed in gut morphology and barrier function in females were beneficial. The lack of adverse compound-related effects indicates that 3'-SL and 2'-FL were well tolerated in rat pups.

In a 90-day toxicity study, 6-week-old Sprague-Dawley rats (10/sex/group) were administered 3'-SL sodium salt (98.8% purity; produced by enzymatic synthesis) at doses of 0, 500, 1,000, or 2,000 mg/kg body weight/day *via* gavage (Kim *et al*., 2018). The animals were observed for mortality, clinical signs, body weight, and food and water consumption, and ophthalmology, urinalysis, hematology,

clinical chemistry, organ weights (brain, pituitary, heart, lung, liver, spleen, kidney, adrenal, testis, prostate, ovary, and uterus), histopathology, and gross pathology parameters were measured. Hematology parameters included red blood cell count, white blood cell count, platelet count, neutrophils, lymphocytes, prothrombin time, and activated partial thromboplastin time. Biochemical parameters included serum alkaline phosphatase, total bilirubin, total protein, albumin, globulin, blood urea nitrogen, total cholesterol, sodium, potassium, calcium, and phosphorus. Histopathological examination included the brain, pituitary, thyroid, parathyroid, thymus, heart, lung with bronchi, trachea, liver, spleen, kidney, adrenal, esophagus, salivary gland, submandibular, sublingual, and parotid gland, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, testis, epididymis, prostate, seminal vesicle, ovary, uterus, vagina, urinary bladder, submandibular and mesenteric lymph nodes, eye and Harderian gland, mammary gland, skin, bone marrow (femur and sternum), tongue, spinal cord, and any tissues showing gross lesions.

No significant, compound-related adverse effects were reported with respect to mortality, body weight, food and water consumption, or clinical signs. Serum glucose levels in mid- and high-dose males were significantly lower than low-dose males and controls, although this effect was considered by the authors to be beneficial and not toxicologically relevant. No other statistically significant effects were reported, and the authors determined a NOAEL of >2,000 mg/kg body weight/day, the highest dose tested, for 3'-SL sodium salt in male and female rats.

In another 90-day study, 7-day-old Sprague-Dawley rats (10/sex/group) were administered 0 (vehicle control or 5,000 mg fructooligosaccharide/kg body weight/day), 1,000, 3,000, or 5,000 mg 3'-SL sodium salt (90.3% purity; produced by microbial fermentation)/kg body weight/day *via* gavage, with additional rats (5/sex/group) from the control, vehicle control, and highest dose groups evaluated after a 4-week recovery period (Phipps *et al.*, 2019b). Physical observations were made throughout the exposure period, and body weights and food and water consumption were recorded. Developmental indices were measured, including pre-weaning auditory and visual function, time to first eye opening, time to air righting reflex, and time to sexual maturity. During Week 11 of the dosing period, animals were assessed using a functional observational battery test, including a spatial learning and memory assessment using the Morris water maze. Ophthalmic examinations were conducted during the final week of dosing. Clinical chemistry and hematological parameters were measured in blood samples collected at the end of the dosing period, and included sodium, potassium, chloride, calcium, inorganic phosphorus, total bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea, creatinine, total protein, albumin, albumin/globulin ratio, triglyceride, total cholesterol, glucose, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count, platelet count, reticulocyte count, red cell distribution width, neutrophils, lymphocytes, monocytes, eosinophils, basophils, large unstained cells, prothrombin time, and activated partial thromboplastin time. Urine was sampled at the end of the dosing and recovery periods (following a 16-hour fast) for determinations of clarity, color, volume, pH, specific gravity, ketones, bilirubin, blood pigments, protein, creatinine, and glucose prior to necropsy. The following organs and tissues were weighed and subject to histopathological examination: adrenal glands, aorta, brain, cecum, colon, duodenum, epididymides, femur, Harderian glands, head, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric and left axillary), esophagus, ovaries, pancreas, pituitary gland, prostate, salivary glands (submandibular, parotid, sublingual), sciatic nerves, seminal vesicles, skeletal muscle, skin (with mammary glands), spinal cord, spleen, sternum, stomach, thymus, thyroid glands (with parathyroids), trachea, urinary bladder, uterus (with cervix), and vagina. No compound-related effects on mortality, clinical signs, ophthalmology, water and food consumption, or body weight were reported. Compared to vehicle controls, a small but significant decrease in body weight was observed for males administered 5,000 mg 3'-SL sodium salt/kg body weight/day (compared to vehicle controls); however, there was no evidence of dose-dependency reported and the study authors considered this effect to be not biologically relevant. Similarly, no compound-related differences were reported in developmental endpoints except

for a significant decrease in forelimb grip strength and rearing counts of females administered 5,000 mg 3'-SL sodium salt/kg body weight/day (compared to vehicle controls), which were not observed to be dose-dependent. The few statistically significant differences in hematology parameters (decreased hemoglobin in low-dose males, decreased hemoglobin and red blood cell count in high-dose females, increased neutrophils in all females administered 3'-SL sodium salt, and decreased prothrombin time in all animals administered 3'-SL sodium salt) were reported to be not dose-dependent and within historical ranges of normal biological variation. Likewise, statistically significant changes in clinical chemistry values (*e.g.*, serum levels of sodium, chloride, urea, creatinine, total protein, albumin, albumin/globulin ratio, triglycerides) were not dose-dependent, non-adverse, and/or sex-specific, and remained within historical control ranges. Therefore, the observed changes in hematology and clinical chemistry parameters were considered by the study authors to be not toxicologically relevant.

Significant reductions were reported with respect to urinary volume, total protein, and total creatinine in males administered 5,000 mg/kg body weight/day, but these changes did not exhibit a dose-dependent response relationship and were not considered toxicologically relevant by the authors due to the individual values generally remaining within historical control ranges. Urinary pH increased in all 3'-SL groups relative to vehicle controls but remained within the historical control range. No statistically significant changes were observed in animals from any treatment group following the recovery period. All differences in organ weights were not associated with a dose- dependent response relationship and were therefore not considered to be the result of 3'-SL sodium salt administration. Based on the results of this study, the authors determined a NOAEL of 5,000 mg/kg body weight/day, the highest dose tested, for 3'-SL sodium salt in male and female rats.

One study in *Helicobacter pylori*-positive rhesus monkeys that was not included in previous GRAS evaluations for 3'-SL sodium salt notified to the U.S. FDA was identified, in which the effects of 3'-SL sodium salt administration on *H. pylori* infection were investigated (Mysore *et al.*, 1999). Rhesus monkeys (6/group) were administered 100 or 500 mg 3'-SL sodium salt/kg body weight/day for 28 or 56 days, respectively. The 3'-SL sodium salt test article used in this study (NE-0080 manufactured by Neose Technologies) was being investigated for use as a drug for use in the treatment of *H. pylori* infection, but was discontinued for this purpose in 2002[7]. No further details regarding the manufacturing process for NE-0080 were identified. Throughout the full duration of the treatment period, the monkeys were subject to gastric endoscopy (with gastric biopsy and *H. pylori* colony count) at 14-day intervals until Day 3 post-treatment, at which point they were subject to gastric endoscopy (with gastric biopsy and *H. pylori* colony count) at 14- or 30-day intervals for a 6-month follow-up period. Blood samples were collected at the same time points for each monkey, and hematology (*i.e.*, total white blood cell count, total red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, primed lymphocyte typing, and differential leukocyte count) and clinical chemistry parameters (*i.e.*, glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, CO_2, calcium, phosphorus, triglycerides, total protein, albumin, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatine phosphokinase, alkaline phosphatase, and total bilirubin) were measured. No adverse effects on hematology or clinical chemistry were reported following consumption of up to 500 mg 3'-SL sodium salt/kg body weight/day for 56 days (Mysore *et al.*, 1999). Thus, the authors concluded that 3'-SL sodium salt was safe when administered at doses of 100 and 500 mg/kg body weight/day for periods of up to 56 days.

[7] Source: http://adisinsight.springer.com/drugs/800005552.

Table 6.4.4.3-1 Summary of Subchronic Studies Conducted with Other 3'-Sialyllactose Preparations

Species	Duration	Test Article [Method]	Dose (mg/kg bw/d)	Outcome Parameters	Results Relevant to Safety	Reference
Rat						
Sprague-Dawley (Crl:CD[SD]) (10/sex/group; 6 weeks old)	28 days	3'-SL sodium salt (98.8% purity; produced by enzymatic synthesis; GeneChem) [gavage]	0, 500, 1,000, or 2,000	Clinical signs, bw, food consumption, urinalysis, organ weights, hematology, clinical chemistry	No compound-related adverse effects on measured parameters.	Kim *et al.* (2018)
Sprague-Dawley (10/sex/group; weanling)	56 days	3'-SL (97.5% purity; method of manufacture NR; Glycom) [diet]	0 or 625[a] (0 or 0.625% in the diet, either alone or in combination with 0.625% 2'-FL)	Bw, food consumption, glucose tolerance, insulin tolerance, intestinal permeability, serum cytokine levels, organ weights, gut microbiota	No compound-related adverse effects on measured parameters.	Chleilat *et al.* (2020)
Sprague-Dawley (Crl:CD[SD]) (10/sex/group; 6 weeks old)	90 days	3'-SL sodium salt (98.8% purity; produced by enzymatic synthesis; GeneChem) [gavage]	0, 500, 1,000, or 2,000	Clinical signs, bw, food and water, ophthalmology, urinalysis, hematology, clinical chemistry, organ weights, gross and histopathology	No compound-related adverse effects on measured parameters. Authors reported a NOAEL of >2,000 mg/kg bw/d for males and females.	Kim *et al.* (2018)
Sprague-Dawley (10/sex/group; 7 days old)	90 days with 4-week recovery period (additional 5/sex/group)	3'-SL sodium salt (90.3% purity; produced by microbial fermentation; Glycom) [gavage]	0 (vehicle or 5,000 mg FOS/kg bw/d), 1,000, 3,000, or 5,000 Recovery period: 0 (vehicle), 0 (5,000 mg FOS/kg bw/d), or 5,000	Clinical signs, mortality, ophthalmology, food and water consumption, bw, developmental indices, FOB, urinalysis, hematology, clinical chemistry, organ weights, gross and histopathology	No compound-related adverse effects on measured parameters. Authors concluded that 3'-SL is safe at levels representative of normal breastfed-infant intake. NOAEL = 5,000 mg/kg bw/d for males and females	Phipps *et al.* (2019b)

Table 6.4.4.3-1 Summary of Subchronic Studies Conducted with Other 3'-Sialyllactose Preparations

Species	Duration	Test Article [Method]	Dose (mg/kg bw/d)	Outcome Parameters	Results Relevant to Safety	Reference
Monkeys						
Helicobacter pylori positive rhesus monkeys (*Macaca mulatta*; n=6; 2 to 9 kg; sex NR; 2 to 15 years old)	28 days	3'-SL sodium salt (purity and method of manufacture NR; Neose Technologies) [mixed with peanut butter or banana]	100 [provided as 33 mg/kg bw tid]	Hematology, clinical chemistry, gastric endoscopy (with gastric biopsy and *H. pylori* colony counts; conducted at 14-day intervals during study period, and at 14- or 30-day intervals for 6-month follow-up period)	No compound-related adverse effects on measured parameters during treatment or follow up (endoscopies were conducted at 14- or 30-day intervals from day 3 post-treatment for a 6-month period) compared to baseline. Authors concluded that 3'-SL is safe over extended time periods.	Mysore *et al.* (1999)
	56 days		500 [provided as 167 mg/kg bw tid]			

2'-FL = 2'-fucosyllactose; 3'-SL = 3'-sialyllactose sodium salt; bw = body weight; d = day; FOB = functional observational battery; FOS = fructooligosaccharides; GeneChem = GeneChem Inc.; Glycom = Glycom A/S; NOAEL = no-observed-adverse-effect level; NR = not reported; tid = three times daily.

[a] Dose calculated using conversion table (U.S. FDA, 1993).

6.4.4.4 Reproductive and Developmental Toxicity

Two gastrointestinal developmental toxicity studies of 3'-SL in piglets were identified in the literature (Jacobi *et al.*, 2016; Monaco *et al.*, 2019), and these are summarized below and included in Table 6.4.4.4-1. These studies have been included in previous GRAS evaluations for 3'-SL sodium salt ingredients that have been notified to the U.S. FDA with no objections (GRNs 766, 880, and 921). Overall, no compound-related adverse effects were reported in piglets administered up to 1,200 mg 3'-SL/kg body weight/day for up to 30 days.

The safety of orally administered 3'-SL sodium salt (>98% purity; produced by enzymatic synthesis; GeneChem), delivered *via* a non-medicated sow-milk replacer formula for 21 days, was evaluated in 2-day-old piglets (6/sex/group; strain NR) (Monaco *et al.*, 2019). Diets were formulated to contain 0, 140, 200, or 500 mg 3'-SL sodium salt/L, and were administered to the piglets 10 times daily *via* a peristaltic pump at 300 or 360 mL diet/kg body weight on Study Days 1 to 5 or 6 to 21, respectively. Body weights of piglets were measured daily in the mornings prior to feeding. On Days 8 and 22 of feeding, blood samples were collected to measure clinical chemistry (calcium, phosphorus, magnesium, sodium, potassium, chloride, glucose, total cholesterol, triglycerides, total protein, albumin, globulin, albumin/globulin ratio, alkaline phosphatase, aspartate transaminase, creatine phosphokinase, glutamate dehydrogenase, gamma glutamyltransferase, blood urea nitrogen, creatinine, total bilirubin, bicarbonate, and anion gap) and coagulation parameters. Urine samples were collected immediately prior to necropsy for urinalysis (pH, protein, glucose, ketones, bilirubin, blood, and urine sediments). Upon necropsy, organs (spleen, stomach, kidneys, heart, lungs, and liver) were weighed and fixed, and the small intestine was excised to measure total intestine length. The length of the large intestine was measured and the cecal and colonic contents were collected to measure pH. Histological analyses were conducted on tissues (stomach, spleen, liver, gallbladder, kidney, cecum, colon, pancreas, mesenteric lymph nodes, lung, heart, duodenum, jejunum, ileum, ileal Peyer's patches, and brain) from the control and high-dose groups.

There were no significant differences among groups in total body weight gain, organ weights, intestinal length, colonic pH, clinical chemistry, coagulation, or hematologic parameters. A significantly increased incidence of crystals in the urine were observed in piglets administered formula containing 500 mg 3'-SL sodium salt/L; however, all 5 samples containing crystals in the 500 mg 3'-SL sodium salt group were classified as having “rare” or “few” crystals, and no other adverse renal or urinary effects were reported. The authors also noted that refrigeration of urine samples can sometimes promote crystal formation. The histological effects reported (lymphoplasmacytic inflammation in the stomach, extramedullary hematopoiesis in cecum, spleen, liver and gallbladder, spleen congestion, glycogen depletion in the liver, kidney hemorrhage, and neutrophilic inflammation in the cecum, ascending and descending colon) were not considered by the study authors to be toxicologically relevant due to the lack of dose-dependence or statistically significant differences from control animals. The authors concluded that there were no dose-dependent adverse effects in the study, and that 3’-SL sodium salt was safe at concentrations up to 500 mg/L in reconstituted formula (Monaco *et al.*, 2019).

An additional study of 3'-SL was identified in the literature in which gastrointestinal parameters were evaluated in piglets. In this study, 1-day-old piglets (9/group, sex, and strain not reported) were provided with 0, 600, or 1,200 mg 3'-SL or 6'-SL/kg body weight/day in formula for 21 days (from PND 2 to 22) and brain sialic acid content and the colonic microbiota were investigated (Jacobi *et al.*, 2016). The source of the 3'-SL and 6'-SL test articles was not reported. In this study, there was no effect of 3'-SL or 6'-SL on feed intake, growth, intestinal pH, or diarrhea scores. The authors reported that both oligosaccharide diets were well tolerated by the pigs across all treatment groups.

Table 6.4.4.4-1 Summary of Gastrointestinal Developmental Studies of Other 3'-Sialyllactose Preparations

Species	Duration	Test Article [Method]	Dose (mg/kg bw/d)	Outcome Parameters	Results Relevant to Safety	Reference
Piglet (6/sex/group; 2 days old; strain NR)	21 days	3′-SL sodium salt (>98% purity; produced by enzymatic synthesis; GeneChem) [non-medicated sow-milk replacer formula, Advance Liqui-Wean]	Dose in mg/kg bw/d NR [0, 140, 200, or 500 mg/L]	Growth, bw gain, organ weights, intestinal length, histopathology, clinical chemistry, hematology, urinalysis	No compound-related, toxicologically relevant adverse effects on measured parameters. Significantly increased incidence of urinary crystals in piglets administered 500 mg 3'-SL sodium salt/L formula, although the amounts of crystals in all 5 samples were classified as "rare" or "few". The study authors did not consider this effect to be toxicologically relevant. 3'-SL was well tolerated, and the authors concluded it was safe at the levels tested.	Monaco *et al.* (2019)
Piglet (crossbred; 9/group; full-term; 1 day old; strain and sex NR)	21 days (PND 2 to 22)	3'-SL (purity and source NR) [formula]	0, 600, or 1,200 [0, 2, or 4 g/L]	Sialic acid content of the brain, microbial composition of digesta, intestinal pH, feed intake, growth, fecal consistency	No compound-related adverse effects on measured parameters. The 3'-SL diet was reported to be well tolerated.	Jacobi *et al.* (2016)

3'-SL = 3'-sialyllactose; bw = body weight; d = day; GeneChem = GeneChem Inc.; NR = not reported; PND = Postnatal Day.

6.4.5 Studies Conducted on HMO Mixtures Containing 3'-SL and/or 6'-SL

6.4.5.1 Overview

Toxicological studies have been conducted on HMO mixtures and reported in the literature. Each HMO mixture contains 3'-SL and/or 6'-SL, and as such, toxicological data on these preparations are relevant to the safety assessment of Kyowa's 6'-SL ingredient. The studies reported by Parschat *et al.* (2020), Obelitz-Ryom *et al.* (2018), and Monaco *et al.* (2018) have been reviewed during previous GRAS evaluations notified to the U.S. FDA and filed as GRNs 880, 881, 921, 922, and 925 (Glycom A/S, 2019a,b; Jennewein Biotechnologie GmbH, 2020a,b,c), to which the FDA responded to each with no questions (U.S. FDA, 2020e,f,g, 2021b,c). Two additional studies reported by Comstock *et al*. (2017) and Yang *et al.* (2018) were identified in the literature search and were not included in GRAS evaluations notified to the U.S. FDA. These studies are further discussed in the sections below.

6.4.5.2 Genotoxicity

The potential genotoxicity of HMO mixtures was evaluated *in vitro* in bacterial and mammalian test systems (Parschat *et al.*, 2020). The studies reported by Parschat *et al.* (2020) were reviewed in GRAS evaluations notified to the U.S. FDA and filed as GRNs 921 and 925 (Jennewein Biotechnologie GmbH, 2020b,c). The results of these studies are summarized in Table 6.4.5.2-1. The consistently negative results reported in *in vitro* studies demonstrate that the tested HMO mixtures lack genotoxic potential.

The evaluations of the potential genotoxicity of an HMO mixture [containing on a dry weight basis 47.1% 2'-FL, 16.0% 3-FL, 23.7% lacto-*N*-tetraose (LNT), 4.1% 3'-SL, 4.0% 6'-SL, and 5.1% other carbohydrates] were conducted using a bacterial reverse mutation assay and an *in vitro* micronucleus test with cultured human peripheral lymphocytes (Parschat *et al.*, 2020). In the bacterial reverse mutation assay, *S.* Typhimurium strains TA98, TA100, TA102, TA1535, and TA1537 were exposed to 0, 5.0, 10.0, 31.6, 100, 316, or 600 mg HMO mixture/plate with or without metabolic activation. In the absence of metabolic activation, sodium azide, 2-nitrofluorene, 9-aminoacridine, and mitomycin C served as positive controls, and in the presence of metabolic activation, benzo[a]pyrene and 2-aminoanthracene served as positive controls. Highly purified water was used as the vehicle and negative control. In the presence and absence of metabolic activation, no changes in mean revertant colony numbers were reported relative to the negative control, and the authors concluded that the HMO mixture was not cytotoxic or mutagenic.

In the *in vitro* micronucleus test conducted by Parschat *et al.* (2020), cultured human peripheral lymphocytes were exposed to 0, 7.5, 15, 30, or 60 mg HMO mixture/mL medium for 4 hours with metabolic activation, and 4 or 24 hours without metabolic activation. Highly purified water was used as the vehicle control. Colchicine and mitomycin C served as the positive controls in the presence of metabolic activation, and cyclophosphamide served as the positive control in the absence of metabolic activation. No indications of chromosomal damage were observed with or without metabolic activation, and the authors concluded that the HMO mixture was not genotoxic.

Table 6.4.5.2-1 Genotoxicity Studies of HMO Mixtures Containing 3'-Sialyllactose and/or 6'-Sialyllactose

Test	Test System/Animal Species	Test Article Concentration/Dose	Results	Reference
***In Vitro* Studies**				
Bacterial reverse mutation test	*Salmonella* Typhimurium TA98, TA100, TA102, TA1535, and TA1537	HMO mixture (containing 4.0% 6'-SL) 0, 5.0, 10.0, 31.6, 100, 316, or 600 mg/plate +/- S9	Negative	Parschat *et al.* (2020)
Micronucleus test	Human peripheral lymphocytes	HMO mixture (containing 4.0% 6'-SL) 0, 7.5, 15, 30, or 60 mg/plate 4 hours: +/- S9 24 hours: - S9	Negative	Parschat *et al.* (2020)

+ S9 = with metabolic activation; - S9 = without metabolic activation; 3'-SL = 3'-sialyllactose sodium salt; 6'-SL = 6'-sialyllactose sodium salt; HMO = human milk oligosaccharide.

6.4.5.3 Subchronic Toxicity

Two repeat-dose studies of HMO mixtures were identified in the literature, including a 90-day study conducted in rats (Parschat *et al.*, 2020) that was reviewed in GRAS evaluations notified to the U.S. FDA and filed as GRNs 921 and 925 without questions (Jennewein Biotechnologie GmbH, 2020b,c; U.S. FDA, 2020g, 2021c) and a 15-day study in piglets (Comstock *et al.*, 2017) which has not been reviewed in a GRAS evaluation notified to the U.S. FDA. These studies are summarized below and in Table 6.4.5.3-1.

In a 13-week oral study, Charles River (SD) rats (10/sex/group) were administered a basal control diet or diet containing a 10% HMO mixture (consisting of 47.1% 2'-fucosyllactose, 16.0% 3'-FL, 23.7% LNT, 4.1% 3'-SL, 4.0% 6'-SL, and 5.1% other carbohydrates, each produced individually *via* fermentation) *ad libitum* for the duration of the test period (Parschat *et al.*, 2020). Actual intake of the HMO mixture for rats administered the test diet was calculated to be 5,670 and 6,970 mg HMO mixture/kg body weight/day for males and females, respectively. Daily observations were made for clinical signs, body weight, and food and water consumption. Ocular and auditory function were examined before the dosing period and 1 week prior to the conclusion of the test. Blood and urine samples were collected at the end of the study period, while organ weights and gross and histopathological examinations were conducted upon necropsy. No mortality was reported throughout the study period, and no compound-related adverse effects were reported with respect to body weight, body weight gain, animal behavior, food and water consumption, hematology, clinical chemistry, urinalysis, organ weights, neurology, or ophthalmology. Based on the results of the study, the authors determined the NOAELs to be 5,670 and 6,970 mg HMO mixture/kg body weight/day for male and female rats, respectively, corresponding to NOAELs of 232 and 286 mg 3'-SL/kg body weight/day and 227 and 279 mg 6'-SL/kg body weight/day for males and females, respectively.

In another study, groups of healthy and rotavirus-infected newborn piglets were fed a control formula (n=16) or formula containing 4 g HMOs/L[8] (n=17) from birth until 15 days of age to measure the effects of HMOs on immune cell populations (Comstock *et al.*, 2017). The piglets were weighed at the time of birth and at the end of the study. The birth weights, final weights, and weight gains were similar for all pigs administered the HMO treatment formula. No other parameters relevant to safety were assessed.

[8] 40% 2'-fucosyllactose (Glycom), 35% lacto-*N*-neotetraose (Glycom), 10% 6'-SL (Carbosynth), 5% 3'-SL (Carbosynth), and 10% free sialic acid (Glycom)

Table 6.4.5.3-1 Summary of Studies Conducted on HMO Mixtures Containing 3'-Sialyllactose and/or 6'-Sialyllactose

Species	Duration	Test Article [Method]	Dose (mg/kg bw/d)	Outcome Parameters	Results Relevant to Safety	Reference
Rat						
Charles River (CD; 10/sex/group; 65 days old)	90 days	HMO mixture[a] (containing 4.1% 3'-SL, 4.0% 6'-SL, produced individually by fermentation) [diet]	M: 0 or 5,670 HMO mix (providing 200 to 280 mg 6'-SL/kg bw/day) F: 0 or 6,970 HMO mix (providing 250 to 320 mg 6'-SL/kg bw/day)	Bw, food and water consumption, clinical signs, neurological measures (reactivity to stimuli, grip strength, locomotor activity), urinalysis, hematology, organ weights, histopathology	No compound-related adverse effects on measured parameters. Authors concluded that these results support the safe use of this HMO mixture.	Parschat *et al.* (2020)
Piglet						
Piglets (strain NR; M and F; 16 or 17/group; newborn)	15 days	HMO[a] (5% 3'-SL, 10% 6'-SL; method of manufacture NR) [formula]	Dose in mg/kg bw/d NR 0 (n=16) or 4 g HMO mixture/L formula (n=17) HMO mixture: [0.4 g 6'-SL/L formula; 0.2 g 3'-SL/L formula] [0.4 g 6'-SL/L formula]	Growth (bw)	No compound-related adverse effects on measured parameters.	Comstock *et al.* (2017)

3'-SL = 3'-sialyllactose sodium salt; 6'-SL = 6'-sialyllactose sodium salt; bw = body weight; F = female; HMO = human milk oligosaccharides; M = male; NR = not reported.
[a] Consisted of 40% 2'-fucosyllactose (Glycom A/S), 35% lacto-*N*-neotetraose (Glycom A/S), 10% 6'-SL (Carbosynth), 5% 3'-SL (Carbosynth), and 10% free sialic acid (Glycom A/S).

6.4.5.4 Reproductive and Developmental Toxicity

Gastrointestinal developmental parameters were evaluated in 3 additional studies of sialyllactose mixtures identified in the literature (Monaco *et al.*, 2018; Obelitz-Ryom *et al.*, 2018; Yang *et al.*, 2018), which are summarized below and included in Table 6.4.5.4-1. The studies reported by Monaco *et al.* (2018) and Obelitz-Ryom *et al.* (2018) were reviewed in GRAS evaluations notified to the U.S. FDA and filed as GRNs 880, 881, 921, and 922 (Glycom A/S, 2019a,b; Jennewein Biotechnologie GmbH, 2020a,b), to which the FDA had no questions (U.S. FDA, 2020e,f,g, 2021b). The study reported by Yang *et al.* (2018) has not been reviewed in a GRAS evaluation notified to the FDA.

Obelitz-Ryom *et al.* (2018) investigated the effects of SL on gut development and colonization in preterm piglets. Caesarean-delivered preterm pigs (18 to 20/group), delivered on Gestation Day 106, were administered 0 or 380 mg SL/L (8.5 g/L Lacprodan SAL-10, Arla Foods Ingredients; 4.5% SL; 3'-SL:6'-SL 6:1; method of manufacture not reported) in unpasteurized Jersey cow's milk daily for 19 days. Clinical condition, growth, colonic microbial diversity, microbial metabolite concentration, villus height and crypt depth, gut function (digestive capacity for lactose), organ weights (proximal small intestine, middle small intestine, distal small intestine, stomach, colon, liver, spleen, heart, lungs, kidneys, adrenals, brain), clinical chemistry (albumin, total protein, alkaline phosphatase, alanine aminotransferase, total bilirubin, cholesterol, creatinine, creatine kinase, iron, phosphate, aspartate aminotransferase, blood urea nitrogen, gamma-glutamyl transferase, calcium, magnesium, sodium, potassium, lactate, and glucose), hematology (white blood cells, red blood cells, hemoglobin, hematocrit, platelets, neutrophils, lymphocytes, monocytes, eosinophils, basophils), and systemic immunity (phagocytic capacity of collected neutrophils to engulf a *Staphylococcus aureus* challenge) were measured as a part of this investigation. No compound-related adverse effects were reported with respect to any measured parameters. The authors reported that the SL supplementation was well tolerated in the artificially reared preterm piglets.

In a 30-day study, the effects of a dietary bovine milk-based formula containing a SL mixture (Lacprodan SAL-10, Arla Foods Ingredients Group; not further specified) on weight gain, gastrointestinal development, microbiota composition, clinical chemistry, and hematology was investigated in piglets (12/group) aged 1 day at study commencement (Monaco *et al.*, 2018). Piglets were administered formula containing 0, 130, 380, or 760 mg SL/L daily. On Days 1 to 4 (PND 2 to 5), piglets were administered 285 mL formula/kg body weight/day, which was increased to 325 mL formula/kg body weight/day starting on Day 5 (PND 6). Weight gain and growth were measured daily. Eight hours after the final feeding, blood samples were collected and clinical chemistry (calcium, phosphorus, magnesium, sodium, potassium, chloride, total protein, albumin, globulin, glucose, total cholesterol, triglycerides, creatinine, urea, total bilirubin, bicarbonate, alkaline phosphatase, aspartate transaminase, gamma-glutamyltransferase, creatine phosphokinase, and glutamate dehydrogenase) and hematology (red blood cells, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, platelets, mean platelet volume, white blood cells, neutrophils, lymphocytes, band, monocytes, eosinophils, basophils, activated partial thromboplastin time, and prothrombin time) parameters were evaluated. Histomorphologic analyses were conducted on the duodenum, jejunum, ileum, and ascending colon (villus height and area, crypt area, colon cuff depth, and cuff area). Growth of the intestinal tract was also evaluated (intestinal length and weight). No compound-related, toxicologically relevant adverse effects were reported with respect to the parameters measured, and the authors noted that supplementation of SL in formula was supportive of normal growth and was well tolerated, as indicated by the lack of adverse effects on piglet development.

In a 35-day study, 3-day-old male piglets (*Sus scrofa* landrace × large white F1; 17/group) were orally administered SL (3'-SL:6'-SL, 5:1; GeneChem Inc.) in milk replacement formula (Feed & Grow, International Co. Ltd. China) at doses of 0 (control) or 1.71 g/L (Yang *et al.*, 2018). Piglets received 285 mL formula/kg body weight/day from PND 3 to 15, and 230 mL formula/kg body weight/day for the remainder of the study duration. Body weight, milk intake, health status (not further specified), and stool consistency were measured daily. Evaluations of intestinal gene and protein expression, intestinal histology, and intestinal immunofluorescence were conducted. A significant decrease in diarrhea incidence and severity was reported in animals administered SL supplemented formula, although no difference was observed in time-to-onset. No compound-related adverse effects were reported with respect to the parameters measured, including growth or clinical signs. The authors considered the observed effects as beneficial to the neonatal piglets; these effects included significantly increased levels of intestinal crypt cell proliferation biomarker Ki67, along with a significant increase in intestinal crypt area, depth, and width in the ileum. The glial cell line-derived neurotrophic factor signaling pathway was also significantly upregulated, which is involved in the self-renewal of epithelial cells.

Table 6.4.5.4-1 Summary of Gastrointestinal Developmental Studies of HMO Mixtures Containing 3'-Sialyllactose and/or 6'-Sialyllactose

Species	Duration	Test Article [Method]	Dose (mg/kg bw/d)	Outcome Parameters	Results Relevant to Safety	Reference
Piglet (18 to 20/group; preterm from GD 106; strain and sex NR)	19 days	SL (8.5 g/L Lacprodan SAL-10, Arla Foods Ingredients; 4.5% SL with 3'-SL:6'-SL 6:1; purity and source NR) [unpasteurized Jersey cow's milk]	Dose in mg/kg bw/d NR [0 or 380 mg/L SL]	Clinical condition, growth, colonic microbial diversity, microbial metabolite concentrations, villus height and crypt depth, gut function, organ weights, clinical chemistry, hematology, systemic immunity	No compound-related adverse effects on measured parameters. The oral SL supplementation was reported to be well tolerated.	Obelitz-Ryom *et al.* (2018)
Piglet (M; 12/group; 1 day old; strain NR)	30 days (PND 2 to 32 or 33)	SL (Lacprodan SAL-10, Arla Foods Ingredients Group; purity and source NR) [bovine milk-based formula]	Dose in mg/kg bw/d NR [0, 130, 380, or 760 mg/L]	Bw gain, gastrointestinal development, microbiota composition, clinical chemistry, hematology	No compound-related adverse effects on measured parameters. SL was reported to be supportive of normal growth and was well tolerated.	Monaco *et al.* (2018)
Piglet (M; *Sus scrofa* landrace × large white F1; 17/group; 3 days old)	35 days	SL (3'-SL:6'-SL 5:1; purity NR; GeneChem Inc.) [milk replacement formula (Feed & Grow, International Co. Ltd. China)]	Dose of SL in mg/kg bw/d NR [0 or 1.71 g/L]	Bw, milk intake, health status (not further specified), stool consistency, intestinal gene and protein expression, intestinal histology, intestinal immunofluorescence	No compound-related adverse effects on measured parameters. SL was well tolerated by neonatal piglets.	Yang *et al.*, 2018

3'-SL = 3'-sialyllactose; 6'-SL = 6'-sialyllactose; bw = body weight; d = day; GD = Gestation Day; M = male; NR = not reported; PND = Post-natal Day; SL = sialyllactose.

6.5 Human Studies

6.5.1 Intervention Studies Conducted with Other 6'-SL Preparations and with the Structurally-Related 3'-SL

One study (Parschat *et al.*, 2021) was identified in the update literature search that was not included in previous GRAS evaluations for 6'-SL sodium salt notified to the U.S. FDA. The study is summarized below and in Table 6.5.1-1.

Parschat *et al.* (2021) reported a multi-center, randomized, controlled, parallel group, GLP-compliant study to assess potential associations between a mixture of 5 HMOs and infant growth over a 16-week period (from ≤14 days of age to 4 months of age), in addition to the safety and tolerability of the HMO mixture. Infants (≤14 days of age at first visit, born at ≥37 weeks and ≤42 weeks of gestational age) were randomized to receive an infant formula containing 5.75 g/L of the 5-HMO mixture (113 subjects; 97 included in full analysis dataset) or an infant formula without additional HMOs (112 subjects; 101 included in full analysis dataset). A breastfeeding reference group (116 subjects; 102 included in full analysis dataset) was also included in the study. Infant formulas were consumed *ad libitum.* Over 6 visits during the 16-week period (Days 14, 28, 56, 84, 112, and 180), the following infant data were recorded and assessed: tolerability (stool frequency and consistency), digestive tolerability (regurgitation, vomiting, flatulence), behavioral parameters (fussiness, crying, awakening at night), adverse effects, body weight, body length, and head circumference. Breast milk samples were collected from the reference group at the second, fourth, and sixth visits. The 5-HMO mixture contained 0.23 g/L 3'-SL and 0.28 g/L 6'-SL, as well as 2'-FL, 3'-FL, and LNT, and the infant formula also contained proteins, lipids, other carbohydrates, vitamins, and nutrients. The control formula contained the same quantities of proteins, lipids, other carbohydrates, vitamins, and nutrients. The mean daily intake volume of infant formula was calculated from the weight of returned packages of infant formula. Mean intakes of 3'-SL and 6'-SL were calculated to be 0.17 and 0.21 g/day, respectively. The total incidence of adverse events was similar between the infant formula groups, and there was no significant difference between the intervention groups and the breastfeeding group. One subject withdrew from the HMO mix group due to severe diarrhea, which was later confirmed to result from allergy to bovine milk protein. Stool frequency declined in all groups over the course of the study. From Day 84 to the end of the study, there were no significant differences in stool frequency between the HMO group and the breastfed reference group, while at the last visit (Day 180) stool frequency was significantly lower in the control formula group compared to the breastfed reference group. No significant differences between the 2 infant formula groups were reported with respect to infant stool consistency, flatulence, or regurgitation or vomiting. From Day 0 to 56 there were significantly more soft stools in the HMO mix group compared to the control formula group, however the breastfed reference group had the greatest number of soft stools. There were no significant differences in mean weight, length, or head circumference between the 2 infant formula groups. Compared to the breastfed reference group, body weight and weight-for-age z-scores (WAZ) in both infant formula groups were significantly greater at Day 180, and body length and length-for-age z-scores in both infant formula groups were significantly higher at Days 112 and 180. There were no significant differences between infant formula groups and breastfed reference controls in head circumference. The authors concluded that "*an infant formula fortified with a mixture of the five most abundant HMOs (2'-FL, 3-FL, LNT, 3'-SL, and 6'-SL) at the concentrations and ratios resembling those in breast milk supports normal infant growth and is safe and well-tolerated for use in healthy term infants*" (Parschat *et al.*, 2021).

Two studies of 3'-SL (NE-080; Neose Technologies Inc., Horsham PA) in adults were identified in the literature and are summarized in Table 6.5.1-1. No details on the source, purity, or manufacturing process for NE-080 were reported in these studies. Both of these studies (Opekun *et al*., 1999; Parente *et al*., 2003) were reviewed in GRAS evaluations notified to the U.S. FDA and filed as GRNs 766, 880, and 921 to which the FDA responded with no questions (GeneChem, Inc., 2018; Glycom A/S, 2019b; Jennewein Biotechnologie GmbH, 2020b; U.S. FDA, 2018c, 2020f,g). In the first study, 6 otherwise healthy men with gastric *H. pylori* infection consumed five 2-g doses of 3'-SL following meals and snacks over the course of a 24-hour period for a total dose of 10 g, and *H. pylori* infection [determined *via* histopathology, positive serology, and a ^{13}C-urea breath test (UBT)], inflammatory response, and serum liver transaminase levels (not further specified) were reported (Opekun *et al.*, 1999). There were no differences from baseline in liver transaminase tests. The second study was a randomized, double-blind, controlled trial in which 60 dyspeptic adult patients with *H. pylori* infection (as determined by UBT values >15) consumed 0, 10, or 20 g 3'-SL (NE-080)/day for 4 weeks (Parente *et al.*, 2003). The authors concluded that 3'-SL was safe and well tolerated due to the nature of the reported adverse events (halitosis, asthenia, epigastric pain, and headache) and their lack of severity, as well as no significant changes in UBT values. The results of these studies support the safety of 3'-SL at doses up to 20 g/day in adults.

Table 6.5.1-1 Summary of Human Studies of Other 6'-Sialyllactose and 3'-Sialyllactose Preparations

Study Population & Design	Duration	Test Article	Dose	Outcome Parameters	Results Relevant to Safety	Reference
Studies in Infants						
341 healthy infants (≤14 days of age at first visit born at ≥37 weeks and ≤42 weeks of gestational age) MC, P, R, DB, C	16 weeks	5HMO-mix [n=97 (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)] Manufactured by Töpfer Control: Infant formula (n=101) without 5HMO mix A breastfed-only group (n=102) served as the reference group	*ad libitum.* 6'-SL mean intake: 0.21 g/day	Anthropometric data, (body weight, body weight gain, body length, head circumference) Digestive tolerability (stool frequency and consistency, regurgitation, vomiting, flatulence) Behavioral parameters (fussiness, crying, awakening at night) Adverse effects	NSD in incidence of AE between formula groups. One subject withdrew from the HMO mix group due to severe diarrhea, which was later confirmed to result from allergy to bovine milk protein. NSD in stool frequency between HMO group and BM group from Days 84 to 180. NSD between infant formula groups in infant stool consistency, flatulence, or regurgitation or vomiting. NSD between infant formula groups in mean weight, length, or head circumference. From Day 0 to 56 there were significantly more soft stools in the HMO mix group compared to the control formula group, however the reference group had the greatest number of soft stools.	Parschat *et al.* (2021)

Table 6.5.1-1 Summary of Human Studies of Other 6'-Sialyllactose and 3'-Sialyllactose Preparations

Study Population & Design	Duration	Test Article	Dose	Outcome Parameters	Results Relevant to Safety	Reference
Studies in Adults						
6 otherwise healthy men with gastric *Heliobacter pylori* infection (33 to 49 years old) Open Label	1 day	3'-SL [method of manufacture and purity NR]	10 g (in 5 divided doses of 2 g each)	*H. pylori* infection (determined *via* histopathology, positive serology, and ^{13}C-urea breath test), inflammatory response, serum liver transaminase levels, adverse effects	No compound-related adverse effects on measured parameters.	Opekun *et al.* (1999)
60 Dyspeptic patients with gastric *Heliobacter pylori* infection (24 M; 36 F; 53 ± 9 years old) R, C, DB	4 weeks	3'-SL [method of manufacture and purity NR]	0, 10, or 20 g/day	*H. pylori* infection (determined *via* histology and ^{13}C-urea breath test), adverse effects	No compound-related adverse effects on measured parameters.	Parente *et al.* (2003)

3'-SL = 3'-sialyllactose; AE = adverse effects; BM = breast milk; C = controlled; DB = double blind; HMO = human milk oligosaccharide; F = females; M = males; MC = multi-center; NR = not reported; NSD = no significant differences; P = parallel; R = randomized.

6.5.2 Observational Studies

Four observational studies were identified in the update literature search (Binia *et al.*, 2021; Cho *et al.*, 2021; Menzel *et al.*, 2021; Saben *et al.*, 2021) in which associations between the consumption of HMOs (including 3'-SL and 6'-SL) and infant growth, adiposity, and/or language development were investigated. These studies were not included in previous GRAS evaluations for 3'-SL or 6'-SL sodium salt notified to the U.S. FDA. The studies are summarized below, and the results do not suggest a concern for safety from the consumption of 3'-SL and 6'-SL at levels present in breast milk.

Menzel *et al.* (2021) conducted a study to evaluate the association between breast milk HMO concentrations present at 3 months postpartum and child growth in 145 breast milk sample-child pairs from 3 months to 7 years of age. A milk sample was collected at 3 months and analyzed for HMO concentrations using liquid chromatography with fluorescence detection (LC-FD). The duration of infant feeding with breast milk was not reported, although it was noted that the milk samples were collected from nursing mothers (implying that the duration of breastfeeding was ≥3 months). Infant parameters (anthropometric measurements, gestational age) at birth were obtained, and the following parameters were recorded at 6 months of age, 1 year of age, and then annually until 7 years of age: height, growth velocity, head circumference, weight, and BMI. Height, weight, and BMI were then converted into standard deviation scores (SDS). The concentrations of 3'-SL and 6'-SL in milk at 3 months were negatively associated with BMI-SDS at all time points assessed, reaching significance in non-secretor mothers at 6 months, 1 year, 3 years, 4 years, and 7 years for 3'-SL and in non-secretor mothers at 3 months, 1 year, 4 years, 5 years, and 6 years for 6'-SL. The concentration of 3'-SL in milk at 3 months postpartum was negatively associated with growth velocity from 4 to 5 years of age but not at other timepoints. No significant associations were reported between concentrations of 3'-SL or 6'-SL and head circumference or height.

Binia *et al.* (2021) evaluated the relationship between HMOs and infant growth and adiposity over the first 4 months of lactation in 357 mother-infant pairs from 7 European countries. Infant anthropometry (weight, length, head circumference, and BMI), infant body composition [(fat mass and fat-free mass (FFM)], and HMO composition were assessed at 6 postpartum time points (*i.e.,* birth and 2, 17, 30, 60, 90, and 120 days) in varying numbers of subjects. Maternal milk was collected following complete expression from a single breast and HMO composition was analyzed by LC-FD. Due to withdrawal from the study for varying reasons, 322 mother-infant pairs were assessed at birth, and 224 mother-infant pairs were assessed at 4 months. Relationships between individual HMO area under the concentration time curve (AUC) over time (Day 2 to Day 120) and infant anthropometry and body composition parameters at 4 months were assessed using Spearman's rank-order correlations. Although the 3'-SL AUC over 4 months was negatively correlated with length, all infants were reported to grow within normal ranges in accordance with World Health Organization (WHO) growth charts, and the authors noted that there was little impact of individual HMOs on anthropometric data, fat mass accretion, or fat mass index. Infants in the weight-for-length z-score gain upper 25th percentile had a higher concentration of 3'-SL than those in the lower 25th percentile for weight-for-length z-score. When the analysis was split by gender, the same findings were reported for males but there were no differences reported in the females. No significant associations were reported for 6'-SL. The study authors concluded that "*individual HMO AUC during the first 4 months appears to have no or only moderate effect on infant growth and body composition during this time of exclusive breastfeeding in term-born, healthy growing infants*" (Binia *et al.*, 2021).

The association between HMO intake and infant growth from 0 to 6 months of age in 194 mother-infant breastfeeding pairs (140 exclusively breastfeeding) was investigated by Saben *et al.* (2021). Maternal milk was collected following complete expression from a single breast 2 months after birth and was analyzed by HPLC. Infants' gestational weight gain was calculated between the subject's first study visit and Week 36 of gestation. Infant weight, length, fat mass, and FFM were measured at 2 and 6 months of age. A WAZ was then calculated using the WHO Child Growth Standards (WHO, 2006). The relationship between HMO intake and infant growth from 2 to 6 months was determined using linear mixed-effects models. In exclusively breastfed infants, 3'-SL intake levels were significantly positively associated with fat mass and WAZ, and 6'-SL intake levels were significantly positively associated with fat mass from 2 to 6 months of age.

In a study of 99 mother-infant pairs with 183 breast milk samples, Cho *et al.* (2021) investigated the association between *alpha*-tetrasaccharide and other HMOs (including 3'-SL and 6'-SL) in breast milk and language development during infancy. The subjects were 2 to 25 months of age at baseline, and were classified as predominantly breastfed when infants consumed less than 4 teaspoons or 20 g/day of other food or liquids (80 subjects) or as mixed breastfed infants when infants consumed more than 50% human milk intake (15 subjects). Breast milk samples were collected at each study visit[9] by complete expression from a single breast and analyzed using LC-FD. The mean duration of breastfeeding was 14.4 ± 4.95 months. The Mullen Scales of Early Learning (MSEL) was used to assess fine motor, gross motor, visual reception, receptive language, and expressive language in the infant subjects. An Early Learning Composite (ELC) score was derived for all the MSEL subdomains, excluding the gross motor domain. The concentration of 6'-SL in breast milk was found to decrease with age, whereas 3'-SL increased with age. There were no significant associations between cognition and 3'-SL without stratification, however, when the effects of age were removed from the HMO levels, there was a significant positive association between age-removed 3'-SL levels and ELC scores. When the specific subdomains were investigated, significant positive associations also were reported between 3'-SL and the receptive and expressive language scores. The effect of age on these scores was investigated but was not reported to be significant. There were no significant associations reported regarding 6'-SL levels and MSEL or ELC scores.

6.5.3 Safety of 6'-SL Sodium Salt in Enteral Tube Feeding Formula

No human studies have been conducted with 6'-SL sodium salt added to formula for enteral tube feeding. HMOs, including 6'-SL, are considered to be non-digestible oligosaccharides (EFSA, 2020a). Therefore, the safety and suitability of 6'-SL sodium salt for use in formula for enteral tube feeding was assessed using data from studies conducted with other non- or poorly-digestible carbohydrates.

Studies conducted to assess the safety/tolerability of other poorly-digestible carbohydrates as components of enteral tube feeding formula in a variety of healthy and vulnerable patient populations were summarized in GRN 897 in response to U.S. FDA Question 8 (DuPont Nutrition and Health, 2019), which is incorporated herein by reference. The notifier considered the results of 17 unique published studies[10] of the safety/tolerability of other poorly-digestible carbohydrates as components of enteral tube feeding formula in a variety of healthy and vulnerable patient populations. An additional study included in the reference list but not in the response to Question 8 of GRN 897 also was identified. These studies are summarized below in Table 6.5.3-1. Briefly, the studies involved administration of partially hydrolyzed guar gum (PHGG), galactomannan, fructooligosaccharides (FOS), galactooligosaccharides (GOS), and FOS/GOS mixtures at

[9] Study visits were conducted irregularly, at time points between 2 and 25 months of age, with some subjects having only 1 visit, and others having up to 4 visits at irregular time intervals and varying subject ages.

[10] Due to the inclusion of a pair of kin studies (Homann *et al.*, 1994, 2004) and a duplication of an additional study (Karakan *et al.*, 2007), a total of 17 unique studies of poorly-digestible carbohydrates in enteral feeding formula were included in GRN 897.

doses up to 63 g/day. The notifier concluded that the safety of the use of 2'-FL (the subject of GRN 897) as an ingredient in enteral tube-feeding formula at levels up to 20 g/kg was supported by the lack of test compound-related adverse effects reported in the identified studies, as well as the Institute of Medicine's conclusion that establishing a tolerable upper intake level for fiber was not necessary (due to the unlikelihood of adverse effects due to excessive consumption of fiber) (U.S. FDA, 2020b). Upon consideration of the information provided by the notifier, the U.S. FDA responded with no questions regarding the GRAS status of 2'-FL under the conditions of use specified in GRN 897, including use in enteral tube feeding formula at levels up to 20 g/L.

Kyowa obtained the original published studies of poorly-digestible carbohydrates in enteral tube feeding formula cited in GRN 897 in order to clarify details of study design and results as presented in GRN 897. In addition, Kyowa conducted a search of the published literature[11] on 27 October 2021 to identify any studies of poorly-digestible carbohydrates in enteral tube feeding formula published since March 2020. One newly identified study of poorly-digestible carbohydrates in enteral tube feeding formula was identified (Chen *et al*., 2021) and is included in Table 6.5.3-1 below.

The 19 unique studies of poorly-digestible carbohydrates cited in Table 6.5.3-1 included studies of PHGG, galactomannan, FOS and/or GOS, or polydextrose in healthy adults or children and adults, children, and infants with a range of chronic or acute medical conditions. In these studies, no test product-related adverse effects were reported with respect to the measured parameters, including those related to clinical outcomes, immune function, fecal characteristics (*e.g.,* frequency or consistency), or standard safety parameters (*i.e.,* hematology, clinical chemistry, or vital signs). Some mild, transient symptoms of gastrointestinal intolerance considered typical and expected following supplementation with soluble fiber (*i.e.*, flatulence, abdominal distension, abdominal pain, diarrhea) were reported in several studies. The reported gastrointestinal symptoms occurred upon supplementation with up to 24 g PHGG/day in post-operative or critically ill adults, consumption of 30 g scFOS/day by healthy adults, or administration of approximately 1.22 g FOS/day to children (1 to 12 years of age) undergoing chemotherapy for stage 1 to 3 cancer (Homann *et al.*, 1994, 2004; Fussell *et al.*, 1996; Garleb *et al.*, 1996; Zheng *et al.*, 2006). In all 4 of these studies, the authors concluded that, overall, the intervention products were well-tolerated and were considered to be beneficial with respect to clinical outcomes (Homann *et al.*, 1994, 2004; Fussell *et al.*, 1996; Garleb *et al.*, 1996; Zheng *et al.*, 2006).

Kyowa agrees with the conclusions presented in GRN 897, *i.e.,* that the results of the identified studies of poorly-digestible carbohydrates at doses up to 63 g/day in enteral tube feeding formula support the safety of 2'-FL for use in enteral tube feeding formula at the intended use level of 20 g/kg. Kyowa also considers that the results of the identified studies of poorly-digestible carbohydrates at doses up to 63 g/day in enteral tube feeding formula support the safety of 6'-SL on the basis that 6'-SL is a non-digestible oligosaccharide (EFSA, 2020a). Kyowa's 6'-SL sodium salt ingredient is proposed for use at a level of 4.1 g/L, which is 1/5th the level concluded to be GRAS for 2'-FL and consistent with the ratio of 6'-SL to 2'-FL present in human breast milk.

[11] The databases searched include: AdisInsight: Trials, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, ToxFile®, Toxicology Abstracts, and Toxicology Abstracts. Terms pertaining to human exposure via tube-feeding formula were used with terms intended to capture poorly-digestible carbohydrates (*i.e.,* names, synonyms, abbreviations, and CAS numbers) included in the notifier's response to FDA's Question 8 on Page 39 GRN 897.

Kyowa concludes that the safety of 6'-SL sodium salt in formula for enteral tube feeding at a use level of 4.1 g/L is supported by the safety profile of the ingredient and the safety of poorly-digestible carbohydrates in general in enteral feeding at levels that exceed the recommended intake of 6'-SL sodium salt from the intended use in formula for enteral tube feeding.

Table 6.5.3-1 Studies from GRN 897

Patient Population and Study Design	Dose or Concentration and Study Duration	Results Relevant to Safety	Reference
Studies of PHGG			
11 healthy men P, R, DB, CO	ETFF providing 0 or 15 g PHGG/day 18 days	• No compound-related adverse effects on fecal wet and dry weights, fecal moisture content, fecal pH, and stool frequency. • No adverse events reported. Authors concluded that "*despite significant differences in mean transit time, few differences in other parameters of bowel function were observed when healthy subjects consumed enteral formula diets containing 0 g of fiber and 15 g of total dietary fiber as modified guar and soy*".	Lampe *et al.* (1992)
12 healthy men (mean age = 29 years) R, CO	Liquid formula diet providing 0 or 42 g PHGG/day 7 days	• Significantly increased colonic transit time (*vs.* washout period or formula without fiber; not considered to be an adverse effect). • No effect on stool consistency or frequency reported.	Meier *et al.* (1993)
10 healthy adults R, DB, CO	ETFF with 42 to 63 g PHGG/day 7 days	• No reports of intolerance. • No adverse effects on hemoglobin, hematocrit, total and differential white blood cell count, Na, K, Mg, Cl, ALT, AST, GGT, alkaline phosphatase, bilirubin, or creatinine.	Alam (1993)
100 postoperative subjects (mean age = 59 to 70 years); 30 administered TEN and 70 administered enteral supplementation P, R, DB, PC	TEN: standard ETFF or ETFF with 24 g PHGG/day Enteral supplementation: standard ETFF or ETFF with 20 g PHGG/day ≥5 days	• Increased but well-tolerated flatulence (both PHGG groups), without bloating or cramping. • Total number of adverse gastrointestinal effects not significantly different between PHGG and standard ETFF groups. The authors reported: "*The total number of GI-side effects was not different in the two groups (17 in each group)*".	Homann *et al.* (1994, 2004)
57 critically ill adults (with recent abdominal surgery/ trauma, cerebral trauma, head/neck surgery, multiple fractures, or vascular surgery) P, R, DB, PC	ETFF with 0 or 14 g PHGG/L formula 5 to 14 days	• No adverse effects with respect to diarrhea, albumin, transthyretin, or flatulence. • Abdominal distension observed significantly more often in PHGG group, although authors noted that this was not clinically significant. • PHGG was generally well-tolerated.	Fussell *et al.* (1996)
12 subjects (age NR) with type 1 diabetes PC, CO	480 mL ETFF consumed over 4 hours (dose or concentration of PHGG NR)	• Monitoring or reporting of adverse effects or adverse events not reported.	Peters and Davidson (1996)

Table 6.5.3-1 Studies from GRN 897

Patient Population and Study Design	Dose or Concentration and Study Duration	Results Relevant to Safety	Reference
25 ICU patients (mean age = 68.5 ± 13.1 years) with severe sepsis and septic shock P, R, DB, PC	Standard ETFF or ETFF with 22 g PHGG/L (daily dose NR) 6 to 21 days	• No adverse effects with respect to diarrhea, sepsis-related mortality, or duration of ICU stay. Authors concluded: "*Fiber treatment was well-tolerated and did not affect glucose control*".	Spapen *et al.* (2001)
20 adult ICU patients (with ≥3 liquid stools/day and a variety of health conditions/injuries) P, R, DB, C	ETFF With 22 to 39 g PHGG/day (22 g PHGG/L) 4 days	• No compound-related adverse effects on number of liquid stools, tolerance, or incidence or severity of gastrointestinal symptoms (including flatulence, vomiting, constipation. • Significant decrease from baseline in number of liquid stools.	Rushdi *et al.* (2004)
Studies of Galactomannan			
20 elderly subjects (bed-ridden) Open-label	ETFF with 7 g galactomannan/day (1st week); dose increased by 7 g/day each week until 4th week (28 g/day) 4 weeks	• No compound-related adverse effects on serum diamine oxidase activity, fecal water content, frequency of normal stools, frequency of bowel movements, number of aerobic bacteria, fecal pH, fecal SCFA, total bacteria or anaerobe counts, body weight, total serum protein, prealbumin, transferrin, retinol-binding protein, total cholesterol, triacylglycerol, iron, copper, or zinc. • No adverse events reported. Authors concluded that soluble dietary fiber is "*useful for controlling spontaneous, favorable bowel movement*".	Nakao *et al.* (2002)
Studies of FOS			
30 patients (mean age = 46.1 ± 14.0 years) with severe acute pancreatitis R, DB, PC	ETFF with 24 g fiber (containing approximately 50% scFOS)/day 2 days	• No compound-related adverse effects on duration of enteral feeding or hospital stay, pancreatitis severity scores, mortality, or overall complications. • Formula was well-tolerated with no reported adverse effects or adverse events.	Karakan *et al.* (2007)
14 children (1 to 15 years of age) with compromised gut function receiving 75 to 100% of calories *via* ETF R, DB, CO	ETFF with 3.5 g FOS/day (3.5 g FOS/L) 14 days	• No compound-related adverse effects with respect to stool quality, vomiting, abdominal pain, or weight gain. Authors concluded: "*This study showed that a peptide-based formula containing fiber was as well-tolerated as a fiber-free formula in a small population of children with gastrointestinal impairments*".	Khoshoo *et al.* (2010)

Table 6.5.3-1 Studies from GRN 897

Patient Population and Study Design	Dose or Concentration and Study Duration	Results Relevant to Safety	Reference
27 healthy college students R, DB, C	ETFF with 0, 15, or 30 g scFOS/day (0, 5, or 10 g/L formula) 14 days	• No compound-related adverse effects on body weight, clinical chemistry, fecal short-chain fatty acids, fecal pH, fecal dry matter, reported adverse effects (nausea, cramping, distension, vomiting, diarrhea, and regurgitation). • Increased flatulence in 30 g/day group (during first 4 days of intervention). • One withdrawal from high-dose group due to unspecified intolerance. • scFOS-containing formulas were well-tolerated. Authors concluded that "*these results indicate that [scFOS] does not compromise serum chemistry profiles, is well-tolerated particularly at an intake of 15 g/d and would serve as a bifidogenic factor when incorporated into a liquid enteral product*".	Garleb *et al.* (1996)
94 critically ill children (1 to 3 years of age) on mechanical ventilation R, DB, PC	Control ETFF or ETFF with 2.6 g oligofructose/inulin and 2.8 g acacia gum/L, DHA, and 5 strains of live microorganisms ≤14 days	• No compound-related adverse effects on caloric intake, abdominal distension, vomiting, stool frequency, or fecal microbiota. Authors concluded that the study product is safe and well-tolerated by children in ICU.	Simakachorn *et al.* (2011)
67 children (1 to 12 years of age) with stage 1 to 3 cancer and undergoing chemotherapy P, R, DB, PC	Standard ETFF or ETFF with FOS (2 g/L; 1.22 ± 0.24 g/day; 60 ± 20 mg/kg bw/day) 13 to 30 days	• No compound-related adverse effects on fecal microbiota, biomarkers of immunologic status (*i.e.,* cytokines and cell counts), nutritional status, weight, blood pressure, heart rate, body temperature, respiratory rate, prognostic inflammatory and nutritional index, stool characteristics, and standard hematological and biochemical parameters. • Transient gastrointestinal effects: rectal discomfort (1/32 in FOS group), mild flatulence (3/32 in FOS group; 2 reported in association with abdominal pain), mild diarrhea (1/32 in FOS group), nausea (12/35 in control group and 11/32 in FOS group). • One adverse event: 1 subject in FOS group had diarrhea and complained of abdominal pain on study Day 3 and was withdrawn from the study (subject had been non-compliant with study protocol from Days 1 to 3). • Authors noted a lack of gastrointestinal discomfort and concluded: "*Both enteral formulas were well-tolerated and accepted*".	Zheng *et al.* (2006)

Table 6.5.3-1 Studies from GRN 897

Patient Population and Study Design	Dose or Concentration and Study Duration	Results Relevant to Safety	Reference
Studies of GOS or GOS/FOS Mixtures			
154 preterm infants (gestational age <33 weeks) P, R, DB, PC, MC	Standard formula or formula with 8 g scGOS:lcFOS (9:1)/L ~8 weeks or until hospital discharge	• No compound-related adverse effects on tolerance; gains in weight, length, or head circumference; stool frequency or characteristics; fecal microbiota; gastrointestinal signs; or overall water balance (based on concentrations of serum sodium and creatinine). Authors concluded: "*Prebiotic supplementation appears safe and may benefit enteral tolerance in the most immature infants*".	Modi *et al.* (2010)
23 elderly subjects (bedridden with a variety of chronic health conditions) P, R, DB, PC	Standard ETFF or ETFF with fermented milk, GOS (4 g/day), and prebiotic bifidogenic growth stimulator (0.4 g/day) 10 weeks	• No compound-related adverse effects with respect to hematology, clinical chemistry, fecal microbiota, antibody response to influenza vaccine, or plasma cytokine levels. • No adverse events reported.	Akatsu *et al.* (2016)
50 preterm neonates with hyperbilirubinemia P, R, DB, PC	Standard ETFF or ETFF with 9:1 ratio of scGOS:icFOS (initially 0.5, increased to 1.5 g/kg bw/day) 1 week	• No adverse effects with respect to bilirubinemia or stool frequency. Authors concluded: "*Prebiotic oligosaccharides increase stool frequency, improve feeding tolerance and reduce bilirubin level in preterm neonates and therefore can be efficacious for the management of neonatal hyperbilirubinemia*".	Armanian *et al.* (2016)
113 infants with gestational age <32 weeks or birth weight <1,500 g P, R, DB, PC	Breast milk or formula alone or with scGOS, lcFOS, and pectin-derived acidic oligosaccharides (dose or concentration NR) 28 days	• No adverse effects on response to influenza vaccination. • Monitoring or reporting of adverse events not reported.	van den Berg *et al.* (2015)
Studies Identified in Literature Search Conducted 27 October 2021			
51 adults with severe acute pancreatitis P, R, single-blind, C	Standard ETFF or ETFF with 20 g polydextrose/day	• No compound-related adverse effects on feeding intolerance, symptoms and signs of gastrointestinal tolerance (abdominal distension, vomiting, diarrhea, constipation, gastrointestinal bleeding, bowel sounds, intra-abdominal pressure), other signs of gastrointestinal health (flatulence, bowel habit, intestinal barrier function, gastrointestinal hormones), or clinical outcomes. • No adverse events reported. Authors concluded: soluble dietary fiber is well-tolerated and improves clinical outcomes in patients with severe acute pancreatitis.	Chen *et al.* (2021)

ALT = alanine aminotransferase; AST = aspartate aminotransferase; C = controlled; Cl = chloride; CO = crossover; DB = double-blind; DHA = docosahexanoic acid; ETFF = enteral tube feeding formula; FOS = fructooligosaccharides; GGT = *gamma*-glutamyltransferase; GOS = galactooligosaccharides; ICU = intensive care unit; K = potassium; lc = long-chain; MC = multi-center;

Table 6.5.3-1 Studies from GRN 897

Patient Population and Study Design	Dose or Concentration and Study Duration	Results Relevant to Safety	Reference

Mg = magnesium; Na = sodium; NR = not reported; P = prospective; PC = placebo-controlled; PHGG = partially hydrolyzed guar gum; R = randomized; sc = short-chain; SCFA = short-chain fatty acids; TEN = total enteral nutrition.

6.6 Other Considerations – Use of 6'-SL Sodium Salt in Combination with Other HMOs or Poorly-Digestible Carbohydrates

Kyowa is not a manufacturer of infant formula; however, the company considers it likely that infant formula manufacturers may use a combination of HMOs, or other poorly digestible carbohydrates, to produce infant formula products that are more compositionally similar to human milk. Kyowa acknowledges that symptoms of gastrointestinal intolerance have been reported upon consumption of large amounts of poorly-digestible carbohydrates, especially in sensitive populations, including infants. Kyowa anticipates that their HMO ingredients, including 6'-SL sodium salt, may be used as ingredients in infant formula in combination with other HMOs in order to provide a variety of HMOs at concentrations that are within the natural variation of concentrations found in human milk. The safety and expected tolerability of the combined intake of different HMOs and other poorly-digestible carbohydrates have been addressed in previous GRAS notices to which the U.S. FDA responded with no questions (GRNs 815, 833, 880, 881, 932, 951) (U.S. FDA, 2019b,c, 2020e,f, 2021a,d).

As noted above in Section 3.2.2.2 the proposed use level of Kyowa's 6'-SL sodium salt in infant formula was chosen by Kyowa to align with the mean concentration of 6'-SL in human milk (*i.e.,* 0.50 g 6'-SL/L). This use level is well within the range of concentrations in human milk, with corresponding intakes from all intended uses within the ranges of infant consumption of 6'-SL from human milk. Other HMOs also are GRAS for use in infant formula (with no questions from the U.S. FDA), including 3'-SL, 2'-FL, 3'-FL, 2'-FL/DFL, LNT, and LNnT, at use levels intended to be reflective of the mean concentrations of the individual HMOs in human milk, taking into account natural variation among women with differing Lewis/Secretor genotypes and at various lactational stages (GRNs 659, 815, 833, 880, 881, 932, 951). Since each individual HMO would be used in infant formula at a level comparable to its mean concentrations in human milk, the combined intake of these HMOs as ingredients in infant formula would be expected to be similar to their intake *via* human milk. The combined intake of the HMOs that are GRAS for use in infant formula would therefore be expected to be safe and well-tolerated based on their history of consumption *via* human milk.

Kyowa notes that the results of a multi-center, randomized, double-blind, controlled intervention study in healthy, singleton, term infants provide support for the safety and tolerability of consumption of combinations of HMOs (*i.e.*, 2'-FL, 3'-FL, LNT, 3'-SL, and 6'-SL) that are individually present at use levels that are within their natural variation in human milk (Parschat *et al.*, 2021; see Section 6.5.1 above). In this study, no significant differences between a group of infants consuming the HMO formula and a group of infants consuming a control formula were reported in incidences of adverse events, infant stool consistency, flatulence, regurgitation, vomiting, mean weight, length, or head circumference. The study authors concluded that "*an infant formula fortified with a mixture of the five most abundant HMOs (2'-FL, 3-FL, LNT, 3'-SL, and 6'-SL) at the concentrations and ratios resembling those in breast milk supports normal infant growth and is safe and well tolerated for use in healthy term infants*" (Parschat *et al.*, 2021).

The use of Kyowa's 6'-SL sodium salt in infant formula is intended to be substitutional to other 6'-SL sodium salt ingredients produced by other manufacturers and currently on the U.S. market; therefore, additive consumption of 6'-SL sodium salt, beyond the estimated consumption levels detailed in Section 3.4 above, is not expected.

Kyowa cannot provide input on the levels of other poorly-digestible carbohydrates that may be used in infant formula in combination with their HMO ingredients, as Kyowa is not a manufacturer of infant formula. However, any new infant formula containing new HMOs, a new HMO combination, or a new combination of HMOs and other poorly-digested carbohydrates in the U.S. would be subject to Section 412 of the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 USC §350(a)) (U.S. FDA, 2021e). According to Section 412(d)(1) of the FFDCA, a manufacturer must notify the U.S. FDA ≥90 days before marketing a new infant formula; this notice must include descriptions of any reformulation or change in processing of the infant formula (U.S. FDA, 2021c). The manufacturer would therefore need to provide the U.S. FDA with information supporting that the combination of poorly-digestible carbohydrates intended to be used in the infant formula would be well-tolerated. Therefore, Section 412 of the FFDCA would ensure that any combination of HMOs would be supported by tolerance and safety testing in infants (U.S. FDA, 2021e).

6.7 Allergenicity

The allergenic potential of Kyowa's 6'-SL sodium salt is expected to be very low. This lack of allergenic potential is supported by analytical data demonstrating that Kyowa's final 6'-SL product does not contain the production strain or residual proteins, both of which are removed during the purification steps of the manufacturing process (*via* microfiltration and ultra-filtration). The absence of the production organism in Kyowa's final 6'-SL ingredient was demonstrated using PCR (see Section 2.3.3.3.1). Kyowa's final 6'-SL sodium salt ingredient is specified to contain ≤10 mg/kg residual protein, and the batch analysis of 5 lots of Kyowa's 6'-SL sodium salt ingredient yielded residual protein levels equal to or below the limit of detection of 1 mg/kg (0.0001%; see Table 2.3.3.1-1). Notably, Kyowa's specification limit of ≤10 mg/kg (*i.e.*, ≤0.001%) is 10-fold lower than the residual protein limit for Glycom's 6'-SL sodium salt (*i.e.*, ≤0.01%) as reported in GRN 881 (Glycom A/S, 2019a) and Jennewein's 6'-SL sodium salt (≤100 µg/g) as reported in GRN 922 (Jennewein Biotechnologie GmbH, 2020a).

Kyowa has conducted 2 tests of their final 6'-SL sodium salt ingredient (Lot C) to detect the presence of milk proteins. These tests were conducted using 2 enzyme-linked immunosorbent assay (ELISA) test kits [FASPEK ELISA II Milk (Casein; Morinaga Institute of Biological Science, Inc.) and FASTKIT ELISA Ver. III MILK (NH Foods Ltd.)], both of which have quantification limits of 1.0 µg/g. Milk proteins were not detected with either ELISA test kit, demonstrating that milk proteins are effectively removed during the purification process and are not present in Kyowa's final 6'-SL sodium salt ingredient.

No published reports of sensitization, case reports of allergic reactions, or allergenicity studies on 6'-SL were identified in a comprehensive and detailed search of the published scientific literature that was conducted through 08 December 2021 to identify studies relevant to the safety of 6'-SL.

Therefore, Kyowa's 6'-SL sodium salt manufactured with a genetically modified strain of E. coli was concluded to be of low allergenic risk.

6.8 Basis for GRAS

The conclusion that 6'-SL sodium salt produced by fermentation using a genetically modified strain of *E. coli* W is GRAS for use as an ingredient in non-exempt infant formula, conventional foods, and foods for special dietary uses is on the basis of scientific procedures.

Kyowa's 6'-SL has been demonstrated to be chemically and structurally equivalent to 6'-SL from bovine milk or colostrum by LC-MS, ^{1}H NMR, and ^{13}C NMR, which has been demonstrated to be structurally and chemically identical to 6'-SL in human milk (Aldredge *et al.*, 2013). On the basis of the chemical and structural identity to 6'-SL from human milk, the intakes of Kyowa's 6'-SL sodium salt under the conditions of intended use in comparison to the natural background dietary exposure to 6'-SL from the consumption of human milk is pivotal in the assessment of the safety of Kyowa's 6'-SL sodium salt ingredient. Natural background dietary intakes of 6'-SL in infants from the consumption of human milk are higher than those estimated under the proposed conditions of use of Kyowa's 6'-SL sodium salt and support the safety of Kyowa's 6'-SL sodium salt ingredient under the proposed conditions of use. As 6'-SL sodium salt intakes from all proposed conditions of use are within background exposure to 6'-SL from human milk in infants, a vulnerable population group, 6'-SL sodium salt is considered to be safe for all population groups.

Kyowa's 6'-SL sodium salt is compositionally similar to other 6'-SL sodium salt ingredients previously concluded to be GRAS and notified to the U.S. FDA without questions (GRN 881, 922 – Glycom A/S, 2019a; Jennewein Biotechnologie GmbH, 2020a; U.S. FDA, 2020e, 2021b). Based on the compositional similarity between Kyowa's 6'-SL sodium salt ingredient and other 6'-SL sodium salt ingredients, the safety of Kyowa's 6'-SL sodium salt ingredient is supported by the results of published preclinical toxicology studies conducted on other 6'-SL sodium salt ingredients produced by microbial fermentation and by the conclusions of various experts qualified by scientific training and experience to evaluate the safety of food ingredients including those used in infant formula (GRN 881, 922 – Glycom A/S, 2019a; Jennewein Biotechnologie GmbH, 2020a; U.S. FDA, 2020e, 2021b) and EFSA (EFSA, 2020a). Additional safety studies published subsequent to the latest GRAS notice for 6'-SL sodium salt submitted to the U.S. FDA also were considered supportive of safety.

The results of unpublished toxicology studies of Kyowa's 6'-SL sodium salt ingredient, published and unpublished studies on the constitutional isomer 3'-SL, and published studies of mixtures containing 3'-SL and/or 6'-SL were considered as corroborative evidence of the safety of Kyowa's 6'-SL sodium salt ingredient.

6.9 GRAS Panel Evaluation

Based on the above data and information presented herein, Kyowa Hakko Bio Co., Ltd. has concluded that the intended uses of 6'-SL sodium salt as an ingredient in non-exempt infant formula, conventional foods, and foods for special dietary uses, as described in Section 1.3 are GRAS, on the basis of scientific procedures.

This GRAS conclusion is based on data generally available in the public domain pertaining to the safety of 6'-SL sodium salt, as discussed herein, and on consensus among a panel of experts (the GRAS Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients. The GRAS Panel consisted of the following qualified scientific experts: Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Robert J. Nicolosi, Ph.D. (University of Massachusetts Lowell; R.J. Nicolosi, LLC), and Steven L. Taylor, Ph.D. (University of Nebraska-Lincoln; Taylor Consulting LLC).

The GRAS Panel, convened by Kyowa, independently and critically evaluated all data and information presented herein, and also concluded that 6'-SL sodium salt is GRAS for use as an ingredient in non-exempt infant formula, conventional foods, and foods for special dietary uses as described in Section 1.3, based on scientific procedures. A summary of data and information reviewed by the GRAS Panel, and evaluation of such data as it pertains to the proposed GRAS uses of 6'-SL sodium salt is presented in Appendix A.

6.10 Conclusion

Based on the above data and information presented herein, Kyowa Hakko Bio Co., Ltd. has concluded that 6'-SL sodium salt is GRAS, on the basis of scientific procedures, for use as an ingredient in non-exempt infant formula, conventional foods, and foods for special dietary uses as described in Section 1.3. General recognition of Kyowa's GRAS conclusion is supported by the unanimous consensus rendered by an independent Panel of Experts (the GRAS Panel), qualified by experience and scientific training, to evaluate the use of 6'-SL sodium salt in food, who similarly concluded that the proposed uses of 6'-SL sodium salt are GRAS on the basis of scientific procedures.

Part 7. §170.255 List of Supporting Data and Information

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APPENDIX A
GRAS Panel Consensus Statement

GRAS Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of 6'-Sialyllactose Sodium Salt for Use in Infant Formula, Conventional Foods, and Foods for Special Dietary Uses

24 June 2021

INTRODUCTION

Kyowa Hakko Bio Co., Ltd. (Kyowa) intends to market 6'-sialyllactose (6'-SL) sodium salt, produced by microbial fermentation using a genetically modified strain of *Escherichia coli* W, as an ingredient for addition to infant formula, specified conventional food products, and foods for special dietary uses in the United States (U.S.). Kyowa convened a panel of independent scientists (GRAS Panel), qualified by their relevant scientific training and experience in the safety evaluation of food ingredients, to conduct a critical and comprehensive evaluation of the available pertinent data and information on 6'-SL sodium salt, and to determine whether the intended uses of Kyowa's 6'-SL sodium salt would be Generally Recognized as Safe (GRAS) based on scientific procedures. For the purposes of the GRAS Panel's evaluation, "safe" or "safety" indicates that there is a reasonable certainty of no harm under the intended conditions of use of the ingredient in foods, as stated in 21 CFR §170.3(i) (U.S. FDA, 2020a). The GRAS Panel consisted of the below-signed qualified scientific experts: Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Robert J. Nicolosi, Ph.D. (University of Massachusetts Lowell; R.J. Nicolosi, LLC), and Steve L. Taylor, Ph.D. (University of Nebraska-Lincoln; Taylor Consulting LLC).

The GRAS Panel was selected and convened in accordance with the U.S. Food and Drug Administration's (FDA's) *Draft Guidance for Industry: Best Practices for Convening a GRAS Panel* (U.S. FDA, 2017). Kyowa confirms that prior to convening the GRAS Panel, all reasonable efforts were made to identify and select a balanced GRAS Panel with expertise in appropriate scientific disciplines deemed necessary for the safety evaluation of 6'-SL sodium salt, and efforts were placed on identifying conflicts of interest or relevant appearance issues that would potentially bias the outcome of the deliberations of the GRAS Panel; no such conflicts of interest or appearance of conflicts were identified. The GRAS Panel received reasonable honoraria as compensation for its time, and honoraria provided to the GRAS Panel were not contingent upon the outcome of the GRAS Panel's deliberations.

The GRAS Panel, independently and collectively, critically examined a comprehensive package of publicly available scientific information and data, both favorable and unfavorable, relevant to the safety evaluation of Kyowa's 6'-SL sodium salt under the intended conditions of use that was presented to the GRAS Panel in a dossier titled "*Documentation Supporting the Evaluation of 6'-Sialyllactose Sodium Salt as Generally Recognized as Safe (GRAS) for Use in Food*" (dated 24 June 2021). Publicly available scientific information and data were compiled from a comprehensive search of the scientific literature through 19 April 2021. The GRAS Panel also reviewed unpublished studies of 6'-SL sodium salt sponsored by Kyowa. Information and data reviewed by the GRAS Panel included information characterizing the identity and purity of the ingredient, the manufacture of the ingredient, product specifications, supporting analytical data, the intended conditions of use, the estimated exposure under the intended conditions of use, the history of safe consumption from human breast milk, and the safety of 6'-SL sodium salt.

Following independent and collective critical evaluation of such data and information, the GRAS Panel unanimously concluded that under the conditions of intended use described herein, 6'-SL sodium salt, manufactured by fermentation using a genetically modified strain of *E. coli* W, meeting appropriate food-grade specifications, and manufactured in accordance with current Good Manufacturing Practice (cGMP), is GRAS on the basis of scientific procedures. A summary of the basis for the GRAS Panel's conclusion is presented below.

IDENTITY, MANUFACTURING, SPECIFICATIONS, AND BATCH ANALYSES

6'-SL is a naturally occurring sialylated oligosaccharide in human milk that is composed of lactose at the reducing terminus and a sialic acid residue at the nonreducing end that is connected to the galactose unit of lactose at the 6 position *via* an α-2,6 linkage (ten Bruggencate *et al.*, 2014; Jacobi *et al*., 2016). The trisaccharide sialyllactose is the predominant sialylated oligosaccharide in human and bovine milk (Goedhart and Bindels, 1994; Nakano, 1999). The predominant forms of sialyllactose are 6'-SL and its constitutional isomer 3'-siallyllactose (3'-SL), which differ by the location of the connection of the sialic acid moiety to the galactose unit of lactose at the 6 or 3 position *via* an α-2,6 linkage or α-2,3 linkage, respectively (ten Bruggencate *et al.*, 2014; Jacobi *et al*., 2016). The chemical and structural identity of Kyowa's 6'-SL ingredient produced by fermentation with a genetically engineered strain of *E. coli* W (Lot C) was evaluated by proton nuclear magnetic resonance spectroscopy (^{1}H NMR), carbon-13 nuclear magnetic resonance spectroscopy (^{13}C NMR), and liquid chromatography–mass spectrometry (LC-MS). The GRAS Panel critically evaluated chromatograms and spectra demonstrating that Kyowa's 6'-SL is chemically and structurally identical to 6'-SL isolated from bovine milk or colostrum (SIGMA-ALDRICH, Lot No. SLCD8825), which has been demonstrated to be structurally and chemically identical to 6'-SL in human milk (Aldredge *et al.*, 2013).

Kyowa's 6'-SL sodium salt ingredient is produced by microbial fermentation from a genetically modified strain of *E. coli* W. The GRAS Panel reviewed data pertaining to the safety of the host organism and critically evaluated the genetic modifications applied to *E. coli* W for the biosynthesis of 6'-SL. The host organism, *E. coli* W, has been deposited in the American Type Culture Collection (ATCC 9637 – ATCC, 2021a), and is 1 of 4 *E. coli* strains designated safe for laboratory use (Archer *et al*., 2011). These safe strains are designated as Risk Group 1 organisms according to biological safety guidelines (Archer *et al*., 2011; ATCC, 2021a), as they are well-characterized and do not cause disease in healthy adult humans (NIH, 2019), and do not colonize the human gut (Bauer *et al*., 2008). *E. coli* W is non-toxigenic and non-pathogenic, as it lacks genes encoding toxins and genes encoding pathogenic determinants have been mutationally inactivated or are missing key components required for pathogenicity (Archer *et al*., 2011).

The host strain *E. coli* W was genetically modified to produce the 6'-SL recombinant production strain, which was optimized to produce 6'-SL *via* the fermentation of glucose and lactose. Host modifications were achieved using a modified lambda red recombinase system (Datsenko and Wanner, 2000), a common technique used to make targeted genetic modifications (including insertions and deletions) in *E. coli* at loci specified by flanking homology regions (Murphy, 1998; Yu *et al.*, 2000; Sharan *et al.*, 2009). Host modifications include the insertion of a total of 5 heterologous gene sequences (encoding glucosamine 6-phosphate *N*-acetyltransferase, *N*-acylglucosamine 2-epimerase, CMP-*N*-acetylneuraminic acid synthetase, α-2,6-sialyltransferase, and *N*-acetylneuraminic acid synthetase) originating from defined donor organisms into the chromosomal DNA of the host organism, *E. coli* W. The gene encoding a glucosamine 6-phosphate *N*-acetyltransferase originates from *Saccharomyces cerevisiae* S288C (ATCC 204508 – ATCC, 2021b). The gene encoding an *N*-acylglucosamine 2-epimerase originates from *Synechocystis* sp. PCC 6803 (ATCC 27184 – ATCC, 2021c). The gene encoding a *N*-acetylneuraminic acid synthetase originates from *Rhodobacter capsulatus* NBRC16581 (NBRC16581 – NBRC, 2001). The gene encoding a

CMP-*N*-acetylneuraminic acid synthetase originates from *Pasteurella multocida* subsp. *multocida* str. Pm70 (ATCC BAA-1909 – ATCC, 2021d). The gene encoding an α-2,6-sialyltransferase originates from *Photobacterium damselae* NBRC 15633 (NBRC15633 – NBRC, 1994). No unspecified DNA is expected to be associated with the transfer of the genes, as the DNA inserts are well-characterized, confirmed to consist of the desired sequences only, and the expression products have well-defined functions in the biosynthesis of 6'-SL and are not associated with any potential toxicity or pathogenic traits of the donor organism. Host modifications also include the deletion of 8 gene sequences which serve as insertion loci for the inserted gene products described above. The final production strain is selected using levansucrase as a counter-selectable marker, an enzyme that catalyzes the hydrolysis of sucrose, preventing the growth of the production organisms in the presence of sucrose (Gay *et al.*, 1983; Mizoguchi *et al*., 2007).

The GRAS Panel critically reviewed details of the manufacturing process of 6'-SL sodium salt, which involves 2 main steps: fermentation and purification. Kyowa has stated that the manufacturing process is controlled by a Hazard Analysis Critical Control Point (HACCP) plan and in accordance with cGMP as established by 21 CFR §117 (U.S. FDA, 2020a). The fermentation components used in the manufacture of 6'-SL sodium salt are food-grade and considered safe and suitable for their intended uses in food and/or were previously determined to be GRAS for their intended use and are used consistent with cGMP requirements. The fermentation media used for culturing the genetically modified strain of *E. coli* W contains nutrient sources and ingredients that are commonly used in microbial growth media. Kyowa also confirmed that all raw materials, and processing aids are of food-grade quality and are used in accordance with an applicable federal regulation or have been concluded to be GRAS for their intended use.

The fermentation process is conducted in chemically defined nutrient media under sterile and controlled conditions (*e.g.*, time, temperature, pH, and feeding rate). Production strain cells obtained from a frozen cell bank are initially cultured in flask seed culture medium followed by a factory seed culture medium. After reaching a specific optical density, the main fermentation medium is first inoculated with factory seed cultures and fermented in the presence of glucose. Following glucose depletion, lactose and glucose are added to the fermentation media and taken up by the production strain for the synthesis of 6'-SL, which is excreted into the media. The production of 6'-SL is terminated *via* heat treatment (sterilization), after which the broth is cooled and acidified.

During the purification processes, the intact cells are removed *via* microfiltration. The resulting solution is passed through a series of cationic resin and anionic resin ion exchangers to remove cations, anions, minerals, and organic impurities. The concentrated solution is decolorized with activated carbon and filtered using an ultra-filtration membrane to remove endotoxins, as well as any residual protein, organic impurities, or production organisms not removed by the cationic/anionic exchange resins. The obtained solution is concentrated, filtered, spray-dried, homogenized, and passed through a sieve to remove foreign materials to obtain the final 6'-SL sodium salt ingredient.

Kyowa has established food-grade physical, chemical, heavy metal, and microbiological specifications for their 6'-SL sodium salt ingredient (see Table A-1 of Attachment A). Specification limits for 6'-SL sodium salt purity and related carbohydrate impurities are similar to those established for other 6'-SL sodium salt ingredients that have been concluded to be GRAS, demonstrating that Kyowa's 6'-SL sodium salt is compositionally similar to other 6'-SL sodium salt ingredients permitted on the U.S. market. Specifically, the GRAS Panel noted that Kyowa's 6'-SL sodium salt has a purity of at least 82% 6'-SL sodium salt on a dry basis, and contains low levels of other carbohydrates (≤1 to ≤9 w/w% for each specified carbohydrate), sodium (≤5% on a dry basis), water (≤10.5 w/w%), and residual protein (≤10 mg/kg). The specification limits for heavy metals and microbial parameters in the final product are in accordance with the requirements for a food-grade quality ingredient. Most specification parameters are evaluated using nationally or

internationally accepted validated methods (United States or Japanese Pharmacopeia; International Organization for Standardization). Kyowa confirmed that internal methods, including the identification and quantification of 6'-SL sodium salt and other carbohydrates and quantification of residual protein, were concluded to be suitable.

The GRAS Panel critically evaluated analytical results and representative impurity profiles of 5 lots of 6'-SL sodium salt (3 of which were non-consecutive), which demonstrate that the manufacturing process produces a consistent product that meets specifications.

Kyowa's final 6'-SL sodium salt ingredient also was assessed for residual production organism and residual production organism-derived DNA in accordance with the European Food Safety Authority's (EFSA's) *Guidance on the characterization of microorganisms used as feed additives or as production organisms* (EFSA, 2018). The results of these analyses on 3 lots of the 6'-SL sodium salt ingredient demonstrate that the production organism is absent and that there is no detectable residual DNA (limit of quantification of 4 µg/kg or 4 ppb) in the final 6'-SL sodium salt ingredient.

The GRAS Panel critically reviewed bulk stability data of 6'-SL sodium salt under accelerated conditions (temperature of 40 ± 2°C; 75 ± 5% relative humidity) and real-time conditions (25 ± 2°C; 60 ± 5% relative humidity). In both studies, 6'-SL sodium salt was stored in polyethylene bags within an aluminum chuck bag, which are similar packaging materials as those intended to be used for the storage and distribution of the commercial product. Parameters evaluated included physicochemical parameters (appearance, color, pH, water activity) and biochemical parameters (purity, carbohydrate profile, water content, and water activity).

The accelerated study is complete, with results available to 6 months and the real-time study is ongoing with results available to 9 months (planned duration of 36 months, equivalent to the predicted shelf-life). The available data demonstrate that 6'-SL sodium salt was stable and remained within specification limits following storage under accelerated and real-time conditions. The water activity of 6'-SL sodium salt was considerably lower than 0.88 at all time points of evaluation and conditions of storage, indicating that microbial growth or toxin formation in Kyowa's 6'-SL sodium salt ingredient is unlikely. The results of the accelerated stability study support a shelf-life of 3 years. The stability of 3'-SL sodium salt under representative intended conditions of use, including infant formula powder, ready-to-drink milk, and yogurt has been previously demonstrated in GRN 766 (GeneChem, Inc., 2018 – GRN 766). The GRAS Panel notes that on the basis that Kyowa's 6'-SL is a structural isomer to 3'-SL and no differences in stability are expected between the isomers, these results support the stability of Kyowa's 6'-SL sodium salt in powdered infant formula, milk, and yogurt when stored under the same conditions. The GRAS Panel also considered stability studies on other structurally and chemically related human milk oligosaccharides (HMOs) to be relevant to the stability of Kyowa's 6'-SL sodium salt ingredient on the basis of their related structures. The results of the stability studies on other related HMOs such as 2'-fucosyllactose (2'-FL), a 2'-fucosyllactose/ difucosyllactose (2'-FL/DFL) mixture, lacto-*N*-neotetraose (LNnT), and sialic acid support the stability of 6'-SL sodium salt in the evaluated food matrices [infant formula, follow-on formula, yogurts, ready-to-drink flavored milk (pasteurized or ultra-high temperature treated [UHT]), citrus fruit drinks, and cereal bars] when stored under the same conditions (GRN 546, 547, 602, 650, 815 – Glycom A/S, 2014a,b, 2015, 2016, 2018; EFSA, 2015a,b, 2017, 2019).

INTENDED USE AND ESTIMATED EXPOSURE

Kyowa's 6'-SL sodium salt ingredient is intended as an alternative to other sources of 6'-SL currently on the U.S. market. 6'-SL sodium salt has previously been concluded to be GRAS for use in term (non-exempt) infant formula and toddler formula, infant and toddler foods, and specified conventional foods (GRN 881; GRN 922 – Glycom A/S, 2019a; Jennewein Biotechnologie GmbH, 2020; U.S. FDA, 2020b, 2021).

Kyowa proposes to use 6'-SL sodium salt in food uses currently permitted for other 6'-SL sodium salt ingredients, as well as in the following uses: breads and baked goods (all varieties), protein drinks, hot breakfast cereals, ready-to-eat breakfast cereals, chewing gum, coffee, tea, milk imitates, beverage whiteners, non-dairy cream, non-dairy yogurt, frozen dairy desserts (including ice cream and frozen yogurt), edible ices, sherbet and sorbet, dairy-based puddings, custards, and mousses, fruit pie filling, "fruit prep" fillings, energy and protein bars, hypoallergenic infant formula, jellies and jams, fruit preserves, and fruit butters, evaporated and condensed milk, formula intended for pregnant women, fruit juices and nectars, canned fruit, fruit-based desserts, vegetable juices and nectars, table-top sweeteners, syrups for flavoring milk beverages, and foods for special dietary use (oral nutritional supplements and enteral tube feeding). Kyowa's 6'-SL sodium salt is proposed for addition to term and hypoallergenic infant formulae to mimic the composition of human milk. The GRAS Panel noted that Kyowa's proposed use levels in term infant formula and hypoallergenic infant formula (0.50 g/L) are within the range of average levels of 6'-SL calculated from studies in which levels of 6'-SL were assessed in the milk of healthy human mothers following the birth of healthy infants. All proposed conditions of use of 6'-SL sodium salt are presented in Table A-2 of Attachment A.

Dietary exposure of 6'-SL sodium salt was assessed using food consumption data available in the 2017-2018 cycle of the U.S. National Center for Health Statistics' National Health and Nutrition Examination Survey (NHANES) (CDC, 2021a,b; USDA, 2021). The GRAS Panel reviewed dietary exposure estimates of 6'-SL sodium salt considering all proposed conditions of use in various U.S. population groups, as well as estimates from conditions of use in term infant formula and toddler formula only in infant and toddler population groups, and estimates from consumption of foods for special dietary uses only.

Considering all proposed food uses, the resulting consumer-only mean and 90th percentile intakes of 6'-SL sodium salt by the total U.S. population (≥2 years of age) were estimated to be 1.95 g/person/day (30 mg/kg body weight/day) and 3.6 g/person/day (64 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean intakes of 6'-SL sodium salt on an absolute basis were determined to be 2.29 g/person/day (28 mg/kg body weight/day), as identified among the elderly, while the highest 90th percentile intakes of 6'-SL sodium salt on an absolute basis were determined to be 4.26 g/person/day (58 mg/kg body weight/day), as identified among female adults. While infants 0 to 6 months of age had the lowest consumer-only intakes on an absolute basis (0.49 and 0.90 g/person/day at the mean and 90th percentile, respectively), infants 7 to <12 months of age had the highest daily mean and 90th percentile intakes on a body weight basis, of 110 mg/kg body weight/day (1.00 g/person/day) and 190 mg/kg body weight/day (1.74 g/person/day), respectively. The mean and 90th percentile consumer-only intakes of 6'-SL sodium salt from use in infant formulas and toddler formula only were highest in infants 0 to 6 months of age on both an absolute and body weight basis, at 61 mg/kg body weight/day (0.39 g/person/day) and 103 mg/kg body weight/day (0.62 g/person/day), respectively.

Under the recommended conditions of use in foods for special dietary uses, use of 6'-SL sodium salt in oral nutritional supplements for ages 2 and up and enteral tube feeding formula for ages 11 and up would result in total daily intakes of 0.84 and 2 g 6'-SL sodium salt /day, respectively, which are less than the highest estimated 90th percentile intakes of 6'-SL sodium salt from all proposed uses. The GRAS Panel noted that foods for special dietary use containing 6'-SL sodium salt are not intended to be consumed in combination with any other supplemental sources of 6'-SL and will be labeled as such. Consumption of 6'-SL sodium salt from foods for special dietary use was therefore concluded to be substitutional and not additive to consumption of 6'-SL sodium salt from other sources.

DATA PERTAINING TO SAFETY

The GRAS Panel noted that Kyowa's 6'-SL has been demonstrated to be chemically and structurally equivalent to 6'-SL from bovine milk or colostrum by LC-MS, ^{1}H NMR, and ^{13}C NMR, which has been demonstrated to be structurally and chemically identical to 6'-SL in human milk (Aldredge *et al.*, 2013). On the basis of the chemical and structural identity to 6'-SL from human milk, the GRAS Panel considered the natural background dietary exposure to 6'-SL from the consumption of human milk to be pivotal in the assessment of the safety of Kyowa's 6'-SL sodium salt ingredient.

The GRAS Panel also noted that the composition of Kyowa's 6'-SL sodium salt is similar to other 6'-SL sodium salt ingredients previously concluded to be GRAS and notified to the U.S. FDA based on their review of specifications for Kyowa's 6'-SL sodium salt produced by a genetically modified strain of *E. coli* W compared to those for other 6'-SL sodium salt ingredients (GRN 881, 922 – Glycom A/S, 2019a; Jennewein Biotechnologie GmbH, 2020; U.S. FDA, 2020b, 2021). Based on the compositional similarity between Kyowa's 6'-SL sodium salt ingredient and other 6'-SL sodium salt ingredients, the GRAS Panel considered that the safety of Kyowa's 6'-SL sodium salt ingredient is supported by the results of published preclinical toxicology and human studies conducted on other 6'-SL sodium salt ingredients produced by microbial fermentation and by the conclusions of various experts qualified by scientific training and experience to evaluate the safety of food ingredients including those used in infant formula (GRN 881, 922 – Glycom A/S, 2019a; Jennewein Biotechnologie GmbH, 2020; U.S. FDA, 2020b, 2021) and EFSA (EFSA, 2020). A comprehensive and detailed search of the published scientific literature (conducted 19 April 2021) was conducted to identify the totality of publicly available data and information relevant to the safety of 6'-SL sodium salt. The GRAS Panel also critically reviewed unpublished toxicology studies of Kyowa's 6'-SL sodium salt ingredient and considered the results corroborative of the safety of the ingredient.

3'-SL and 6'-SL are constitutional isomers wherein the sialic acid moiety is connected to the galactose unit of lactose at the 3 or 6 position *via* an α-2,3 linkage or α-2,6 linkage, respectively (ten Bruggencate *et al.*, 2014; Jacobi *et al*., 2016). Due to the structural similarity between 6'-SL and 3'-SL, published scientific literature on 3'-SL and mixtures containing 6'-SL and/or 3'-SL, as well as unpublished toxicology studies conducted by Kyowa on their 3'-SL sodium salt ingredient were considered as corroborative evidence of the safety of Kyowa's 6'-SL sodium salt ingredient.

Consistent with the requirements of the GRAS standard, conclusions on the GRAS status of 6'-SL sodium salt have considered all publicly available sources of information including favorable and potentially unfavorable information. Based on Kyowa's search of the literature, the company is not aware of published studies to suggest 6'-SL sodium salt is unsafe for use as a food ingredient.

History of Safe Consumption

The GRAS Panel noted that 6'-SL has an established history of safe consumption by breastfed infants, as 6'-SL is one of the predominant forms of sialyllactose in human milk (ten Bruggencate *et al.*, 2014; Jacobi *et al*., 2016). The levels of 6'-SL in human milk have been quantified by many investigators, with highly variable concentrations reported within and between studies. The concentration of 6'-SL has been reported by most authors to decrease as lactation progresses, but to be unaffected by maternal diet, age, parity, ethnicity, obesity, smoking, mode of delivery, gestational age, or birth weight (Asakuma *et al.*, 2007; Eckhardt *et al.*, 2016; Azad *et al*., 2018).

The GRAS Panel noted that the levels of 6'-SL measured in the milk of healthy human mothers following the birth of healthy, full-term infants in studies identified in the literature search ranged from 39 to 1,770 mg/L in transitional and mature human milk. The average level of 6'-SL in transitional and mature milk from mothers who had given birth to full-term infants was calculated to be 345 mg/L. The GRAS Panel further noted that Thurl *et al.* (2017) conducted a systematic review of levels of individual HMOs in human breast milk from healthy mothers and reported that the mean concentration of 6'-SL in milk from Secretor mothers who gave birth to term infants was 0.64 g/L [95% confidence interval (CI): 0.38–0.91 g/L] and the mean concentration in milk from mothers regardless of Secretor status who gave birth to term infants was 0.35 g/L (95% CI: 0.29–0.42). Despite a lower calculated mean when including milk regardless of Secretor status, there was no significant difference between the level of 6'-SL in milk from Secretor and non-Secretor mothers of term infants.

Exposure to 6'-SL on a body weight basis was calculated based on the range of maternal milk levels (*i.e.*, 39 to 1,770 mg/L) and the highest calculated average from the results of multiple studies for term infants (*i.e.*, 640 mg/L) described above, assuming a standard infant body weight of 6.7 kg (WHO Growth Chart[1]; average of 50th percentile for boys and girls at 4 months), an average milk consumption of 800 mL/day and a high-level milk consumption of 1.2 L/day (Butte *et al.*, 2002; da Costa *et al.*, 2010; Nielsen *et al.*, 2011; EFSA, 2013). The resulting mean intake of 6'-SL from transitional and mature human milk by infants was determined to range between 5 and 211 mg/kg body weight/day, with a maximum intake of up to 317 mg/kg body weight/day from the upper range of the reported mean concentrations of 6'-SL and high-level consumption of human milk.

The GRAS Panel compared the estimated daily intake of 6'-SL sodium salt in infants resulting from the proposed conditions of use to that from human milk. Mean consumer-only intakes from all proposed conditions of use in infants 0 to <12 months (74 to 110 mg/kg body weight/day) are within the average range of 6'-SL intakes resulting from the mean consumption of breast milk (5 to 211 mg/kg body weight/day), whereas 90th percentile intakes of 6'-SL sodium salt (119 to 190 mg/kg body weight/day) are below the maximum estimated daily intake of 6'-SL from the upper range of the reported mean concentrations of 6'-SL and high-level consumption of human milk (317 mg/kg body weight/day). 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 110 and 190 mg/kg body weight/day, respectively. Considering exposure from infant formulas and toddler formula only, mean and 90th percentile consumer-only intakes of 6'-SL sodium salt of up to 61 and 103 mg/kg body weight/day, respectively, are within the average range of 6'-SL intakes from the mean consumption of human milk of 5 to 211 mg/kg body weight/day, and below maximum 6'-SL intakes from the high-level consumption of human milk of 317 mg/kg body weight/day. The GRAS Panel noted that natural background dietary intakes of 6'-SL from the consumption of human milk are higher than those estimated under the proposed conditions of use of Kyowa's 6'-SL sodium salt and support

[1] https://www.cdc.gov/growthcharts/who_charts.htm.

the safety of Kyowa's 6'-SL sodium salt ingredient under the proposed conditions of use. As 6'-SL intakes from all proposed conditions of use are within background exposure from human milk in infants, a vulnerable population group, 6'-SL is considered to be safe for all population groups.

The GRAS Panel also considered additive exposure from complementary foods supplemented with 6'-SL sodium salt by breastfed infants and noted that breastfed infants are not expected to be high consumers of both 6'-SL from breast milk and 6'-SL from complementary foods since as consumption of complementary foods increase, consumption of breast milk decreases, such that additive exposure will be occasional and transient. The GRAS Panel concluded that no safety concerns are anticipated due to consumption of complementary foods supplemented with 6'-SL sodium salt by breastfed infants.

Absorption, Distribution, Metabolism, and Excretion (ADME)

The GRAS Panel noted that the absorption, distribution, metabolism, and excretion (ADME) of 6'-SL has been previously reviewed in GRAS Notices for 6'-SL ingredients submitted to the U.S. FDA (GRN 881, 922 – Glycom A/S, 2019a; Jennewein Biotechnologie GmbH, 2020) and by the EFSA Panel on Nutrition, Novel Foods and Food Allergens (EFSA, 2020). HMOs, including 6'-SL, are considered to be non-digestible oligosaccharides that "*do not undergo any significant digestion in the upper gastrointestinal tract*" (EFSA, 2020). HMOs in general are fermented in the colon by the intestinal microbiota, with 40 to 97% of ingested HMOs excreted unchanged in the feces of breastfed infants, and up to 2% excreted unchanged in the urine (EFSA, 2020). Breastfed infants were reported to excrete up to 3 mg/day of individual oligosaccharides following consumption of 150 mg oligosaccharides/feed and it was reported that approximately 4% of the amount of 6'-SL consumed *via* breast milk was excreted in the urine (EFSA, 2020). As Kyowa's 6'-SL is structurally and chemically identical to 6'-SL that is naturally present in human milk, the absorption of 6'-SL from the use of Kyowa's 6'-SL ingredient would also be limited and not different from the absorption of 6'-SL from the natural background dietary exposure from human breast milk. Kyowa's 6'-SL ingredient would be similarly fermented by the intestinal microbiota or excreted unchanged in the feces.

The specification limits for other carbohydrates in Kyowa's 6'-SL sodium salt produced with a genetically modified strain of *E. coli* W are comparable to those for 6'-SL sodium salt ingredients notified to the U.S. FDA as GRAS for their intended uses (GRN 881, 922 – Glycom A/S, 2019a; Jennewein Biotechnologie GmbH, 2020; U.S. FDA, 2020b, 2021). The other carbohydrates (*N*-acetyl D-neuraminic acid, glucose, lactose, and 3'-SL) are naturally occurring components of human milk, a breakdown product of the naturally occurring milk sugar lactose, or an isomerization product of 6'-SL formed when the terminal glucose moiety isomerizes into fructose (EFSA, 2020). It is expected that 6'-sialyllactulose would be present at a similar ratio to 6'-SL as the contents of lactulose to lactose in heat-treated human milk (Beach and Menzies, 1983; Schuster-Wolff-Bühring *et al.*, 2010; Gómez de Segura *et al.*, 2012), and as such, would have a history of safe consumption as a component of heat-treated human milk. Furthermore, the ADME profile of 6'-sialyllactulose and the other naturally-occurring carbohydrates following the consumption of Kyowa's 6'-SL sodium salt is not expected to differ from the ADME profile of these compounds from human milk.

Toxicological Studies

Subchronic and Chronic Studies

Kyowa's 6'-SL Sodium Salt

The potential subchronic toxicity of Kyowa's 6'-SL sodium salt administered by gavage to Crl:CD(SD) rats was evaluated in a 90-day repeat dose toxicity study (Tsuboi, 2021a [unpublished]) conducted in compliance with the Organisation for Economic Co-operation and Development (OECD) principles of Good Laboratory Practice (GLP) (OECD, 1998) and according to OECD Test Guideline 408 (OECD, 2018). Groups of 10 male and 10 female Crl:CD(SD) rats received 0 (distilled water for injection), 542, 1,084, or 2,168 mg 6'-SL sodium salt/kg body weight/day[2], by gavage at a dose volume of 10 mL/kg body weight for 90 days (purity of 90% on a dry basis). Evaluated safety parameters included clinical signs, body weight, food intake, sensory reactivity, grip strength, locomotor activity, ophthalmology, urinalysis, hematology, blood chemistry, blood coagulation, estrus cycle of all females, and gross and histological pathology. No statistically significant, toxicologically relevant, test item-related adverse effects were reported, and the no-observed-adverse-effect level (NOAEL) was concluded by the study authors to be 2,168 mg/kg body weight/day (the highest dose tested). The GRAS Panel noted that the results of this study are consistent with the published literature and concluded that the results of this study corroborate the safety of Kyowa's 6'-SL sodium salt ingredient.

Other 6'-SL Preparations

The GRAS Panel critically reviewed two 90-day repeat-dose studies of 6'-SL sodium salt conducted in rats.

No compound-related, toxicologically relevant, adverse effects on clinical signs, body weight, food or water consumption, ophthalmology, urinalysis, hematology, clinical chemistry, organ weights, or gross and histopathology were reported following the administration of 6'-SL sodium salt (98.8% purity; produced by enzymatic synthesis; GeneChem, Inc.) to 6- to 7-week-old Sprague-Dawley rats (11/sex/group) by gavage at doses of 0 (purified water), 1,000, 2,500, or 5,000 mg/kg body weight/day (Gurung *et al*., 2018) or following the administration of 6'-SL sodium salt (96.8% purity; Glycom A/S) to 7-day old neonatal Sprague-Dawley rats (10/sex/group) at doses of 0 (vehicle control), 0 (5,000 mg fructooligosaccharides/kg body weight/day reference control), 1,000, 3,000, or 5,000 mg/kg body weight/day *via* gavage (Phipps *et al.*, 2019a). In the study reported by Phipps *et al.* (2019a), minor differences were observed in time to completion of balano-preputial separation, completion of vaginal opening, and body weight at vaginal opening; however, these findings were not dose-dependent. Pre-weaning development, animal behavior, and Morris maze performance were comparable across groups. Statistically significant increases in overall mean ulna growth in all male 6'-SL sodium salt groups compared to controls were considered to be unrelated to the test article due to the lack of a dose response relationship, the small magnitude of the differences, and the lack of any differences observed in the female groups. Based on the lack of compound-related adverse effects, the authors of each study determined a NOAEL of 5,000 mg/kg body weight/day, the highest dose tested, for 6'-SL sodium salt in male and female rats.

[2] Doses were planned to be 0, 500, 1,000, and 2,000 mg/kg body weight/day; however, due to a correction in the analysis of the purity of the test article, which resulted in a higher purity value than initially reported, the doses used in the study were calculated to be 0, 542, 1,084, and 2,168 mg/kg body weight/day.

Kyowa's 3'-SL Sodium Salt

The potential subchronic toxicity of Kyowa's 3'-SL sodium salt administered by gavage to Crl:CD(SD) rats was evaluated in a 90-day repeat dose toxicity study (Tsuboi, 2021b [unpublished]) conducted in compliance with the OECD principles of GLP (OECD, 1998) and according to OECD Test Guideline 408 (OECD, 2018). Groups of 10 male and 10 female Crl:CD(SD) rats received 0 (distilled water for injection), 502, 1,003, or 2,007 mg 3'-SL sodium salt/kg body weight/day[3] at a dose volume of 10 mL/kg body weight for 90 days (purity value of 93% on a dry basis). Evaluated safety parameters included clinical signs, body weight, food intake, sensory reactivity, grip strength, locomotor activity, ophthalmology, urinalysis, hematology, blood chemistry, blood coagulation, estrus cycle of all females, and gross and histological pathology. No statistically significant, toxicologically relevant, test item-related adverse effects were reported, and the NOAEL was concluded by the study authors to be 2,007 mg/kg body weight/day (the highest dose tested). The GRAS Panel noted that the results of this study are consistent with the published literature and concluded that the results of this study corroborate the safety of Kyowa's 6'-SL sodium salt ingredient.

Other 3'-SL Preparations

The GRAS Panel critically reviewed 5 publications including 6 repeat-dose studies of other 3'-SL preparations in rats and monkeys.

No compound-related, toxicologically relevant, adverse effects on clinical signs, body weight, food consumption, urinalysis, hematology, or clinical chemistry were reported following the administration of 3'-SL sodium salt (98.8% purity; produced by enzymatic synthesis; GeneChem, Inc.) to 6-week-old Sprague-Dawley (Crl:CD[SD]) rats (10/sex/group) at doses of 0, 500, 1,000, or 2,000 mg/kg body weight/day *via* gavage for 28 days (Kim *et al*., 2018).

No compound-related, toxicologically relevant, adverse effects on clinical signs, body weight, food or water consumption, ophthalmology, urinalysis, hematology, clinical chemistry, organ weights, or gross and histopathology were reported following the administration of 3'-SL sodium salt (98.8% purity; produced by enzymatic synthesis) to 6-week-old Sprague-Dawley rats (10/sex/group) at doses of 0, 500, 1,000, or 2,000 mg/kg body weight/day *via* gavage for 90 days (Kim *et al*., 2018) or following the administration of 0 (vehicle control or 5,000 mg fructooligosaccharide/kg body weight/day), 1,000, 3,000, or 5,000 mg 3'-SL sodium salt (90.3% purity; produced by microbial fermentation)/kg body weight/day *via* gavage to 7-day-old Sprague-Dawley rats (10/sex/group) for 90 days (Phipps *et al.*, 2019b). In the study reported by Phipps *et al.* (2019b), no compound-related differences were reported in developmental endpoints except for a significant decrease in forelimb grip strength and rearing counts of females administered 5,000 mg 3'-SL sodium salt/kg body weight/day (compared to vehicle controls), which were not observed to be dose-dependent. The NOAEL was determined by the authors of each study to be the highest dose tested: 2,000 mg/kg body weight/day (Kim *et al*., 2018) and 5,000 mg/kg body weight/day (Phipps *et al.*, 2019b) for 3'-SL sodium salt in male and female rats.

[3] Doses were planned to be 0, 500, 1,000, and 2,000 mg/kg body weight/day and calculated using a preliminary Certificate of Analysis with one significant digit; however, upon re-calculation using the rounded assay value reported on the final Certificate of Analysis, the doses were calculated to be 0, 502, 1,003, and 2,007 mg/kg body weight/day.

In a 56-day toxicity study, weanling Sprague-Dawley rats (10/sex/group) were administered diets providing 3'-SL (97.5% purity; method of manufacture not reported) at doses of 0 or 625 mg/kg body weight/day, or 625 mg 3'-SL/kg body weight/day in combination with 625 mg 2'-FL (96.1% purity; method of manufacture not reported)/kg body weight/day (Chleilat *et al.*, 2020). Body weight and food intake were measured weekly, and fecal samples were collected for microbial profiling. At the end of the dosing period, lean mass, fat mass, body fat percent, bone mineral content, bone mineral density, intestinal permeability, serum cytokines, and gastrointestinal organ weights were measured. A significant decrease in body weight was measured in males who were administered 3'-SL in the diet relative to the controls, but this finding was only significant on test completion and not throughout the exposure. Females administered 3'-SL consumed significantly more food than the controls at the beginning of dosing; however, they consumed significantly less food than the controls by test completion. Serum leptin levels were significantly lower in rats that consumed the 3'-SL diet. The weight of the cecum from females administered the 3'-SL + 2'-FL mixture diet was significantly higher than controls. Conversely, female colon weight was significantly lower in the 3'-SL + 2'-FL group compared to the controls. Gut barrier permeability of females administered HMO diets was reduced relative to control animals. No statistically significant adverse effects were reported, and the authors reported that the changes observed in gut morphology and barrier function in females were beneficial. The lack of adverse compound-related effects indicates that 3'-SL and 2'-FL were well tolerated in rat pups.

The GRAS Panel reviewed 1 study in *Helicobacter pylori*-positive rhesus monkeys, in which the effects of 3'-SL sodium salt administration on *H. pylori* infection were investigated (Mysore *et al.*, 1999). Rhesus monkeys (6/group) were administered 100 or 500 mg 3'-SL sodium salt/kg body weight/day for 28 or 56 days, respectively. The 3'-SL sodium salt test article used in this study (NE-0080 manufactured by Neose Technologies) was being investigated for use as a drug for use in the treatment of *H. pylori* infection, but was discontinued for this purpose in 2002[4]. Throughout the full duration of the treatment period, the monkeys were subject to gastric endoscopy (with gastric biopsy and *H. pylori* colony count) at 14-day intervals until Day 3 post-treatment, at which point they were subject to gastric endoscopy (with gastric biopsy and *H. pylori* colony count) at 14- or 30-day intervals for a 6-month follow-up period. Blood samples were collected at the same time points for each monkey, and hematology, and clinical chemistry parameters were measured. No adverse effects on hematology or clinical chemistry were reported following consumption of up to 500 mg 3'-SL sodium salt/kg body weight/day for 56 days (Mysore *et al.*, 1999). Thus, the authors concluded that 3'-SL sodium salt was safe when administered at doses of 100 and 500 mg/kg body weight/day for periods of up to 56 days.

HMO Mixtures Containing 6'-SL or 3'-SL

The GRAS Panel critically reviewed 2 repeat-dose studies of HMO mixtures, including a 90-day study conducted in rats (Parschat *et al.*, 2020) and a 15-day study in piglets (Comstock *et al*., 2017).

In a 13-week oral study, Charles River (SD) rats (10/sex/group) were administered a basal control diet or diet containing a 10% HMO mixture [consisting of 47.1% 2'-FL, 16.0% 3'-fucosyllactose (3'-FL), 23.7% lacto-*N*-tetraose (LNT), 4.1% 3'-SL, 4.0% 6'-SL, and 5.1% other carbohydrates, each produced individually *via* fermentation] *ad libitum* for the duration of the test period (Parschat *et al.*, 2020). Actual intake of the HMO mixture for rats administered the test diet was calculated to be 5,670 and 6,970 mg HMO mixture/kg body weight/day for males and females, respectively. No mortality was reported throughout the study period, and no compound-related adverse effects were reported with respect to body weight, body weight gain, animal behavior, food and water consumption, hematology, clinical chemistry,

[4] Source: http://adisinsight.springer.com/drugs/800005552.

urinalysis, organ weights, neurology, or ophthalmology. Based on the results of the study, the authors determined the NOAELs to be 5,670 and 6,970 mg HMO mixture/kg body weight/day for male and female rats, respectively, corresponding to NOAELs of 232 and 286 mg 3'-SL/kg body weight/day and 227 and 279 mg 6'-SL/kg body weight/day for males and females, respectively.

In another study, groups of healthy and rotavirus-infected newborn piglets were fed a control formula (n=16) or formula containing 4 g HMOs/L[5] (n=17) from birth until 15 days of age to measure the effects of HMOs on immune cell populations (Comstock *et al*., 2017). The piglets were weighed at the time of birth and at the end of the study. The birth weights, final weights, and weight gains were similar for all pigs administered the HMO treatment formula. No other parameters relevant to safety were assessed.

Reproductive and Developmental Studies

The GRAS Panel critically reviewed 2 gastrointestinal developmental toxicity studies of 6'-SL, 2 gastrointestinal developmental toxicity studies of 3'-SL, and 3 gastrointestinal developmental toxicity studies of sialyllactose (SL) mixtures in piglets.

The safety of orally administered 6'-SL sodium salt (>98% purity; produced by enzymatic synthesis; GeneChem, Inc.), delivered *via* a non-medicated sow-milk replacer formula for 21 days, was evaluated in 2-day-old piglets (6/sex/group; strain not reported) (Monaco *et al.*, 2020). Diets were formulated to contain 0, 300, 600, or 1,200 mg 6'-SL sodium salt/L, and were administered to the piglets 10 times daily *via* a peristaltic pump at 300 or 330 mL diet/kg body weight on Study Days 1 to 5 or 6 to 21, respectively. There were no significant differences among groups in total body weight gain, food consumption, intestinal length, organ weights, colonic pH, coagulation parameters, blood chemistry, hematology, and urinalysis parameters. The histological effects reported in the high-dose 6'-SL sodium salt group were comparable to control piglets and not considered to be toxicologically relevant by the study authors. The authors concluded that there were no dose-dependent adverse effects in the study, and that 6'-SL sodium salt was well tolerated and supported normal growth and development at concentrations up to 1,200 mg/L in reconstituted formula.

In a study with the same design, the safety of orally administered 3'-SL sodium salt (>98% purity; produced by enzymatic synthesis; GeneChem, Inc.), delivered *via* a non-medicated sow-milk replacer formula for 21 days, was evaluated in 2-day-old piglets (6/sex/group; strain not reported) (Monaco *et al.*, 2019). Diets were formulated to contain 0, 140, 200, or 500 mg 3'-SL sodium salt/L, and were administered to the piglets 10 times daily *via* a peristaltic pump at 300 or 360 mL diet/kg body weight on Study Days 1 to 5 or 6 to 21, respectively. There were no significant differences among groups in total body weight gain, organ weights, intestinal length, colonic pH, clinical chemistry, coagulation, or hematologic parameters. A significantly increased incidence of crystals in the urine were observed in piglets administered formula containing 500 mg 3'-SL sodium salt/L; however, all 5 samples containing crystals in the 500 mg 3'-SL sodium salt group were classified as having “rare” or “few” crystals, and no other adverse renal or urinary effects were reported. The authors also noted that refrigeration of urine samples can sometimes promote crystal formation. The histological effects reported were not considered by the study authors to be toxicologically relevant due to the lack of dose-dependence or statistically significant differences from control animals. The authors concluded that there were no dose-dependent adverse effects in the study, and that 3'-SL sodium salt was safe at concentrations up to 500 mg/L in reconstituted formula (Monaco *et al.*, 2019).

[5] 40% 2'-fucosyllactose (Glycom A/S), 35% lacto-*N*-neotetraose (Glycom A/S), 10% 6'-SL (Carbosynth), 5% 3'-SL (Carbosynth), and 10% free sialic acid (Glycom A/S).

An additional study in which the effects of 3'-SL and 6'-SL on gastrointestinal parameters were evaluated individually in piglets was identified in the literature. In this study, 1-day-old piglets (9/group, sex, and strain not reported) were provided up to 1,200 mg 3'-SL or 6'-SL/kg body weight/day in formula for 21 days (from PND 2 to 22) and brain sialic acid content and the colonic microbiota were investigated (Jacobi *et al.*, 2016). The source of the 3'-SL and 6'-SL test articles was not reported. In this study, there was no effect of 3'-SL or 6'-SL on feed intake, growth, intestinal pH, or diarrhea scores. The authors reported that both oligosaccharide diets were well tolerated by the pigs across all treatment groups.

In the studies conducted with SL mixtures, no compound-related adverse effects on clinical condition, growth, body weight gain, clinical chemistry, hematology, organ weights, or stool consistency were reported following the provision of milk or milk-based formula supplemented with 0.13, 0.38, 0.76, or 1.71 g SL/L to piglets aged 1 to 3 days old or preterm piglets from Gestational Day 106 for periods of 19 to 35 days (Monaco *et al.*, 2018; Obelitz-Ryom *et al.*, 2018; Yang *et al.*, 2018). Additionally, no compound-related adverse effects on colonic microbial diversity, microbial metabolite concentrations, villus height and crypt depth, or gut function were reported and the SL mixtures were reported to be well tolerated by the study authors. The GRAS Panel considered that the results of the studies on SL mixtures were corroborative of the safety of Kyowa's 6'-SL sodium salt.

Genotoxicity Studies

The GRAS Panel critically evaluated the results of a bacterial reverse mutation test conducted with Kyowa's 6'-SL sodium salt (90% assay) which was performed in compliance with the OECD principles of GLP (OECD, 1998) and according to OECD Test Guideline 471 (OECD, 1997) (Oguma, 2020a [unpublished]). *Salmonella* Typhimurium strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2 uvrA were incubated with Kyowa's 6'-SL sodium salt at concentrations up to 5,000 µg/plate in the absence and presence of external metabolic activation (S9 mix), using the pre-incubation method. There was no evidence of mutagenicity, in the absence or presence of metabolic activation. No growth inhibition or precipitation of the test substance was observed. Based on the results of the study, the study authors concluded that 6'-SL sodium salt is non-mutagenic at concentrations up to 5,000 µg/plate (the OECD Test Guideline 471 maximum recommended concentration).

The potential clastogenicity and aneugenicity of Kyowa's 6'-SL (90% assay) was evaluated in an *in vivo* micronucleus test with ICR mice (Inasa Branch, Japan SLC, Inc.) conducted in in compliance with the OECD principles of GLP (OECD, 1998) and OECD Test Guideline 474 (OECD, 2016) (Kikuchi, 2020a [unpublished]). In the main study, male ICR mice (5/group) were administered 6'-SL sodium salt by gavage twice (at a 24-hour interval) at doses up to 2,000 mg/kg body weight. No clinical signs or abnormalities and no statistically significant changes in the body weights of any animal were observed in the test substance, negative, and positive control groups. No significant changes in micronucleated immature erythrocytes (MNIME) frequency were observed between the test substance and negative control groups. No significant difference in the proportion of immature erythrocytes (IMEs) among total erythrocytes was observed among the study groups. Based on the results of this study, 6'-SL sodium salt was concluded to have no potential for induction of chromosomal aberrations in male ICR mice at doses up to 2,000 mg/kg body weight.

The GRAS Panel noted that the results of these 2 studies are consistent with those reported by other investigators and further corroborate the conclusion that 6'-SL sodium salt is not genotoxic.

The potential genotoxicity of other 6'-SL sodium salt preparations has previously been evaluated *in vitro* in bacterial reverse mutation tests, a chromosome aberration test in Chinese hamster lung cells, and a micronucleus test conducted with human peripheral blood lymphocytes (Gurung *et al.*, 2018; Phipps *et al.*, 2019a) and *in vivo* in a micronucleus test conducted with ICR mice (Gurung *et al.*, 2018). The GRAS Panel noted that the consistently negative results reported in *in vitro* and *in vivo* studies demonstrate that 6'-SL sodium salt lacks genotoxic potential.

The GRAS Panel also critically evaluated the results of genotoxicity studies conducted with the structural isomer 3'-SL and with HMO mixtures.

Kyowa's 3'-SL sodium salt (92.8% assay) was non-mutagenic in the absence and presence of metabolic activation (S9 mix) at concentrations up to 5,000 µg/plate (the OECD Test Guideline 471 maximum recommended concentration) in a bacterial reverse mutation test that was performed in compliance with the OECD principles of GLP (OECD, 1998) and according to OECD Test Guideline 471 (OECD, 1997) (Oguma, 2020b [unpublished]). In an *in vivo* micronucleus test with ICR mice (Inasa Branch, Japan SLC, Inc.) conducted in compliance with the OECD principles of GLP (OECD, 1998) and OECD Test Guideline 474 (OECD, 2016), Kyowa's 3'-SL sodium salt (92.8% assay) was concluded to have no potential for induction of chromosomal aberrations in male ICR mice at doses up to 2,000 mg/kg body weight (Kikuchi, 2020b [unpublished]).

The GRAS Panel noted that the results of these 2 studies are consistent with those reported by other investigators and further corroborate the conclusion that 6'-SL sodium salt is not genotoxic.

The potential genotoxicity of other 3'-SL sodium salt preparations has previously been evaluated *in vitro* in bacterial reverse mutation tests, a chromosome aberration test in Chinese hamster lung cells, and a micronucleus test conducted with human peripheral blood lymphocytes (Kim *et al*., 2018; Phipps *et al.*, 2019b) and *in vivo* in a micronucleus test conducted with ICR mice (Kim *et al*., 2018). The GRAS Panel noted that the consistently negative results reported in *in vitro* and *in vivo* studies demonstrate that 3'-SL sodium salt lacks genotoxic potential and corroborate the conclusion that 6'-SL sodium salt lacks genotoxic potential.

The potential genotoxicity of HMO mixtures has previously been evaluated *in vitro* in a bacterial reverse mutation assay and a micronucleus test conducted with human peripheral lymphocytes (Parschat *et al.*, 2020). The GRAS Panel noted that the consistently negative results reported in *in vitro* studies demonstrate that the tested HMO mixtures lack genotoxic potential and corroborate the conclusion that 6'-SL sodium salt lacks genotoxic potential.

Human Studies

No studies in humans conducted with 6'-SL were identified in the literature; however, 2 studies of 3'-SL (NE-080; Neose Technologies Inc., Horsham PA) in adults were identified and were considered by the GRAS Panel to be supportive of the safety of 6'-SL. No details on the source, purity, or manufacturing process for NE-080 were reported in these studies. In the first study, 6 otherwise healthy men with gastric *H. pylori* infection consumed five 2-g doses of 3'-SL following meals and snacks over the course of a 24-hour period for a total dose of 10 g, and *H. pylori* infection [determined *via* histopathology, positive serology, and a ^{13}C-urea breath test (UBT)], inflammatory response, and serum liver transaminase levels (not further specified) were reported (Opekun *et al.*, 1999). There were no differences from baseline in liver transaminase tests. The second study was a randomized, double-blind, controlled trial in which 60 dyspeptic adult patients with *H. pylori* infection (as determined by UBT values >15) consumed 0, 10, or

20 g 3'-SL (NE-080)/day for 4 weeks (Parente *et al.*, 2003). The authors concluded that 3'-SL was safe and well tolerated due to the nature of the reported adverse events (halitosis, asthenia, epigastric pain, and headache) and their lack of severity, as well as no significant changes in UBT values. The results of these studies support the safety and tolerability of 3'-SL and 6'-SL at doses up to 20 g/day in adults.

Safety of 6'-SL Sodium Salt in Hypoallergenic Infant Formula

The GRAS Panel noted that no studies have been conducted in infants with 6'-SL sodium salt added to hypoallergenic infant formulas. As previously discussed, 6'-SL is an HMO, which is a diverse group of structurally related oligosaccharides present in human breast milk. 6'-SL consists of glucose and galactose with NeuAc bound to galactose *via* an α-2,6 linkage, while the related HMO 2'-FL consists of glucose and galactose with fucose bound to galactose *via* an α-1,2 linkage. Given the close chemical and structural similarity between 6'-SL and the related HMO 2'-FL, as well as their common natural presence in human breast milk, the GRAS Panel considered that studies conducted with 2'-FL were relevant using a "read-across" approach to assess the safety and suitability of 6'-SL sodium salt for use in hypoallergenic infant formula. Studies conducted with 2'-FL in term infants with cow's milk protein allergy (CMPA), suspected food protein allergy, persistent feeding intolerance, or other conditions warranting the use of extensively hydrolyzed infant formula were identified and are summarized below.

The GRAS Panel critically evaluated a clinical study assessing the allergenic potential, tolerability, and safety of a whey-based extensively hydrolyzed formula (EHF) supplemented with 2'-FL (1.0 g/L) and LNnT (0.5 g/L) in infants and children 2 months to 4 years of age with CMPA (Nowak-Wegrzyn *et al*., 2019). The risk of hypersensitivity was evaluated in a crossover double-blind placebo-controlled food challenge, where the placebo control formula was a commercially available hypoallergenic EHF without HMOs (Althéra®, Nestlé Health Science, Vevey, Switzerland). The sample size was calculated to meet the American Academy of Pediatrics (AAP) criteria for assessing hypoallergenicity of infant formulas, where, at minimum, it must be demonstrated with 95% confidence that 90% (95% lower bound CI ≥90%) of infants with documented CMPA will not react with defined symptoms (AAP, 2000). Following an initial lip dose challenge, oral doses of the assigned EHF were administered at 10- to 15-minute intervals providing a total volume of 180 mL (subjects ≤1 year of age) or 240 mL (subjects >2 years of age). The alternate EHF was administered 2 to 7 days later. Subjects were observed for a 1-hour period post-administration, where allergic signs or symptoms were documented and assessed according to pre-defined pass/fail criteria. An open challenge during a period of 7 to 9 days was also conducted, as recommended by the AAP to detect late-onset reactions, during which allergic symptoms, clinical parameters, and adverse events were recorded. The study authors reported 1 allergic reaction to the test formula and 1 allergic reaction to the control formula in both the modified intention-to-treat cohort (n = 63 of 64; 98.4%; 95% CI lower bound 92.8%) and the per protocol cohort (n = 60 of 61; 98.4%; 95% CI lower bound 92.5%). No treatment-related gastrointestinal symptoms or adverse events were reported. The study authors concluded that the hypoallergenicity of the EHF supplement with 2'-FL and LNnT was confirmed according to AAP criteria.

Ramirez-Farias *et al*. (2021) conducted a Good Clinical Practice (GCP)-compliant multicenter study in 47 infants (0 to 60 days of age) with suspected food protein allergy, persistent feeding intolerance, or other conditions warranting the use of extensively hydrolyzed infant formula. All infants were administered formula containing 2'-FL (0 or 0.2 g/L; source not reported) for 2 months, with 36 of the 48 enrolled infants completing the study. Measures of growth as well as daily formula intake, stool observations, and adverse events were recorded throughout the study. No adverse effects were reported with respect to growth or between-group differences in the incidence of adverse effects, and the study authors concluded that the formula containing 2'-FL was well tolerated and safe.

Safety of 6'-SL Sodium Salt in Enteral Tube Feeding Formula

The GRAS Panel noted that no human studies have been conducted with 6'-SL sodium salt added to formula for enteral tube feeding. Given that HMOs, including 6'-SL, are considered to be non-digestible oligosaccharides (EFSA, 2020), the GRAS Panel assessed the safety and suitability of 6'-SL sodium salt for use in formula for enteral tube feeding using data from studies conducted with other non- or poorly-digestible carbohydrates.

The GRAS Panel critically reviewed the results of 19 published studies of the safety/tolerability of other poorly-digestible carbohydrates as components of enteral tube feeding formula (at doses up to 63 g/day) that were considered *in lieu* of relevant safety or tolerability studies of 2'-FL that were submitted to the U.S. FDA in response to questions on GRN 897 (DuPont Nutrition and Health, 2019). The GRAS Panel concurred with the notifier's conclusion that the safety of the use of 2'-FL as an ingredient in enteral tube-feeding formula at levels up to 20 g/kg is supported by the lack of test compound-related adverse effects reported in these 19 studies, as well as the Institute of Medicine's conclusion that establishing a tolerable upper intake level for fiber is not necessary. The GRAS Panel noted that upon consideration of the information provided by the notifier, the FDA responded with no questions regarding the GRAS status of 2'-FL under the conditions of use specified in GRN 897, including use in enteral tube feeding formula at levels up to 20 g/L (U.S. FDA, 2020c).

The GRAS Panel noted that Kyowa's 6'-SL sodium salt ingredient is proposed for use at a level of 4.1 g/L, which is approximately one-fifth the level concluded to be GRAS for 2'-FL and consistent with the ratio of 6'-SL to 2'-FL present in human breast milk. The GRAS Panel concluded that the safety of 6'-SL sodium salt in formula for enteral tube feeding at a use level of 4.1 g/L is supported by the safety profile of the ingredient and the safety of poorly-digestible carbohydrates in general in enteral feeding at levels that exceed the recommended intake of 6'-SL sodium salt from the intended use in formula for enteral tube feeding.

Allergenicity

Possible transfer of protein originating from the fermentation broth is controlled during the manufacturing process through the removal of production organism from the fermentation media and through downstream processing of the media during the purification processes. The GRAS Panel noted that analytical data demonstrating the absence of the production organism and production organism-derived DNA in the final 6'-SL sodium salt ingredient support the effective removal of these potential impurities from the final ingredient. The GRAS Panel also noted that the purification processes have been demonstrated to remove residual protein to a level that is well below Kyowa's specification for residual protein (10 mg/kg) and below the limit of detection of 1 mg/kg (0.0001%) using dot blot analysis. The results of analysis of the final 6'-SL sodium salt ingredient using 2 enzyme-linked immunosorbent assay (ELISA) test kits [FASPEK ELISA II Milk (Casein; Morinaga Institute of Biological Science, Inc.) and FASTKIT ELISA Ver. III MILK (NH Foods Ltd.)], with quantification limits of 1.0 µg/g, demonstrate that milk proteins are effectively removed during the purification process and are not present in Kyowa's final 6'-SL sodium salt ingredient. In addition, no published reports of sensitization, case reports of allergic reactions, or allergenicity studies on 6'-SL were identified in a comprehensive and detailed search of the published scientific literature that was conducted on 19 April 2021 to identify studies relevant to the safety of 6'-SL sodium salt. The GRAS Panel considered Kyowa's 6'-SL sodium salt manufactured with a genetically modified strain of *E. coli* W to be of low allergenic risk and noted that the low allergenic risk of Kyowa's 6'-SL sodium salt supports its safe addition to exempt hypoallergenic infant formula in the U.S.

CONCLUSION

We, the undersigned, independent, qualified members of the Generally Recognized as Safe (GRAS) Panel, have independently and collectively, critically evaluated the data and information summarized above that is pertinent to the safety of the proposed uses of 6'-SL sodium salt. We unanimously conclude that the proposed uses in infant formula, conventional foods, and foods for special dietary uses specified herein of Kyowa's 6'-SL sodium salt produced by microbial fermentation by a genetically modified strain of *E. coli* W, meeting appropriate food grade specifications and produced in accordance with current good manufacturing practice, are GRAS based on scientific procedures.

It is our professional opinion that other qualified experts would concur with this conclusion.

Professor Emeritus Joseph F. Borzelleca, Ph.D. Virginia Commonwealth University School of Medicine	14 July 2021 Date
Professor Emeritus Robert J. Nicolosi, Ph.D. University of Massachusetts Lowell	Date
Prof Emeritus Steve L. Taylor, Ph.D. University of Nebraska-Lincoln	Date

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Virginia Commonwealth University School of Medicine

Date

[redacted]

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07/14/2021
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Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	Section §	Last Amended	Section Title
117—Current good manufacturing practice, hazard analysis, and risk-based preventive controls for human food	117	5-1-20	[full section]
130 to 169 [Food Standards]	—	5-1-20	—
170—Food additives	170.3	4-1-19	Definitions

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ATTACHMENT A:
Specifications and Intended Conditions of Use

Table A-1 Chemical and Microbiological Specifications for 6'-Sialyllactose Sodium Salt

Specification Parameter	Specification	Method
Organoleptic		
Appearance	Powder	Visual observation
Color	White to off-white	General Notice, JP 17[a]
Physicochemical		
Identification	RT of standard ± 3%	HPLC-CAD (internal method)
Purity (6'-SL)	≥82% dry basis	HPLC-CAD (internal method)
Water	≤10.5 w/w%	JP 2.48[a]
Sodium (Assay)	≤5.0% dry basis	USP 233[b]
Residual protein	≤10 mg/kg	Dot-blot (internal method)
pH (20˚C, 5% solution)	4.0 to 9.0	JP 2.54[a]
Other Carbohydrates		
N-Acetyl D-neuraminic acid	≤9 w/w%	HPLC-CAD (internal method)
D-glucose	≤3 w/w%	HPLC-PAD (internal method)
D-lactose	≤3 w/w%	HPLC-PAD (internal method)
6'-Sialyllactulose	≤5 w/w%	HPLC-CAD (internal method)
3'-Sialyllactose sodium salt	≤1 w/w%	HPLC-CAD (internal method)
Heavy Metals		
Arsenic	≤0.2 mg/kg	USP 233[b]
Cadmium	≤0.2 mg/kg	USP 233[b]
Lead	≤0.2 mg/kg	USP 233[b]
Mercury	≤0.2 mg/kg	USP 233[b]
Iron	≤10 mg/kg	USP 233[b]
Microbiological		
Aerobic plate count	≤1,000 CFU/g	ISO 4833-1:2013
Molds	≤100 CFU/g	ISO 21527-2:2008
Yeasts	≤100 CFU/g	ISO 21527-2:2008
Salmonella	Negative in 100 g	ISO 6579-1:2017
Enterobacteriaceae	Negative in 10 g	ISO 21528-1:2017
Cronobacter spp. *(Enterobacter sakazakii)*	Negative in 100 g	ISO 22964:2017
Listeria monocytogenes	Negative in 25 g	ISO 11290-1:2017
Bacillus cereus	≤50 CFU/g	ISO 7932:2004
Residual endotoxins	≤10 EU/mg	JP 4.01 (kinetic-turbidimetric method)[a]

6'-SL = 6'-sialyllactose; CFU = colony forming units; EU = endotoxin units; HPLC-CAD = high-performance liquid chromatography coupled with charged aerosol detection; HPLC-PAD = high-performance liquid chromatography coupled with pulsed amperometric detection; ISO = International Organization for Standardization; JP = Japanese Pharmacopeia; RT = retention time; USP = United States Pharmacopeia.

[a] Method is consistent with the compendial method specified in 17th edition of the Japanese Pharmacopeia (2016).

[b] Method is consistent with the compendial method specified in the United States Pharmacopeia 35th revision (2011).

Table A-2 Summary of the Individual Proposed Food Uses and Use Levels for 6'-Sialyllactose Sodium Salt in the U.S.

Food Category (21 CFR §170.3) U.S. FDA, 2020a)	Food Uses[a,b]	Use Levels (g/L or g/kg)
Baked Goods and Baking Mixes	**Breads and baked goods, incl. gluten-free**	10
Beverages and Beverage Bases	Soft drinks (regular and diet)[c]	0.25
	Enhanced, fortified, and flavored waters (incl. carbonated waters)[c]	0.25
	Non-milk-based meal replacement drinks	1.0
	Sports, isotonic, and energy drinks	0.5
	Protein drinks	1.0
Breakfast Cereals	**Hot breakfast cereals (*e.g.*, oatmeal, grits), instant and RTE**	1.0
	RTE breakfast cereals	
	Puffed cereals	17
	High-fiber cereals	6.2
	Biscuit-type cereals	4.2
Chewing Gum	**Chewing gum**	62
Coffee and Tea	**Coffee**	2.1
	Tea	2.1
Dairy Product Analogs	**Milk substitutes such as soy milk and imitation milks**	0.25
	Beverage whiteners	125
	Non-dairy cream	125
	Non-dairy yogurt	2.2
Frozen Dairy Desserts	**Frozen desserts incl. ice creams and frozen yogurts, frozen novelties**	3.5
Fruit and Water Ices	**Edible ices, sherbet, and sorbet**	3.5
Gelatins, Puddings, and Fillings	**Dairy-based puddings, custards, and mousses**[d]	3.5
	Fruit pie filling	2.9
	"Fruit Prep" such as fruit filling in bars, cookies, yogurt, and cakes	6.25
Grain Products and Pastas	Cereal and granola bars incl. **energy, protein,** and meal replacement bars[e]	10
Infant and Toddler Foods	Term infant formula[f]	0.50 (as consumed)
	Toddler formula[f]	0.50 (as consumed)
	Hypoallergenic infant formula	0.50 (as consumed)
	Other baby foods for infants and young children	2.5
	Hot cereals (dry and RTE)[g]	2.3
	Other drinks for young children, incl. yogurt and juice beverages identified as "baby drinks"[h]	0.25 to 2.1
	Desserts incl. fruit desserts, cobblers, yogurt/fruit combinations ("junior type desserts")[g]	2.3
	Baby crackers, pretzels, cookies, and snack items[g]	12
Jams and Jellies	**Jellies and jams, fruit preserves, and fruit butters**	12
Milk, Whole, and Skim	Unflavored pasteurized and sterilized milk (whole milk, reduced-fat milk, low-fat milk, non-fat milk; including powdered milks, reconstituted)	0.5

Table A-2 Summary of the Individual Proposed Food Uses and Use Levels for 6'-Sialyllactose Sodium Salt in the U.S.

Food Category (21 CFR §170.3) U.S. FDA, 2020a)	Food Uses[a,b]	Use Levels (g/L or g/kg)
Milk Products	Buttermilk[i]	0.25
	Flavored milk[i]	0.25
	Evaporated and condensed milk	0.25
	Milk-based meal replacement beverages for weight reduction	1.0
	Yogurt	5.0
	Formula intended for pregnant women ("mum" formulas, -9 to 0 months)[j]	12.5
Processed Fruits and Fruit Juices	Fruit flavored drinks and ades[k]	0.25
	Fruit juices	0.25
	Fruit nectars	0.25
	Canned fruit	3.5
	Fruit-based desserts	3.5
Processed Vegetables and Vegetable Juices	**Vegetable juices and nectars**	0.25
Sugar Substitutes	**Table-top sweeteners**	62
Sweet Sauces, Toppings, and Syrups	**Syrups used to flavor milk beverages**	1.5
Foods For Special Dietary Use	**Oral nutritional supplements and enteral tube feeding (11 years and older)[l]**	4.1[m]

6'-SL = 6'-sialyllactose; CFR = *Code of Federal Regulations*; GRAS = Generally Recognized as Safe; incl. = including; NHANES = National Health and Nutrition Examination Survey; RTE = ready-to-eat; U.S. = United States.

[a] 6'-SL are 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 (U.S. FDA, 2020a).

[b] Additional food uses proposed by Kyowa that have not been previously concluded as GRAS and notified to the U.S. FDA are **bolded.**

[c] The use of 6'-SL sodium salt in soft drinks and enhanced, fortified, and flavored waters was previously concluded to be GRAS at a use level of 0.50 g/L.

[d] Includes gelatin desserts.

[e] The use of 6'-SL sodium salt was previously concluded to be GRAS in cereal and granola bars at a use level of 5 g/kg and in meal replacement bars at a use level of 10 g/kg. Kyowa now proposes to also use 6'-SL sodium salt in energy and protein bars and at a use level of 10 g/kg for all bar types.

[f] The use of 6'-SL sodium salt was previously concluded to be GRAS in term infant formula at a use level of 0.4 g/L and toddler formula at a use level of 0.3 g/L.

[g] The use of 6'-SL sodium salt was previously concluded to be GRAS in baby foods at a use level of 2.5 g/kg.

[h] The use of 6'-SL sodium salt was previously concluded to be GRAS in drinks for young children at a use level of 0.3 g/L.

[i] The use of 6'-SL sodium salt was previously concluded to be GRAS in buttermilk and flavored milk at a use level of 0.5 g/L.

[j] Food codes for "mum formulas" were not available in the 2017-2018 NHANES. This intended use is excluded from the calculation of estimated daily intakes due to absence of consumption data.

[k] The use of 6'-SL sodium salt was previously concluded to be GRAS in fruit flavored drinks and ades at a use level of 0.5 g/L.

[l] Foods for special dietary use were assessed separately from the intended food uses of 6'-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 6'-SL sodium salt from foods for special dietary use is, therefore, not expected to be cumulative to other dietary sources.

[m] Use level of 4.1 g/L represents the level of 6'-SL sodium salt in the final, ready to consume product.

KYOWA HAKKO BIO CO., LTD.
4-10-2, Nakano, Nakano-ku, Tokyo 164-0001, Japan
Tel: +81-3-6625-0480 Fax: +81-3-5343-1160
http://www.kyowahakko-bio.co.jp/

December 7, 2022

Dr. Ellen Anderson
Regulatory Review Scientist
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

Dear Dr. Anderson,

Re: GRAS Notice No. GRN 001053

In response to your email of November 19, 2022, below are our responses to your request for additional information regarding GRN 001053. FDA's questions are italicized text and our responses are in plain text.

We hope the responses to your questions are satisfactory. We are looking forward to your completed evaluation. If you have any further questions or need clarification, please reach out to me at saori.akizuki@kyowa-kirin.co.jp.

Yours sincerely,

Saori Akiduki, PhD
Assistant Manager
KYOWA HAKKO BIO CO., LTD.
Research & Business Development Department
External Relations Division

Nakano Central Park South, Nakano 4-10-2
Nakano-ku, Tokyo 164-0001, Japan

saori.akizuki@kyowa-kirin.co.jp

Response to Questions from U.S. FDA – GRAS Notice No. GRN 001053 – 6'-Sialyllactose (6'-SL) Sodium Salt

OVERVIEW

Kyowa Hakko Bio Co., Ltd. (Kyowa) presents the following responses to the United States (U.S.) Food and Drug Administration's (FDA's) letter dated 18 November 2022, pertaining to questions from the Agency on the Generally Recognized as Safe (GRAS) uses of 6'-sialyllactose (6'-SL) sodium salt described in GRAS Notice No. GRN 001053.

RESPONSES

Question 1

1. *The intended uses of 6'-SL sodium salt described in the notice include use in non-exempt, infant formula for term infants. Please identify the protein source(s) included in the intended infant formula (e.g., cow milk-based, soy-based).*

Response 1

As Kyowa is not a manufacturer of infant formula, the protein sources that will be included in infant formula products to which 6'-SL sodium salt may be added will be determined by the infant formula manufacturer; however, it is anticipated that 6'-SL sodium salt may be used with any protein source that is permitted for use in infant formula products in the U.S. The major protein sources used in infant formula products marketed in the U.S. include cow's milk protein–based products and soy protein–based products.

Question 2

2. *In section 3.3 on page 51 of the notice, it states, "Kyowa intends to market 6'-SL sodium salt as a nutritional ingredient for use in non-exempt term infant formula, as well as specified foods and beverages as defined under 21 CFR §170.3(n) …" We note that 6'-SL sodium salt does not meet the regulatory definition of a "nutrient" in infant formula under 21 CFR 106.3. We also note that, while there is no regulatory definition of "nutrient" or "nutritional ingredient" for conventional foods and beverages, FDA refers to the Dietary Reference Intakes of the National Academy of Medicine (formerly the Institute of Medicine) in 21 CFR Part 101. In our review of the notice, we would refer to 6'-SL sodium salt as an ingredient but not a "nutritional ingredient." For the administrative record, please revise this statement with regards to 6'-SL sodium salt being described as a "nutritional ingredient."*

Response 2

Kyowa agrees with the U.S. FDA that 6'-SL sodium salt should be referred to as an ingredient rather than a nutritional ingredient. Section 3.3 is updated as follows:

> *"Kyowa intends to market 6'-SL sodium salt as an ingredient for use in non-exempt term infant formula, as well as specified foods and beverages as defined under 21 CFR §170.3(n)…"*

Question 3

3. *We note that the intended uses of 6'-SL sodium salt in oral nutritional supplements and enteral tube feeding uses are listed together in Table 1.3-1 on page 8 of the notice with a maximum use level of 4.1 g/L. However, the use level for oral nutritional supplements is described in the text of page 6 as 0.42 g per 250 mL serving, which would be equivalent to 1.68 g/L. Please clarify the intended maximum use level for this category.*

Response 3

Kyowa's intended use level in oral nutrition products is based on oral nutrition products containing 2'-fucosyllactose (2'-FL) that are already on the U.S. market, with the use level adjusted for the ratio of 6'-SL to 2'-FL in human milk (*i.e.*, 6'-SL is present at approximately one-fifth the level of 2'-FL; thus, Kyowa's proposed use level for 6'-SL sodium salt is approximately one-fifth that of 2'-FL [see GRN 001051 for 2'-FL submitted by Kyowa]). The typical conditions of use, as described in Section 1.3 (page 6) of the notice, were 0.42 g 6'-SL sodium salt/45 g powdered serving or 250 mL ready to consume product, which is equivalent to 1.68 g 6'-SL sodium salt/L. Therefore, the maximum use level for 6'-SL sodium salt in oral nutritional supplements is 1.68 g/L. The use level in enteral tube feeding formula is based on the intended use of 2'-FL in enteral tube-feeding formulas for patients ≥11 years (20 g/L) that was concluded to be GRAS and notified to the Agency without questions in GRN 897 (DuPont Nutrition and Health, 2019 – U.S. FDA, 2020a), with the use level adjusted for the ratio of 6'-SL to 2'-FL in human milk (*i.e.*, 6'-SL is present at approximately one-fifth the level of 2'-FL; thus, Kyowa's proposed use level for 6'-SL sodium salt is approximately one-fifth that of 2'-FL). Therefore, Kyowa's intended use of 6'-SL sodium salt in enteral tube feeding formula is intended for ages 11 and up and is proposed at a use level of 4.1 g/L in the final, ready to consume product.

Question 4

4. *Kyowa intends to use 6'-SL sodium salt in food categories not previously included among the intended uses of 6'-SL sodium salt in previous GRAS notices submitted to FDA. Some of the new food categories have standards of identity as listed in 21 CFR Parts 131 (milk and cream), 136 (bakery products), and 145 (canned fruits). A footnote to Table 1.3-1 states that "6'-SL are intended for use in unstandardized products where standards of identity … do not permit its addition in standardized products." However, it is not clear what foods would be included among the expanded uses in Table 1.3-1. Please address the following:*

Question 4a

a. Kyowa proposes use levels of 10 g/kg in breads and baked goods (including gluten free). Many of these foods have standards of identity (21 CFR Part 136) that may preclude use of 6'-SL sodium salt in these foods. Please clarify which subcategories of baked goods are likely to contain 6'-SL sodium salt and if standardized foods were excluded from the estimates of dietary exposure presented in the notice.

Response 4a

"Breads and baked goods (including gluten-free varieties)" includes both bakery products with standards of identity that do not restrict the addition of 6'-SL sodium salt, as well as unstandardized products. For example, the standard of identity for bread, rolls, and buns (*i.e.*, white, enriched, milk, raisin, and whole wheat) laid out in 21 CFR 136.110(c)(18) states that "*other ingredients that do not change the basic identity or adversely affect the physical and nutritional characteristics of the food*" may be added to these products. All other varieties of bread, rolls, and buns with standards of identity must conform to the requirements prescribed under Part 136.110 (U.S. FDA, 2021a). It is expected that the addition of 6'-SL sodium salt to bread products with standards of identity would comply with 21 CFR 136.110(c)(18) since its addition would not change the identity or the physical and nutritional characteristic of the bread product. Examples of unstandardized food included for "Breads and baked goods, including gluten-free" includes bagels, muffins, biscuits, cakes, cookies, pies, doughnuts, crackers, pancakes, waffles, French toast, and similar baked good products (U.S. FDA, 2021a). Food codes pertaining to the standardized and unstandardized breads and baked goods included in the exposure assessment are detailed in Appendix A.

Question 4b

b. We note that the use levels of 6'-SL sodium salt are up to 3.5 g/kg in canned fruit. The specific standardized canned fruits are listed in 21 CFR Part 145, and many canned fruits have standards of identity that may preclude use of 6'-SL sodium salt in this food category. Please clarify the intended use (e.g., provide examples of nonstandardized foods within this category), use level, and the specific types of foods considered in the dietary exposure estimates of 6'-SL sodium salt from this food category.

Response 4b

6'-SL sodium salt is intended for use in unstandardized canned fruits such as canned kumquat, canned orange, canned apple, canned lychee, canned papaya, canned cranberry, and canned rhubarb (see Appendix A for the list of food codes). Food codes for canned fruits with standards of identity were included in the exposure assessment to generate conservative estimates for the intake of 6'-SL sodium salt.

Question 4c

c. Kyowa proposes use levels for 6'-SL sodium salt of up to 0.25 g/L in evaporated and condensed milks. The standards of identity for evaporated milk (21 CFR 131.130), concentrated milk (21 CFR 131.115), and sweetened condensed milk (21 CFR 131.120) do not include use of 6'-SL sodium salt in these foods. Please clarify the intended use in this food category, including the types of foods used in the estimates of dietary exposure.

Response 4c

Under 21 CFR 131.130, evaporated milk is defined as the liquid food obtained by partial removal of water only from milk that contains not less than 6.5% by weight of milk fat (U.S. FDA, 2021a). Under 21 CFR 131.115, concentrated milk is defined as the liquid food obtained by partial removal of water from milk with a milk fat content of not less than 7.5% (U.S. FDA, 2021a). Under 21 CFR 131.120, sweetened condensed milk is defined as the food obtained by partial removal of water only from a mixture of milk and safe and suitable nutritive carbohydrate sweeteners that contains not less than 8% milk fat (U.S. FDA, 2021a). There are no provisions for optional ingredients in the standards of identity for evaporated milk, concentrated milk, or sweetened condensed milk that would permit the addition of 6'-SL sodium salt. Therefore, 6'-SL sodium salt is intended for use in unstandardized evaporated and condensed milk products such as the low-fat and fat-free varieties that would not meet the standards of identity detailed above. Food codes for the standardized products were included in the exposure assessment as a conservative measure (see Appendix A).

Question 5

5. *6'-SL sodium salt is intended to be used in formula intended for pregnant women ("mum" formulas, -9 to 0 months). Kyowa states that dietary exposure was not determined for this intended use due to a lack of food codes and consumption data for this food category in the 2017-2018 NHANES. Does Kyowa consider dietary exposure from this use to be additive to or substitutional for other uses of 6'-SL sodium salt? Please clarify whether you consider these formulas to be a type of milk-based meal replacement or a different type of food product.*

Response 5

Formulas intended for pregnant women ("mum formulas") are not intended to be used as meal replacements, but rather consumed in a manner similar to nutritional drinks or other nutrient-enriched milk beverages. On this basis, it is expected that this use would be primarily substitutional to other uses such as "flavored milk" and "protein drinks".

Question 6

6. *In section 1.2 on page 5 of the notice, "6'-SL sodium salt" is given as an abbreviation for 6'-siallylactose sodium salt, indicating that 6'-SL alone refers to a free acid form of the ingredient. Please clarify whether the specification parameter listed as "Purity (6'-SL)" in Table 2.3.1-1 refers to the purity of 6'-siallylactose as a free acid or as a sodium salt.*

Response 6

The specification listed as "Purity (6'-SL)" in Table 2.3.1-1 refers to the purity of 6'-sialyllactose as a sodium salt. The specification should be listed as "Purity (6'-SL sodium salt)".

Question 7

7. *The cited method for determination of residual protein is described as the dot-blot (internal) method. In section 2.3.1 on page 20 of the notice, it is noted that the method was developed and concluded to be suitable for its intended use by Kyowa. The results of batch analyses demonstrate that protein levels are consistently ≤1 mg/kg; however, the specification for residual protein is ≤10 mg/kg. For clarity, please provide a brief description of the dot blot (internal) method and its validation.*

Response 7

The Dot-Blot method has been validated for its intended use. A brief summary of the method follows.

Test Method: Prepare 100 mg/mL sample solution and 0.1 µg/mL bovine serum albumin (BSA) standard solution. The proteins in 1 mL of the sample and standard solutions are collected on a polyvinylidene difluoride (PVDF) membrane using a Bio-Dot device, stained with Amido Black solution, and the color of the BSA standard solution (equivalent to 1 part per million [ppm]) is used as the comparison color.

Evaluation Criteria: The color of the sample solution is not darker than that of the standard solution (1 ppm) (coloration is confirmed visually). If the color of the sample solution is darker than that of the standard solution, dilute the sample solution 10-fold and repeat the measurement.

No proteins have been detected in the batch analyses for Kyowa's 6'-SL sodium salt ingredient. Sources of potential protein include the production microorganism. 6'-SL is secreted into the medium by the production organism and intact cells are removed with a ceramic filter, followed by purification with a series of cationic resin and anionic resin ion exchangers and a series of microfiltration steps and an ultrafiltration membrane (molecular weight cut-off of 6,000 Da), which effectively remove any residual microorganism, endotoxins, and residual proteins. Batch analyses data demonstrate the reliability of the purification processes to remove residual proteins, as results for all batches were below the limit of quantification (LOQ) (≤1 mg/kg) of the Dot-Blot method.

Despite the sensitivity of the Dot-Blot method, it is more technically challenging than other colorimetric methods (*e.g*., Bradford assay). Although the Bradford assay is less sensitive than the Dot-Blot method, there are no safety concerns with the low-level residual protein that may be present in the ingredient. Therefore, Kyowa has changed analytical methods to the Bradford assay, which is an internationally recognized standard method. The test for residual proteins in 6'-SL sodium salt was conducted as a limit test at 100 ppm, and no residual proteins were detected in 4 lots of Kyowa's 6'-SL sodium salt. The results are provided in Table 7-1 below, and for clarity, an updated Table 2.3.3.1-1 has been provided below with the results for Lot C added in red font.

Table 7-1 Summary of Residual Protein Analyses for the Final 6'-SL Sodium Salt Powdered Ingredient Produced with a Genetically Modified Strain of *Escherichia coli* W

Specification Parameter	Specification	Methods of Analysis	Manufacturing Lot			
			C	H	I	J
Residual proteins (mg/kg)	≤100	Bradford method	≤100[a]	≤100[a]	≤100[a]	≤100[a]

6'-SL = 6'-sialyllactose; ppm = parts per million.
[a] Evaluated using a limit test at 100 ppm.

Table 2.3.3.1-1 Summary of Batch Analyses for the Final 6'-Sialyllactose Sodium Salt Powdered Ingredient Produced with a Genetically Modified Strain of *Escherichia coli* W

Specification Parameter	Specification	Methods of Analysis	Manufacturing Lot				
			A	B	C	D	E
Properties							
Appearance	Powder	Visual observation	Complies	Complies	Complies	Complies	Complies
Color	White to off-white	JP 17; General Notice[a]	Complies	Complies	Complies	Complies	Complies
Identification	RT of standard ± 3%	HPLC-CAD (internal method 3)	Complies	Complies	Complies	Complies	Complies
Purity	≥82% dry basis	HPLC-CAD (internal method 3)	87	92	90	92	92
Purity as free acid	Not established[b]	By calculation[c]	84.08	88.92	86.98	88.92	88.92
Water	≤10.5 w/w%	JP 2.48[a]	5.3	5.0	5.4	5.6	5.0
Sodium	≤5.0% dry basis	USP 233[d]	3.8	3.8	3.8	3.7	3.8
pH (25˚C, 5% solution)	4.0 to 9.0	JP 2.54[a]	6.4	6.5	6.5	6.5	6.5
Residual proteins	NS	Dot-blot (internal method)	≤1	≤1	≤1	≤1	≤1
Residual proteins	≤100 mg/kg	Bradford method	NT	NT	≤100[e]	NT	NT
Other Carbohydrates							
NeuAc	≤9% w/w	HPLC-CAD (internal method 3)[f]	5.1	3.5	4.9	4.3	5.4
D-Glucose	≤3% w/w	HPLC-PAD (internal method 2)[g]	ND	ND	ND	ND	ND
D-Lactose	≤3% w/w	HPLC-PAD (internal method 2)[g]	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05
6'-Sialyllactulose	≤5% w/w	HPLC-CAD (internal method 3)[f]	0.4	0.4	0.5	0.5	0.4
3'-Sialyllactose sodium salt	≤1% w/w	HPLC-CAD (internal method 3)[f]	ND	ND	ND	ND	ND
Mass balance	NA	By calculation[h]	93.5	96.7	96.3	97.5	98.6
Heavy Metals							
Arsenic	≤0.2 mg/kg	USP 233[d,i]	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05
Cadmium	≤0.2 mg/kg	USP 233[d, i]	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05
Lead	≤0.2 mg/kg	USP 233[d, i]	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05
Mercury	≤0.2 mg/kg	USP 233[d, i]	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05
Iron	≤10 mg/kg	USP 233[d, i]	0.3	0.2	0.3	0.3	0.6

Table 2.3.3.1-1 Summary of Batch Analyses for the Final 6'-Sialyllactose Sodium Salt Powdered Ingredient Produced with a Genetically Modified Strain of *Escherichia coli* W

Specification Parameter	Specification	Methods of Analysis	Manufacturing Lot				
			A	B	C	D	E

HPLC-CAD = high-performance liquid chromatography coupled with charged aerosol detection; HPLC-PAD = high-performance liquid chromatography coupled with pulsed amperometric detection; JP = Japanese Pharmacopeia; LOD = limit of detection; LOQ = limit of quantification; NA = not applicable; ND = not detected; NeuAc = *N*-acetyl D-neuraminic acid; NS = not specified; NT = not tested; RT = retention time; USP = United States Pharmacopeia.
[a] Method is consistent with the compendial method specified in 17th edition of the Japanese Pharmacopeia (2016).
[b] No specification limit established as purity as free acid was calculated for the purposes of calculating mass balance.
[c] Purity as free acid was calculated as Purity * Mw 6'-SL (633.55)/Mw 6'-SL Na (655.53).
[d] Method is consistent with the compendial method specified in the United States Pharmacopeia 35th revision (2011).
[e] Evaluated using a limit test at 100 ppm.
[f] LOD for NeuAc, 6'-Sialyllactulose, and 3'-sialyllactose sodium salt is 0.01 w/w% and LOQ for NeuAc, 6'-Sialyllactulose, and 3'-sialyllactose sodium salt is 0.2 w/w% as 6'-Sialyllactose sodium salt.
[g] LOD for D-glucose and D-lactose is 0.02 w/w% and LOQ for D-glucose and D-lactose is 0.05 w/w% as D-lactose.
[h] Mass balance = sum of purity as free acid, sodium, NeuAc, D-glucose, D-lactose, 6'-sialyllactulose, 3'-sialyllactose sodium salt. Results that were ND were replaced with the respective LOD values. Results that were ≤ LOQ were replaced with the LOQ values.
[i] LOQ for heavy metals (*i.e.*, arsenic, cadmium, lead, and mercury) is 0.05 mg/kg.

In light of the request, Kyowa proposes to establish the residual protein specification at ≤100 mg/kg using the Bradford method. Please note that the temperature for the pH analysis was incorrectly reported as 20°C in the dossier; the correct temperature for the analysis is 25°C, and this has been updated below.

Additionally, it was determined that the related carbohydrates 6'-sialyllactulose (6SLu) and 3'-sialyllactose (3'-SL) sodium salt could not be separated in the high-performance liquid chromatography (HPLC) chromatograms. Therefore, Kyowa is planning to delete the individual parameters for 6SLu and 3'-SL sodium salt from the specifications and replace them with a new parameter for 6SLu and 3'-SL sodium salt combined, with a specification limit of ≤5 w/w%. This has been updated below.

Table 2.3.1-1 is reproduced and updated below, with changes in red font.

Table 2.3.1-1 Chemical Specifications for 6'-Sialyllactose Sodium Salt Produced with a Genetically Modified Strain of *Escherichia coli* W

Specification Parameter	Specification	Method
Organoleptic		
Appearance	Powder	Visual observation
Color	White to off-white	General Notice, JP 17[a]
Physicochemical		
Identification	RT of standard ± 3%	HPLC-CAD (internal method)
Purity (6'-SL sodium salt)	≥82% dry basis	HPLC-CAD (internal method)
Water	≤10.5 w/w%	JP 2.48[a]
Sodium (Assay)	≤5.0% dry basis	USP 233[b]
Residual protein	≤100 mg/kg	Bradford
pH (25˚C, 5% solution)	4.0 to 9.0	JP 2.54[a]
Other Carbohydrates		
N-Acetyl D-neuraminic acid	≤9 w/w%	HPLC-CAD (internal method)
D-glucose	≤3 w/w%	HPLC-PAD (internal method)
D-lactose	≤3 w/w%	HPLC-PAD (internal method)

Table 2.3.1-1 Chemical Specifications for 6'-Sialyllactose Sodium Salt Produced with a Genetically Modified Strain of *Escherichia coli* W

Specification Parameter	Specification	Method
6'-Sialyllactulose and 3'-sialyllactose sodium salt	≤5 w/w%	HPLC-CAD (internal method)
Heavy Metals		
Arsenic	≤0.2 mg/kg	USP 233[b]
Cadmium	≤0.2 mg/kg	USP 233[b]
Lead	≤0.2 mg/kg	USP 233[b]
Mercury	≤0.2 mg/kg	USP 233[b]
Iron	≤10 mg/kg	USP 233[b]

6'-SL = 6'-sialyllactose; HPLC-CAD = high-performance liquid chromatography coupled with charged aerosol detection; HPLC-PAD = high-performance liquid chromatography coupled with pulsed amperometric detection; JP = Japanese Pharmacopeia; RT = retention time; USP = United States Pharmacopeia.
[a] Method is consistent with the compendial method specified in 17th edition of the Japanese Pharmacopeia (2016).
[b] Method is consistent with the compendial method specified in the United States Pharmacopeia 35th revision (2011).

Question 8

8. *On page 38 of the notice, it states, "Kyowa selected a use level of 0.50 g 6'-SL sodium salt/L in non-exempt term infant formula, as this use level was mid-range of the calculated average from all identified studies and mean levels determined in the review publication by Thurl et al. (2017)." Kyowa further states "the overall average levels" to be 0.345 and 0.64 g/L for human milk samples from non-secretor and secretor mothers. We note that these "overall average levels" cited from Thurl et al. (2017) reflect datasets from all samples that include colostrum, transitional milk and mature milk. We further note that this systematic review as well as more recent systematic reviews (i.e., Soyyilmaz et al. 2021 and Zhou et al. 2021) show that the levels of 6'-SL, on average, significantly decrease with period of lactation when datasets are stratified. Thus, on average, breastfed infants older than 30 days are exposed to levels close to or below 0.4 g/L, the use level previously proposed in GRN 000881 and 000922[1]. Additionally, another systematic review (Conze et al. 2022) also concluded that the weighted mean level of 6'-SL was 0.39 g/L, again closely matching the use level proposed in GRN 000881 and 000922. Given mature milk is generally thought to be the most appropriate human milk reference for formulation of infant formula (Wells, 1996), the relevance of a dataset from colostrum or transitional milk for supporting an appropriate and safe use level of 6'-SL sodium salt in infant formula, whose consumers include those aged up to 12 months, is unclear. We further note that we are not aware of the existence of a clinical study of infant formula supplemented with 6'-SL at a constant use level above 0.3 g/L.[2] Please provide additional narrative, based on generally available and generally accepted data, that a use level of 0.5 g/L 6'-SL sodium salt in infant formula can be concluded to be GRAS.*

   [1] We note that EFSA's 2020 evaluation on the safety of 6'-SL was based on 0.4 g/L in infant formula and 0.3 g/L in follow-on formula (EFSA J 2020, 18: 6097).

   [2] The only currently published clinical studies with 6'-SL in infant formula used ~0.3 g/L as the use level (as part of a mixture of HMOs; Parschat et al. 2021, Nutrients 13: 2871; Lasekan et al. 2022, Nutrients 14: 2625).

Response 8

Kyowa wishes to amend the use level to 0.4 g 6'-SL sodium salt/L in non-exempt term infant formula, which has previously been concluded to be GRAS (GRN 881 and 922) and is supported by recent systematic reviews (Glycom A/S, 2019; Jennewein Biotechnologie GmbH, 2020; U.S. FDA, 2020b, 2021b).

Question 9

9. *In Table 1.3-1 on page 8 of the notice, a proposed food use for 6'-SL sodium salt is in formula intended for pregnant women ("mum" formulas, -9 to 0 months). However, there is no accompanying narrative that supports the safe consumption of 6'-SL sodium salt in a product specifically targeted to pregnant women who are a vulnerable subpopulation. We are also unaware of any published study that specifically examined the safety and tolerance of 6'-SL sodium salt when consumed by pregnant women. Please provide a narrative that discusses the consumption of 6'-SL sodium salt by pregnant women and why this is not expected to be a safety concern. As part of this discussion, we suggest including information on how the gut microbiome changes during pregnancy and if this impacts the absorption, distribution, metabolism, or excretion of 6'-SL sodium salt in this subpopulation.*

Response 9

As discussed in Section 1.3 of GRN 001053, Kyowa proposed to use their 6'-SL sodium salt ingredient in the same food uses as previously concluded to be GRAS by other notifiers; however, Kyowa intends to use 6'-SL sodium salt at different use levels in several food uses. Kyowa's proposed use level of 6'-SL sodium salt in "formula intended for pregnant women ('mum' formulas, -9 to 0 months)" is 1.25 g/100 g (equivalent to 12.5 g/kg). The proposed food use of "Meal replacement drinks for adults (including dairy and non-dairy drinks for weight reduction); including formulas for pregnant women" at a use level of 2.28 g/L has previously been concluded to be GRAS and was notified to the FDA without questions in GRN 1016 (Chr. Hansen, Inc., 2021 – U.S. FDA, 2022). Kyowa reviewed GRN 1016 to identify a narrative supporting safety of this use; however, no supporting safety information specific to consumption of 6'-SL by pregnant or lactating women was provided in GRN 1016 (Chr. Hansen, Inc., 2021 – U.S. FDA, 2022).

The gastrointestinal microbiota has been reported to differ between pregnant and non-pregnant women, although a high degree of variability exists in both populations (Koren *et al.*, 2012). The optimal gut microbiome is not known; however, decreased diversity has been linked to a disruption of the normal gut microbiota and a higher diversity has been suggested to correlate with a healthier microbiome (Maher *et al.*, 2020). In a recent systematic review, the gut microbiome of pregnant women was reported to be influenced by maternal diet in all 5 studies identified (Maher *et al.*, 2020). Four of the identified studies evaluated the effects of dietary carbohydrate intake on maternal gut microbiota, and in all 4 studies higher dietary fiber intake was positively associated with increased gut microbiota diversity and richness. Although 6'-SL is not a dietary fiber, it is a poorly-digestible carbohydrate that is resistant to hydrolysis by digestive enzymes in the upper digestive tract and is either partially fermented by the intestinal microbiota or excreted unchanged in the feces (EFSA, 2020). Given that consumption of 6'-SL is associated with the establishment of gut microbiota in infants (Nakano, 1999; German *et al.*, 2008; ten Bruggencate *et al.*, 2014; Vasquez *et al.*, 2017), that no adverse effects on the gut microbiota were reported in animal and human studies of 6'-SL ingredients included in GRN 001053, and that consumption of dietary fiber did not have an adverse effect on the gut microbiota of pregnant women, no adverse effects on gut microbiota diversity would be expected from the consumption of 6'-SL sodium salt.

Pregnant or lactating women, and women seeking to become pregnant, are often excluded from clinical studies, typically based on a need to reduce between-subject variability in the study population and the fact that it is standard practice to avoid this population group in clinical trials. As such, no studies were identified in which 6'-SL sodium salt was consumed by pregnant women. However, as discussed above, 6'-SL is a poorly-digestible carbohydrate and studies conducted with other poorly-digestible carbohydrates in pregnant women can be used to support the safe use of 6'-SL sodium salt in this population group. In a recent meta-analysis of studies involving supplementation of pregnant women with probiotics or prebiotics (*i.e.*, galactooligosaccharides [GOS] or fructooligosaccharides [FOS]), the authors reported no serious health concerns regarding maternal or infant health and concluded that prebiotics are "*safe to use during and after pregnancy and lactation*" (Sheyholislami and Connor, 2021).

Based on the safe consumption of other poorly-digestible carbohydrates by pregnant women, the lack of observations indicative of potential adverse effects reported in any of the pre-clinical studies or human studies in infants included in GRN 001053, as well as the increased permeability and sensitivity of the infant gastrointestinal tract compared to adults, no adverse effects attributable to the consumption of 6'-SL sodium salt by pregnant women are anticipated to occur. As 6'-SL sodium salt intakes from all proposed conditions of use in GRN 001053 are within the range of background exposure from human milk in infants, a vulnerable population group, 6'-SL sodium salt is considered to be safe for all population groups, including pregnant women.

Question 10

10. One of Kyowa's stated intended uses for 6'-SL sodium salt is in enteral tube feeds. We note that consumers requiring enteral tube feeds consist of vulnerable subpopulations suffering from a range of ailments that may preclude assessing safety of ingredients such as 6'-SL sodium salt in those specific subpopulations. Please clarify if the population expected to receive 6'-SL sodium salt through enteral tube feeds will be under the care of a physician and/or other medical supervision.

Response 10

Kyowa is not a final food product manufacturer; however, the company anticipates that people who receive 6'-SL sodium salt by enteral tube feeding would use the product under the guidance of their physician or health care professional.

Question 11

11. On page 105 of the notice, it states, "Kyowa's 6'-SL sodium salt ingredient is proposed for use [in formula for enteral tube feeding] at a level of 4.1 g/L, which is 1/5th the level concluded to be GRAS for 2'-FL and consistent with the ratio of 6'-SL to 2'-FL present in human breast milk." This statement implies that 6'-SL sodium salt is GRAS at a level of 4.1 g/L for use in formula for enteral tube feeding only in the presence of 2'-FL. If this is the case, the condition of use should be specified as such. Please provide an explanation.

Response 11

Kyowa wishes to clarify that for all intended uses of 6'-SL sodium salt included in GRN 001053, it is not intended that 6'-SL sodium salt be used exclusively in the presence of 2'-FL. Kyowa confirms that 6'-SL sodium salt, independent of the presence of 2'-FL, is therefore GRAS for use at a level of 4.1 g/L in formula for enteral tube feeding and for all proposed uses as indicated in GRN 001053 (with the exception of the amendment of the use level in non-exempt term infant formula, as indicated in the response to Question 8 above).

Question 12

12. Please state for the administrative record if the production strain has been deposited in a recognized cell culture collection, and if so, please provide the deposit designation.

Response 12

Kyowa has deposited the production strain at the National Biological Resource Center (NBRC). The deposition number is NITE SD_00489.

Question 13

13. Please state if there are any components derived from major allergens used in the production and formulation of 6'-SL sodium salt and state if they will be in the final formulation. If there are none, please provide at statement confirming the lack of any allergens.

Response 13

None of the raw materials used in the production and formulation of 6'-SL sodium salt are derived from major allergens (other than lactose derived from milk). Therefore, no materials derived from major allergens or allergens are present in the final product.

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

Part	Section §	Section Title
131—Milk and Cream	131.115	Concentrated milk
	131.120	Sweetened condensed milk
	131.130	Evaporated milk
136—Bakery products	136.110	Bread, rolls, and buns

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Appendix A
Representative Food Codes for Proposed Food Uses of 6'-Sialyllactose Sodium Salt in the U.S. (2017-2018 NHANES Data)

Representative Food Codes for Proposed Food Uses of 6'-Sialyllactose Sodium Salt in the U.S. (2017-2018 NHANES Data)

Baked Goods and Baking Mixes

Breads and baked goods, including gluten-free
[6'-SL Sodium Salt] = 1 g/100 g

13252600	Tiramisu
51000100	Bread, NS as to major flour
51000110	Bread, NS as to major flour, toasted
51000180	Bread, made from home recipe or purchased at a bakery, NS as to major flour
51000190	Bread, made from home recipe or purchased at a bakery, toasted, NS as to major flour
51000200	Roll, NS as to major flour
51000300	Roll, hard, NS as to major flour
51000400	Roll, bran, NS as to type of bran
51101000	Bread, white
51101010	Bread, white, toasted
51101050	Bread, white, made from home recipe or purchased at a bakery
51101060	Bread, white, made from home recipe or purchased at a bakery, toasted
51102010	Bread, white with whole wheat swirl
51102020	Bread, white with whole wheat swirl, toasted
51105010	Bread, Cuban
51105040	Bread, Cuban, toasted
51106010	Bread, native, water, Puerto Rican style
51106020	Bread, native, water, toasted, Puerto Rican style
51106200	Bread, lard, Puerto Rican style
51106210	Bread, lard, toasted, Puerto Rican style
51106300	Bread, caressed, Puerto Rican style
51106310	Bread, caressed, toasted, Puerto Rican style
51107010	Bread, French or Vienna
51107040	Bread, French or Vienna, toasted
51108010	Focaccia, Italian flatbread, plain
51108100	Naan, Indian flatbread
51109010	Bread, Italian, Grecian, Armenian
51109040	Bread, Italian, Grecian, Armenian, toasted
51109100	Bread, pita
51109110	Bread, pita, toasted
51109150	Bread, pita with fruit
51109200	Bread, pita with fruit, toasted
51111010	Bread, cheese
51111040	Bread, cheese, toasted
51113010	Bread, cinnamon

51113100	Bread, cinnamon, toasted
51115010	Bread, cornmeal and molasses
51115020	Bread, cornmeal and molasses, toasted
51119010	Bread, egg, Challah
51119040	Bread, egg, Challah, toasted
51121015	Garlic bread, NFS
51121025	Garlic bread, from fast food / restaurant
51121035	Garlic bread, from frozen
51121045	Garlic bread, with parmesan cheese, from fast food / restaurant
51121055	Garlic bread, with parmesan cheese, from frozen
51121065	Garlic bread, with melted cheese, from fast food / restaurant
51121075	Garlic bread, with melted cheese, from frozen
51121110	Bread, onion
51121120	Bread, onion, toasted
51122000	Bread, reduced calorie and/or high fiber, white or NFS
51122010	Bread, reduced calorie and/or high fiber, white or NFS, toasted
51122100	Bread, reduced calorie and/or high fiber, white or NFS, with fruit and/or nuts
51122110	Bread, reduced calorie and/or high fiber, white or NFS, with fruit and/or nuts, toasted
51122300	Bread, white, special formula, added fiber
51122310	Bread, white, special formula, added fiber, toasted
51123010	Bread, high protein
51123020	Bread, high protein, toasted
51127010	Bread, potato
51127020	Bread, potato, toasted
51129010	Bread, raisin
51129020	Bread, raisin, toasted
51130510	Bread, white, low sodium or no salt
51130520	Bread, white, low sodium or no salt, toasted
51133010	Bread, sour dough
51133020	Bread, sour dough, toasted
51134000	Bread, sweet potato
51134010	Bread, sweet potato, toasted
51135000	Bread, vegetable
51135010	Bread, vegetable, toasted
51140100	Bread, dough, fried
51150000	Roll, white, soft
51153000	Roll, white, hard
51154010	Roll, white, hot dog bun
51154100	Roll, white, hamburger bun
51154510	Roll, diet
51154550	Roll, egg bread
51154600	Roll, cheese
51155000	Roll, French or Vienna

51156500	Roll, garlic
51157000	Roll, white, hoagie, submarine
51158100	Roll, Mexican, bolillo
51159000	Roll, sour dough
51165000	Coffee cake, yeast type
51180010	Bagel
51180030	Bagel, with raisins
51180080	Bagel, with fruit other than raisins
51183990	Breadsticks, NFS
51184000	Breadsticks, hard, NFS
51184100	Breadsticks, hard, reduced sodium
51184200	Breadsticks, soft, NFS
51184210	Breadsticks, soft, from fast food / restaurant
51184220	Breadsticks, soft, from frozen
51184230	Breadsticks, soft, with parmesan cheese, from fast food / restaurant
51184240	Breadsticks, soft, with parmesan cheese, from frozen
51184250	Breadsticks, soft, topped with melted cheese
51184260	Breadsticks, soft, stuffed with melted cheese
51185000	Croutons
51186010	Muffin, English
51186100	Muffin, English, with raisins
51186130	Muffin, English, cheese
51186160	Muffin, English, with fruit other than raisins
51187000	Melba toast
51187020	Anisette toast
51188500	Zwieback toast
51300050	Bread, whole grain white
51300060	Bread, whole grain white, toasted
51300100	Bagel, whole grain white
51300110	Bread, whole wheat
51300120	Bread, whole wheat, toasted
51300140	Bread, whole wheat, made from home recipe or purchased at bakery
51300150	Bread, whole wheat, made from home recipe or purchased at bakery, toasted
51300175	Bread, chappatti or roti, wheat
51300180	Bread, puri, wheat
51300185	Bread, paratha, wheat
51300210	Bread, whole wheat, with raisins
51300220	Bread, whole wheat, with raisins, toasted
51300300	Bread, sprouted wheat
51300310	Bread, sprouted wheat, toasted
51301010	Bread, wheat or cracked wheat
51301020	Bread, wheat or cracked wheat, toasted
51301040	Bread, wheat or cracked wheat, made from home recipe or purchased at bakery

51301050 Bread, wheat or cracked wheat, made from home recipe or purchased at bakery, toasted
51301120 Bread, wheat or cracked wheat, with raisins
51301130 Bread, wheat or cracked wheat, with raisins, toasted
51301510 Bread, wheat or cracked wheat, reduced calorie and/or high fiber
51301520 Bread, wheat or cracked wheat, reduced calorie and/or high fiber, toasted
51301540 Bread, French or Vienna, whole wheat
51301550 Bread, French or Vienna, whole wheat, toasted
51301600 Bread, pita, whole wheat
51301610 Bread, pita, whole wheat, toasted
51301620 Bread, pita, wheat or cracked wheat
51301630 Bread, pita, wheat or cracked wheat, toasted
51301700 Bagel, wheat
51301750 Bagel, whole wheat
51301800 Bagel, wheat, with raisins
51301805 Bagel, whole wheat, with raisins
51301820 Bagel, wheat, with fruit and nuts
51301900 Bagel, wheat bran
51302500 Muffin, English, wheat bran
51302520 Muffin, English, wheat bran, with raisins
51303010 Muffin, English, wheat or cracked wheat
51303030 Muffin, English, whole wheat
51303050 Muffin, English, wheat or cracked wheat, with raisins
51303070 Muffin, English, whole wheat, with raisins
51303100 Muffin, English, whole grain white
51306000 Breadsticks, hard, whole wheat
51320010 Roll, wheat or cracked wheat
51320060 Roll, wheat or cracked wheat, hot dog bun
51320070 Roll, wheat or cracked wheat, hamburger bun
51320500 Roll, whole wheat
51320550 Roll, whole wheat, hot dog bun
51320560 Roll, whole wheat, hamburger bun
51320700 Roll, whole grain white
51320710 Roll, whole grain white, hot dog bun
51320720 Roll, whole grain white, hamburger bun
51401010 Bread, rye
51401020 Bread, rye, toasted
51401030 Bread, marble rye and pumpernickel
51401040 Bread, marble rye and pumpernickel, toasted
51401200 Muffin, English, rye
51404010 Bread, pumpernickel
51404020 Bread, pumpernickel, toasted
51404500 Bagel, pumpernickel
51404550 Muffin, English, pumpernickel

51407010	Bread, black
51407020	Bread, black, toasted
51420000	Roll, rye
51421000	Roll, pumpernickel
51501010	Bread, oatmeal
51501020	Bread, oatmeal, toasted
51501040	Bread, oat bran
51501050	Bread, oat bran, toasted
51501080	Bagel, oat bran
51502010	Roll, oatmeal
51503000	Muffin, English, oat bran
51503040	Muffin, English, oat bran, with raisins
51601010	Bread, multigrain, toasted
51601020	Bread, multigrain
51601210	Bread, multigrain, with raisins
51601220	Bread, multigrain, with raisins, toasted
51602010	Bread, multigrain, reduced calorie and/or high fiber
51602020	Bread, multigrain, reduced calorie and/or high fiber, toasted
51620000	Roll, multigrain
51620020	Roll, multigrain, hot dog bun
51620030	Roll, multigrain, hamburger bun
51630000	Bagel, multigrain
51630100	Bagel, multigrain, with raisins
51630200	Muffin, English, multigrain
51801010	Bread, barley
51801020	Bread, barley, toasted
51804010	Bread, soy
51804020	Bread, soy, toasted
51805010	Bread, sunflower meal
51805020	Bread, sunflower meal, toasted
51806010	Bread, rice
51806020	Bread, rice, toasted
51807000	Injera, Ethiopian bread
51808000	Bread, gluten free
51808010	Bread, gluten free, toasted
51808050	Breadsticks, hard, gluten free
51808100	Roll, gluten free
52101000	Biscuit, NFS
52101040	Crumpet
52102040	Biscuit, from refrigerated dough
52103000	Biscuit, from fast food / restaurant
52104010	Biscuit, home recipe
52104040	Biscuit, wheat

52104100 Biscuit, cheese
52104200 Biscuit with fruit
52105100 Scone
52105200 Scone, with fruit
53100050 Cake batter, raw, chocolate
53100070 Cake batter, raw, not chocolate
53100100 Cake or cupcake, NS as to type
53101100 Cake, angel food, without icing or filling
53101200 Cake, angel food, with icing or filling
53101250 Cake, angel food, with fruit and icing or filling
53102100 Cake or cupcake, applesauce, without icing or filling
53102200 Cake or cupcake, applesauce, with icing or filling
53102600 Cake or cupcake, banana, without icing or filling
53102700 Cake or cupcake, banana, with icing or filling
53102800 Cake or cupcake, Black Forest
53103000 Cake, Boston cream pie
53104100 Cake or cupcake, carrot, without icing or filling
53104260 Cake or cupcake, carrot, with icing or filling
53104300 Cake, carrot, diet
53104400 Cake or cupcake, coconut, with icing or filling
53104500 Cheesecake
53104550 Cheesecake with fruit
53104600 Cheesecake, chocolate
53105270 Cake or cupcake, chocolate, devil's food or fudge, with icing or filling
53105275 Cake or cupcake, chocolate, devil's food or fudge, without icing or filling
53105300 Cake or cupcake, German chocolate, with icing or filling
53105500 Cake, chocolate, with icing, diet
53106500 Cake, cream, without icing or topping
53108200 Snack cake, chocolate, with icing or filling
53108220 Snack cake, chocolate, with icing or filling, reduced fat and calories
53109200 Snack cake, not chocolate, with icing or filling
53109220 Snack cake, not chocolate, with icing or filling, reduced fat and calories
53109300 Cake, Dobos Torte
53110000 Cake, fruit cake, light or dark, holiday type cake
53111000 Cake or cupcake, gingerbread
53112100 Ice cream cake
53113000 Cake, jelly roll
53114000 Cake or cupcake, lemon, without icing or filling
53114100 Cake or cupcake, lemon, with icing or filling
53115100 Cake or cupcake, marble, without icing or filling
53115200 Cake or cupcake, marble, with icing or filling
53115310 Cake or cupcake, nut, without icing or filling
53115320 Cake or cupcake, nut, with icing or filling

53115410 Cake or cupcake, oatmeal
53115450 Cake or cupcake, peanut butter
53116000 Cake, pound, without icing or filling
53116020 Cake, pound, with icing or filling
53116270 Cake, pound, chocolate
53116350 Cake, pound, Puerto Rican style
53116390 Cake, pound, reduced fat, cholesterol free
53116500 Cake or cupcake, pumpkin, without icing or filling
53116510 Cake or cupcake, pumpkin, with icing or filling
53116550 Cake or cupcake, raisin-nut
53116570 Cake, Ravani
53116600 Cake, rice flour, without icing or filling
53116650 Cake, Quezadilla, El Salvadorian style
53117100 Cake or cupcake, spice, without icing or filling
53117200 Cake or cupcake, spice, with icing or filling
53118100 Cake, sponge, without icing or filling
53118200 Cake, sponge, with icing or filling
53118300 Cake, sponge, chocolate
53118410 Rum cake, without icing
53118500 Cake, torte
53118550 Cake, tres leche
53119000 Cake, pineapple, upside down
53120270 Cake or cupcake, white, with icing or filling
53120275 Cake or cupcake, white, without icing or filling
53121270 Cake or cupcake, yellow, with icing or filling
53121275 Cake or cupcake, yellow, without icing or filling
53122070 Cake, shortcake, biscuit type, with whipped cream and fruit
53122080 Cake, shortcake, biscuit type, with fruit
53123070 Cake, shortcake, sponge type, with whipped cream and fruit
53123080 Cake, shortcake, sponge type, with fruit
53123500 Cake, shortcake, with whipped topping and fruit, diet
53124110 Cake or cupcake, zucchini
53200100 Cookie, batter or dough, raw
53201000 Cookie, NFS
53202000 Cookie, almond
53203000 Cookie, applesauce
53203500 Cookie, biscotti
53204000 Cookie, brownie, NS as to icing
53204010 Cookie, brownie, without icing
53204100 Cookie, brownie, with icing or filling
53204840 Cookie, brownie, reduced fat, NS as to icing
53204860 Cookie, brownie, fat free, NS as to icing
53205250 Cookie, butterscotch, brownie

53205260 Cookie, bar, with chocolate
53206000 Cookie, chocolate chip
53206020 Cookie, chocolate chip, made from home recipe or purchased at a bakery
53206030 Cookie, chocolate chip, reduced fat
53206100 Cookie, chocolate chip sandwich
53206500 Cookie, chocolate, made with rice cereal
53206550 Cookie, chocolate, made with oatmeal and coconut, no bake
53207000 Cookie, chocolate or fudge
53207020 Cookie, chocolate or fudge, reduced fat
53207050 Cookie, chocolate, with chocolate filling or coating, fat free
53208000 Cookie, marshmallow, chocolate-covered
53208200 Cookie, marshmallow pie, chocolate covered
53209005 Cookie, chocolate, with icing or coating
53209010 Cookie, sugar wafer, chocolate-covered
53209015 Cookie, chocolate sandwich
53209020 Cookie, chocolate sandwich, reduced fat
53209100 Cookie, chocolate, sandwich, with extra filling
53209500 Cookie, chocolate and vanilla sandwich
53210000 Cookie, chocolate wafer
53210900 Cookie, graham cracker with chocolate and marshmallow
53211000 Cookie bar, with chocolate, nuts, and graham crackers
53215500 Cookie, coconut
53220000 Cookie, fruit-filled bar
53220010 Cookie, fruit-filled bar, fat free
53220030 Cookie, fig bar
53220040 Cookie, fig bar, fat free
53222010 Cookie, fortune
53222020 Cookie, cone shell, ice cream type, wafer or cake
53223000 Cookie, gingersnaps
53223100 Cookie, granola
53224000 Cookie, ladyfinger
53224250 Cookie, lemon bar
53225000 Cookie, macaroon
53226000 Cookie, marshmallow, with coconut
53226500 Cookie, marshmallow, with rice cereal, no bake
53226550 Cookie, marshmallow, with rice cereal and chocolate chips
53226600 Cookie, marshmallow and peanut butter, with oat cereal, no bake
53228000 Cookie, meringue
53230000 Cookie, molasses
53231000 Cookie, Lebkuchen
53231400 Cookie, multigrain, high fiber
53233000 Cookie, oatmeal
53233010 Cookie, oatmeal, with raisins

53233040 Cookie, oatmeal, reduced fat, NS as to raisins
53233050 Cookie, oatmeal sandwich, with creme filling
53233060 Cookie, oatmeal, with chocolate chips
53233080 Cookie, oatmeal sandwich, with peanut butter and jelly filling
53233100 Cookie, oatmeal, with chocolate and peanut butter, no bake
53234000 Cookie, peanut butter
53234100 Cookie, peanut butter, with chocolate
53234250 Cookie, peanut butter with rice cereal, no bake
53235000 Cookie, peanut butter sandwich
53235500 Cookie, with peanut butter filling, chocolate-coated
53235600 Cookie, Pfeffernusse
53236000 Cookie, Pizzelle
53236100 Cookie, pumpkin
53237000 Cookie, raisin
53237010 Cookie, raisin sandwich, cream-filled
53237500 Cookie, rum ball, no bake
53238000 Cookie, sandwich-type, not chocolate or vanilla
53239000 Cookie, shortbread
53239010 Cookie, shortbread, reduced fat
53239050 Cookie, shortbread, with icing or filling
53239100 Pocky
53240000 Cookie, animal
53240010 Cookie, animal, with frosting or icing
53241500 Cookie, butter or sugar
53241510 Marie biscuit
53241600 Cookie, butter or sugar, with fruit and/or nuts
53242000 Cookie, sugar wafer
53242500 Cookie, toffee bar
53243000 Cookie, vanilla sandwich
53243010 Cookie, vanilla sandwich, extra filling
53243050 Cookie, vanilla sandwich, reduced fat
53244010 Cookie, butter or sugar, with chocolate icing or filling
53244020 Cookie, butter or sugar, with icing or filling other than chocolate
53246000 Cookie, tea, Japanese
53247000 Cookie, vanilla wafer
53247050 Cookie, vanilla wafer, reduced fat
53247500 Cookie, vanilla with caramel, coconut, and chocolate coating
53251100 Cookie, rugelach
53260030 Cookie, chocolate chip, sugar free
53260200 Cookie, oatmeal, sugar free
53260300 Cookie, sandwich, sugar free
53260400 Cookie, sugar or plain, sugar free
53260500 Cookie, sugar wafer, sugar free

53260600 Cookie, peanut butter, sugar free
53261000 Cookie, gluten free
53270100 Cookies, Puerto Rican style
53300100 Pie, NFS
53300170 Pie, individual size or tart, NFS
53300180 Pie, fried, NFS
53301000 Pie, apple, two crust
53301070 Pie, apple, individual size or tart
53301080 Pie, apple, fried pie
53301500 Pie, apple, one crust
53302000 Pie, apricot, two crust
53302070 Pie, apricot, individual size or tart
53302080 Pie, apricot, fried pie
53303000 Pie, blackberry, two crust
53303070 Pie, blackberry, individual size or tart
53303500 Pie, berry, not blackberry, blueberry, boysenberry, huckleberry, raspberry, or strawberry; two crust
53303510 Pie, berry, not blackberry, blueberry, boysenberry, huckleberry, raspberry, or strawberry; one crust
53303570 Pie, berry, not blackberry, blueberry, boysenberry, huckleberry, raspberry, or strawberry, individual size or tart
53304000 Pie, blueberry, two crust
53304070 Pie, blueberry, individual size or tart
53305000 Pie, cherry, two crust
53305010 Pie, cherry, one crust
53305070 Pie, cherry, individual size or tart
53305080 Pie, cherry, fried pie
53305700 Pie, lemon, not cream or meringue
53305720 Pie, lemon, not cream or meringue, individual size or tart
53305750 Pie, lemon, fried pie
53306000 Pie, mince, two crust
53307000 Pie, peach, two crust
53307050 Pie, peach, one crust
53307070 Pie, peach, individual size or tart
53307080 Pie, peach, fried pie
53307500 Pie, pear, two crust
53307570 Pie, pear, individual size or tart
53308000 Pie, pineapple, two crust
53308070 Pie, pineapple, individual size or tart
53309000 Pie, raisin, two crust
53309070 Pie, raisin, individual size or tart
53310000 Pie, raspberry, one crust
53310050 Pie, raspberry, two crust

53311000 Pie, rhubarb, two crust
53312000 Pie, strawberry, one crust
53313000 Pie, strawberry-rhubarb, two crust
53314000 Pie, strawberry, individual size or tart
53340000 Pie, apple-sour cream
53340500 Pie, cherry, made with cream cheese and sour cream
53341000 Pie, banana cream
53341070 Pie, banana cream, individual size or tart
53341500 Pie, buttermilk
53341750 Pie, chess
53342000 Pie, chocolate cream
53342070 Pie, chocolate cream, individual size or tart
53343000 Pie, coconut cream
53343070 Pie, coconut cream, individual size or tart
53344000 Pie, custard
53344070 Pie, custard, individual size or tart
53344200 Mixed fruit tart filled with custard or cream cheese
53344300 Dessert pizza
53345000 Pie, lemon cream
53345070 Pie, lemon cream, individual size or tart
53346000 Pie, peanut butter cream
53346500 Pie, pineapple cream
53347000 Pie, pumpkin
53347070 Pie, pumpkin, individual size or tart
53347500 Pie, sour cream, raisin
53347600 Pie, squash
53348000 Pie, strawberry cream
53348070 Pie, strawberry cream, individual size or tart
53360000 Pie, sweet potato
53365000 Pie, vanilla cream
53370000 Pie, chiffon, not chocolate
53371000 Pie, chiffon, chocolate
53373000 Pie, black bottom
53381000 Pie, lemon meringue
53381070 Pie, lemon meringue, individual size or tart
53382000 Pie, chocolate-marshmallow
53385000 Pie, pecan
53385070 Pie, pecan, individual size or tart
53385500 Pie, oatmeal
53386000 Pie, pudding, flavors other than chocolate
53387000 Pie, Toll house chocolate chip
53390000 Pie, shoo-fly
53390100 Pie, tofu with fruit

53391000 Pie shell
53391100 Pie shell, graham cracker
53391150 Pie shell, chocolate wafer
53391200 Vanilla wafer dessert base
53400200 Blintz, cheese-filled
53400300 Blintz, fruit-filled
53410100 Cobbler, apple
53410200 Cobbler, apricot
53410300 Cobbler, berry
53410500 Cobbler, cherry
53410800 Cobbler, peach
53410850 Cobbler, pear
53410880 Cobbler, plum
53410900 Cobbler, rhubarb
53415100 Crisp, apple, apple dessert
53415120 Fritter, apple
53415200 Fritter, banana
53415220 Fritter, berry
53415300 Crisp, blueberry
53415400 Crisp, cherry
53415500 Crisp, peach
53430000 Crepe, NS as to filling
53430100 Crepe, chocolate filled
53430200 Crepe, fruit filled
53441210 Basbousa
53520000 Doughnut, NFS
53520100 Doughnut, cake type, plain
53520120 Doughnut, chocolate
53520130 Doughnut, cake type, powdered sugar
53520135 Doughnut, cake type, with icing
53520140 Doughnut, cake type, chocolate icing
53520160 Doughnut, chocolate, with chocolate icing
53520170 Doughnut holes
53520200 Churros
53520510 Beignet
53521110 Doughnut, yeast type
53521130 Doughnut, yeast type, with chocolate icing
53521140 Doughnut, jelly
53521210 Doughnut, custard-filled
53521230 Doughnut, custard-filled, with icing
53610100 Coffee cake, crumb or quick-bread type
53610170 Coffee cake, crumb or quick-bread type, with fruit
53610200 Coffee cake, crumb or quick-bread type, cheese-filled

54001000 Crackers, NFS
54102010 Graham crackers
54102015 Graham crackers (Teddy Grahams)
54102020 Graham crackers, chocolate covered
54102050 Crackers, oatmeal
54102060 Crackers, Cuban
54102100 Graham crackers, reduced fat
54102200 Graham crackers, sandwich, with filling
54103000 Crackers, breakfast biscuit
54200100 Crackers, butter, reduced sodium
54201010 Crackers, matzo, reduced sodium
54202020 Crackers, saltine, reduced sodium
54204020 Crackers, wheat, reduced sodium
54204030 Crackers, woven wheat, reduced sodium
54301010 Crackers, butter, plain
54301020 Crackers, butter, flavored
54301030 Crackers, butter (Ritz)
54301100 Crackers, butter, reduced fat
54304000 Crackers, cheese
54304005 Crackers, cheese (Cheez-It)
54304020 Crackers, cheese (Goldfish)
54304100 Crackers, cheese, reduced fat
54304110 Crackers, cheese, reduced sodium
54304150 Crackers, cheese, whole grain
54305010 Crackers, crispbread
54305020 Crackers, flatbread
54307000 Crackers, matzo
54308000 Crackers, milk
54313000 Crackers, oyster
54318500 Rice cake
54319000 Crackers, rice
54319005 Crackers, rice and nuts
54319020 Popcorn cake
54319500 Rice paper
54325000 Crackers, saltine
54325010 Crackers, saltine, reduced fat
54325060 Crackers, saltine, multigrain
54326000 Crackers, multigrain
54328000 Crackers, sandwich
54328100 Crackers, sandwich, peanut butter filled
54328105 Crackers, sandwich, peanut butter filled (Ritz)
54328110 Crackers, sandwich, reduced fat, peanut butter filled
54328120 Crackers, whole grain, sandwich, peanut butter filled

54328200 Crackers, sandwich, cheese filled
54328210 Crackers, sandwich, cheese filled (Ritz)
54336000 Crackers, water
54336100 Crackers, wonton
54337010 Crackers, woven wheat
54337020 Crackers, woven wheat, plain (Triscuit)
54337030 Crackers, woven wheat, flavored (Triscuit)
54337060 Crackers, woven wheat, reduced fat
54338000 Crackers, wheat
54338010 Crackers, wheat, plain (Wheat Thins)
54338020 Crackers, wheat, flavored (Wheat Thins)
54338100 Crackers, wheat, reduced fat
54339000 Crackers, corn
54340100 Crackers, gluten free, plain
54340110 Crackers, gluten free, flavored
54402700 Pita chips
54440010 Bagel chips
55100005 Pancakes, NFS
55100010 Pancakes, plain, from frozen
55100015 Pancakes, plain, reduced fat, from fozen
55100020 Pancakes, with fruit, from frozen
55100025 Pancakes, with chocolate, from frozen
55100030 Pancakes, whole grain, from frozen
55100035 Pancakes, whole grain, reduced fat, from frozen
55100040 Pancakes, gluten free, from frozen
55100050 Pancakes, plain, from fast food / restaurant
55100055 Pancakes, with fruit, from fast food / restaurant
55100060 Pancakes, with chocolate, from fast food / restaurant
55100065 Pancakes, whole grain, from fast food / restaurant
55100070 Pancakes, whole grain and nuts, from fast food / restaurant
55100080 Pancakes, from school, NFS
55101000 Pancakes, plain
55101015 Pancakes, plain, reduced fat
55103000 Pancakes, with fruit
55103020 Pancakes, pumpkin
55103100 Pancakes, with chocolate
55105000 Pancakes, buckwheat
55105100 Pancakes, cornmeal
55105200 Pancakes, whole grain
55105205 Pancakes, whole grain, reduced fat
55106000 Pancakes, gluten free
55200010 Waffle, NFS
55200020 Waffle, plain, from frozen

55200030 Waffle, plain, reduced fat, from frozen
55200040 Waffle, fruit, from frozen
55200050 Waffle, chocolate, from frozen
55200060 Waffle, whole grain, from frozen
55200070 Waffle, whole grain, reduced fat, from frozen
55200080 Waffle, whole grain, fruit, from frozen
55200090 Waffle, gluten free, from frozen
55200100 Waffle, plain, from fast food / restaurant
55200110 Waffle, chocolate, from fast food / restaurant
55200120 Waffle, fruit, from fast food / restaurant
55200130 Waffle, whole grain, from fast food / restaurant
55200200 Waffle, from school, NFS
55201000 Waffle, plain
55203000 Waffle, fruit
55203600 Waffle, chocolate
55203700 Waffle, cinnamon
55204000 Waffle, cornmeal
55205000 Waffle, whole grain
55208000 Waffle, gluten free
55211050 Waffle, plain, reduced fat
55212000 Waffle, whole grain, reduced fat
55300010 French toast, NFS
55300020 French toast, plain, from frozen
55300030 French toast, whole grain, from frozen
55300040 French toast, gluten free, from frozen
55300050 French toast, plain, from fast food / restaurant
55300055 French toast, whole grain, from fast food / restaurant
55300060 French toast, from school, NFS
55301000 French toast, plain
55301010 French toast, plain, reduced fat
55301015 French toast, whole grain
55301020 French toast, whole grain, reduced fat
55301025 French toast, gluten free
55301030 French toast sticks, NFS
55301031 French toast sticks, plain, from frozen
55301040 French toast sticks, plain, from fast food / restaurant
55301048 French toast sticks, from school, NFS
55301050 French toast sticks, plain
55301055 French toast sticks, whole grain
55310100 Fried bread, Puerto Rican style
55400010 Crepe, NFS
55401000 Crepe, plain
55501000 Chinese pancake

55610300 Dumpling, plain
55702100 Dosa (Indian), plain
91550100 Coconut cream cake, Puerto Rican style

Beverages and Beverage Bases

Soft drinks (regular and diet)
[6'-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

Enhanced, fortified, or flavored waters (including carbonated waters)
[6'-SL Sodium Salt] = 0.025 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)

Non-milk-based meal replacement drinks
[6'-SL Sodium Salt] = 0.1 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 7
[6'-SL Sodium Salt] = 0.7 g/100 g

95201600	Nutritional powder mix (Isopure)
95201700	Nutritional powder mix (Kellogg's Special K20 Protein Water)

Sports, isotonic, or energy drinks
[6'-SL Sodium Salt] = 0.05 g/100 g

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

Foods adjusted for being present in dried form
Reconstitution factor of 16.625
[6'-SL Sodium Salt] = 0.83 g/100 g

92900300	Sports drink, dry concentrate, not reconstituted

Protein drinks
[6'-SL Sodium Salt] = 0.1 g/100 g

Foods adjusted for being present in dried form
Reconstitution factor of 6 to 10
[6'-SL Sodium Salt] = 0.6 to 1.0 g/100 g

95201200	Nutritional powder mix (EAS Whey Protein Powder)
95201300	Nutritional powder mix (EAS Soy Protein Powder)
95201500	Nutritional powder mix, high protein (Herbalife)
95210020	Nutritional powder mix, high protein (Slim Fast)
95220010	Nutritional powder mix, high protein, NFS
95230000	Nutritional powder mix, whey based, NFS
95230010	Nutritional powder mix, protein, soy based, NFS
95230020	Nutritional powder mix, protein, light, NFS
95230030	Nutritional powder mix, protein, NFS

Breakfast Cereals

Hot breakfast cereals (*e.g.*, oatmeal, grits), instant and RTE
[6'-SL Sodium Salt] = 0.1 g/100 g

56200300	Cereal, cooked, NFS
56200990	Grits, NS as to regular, quick, or instant, NS as to fat
56201000	Grits, NS as to regular, quick, or instant, no added fat
56201040	Grits, NS as to regular, quick, or instant, fat added
56201050	Grits, regular or quick, made with water, NS as to fat
56201051	Grits, regular or quick, made with water, no added fat
56201052	Grits, regular or quick, made with water, fat added
56201055	Grits, regular or quick, made with milk, NS as to fat
56201056	Grits, regular or quick, made with milk, no added fat
56201057	Grits, regular or quick, made with milk, fat added
56201065	Grits, regular or quick, made with non-dairy milk, NS as to fat
56201066	Grits, regular or quick, made with non-dairy milk, no added fat
56201067	Grits, regular or quick, made with non-dairy milk, fat added
56201090	Grits, with cheese, NS as to fat
56201091	Grits, with cheese, no added fat
56201092	Grits, with cheese, fat added
56201210	Grits, instant, made with water, no added fat
56201220	Grits, instant, made with water, fat added
56201230	Grits, instant, made with water, NS as to fat
56201340	Grits, instant, made with milk, fat added
56201342	Grits, instant, made with milk, no added fat

56201344	Grits, instant, made with milk, NS as to fat
56201350	Grits, instant, made with non-dairy milk, NS as to fat
56201355	Grits, instant, made with non-dairy milk, no added fat
56201360	Grits, instant, made with non-dairy milk, fat added
56201515	Cornmeal mush, NS as to fat
56201516	Cornmeal mush, no added fat
56201517	Cornmeal mush, fat added
56201540	Cornmeal, Puerto Rican Style
56201600	Masa harina, cooked
56202900	Oatmeal, from fast food, plain
56202905	Oatmeal, from fast food, maple flavored
56202910	Oatmeal, from fast food, fruit flavored
56202920	Oatmeal, from fast food, other flavors
56202960	Oatmeal, NS as to regular, quick, or instant, NS as to fat
56203000	Oatmeal, NS as to regular, quick, or instant, no added fat
56203040	Oatmeal, NS as to regular, quick, or instant, fat added
56203055	Oatmeal, regular or quick, made with water, NS as to fat
56203056	Oatmeal, regular or quick, made with water, no added fat
56203057	Oatmeal, regular or quick, made with water, fat added
56203065	Oatmeal, regular or quick, made with milk, NS as to fat
56203066	Oatmeal, regular or quick, made with milk, no added fat
56203067	Oatmeal, regular or quick, made with milk, fat added
56203075	Oatmeal, regular or quick, made with non-dairy milk, NS as to fat
56203076	Oatmeal, regular or quick, made with non-dairy milk, no added fat
56203077	Oatmeal, regular or quick, made with non-dairy milk, fat added
56203085	Oatmeal, instant, plain, made with water, NS as to fat
56203086	Oatmeal, instant, plain, made with water, no added fat
56203087	Oatmeal, instant, plain, made with water, fat added
56203095	Oatmeal, instant, plain, made with milk, NS as to fat
56203096	Oatmeal, instant, plain, made with milk, no added fat
56203097	Oatmeal, instant, plain, made with milk, fat added
56203105	Oatmeal, instant, plain, made with non-dairy milk, NS as to fat
56203106	Oatmeal, instant, plain, made with non-dairy milk, no added fat
56203107	Oatmeal, instant, plain, made with non-dairy milk, fat added
56203125	Oatmeal, instant, maple flavored, NS as to fat
56203130	Oatmeal, instant, maple flavored, no added fat
56203135	Oatmeal, instant, maple flavored, fat added
56203150	Oatmeal, instant, fruit flavored, NS as to fat
56203155	Oatmeal, instant, fruit flavored, no added fat
56203160	Oatmeal, instant, fruit flavored, fat added
56203170	Oatmeal, instant, other flavors, NS as to fat
56203175	Oatmeal, instant, other flavors, no added fat
56203180	Oatmeal, instant, other flavors, fat added

56203500	Oatmeal, reduced sugar, plain, NS as to fat
56203510	Oatmeal, reduced sugar, plain, no added fat
56203520	Oatmeal, reduced sugar, plain, fat added
56203540	Oatmeal, made with milk and sugar, Puerto Rican style
56203550	Oatmeal, reduced sugar, flavored, NS as to fat
56203555	Oatmeal, reduced sugar, flavored, no added fat
56203560	Oatmeal, reduced sugar, flavored, fat added
56203600	Oatmeal, multigrain, NS as to fat
56203610	Oatmeal, multigrain, no added fat
56203620	Oatmeal, multigrain, fat added
56205050	Rice, cream of, cooked, no added fat
56205080	Rice, creamed, made with milk and sugar, Puerto Rican style
56205090	Rice, cream of, cooked, fat added
56205092	Rice, cream of, cooked, NS as to fat
56205094	Rice, cream of, cooked, made with milk
56206990	Cream of wheat, NS as to regular, quick, or instant, NS as to fat
56207000	Cream of wheat, NS as to regular, quick, or instant, no added fat
56207005	Cream of wheat, NS as to regular, quick, or instant, fat added
56207015	Cream of wheat, regular or quick, made with water, NS as to fat
56207016	Cream of wheat, regular or quick, made with water, no added fat
56207017	Cream of wheat, regular or quick, made with water, fat added
56207021	Cream of wheat, regular or quick, made with milk, NS as to fat
56207022	Cream of wheat, regular or quick, made with milk, no added fat
56207023	Cream of wheat, regular or quick, made with milk, fat added
56207025	Cream of wheat, regular or quick, made with non-dairy milk, NS as to fat
56207026	Cream of wheat, regular or quick, made with non-dairy milk, no added fat
56207027	Cream of wheat, regular or quick, made with non-dairy milk, fat added
56207030	Cream of wheat, instant, made with water, no added fat
56207050	Wheat, cream of, cooked, made with milk and sugar, Puerto Rican style
56207060	Cream of wheat, instant, made with water, fat added
56207070	Cream of wheat, instant, made with water, NS as to fat
56207094	Cream of wheat, instant, made with milk, fat added
56207095	Cream of wheat, instant, made with milk, no added fat
56207096	Cream of wheat, instant, made with milk, NS as to fat
56207101	Cream of wheat, instant, made with non-dairy milk, NS as to fat
56207102	Cream of wheat, instant, made with non-dairy milk, no added fat
56207103	Cream of wheat, instant, made with non-dairy milk, fat added
56207190	Whole wheat cereal, cooked, NS as to fat
56207200	Whole wheat cereal, cooked, no added fat
56207210	Whole wheat cereal, cooked, fat added
56207370	Wheat cereal, chocolate flavored, cooked
56208500	Oat bran cereal, cooked, no added fat
56208510	Oat bran cereal, cooked, fat added

56208520 Oat bran cereal, cooked, NS as to fat
56209000 Cream of rye
58174000 Upma, Indian breakfast dish
75217520 Hominy, cooked

RTE breakfast cereals – Puffed cereals
[6'-SL Sodium Salt] = 1.7 g/100 g

57124200 Cereal, chocolate flavored, frosted, puffed corn
57126000 Cereal (Kellogg's Cocoa Krispies)
57128000 Cereal (General Mills Cocoa Puffs)
57132000 Cereal (General Mills Chex Corn)
57137000 Cereal, corn puffs
57151000 Cereal, crispy rice
57216000 Cereal, frosted rice
57301500 Cereal (Kashi 7 Whole Grain Puffs)
57303100 Cereal (General Mills Kix)
57303105 Cereal (General Mills Honey Kix)
57306500 Cereal (Malt-O-Meal Golden Puffs)
57326000 Cereal (Barbara's Puffins)
57335550 Cereal (General Mills Reese's Puffs)
57336000 Cereal (General Mills Chex Rice)
57337000 Cereal, rice flakes
57339000 Cereal (Kellogg's Rice Krispies)
57339500 Cereal (Kellogg's Rice Krispies Treats Cereal)
57340000 Cereal, puffed rice
57347000 Cereal (Kellogg's Corn Pops)
57407100 Cereal (General Mills Trix)
57416000 Cereal, puffed wheat, plain
57416010 Cereal, puffed wheat, sweetened

RTE breakfast cereals – High-fiber cereals
[6'-SL Sodium Salt] = 0.62 g/100 g

57000100 Cereal, oat, NFS
57100100 Cereal, ready-to-eat, NFS
57101000 Cereal (Kellogg's All-Bran)
57103000 Cereal (Post Alpha-Bits)
57103100 Cereal (General Mills Cheerios Apple Cinnamon)
57104000 Cereal (Kellogg's Apple Jacks)
57106060 Cereal (General Mills Cheerios Banana Nut)
57106260 Cereal (General Mills Cheerios Berry Burst)
57117000 Cereal (Quaker Cap'n Crunch)

57117500 Cereal (Quaker Christmas Crunch)
57119000 Cereal (Quaker Cap'n Crunch's Crunchberries)
57120000 Cereal (Quaker Cap'n Crunch's Peanut Butter Crunch)
57123000 Cereal (General Mills Cheerios)
57124030 Cereal (General Mills Chex Chocolate)
57124050 Cereal (General Mills Chex Cinnamon)
57124100 Cereal (General Mills Cheerios Chocolate)
57124300 Cereal (General Mills Lucky Charms Chocolate)
57125000 Cereal (General Mills Cinnamon Toast Crunch)
57125010 Cereal (General Mills 25% Less Sugar Cinnamon Toast Crunch)
57125900 Cereal (General Mills Honey Nut Clusters)
57127000 Cereal (Post Cocoa Pebbles)
57130000 Cereal (General Mills Cookie Crisp)
57134000 Cereal, corn flakes
57135000 Cereal (Kellogg's Corn Flakes)
57139000 Cereal (General Mills Count Chocula)
57143500 Cereal (Post Great Grains, Cranberry Almond Crunch)
57148000 Cereal (Kellogg's Crispix)
57206700 Cereal (General Mills Fiber One)
57206710 Cereal (General Mills Fiber One Honey Clusters)
57206715 Cereal (General Mills Fiber One Raisin Bran Clusters)
57211000 Cereal (General Mills Frankenberry)
57213000 Cereal (Kellogg's Froot Loops)
57213010 Cereal (Kellogg's Froot Loops Marshmallow)
57213850 Cereal (General Mills Cheerios Frosted)
57214000 Cereal (Kellogg's Frosted Mini-Wheats)
57221700 Cereal, fruit rings
57221810 Cereal (General Mills Cheerios Fruity)
57223000 Cereal (Post Fruity Pebbles)
57230000 Cereal (Post Grape-Nuts)
57231200 Cereal (Post Great Grains Raisins, Dates, and Pecans)
57237100 Cereal (Post Honey Bunches of Oats Honey Roasted)
57237200 Cereal (Post Honey Bunches of Oats with Vanilla Bunches)
57237300 Cereal (Post Honey Bunches of Oats with Almonds)
57238000 Cereal (Post Honeycomb)
57240100 Cereal (General Mills Chex Honey Nut)
57241000 Cereal (General Mills Cheerios Honey Nut)
57241200 Cereal (Post Shredded Wheat Honey Nut)
57243000 Cereal (Kellogg's Honey Smacks)
57301505 Cereal (Kashi Autumn Wheat)
57301510 Cereal (Kashi GOLEAN)
57301511 Cereal (Kashi GOLEAN Crunch)
57301512 Cereal (Kashi GOLEAN Crunch Honey Almond Flax)

57301530 Cereal (Kashi Heart to Heart Honey Toasted Oat)
57303200 Cereal (Kellogg's Krave)
57304100 Cereal (Quaker Life)
57305100 Cereal (General Mills Lucky Charms)
57305150 Cereal, frosted oat cereal with marshmallows
57305160 Cereal (Malt-O-Meal Blueberry Muffin Tops)
57305165 Cereal (Malt-O-Meal Cinnamon Toasters)
57305170 Cereal (Malt-O-Meal Coco-Roos)
57305174 Cereal (Malt-O-Meal Colossal Crunch)
57305175 Cereal (Malt-O-Meal Cocoa Dyno-Bites)
57305180 Cereal (Malt-O-Meal Corn Bursts)
57305210 Cereal (Malt-O-Meal Frosted Flakes)
57305300 Cereal (Malt-O-Meal Fruity Dyno-Bites)
57305400 Cereal (Malt-O-Meal Honey Graham Squares)
57305500 Cereal (Malt-O-Meal Honey Nut Toasty O's)
57305600 Cereal (Malt-O-Meal Marshmallow Mateys)
57306700 Cereal (Malt-O-Meal Toasted Oat Cereal)
57306800 Cereal (Malt-O-Meal Tootie Fruities)
57308400 Cereal (General Mills Cheerios Multigrain)
57316380 Cereal (General Mills Cheerios Oat Cluster Crunch)
57316385 Cereal (General Mills Cheerios Protein)
57316710 Cereal (Quaker Honey Graham Oh's)
57327450 Cereal (Quaker Toasted Oat Bran)
57327500 Cereal (Quaker Oatmeal Squares)
57341200 Cereal (Kellogg's Smart Start Strong)
57341300 Cereal (Kellogg's Smorz)
57344000 Cereal (Kellogg's Special K)
57344001 Cereal (Kellogg's Special K Blueberry)
57344005 Cereal (Kellogg's Special K Chocolatey Delight)
57344010 Cereal (Kellogg's Special K Red Berries)
57344015 Cereal (Kellogg's Special K Fruit & Yogurt)
57344020 Cereal (Kellogg's Special K Vanilla Almond)
57344025 Cereal (Kellogg's Special K Cinnamon Pecan)
57348000 Cereal, frosted corn flakes
57349000 Cereal (Kellogg's Frosted Flakes)
57355000 Cereal (Post Golden Crisp)
57408100 Cereal (Uncle Sam)
57411000 Cereal (General Mills Chex Wheat)
57417000 Cereal (Post Shredded Wheat)
57418000 Cereal (General Mills Wheaties)

RTE breakfast cereals – Biscuit-type cereals
[6'-SL Sodium Salt] = 0.42 g/100 g

57106050	Cereal (Post Great Grains Banana Nut Crunch)
57143000	Cereal (Kellogg's Cracklin' Oat Bran)
57207000	Cereal, bran flakes
57208000	Cereal (Kellogg's All-Bran Complete Wheat Flakes)
57209000	Cereal (Post Bran Flakes)
57224000	Cereal (General Mills Golden Grahams)
57227000	Cereal, granola
57228000	Granola, homemade
57229000	Cereal (Kellogg's Low Fat Granola)
57308190	Cereal, muesli
57309100	Cereal (Nature Valley Granola)
57316450	Cereal (General Mills Oatmeal Crisp with Almonds)
57320500	Cereal (Quaker Granola with Oats, Honey, and Raisins)
57321900	Cereal (Nature's Path Organic Flax Plus)
57329000	Cereal, raisin bran
57330000	Cereal (Kellogg's Raisin Bran)
57330010	Cereal (Kellogg's Raisin Bran Crunch)
57331000	Cereal (Post Raisin Bran)
57332100	Cereal (General Mills Raisin Nut Bran)
57401100	Cereal, toasted oat

Chewing Gum

Chewing gum
[6'-SL Sodium Salt] = 6.2 g/100 g

91800100	Chewing gum, NFS
91801000	Chewing gum, regular
91802000	Chewing gum, sugar free

Coffee and Tea

Coffee
[6'-SL Sodium Salt] = 0.21 g/100 g

92171000	Coffee, bottled/canned
92171010	Coffee, bottled/canned, light

Tea
[6'-SL Sodium Salt] = 0.21 g/100 g

92309000 Tea, iced, bottled, black
92309010 Tea, iced, bottled, black, decaffeinated
92309020 Tea, iced, bottled, black, diet
92309030 Tea, iced, bottled, black, decaffeinated, diet
92309040 Tea, iced, bottled, black, unsweetened
92309050 Tea, iced, bottled, black, decaffeinated, unsweetened
92309500 Tea, iced, bottled, green
92309510 Tea, iced, bottled, green, diet
92309520 Tea, iced, bottled, green, unsweetened

Dairy Product Analogs

Milk substitutes such as soy milk and imitation milks
[6'-SL Sodium Salt] = 0.025 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%
[6'-SL Sodium Salt] = 0.011 to 0.021 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
92161002	Coffee, Cappuccino, with non-dairy milk
92162002	Coffee, Cappuccino, decaffeinated, with non-dairy milk

Beverage whiteners
[6'-SL Sodium Salt] = 12.5 g/100 g

12200100	Coffee creamer, NFS
12210200	Coffee creamer, liquid
12210210	Coffee creamer, liquid, flavored
12210260	Coffee creamer, liquid, fat free
12210270	Coffee creamer, liquid, fat free, flavored
12210280	Coffee creamer, liquid, fat free, sugar free, flavored
12210310	Coffee creamer, liquid, sugar free, flavored
12210400	Coffee creamer, powder
12210420	Coffee creamer, powder, flavored
12210430	Coffee creamer, powder, fat free
12210440	Coffee creamer,powder, fat free, flavored
12210505	Coffee creamer,powder, sugar free, flavored

Non-dairy cream
[6'-SL Sodium Salt] = 12.5 g/100 g

12210520 Coffee creamer, soy, liquid
42402010 Coconut cream, canned, sweetened

Non-dairy yogurt
[6'-SL Sodium Salt] = 0.22 g/100 g

41420380 Yogurt, soy
42401100 Yogurt, coconut milk

Frozen Dairy Desserts and Mixes

Frozen desserts including ice creams and frozen yogurts, frozen novelties
[6'-SL Sodium Salt] = 0.35 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
13110000 Ice cream, NFS
13110100 Ice cream, vanilla
13110102 Ice cream, vanilla, with additional ingredients
13110110 Ice cream, chocolate
13110112 Ice cream, chocolate, with additional ingredients
13110200 Ice cream, soft serve, vanilla
13110210 Ice cream, soft serve, chocolate
13110460 Gelato, vanilla
13110470 Gelato, chocolate
13120050 Ice cream bar, vanilla
13120100 Ice cream bar, vanilla, chocolate coated
13120110 Ice cream candy bar
13120140 Ice cream bar, chocolate
13120500 Ice cream sandwich, vanilla
13120510 Ice cream sandwich, chocolate
13120550 Ice cream cookie sandwich

13120730 Ice cream cone, scooped, vanilla
13120735 Ice cream cone, scooped, vanilla, waffle cone
13120740 Ice cream cone, NFS
13120770 Ice cream cone, scooped, chocolate
13120775 Ice cream cone, scooped, chocolate, waffle cone
13120782 Ice cream cone, soft serve, vanilla
13120784 Ice cream cone, soft serve, chocolate
13120786 Ice cream cone, soft serve, vanilla, waffle cone
13120788 Ice cream cone, soft serve, chocolate, waffle cone
13120790 Ice cream cone, vanilla, prepackaged
13120792 Ice cream cone, chocolate, prepackaged
13120800 Ice cream soda, flavors other than chocolate
13120810 Ice cream soda, chocolate
13121000 Ice cream sundae, NFS
13121100 Ice cream sundae, fruit topping
13121120 Banana split
13121300 Ice cream sundae, hot fudge topping
13121400 Ice cream sundae, caramel topping
13126000 Ice cream, fried
13130100 Light ice cream, NFS
13130300 Light ice cream, vanilla
13130310 Light ice cream, chocolate
13130700 Soft serve, blended with candy or cookies, from fast food
13135000 Light ice cream sandwich, vanilla
13135010 Light ice cream sandwich, chocolate
13140000 Light ice cream bar, vanilla
13140100 Light ice cream bar, vanilla, chocolate coated
13140115 Light ice cream bar, chocolate
13140700 Creamsicle
13140710 Creamsicle, light
13140900 Fudgesicle
13142100 Light ice cream cone, vanilla, prepackaged
13142110 Light ice cream cone, chocolate, prepackaged
13161600 Fudgesicle, light

Fruit and Water Ices

Edible ices, sherbet, and sorbet
[6'-SL Sodium Salt] = 0.35 g/100 g

13150000	Sherbet, all flavors
63420105	Frozen fruit juice bar
63420205	Frozen fruit juice bar, no sugar added
63430150	Sorbet
91601000	Italian Ice
91601010	Italian Ice, no sugar added
91610900	Popsicle, NFS
91611000	Popsicle
91611100	Popsicle, no sugar added
91612000	Freezer pop
91621000	Snow cone
91621050	Snow cone, no sugar added

Gelatins, Puddings, and Fillings

Dairy-based puddings, custards, and mousses
[6'-SL Sodium Salt] = 0.35 g/100 g

13200110	Pudding, chocolate, NFS
13210110	Pudding, bread
13210280	Pudding, flavors other than chocolate, NFS
13210300	Custard
13210350	Flan
13210370	Creme brulee
13210410	Pudding, rice
13210450	Firni, Indian pudding
13210520	Pudding, tapioca, made from dry mix
13220110	Pudding, flavors other than chocolate, made from dry mix
13220120	Pudding, chocolate, made from dry mix
13220210	Pudding, flavors other than chocolate, made from dry mix, sugar free
13220220	Pudding, chocolate, made from dry mix, sugar free
13230110	Pudding, flavors other than chocolate, ready-to-eat
13230120	Pudding, flavors other than chocolate, ready-to-eat, sugar free
13230130	Pudding, chocolate, ready-to-eat
13230140	Pudding, chocolate, ready-to-eat, sugar free
13230500	Pudding, tapioca, ready-to-eat
13241000	Banana pudding
13250000	Mousse
13252200	Milk dessert or milk candy, Puerto Rican style

13252500 Barfi or Burfi, Indian dessert
13252590 Trifle
91560100 Haupia

Fruit pie filling
[6'-SL Sodium Salt] = 0.29 g/100 g

61113500 Lemon pie filling
63101210 Apple pie filling
63113030 Cherry pie filling
63203700 Blueberry pie filling

"Fruit Prep" such as fruit filling in bars, cookies, yogurt, and cakes
[6'-SL Sodium Salt] = 0.625 g/100 g

Mixed foods containing fruit filling
Adjusted for fruit filling content of 26.3 to 61.2%
[6'-SL Sodium Salt] = 0.164 to 0.383 g/100 g

53440000 Strudel, apple
53440300 Strudel, berry
53440500 Strudel, cherry
53440700 Strudel, peach
53440800 Strudel, cheese and fruit
53450000 Turnover or dumpling, apple
53450300 Turnover or dumpling, berry
53450500 Turnover or dumpling, cherry
53450800 Turnover or dumpling, lemon
53451000 Turnover or dumpling, peach
53451500 Turnover, guava
53451750 Turnover, pumpkin
53452100 Pastry, fruit-filled
53453150 Empanada, Mexican turnover, fruit-filled
53453170 Empanada, Mexican turnover, pumpkin

Grain Products and Pastas

Cereal and granola bars including energy, protein, and meal replacement bars
[6'-SL Sodium Salt] = 1 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
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

[6'-SL Sodium Salt] = 0.05 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

[6'-SL Sodium Salt] = 0.05 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)
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)
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)

Hypoallergenic infant formula

[6'-SL Sodium Salt] = 0.05 g/100 g

11710050	Infant formula, NS as to form (Similac Expert Care Alimentum)
11710051	Infant formula, ready-to-feed (Similac Expert Care Alimentum)
11710053	Infant formula, powder, made with water, NFS (Similac Expert Care Alimentum)
11710054	Infant formula, powder, made with tap water (Similac Expert Care Alimentum)
11710055	Infant formula, powder, made with plain bottled water (Similac Expert Care Alimentum)
11710056	Infant formula, powder, made with baby water (Similac Expert Care Alimentum)
11740310	Infant formula, NS as to form (Enfamil Nutramigen)

11740311 Infant formula, ready-to-feed (Enfamil Nutramigen)
11740312 Infant formula, liquid concentrate, made with water, NFS (Enfamil Nutramigen)
11740313 Infant formula, powder, made with water, NFS (Enfamil Nutramigen)
11740320 Infant formula, NS as to form (PurAmino)
11740323 Infant formula, powder, made with water, NFS (PurAmino)
11740400 Infant formula, NS as to form (Enfamil Pregestimil)
11740401 Infant formula, ready-to-feed (Enfamil Pregestimil)
11740403 Infant formula, powder, made with water, NFS (Enfamil Pregestimil)

Other baby foods for infants and young children
[6'-SL Sodium Salt] = 0.25 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

Hot cereals (dry and RTE)

[6'-SL Sodium Salt] = 0.23 g/100 g

56210000 Cereal, nestum
57805090 Rice cereal with mixed fruits, baby food, dry, instant
57806050 Multigrain, whole grain cereal, baby food, dry, instant
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

Foods adjusted for being present in dried form
Reconstitution factor of 8.33
[6'-SL Sodium Salt] = 1.92 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
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
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 drinks for young children, including yogurt and juice beverages identified as "baby drinks"
[6'-SL Sodium Salt] = 0.21 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

Desserts including fruit desserts, cobblers, yogurt/fruit combinations ("junior type desserts")

[6'-SL Sodium Salt] = 0.23 g/100 g

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

Baby crackers, pretzels, cookies, and snack items

[6'-SL Sodium Salt] = 1.2 g/100 g

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

Jams and Jellies

Jellies and jams, fruit preserves, and fruit butters
[6'-SL Sodium Salt] = 1.2 g/100 g

91401000 Jelly, all flavors
91402000 Jam, preserve, all flavors
91403000 Fruit butter, all flavors
91404000 Marmalade, all flavors
91405000 Jelly, sugar free, all flavors
91405500 Jelly, reduced sugar, all flavors
91406000 Jam, preserve, marmalade, sugar free, all flavors
91406500 Jam, preserve, marmalade, sweetened with fruit juice concentrates, all flavors
91406600 Jam, preserve, marmalade, reduced sugar, all flavors
91407100 Guava paste
91407120 Sweet potato paste
91407150 Bean paste, sweetened

Milk, Whole, and Skim

Unflavored pasteurized and sterilized milk
[6'-SL Sodium Salt] = 0.05 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%
[6'-SL Sodium Salt] = 0.021 to 0.042 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
92161000 Coffee, Cappuccino
92161001 Coffee, Cappuccino, nonfat
92162000 Coffee, Cappuccino, decaffeinated
92162001 Coffee, Cappuccino, decaffeinated, nonfat

Foods adjusted for being present in dried form
Reconstitution factor of 11
[6'-SL Sodium Salt] = 0.55 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
[6'-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
[6'-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
[6'-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

Evaporated and condensed milk
[6'-SL Sodium Salt] = 0.025 g/100 g

11210050 Milk, evaporated, NS as to fat content
11211050 Milk, evaporated, whole
11211400 Milk, evaporated, reduced fat (2%)
11212050 Milk, evaporated, fat free (skim)
11220000 Milk, condensed, sweetened

Milk-based meal replacement beverages for weight reduction
[6'-SL Sodium Salt] = 0.1 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)
95120000	Nutritional drink or shake, ready-to-drink, NFS
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
[6'-SL Sodium Salt] = 0.6 to 1.0 g/100 g

95201000	Nutritional powder mix (Carnation Instant Breakfast)
95201010	Nutritional powder mix, sugar free (Carnation Instant Breakfast)
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)
95220000	Nutritional powder mix, NFS

Yogurt
[6'-SL Sodium Salt] = 0.5 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

Processed Fruits and Fruit Juices

Fruit flavored drinks and ades
[6'-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
92512050 Frozen daiquiri mix, from frozen concentrate, reconstituted
92512090 Pina Colada, nonalcoholic
92512110 Margarita mix, nonalcoholic
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
92801000 Wine, nonalcoholic
92802000 Wine, light, nonalcoholic
92803000 Nonalcoholic malt beverage
92804000 Shirley Temple

<u>Foods adjusted for being present in dried form</u>
Reconstitution factor of 4 to 10.23
[6'-SL Sodium Salt] = 0.1 to 0.256 g/100 g

92511000 Lemonade, frozen concentrate, not reconstituted
92512040 Frozen daiquiri mix, 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

Fruit juices and nectars
[6'-SL Sodium Salt] = 0.025 g/100 g

61201020 Grapefruit juice, 100%, NS as to form
61201220 Grapefruit juice, 100%, canned, bottled or in a carton
61201225 Grapefruit juice, 100%, with calcium added
61201620 Grapefruit juice,100%, frozen, reconstituted
61210000 Orange juice, 100%, NFS
61210220 Orange juice, 100%, canned, bottled or in a carton
61210250 Orange juice, 100%, with calcium added, canned, bottled or in a carton
61210620 Orange juice, 100%, frozen, reconstituted
61210820 Orange juice, 100%, with calcium added, frozen, reconstituted
61213220 Tangerine juice, 100%
61213800 Fruit juice blend, citrus, 100% juice
61213900 Fruit juice blend, citrus, 100% juice, with calcium added
64100100 Fruit juice, NFS
64100110 Fruit juice blend, 100% juice
64100200 Cranberry juice blend, 100% juice
64100220 Cranberry juice blend, 100% juice, with calcium added
64101010 Apple cider
64104010 Apple juice, 100%
64104030 Apple juice, 100%, with calcium added
64104600 Blackberry juice, 100%
64104610 Blueberry juice
64105400 Cranberry juice, 100%, not a blend
64116020 Grape juice, 100%
64116060 Grape juice, 100%, with calcium added
64120010 Papaya juice, 100%
64121000 Passion fruit juice, 100%
64124020 Pineapple juice, 100%
64126000 Pomegranate juice, 100%
64132010 Prune juice, 100%
64132500 Strawberry juice, 100%
64133100 Watermelon juice, 100%
64134015 Fruit smoothie, with whole fruit, no dairy
64134020 Fruit smoothie, with whole fruit, no dairy, added protein
64134025 Fruit smoothie, with whole fruit, non-dairy
64134030 Fruit smoothie juice drink, no dairy
64134100 Fruit smoothie, light
64134200 Fruit smoothie, bottled

64200100 Fruit nectar, NFS
64201010 Apricot nectar
64201500 Banana nectar
64202010 Cantaloupe nectar
64203020 Guava nectar
64204010 Mango nectar
64205010 Peach nectar
64210010 Papaya nectar
64213010 Passion fruit nectar
64215010 Pear nectar
64221010 Soursop, nectar
75200700 Aloe vera juice drink
78101000 Vegetable and fruit juice, 100% juice, with high vitamin C
78101100 Fruit and vegetable smoothie, with dairy
78101110 Fruit and vegetable smoothie, added protein
78101115 Fruit and vegetable smoothie, non-dairy
78101118 Fruit and vegetable smoothie, non-dairy, added protein
78101120 Fruit and vegetable smoothie, bottled
78101125 Fruit and vegetable smoothie, no dairy
95342000 Fruit juice, acai blend

Foods adjusted for being present in dried form
Reconstitution factor of 4
[6'-SL Sodium Salt] = 0.1 g/100 g

61210720 Orange juice, 100%, frozen, not reconstituted

Canned fruit
[6'-SL Sodium Salt] = 0.35 g/100 g

61101200 Grapefruit, canned
61122300 Orange, canned, NFS
61122320 Orange, canned, juice pack
61122330 Orange, canned, in syrup
63103110 Apricot, canned
63115110 Cherries, canned
63119110 Fig, canned
63129030 Mango, canned
63133100 Papaya, canned
63135110 Peach, canned, NFS
63135140 Peach, canned, in syrup
63135170 Peach, canned, juice pack
63137110 Pear, canned, NFS
63137140 Pear, canned, in syrup

63137170 Pear, canned, juice pack
63141110 Pineapple, canned, NFS
63141140 Pineapple, canned, in syrup
63141170 Pineapple, canned, juice pack
63143110 Plum, canned
63147110 Rhubarb
63203110 Bluberries, canned
63207110 Cranberry sauce
63223110 Strawberries, canned
63311110 Fruit cocktail, canned, NFS
63311140 Fruit cocktail, canned, in syrup
63311170 Fruit cocktail, canned, juice pack

Fruit-based desserts
[6'-SL Sodium Salt] = 0.35 g/100 g

63301010 Ambrosia
63401010 Apple salad with dressing
63401060 Apple, candied
63401070 Fruit, chocolate covered
63402950 Fruit salad, excluding citrus fruits, with salad dressing or mayonnaise
63402960 Fruit salad, excluding citrus fruits, with whipped cream
63402970 Fruit salad, excluding citrus fruits, with nondairy whipped topping
63402980 Fruit salad, excluding citrus fruits, with marshmallows
63402990 Fruit salad, including citrus fruits, with pudding
63403000 Fruit salad, excluding citrus fruits, with pudding
63403010 Fruit salad, including citrus fruits, with salad dressing or mayonnaise
63403020 Fruit salad, including citrus fruit, with whipped cream
63403030 Fruit salad, including citrus fruits, with nondairy whipped topping
63403040 Fruit salad, including citrus fruits, with marshmallows

Processed Vegetables and Vegetable Juices

Vegetable juices and nectars
[6'-SL Sodium Salt] = 0.025 g/100 g

73105000 Beet juice
73105010 Carrot juice, 100%
74301100 Tomato juice, 100%
74301150 Tomato juice, 100%, low sodium
74302000 Tomato juice cocktail
74303000 Tomato and vegetable juice, 100%
74303100 Tomato and vegetable juice, 100%, low sodium

75132000	Mixed vegetable juice
75132100	Celery juice
78101130	Vegetable smoothie

Sugar Substitutes

Table-top sweeteners
[6'-SL Sodium Salt] = 6.2 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

Sweet Sauces, Toppings, and Syrups

Syrups used to flavor milk beverages
[6'-SL Sodium Salt] = 0.15 g/100 g

91301130	Strawberry drink syrup

From: 秋月さおり Saori Akizuki
To: Anderson, Ellen
Cc: 秋月さおり Saori Akizuki
Subject: [EXTERNAL] RE: GRN 001053
Date: Monday, April 24, 2023 9:35:19 PM
Attachments: image003.png
Importance: High

Dear Dr. Anderson
We would like to express our sincere appreciation for taking the time to share your valuable review of GRN001053 with us.
Kyowa recognizes the FDA's recent "Closer to Zero" initiative (U.S. FDA, 2023). In support of this initiative, Kyowa proposes new specification limits for arsenic, lead, cadmium, and mercury of 0.1 mg/kg (each individually).
Thank you very much in advance.

Sincerely yours,
Saori Akiduki

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Saori Akiduki, PhD
Assistant Manager

KYOWA HAKKO BIO CO., LTD
Research & Business Development Department
External Relations Division

Nakano Central Park South, Nakano 4-10-2
Nakano-ku, Tokyo 164-0001, Japan
Direct: +81-80-4935-3378
saori.akizuki@kyowa-kirin.co.jp

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From: Anderson, Ellen <Ellen.Anderson@fda.hhs.gov>
Sent: Friday, April 21, 2023 11:16 PM
To: 秋月さおり Saori Akizuki <saori.akizuki@kyowa-kirin.co.jp>
Subject: GRN 001053

Dear Dr. Akiduki,

We are finishing up our response to GRN 001053 and discovered an issue with the specified limits for heavy metal that we inadvertently overlooked during our review. The limits for heavy metals are established as ≤ 0.2 mg/kg each. However, the analytical results from five non-consecutive batches presented in Table 2.3.1-1 on page 20 demonstrate that the levels of heavy metals are below the limit of

quantitation of the method (i.e., 0.05 mg/kg). Additionally, we note that FDA's "Closer to Zero" initiative focuses on reducing dietary exposure to arsenic, lead, cadmium, and mercury from foods consumed by infants and young children. Therefore, we request that you consider lowering the limits for heavy metals to at least 0.1 mg/kg each.

We would appreciate a response to this request at your earliest convenience. We apologize for the delay in bringing this to your attention.

Sincerely,
Ellen

Ellen Anderson (she/her/hers)
Regulatory Review Scientist

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1309
ellen.anderson@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION