# GRAS NOTICE FOR 2'-FUCOSYLLACTOSE

**SUBMITTED TO:**
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
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**DATE:**
03 March 2022

# GRAS Notice for 2'-Fucosyllactose

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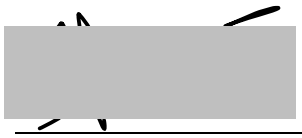
# GRAS Notice for 2'-Fucosyllactose

## Part 1. § 170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §§170.203 through 170.285, Inbiose N.V. (Inbiose) hereby informs the United States (U.S.) Food and Drug Administration (FDA) that the intended uses of 2'-fucosyllactose (2'-FL), as manufactured by Inbiose, in non-exempt term infant formula and various conventional food and beverage products as described in Section 1.3 below, are not subject to the premarket approval requirements of the *Federal Food, Drug, and Cosmetic Act* based on Inbiose's view that these notified uses of 2'-FL are Generally Recognized as Safe (GRAS). To the best of our knowledge, the data and information presented in this Notice represents a complete and balanced submission that is representative of the generally available literature. Inbiose considered all unfavorable as well as favorable information that is publicly available and/or known to Inbiose and that is pertinent to the evaluation of the safety and GRAS status of 2'-FL as a food ingredient for addition to non-exempt term infant formula and various conventional food and beverage products, as described herein.

Signed,



March 03, 2022

Joeri Beauprez
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Date

### 1.1 Name and Address of Notifier

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### 1.2 Common Name of Notified Substance

2'-Fucosyllactose; 2'-FL

### 1.3 Conditions of Use

Inbiose's 2'-FL is intended for use as an ingredient in non-exempt term infant formula at a use level of 2.4 g/L, and in the same food categories and use levels as those described in previous GRAS Notices (Glycosyn, LLC and Friesland Campina Domo B.V., 2017; DuPont Nutrition & Health, 2017, 2019; U.S. FDA, 2018a,b, 2020).

A summary of the proposed food categories and use levels is presented in Table 1.3-1 below. The intended conditions of use for Inbiose's 2'-FL will be fully substitutional to those described in GRAS Notice (GRN) 735 and 897 (GRN 735 – U.S. FDA, 2018a; GRN 897 – U.S. FDA, 2020).

**Table 1.3-1 Summary of the Individual Uses and Maximum Use Levels for 2'-FL Previously Determined to be GRAS in the U.S.**

| Food Category (21 CFR §170.3) (U.S. FDA, 2021a) | Proposed Food Use | Maximum Use Levels (g/kg or g/L)[a] | | |
|---|---|---|---|---|
| | | Inbiose | GRN 735 | GRN 897 |
| Beverages and Beverage Bases | Meal Replacement Drinks, for Weight Reduction[b] | 5 | - | 5 |
| | Sports, Isotonic, and Energy Drinks, Soft Drinks, Enhanced or Fortified Waters, Fruit-based Ades | 1.2 | 0.8 | 1.2 |
| Infant and Toddler Foods | Non-exempt Term Infant Formulas | 2.4 | 2.4 | 2.4 [c] |
| | Toddler Formulas | 2.4 | 2.4 | 2.4 [c] |
| | Other Baby Foods for Infants and Young Children | 57 | 57 | 12 [c] |
| | Other Drinks for Young Children | 10 | 10 | 1.2 [c] |
| Breakfast Cereals | Hot Cereals | 31 | 4.8 | 31 |
| | Ready-to-eat Cereals | 80 | 80 | 40 |
| Grain Products and Pastas | Meal Replacement Bars, for Weight Reduction | 30 | 12 | 30 |
| | Cereal and Granola Bars | 30 | 12 | 30 |
| Milk Products | Buttermilk* | 1.2 | - | 1.2 |
| | Flavored Milk | 1.2 | 1.2 | 1.2 |
| | Milk-Based Meal Replacement Drinks, for Weight Reduction[b] | 5 | 1.2 | 5 |
| | Yogurt* | 12 | 5.3 | 12 |
| | Formula intended for pregnant women ("mum" formulas; -9 to 0 months) | 60 | 60 | - |
| Dairy Product Analogs | Imitation Milks | 1.2 | 1.2 | 1.2 |
| | Non-dairy Yogurt | 1.2 | - | 1.2 |
| Processed Fruits and Fruit Juices | Fruit Juices, Drinks, Nectars | 1.2 | 1.2 | 1.2 |
| Processed Vegetables and Vegetable Juices | Vegetable Juices | 1.2 | - | 1.2 |
| Foods for Special Dietary Use | Oral Nutritional Food Supplements and Enteral and Oral Tube-feeding Formulas for Patients ≥11 Years | 20 | 20 | 20 |

2'-FL = 2'-fucosyllactose; CFR = *Code of Federal Regulations*; GRAS = Generally Recognized as Safe; GRN = GRAS Notice; U.S. = United States.

[a] Proposed maximum use levels are presented as g/kg for solids and as g/L for liquids.

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

[c] These intended uses were incorporated by reference to GRN 749.

* Inbiose's 2'-FL is only intended for use in unstandardized products and not in foods where standards of identity exist that preclude its addition.

## 1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a)(b) of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2021b), Inbiose has concluded that the intended uses of 2'-FL 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 FDA upon request, or will be available for review and copying at reasonable times at the offices of:

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

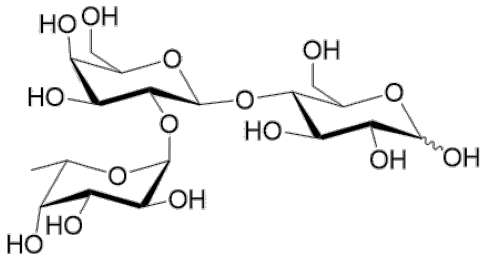
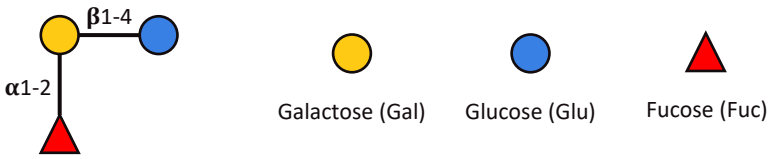
Should the FDA have any questions or additional information requests regarding this Notification, Inbiose will supply these data and information upon request.

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

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

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

## 2.1 Identity

| | |
|---|---|
| Common Name: | 2'-Fucosyllactose |
| Abbreviation: | 2'-FL |
| International Union of Pure and Applied Chemistry (IUPAC) Name: | α-L-Fucopyranosyl-(1→2)-β-Dgalactopyranosyl-(1→4)-D-glucopyranose |
| Chemical Abstracts Service (CAS) Number: | 41263-94-9 |
| Chemical Formula: | $C_{18}H_{32}O_{15}$ |
| Molecular Weight: | 488.436 g/mol |
| Chemical Structure: |  |
| Schematic Representation: |  |

### 2.1.1 Chemical and Physical Characteristics

2'-FL is an abundant human milk oligosaccharide (HMO), comprised of galactose, glucose, and fucose, and manufactured by Inbiose using fermentation with a genetically modified strain of *Escherichia coli* K-12 MG1655. The final product is a purified white powder containing ≥94% 2'-FL, and small quantities of lactose and other related carbohydrates.

The identity of Inbiose's 2'-FL has been confirmed by nuclear magnetic resonance (NMR), by comparison with a 2'-FL reference standard (Product no: 35/08, IsoSep AB, Sweden) derived from human milk. Based on NMR, the Inbiose 2'-FL is structurally identical to the IsoSep reference. All major signals in the $^{1}$H-NMR spectra of 2'-FL were identical among materials isolated from Inbiose's 2'-FL and IsoSep AB reference, and identical to $^{1}$H-NMR spectra reported in the literature (Ishizuka *et al.*, 1999; Kjærulff, 2014; van Leeuwen *et al.*, 2014). The typical shifts of the anomeric protons/carbons and those of the methyl group of the fucose moiety further confirm the 2'-FL structure.

## 2.2 Manufacturing

### 2.2.1 Production Microorganism

#### *2.2.1.1 Host Organism*

The host organism is *Escherichia coli* K-12 strain MG1655, which is the same host organism as described in GRN 749, 897, and 951 (U.S. FDA, 2018b, 2020, 2021c). The taxonomy of the species is as follows:

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

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

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

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

- Wild-type *E. coli* is an inhabitant of the human colon;
- Studies have demonstrated that *E. coli* K-12 is a debilitated strain, defective in at least 3 cell wall characteristics that are important for colonization. As a result, *E. coli* K-12 is unable to colonize the human intestinal tract under normal conditions. Even in germ-free mice, *E. coli* K-12 is a poor colonizer;
- Experimental evidence has strongly suggested that indigenous intestinal microorganisms have a large competitive advantage over *E. coli* K-12 strains;
- *E. coli* K-12 lacks the ability to produce toxins that affect humans. There is no record in the literature of *E. coli* K-12 enterotoxin-induced disease in fermentation workers; and
- *E. coli* K-12 has a history of safe commercial use. Its derivative strains are currently used in many industrial applications, including the production of specialty substances L-aspartic, inosinic, and adenylic acids, which the human body produces, and FDA-approved human drugs such as insulin and somatostatin.

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

#### *2.2.1.2 Production Strain*

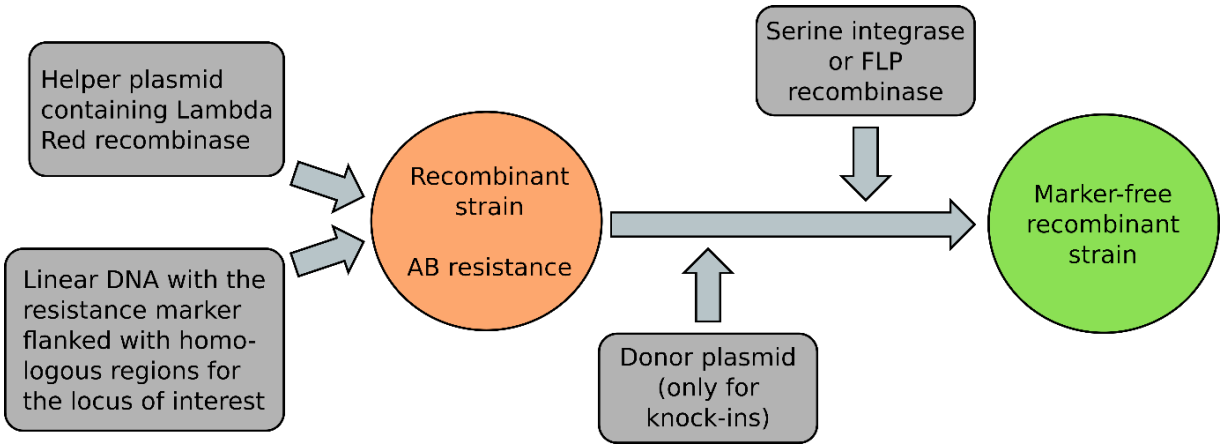
Several modifications, like gene knock-outs, gene insertions, and the addition of a production plasmid, were performed on *E. coli* K-12 strain MG1655 to create a 2'-FL production strain. This *E. coli* production strain is derived from the same parental strain as those that were previously assessed as part of GRNs 749 and 897. A production strain, INB-2FL_03, has been developed, through which the safety was assessed.

[1] https://www.ncbi.nlm.nih.gov/nuccore/545778205/.
[2] https://www.atcc.org/~/ps/47076.ashx.

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

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

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




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

All heterologous genes introduced into INB-2FL_03 were produced by DNA synthesis and were based on well-known annotated genomes from the respective donor organism. As such, no PCR techniques were used, indicating that there is no risk of undesirable or unintended genes from the donor organism being introduced to the production host. If needed, the heterologous genes were codon-optimized using bio-informatic tools. Additionally, before and after introducing these heterologous genes into the genome of the production host organism, a full Sanger sequencing of the transcription units was performed to ensure their identity.

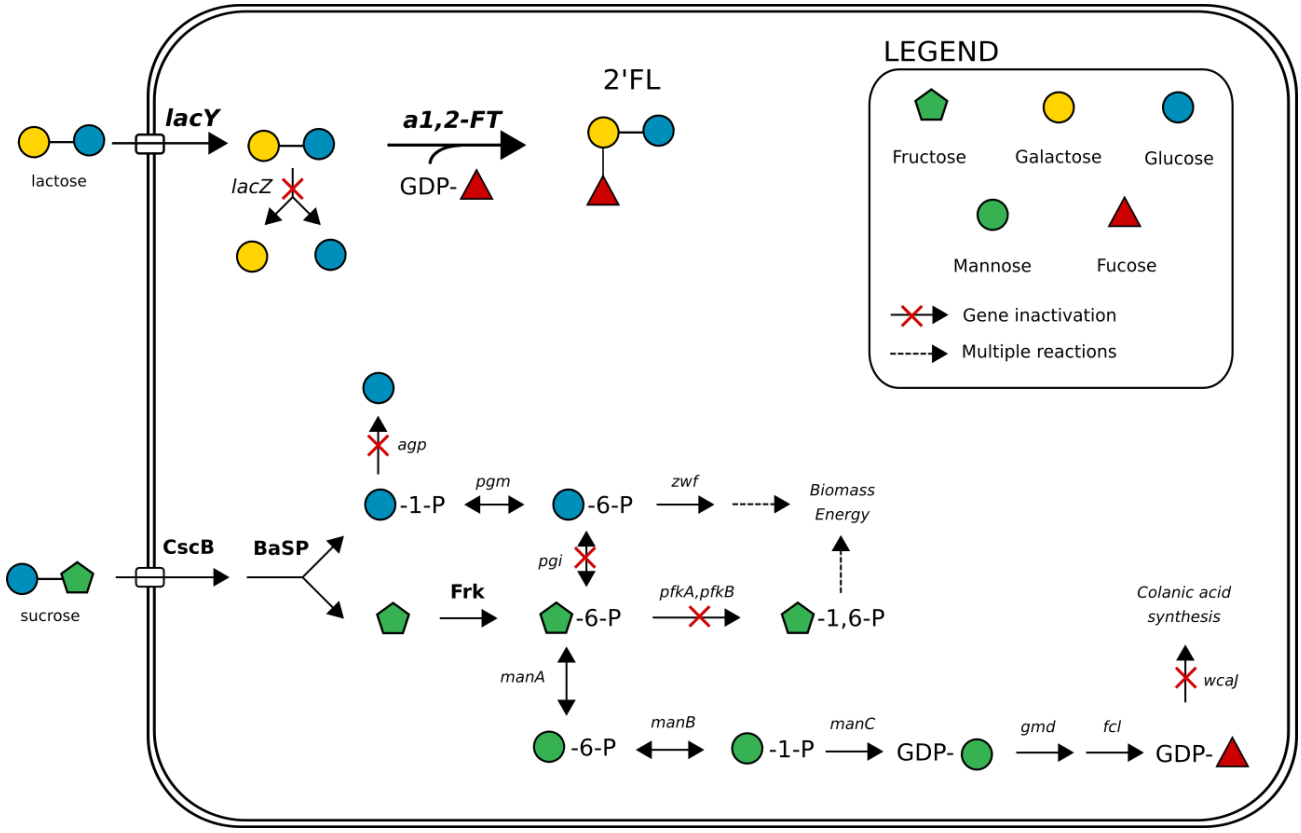
The host organism *E. coli* K-12 strain MG1655 was modified by genomic knock-outs and knock-ins by using the methods, as described above, to obtain efficient biosynthesis of 2'-FL (see Table 2.2.1.2-1 and Figure 2.2.1.2-2).

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

| Origin | Function |
|---|---|
| *Escherichia coli* | Lactose permease |
| *Escherichia coli* | Sucrose permease |
| *Bifidobacterium adolescentis* | Sucrose phosphorylase |
| *Zymomonas mobilis* | Fructokinase |
| *Helicobacter* sp. | α(1,2)-fucosyltransferase |

Knock-outs were performed to avoid breakdown of lactose, improve the flux towards guanosine diphosphate (GDP)-fucose, and avoid the production of unwanted metabolic by-products. This strain was further modified to biosynthesize 2'-FL by the introduction of genes throughout the genome (see Table 2.2.1.2-1). In addition to the chromosomal modifications, a plasmid was also introduced in the production host INB-2FL_03 for overexpression of a fucosyltransferase gene from *Helicobacter sp.* No antibiotic resistance genes were present on the plasmid. The whole vector was synthesized *de novo* and is named pINB-2FL_03. After strain construction, colony PCR, Sanger sequencing, and WGS checks were performed to verify all genetic modifications introduced in the 2'-FL production strain. Production strain INB-2FL_03 does not contain any antibiotic resistant marker on the plasmid or introduced inside its genome.

**Figure 2.2.1.2-2 Schematic Overview of the 2'-FL Biosynthetic Pathway in INB-2FL_03 using Sucrose as Carbon Source**




2'FL = 2'-Fucosyllactose; a1,2-FT = a1,2-fucosyltransferase; agp = glucose-1-phosphatase; BaSP = sucrose phosphorylase; CscB = sucrose permease; fcl = GDP-L-fucose synthase; Frk = fructokinase; GDP = guanosine diphosphate; gmd = GDP-mannose 4,6-dehydratase; lacY = lactose permease; lacZ = β-galactosidase; manA = mannose-6-phosphate isomerase; manB = phosphomannomutase; manC = mannose-1-phosphate guanylyltransferase; P = phosphate; pfkA = 6-phosphofructokinase 1; pfkB = 6-phosphofructokinase 2; pgi = glucose-6-phosphate isomerase; pgm = phosphoglucomutase; wcaJ = UDP-glucose:undecaprenyl-phosphate glucose-1-phosphate transferase; zwf = NADP+-dependent glucose-6-phosphate dehydrogenase.

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

## 2.2.2 Raw Materials, Processing Aids, and Equipment Specifications

2'-FL is manufactured by Inbiose in compliance with current Good Manufacturing Practice (cGMP) and/or principles of Hazard Analysis and Critical Control Points (HACCP) and/or Food Safety System Certification (FSSC 22000).

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

The manufacture of 2'-FL is largely comparable to the production processes previously evaluated for other HMOs produced by microbial fermentation involving construction of a production organism engineered to synthesize human milk oligosaccharides from lactose, with large-scale fermentation and downstream processing to isolate the human milk oligosaccharide. All additives, processing aids, and food contact articles used during manufacturing are permitted by federal regulation, have been previously concluded to be GRAS for their respective uses, or have been the subject of an effective food contact notification.

### 2.2.3 2'-FL Manufacturing Process

In summary, the manufacturing method for 2'-FL entails a fermentation process with a K-12-based production host (see Section 2.2.1) that produces 2'-FL. This host produces 2'-FL through the utilization of a carbon source (sucrose), combined with lactose in a minimal medium. The product is released into the medium. The remaining intracellular 2'-FL is released after pasteurization. The broth is then subjected to downstream purification and concentration processes to isolate 2'-FL, to remove impurities originating from fermentation (*e.g.*, minerals, substrates, proteins, and other cellular matter) followed by drying (see Figures 2.2.3-1 and 2.2.3-2 below).

In the first step, biomass is removed together with cell components and large molecules (DNA, protein, and lipopolysaccharides). After removal of larger particles, the color is removed using activated charcoal. Subsequently, the salts present in the medium are removed, which are cations (*e.g.*, magnesium, calcium, and ammonium) and anions (*e.g.*, phosphate and sulfate), which are minerals used for growth of the microorganism. Leftover water is removed from the product mainly through evaporation and the product is filtered again to ensure the microbial specification before drying.

**Figure 2.2.3-1 Fermentation Process**

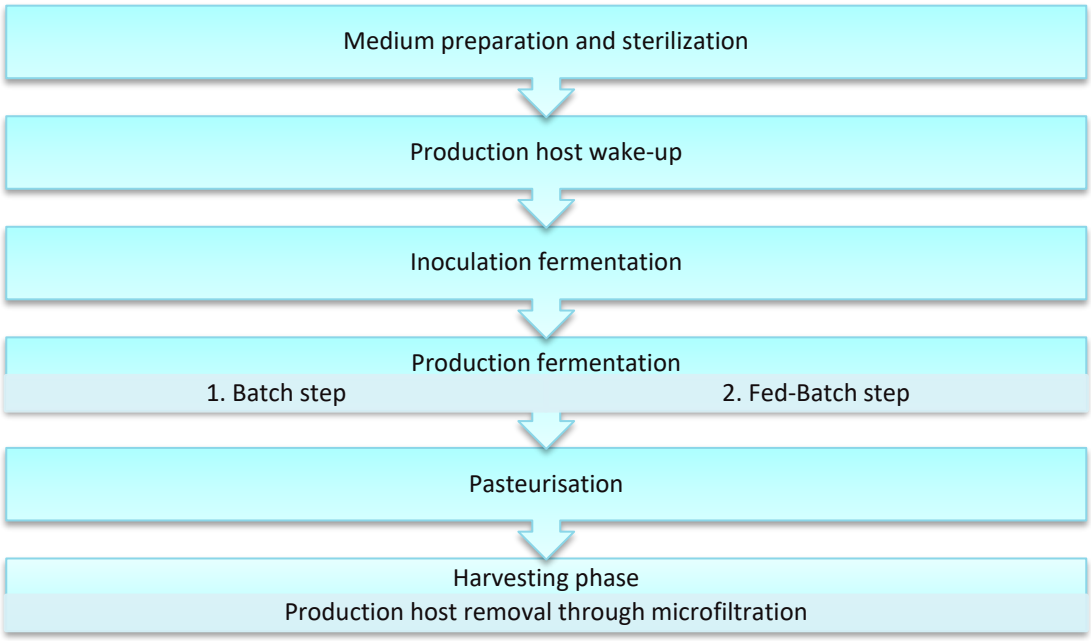
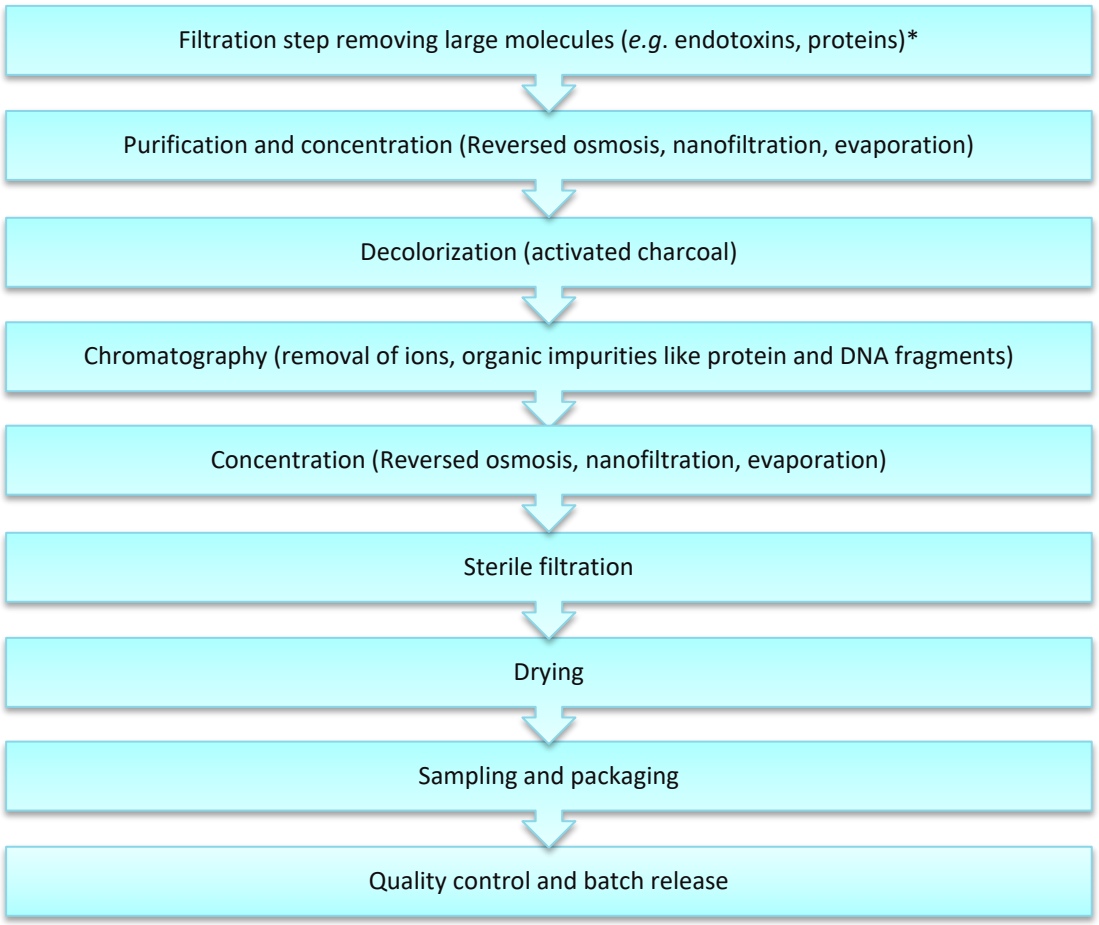


**Figure 2.2.3-2 Purification Process**




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

## 2.3 Product Specifications and Batch Analyses

### 2.3.1 Specifications

To ensure consistent product quality, Inbiose has established a set of specifications for 2'-FL, which includes the acceptability criteria for purity of 2'-FL and the presence of other carbohydrates, chemical parameters, heavy metals, and microbial contaminants, and confirms the absence of the genetically modified production strain and any related endotoxins. The specifications proposed for 2'-FL are presented in Table 2.3.1-1. All parameters are determined using compendial or validated methods.

**Table 2.3.1-1 Product Specifications for Inbiose's 2'-FL**

| Parameter | Specification | Method of Analysis |
|---|---|---|
| ***Identification*** | | |
| Appearance (Color) | White | Visual |
| Appearance (Form) | Powder | Visual |
| Appearance in solution | Clear, colorless to slightly yellow | Visual |

**Table 2.3.1-1 Product Specifications for Inbiose's 2'-FL**

| Parameter | Specification | Method of Analysis |
|---|---|---|
| Identity (2'-FL) | Conform to reference standard, 2'-FL derived from human milk | NMR |
| ***Chemical Specifications*** | | |
| Moisture | NMT 5.0% | Karl-Fischer, volumetric |
| pH (20°C, 10% solution) | 3.0 to 7.5 | Eurofins' internal method, potentiometry |
| Protein | NMT 100 µg/g | Roti®Nanoquant |
| Ash | NMT 0.5% | NEN 6810 |
| Endotoxins | NMT 10 E.U./mg | Ph. Eur. 2.6.14 |
| ***Carbohydrates (% DM)*** | | |
| 2'-FL | NLT 94% | UHPLC-RI |
| Sum of other carbohydrates[a] | NMT 5.0% | UHPLC-RI |
| Lactose | NMT 5.0% | UHPLC-RI |
| Difucosyllactose | NMT 5.0% | UHPLC-RI |
| ***Heavy Metals*** | | |
| Arsenic | NMT 0.2 mg/kg | ICP-MS |
| Cadmium | NMT 0.05 mg/kg | ICP-MS |
| Lead | NMT 0.05 mg/kg | ICP-MS |
| Mercury | NMT 0.5 mg/kg | ICP-MS |
| ***Microbiological Contaminants*** | | |
| Total aerobic mesophilic plate count | NMT 1,000 CFU/g | ISO 4833 |
| Yeast | NMT 100 CFU/g | ISO 7954 |
| Mold | NMT 100 CFU/g | ISO 7954 |
| *Enterobacteriaceae* | Absent in 10 g | ISO 21528-1 |
| *Salmonella* spp. | Absent in 25 g | ISO 6579-1 |
| *Cronobacter sakazakii* | Absent in 25 g | ISO/TS 22964 |
| *Listeria monocytogenes* | Absent in 25 g | AFNOR EGS 38/05-03/17 |
| *Bacillus cereus* | NMT 50 CFU/g | ISO 7932 |

2'-FL = 2'-fucosyllactose; AFNOR = Association Française de Normalisation; CFU = colony forming units; DM = dry matter; E.U. = endotoxin units; EGS = Eurofins GeneScan; GRN = Generally Recognized as Safe Notice; ICP-MS = inductively coupled plasma mass spectrometry; ISO = International Organization for Standardization; NEN = Royal Netherlands Standardization Institute; NLT = not less than; NMR = nuclear magnetic resonance; NMT = not more than; Ph. Eur. = European Pharmacopoeia; UPLC-RI = ultra-high performance liquid chromatography coupled with refractive index detector.

[a] Sum of other carbohydrates, such as 3-fucosyllactose, 2-fucosyl-D-lactulose, fucosyl-galactose, glucose/galactose, fucose, sorbitol/galactitol, mannitol, and trihexose.

## 2.3.2 Batch Analysis

Results for the analyses of 3 non-consecutive batches of 2'-FL are summarized in Table 2.3.2-1. The data demonstrate that the production process as described in Section 2.2 results in a consistent product that meets the established product specifications.

**Table 2.3.2-1 Analytical Data Obtained from 3 Batches of 2'-FL**

| Parameter | Specification | Lot Nos. | | |
|---|---|---|---|---|
| | | HeraD03 | HeraD04 | HeraD07 |
| ***Identification*** | | | | |
| Appearance (color) | White | White | White | White |
| Appearance (form) | Powder | Powder | Powder | Powder |
| Appearance in solution | Clear, colorless to slightly yellow | Clear, colorless to slightly yellow | Clear, colorless to slightly yellow | Clear, colorless to slightly yellow |
| pH (20°C, 10% solution) | 3.0 to 7.5 | 6.8 | 5.5 | 5.3 |
| ***Carbohydrates, water free (%DM)*** | | | | |
| 2'-FL | ≥94 | 97.5 | 97.3 | 98.4 |
| Lactose | ≤5.0 | 2.1 | 2.3 | 1.0 |
| Difucosyllactose (DFL) | ≤5.0 | 0.13 | 0.13 | 0.13 |
| Sum of other carbohydrates[a] | ≤5.0 | 0.30 | 0.29 | 0.51 |
| ***Chemical Analysis*** | | | | |
| Water content, volumetric (% w/w) | ≤5.0 | 3.5 | 4.3 | 4.2 |
| Protein content (µg/g) | ≤100 | <25 | <25 | <25 |
| Total ash (%) | ≤0.5 | <0.1 | <0.1 | |
| Endotoxin (E.U./g) | ≤10,000 | 230 | 62.2 | <50 |
| ***Heavy Metals*** | | | | |
| Arsenic (mg/kg) | ≤0.02 | <0.01 | <0.01 | <0.01 |
| Cadmium (mg/kg) | ≤0.05 | <0.005 | <0.005 | <0.005 |
| Lead (mg/kg) | ≤0.05 | <0.01 | <0.01 | <0.01 |
| Mercury (mg/kg) | ≤0.5 | <0.01 | <0.01 | <0.01 |
| ***Microbiological Contaminants*** | | | | |
| Standard plate count (CFU/g) | ≤1,000 | 30 | <10 | <10 |
| Yeast (CFU/g) | ≤100 | <10 | <10 | <10 |
| Mold (CFU/g) | ≤100 | <10 | <10 | <10 |
| Coliform / *Enterobacteriaceae* | Absent in 10 g | Absent | Absent | Absent |
| *Salmonella* spp. | Absent in 25 g | Absent | Absent | Absent |
| *Cronobacter (Enterobacter) sakazakii* | Absent in 25 g | Absent | Absent | Absent |
| *Listeria monocytogenes* | Absent in 25 g | Absent | Absent | Absent |
| *Bacillus cereus* (CFU/g) | ≤50 | <10 | <10 | <10 |

2'-FL = 2'-fucosyllactose; CFU = colony forming units; DM = dry matter; E.U. = endotoxin units; GRN = Generally Recognized as Safe Notice

[a] Sum of other carbohydrates, such as 3-fucosyllactose, 2-fucosyl-D-lactulose, fucosyl-galactose, glucose/galactose, fucose, sorbitol/galactitol, mannitol, and trihexose.

### 2.3.3 Microbiological Endotoxins and Residual Protein Analysis

The content of endotoxins and residual proteins in the 2'-FL product is determined by methods with high sensitivity [Protein content: Roti®Nanoquant method, based on the Bradford assay; and Endotoxins: kinetic-chromogenic test (Method D) described in the European Pharmacopoeia] to ensure the consistency and quality of the 2'-FL product.

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

### 2.3.4 Residual DNA Analysis

To ensure the absence of residual DNA of the production organism, PCR tests were performed on 3 regulatory batches of INB-2FL_03. A short subsequence of the inserted fucosyltransferase gene (derived from *Helicobacter* sp.) on the plasmid and a subsequence of the sucrose phosphorylase gene (derived from *Bifidobacterium adolescentis*) on the genome were targeted to check for residual DNA in the product. For every batch, the analysis was performed in triplicate together with 3 types of positive controls and 1 negative control. The analysis of all regulatory batches of 2'-FL showed no detectable levels of residual DNA in the final product. The limit of detection for the PCR method is 10 ng DNA per gram 2'-FL as recommended in European Food Safety Authority (EFSA) guidelines (EFSA, 2018).

## 2.4 Stability

The stability of Inbiose's 2'-FL is supported by the real-time and accelerated stability studies summarized in GRNs 546, 735, 749, and 987. The compositional similarities between Inbiose's 2'-FL and other 2'-FL preparations (see Section 6.3) indicate that the stability of the ingredients will be similar. A summary of the real-time and accelerated stability studies, as described in GRNs 546, 735, 749, and 987, is provided below (Glycom A/S, 2014; U.S. FDA, 2015a, 2018a,b, 2021d; DuPont Nutrition & Health, 2017; Glycosyn, LLC and Friesland Campina Domo B.V., 2017; Amyris, Inc., 2020). Additionally, the stability of 2'-FL has been tested in studies conducted under some of the intended conditions of use, which further supports the stability of 2'-FL as an ingredient in food and beverages matrices, which were included in GRN 546 (Glycom A/S, 2014; U.S. FDA, 2015a).

The bulk stability of crystalline, chemically synthesized, Glycom's 2'-FL ingredient was evaluated in a 36-month real-time test and a 6-month accelerated test, as described in Section II.D.1 of GRN 546. In the real-time study [25°C, 60% relative humidity (RH)], no significant change was observed in the 2'-FL content or microbiological parameters of the stored sample and only a minor increase in water content (remaining within acceptable defined product specifications) was measured at the 18-month time point. Results from the 6-month accelerated stability study (40°C, 75% RH) also indicate that 2'-FL does not undergo significant degradation under the described storage conditions. No unknown degradation products were measured following HPLC analysis of the 2'-FL following accelerated storage.

As described in Section 7 and Appendix C of GRN 749, the shelf life of DuPont's 2'-FL was assessed *via* a 6-month accelerated stability study (40°C, 75% RH). The results of this study indicated no significant changes in the evaluated carbohydrate content (*i.e.*, 2'-FL, difucosyllactose, lactose, and other unspecified carbohydrates), moisture content, and microbiological parameters (*i.e.*, standard plate count, yeast and mold, coliform/*Enterobacteriaceae*, *Salmonella* spp., and *Cronobacter sakazakii*) in 3 representative batches following storage for up to 6 months. A minor degree of degradation was reported in the purity of 1 sample and the moisture content slightly increased over the test period due to the hygroscopic nature of the ingredient. No other changes were noted.

As described in Section E.2 of GRN 735, a range of chemical and microbiological specification parameters was tested, along with the overall purity of FrieslandCampina's 2'-FL ingredient, in a 6-month accelerated storage (40°C, 75% RH) and an ongoing 36-month real-time study (25°C, 60% RH). The results from the accelerated stability study indicated no changes in the appearance of the ingredient or the evaluated chemical (*i.e.*, 2'-FL, lactose, allo-lactose, glucose, ash, and water content) and microbiological parameters. The 6-month interim results from the real-time study confirmed that 2'-FL is stable when stored at ambient room temperatures.

Similarly, the stability of Amyris's 2'-FL was assessed in a study conducted under accelerated (40°C, 75% RH) conditions for 13 weeks (Section 2.6; GRN 987). Under these conditions, 2'-FL content remained relatively unchanged along with the other measured parameters (*i.e.*, moisture, 2'-fucosyllactitol, and other minor carbohydrate components).

The stability of 2'-FL has also been assessed under the intended conditions of use. The 2'-FL ingredient produced by Glycom *via* chemical synthesis (compositionally comparable to 2'-FL produced from fermentation) was assessed in powdered infant formula, as described in Section II.D.2 of GRN 546. Glycom's 2'-FL added to a powdered infant formula supplemented with other human-identical milk oligosaccharides (*i.e.*, lacto-*N*-neotetraose), containing a range of other ingredients (*i.e.*, salts, carbohydrates, and proteins), was observed to be stable for up to 18 months of storage at various temperatures (4°C, 20°C, 30°C, and 37°C). The results from additional stability testing of Glycom's 2'-FL in yogurts, ready-to-drink flavored milk, and citrus fruit beverages also indicated that 2'-FL was stable in a range of different products, with no loss in 2'-FL content following typical processing and storage conditions, for up to 28 days post-processing.

Other recently filed GRNs (*e.g.*, GRNs 897 and 929) similarly reflect on stability tests that have been conducted and reported in these earlier filings (DuPont Nutrition & Health, 2019; Jennewein Biotechnologie GmbH, 2020; U.S. FDA, 2021e).

These results demonstrate that 2'-FL is not significantly degraded when stored under the tested conditions and is anticipated to be stable in most food matrices.

# Part 3. § 170.235 Dietary Exposure

## 3.1 Estimated Intake of 2'-FL

As described in Section 3.2 of GRN 897, the estimated intake of 2'-FL as an ingredient in term infant formula (0 to 12 months), toddler formula, and other food and beverage products has been estimated using dietary survey data in the U.S. population. The intake of 2'-FL described in GRN 897 was estimated based on food consumption records collected from the What We Eat in America (WWEIA) component of the National Center for Health Statistics' 2013-2014 National Health and Nutrition Examination Surveys (NHANES) conducted in 2013-2014 and 2015-2016 (CDC, 2015, 2016, 2018; USDA, 2016), incorporating the product uses (*i.e.*, infant and toddler formulas, foods, and drinks) and intake estimates originally presented in GRN 749. Based on the proposed food uses, approximately 81% of the evaluated population groups consisted of eligible 2'-FL consumers. Infants (7 to <12 months) were established to represent the highest mean and 90$^{th}$ percentile all-user consumption of 2'-FL on an absolute basis, at 4.63 and 8.36 g/person/day (*i.e.*, 520.2 and 987.1 mg/kg body weight/day), respectively. Inbiose's 2'-FL ingredient will be fully substitutional to the GRAS infant and toddler food uses described in GRN 897, and this intakes estimate is therefore considered to remain unchanged. The summary of the estimated dietary intake of 2'-FL in the U.S. population, as described in GRN 897, is provided in Table 3.1-1 (U.S. FDA, 2020). Furthermore, due to the overall intended conditions of use for Inbiose's 2'-FL being fully substitutional to those described in both GRN 735 and 897, a summary of estimated dietary intake of 2'-FL, as described in GRN 735, is provided in Table 3.1-2 (U.S. FDA, 2018a).

**Table 3.1-1 Summary of the Estimated Daily Intake of 2'-FL[a] from All Proposed Food Uses in the U.S. by Population Group in GRN 897 (2009-2016 NHANES Data)[b]**

| Population Group | Age Group (Years, Unless Otherwise Specified) | Per User Intakes (g/day) | | Per User Intakes (mg/kg bw/day) | |
|---|---|---|---|---|---|
| | | Mean | 90th Percentile | Mean | 90th Percentile |
| Infants | 0 to 6 months | 2.93 | 5.29 | 449.7 | 712.4 |
| Infants | 7 to 12 months | 4.63 | 8.36 | 520.2 | 987.1 |
| Toddlers | 13 to 36 months | 1.12 | 1.97 | 84.9 | 146.0 |
| Children | 3 to 12 | 1.6 | 3.5 | 60 | 140 |
| Adolescents | 13 to 18 | 1.7 | 3.9 | 30 | 60 |
| Adults | 19 to 49 | 2.2 | 5.2 | 30 | 70 |
| Adults | 50 and up | 2.5 | 5.9 | 30 | 80 |
| Total Population | 3 and up | 2.2 | 5.0 | 40 | 100 |

2'-FL = 2'-fucosyllactose; bw = body weight; GRAS = Generally Recognized as Safe; GRN = GRAS Notice; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

[a] Intake data expressed as wet weight of ingredient under the proposed conditions of intended use.

[b] Table adapted from GRN 897 (U.S. FDA, 2020); full intake assessment reported in GRN 897 GRAS determination.

**Table 3.1-2 Summary of the Estimated Daily Intake of 2'-FL[a] from All Proposed Food Uses in the U.S. by Population Group in GRN 735 (2013-2014 NHANES Data)[b]**

| Population Group | Age Group (Years, Unless Otherwise Specified) | Per User Intakes (g/day) | | Per User Intakes (mg/kg bw/day) | |
|---|---|---|---|---|---|
| | | Mean | 90th Percentile | Mean | 90th Percentile |
| Infants | 0 to 5 months | 1.10 | 2.75 | 181 | 477 |
| Infants | 6 to 11 months | 2.14 | 3.86 | 244 | 441 |
| Toddlers | 12 to 35 months | 1.83 | 2.97 | 148 | 243 |
| Children | 3 to 11 | 1.96 | 3.53 | 75 | 147 |
| Female Teenagers | 12 to 19 | 1.47 | 2.95 | 24 | 52 |
| Male Teenagers | 12 to 19 | 1.85 | 4.16 | 29 | 67 |
| Women of Child-Bearing Age | 16 to 45 | 1.22 | 2.82 | 18 | 42 |
| Female Adults | 20 years and up | 1.32 | 2.96 | 19 | 42 |
| Male Adults | 20 years and up | 1.59 | 3.81 | 19 | 26 |
| Elderly | 65 years and up | 1.76 | 3.74 | 24 | 53 |
| Total Population | All ages | 1.55 | 3.41 | 32 | 76 |

2'-FL = 2'-fucosyllactose; bw = body weight; GRAS = Generally Recognized as Safe; GRN = GRAS Notice; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

[a] Intake data expressed as wet weight of ingredient under the proposed conditions of intended use.

[b] Table adapted from GRN 735 (U.S. FDA, 2018a); full intake assessment reported in GRN 735 GRAS determination.

# Part 4. § 170.240 Self-Limiting Levels of Use

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

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

Not applicable.

# Part 6. § 170.250 Narrative and Safety Information

## 6.1 Introduction

The first GRAS conclusion notified to the FDA for 2'-FL (produced by chemical synthesis) was submitted by Glycom in 2014 (GRN 546; U.S. FDA, 2015a). A subsequent GRAS notification for 2'-FL produced by Glycom using fermentation technology was submitted to the FDA and filed by the agency without objection in 2016 under GRN 650 (GRN 650; U.S. FDA, 2016). A critical and comprehensive review of the publicly available data and information pertaining to the safety of 2'-FL for use as an ingredient in non-exempt infant formula, and various food and beverage products across multiple categories, was presented in these Notices, and the published information pertinent to safety of 2'-FL presented by Glycom has served as the basis for subsequent GRAS conclusions for similar 2'-FL preparations (Glycom A/S, 2014, 2018; Jennewein Biotechnologie GmbH, 2015, 2020; U.S. FDA, 2015a,b, 2016, 2018a,b, 2019a,b, 2020, 2021e,f; DuPont Nutrition & Health, 2017, 2019; Glycosyn, LLC and Friesland Campina Domo B.V., 2017; BASF SE, 2019). To date, the majority of 2'-FL preparations that have been evaluated under the GRAS procedure have been synthesized by microbial fermentation using genetically modified strains of *E. coli* K-12. Downstream processing using various purification techniques (*e.g.*, chromatography, ion-exchange, nano-filtration, carbon filtration, solvent crystallization) are then applied to produce 2'-FL products that are typically >90% purity. Compositional differences between various 2'-FL preparations are largely limited to differences in the concentrations of residual lactose, and other related sugars; however, these compounds have been demonstrated to be present in human milk or within infant formula and therefore these minor differences in the impurity profiles between various preparations have not been of safety concern. A large number of toxicological evaluations have been conducted using 2'-FL preparations from different manufacturers without evidence of toxicity at the highest doses tested, further supporting the view that residues of these carbohydrate impurities are of low toxicity potential. Within the previous GRAS Notices, data and information supporting the GRAS use of 2'-FL as an ingredient in infant formula and other foods have been critically reviewed by a number of qualified scientific experts, including the FDA, and are publicly available. Additionally, EFSA has issued multiple opinions supporting the safe use of 2'-FL as an ingredient in a variety of foods, including infant and follow-on formula either alone or in combination with other HMOs (EFSA, 2015a,b, 2019).

2'-FL is the most abundant HMO occurring naturally in human breast milk and approximately 85% of the world population is exposed to 2'-FL from human milk (Thurl *et al*., 2017; Menzel *et al*., 2020; Liu *et al*., 2021; Plows *et al*., 2021; Soyyılmaz *et al*., 2021). As reported in Section 3.B of GRN 932, concentrations of 2'-FL in human milk can range from 0.22 to 8.4 g/L, varying depending on the mother's genotype and the lactation stage of the pregnancy (Advanced Protein Technologies, Corp., 2020). These 2'-FL levels in human breast milk have been measured in a wide range of previously-evaluated studies (Grollman and Ginsburg, 1967; Thurl *et al.*, 1996, 2010, 2017; Chaturvedi *et al.*, 1997, 2001; Coppa *et al.*, 1999, 2011; Kunz *et al.*, 1999; Nakhla *et al.*, 1999; Erney *et al.*, 2000, 2001; Sumiyoshi *et al.*, 2003; Morrow *et al.*, 2004; Musumeci *et al.*, 2006; Asakuma *et al.*, 2008, 2011; Leo *et al.*, 2009, 2010; Gabrielli *et al.*, 2011; Galeotti *et al.*, 2012, 2014; Bao *et al.*, 2013; Castanys-Munoz *et al.*, 2013; Smilowitz *et al.*, 2013; Goehring *et al.*, 2014; Hong *et al.*, 2014; Marx *et al.*, 2014; Balogh *et al.*, 2015; Austin *et al.*, 2016, 2019; Donovan and Comstock, 2016; McGuire *et al.*, 2017; Samuel *et al*., 2019).

Recent studies support the previous data demonstrating that 2'-FL levels in breast milk decrease throughout lactation (Ferreira *et al*., 2020; Lagström *et al*., 2020; Lefebvre *et al*., 2020; Gu *et al*., 2021; Liu *et al*., 2021; Menzel *et al*., 2021; Plows *et al*., 2021; Siziba *et al*., 2021; Soyyılmaz *et al*., 2021; Zhou *et al*., 2021). The results of these studies are summarized in Table 6.1-1.

In a prospective population-based birth cohort study in Turku, Finland performed by Lagström et al. (2020), breast milk samples of 802 mothers were collected at 3 months post-partum and analyzed. The median concentration of 2'-FL in all milk samples was 2.72 g/L [number of samples (N)=802], 2.90 g/L in the secretor group (N=699), and 0.02 g/L in the non-secretor group (N=103) [the original data, which were reported in (nmol/ml), were converted into (g/L) using a molecular weight of 2'-FL of 448.436 g/mol] (Lagström *et al*., 2020). Similar results were observed in the study conducted by Menzel *et al*. (2020), who collected milk samples from German mothers at 3 months after delivery. The levels of 2'-FL in breast milk were 2.04 g/L (median, N=124) in secretor and 0.01 g/L (median, N=21) in non-secretor mothers. A median level of 2'-FL of 1.91 g/L was obtained from secretor and non-secretor samples (N=145). In another study, Gu *et al*. (2021) reported levels of 2'-FL in breast milk collected from 68 mothers in the Nijmegen−Arnhem region in the Netherlands. In total 180 samples were analyzed (N=138 for secretors and N=42 for non-secretors, Lewis +) at 2, 6, and 12 weeks postpartum. 2'-FL levels in secretors decreased from 1.22 g/L at 2 weeks (N=48) to 1.03 g/L at 6 weeks (N=48) and then to 0.91 g/L at 12 weeks (N=42). For non-secretors, the reported 2'-FL concentrations were 0 g/L for all three timepoints.

Soyyılmaz *et al*. (2021) investigated the concentration of diverse HMOs, including 2'-FL, in milk samples from mothers around the world. This meta-analysis data showed that 2'-FL significantly decreases from colostrum (3.18 g/L, 0 to 5 days) to transitional milk (2.07 g/L, 6 to 14 days), and from mature milk (2.28 g/L, 15 to 90 days) to late milk (1.65 g/L days, >90 days). Another systematic review, Thum *et al.* (2021), examined the concentration of HMOs in different geographic locations. Overall, the levels of 2'-FL at the first timepoint (0 to 7 days) ranged from 1.70 to 3.70 g/L, from 0.22 to 3.37 g/L in 5 to 15 days and from 0.98 to 3.02 g/L in >1 month. These results were in line with Soyyılmaz *et al*. (2021).

Ferreira *et al*. (2020) described the variation of 2'-FL concentration up to 4 months postpartum in milk samples of Brazilian mothers who delivered the pre-term babies. The results showed that the median of 2'-FL concentration in analyzed samples of the breast milk was 2.26 g/L in the lactation period of 2 to 50 days and then decreased to 1.91 g/L during the lactational period of 88 to 119 days [the original data, which were reported in (mmol/ml), were converted into (g/L) using a molecular weight of 2'-FL of 448.436 g/mol]. Zhou *et al*. (2021) summarized the concentrations of HMOs in the Chinese population. The concentration of 2'-FL declined gradually during lactation, from 2.93 g/L at 0 to 7 days postpartum to 0.92 g/L at >121 days postpartum. In another recent study of Liu *et al*. (2021), a total of 488 breast milk samples were collected from 335 healthy Chinese mothers at 5 different timepoints. 2'-FL levels remained relatively high in breast milk throughout the full duration of the study. The highest 2'-FL concentrations were reported in the colostrum (2.89 g/L, 0 to 5 days) and then continuously decreased to 1.01 g/L in the late milk (300 to 400 days postpartum).

Plows *et al*. (2021) investigated the change of 2'-FL levels up to 24 months postpartum. The results showed that 2'-FL concentration in breast milk samples collected from Hispanic mothers in a longitudinal cohort study decreased from 3.48 g/L at 1 month postpartum (N=183) to 2.49 g/L at 24 months postpartum (N=26) in samples collected from secretor mothers. For non-secretors, low levels of 2'-FL were reported and remained stable during the study (range of medians: 0.01 to 0.05 g/L). These results are supported by the previous study conducted by Lefebvre *et al.* (2020). In another birth cohort study conducted in south of Germany, Siziba *et al*. (2021) investigated the trajectories of absolute HMO concentrations during lactation. A total of 66 lactating mothers had HMO data available from samples at all three time points. The absolute mean 2'-FL concentration in milk decreased over the first year of lactation; from 2.45 g/L at 6 weeks to 1.65 g/L and 1.43 g/L at 6 months and 12 months of lactation, respectively.

Overall, the 2'-FL levels up to 3.84 g/L were reported in the latest studies (published in 2020 and 2021, Table 6.1-1). As such, the use of 2'-FL as an ingredient in non-exempt term infant formula at levels up to 2.4 g/L is within the reported range that infants are exposed to following the ingestion of human milk.

**Table 6.1-1 Levels of 2'-FL in Human Breast Milk Reported in Studies Published Between 2020-2021**

| Reference Study | Quantification Method | Gestation | Secretor Status | Lewis | Lactation Periods | No. of Samples | Mean Concentration ± SD (g 2'-FL/L) | Median of 2'-FL Concentration (Q1, Q3) (g/L) | Site |
|---|---|---|---|---|---|---|---|---|---|
| Thum *et al*. (2021) | UHPLC-FL, HPLC-MRM-MS, HPAEC-PAD, CE-LIF, HPLC-FL, NMR, UHPLC/QqQ-MS, LC-MS, HPLC-UV, CE-UV | Term | N/A* | Se+ and Se- | 0–7 days | N/A | Range: 1.70±1.11 to 3.70±1.94 | N/A | Japan, China, Malaysia, Singapore, Spain, Germany, France, Italy, Norway, Portugal, Romania, Sweden, Finland, Nederland, USA, Mexico, Malawi, South Africa, Samoa, |
| | | | | | 5–15 days | N/A | Range: 0.22±0.37 to 3.37 | N/A | |
| | | | | | 11–30 days | N/A | Range: 1.37±1.12 to 3.02 | N/A | |
| | | | | | 2 months | N/A | Range: 1.18±1.02 to 2.82 | N/A | |
| | | | | | 3 months | N/A | Range: 0.98±0.89 to 2.21±0.71 | N/A | |
| Siziba *et al*. (2021) | LC-MS | Term | Se+ | N/A | 6 weeks | 66 | 2.45±1.32 | N/A | Germany |
| | | | | | 6 months | | 1.65±1.08 | | |
| | | | | | 12 months | | 1.43±0.85 | | |
| Soyyılmaz *et al*. (2021) | HPLC-FL, HPAEC-PAD, LC-MS/MRM, CE, PC, NMR, nano-LC-chip-TOF (time of flight) | Term | Se+ and Se- | N/A | 0–5 days | 1,101 | 3.18 | N/A | Europe, Asia, North America, Latin America, Asia Pacific, Middle East |
| | | | | | 6–14 days | 789 | 2.07 | | |
| | | | | | 15–90 days | 4,048 | 2.28 | | |
| | | | | | >90 days | 1951 | 1.65 | | |
| Liu *et al*. (2021) | HPAEC-PAD | Term | Se+ and Se- | N/A | 0–5 days | 96 | N/A | 2.89(1.71, 4.34) | China |
| | | | | | 10–15 days | 96 | | 2.16(1.67, 2.81) | |
| | | | | | 40–45 days | 104 | | 2.06(1.38, 2.69) | |
| | | | | | 200–400 days | 100 | | 1.03(0.60, 1.52) | |
| | | | | | 300–400 days | 92 | | 1.01(0.60, 1.48) | |
| Menzel *et al*. (2021) | LC-FL | Term | Se+ and Se- | N/A | 3 months | 145 | N/A | 1.93(1.31, 2.41) | Germany |
| | | | Se+ | | | 124 | | 2.04 (1.53, 2.72) | |
| | | | Se- | | | 21 | | 0.007 (0.006, 0.010) | |

**Table 6.1-1 Levels of 2'-FL in Human Breast Milk Reported in Studies Published Between 2020-2021**

| Reference Study | Quantification Method | Gestation | Secretor Status | Lewis | Lactation Periods | No. of Samples | Mean Concentration ± SD (g 2'-FL/L) | Median of 2'-FL Concentration (Q1, Q3) (g/L) | Site |
|---|---|---|---|---|---|---|---|---|---|
| Plows *et al*. (2021) | HPLC–FL | Term | Se+ | N/A | 1 month | 183 | N/A | 3.48(2.42, 4.34) | Hispanic, the U.S. |
| | | | Se+ | | 6 months | 104 | | 3.84(2.80, 4.95) | |
| | | | Se+ | | 12 months | 76 | | 3.35(2.67, 4.14) | |
| | | | Se+ | | 18 months | 54 | | 3.1(1.95, 3.85) | |
| | | | Se+ | | 24 months | 26 | | 2.49(1.66, 3.73) | |
| | | | Se- | | 1 month | 24 | | 0.02(0.01, 0.05) | |
| | | | Se- | | 6 months | 15 | | 0.05(0.02, 0.06) | |
| | | | Se- | | 12 months | 7 | | 0.03(0.02, 0.06) | |
| | | | Se- | | 18 months | 5 | | 0.03(0.02, 0.03) | |
| | | | Se- | | 24 months | 2 | | 0.01(0.01, 0.02) | |
| Gu *et al*. (2021) | PGC-LC-MS | Term | Se+ | Le+ | 2 weeks | 48 | 1.22±0.36 | N/A | Netherlands |
| | | | Se+ | | 6 weeks | 48 | 1.03±0.3 | | |
| | | | Se+ | | 12 weeks | 42 | 0.91±0.26 | | |
| | | | Se- | | 2 weeks | 16 | 0±0 | | |
| | | | Se- | | 6 weeks | 13 | 0±0 | | |
| | | | Se- | | 12 weeks | 13 | 0±0 | | |
| Zhou *et al*. (2021) | UHPLC, HPLC, HPLC-MS, UHPLC-QqQ-MS, UHPLC-FL, UHPLC-MS-MS | N/A | Se+ and Se- | N/A | 1–7 days | N/A | 2.93±1.08 | N/A | Meta-analysis, China |
| | | | | | 8–14 days | | 1.83±0.57 | | |
| | | | | | 15–60 days | | 1.71±0.45 | | |
| | | | | | 61–120 days | | 1.19±0.26 | | |
| | | | | | >121 days | | 0.92±0.19 | | |
| Lagström *et al*. (2020) | HPLC-MS | Term | Se+ and Se- | N/A | 3 months | 802 | N/A | 2.72(1.98, 3.53) | Finland |
| | | | Se+ | | | 699 | | 2.89(2.23, 3.69) | |
| | | | Se- | | | 103 | | 0.02(0.01, 0.05) | |

**Table 6.1-1 Levels of 2'-FL in Human Breast Milk Reported in Studies Published Between 2020-2021**

| Reference Study | Quantification Method | Gestation | Secretor Status | Lewis | Lactation Periods | No. of Samples | Mean Concentration ± SD (g 2'-FL/L) | Median of 2'-FL Concentration (Q1, Q3) (g/L) | Site |
|---|---|---|---|---|---|---|---|---|---|
| Lefebvre *et al*. (2020) | UHPLC-FL | N/A | Se- | Le+ | 3 months | N/A | 0.01 | 0.01 | Germany |
| | | | Se- | Le+ | 6 months | | 0.00 | 0.00 | |
| | | | Se- | Le+ | 12 months | | 0.02 | 0.00 | |
| | | | Se+ | Le- | 3 months | | 3.32 | 3.37 | |
| | | | Se+ | Le- | 6 months | | 3.05 | 3.23 | |
| | | | Se+ | Le- | 12 months | | 3.47 | 3.80 | |
| | | | Se+ | Le+ | 3 months | | 2.12 | 2.00 | |
| | | | Se+ | Le+ | 6 months | | 1.80 | 1.69 | |
| | | | Se+ | Le+ | 12 months | | 1.56 | 1.43 | |
| Ferreira *et al*. (2020) | HPLC-FL | Preterm | Se+ and Se- | N/A | 2–8 days | 52 | N/A | 2.26(1.18, 2.91) | Brazil |
| | | | | | 28–50 days | 75 | | 2.26(1.48, 2.62) | |
| | | | | | 88–119 days | 46 | | 1.91(1.33, 2.76) | |

2'-FL = 2'-fucosyllactose; CE-LIF = capillary electrophoresis with laser-induced fluorescence detection; CE-UV = capillary electrophoresis with ultraviolet detection, paper chromatography; HPAEC-PAD = high-performance anion-exchange chromatography with pulsed amperometry detection; HPLC-FL = high performance liquid chromatography with fluorescence detection; HPLC-MRM-MS = high performance liquid chromatography-multiple reaction monitoring-mass spectrometry; HPLC-MS = high performance liquid chromatography with mass spectrometry; HPLC-UV = high performance liquid chromatography with ultraviolet detection; LC-FL = liquid chromatography with fluorescence detection; LC-MS = liquid chromatography with mass spectrometry; Le+ = Lewis positive; Le- = Lewis negative; MRM = multiple reaction monitoring; N/A = not available; NMR = nuclear magnetic resonance, or nano-LC-chip-TOF (time of flight); PC = paper chromatography; PGC-LC-MS = porous graphitized carbon–liquid chromatography–mass spectrometry; SD = standard deviation; Se+ = secretor; Se- = non-secretor; UHPLC-FL = ultra-high-performance liquid chromatography with fluorescence detection; UHPLC-MS-MS = ultra-high-performance liquid chromatography with tandem mass spectrometry; UHPLC/QqQ-MS = ultra-high-performance liquid chromatography coupled with triple quadrupole mass spectrometry; U.S. = United States.

Based on the equivalence of Inbiose's 2'-FL to other 2'-FL preparations with GRAS status, publicly available data and information establishing the GRAS status of 2'-FL are therefore incorporated by reference to previous GRAS evaluations in the sections below (U.S. FDA, 2015a,b, 2016, 2018a,b, 2019a,b, 2020, 2021e,f). Since the most recent GRAS conclusion for which the FDA has issued a letter of "no questions" was notified to the FDA in March 2020, an updated comprehensive search of the publicly available scientific literature was conducted to identify new information relevant to the safety of 2'-FL published through 14 January 2022. The following databases were accessed: AdisInsight: Trials, AGRICOLA, AGRIS, Allied & Complementary Medicine, BIOSIS Toxicology, BIOSIS Previews, CAB ABSTRACTS, Embase, Foodline: SCIENCE, FSTA, MEDLINE, NTIS: National Technical Information Service, Toxicology Abstracts, and ToxFile. A summary of the historical basis for the GRAS determination of 2'-FL and any newly identified studies relevant to the safety of Inbiose's 2'-FL are provided below.

## 6.2 Absorption, Distribution, Metabolism and Excretion

As discussed previously, 2'-FL produced by microbial fermentation is structurally identical to the 2'-FL found in human milk and will be physiologically equivalent in terms of absorption, distribution, metabolism, and excretion. Therefore, the metabolism of this HMO, when added to infant formula, is expected to be identical to those of other HMOs naturally present in human breast milk.

The metabolism of HMOs, including 2'-FL, has been previously described in detail (U.S. FDA, 2015a,b, 2016, 2018a,b, 2019a,b, 2020, 2021e,f). Briefly, HMOs are resistant to enzymatic hydrolysis and are therefore not significantly digested in the upper gastrointestinal tract (Brand-Miller *et al*., 1998; Engfer *et al*., 2000; Rudloff and Kunz, 2012; EFSA, 2019).

Only minor structural changes of the HMOs were observed after *in vitro* digestion of HMOs using artificial gastric fluid (porcine intestinal brush border membranes within the physiologic range of incubation time, pH, and enzyme activity) (Gnoth *et al.*, 2000). As a result, intact 2'-FL can reach the large intestine, where it is partially metabolized by microbiota into short-chain fatty acids (Salli *et al*., 2019; Van den Abbeele *et al*., 2021). The effects of HMOs on gastrointestinal bacterial growth are bacterial strain- and HMO structure-dependent. Different growth patterns were observed for different bacteria strains when exposed to the same HMOs *in vitro* (Cheng *et al.*, 2021; Van den Abbeele *et al*., 2021). *Bifidobacteriaceae* were shown to be a major group of bacteria involved in the fermentation of 2'-FL (Bunesova *et al*., 2016; Van den Abbeele *et al.,* 2021). A large portion of undigested 2'-FL (ranging from 40 to 97%) was shown to be excreted in the feces (Chaturvedi *et al*., 2001; Coppa *et al*., 2001). Only a small fraction of neutral HMOs including 2'-FL was suggested to be transported transcellularly by receptor-mediated transcytosis, and/or by paracellular flux (Gnoth *et al*., 2001). Indeed, less than 1% of ingested 2'-FL was found to be systematically available in breast-fed infants or infants fed with formula supplemented with 2'-FL (Goehring *et al*., 2014; Marriage *et al*., 2015). A small fraction of absorbed HMOs was excreted unchanged or only slightly metabolized in the urine of breast-fed infants, at levels that correlate with their dietary intake from breast milk (Rudloff *et al*., 2012; Goehring *et al*., 2014).

## 6.3 Toxicological Studies

Pivotal data and information related to the safety of 2'-FL has been discussed previously and is hereby incorporated by reference to Part IV.B.5 of GRN 546, Section 6.3 of GRN 571, Section IV.E of GRN 650, Part 6 Section B.4 of GRN 735, and Section 6.C of GRN 932 (Glycom A/S, 2014, 2016; U.S. FDA, 2015a,b, 2016, 2018a, 2021f; Jennewein Biotechnologie GmbH, 2015; Glycosyn, LLC and Friesland Campina Domo B.V., 2017; Advanced Protein Technologies, Corp., 2020). Analytical data of Inbiose's 2'-FL product establish the ingredient as chemically identical to its 2'-FL counterpart in human breast milk (see Section 2.1.1). For this notification, the specification of Inbiose's 2'-FL product was compared with other high-purity 2'-FL products previously approved as GRAS (*i.e.*, GRN 546, GRN 571, GRN 650, GRN 735, GRN 749, GRN 897, GRN 932). Based on the carbohydrate analytical data, 2'-FL produced by Inbiose is of equal or greater purity when compared to other high-purity 2'-FL preparations that have previously been determined to be GRAS, and thus, the studies characterizing the toxicity and safety of 2'-FL in animal models are considered relevant to the safety assessment of Inbiose's ingredient (see Table 6.3-1).

No evidence of toxicity related to the administration of 2'-FL has been reported in a wide range of previous 2'-FL GRAS Notice submissions (GRN 546, GRN 571, GRN 650, GRN 735, and GRN 932). Several new studies were identified that had been conducted to evaluate the potential toxicological or genotoxic effects of 2'-FL since the most recent 2'-FL GRAS determination to receive a "no questions" letter from the FDA was prepared (*i.e.*, GRN 932); however, no data were identified that would affect the overall conclusion of safety for the 2'-FL ingredient, as established in previous GRNs.

Therefore, in the absence of any safety concerns from previously evaluated and newly identified preclinical safety and genotoxicity studies, it is reasonable to assume that Inbiose's high-purity 2'-FL ingredient (with comparable low levels of carbohydrate impurities to other high-purity 2'-FL products already considered to be GRAS) is safe for use in non-exempt term infant formulas at use levels up to 2.4 g 2'-FL/L and other food and beverage uses within the general population at levels up to 1.2 g/L or 40 g/kg, consistent with uses listed in other GRNs (GRN 546, GRN 571, GRN 650, GRN 735, GRN 749, GRN 897, GRN 932).

The specifications of Glycom A/S's (Glycom's) 2'-FL (GRN 546 and 650), Jennewein Biotechnologie, GmbH's (Jennewein's) 2'-FL (GRN 571), Glycosyn, LLC and Friesland Campina Domo B.V.'s (Glycosyn/FreislandCampina's) 2'-FL (GRN 735), DuPont Nutrition & Health's (DuPont's) 2'-FL (GRN 749 and 897), and Advanced Protein Technologies Corp.'s (APTech's) 2'-FL (GRN 932) are presented in Table 6.3-1 below for comparison (Glycom A/S, 2014, 2016; Jennewein Biotechnologie, GmbH, 2015; U.S. FDA, 2015a,b, 2016, 2018a,b, 2020, 2021f; Glycosyn, LLC and Friesland Campina Domo B.V., 2017; DuPont Nutrition & Health, 2017, 2019; Advanced Protein Technologies, Corp., 2020).

**Table 6.3-1 Product Specifications for Inbiose's 2'-FL in Comparison to Other High-Purity 2'-FL Preparations Used in Toxicological Studies Reported in GRNs 546, 571, 650, 735, 749, 897, and 932**

| Parameter | Proposed Specifications for Inbiose's 2'-FL from Microbial Fermentation with *E. coli* K-12 (MG1655 strain) | Specifications Reported for Other 2'-FL Products | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Glycom's 2'-FL from Chemical Synthesis (GRN No. 546) | Jennewein's 2'-FL from Microbial Fermentation with *E. coli* BL21 (DE3 strain) (GRN No. 571) | Glycom's 2'-FL from Microbial Fermentation with *E. coli* K12 (DH1 strain) (GRN No. 650) | Glycosyn/FC's 2'-FL from Microbial Fermentation with *E. coli* K12 (GI724 strain) (GRN No. 735) | DuPont's 2'-FL from Microbial Fermentation with *E. coli* K-12 (MG1655 strain) (GRN No. 749) | DuPont's 2'-FL from Microbial Fermentation with *E. coli* K-12 (MG1655 strain) (GRN No. 897) | APTech's 2'-FL from Microbial Fermentation with *C. glutamicum* (APC199 strain) (GRN No. 932) |
| ***Identification*** | | | | | | | | |
| Appearance (Color) | White | White to off-white | White to ivory | White to off-white | White | White to off-white/ivory | White/off-white | White to off-white/ivory |
| Appearance (Form) | Powder | Powder | Powder | Powder or agglomerates | Homogenous powder | Dry powder | Dry powder | Dry powder |
| Appearance in solution | Clear, colorless to slightly yellow | NS | NS | NS | NS | Clear, colorless to slightly yellow | NMT 300 ICUMSA | Clear, colorless to slightly yellow |
| Identity (2'-FL) | Conform to reference standard, 2'-FL derived from human milk | Retention time of main component corresponds to ± 3% | Chemical identity confirmed by NMR and LC-MS/MS | Retention time of main component corresponds to ± 3% | Chemical identity confirmed by NMR | Conform to reference standard, 2'-FL derived from human milk | Conform to reference standard, 2'-FL derived from human milk | Conform to reference standard, 2'-FL derived from human milk and synthetic 2'-FL |
| ***Chemical Specifications*** | | | | | | | | |
| Moisture | NMT 5.0% | NMT 9.0% | NMT 9.0% | NMT 5.0% | NMT 5.0% | NMT 9.0% | NMT 5.0% | NMT 9.0% |
| pH | 3.0 to 7.5 (20°C, 10% solution) | 3.0 to 7.5 (20°C in 5% solution) | NS | 3.2 to 5.0 (20°C in 5% solution) | 3.0 to 7.5 (10% solution) | NS | NS | NS |
| Protein | NMT 100 µg/g | 0.1% | NMT 100 µg/g | 0.01% | NMT 0.01% | NMT 100 µg/g | NMT 100 µg/g | NMT 100 µg/g |
| Ash (%) | NMT 0.5 | NMT 0.2 | NMT 0.5 | NMT 1.5 | NMT 0.2 | NMT 0.5 | NMT 0.5 | NMT 0.5 |
| Endotoxins (E.U./mg) | NMT 10 | NMT 50 | NMT 0.3 | NS | NMT 10 | NMT 0.3 | NMT 0.3 | NMT 0.1 |
| ***Carbohydrates (% DM)*** | | | | | | | | |
| 2'-FL | NLT 94% | NLT 95.0% | NLT 90.0% | NLT 94.0% | NLT 90.0% | NLT 82.0% | NLT 96.0% | NLT 94.0% |
| Sum of carbohydrates[a] | NS | NS | NS | NLT 96.0%[a] | NS | NS | NS | NS |
| Lactose | NMT 5.0% | NS | NMT 5.0% | NMT 3.0% | NMT 3.0% | NMT 8.0% | NMT 5.0% | NMT 5.0% |

**Table 6.3-1 Product Specifications for Inbiose's 2'-FL in Comparison to Other High-Purity 2'-FL Preparations Used in Toxicological Studies Reported in GRNs 546, 571, 650, 735, 749, 897, and 932**

| Parameter | Proposed Specifications for Inbiose's 2'-FL from Microbial Fermentation with *E. coli* K-12 (MG1655 strain) | Specifications Reported for Other 2'-FL Products | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Glycom's 2'-FL from Chemical Synthesis (GRN No. 546) | Jennewein's 2'-FL from Microbial Fermentation with *E. coli* BL21 (DE3 strain) (GRN No. 571) | Glycom's 2'-FL from Microbial Fermentation with *E. coli* K12 (DH1 strain) (GRN No. 650) | Glycosyn/FC's 2'-FL from Microbial Fermentation with *E. coli* K12 (GI724 strain) (GRN No. 735) | DuPont's 2'-FL from Microbial Fermentation with *E. coli* K-12 (MG1655 strain) (GRN No. 749) | DuPont's 2'-FL from Microbial Fermentation with *E. coli* K-12 (MG1655 strain) (GRN No. 897) | APTech's 2'-FL from Microbial Fermentation with *C. glutamicum* (APC199 strain) (GRN No. 932) |
| Fucose | NS | NS | NMT 3.0% | NMT 1.0% | NMT 2.0% | NS | NS | NMT 3.0% |
| Allo-lactose | NS | NS | NS | NS | NMT 2.0% | NS | NS | NS |
| Glucose | NS | NS | NMT 3.0% | NS | NMT 2.0% | NS | NS | NMT 3.0% |
| Galactose | NS | NS | NMT 3.0% | NS | NMT 2.0% | NS | NS | NMT 3.0% |
| Difucosyllactose (DFL) | NMT 5.0% | NS | NMT 5.0% | NMT 1.0% | NS | NMT 7.0% | NMT 5.0% | NMT 5.0% |
| 2'-Fucosyl-D-lactulose | NS | NS | NS | NMT 1.0% | NS | NS | NS | NS |
| 3-fucosyllactose | NS | NS | NMT 5.0% | NS | NS | NS | NS | NMT 5.0% |
| Fucosyl-galactose | NS | NS | NMT 3.0% | NS | NS | NS | NS | NMT 3.0% |
| Sum of other carbohydrates[b] | NMT 5.0%[b] | NS | NS | NS | NS | NMT 6.0%[b] | NMT 5.0%[b] | NS |
| ***Heavy Metals*** | | | | | | | | |
| Arsenic (mg/kg) | NMT 0.2 | NS | NMT 0.2 | NS | NMT 0.1 | NMT 0.2 | NMT 0.2 | NMT 0.1 |
| Cadmium (mg/kg) | NMT 0.05 | NS | NMT 0.1 | NS | NMT 0.01 | NMT 0.05 | NMT 0.05 | NMT 0.01 |
| Aluminum (mg/kg) | NS | NS | NS | NS | NMT 4.8 | NS | NS | NS |
| Lead (mg/kg) | NMT 0.05 | NMT 0.8 | NMT 0.02 | NMT 0.1 | NMT 0.05 | NMT 0.05 | NMT 0.05 | NMT 0.02 |
| Mercury (mg/kg) | NMT 0.5 | NS | NMT 0.5 | NS | NMT 0.05 | NMT 0.5 | NMT 0.5 | NMT 0.05 |
| ***Microbiological Contaminants*** | | | | | | | | |
| Total aerobic mesophilic plate count (CFU/g) | NMT 1,000 | NMT 500 | NMT 10,000 | NMT 500 | NMT 3,000 | NMT 1,000 | NMT 1,000 | NMT 500 |
| Yeast (CFU/g) | NMT 100 | NMT 10 | NMT 100[c] | NMT 10 | NMT 10 | NMT 100 | NMT 100[c] | NMT 100[c] |
| Mold (CFU/g) | NMT 100 | NMT 10 | - | NMT 10 | NMT 10 | NMT 100 | - | - |
| *Enterobacteriaceae* | Absent in 10 g | Absent in 10 g | Absent in 11 g | Absent in 10 g | Absent in 10 g | Absent in 10 g | Absent in 10 g | NS |
| *Salmonella* spp. | Absent in 25 g | Absent in 25 g | Absent in 100 g | Absent in 25 g | Absent in 25 g | Absent in 100 g | Absent in 750 g | Absent in 25 g |

**Table 6.3-1 Product Specifications for Inbiose's 2'-FL in Comparison to Other High-Purity 2'-FL Preparations Used in Toxicological Studies Reported in GRNs 546, 571, 650, 735, 749, 897, and 932**

| Parameter | Proposed Specifications for Inbiose's 2'-FL from Microbial Fermentation with *E. coli* K-12 (MG1655 strain) | Specifications Reported for Other 2'-FL Products | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Glycom's 2'-FL from Chemical Synthesis (GRN No. 546) | Jennewein's 2'-FL from Microbial Fermentation with *E. coli* BL21 (DE3 strain) (GRN No. 571) | Glycom's 2'-FL from Microbial Fermentation with *E. coli* K12 (DH1 strain) (GRN No. 650) | Glycosyn/FC's 2'-FL from Microbial Fermentation with *E. coli* K12 (Gl724 strain) (GRN No. 735) | DuPont's 2'-FL from Microbial Fermentation with *E. coli* K-12 (MG1655 strain) (GRN No. 749) | DuPont's 2'-FL from Microbial Fermentation with *E. coli* K-12 (MG1655 strain) (GRN No. 897) | APTech's 2'-FL from Microbial Fermentation with *C. glutamicum* (APC199 strain) (GRN No. 932) |
| *Cronobacter sakazakii* | Absent in 25 g | Absent in 10 g | Absent in 100 g | Absent in 10 g | Absent in 25 g | Absent in 100 g | Absent in 300 g | Absent in 10 g |
| *Listeria monocytogenes* | Absent in 25 g | Absent in 25 g | NS | Absent in 25 g | NS | Absent in 25 | Absent in 25 | NS |
| *Bacillus cereus* (CFU/g) | NMT 50 | NMT 50 | NS | NMT 50 | NMT 100 | NMT 10 | NMT 10 | NS |

2'-FL = 2'-fucosyllactose; CFU = colony forming units; E.U. = endotoxin units; GRN = Generally Recognized as Safe Notice; ICUMSA = International Commission for Uniform Methods of Sugar Analysis; LC-MS/MS = liquid chromatography with tandem mass spectrometry; NLT = not less than; NMR = nuclear magnetic resonance; NMT = not more than; NS = not specified.

[a] Sum of carbohydrates (also referred to as sum of human-identical milk saccharides in GRN 650) is defined as the sum of 2'-FL, lactose, difucosyllactose, and fucose.

[b] Sum of other carbohydrates, such as 3-fucosyllactose, 2-fucosyl-D-lactulose, fucosyl-galactose, glucose/galactose, fucose, sorbitol/galactitol, mannitol, and trihexose.

[c] Specification for yeast and mold is combined.

### 6.3.1 Genotoxicity Studies Conducted with Other 2'-FL Preparations

As described in Section 6.3 above, pivotal genotoxicity data related to 2'-FL has been previously evaluated in many GRNs. The product specifications defined for Inbiose's 2'-FL ingredient are considered to be comparable to other high-purity 2'-FL products previously approved as GRAS (see Table 6.3-1); thus, studies characterizing the genotoxicity of these other preparations are considered relevant to the safety assessment of Inbiose's ingredient. No evidence of genotoxicity related to 2'-FL has been reported in any previous 2'-FL GRN submission (*i.e.*, GRN 546, GRN 571, GRN 650, GRN 735, GRN 749, GRN 897, GRN 932). Studies conducted with a 2'-FL mixture with difucosyllactose (DFL) have also been included in the summary Table 6.3.1-1, below, as they were presented in GRN 815 (Glycom A/S, 2018; U.S. FDA, 2019a).

**Table 6.3.1-1 Summary of Genotoxicity Studies Conducted with 2'-FL Preparations Previously Concluded to be GRAS**

| Type of Study | Species and Strain or Cell Type | Length of Study | 2'-FL Concentration | Result | Reference |
|---|---|---|---|---|---|
| **Studies conducted with Glycom's 2'-FL (purity 99%) produced by chemical synthesis (GRN 546)** | | | | | |
| Bacterial reverse mutation test according to OECD TG 471 (OECD, 1997a) | *Salmonella* Typhimurium TA98, TA100, TA102, TA1535, and TA1537) | Plate incorporation (± S9) and pre-incubation methods (± S9) | 2'-FL dissolved in water up to 5,000 µg/plate (± S9) | 2'-FL was non-mutagenic under the conditions of this test. | Coulet *et al.* (2013) |
| *In vitro* mammalian cell gene mutation test according to OECD TG 476 (OECD, 1997b) | L5178Y tk +/- mouse lymphoma cells | Short-term treatment: 4 hours (± S9); Long-term treatment: 24 hours (- S9) | Short-term treatment: 492 to 5,000 µg/mL for 4 hours (± S9); Long-term treatment: 1.7 to 5,000 µg/mL for 24 hours (-S9) | 2'-FL was non-mutagenic in L5178Y tk +/- mouse lymphoma cells. | |
| **Studies conducted with Jennewein´s 2'-FL (purity 92.4%) produced by microbial fermentation (GRN 571)** | | | | | |
| Bacterial reverse mutation assay according to OECD TG 471 (OECD, 1997a) | *S.* Typhimurium TA98, TA100, TA102, TA1535, and TA1537 | Plate incorporation assay (± S9) and pre-incubation method (± S9) | 2'-FL dissolved in DMSO within the concentration range of 31.6 to 5,000 µg/plate (± S9) | 2'-FL is not cytotoxic or mutagenic under the conditions of this study. | Lauenstein, 2014a [unpublished]; Report No. 30512 (in: Appendix M2 of GRN 571) |
| *In vivo* bone marrow micronucleus test in rat, according to OECD TG 474 (OECD, 1997c) | Groups of 5 male and 5 female (Crl:CD(SD)) rats | 24 hours (vehicle and 3 dose groups) and 48 hours (vehicle control and high-dose-treated animals) | 2'-FL dissolved in vehicle (0.8% aqueous HPMC) at 500, 1,000 or 2,000 mg/kg bw, positive control (cyclophosphamide (27 mg/kg bw i.p.) | 2'-FL did not produce signs of systemic acute toxicity and was mutagenic nor genotoxic under the conditions of this study. | Lauenstein, 2014b [unpublished]; Report No. 30513 (in: Appendix M1 of GRN 571) |

**Table 6.3.1-1 Summary of Genotoxicity Studies Conducted with 2'-FL Preparations Previously Concluded to be GRAS**

| Type of Study | Species and Strain or Cell Type | Length of Study | 2'-FL Concentration | Result | Reference |
|---|---|---|---|---|---|
| **Studies conducted with Glycom´s 2'-FL (purity 97.6%) produced by microbial fermentation (GRN 650)** | | | | | |
| Bacterial reverse mutation assay according to OECD TG 471 (OECD, 1997a) | *S.* Typhimurium TA98, TA100, TA1535, and TA1537; *Escherichia coli* WP2uvrA (± S9) | Plate incorporation (± S9) and pre-incubation methods (± S9) | 2'-FL dissolved in water at concentration range of 52-5,000 µg/plate (plate incorporation) and 492-5,000 µg/plate (pre-incubation method) | 2'-FL was non-mutagenic under the conditions of this test. | Verspeek-Rip (2015) [unpublished], cited in: GRN 650 |
| *In vitro* micronucleus assay according to OECD TG 487 (OECD, 2016) | Peripheral human lymphocytes | Short-term treatment: 3 hours (± S9); Long-term treatment: 24 hours (- S9) | 2'-FL dissolved in water at concentrations of 512, 1,600, or 2,000 µg/mL in short- and long-term treatment | 2'-FL was non-clastogenic and non-aneugenic in human lymphocytes under the conditions of the assay. | Verbaan (2015) [unpublished], cited in: GRN 650 |
| **Studies conducted with Friesland Campina´s 2'-FL (purity 94%) produced by microbial fermentation (GRN 735)** | | | | | |
| Bacterial reverse mutation assay according to OECD TG 471 (OECD, 1997a) | *S.* Typhimurium TA1535, TA1537, TA98, TA100; *E. coli* WP2 uvrA (± S9) | Plate incorporation method | 2'-FL dissolved in PBS tested at the concentration range of 62 to 5,000 µg/plate | 2'-FL was non-mutagenic under the conditions of this test. | van Berlo *et al*. (2018) |
| *In vitro* micronucleus assay according to OECD TG 487 (OECD, 2016) | Peripheral human lymphocytes | Long-term treatment: 24 hours (- S9); Short-term treatment: 4 hours (± S9) | Long-term (24 hours - S9) and short-term treatment (4 hours ± S9): 2'-FL dissolved in culture medium (RPMI1640) at concentration range of 500, 1,000, and 2,000 µg/ml | 2'-FL was non-genotoxic under the conditions of this test. | |
| **Studies conducted with APTech's 2'-FL (purity ≥94%) produced by microbial fermentation (GRN 932)** | | | | | |
| Bacterial reverse mutation assay according to OECD TG 471 (OECD, 1997a) | *S.* Typhimurium TA1535, TA1537, TA98, TA100; *E. coli* WP2 uvrA (± S9) | 1st and 2nd main studies (± S9) – methods not specified | 2'-FL (vehicle type not specified) tested at the concentration range of 313-5,000 µg/plate | 2'-FL was non-mutagenic under the conditions of this test. | Hong, 2019a [unpublished] Report No. B18674; cited in: Appendix J of GRN 932 |
| *In vitro* Mammalian Chromosomal Aberration Test | Chinese hamster lung (CHL/IU) cells (± S9) | Long-term treatment: 24 hours (- S9); Short-term treatment: 6 hours (± S9) | 2'-FL dissolved in DMSO at concentrations of 1,250, 2,500, and 5,000 ug/mL | 2'-FL was non-clastogenic under the conditions of this study. | Hong, 2019b [unpublished] Report No. B18675; cited in: Appendix J of GRN 932 |

**Table 6.3.1-1 Summary of Genotoxicity Studies Conducted with 2'-FL Preparations Previously Concluded to be GRAS**

| Type of Study | Species and Strain or Cell Type | Length of Study | 2'-FL Concentration | Result | Reference |
|---|---|---|---|---|---|
| *In vivo* Micronucleus Test in ICR mice | Groups of 5 CrlOri:CD1(ICR), SPF male mice | Twice at 24- hour intervals | 2'-FL dissolved in saline tested at 0, 2,500 mg/kg, 5,000 and 7,500 mg/kg | All doses were well-tolerated, and no clinical signs were observed. 2'-FL did not induce micronuclei in the bone marrow cells of mice under the conditions of this study. | Hong, 2019c [unpublished] Report No. B18676; cited in: Appendix J of GRN 932 |
| **Studies Conducted with Glycom's 2'-FL/DFL mixture (containing 75% 2'-FL) mixture produced by microbial fermentation (GRN 815)** | | | | | |
| Bacterial reverse mutation assay according to OECD TG 471 (OECD, 1997a) | *S.* Typhimurium TA1535, TA1537, TA98, TA100; *E. coli* WP2 uvrA (± S9) | Plate incorporation (± S9) and pre-incubation methods (± S9) | 2'-FL/DFL mixture up to 5,000 µg/plate for both methods<br><br>(Test article contained only 75% 2'-FL by weight) | 2'-FL/DFL test article was non-mutagenic under the conditions of this test. | Phipps *et al.* (2018) |
| *In vitro* micronucleus assay according to OECD TG 487 (OECD, 2016) | Human lymphocytes | Long-term treatment: 24 hours (- S9); Short-term treatment: 3 hours (± S9) | Long-term (24 hours - S9) and short-term treatment (4 hours ± S9): 2'-FL/DFL mixture at concentration range of 500, 1,000, and 2,000 µg/mL<br><br>(Test article contained only 75% 2'-FL by weight) | 2'-FL/DFL test article was non-genotoxic under the conditions of this test. | |

+ S9 = with metabolic activation; - S9 = without metabolic activation; 2'-FL = 2'-fucosyllactose; bw = body weight; DFL = difucosyllactose; DMSO = dimethylsulfoxide; GRAS = Generally Recognized as Safe; GRN = GRAS Notice; HPMC = hydroxypropylmethylcellulose; i.p. = intraperitoneally; OECD = Organisation for Economic Co-operation and Development; PBS = phosphate-buffered saline; SPF = specific-pathogen-free; TG = Test Guideline.

In addition, 2 new tests of genotoxicity were identified in the updated search of the scientific literature for studies published after the submission of GRN 932. Both tests were conducted as part of the same study to evaluate the safety of an HMO mixture containing 2'-FL with lacto-*N*-fucopentaose I; however, the results from both tests do not affect the overall conclusion of safety established in previous GRAS Notices.

In the recently conducted bacterial reverse mutation test, the potential mutagenicity of an HMO mixture containing 2'-FL and lacto-*N*-fucopentaose I (LNFP-I) was evaluated *in vitro* with *S.* Typhimurium strains TA98, TA100, TA1535, and TA1537, or *E. coli* strain WP2 *uvrA* (pKM101) (Phipps *et al*., 2021). The evaluated mixture contained 31.5% (w/w) 2'-FL and 59.4% (w/w) LNFP-I and was tested at concentrations of 5.55, 16.65, 55.5, 166.5, 555, 1,665, or 5,550 µg/plate when corrected for LNFP-I and 2'-FL content (approximately 91%) of the test article by weight. No evidence of cytotoxicity or precipitate was observed in plate incorporation or preincubation assays, with or without metabolic activation. Mean revertant colony counts remained consistent across the test article groups and controls at any dose level, with or without metabolic activation. The authors therefore concluded that the test article (containing 31.5% 2'-FL w/w) was not mutagenic.

Additionally, an *in vitro* mammalian cell micronucleus test was conducted using the same HMO mixture (*i.e.*, containing 31.5% and 59.4% 2'-FL and LNFP-I, respectively) with human lymphocytes (Phipps *et al*., 2021). Concentrations ranging from 0.5 to 2,220 µg/plate of the HMO mixture were tested in the absence or presence of metabolic activation for short- (3 hours) and long-term exposures (20 hours). Micronucleus analysis and cytotoxicity were evaluated following the defined exposure periods. No evidence of cytotoxicity and no statistically significant increase in the percentage of micronucleated cells (relative to vehicle controls) were observed at any tested concentration. The authors concluded that the HMO mixture (containing 31.5% 2'-FL w/w) was not clastogenic nor aneugenic at the levels tested.

### 6.3.2 Toxicological Studies Conducted with 2'-FL Preparations Previously Concluded to be GRAS

As described in Section 6.3 above, pivotal toxicity data related to the 2'-FL ingredient has been evaluated in many previous GRNs. The product specifications defined for Inbiose's 2'-FL ingredient are considered to be comparable to other high-purity 2'-FL products previously approved as GRAS (see Table 6.3-1); thus, studies characterizing the toxicity of these other preparations are considered relevant to the safety assessment of Inbiose's ingredient. No test article related toxicity has been reported in any previous 2'-FL GRN submission (*i.e.*, GRN 546, GRN 571, GRN 650, GRN 735, GRN 749, GRN 897, and GRN 932). The toxicological studies in GRN 749 and 897 are briefly summarized below in Table 6.3.2-1, and the summaries are presented in the following sections. Studies conducted with a 2'-FL mixture with DFL (as reported in GRN 815) have also been included.

**Table 6.3.2-1 Summary of Toxicological Studies Conducted with 2'-FL Preparations Previously Concluded to be GRAS**

| Type of Study | Species and Strain | Length of Study | 2'-FL Dose and Route of Administration | Result | Reference |
|---|---|---|---|---|---|
| **Studies conducted with Glycom's 2'-FL (purity 99%) produced by chemical synthesis (GRN 546)** | | | | | |
| 14-day oral toxicity study | Groups of 5 male and 5 female Wistar Crl:WI (Han) rats | 14 days (PND 7 to 20) | 0, 2,000, 5,000, or 7,500 mg 2'-FL/kg bw/day, and 7,500 FOS mg/kg bw/day (reference item) | Due to marked clinical signs (transient lower body weight gain and liquid feces) as well as 2 unexplained deaths at 7,500 mg/kg bw/day, 6,000 mg/kg bw/day was the highest recommended dose in the subchronic study. | Coulet *et al.* (2013) |
| 90-day oral toxicity study according to OECD TG 408 (OECD, 1998) | Groups of 10 male and 10 female Wistar Crl:WI (Han) rats | At least 90 days (PND 7 to 97) | 0, 2,000, 5,000, or 6,000 mg 2'-FL/kg bw/day 2'-FL), and 6,000 mg/kg bw/day FOS (reference item) | 2'-FL was well-tolerated and did not elicit any adverse effects up to 5,000 mg/kg bw/day. The NOAEL of 5,000 mg/kg bw/day was considered. | |
| **Studies conducted with Jennewein's 2'-FL (purity from 94.1% to 97.9%) produced by microbial fermentation (GRN 571)** | | | | | |
| 7-day dietary toxicity pilot study | Groups of 10 four-week-old female (Crl:CD(SD)) rats | 7 days | Oral – dietary; 10% 2'-FL in feed and untreated diet *ad libitum* | 2'-FL was well-tolerated. No mortalities were observed and there were no adverse test item-related effects. 10% 2'-FL in diet was recommended for the subchronic treatment | Leuschner, 2014 [unpublished] Report No. 30566; (in: GRN 571: Appendix M3) |
| 90-day oral diet rat study according to OECD TG 408 (OECD, 1998) | Groups of 10 4-week-old male and female rats (Crl:CD(SD)); Additional groups of 3 and 9 animals per sex included in the control (0%) and treatment (10%) groups, respectively, used exclusively for blood sampling | 90 days | Oral – dietary; 10% 2'-FL in feed and untreated diet *ad libitum* | NOAEL of 7,660 mg/kg bw/day (corresponding to 10% dietary concentration of 2'-FL) was determined. | Hansen, 2014 [unpublished]; Report No. 30514 (in: Appendix M2 of GRN 571) |

**Table 6.3.2-1 Summary of Toxicological Studies Conducted with 2'-FL Preparations Previously Concluded to be GRAS**

| Type of Study | Species and Strain | Length of Study | 2'-FL Dose and Route of Administration | Result | Reference |
|---|---|---|---|---|---|
| 3-week pre-clinical study of 2'-FL in farm piglets | 27 male and 21 female domestic Yorkshire crossbred swine farm piglets | 20 days from Day 2 of lactation | Oral – dietary; 0, 200, 500, or 2,000 mg/L corresponding to doses of 0, 29.37, 72.22, or 291.74 mg/kg bw/day in males and 0, 29.30, 74.31, and 298.99 mg/kg bw/day in females, respectively | The dietary exposure to 2'-FL at concentrations up to 2,000 mg/L (up to 292 mg/kg bw/day in males and 299 mg/kg bw/day in females) was well-tolerated and supported normal growth patterns in neonatal piglets with no adverse effects. | Hanlon and Thorsrud, 2014 [published] |
| **Studies Conducted with Glycom's 2'-FL (purity 97.6%) produced by microbial fermentation (GRN 650)** | | | | | |
| 90-day oral toxicity study according OECD TG 408 (OECD, 1998) | Groups of 10 male and 10 female Wistar Crl:WI (Han) rats | Oral gavage from PND 7 till at least PND 97 | 0 (water), 2,000, 4,000, or 5,000 mg 2'-FL/kg bw/day, or the reference compound, FOS, at a dose concertation of 5,000 mg/kg bw/day | NOAEL of 5,000 mg/kg bw/day, the highest dose tested, was determined. | Penard, 2015 [unpublished] (cited in: GRN 650) |
| **Studies Conducted with Friesland Campina's 2'-FL (purity 94%) produced by microbial fermentation (GRN 735)** | | | | | |
| 90-day oral diet rat study according to OECD TG 408 (OECD, 1998) | Groups of 10 male and 10 female Crl:WI(Han) rats | 90 days | Oral – dietary; 0% (controls), 3%, 6%, and 10% (2'-FL w/w) *ad libitum* | NOAEL of ≥7,250 and ≥7,760 mg 2'-FL /kg bw/day for males and females, respectively (corresponding to 10% dietary concentration of 2'-FL), was determined. | van Berlo *et al.*, 2018 |
| **Studies Conducted with APTech's 2'-FL (purity ≥94%) produced by microbial fermentation (GRN 932)** | | | | | |
| Single oral dose toxicity study in juvenile rats | Groups of 5 male and female neonatal/juvenile Sprague-Dawley Crl:CD(SD) rats | Oral gavage on PND 7 | 0 (water) 2,500, 5,000, and 7,500 mg 2'-FL/kg bw | One death at 7,500 mg/kg bw was considered as natural death. The body weight gain was significantly suppressed (males at 7,500 mg/kg). No test item-related gross findings at any dose level. The $LD_{50}$ was concluded to be greater than 7,500 mg/kg bw. | Han, 2019a [unpublished] Report No. B18672 (cited in: Appendix J of GRN 932) |

**Table 6.3.2-1 Summary of Toxicological Studies Conducted with 2'-FL Preparations Previously Concluded to be GRAS**

| Type of Study | Species and Strain | Length of Study | 2'-FL Dose and Route of Administration | Result | Reference |
|---|---|---|---|---|---|
| 90-day repeated oral dose toxicity study with a 4-week recovery period in juvenile rats | Groups of 10 male and female neonatal/juvenile Sprague-Dawley Crl:CD(SD) rats; Additional recovery groups of 5 male and female rats received 0 or 7,500 2'-FL mg/kg bw/day | Oral gavage from PND 7 for 90 days | 0 (water), 2,500, 5,000, or 7,500 mg 2'-FL/kg bw/day | NOAEL of 7,500 mg/kg bw/day, the highest dose tested, was determined. | Han, 2019b [unpublished] Report No. B18673 (cited in: Appendix J of GRN 932) |
| **Studies conducted with Glycom's 2'-FL/DFL mixture (containing 75% 2'-FL) produced by microbial fermentation (GRN 815)** | | | | | |
| 14-day repeated oral dose toxicity study in neonatal rats | Groups of 8 male and female neonatal rats [strain NR] | Oral gavage for 14 days | 0 (water), 4,000, or 5,000 mg/kg bw/day<br><br>(Test article contained only 75% 2'-FL by weight) | No test item related deaths. One death at 5,000 mg/kg bw/day was considered incidental. NOAEL of 5,000 mg/kg bw/day, the highest dose tested, was determined. | Flaxmer, 2017 Report No. RW47RS (cited in: Section 6.3.4 of GRN 815) |
| 90-day repeated oral dose toxicity study with a 4-week recovery period in juvenile rats | Groups of 10 male and female neonatal/juvenile Sprague-Dawley Crl:CD(SD) rats; Additional recovery groups of 5 male and female rats received 0 or 5,000 mg test article/kg bw/day | Oral gavage from PND 7 for 90 days | 0 (water), 1,000, 3,000, or 5,000 mg/kg bw/day<br><br>(Test article contained only 75% 2'-FL by weight) | NOAEL of 5,000 mg/kg bw/day, the highest dose tested, was determined. | Phipps *et al*. (2018) |

2'-FL = 2'-fucosyllactose; bw = body weight; DFL = difucosyllactose; FOS = fructo-oligosaccharide; GRAS = Generally Recognized as Safe; GRN = GRAS Notice; $LD_{50}$ = median lethal dose; NOAEL = no-observed-adverse-effect level; NR = not reported; OECD = Organisation for Economic Co-operation and Development; PND = Postnatal Day; TG = Test Guideline.

In addition to the previously evaluated studies, 2 new toxicological studies conducted with 2'-FL were identified in the updated search of the scientific literature for studies published after the submission of GRN 932. No toxicologically relevant findings were reported in the newly identified literature and therefore these studies do not affect the overall conclusion of safety established in previous GRAS Notices. The 2 new studies are briefly summarized below.

In a 21-day study conducted with 2-day old neonatal piglets, an HMO mixture [containing 2'-FL, 3-FL, LNT, 3'-sialyllactose (3'-SL), and 6'-sialyllactose (6'-SL)] was administered at dose levels of either 5.75 or 8.0 g/L and the following safety endpoints were measured: mortality, clinical observations, body weight, food consumption, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), gross necropsy findings, organ weights, and histopathologic examinations (Hanlon, 2020). The evaluated HMO mixture contained 49.1% 2'-FL by weight. All animals aside from 1 male piglet survived to the end of the dosing period. No toxicologically-relevant effects were noted in any piglets who received the liquid diet containing the HMO test article, relative to the control animals, and the author therefore concluded that the study supports the safety of this HMO mixture (containing 2'-FL) added to infant formula products.

In a subchronic toxicity assessment of a novel HMO mixture containing 2'-FL and LNFP-I (31.5% and 59.4% 2'-FL and LNFP-I, respectively), Sprague-Dawley rats were administered the test substance at dose levels of 1,000, 3,000, or 5,000 mg/kg body weight/day *via* gavage for a 90-day test period (Phipps *et al*., 2021). The low- and mid-dose groups contained 10 males and 10 females per group, whereas the high-dose, vehicle control, and reference control groups contained 15 males and 15 females each. General clinical observations of morbidity and mortality were conducted twice daily and ophthalmic observations were made during the final dosing week. Body weight and food consumption were monitored throughout the dosing period, and physiological development was assessed using a functional observational battery test that included grip strength, auditory function, visual function, and spatial learning during the 12$^{th}$ week of dosing. Hematological, coagulation, and clinical biochemistry parameters were measured in blood samples collected at the end of the dosing period. Similarly, urine samples were collected for urinalysis at the end of the dosing and recovery periods. The rats were then weighed, killed, and necropsied, and individual organs were weighed before histopathological analysis. Nine deaths occurred throughout the study; however, as the majority of these deaths occurred in the reference control group (5), followed by the 2 that occurred in the mid-dose group, the 1 in the high-dose group, and the 1 that was attributed to a dosing error, the deaths were not considered to be related to the administration of the test article. No biologically relevant changes were observed in body weight, food consumption, or physiological development. No test-article related adverse effects were observed at any dose level, and therefore the authors concluded that the no-observed-adverse-effect level for the 2'-FL and LNFP-I mixture is 5,000 mg/kg body weight/day.

## 6.4 Human Studies

### 6.4.1 Previously Reviewed Clinical Studies of 2'-FL

Pivotal safety data and information provided from studies of the addition of 2'-FL to infant formula have been discussed previously and are hereby incorporated primarily by reference to Section VIII Part 6.B of GRN 749, Section 6.3 of GRN 897, and Section 6.C.5 of GRN 932 (U.S. FDA, 2018b, 2020, 2021f). Based on analytical data presented demonstrating that 2'-FL produced by Inbiose is of equal or greater purity to other 2'-FL preparations previously determined to be GRAS, studies characterizing the safety of these 2'-FL preparations in humans are considered relevant to the safety assessment of Inbiose's ingredient.

The results confirm that 2'-FL is well-tolerated in infants when provided at concentrations within the normal range measured in human milk. 2'-FL also is well-tolerated in health adult subjects. Adverse effects of high intakes of 2'-FL are similar to those observed with other sources of dietary fiber (*e.g*., gastrointestinal discomfort) and is self-limiting.

The clinical studies previously included in GRNs 546, 571, 650, 735, 749, 815, 852, 897, 929, and 932 are briefly summarized below in Table 6.4.1-1.

**Table 6.4.1-1 Summary of Human Studies to Support the Safety of Inbiose's 2'-FL**

| Type of Study | Population | Length of Study | Dose | Result | Reference |
|---|---|---|---|---|---|
| Prospective, controlled, growth and tolerance Study, R | 254 term infants enrolled by Day of Life 5, and exclusively fed with formula or human milk since birth | 4 months (enrolled by Day of Life 5 until 119) | Formula containing:<br>• 2.4 g /L GOS (n=68) (control formula)<br>• 0.2 g/L 2'-FL and 2.2 g/L GOS (n=62)<br>• 1.0 g/ L 2'-FL and 1.4 g/L of GOS (n=59)<br><br>Breastfed reference group (n=65) | No significant difference between groups in the percentage of study participants that experienced adverse events.<br><br>The formulas were well-tolerated and that there was comparable average stool consistency, number of stools per day, and percent of feedings associated with spitting up or vomiting. | Marriage *et al.* (2015) |
| DB, R, PC, PD | 100 healthy adult volunteers | 2 weeks | Single daily doses of 2'-FL, LNnT, or a combination of 2'-FL and LNnT at a ratio of 2:1 at 5, 10, or 20 g/day<br><br>Single daily dose of 2'-FL or LNnT alone (at 5, 10, or 20 g/day), or a combination of 2'-FL and LNnT (5, 10, or 20 g/day at a ratio of 2:1)<br><br>Placebo: Glucose | No clinically relevant changes in hematological or biochemical parameters were observed.<br><br>2'-FL was well-tolerated, and no changes in bowel habits *versus* control were noted.<br><br>The results support that 2'-FL is safe and well-tolerated in healthy adults. | Elison *et al.* (2016) |
| Blinded, controlled, R, MC, PD | 175 healthy, full-term infants (0 to 6 months of age) | Time of enrollment until 12 months of age | Standard infant formula supplemented with 2'-FL (at a target concentration of 1.0 g/L reconstituted formula) in combination with LNnT (at a target concentration of 0.5 g LNnT/L reconstituted formula) | The results indicated that formula containing 2'-FL and LNnT was safe and well-tolerated, and supported age-appropriate growth. | Puccio *et al.* (2017) |
| Prospective, DB, R, PC, MC | 131 healthy term infants | 5 weeks | Infant formula containing either 0 (formula control), 0.2 g 2'-FL/L and 0.2 g scFOS/L, or human breast milk (control) | The results indicated that infant formula containing 2'-FL was safe and well-tolerated and bared no significant difference from the human breast milk group. | Kajzer *et al.* (2016); Reverri *et al.* (2018) |

**Table 6.4.1-1 Summary of Human Studies to Support the Safety of Inbiose's 2'-FL**

| Type of Study | Population | Length of Study | Dose | Result | Reference |
|---|---|---|---|---|---|
| Cross-over DB, PC, FC | 67 infants (2 months to 4 years of age) with documented cow milk protein allergy | 1 week (7 to 9 days) | A formula supplemented with 1.0 g/L 2'-FL and 0.5 g/L LNnT; minimum of 240 mL daily<br><br>Control: A formula (hypoallergenic, whey-based, extensively hydrolyzed formula without HMOs) | The results indicated that 2'-FL does not provoke allergic responses in cow milk protein allergy infants. | Nowak-Wegrzyn *et al.* (2019) |
| DB, R, PC, MC | 63 infants (14 days of age) | 6 weeks | A formula of partially-hydrolyzed, 100% whey protein with 0 or 0.25 g 2'-FL/L per day | The results indicated that formula containing 2'-FL was safe and well-tolerated. | Storm *et al.* (2019) |

2'-FL = 2'-fucosyllactose; DB = double-blind; FC = food challenge; GOS = galacto oligosaccharides; HMO = human milk oligosaccharide; LNnT = lacto-*N*-neotetraose; MC = multi-center; PC = placebo-controlled; PD = parallel design; R = randomized; scFOS = short-chain fructo-oligosaccharides.

## 6.4.2 Newly Identified Clinical Studies of 2'-FL

Eleven new clinical studies of 2'-FL, or HMO mixtures containing 2'-FL, were identified in the updated search of the scientific literature for studies published after the submission of GRN 932. No evidence of toxicity related to the oral consumption of 2'-FL was reported in the additional identified studies. Therefore, none of the identified studies summarized in the sections below are expected to affect the overall conclusion of safety established in previous GRNs.

### *6.4.2.1 Studies Conducted with 2'-FL*

In a non-randomized, single-group, multicenter study conducted in infants (0 to 60 days of age) with suspected food protein allergy or persistent feeding intolerance, participants were provided a hypoallergenic casein-based powdered extensively hydrolyzed formula containing 0.2 g 2'-FL/L for 2 months (Ramirez-Farias *et al*., 2021). Thirty-six infants completed the study and changes in primary outcomes were compared to measured values at enrollment (baseline). The primary outcomes of growth (weight, length, and head circumference), formula intake, tolerance measures, and clinical symptoms were assessed. A statistical improvement in weight-for-age z-score was observed over the course of the study. Persistent clinical symptoms either improved, resolved, or remained the same as they were at test initiation following 60 days of 2'-FL administration. Adverse events were observed in 15 infants throughout the study; however, these were generally mild and were not deemed related to the test article by the study investigators. The study authors concluded that 2'-FL, when added to extensively hydrolyzed infant formula, was safe and well-tolerated.

The safety of 2'-FL when used in young children formulas was evaluated as part of a study conducted by Leung *et al.* (2020) to investigate the effects of 3 different formulations on respiratory and gastrointestinal infections in toddlers. In this randomized, controlled, double-blind, parallel-group clinical trial, 2 independent groups of subjects (labelled YCF-A and YCF-C; n=114) received 2 different young children formulae containing 3.0 g 2'-FL/L for a test duration of 6 months. While the primary outcome measured as part of this study were upper respiratory and gastrointestinal tract infections, anthropometric data (*i.e.*, weight-for-age and height-for-age z-scores) for common growth metrics were also measured. No changes in anthropometric data were observed in toddlers who received young children formulae supplemented with 3.0 g 2'-FL/L. Additionally, adverse event occurrence was similar for each young children formulae relative to the reference formula group. The study authors concluded that the young children formulae tested, including those that contained 3.0 g 2'-FL/L, are safe and well-tolerated for use in toddlers.

In a pilot clinical trial included as part of a larger study on the effect of 2'-FL on gut microbiota composition, 20 adult participants (12 completed the study), who had previously been diagnosed with irritable bowel syndrome (IBS), ulcerative colitis, Crohn's disease, or celiac disease, were provided with 40 g of a nutritional formula (containing 2 g 2'-FL) to be consumed twice daily in a reconstituted beverage for 6 weeks for a dose of 4.0 g 2'-FL/day (Ryan *et al*., 2021). Participants were asked to complete questionnaires to characterize their individual gastrointestinal quality of life, inflammatory bowel disease, and digestive system frequency at baseline and at the end of the 6-week test period. Following the test administration period, participants reported an overall improved gastrointestinal quality of life (exceeding the minimal clinically important difference; 15.1 units on the Gastrointestinal Quality of Life index total score) and no serious test-article related adverse events. The presented data indicate that 2'-FL is safe for use in adult populations with chronic gastrointestinal conditions.

#### *6.4.2.2 Studies Conducted with HMO Mixtures Containing 2'-FL*

In an open-label prospective study, healthy term infants (7 days to 2 months of age) were separated into 3 groups and were exclusively breastfed (n=45), fed commercial 100% whey infant formula containing both 1.0 g/L 2'-FL (n=63) and 0.5 g/L lacto-*N*-neotetraose (LNnT) and *Lactobacillus reuteri* (DSM 17938), or fed a mixture of trial formula and breast milk (n=48) for 8 weeks (Román Riechmann *et al*., 2020). Primarily, measures of growth (*i.e*., weight, length, and head circumference) and gastrointestinal tolerance (assessed *via* an Infant Gastrointestinal Symptom Questionnaire) were monitored. Any adverse events that occurred throughout the study were also recorded and assessed for duration, intensity, and frequency. No significant differences were observed in anthropometric growth measures, gastrointestinal tolerance, or adverse events at the end of the 8-week test period between the 3 infant groups. Infant formula containing 1.0 g 2'-FL/L (and 0.5 g LNnT/L) was reported to be well-tolerated by the study authors.

In a prospective, open-label, single-arm clinical trial study of treatment options for IBS, 245 adults with a clinical IBS diagnosis who fulfilled the Rome IV criteria[4] for IBS were provided with 5 g of Glycom's 2'-FL/LNnT mixture (97% purity; 4:1 mass ratio) daily over a 12-week intervention period (Palsson *et al*., 2020). Abnormal bowel movements (as measured by the Bristol Stool Form Scale) were measured as the primary outcome of the study. Severity and/or frequency of abdominal pain, bloating, gastrointestinal symptoms, or IBS-related quality of life was compared to baseline levels for each participant. Any adverse events were also noted throughout the course of the study. Frequency of abnormal stool consistency was significantly decreased from baseline following the 12-week intervention. The severity of IBS symptoms experienced by participants who received the HMO mixture was also significantly reduced relative to

[4] https://theromefoundation.org/rome-iv/rome-iv-criteria/

baseline levels. Common side effects of mild gastrointestinal symptoms such as flatulence, abdominal pain and discomfort, and distension were reported throughout the study; however, no serious adverse events were considered to be related to the intervention. The study participants reported that the intervention was well-tolerated, and no safety concerns were identified with its use.

In the subsequent study, the effect of human milk oligosaccharide (Glycom's 2'-FL/LNnT blend) supplementation on adults with IBS was investigated in a parallel, randomized, double-blind, and placebo-controlled study (Iribarren *et al*., 2020). Sixty adult patients (male and female) with IBS symptoms of at least moderate severity were provided placebo (powdered glucose), 5 g 2'-FL/LNnT, or 10 g 2'-FL/LNnT for daily consumption over a 4-week treatment period. The HMO mixture contained 2'-FL and LNnT in a 4:1 ratio. Measurements were taken of body weight and height at baseline and upon test completion, and any adverse events or changes in medication and diet were recorded. Patients were also required to complete a questionnaire to assess severity of gastrointestinal and psychological symptoms. No significant differences were observed between treatment groups related to the severity of gastrointestinal symptoms or their occurrence. No significant adverse events were reported in the study. Thus, daily intake of 5 and 10 g 2'-FL/LNnT was concluded by the study authors to be well-tolerated by adults with IBS.

The effects of HMO supplementation on feeding tolerance, growth, and safety in preterm infants was investigated in a double-blind, placebo-controlled trial conducted with a liquid HMO supplement comprising of 2'-FL and LNnT in a 10:1 ratio (Hascoët *et al*., 2021 [abstract only]). Groups of 43 preterm infants (23 to 33 weeks' gestation) received either 0.374 g/kg body weight/day of the HMO supplement or 0.140 g/kg body weight/day of a glucose placebo. The primary measured outcome of this study was feeding tolerance, which was measured *via* days to reach full enteral feeding from birth. Secondarily, Fenton growth standards were used to calculate anthropometric z-scores, and gastrointestinal tolerance parameters (including adverse events) were also measured. There was no significant difference between the two groups in any of the measured parameters, aside from increased length-for-age and head circumference-for-age z-scores in the HMO test groups. No statistical difference between the groups was noted for gastrointestinal tolerance measures or adverse events, and the researchers concluded that HMO supplementation is safe and well-tolerated.

As a component of a prospective, randomized, double-blinded, placebo-controlled clinical trial to evaluate the effect of HMOs on intestinal bacteria, Fonvig *et al.* (2021) evaluated the safety of daily HMO intake in young overweight children (age 6 to 12) over an 8-week test period. Groups of 25 children at test initiation were provided with 4.5 g/day of either a placebo, 2'-FL/day, or a 4:1 2'-FL/LNnT mixture. Safety was assessed *via* the measurements of a range of biochemical markers (*i.e.*, of inflammatory blood markers, gut barrier integrity proteins, metabolic biomarkers) and occurrence of adverse events or gastrointestinal symptoms. There were no significant differences measured in any of the biochemical parameters and the occurrence of adverse events. Neither test article was observed to induce digestive tolerance issues as assessed by the Gastrointestinal Symptoms Rating Scale. Minor differences in bloating were noted between groups at the trial's midway point, which were considered by the authors to be clinically irrelevant. The authors therefore concluded that 2'-FL administered either on its own or in a mixture containing LNnT is safe and well-tolerated in young children.

In a double-blind, randomized, controlled, multi-country trial of infant formula supplemented with 2'-FL and 3'-galactosyllactose (3'-GL), Vandenplas *et al.* (2020) provided 215 formula-fed infants (≤14 days of age) with either a partially fermented infant formula (control) or the control formula containing 100 mg 2'-FL/100 mL and 15 mg 3'-GL/100 mL until they reached 17 weeks of age. A group of fully breastfed infants were included as an additional reference group. Weight gain was the primary parameter of interest in this study; however, other growth parameters (*i.e.*, length, head circumference, and anthropometric z-scores), gastrointestinal tolerance, and safety outcomes (*i.e.*, adverse events) were also measured. No significant difference was noted between the infants from either test group in any of the measured parameters, and the study authors concluded that 2'-FL and 3'-GL are safe and well-tolerated in healthy term infants.

In a multicenter study conducted to evaluate the safety of a diverse blend of 5 HMOs in term infant formula, 686 healthy formula-fed infants (ranging from ≥7 to ≤21 days old) were provided with infant formula containing 0, 1.5, or 2.5 g HMOs/L until the participants were 6 months of age. The 5-HMO blend contained 2'-FL, 2',3-difucosyllactose, lacto-neotetraose (LNT), 3'-SL, and 6'-SL at unspecified levels (Bauer *et al.*, 2021 [abstract only]). Ninety-six breastfed infants were included as an additional reference group. Measured parameters included weight gain, anthropometric measures, stooling pattern, gastrointestinal tolerance, and adverse events. Weight gain was considered non-inferior (*i.e.*, lower bound of the 95% confidence interval was above the non-inferiority margin of -3 g/day) in the test groups relative to the control group. All other measured parameters were comparable between the test, control, and breastfed reference groups. The blend of 5 HMOs was concluded to be safe and well-tolerated as well as supportive of age-appropriate growth.

The tolerability, safety, and effect on growth of an HMO mixture was recently evaluated in a randomized, multicenter, controlled, parallel nutritional noninferiority study conducted with term infants (Parshat *et al*., 2021). Two hundred and twenty-five healthy term infants were randomly assigned to groups who received infant formula either without (control) or with a mixture containing 5 HMOs (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). An additional group of breastfed infants was included as a reference group. Measured growth parameters included weight, length, and head circumference. Safety was measured *via* occurrence of adverse events, while tolerability and behavioral parameters were measured *via* stool frequency and consistency, gurgitation, vomiting, and flatulence, and fussiness, crying, and awakening at night. No significant difference was observed in any of the tolerability, safety, or growth parameters, aside from softer stool and increased stool frequency in infants who received the HMO mix. The authors concluded that the HMO mixture is safe and well-tolerated by healthy term infants.

In a very recently published study conducted by Vandenplas *et al.* (2022), full-term infants aged 0 to 6 months with physician-diagnosed cow's milk protein allergy (CMPA) received 100% whey-based extensively hydrolyzed formula (EHF) supplemented with 2'-FL at a concentration of 1.0 g/L and LNnT at 0.5 g/L. This study was designed as a controlled, double-blind, randomized, multicenter, interventional clinical trial. The control formula group received EHF without the addition of HMOs. Body weight, length, and head circumference were measured monthly for 4 months (primary study endpoint), after 6 months, and at the age of 12 months. HMO-supplemented formula was observed to support normal growth in infants with CMPA. No significant group differences in anthropometric parameters were noted, and therefore, both formulas were considered safe and well-tolerated by the study authors.

## 6.5 Allergenicity

To determine if proteins from the newly introduced recombinant protein sequences in production host INB-2FL_03 could be excreted into the extracellular space during 2'-FL production, bioinformatic analysis of the introduced protein sequences was conducted to check for potential signal peptide (SignalP) sequences targeting for protein excretion (SignalP 5.0; Armenteros *et al*., 2019). Since no SignalP sequences were identified in the introduced sequences, none of the resultant recombinant proteins could be identified as an excreted protein. In addition, the complete cell debris is separated from the final 2'-FL product during the various purification steps included in 2'-FL manufacturing.

The potential allergenic activity of the newly introduced recombinant proteins in *E. coli K-12 MG1655*, introduced to obtain production host INB-2FL_03, was assessed by using the Allergen Online Tool (V21, released on 14 February 2021) of the University of Nebraska – Lincoln (FARRP, 2021). The most current version of the database contained 2233 putative allergen sequences. Potential allergenicity was evaluated by scanning each possible 80-amino acid segment of the recombinant protein (sliding window) to the database, and therefore looking for matches of at least 35% identity. No sequence alerts from potential allergens were identified for the recombinant proteins in INB-2FL_03.

Since lactose is used as substrate in the 2'-FL production process and small amounts of residual lactose are present in the final product, the label “contains milk”, in accordance with the requirements of the *Food Allergy, Labelling and Consumer Protection Act of 2004*, must be added (U.S. FDA, 2004).

## 6.6 General Recognition

As discussed, the use of 2'-FL as an ingredient in non-exempt term infant formula at levels up to 2.4 g/L and in various conventional food products has been evaluated by multiple experts, qualified through scientific training and experience, in the safety evaluation of food and infant formula ingredients (GRNs 546, 571, 650, 735, 749, and 897). The use of 2'-FL in infant formula at concentrations up to 1.2 g/L (alone or in combination with other HMOs) and various food products also has been the subject of comprehensive evaluations by multiple authoritative bodies, including EFSA (EFSA, 2015a,b, 2019). As Inbiose has demonstrated that 2'-FL manufactured by the company is qualitatively and quantitatively highly similar to 2'-FL ingredients that have been the subject of previous GRAS evaluations and global novel food approvals, and is intended for use in the same foods and at the same use levels as those concluded to be GRAS, conclusions on the safety of 2'-FL for these uses issued by various experts and scientific bodies forms a basis for general recognition of Inbiose’s GRAS conclusion. Convening of a GRAS Panel was therefore not considered necessary to support a GRAS conclusion on the basis that this HMO ingredient has been evaluated by multiple GRAS Panels and authoritative bodies, including the FDA and EFSA.

## 6.7 Conclusion

Based on the above data and information presented herein, Inbiose has concluded that 2'-FL is GRAS, on the basis of scientific procedures, for use in non-exempt term infant formula and specified conventional food and beverage products as described in Section 1.3.

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

# Part 7. § 170.255 List of Supporting Data and Information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March 17, 2023

Ellen T. Anderson
Regulatory Review Scientist
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration

**Regarding: Response to FDA Questions related to GRAS Notice No. GRN 001091**

Dear Dr. Anderson,

In reference to your letter dated March 6, 2023, regarding Inbiose's GRAS Notice GRN 001091 for the intended uses of 2'-fucosyllactose (2'-FL), I am pleased to provide you with our responses to the Agency's questions in the following document.

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

Kind regards,

Joeri Beauprez
Chief Scientific Officer

**Question 1.** The intended uses of 2′-FL described in the notice include use in non-exempt infant formula for term infants. Please clarify the intended source of the infant formula protein base (e.g., milk, soy, whey) into which 2′-FL would be added.

Inbiose is the bulk ingredient manufacturer of this product, and therefore does not control the specific protein source that infant formula manufacturers may choose to use during the formulation of end use products. Protein sources used in the manufacture of infant formulae are defined by the infant formula manufacturer. Therefore, it is reasonable to expect that Inbiose's HMO ingredients, including 2′-FL, may be used in any of the available protein bases (*e.g.*, milk, soy, whey) that are currently used to manufacture non-exempt infant formula products.

**Question 2.** The intended use level for "formula intended for pregnant women" is listed as 60 g/kg (Table 1.3-1, page 4) with a citation to GRN 000735. We note that the use level for this category in GRN 000735 was 0.6 g/100g, which would be equivalent to 6 g/kg. Please clarify Inbiose's intended use level for this category.

Thank you for the opportunity to clarify. The intended use level for "formula intended for pregnant women" was erroneously listed as 60 g/kg (Table 1.3-1, page 4 of this GRN 001091). Inbiose clarifies that the intended use level for "formula intended for pregnant women" is **6.0** g/kg, which is consistent with the use level of the same food category in GRN 000735.

**Question 3.** We note that the specified maximum level of arsenic presented in Table 2.3.1-1 (≤ 0.2 mg/kg, page 14) does not match the specified maximum in Table 2.3.2-1 (≤ 0.02 mg/kg, page 15). Please provide a correction for the arsenic specification.

Further, we note that the batch results for the three lots of 2′-FL provided in your notice demonstrate that arsenic levels are consistently below 0.01 mg/kg. Therefore, we request that specifications be as low as possible and reflect results of batch analyses for an ingredient produced in accordance with current GMPs.

Thank you for indicating this inconsistency between Tables 2.3.1-1 and 2.3.2-1. The arsenic value indicated in Table 2.3.2-1 (i.e. ≤ 0.02 mg/kg) is incorrect. The proposed maximum level for arsenic is ≤0.2 mg/kg (as presented in Table 2.3.1-1, page 14 of the GRN 001091).

Inbiose highlights that the arsenic specification proposed for this GRAS notice (i.e. ≤0.2 mg/kg) is in line with other HMOs that have been notified to the U.S. FDA without objection from the agency (e.g. GRNs: 001016, 001015, 001014, 000951, 000929, 000925, 000923, 000922). The arsenic specifications reported in these GRN notices are specified in Table 1 below.

In addition, the recent applications GRN 001059, 001037, 001035 and 001034 do not provide specification limits for arsenic (see Table 1), but only provides a specification for lead.

**Table 1.** Arsenic and Mercury specifications reported in previous GRN notices#

| GRN no. | Date of filing | Date of closure | FDA's Letter | Notifier | As specifications (mg/kg) | Hg specifications (mg/kg) | Reference |
|---|---|---|---|---|---|---|---|
| 001091 | Sep 30, 2022 | On-going | N/A | INBIOSE | ≤0.2 | ≤0.5 | Current GRN notice |
| 001059 | Jun 8, 2022 | Dec 2, 2022 | "No questions" | Glycom | NS | NS | Specs: Table 2.3.1-4, p. 10 |
| 001037 | Feb 11, 2022 | Nov 7, 2022 | "No questions" | Glycom | NS | NS | Specs: Table 2.3.1-1, p 13-14 |
| 001035 | Jan 28, 2022 | Jan 19, 2023 | "No questions" | Glycom | NS | NS | Specs: Table 2.3.1-1, p 14 |
| 001034 | Feb 9, 2022 | Oct 21, 2022 | "No questions" | Glycom | NS | NS | Specs: Table 2.4-1, p 11-12 |
| 001016 | Sep 24, 2021 | Jul 15, 2022 | "No questions" | Hansen | ≤0.2 | ≤0.5 | Specs: Table 2, p. 8 |
| 001015 | Sep 24, 2021 | Jul 15, 2022 | "No questions" | Hansen | ≤0.2 | ≤0.5 | Specs: Table 2, pp. 8-9 |
| 001014 | Oct 18, 2021 | Jul 15, 2022 | "No questions" | Hansen | ≤0.2 | ≤0.5 | Specs: Table 2, pp. 10-11 |
| 000951 | Oct 02, 2021 | Aug 12, 2021 | "No questions" | Danisco | ≤0.2 | ≤0.1 | Specs: Table 3, p. 17 |
| 000929 | Jun 19, 2020 | Feb 26, 2021 | "No questions" | Jennewein | ≤0.2 | ≤0.5 | Specs: Table 4, p. 7 |
| 000925 | May 15, 2020 | Feb 08, 2021 | "No questions" | Jennewein | ≤0.2 | ≤0.5 | Specs: Table 3, p. 11 |
| 000923 | May 14, 2020 | Feb 02, 2021 | "No questions" | Jennewein | ≤0.2 | ≤0.5 | Specs: Table 3, p. 11 |
| 000922 | June 3, 2020. | Apr 23, 2021 | "No questions" | Jennewein | ≤0.2 | ≤0.5 | Specs: Table 3, p. 7 |
| 000921 | May 14, 2020 | Oct 30, 2020 | "No questions" | Jennewein | ≤0.2 | ≤0.5 | Specs: Table 3, p. 12 |
| 000919 | May 12, 2020 | Oct 30, 2020 | "No questions" | Jennewein | ≤0.2 | ≤0.5 | Specs: Table 3, p. 14-15 |

# HMOs indicated in this table, likewise Inbiose's 2´-FL product, were produced from fermentation processes using *Escherichia coli* strains, and using similar production techniques.
N/A: Not Available
NS: Not Specified

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

Inbiose would like to indicate that the reduced specification of arsenic of **≤0.1** mg/kg is lower than the acceptance criteria indicated in the Food Chemical Codex (FCC) monograph for 2'-FL (effective since December 1, 2022) for arsenic impurity (no more than 0.2 mg/kg, calculated on the anhydrous basis).

**Question 4.** We note that the specified maximum level for mercury (≤ 0.5 mg/kg) is significantly higher than the reported levels for the three batch analyses provided in Table 2.3.2-1 (page 15), which demonstrated mercury levels to be consistently below 0.01 mg/kg. Therefore, we request that specifications be as low as possible and reflect results of batch analyses for an ingredient produced in accordance with current GMPs.

Such as the specification proposed for arsenic, the specified maximum level for mercury in this GRAS notice 001091 (i.e. ≤0.5 mg/kg) is in line with most of other HMOs (e.g. GRNs: 001016, 001015, 001014, 000929, 000925, 000923, 000922) that have been notified to the U.S. FDA without objection from the agency.
In addition, the recent applications (GRN 001059, 001037, 001035, 001034) do not provide specification limits for mercury, but only specified maximum level for lead. The mercury specifications reported in these GRN notices are indicated in Table 1 above (see the response to the FDA Question 3).

Nevertheless, Inbiose agrees to reduce the proposed specifications of mercury (from initially proposed ≤0.5 mg/kg to **≤0.25** mg/kg).

**Question 5.** The method cited in Table 2.3.1-1 (page 14) for the determination of *Cronobacter sakazakii* is ISO/TS 22964 and the specification is listed as absent in 25 g. We note that the current method ISO 22964:2017 (Microbiology of the food chain — Horizontal method for the detection of *Cronobacter* spp.) is validated for test portions of 10 g. Please clarify if the method used by Inbiose has been validated for the specified sample size.

Inbiose clarifies that the test method utilized to evaluate the presence of *Cronobacter* spp. in the final 2´-FL ingredient is ISO 22964:2017, which has been validated by the external testing facility contracted by Inbiose for use in test portions of 25 g. This version of the ISO method, "Microbiology of the Food Chain – Horizontal Method for the Detection of *Cronobacter* spp." tests for the presence of *Cronobacter* spp., which includes *C. sakazakii*. The description for the *Cronobacter* specification parameter included in this GRN should therefore be described as "*Cronobacter* spp." instead of "*Cronobacter (Enterobacter) sakazakii*".

Inbiose confirms that *Cronobacter* spp. was not detected in any 2´-FL batches analyzed and included in this GRN 001091, therefore, no further identification for *C. sakazakii* were performed.

However, if the presence of *Cronobacter* spp. is confirmed in any future production batch of Inbiose's 2´-FL, the batch would subsequently be rejected and an additional step would be performed to determine if the isolate contains *C. sakazakii.*

**Question 6.** On page 4 of the notice, it states that the intended uses of 2'-FL are fully substitutional to those described in GRNs 000735 and 000897 and provides Table 1.3-1 that summarizes the intended uses that were described in the respective GRAS notices. We note that some of the categories and maximum use levels differ between these two notices, and therefore the cumulative dietary exposure of the combined uses will differ compared to either of the estimated dietary exposures that were presented in the respective GRAS notices. We request that the notifier provide estimates of the cumulative dietary exposures based on the combined intended uses described in their notice (i.e., infant formula, infant and toddler foods, and other food categories) that includes estimates of dietary exposure to 2'-FL for infants, toddlers (1-3

years), children (4-10 years) and the total consuming population ages 2 years and older on a

Thank you for the opportunity to clarify and to provide additional data. A summary of the intended uses of GRN 001091 are provided in Table 2 below. Notably, in alignment with the statements referenced in the question above, the uses of Inbiose's 2'-FL listed in Table 2 are intended be fully substitutional to those described in GRNs 000735 and 000897, both of which have previously been notified as GRAS to the U.S. FDA, receiving a "no questions" letter from the Agency. All food codes included in the cumulative intake assessment below are provided in Appendix A.

**Table 2 Summary of the Individual Proposed Food Uses and Maximum Use Levels Notified in GRNs 735 and 897 and Proposed for Inbiose's 2'-Fucosyllactose in the U.S.**

| Food Category (21 CFR §170.3) (U.S. FDA, 2021a) | Proposed Food Use[a] | Maximum Use Levels (g/kg or g/L)[b] | | |
|---|---|---|---|---|
| | | Inbiose | GRN 735 | GRN 897 |
| Beverages and Beverage Bases | Non-dairy smoothies & meal replacement beverages[c] | 5 | - | 5 |
| | Sports, Isotonic, and Energy Drinks, Enhanced or Fortified Waters | 1.2 | 0.8 | 1.2 |
| Breakfast Cereals | Hot Cereals | 31 | 4.8 | 31 |
| | Ready-to-eat Cereals (all types) | 80.0 (puffed)<br>40.0 (high fiber)<br>40.0 (biscuit type) | 80.0 (puffed)<br>30.0 (high fiber)<br>20.0 (biscuit type) | 40 (all types) |
| Dairy Product Analogs | Milk substitutes such as soy milk and imitation milks | 1.2 | 1.2 | 1.2 |
| | Non-dairy Yogurts | 12 | - | 12 |
| Frozen Dairy Desserts and Mixes | Frozen desserts including ice creams and frozen yogurts, frozen novelties | 17 | 17 | - |
| Gelatins, puddings and Fillings | Dairy-based puddings, custards and mouses | 17 | 17 | - |
| | Fruit pie filling | 14.1 | 14.1 | - |
| | "Fruit prep" such as fruit filling in bars, cookies, yogurt and cakes | 30 | 30 | - |
| Grain Products and Pastas | Cereal bars, nutrition bars, & Meal Replacement Bars | 30 | 12 | 30 |
| Infant and Toddler Foods | Non-exempt Term Infant Formulas | 2.4 | 2.4 | 2.4 |
| | Toddler Formulas | 2.4 | 2.4 | 2.4 |
| | Other Baby Foods for Infants and Young Children | 12 | Up to 10.9[d] | 12 |
| | Yogurt and juice beverages identified as "baby" drinks | 10 | 10 | 1.2 |
| | Baby crackers, pretzels, cookies, and snack items | 57 | 57 | 12[e] |
| Jams and Jellies, commercial | Jellies and jams, fruit preserves, and fruit butters | 60 | 60 | - |
| Milk, Whole, and Skim | Unflavored pasteurized and sterilized milk (whole milk, reduced-fat milk, low-fat milk, non-fat milk; including powdered milks, reconstituted) | 1.2 | 1.2 | - |

| | | | | |
|---|---|---|---|---|
| Milk Products | Fermented & flavored milk, RTD & mixes | 1.2 | 1.2 | 1.2 |
| | Dairy smoothies & meal replacement beverages[c] | 5 | 1.2 | 5 |
| | Yogurt | 12 | 5.3 | 12 |
| | Formula intended for pregnant women("mum" formulas; -9 to 0 months)[f] | 6.0 | 6.0 | - |
| Processed Fruits and Fruit Juices | Fruit Juices, Drinks, Nectars | 1.2 | 1.2 | 1.2 |
| Processed Vegetables and Vegetable Juices | Vegetable Juices | 1.2 | - | 1.2 |
| Sweet sauces, Toppings, and Syrups | Syrups used to flavor milk beverages | 7 | 7 | - |
| Foods for Special Dietary Use | Oral Nutritional Food Supplements and Enteral and Oral Tube-feeding Formulas for Patients ≥11Years[g] | 20 | 20 | 20 |

2'-FL = 2'-fucosyllactose; CFR = *Code of Federal Regulations*; GRAS = Generally Recognized as Safe; GRN = GRAS Notice; U.S. = United States.

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

[b] Proposed maximum use levels are presented as g/kg for solids and as g/L for liquids and expressed on a 2'-FL basis in the final food, as consumed.

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

[d] Inclusive of "hot cereals for babies" and "junior type desserts" as reported in GRN 735

[e] Reported as "Infant and toddler foods" in GRN 897

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

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

Table 3 summarizes the estimated cumulative intake of 2'-FL on an absolute basis (g/person/day) for each of the requested population groups. Table 4 presents these data on a per kilogram body weight basis (mg/kg body weight/day). The percentage of consumers was high among all age groups evaluated in the current intake assessment; more than 68.9% of the population groups consisted of consumers of these food products (see Table 3). 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 90$^{th}$ percentile consumer-only intakes of 2'-FL were determined to be 2.6 and 5.6 g/person/day, respectively. Of the individual population groups, the infants 7 to 12 months of age were determined to have the greatest mean and 90$^{th}$ percentile consumer-only intakes of 2'-FL on an absolute basis, at 3.4 and 6.3 g/person/day (see Table 3).

**Table 3 Summary of the Estimated Cumulative Daily Intake of 2'-FL Based on Select Maximum Conditions of Use Previously Determined to be GRAS in the U.S. by Population Group (2017-2018 NHANES Data)**

| Population Group | Age Group | *Per Capita* Intake (g/day) | | Consumer-Only Intake (g/day) | | | |
|---|---|---|---|---|---|---|---|
| | | Mean | 90th Percentile | % | n | Mean | 90th Percentile |
| Infants | 0 to 6 m | 1.5 | 3.3 | 68.9 | 128 | 2.2 | 3.8 |
| Infants | 7 to <12 m | 3.4 | 6.3 | 100 | 124 | 3.4 | 6.3 |
| Toddlers | 1 to 3 y | 2.6 | 4.9 | 99.2 | 411 | 2.6 | 4.9 |
| Children | 4 to 10 y | 2.3 | 4.3 | 99.3 | 774 | 2.3 | 4.3 |
| Total population | ≥2 y | 2.3 | 5.3 | 90.2 | 5,586 | 2.6 | 5.6 |

2'-FL = 2'-Fucosyllactose; GRAS = Generally Recognized as Safe; m = months; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States; y = years.

On a body weight basis, the total population (2 years and older) mean and 90th percentile consumer-only intakes of 2'-FL were determined to be 44 and 103 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 378 and 662 mg/kg body weight/day, respectively (see Table 4).

**Table 4 Summary of the Estimated Cumulative Daily Per Kilogram Body Weight Intake of 2'-FL Based on Select Maximum Conditions of Use Previously Determined to be GRAS in the U.S. by Population Group (2017-2018 NHANES Data)**

| Population Group | Age Group | *Per Capita* Intake (mg/kg bw/day) | | Consumer-Only Intake (mg/kg bw/day) | | | |
|---|---|---|---|---|---|---|---|
| | | Mean | 90th Percentile | % | n | Mean | 90th Percentile |
| Infants | 0 to 6 m | 223 | 495 | 68.9 | 128 | 324 | 499 |
| Infants | 7 to <12 m | 378 | 662 | 100 | 124 | 378 | 662 |
| Toddlers | 1 to 3 y | 192 | 380 | 99.2 | 401 | 193 | 380 |
| Children | 4 to 10 y | 85 | 163 | 99.3 | 772 | 86 | 163 |
| Total population | ≥2 y | 40 | 96 | 90.2 | 5,534 | 44 | 103 |

2'-FL = 2'-Fucosyllactose; bw = body weight; GRAS = Generally Recognized as Safe; m = months; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States; y = years.

As described above, the use of Inbiose's 2'-FL is intended to be entirely substitutional for other forms of 2'-FL that are currently considered GRAS. Importantly, the intended food uses and use levels of 2' FL described above have not been expanded beyond what has already been concluded to be GRAS. Therefore, while it is recognized that these estimated intake values may be slightly different from previous GRNs that have received "no questions" letters from the agency, the uses of Inbiose's ingredient in the proposed manner are fully substitutional to current GRAS uses and will not lead to an increase intake of 2'-FL in the U.S. population.

**Question 7.** On page 40 of the notice, it states, "Adverse effects of high intake of 2'-FL are similar to those observed with other sources of dietary fiber (e.g., gastrointestinal discomfort) and is self-limiting." Please clarify this statement within the context of the following: 1) FDA has not defined 2'-FL, or any other human milk oligosaccharide, to be classified as dietary fiber, and therefore, consumers are not likely to associate 2'-FL as dietary fiber, and 2) since the uses involve infants, it is not clear why high intake would be considered "self-limiting".

Thank you for providing us with the opportunity to bring clarity regarding this sentence. The sentence is indeed incorrect and Inbiose did not intend to imply that the HMO, 2´-FL, is a dietary fiber ingredient. Instead, the purpose of this statement was to highlight the parallel between the self-limiting nature of 2´-FL and other ingredients, such as dietary fibers, which are considered self-limiting due to the potential for gastrointestinal discomfort to occur at the highest levels of intake. While 2´-FL no 2´-FL -related adverse effects were noted up to the highest tested doses in a wide range of animal studies (pages 33-40 of the GRAS notification) and clinical trials (pages 41-45 of the GRAS Notification), sporadic individual gastrointestinal symptoms, such as an increase in passing gas and bloating, have been observed at some of the highest dose levels tested in humans (e.g. Vandenplas et al, 2020; Fonvig *et al.*, 2021).

gastrointestinal discomfort. Even so, similar rates of adverse effect occurrence have been identified across HMO-test formula, breast fed, and controlled reference groups in clinical trials.

> Rewritten, the identified sentence from page 40 of the Notice could be rewritten, as follows: "*Gastrointestinal effects, such as an , may occur at high intake levels of 2´-FL, which could be perceived as gastrointestinal discomfort in some individuals*."

References:

Fonvig CE, Amundsen ID, Vigsnæs LK, Sørensen N, Frithioff-Bøjsøe C, Christiansen M, et al. (2021). Human milk oligosaccharides modulate fecal microbiota and are safe for use in children with overweight: An RCT. J Pediatr Gastroenterol Nutr 73(3):408-414. DOI:10.1097/mpg.0000000000003205.

Vandenplas Y, de Halleux V, Arciszewska M, Lach P, Pokhylko V, Klymenko V, et al. (2020). A partly fermented infant formula with postbiotics including 3'-GL, specific oligosaccharides, 2'-FL, and milk fat supports adequate growth, is safe and well-tolerated in healthy term infants: a double-blind, randomised, controlled, multi-country trial (On Behalf of the Voyage Study Group). Nutrients 12(11):3560 [17pp, plus supplementary data]. DOI:10.3390/nu12113560.

**Question 8.** We note that the Food Chemicals Codex (FCC) recently published a monograph for 2'-FL (effective December 1, 2022). Please discuss whether the specifications provided in your notice (Table 6.3-1, page 31) meet the FCC specifications. In particular, we note that FCC specifications include a limit for 2'-fucosyl-D-lactulose of not more than 2% on an anhydrous basis.

Inbiose confirms that the specification in Table 6.3-1 (page 31 of this GRN 001091) meets the FCC specification for 2´-FL. In particular, 2'-fucosyl-D-lactulose is no more than 2% w/w (calculated on the anhydrous basis).

**Question 9.** On page 28 of the notice, it states that an updated literature search was conducted through January 2022. Please confirm for the administrative record that no new publicly available data have been found since that date that could be perceived as counter to your GRAS conclusion. If such data is found, please discuss their implications on your GRAS conclusion.

To address this question, Inbiose has conducted an updated literature search through March 07 2023 to identify any new publicly available data pertaining to the safety of 2'-FL that have been published since the original literature search was conducted in January 2022. No new data were identified in the updated search of the published literature that could be perceived as counter to Inbiose's 2'-FL GRAS conclusion; however, several new clinical studies were identified in support of the GRAS conclusion. While the results from these studies are not counter to the GRAS conclusion, these studies are summarized in Table 5 (Summaries of Newly Identified Clinical Trials Conducted with 2´-FL sodium salt), for completeness. Briefly, 2'-FL (in combination with another HMOs) was not observed to elicit adverse effects in humans. Results from these recently published studies support that 3'-SL is safe and well tolerated. Inbiose therefore maintains that this 2'-FL ingredient is GRAS, on the basis of scientific procedures, for use in non-exempt term infant formula and specified conventional food and beverage products, as described in the GRAS Notification.

Lasekan et al. (2022) conducted a randomized, controlled, multicenter, double-blind, parallel feeding growth and tolerance study to investigate the growth and gastrointestinal tolerance of milk-based infant formula supplemented with 5 HMOs in healthy term babies. The 5-HMO blend content used in this study was exactly the same as used by Parschat et al. (2021) , i.e. 3.0 g/L of 2'-FL, 0.8 g/L of 3-FL, 1.5 g/L of LNT, 0.2 g/L of 3'-SL and 0.3 g/L of 6'-SL. Infants were randomized to receive either a control (N = 129) or an experimental formula with blend of 5 HMOs (N = 130) through approximately 4 months of age. Breastfed infants (N = 104) were included as a reference group, as well. Weight, length, head circumference (HC), mean rank stool consistency (MRSC) number of stools per day and a percentage of feedings with spit-up/vomit associated with feeding were measured from day (D) 14 to D119. No differences were observed among the three groups for weight gain per day from D14 to D119 ($p \geq 0.337$). Infants fed with experimental formula had softer, more frequent and yellow stools, and were similar to the reference group. There were no differences between serious and non-serious adverse events among the three groups. The blend of 5 HMOs was concluded to be safe and well-tolerated as well as supportive of normal growth. These results were in line with data published by Parschat et al. (2021).

In a randomized, controlled, double-blind trial, Bosheva et al. (2022) investigated the gut maturation effects (microbiota, metabolites, and selected maturation markers) of an infant formula containing a specific blend of five HMOs (0.87, 0.10, 0.29, 0.11 and 0.14 g/L for 2'-FL, DFL, LNT, 3'-SL and 6'-SL, respectively). Healthy full-term infants were assigned to control group (CG) fed a standard infant formula without HMOs, test group 1 (TG1) and test group 2 (TG2) fed with the same standard infant formula containing the five-HMO blend at a concentration of 1.5 g/L and 2.5 g/L, respectively. A non-

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

Wallingford et al. (2022) conducted a double-blinded randomized controlled study to assess the growth and tolerance of commercial infant formula supplemented with GOS and FOS and containing 2´-FL (1 g/L). In addition, specific pathways of utilization of 2´-FL particularly by *Bifidobacterium* spp. were investigated. In total, 176 infants completed the 16-week study. Briefly, the healthy term Infants (37–42 weeks of gestation, APGAR score ≥ 7) were allocated to test (N=56) and control (N=41) groups and received GOS/FOS-containing infant formula with or without 2´-FL (1g/L), respectively. The breast-fed group (N=79) served as a reference group. The weight gain was measured as total grams gained over the 16-weeks. The weight, length, and head circumference of the infants were measured at baseline and 2, 4, 6, 8, 12, and 16 weeks. The fecal samples were collected at day 1 and day 112 for microbiome analysis. Overall, there were no effects on the growth of infants fed with GOS/FOS infant formula supplemented with 2´-FL when compared to other groups. No significant differences in occurrences of the AEs between two groups were noted. Slightly greater incidence of gastrointestinal disorders was observed in two formula groups when compared to the reference breastfed group, however, the two formula groups had the same incidence. None of the reported serious AEs were confirmed to be related with the 2´-FL treatment.

Giorgetti et al. (2023) investigated whether consumption of 3.0 g HMO, given as a mixture of 2.0 g 2'-FL and 1.0 g LNnT, enhances fractional iron absorption from ferrous fumarate (FeFum)-fortified maize porridge in partially breastfed Kenyan infants. 55 infants were recruited and were fed with different test meals: A, B, and C. All test meals were made of different molecules of interest: test meal A, FeFum control test meal with 5.0 mg iron as FeFum (added as 2.5 mg $^{56}$Fe + 2.5 mg $^{54}$Fe); test meal B, FeFum + GOS with 5.0 mg iron as FeFum (added as 2.5 mg $^{56}$Fe + 2.5 mg $^{58}$Fe) and 3.0 g of GOS (added as 4.0 g of Vivinal GOS-75 Powder); and test meal C, FeFum + HMO, with 5.0 mg iron as FeFum (added as 2.5 mg $^{56}$Fe + 2.5 mg $^{57}$Fe) and 3.0 g HMO (added as 2.0 g 2'-FL and 1.0 g LNnT)). On alternate test meal days (days 2, 4, and 6), 30 infants were fed 3 different test meals (A, B and C). The other 25 infants consumed 2 different test meals (A and C) on alternate test meal days at days 2 and 4. Blood samples of infants were collected, and hemoglobin (Hb) concentration was analyzed to be used as an indicator of Iron deficiency anemia. Maternal HMOs profiles were also analyzed. No significant linear or monotonic correlation between fractional iron absorption and HMO concentration was observed. The authors concluded that single dose consumption of HMOs did not enhance iron absorption, nor increase iron dialyzability *in vitro*.

References:

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Lasekan, J., Choe, Y., Dvoretskiy, S., Devitt, A., Zhang, S., Mackey, A., Wulf, K., Buck, R., Steele, C., Johnson, M., & Baggs, G. (2022). Growth and Gastrointestinal Tolerance in Healthy Term Infants Fed Milk-Based Infant Formula Supplemented with Five Human Milk Oligosaccharides (HMOs): A Randomized Multicenter Trial. Nutrients, 14(13), 2625. https://doi.org/10.3390/nu14132625

Parschat, K., Melsaether, C., Jäpelt, K. R., & Jennewein, S. (2021). Clinical Evaluation of 16-Week Supplementation with 5HMO-Mix in Healthy-Term Human Infants to Determine Tolerability, Safety, and Effect on Growth. Nutrients, 13(8), 2871. https://doi.org/10.3390/nu13082871

Wallingford, J. C., Neve Myers, P., & Barber, C. M. (2022). Effects of addition of 2-fucosyllactose to infant formula on growth and specific pathways of utilization by Bifidobacterium in healthy term infants. Frontiers in nutrition, 9, 961526. https://doi.org/10.3389/fnut.2022.96152

**Table 5. Summaries of Newly Identified Clinical Trials Conducted with 2´-FL**

| Type of Study | Population | Length of Study | Dose | Outcome | Reference |
|---|---|---|---|---|---|
| **Randomized, double-blind, controlled parallel feeding growth trial** | Healthy term infants (gestational age 37–42 weeks) between 0 and 14 days of age with a birth weight ≥ 2490 g. | Time of enrolment at ≤14 Days (D) of age until D119 or up to D183 | Control milk-based formula (CF; n = 129);<br><br>experimental formula (EF; N = 130) containing five HMOs (5.75 g/L; 2'-FL (3.0 g/L), 3-FL (0.8 g/L), LNT (1.5 g/L), 3'-SL (0.2 g/L) and 6'-SL (0.3 g/L));<br><br>reference group: human milk (HM; N = 104) | No significant differences among the three groups for weight gain per day and gains in weight and length ($p \geq 0.05$) from D14 to D119.<br>Color of stool, its consistency and frequency per day were more similar between EF and HM groups.<br>Serious and non-serious adverse events were not different among groups.<br>The results indicated that EF containing five HMOs was safe and well-tolerated and supported age-appropriate growth. | Lasekan et al., 2022 |
| **Randomized, controlled, double-blind trial** | Healthy full-term infants (7–21 days old) | Time of enrolment: a couple weeks of age (baseline) up to 6 months | Test group 1 (TG1) fed standard infant formula with a concentration of 1.5 g/L of the five-HMO blend: 0.87, 0.10, 0.29, 0.11 and 0.14 g/L for 2'-FL, DFL, LNT, 3'-SL and 6'-SL, respectively. (N = 230)<br><br>Test group 2 (TG2) fed standard IF with a concentration of 2.5 g/L of the five-HMO blend: 1.45, 0.14, 0.48, 0.18 and 0.24 g/L for 2'- | Relative abundance of *Bifidobacterium longum* subsp. *infantis (B. infantis)* was higher in TGs vs. CG.<br>At both post-baseline visits, toxigenic *Clostridioides difficile* abundance was 75–85% lower in TGs vs. CG ($P < 0.05$) and comparable with HMG.<br>At 3 months, TGs (vs. CG) had higher secretory immunoglobulin A (sIgA) and lower alpha-1-antitrypsin ($P < 0.05$). | Bosheva et al., 2022 |

| Type of Study | Population | Length of Study | Dose | Outcome | Reference |
|---|---|---|---|---|---|
| | | | FL, DFL, LNT, 3'-SL and 6'-SL, respectively (N = 230).<br><br>Control group (CG): standard cow's milk-based infant formula (N = 233)<br><br>Placebo: standard IF without HMOs (HMG) (N = 96) | | |
| **Double-blinded randomized controlled study** | Healthy term (37–42 weeks of gestation) infants | Time of enrolment at ≤28 days up to 16 weeks | Test group: milk-based formula supplemented with Galactooligosaccharide (GOS), Fructooligosaccharide (FOS) supplemented with 1 g/L 2'-FL, (N=56)<br><br>Control group: milk-based control formula supplemented with GOS and FOS without 2'-FL (N=41)<br><br>Breastfed group (N=79) | There were no effects on the growth or occurrence of adverse events (AEs) in the test group formula when compared to the control group.<br><br>Both test and control formula groups had significantly greater richness of metagenomic species (MGS) ($p < 0.05$) and Shannon diversity ($p < 0.05$) after 16 weeks of feeding when compared with the breastfed group, but did not differ from each other.<br>In a test group, a microbiome shift was indicated towards the direction of breastfed infant microbiota. | Wallingford et al., 2022 |

| Type of Study | Population | Length of Study | Dose | Outcome | Reference |
|---|---|---|---|---|---|
| **Single-blinded, randomized, prospective crossover study** | Infant age 8–12 months | 20 days | Test meal A (FeFum control test meal) 5.0 mg iron as FeFum (added as 2.5 mg $^{56}Fe$ + 2.5 mg $^{54}Fe$); test meal B (FeFum + GOS) 5.0 mg iron as FeFum (added as 2.5 mg $^{56}Fe$ + 2.5 mg $^{58}Fe$) and 3.0 g of GOS (added as 4.0 g of Vivinal GOS-75 Powder); and test meal C (FeFum + HMO) 5.0 mg iron as FeFum (added as 2.5 mg $^{56}Fe$ + 2.5 mg $^{57}Fe$) and 3.0 g HMO (added as 2.0 g 2'-FL and 1.0 g LNnT))<br><br>30 infants consumed 3 different test meals (A, B, and C) on alternate test meal days (days 2, 4, and 6) and 25 infants consumed 2 different test meals (A and C) on alternate test meal days (days 2 and 4); infants in each group were randomly allocated to meal sequence (ABC, ACB, BAC, BCA, CAB, CBA, AC or CA) | Maternal HMO profile did not predict FIA or modulate the effects of GOS or HMO on FIA.<br><br>No significant difference was found between FIA of the FeFum+HMO group and that of the FeFum group (-0.5,CI: -3.1, 2.2; P=0.923). Consumption of a single low dose of 3.0 g HMOs did not enhance FIA from maize porridge fortified with FeFum, nor did it increase iron dialyzability *in vitro*. | Giorgetti et al., 2023 |

## Appendix A
## Representative Food Codes for Cumulative Dietary Exposure Assessment of 2'-fucosyllactose in the U.S. (2017-2018 NHANES Data)

# Representative Food Codes for Cumulative Dietary Exposure Assessment of 2'-fucosyllactose in the U.S. (2017-2018 NHANES Data)

## Beverages and Beverage Bases

**Non-dairy smoothies & meal replacement beverages**
[2´-FL] = 0.5 g/100 g

| | |
|---|---|
| 11553100 | Fruit smoothie, NFS |
| 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 |
| 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 |
| 78101130 | Vegetable smoothie |
| 95120050 | Nutritional drink or shake, liquid, soy-based |

Foods adjusted for being present in dried form
*Reconstitution factor of 6.5*
[2´-FL] = 3.25 g/100 g

| | |
|---|---|
| 95201300 | Nutritional powder mix (EAS Soy Protein Powder) |
| 95230010 | Nutritional powder mix, protein, soy based, NFS |

**Sports, Isotonic, and Energy Drinks; Enhanced or Fortified Waters**
[2´-FL] = 0.12 g/100 g

| | |
|---|---|
| 92410110 | Carbonated water, sweetened |
| 92410250 | Carbonated water, sweetened, with low-calorie or no-calorie sweetener |
| 94100200 | Water, bottled, sweetened, with low calorie sweetener |
| 94100300 | Water, bottled, flavored (Capri Sun Roarin' Waters) |
| 94210100 | Water, bottled, flavored (Propel Water) |
| 94210200 | Water, bottled, flavored (Glaceau Vitamin Water) |
| 94210300 | Water, bottled, flavored (SoBe Life Water) |
| 94220215 | Water, bottled, flavored, sugar free (Glaceau Vitamin Water) |
| 94220310 | Water, bottled, flavored, sugar free (SoBe) |
| 95310200 | Energy drink (Full Throttle) |

95310400 Energy drink (Monster)
95310500 Energy drink (Mountain Dew AMP)
95310550 Energy drink (No Fear)
95310555 Energy drink (No Fear Motherload)
95310560 Energy drink (NOS)
95310600 Energy drink (Red Bull)
95310700 Energy drink (Rockstar)
95310750 Energy drink (SoBe Energize Energy Juice Drink)
95310800 Energy drink (Vault)
95311000 Energy Drink
95312400 Energy drink, low calorie (Monster)
95312410 Energy drink, sugar free (Monster)
95312500 Energy drink, sugar free (Mountain Dew AMP)
95312550 Energy drink, sugar free (No Fear)
95312555 Energy drink, sugar-free (NOS)
95312560 Energy drink (Ocean Spray Cran-Energy Juice Drink)
95312600 Energy drink, sugar-free (Red Bull)
95312700 Energy drink, sugar free (Rockstar)
95312800 Energy drink, sugar free (Vault)
95312900 Energy drink (XS)
95312905 Energy drink (XS Gold Plus)
95313200 Energy drink, sugar free
95320200 Sports drink (Gatorade G)
95320500 Sports drink (Powerade)
95321000 Sports drink, NFS
95322200 Sports drink, low calorie (Gatorade G2)
95322500 Sports drink, low calorie (Powerade Zero)
95323000 Sports drink, low calorie
95330100 Fluid replacement, electrolyte solution
95330500 Fluid replacement, 5% glucose in water

<u>Foods adjusted for being present in dried form</u>
*Reconstitution factor of 16.65*
[2´-FL] = 2.00 g/100 g

92900300 Sports drink, dry concentrate, not reconstituted

## Breakfast Cereals

**<u>Hot cereals</u>**
[2´-FL] = 3.1 g/100 g

56200300 Cereal, cooked, NFS
56200390 Barley, NS as to fat

| | |
|---|---|
| 56200400 | Barley, no added fat |
| 56200410 | Barley, fat added |
| 56200490 | Buckwheat groats, NS as to fat |
| 56200500 | Buckwheat groats, no added fat |
| 56200510 | Buckwheat groats, fat added |
| 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 |
| 56201990 | Millet, NS as to fat |
| 56202000 | Millet, no added fat |
| 56202100 | Millet, fat added |
| 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
56207110 Bulgur, no added fat
56207120 Bulgur, fat added
56207130 Bulgur, NS as to fat
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
56210000 Cereal, nestum
58174000 Upma, Indian breakfast dish
75217520 Hominy, cooked

**Ready-to-eat Cereals (high fiber and biscuit types)**
[2´-FL] = 4.0 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)
57106050 Cereal (Post Great Grains Banana Nut Crunch)
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)
57143000 Cereal (Kellogg's Cracklin' Oat Bran)
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)
57207000 Cereal, bran flakes
57208000 Cereal (Kellogg's All-Bran Complete Wheat Flakes)
57209000 Cereal (Post Bran Flakes)
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)
57224000 Cereal (General Mills Golden Grahams)
57227000 Cereal, granola
57228000 Granola, homemade
57229000 Cereal (Kellogg's Low Fat Granola)
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)
57308190 Cereal, muesli
57308400 Cereal (General Mills Cheerios Multigrain)
57309100 Cereal (Nature Valley Granola)
57316380 Cereal (General Mills Cheerios Oat Cluster Crunch)
57316385 Cereal (General Mills Cheerios Protein)
57316450 Cereal (General Mills Oatmeal Crisp with Almonds)
57316710 Cereal (Quaker Honey Graham Oh's)
57320500 Cereal (Quaker Granola with Oats, Honey, and Raisins)
57321900 Cereal (Nature's Path Organic Flax Plus)
57327450 Cereal (Quaker Toasted Oat Bran)
57327500 Cereal (Quaker Oatmeal Squares)
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)
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)
57401100 Cereal, toasted oat
57408100 Cereal (Uncle Sam)
57411000 Cereal (General Mills Chex Wheat)
57417000 Cereal (Post Shredded Wheat)
57418000 Cereal (General Mills Wheaties)

**Ready-to-eat Cereals (Puffed)**
[2´-FL] = 8.0 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

## Dairy Product Analogs

**Milk substitutes such as soy milk and imitation milks**
[2´-FL] = 0.12 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 |
| 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 |
| 11519215 | Strawberry milk, non-dairy |
| 42401010 | Coconut milk, used in cooking |

<u>Mixed foods containing Milk substitutes such as soy milk and imitation milks</u>
*Adjusted for milk substitute content of 42.2 to 95.7%*
[2´-FL] = 0.051 to 0.115 g/100 g

| | |
|---|---|
| 11513375 | Chocolate milk, made from reduced sugar mix with non-dairy milk |
| 11513395 | Chocolate milk, made from no sugar added dry mix with non-dairy milk (Nesquik) |
| 11513310 | Chocolate milk, made from dry mix with non-dairy milk |
| 11513385 | Chocolate milk, made from dry mix with non-dairy milk (Nesquik) |
| 11514360 | Hot chocolate / Cocoa, made with no sugar added dry mix and non-dairy milk |
| 11514150 | Hot chocolate / Cocoa, made with dry mix and non-dairy milk |
| 92101913 | Coffee, Latte, decaffeinated, with non-dairy milk |
| 92101903 | Coffee, Latte, with non-dairy milk |
| 11512030 | Hot chocolate / Cocoa, ready to drink, made with non-dairy milk |
| 92101919 | Coffee, Latte, decaffeinated, with non-dairy milk, flavored |
| 92101906 | Coffee, Latte, with non-dairy milk, flavored |
| 11512120 | Hot chocolate / Cocoa, ready to drink, made with non-dairy milk and whipped cream |
| 92101975 | Coffee, Cafe Mocha, decaffeinated, with non-dairy milk |
| 92101960 | Coffee, Cafe Mocha, with non-dairy milk |
| 92102512 | Coffee, Iced Latte, decaffeinated, with non-dairy milk |
| 92102502 | Coffee, Iced Latte, with non-dairy milk |
| 92162002 | Coffee, Cappuccino, decaffeinated, with non-dairy milk |
| 92161002 | Coffee, Cappuccino, with non-dairy milk |
| 92102515 | Coffee, Iced Latte, decaffeinated, with non-dairy milk, flavored |
| 92102505 | Coffee, Iced Latte, with non-dairy milk, flavored |
| 92102612 | Coffee, Iced Cafe Mocha, decaffeinated, with non-dairy milk |
| 92102602 | Coffee, Iced Cafe Mocha, with non-dairy milk |
| 92101933 | Frozen coffee drink, decaffeinated, with non-dairy milk |

92101923 Frozen coffee drink, with non-dairy milk
92102080 Frozen mocha coffee drink, decaffeinated, with non-dairy milk
92102020 Frozen mocha coffee drink, with non-dairy milk
92101938 Frozen coffee drink, decaffeinated, with non-dairy milk and whipped cream
92101928 Frozen coffee drink, with non-dairy milk and whipped cream
92102110 Frozen mocha coffee drink, decaffeinated, with non-dairy milk and whipped cream
92102050 Frozen mocha coffee drink, with non-dairy milk and whipped cream

**Non-dairy yogurt**
[2´-FL] = 1.2 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**
[2´-FL] = 1.7 g/100 g

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
13150000 Sherbet, all flavors
13161600 Fudgesicle, light

## Gelatins, puddings and Fillings

**Dairy-based puddings, custards and mouses**
[2´-FL] = 1.7 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 |
| 13252600 | Tiramisu |
| 91550100 | Coconut cream cake, Puerto Rican style |
| 91560100 | Haupia |

**Fruit pie filling**
[2´-FL] = 1.41 g/100 g

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

Mixed foods containing Fruit pie filling
*Adjusted for Fruit pie filling content of 35.7 to 61.2%*
[2´-FL] = 0.50 to 0.86 g/100 g

| | |
|---|---|
| 53410200 | Cobbler, apricot |
| 53410880 | Cobbler, plum |
| 53410500 | Cobbler, cherry |
| 53410800 | Cobbler, peach |
| 53410850 | Cobbler, pear |
| 53410300 | Cobbler, berry |
| 53410900 | Cobbler, rhubarb |
| 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
53345000 Pie, lemon cream
53345070 Pie, lemon cream, individual size or tart
53346500 Pie, pineapple cream
53347000 Pie, pumpkin
53347070 Pie, pumpkin, individual size or tart

53348000 Pie, strawberry cream
53348070 Pie, strawberry cream, individual size or tart
53381000 Pie, lemon meringue
53381070 Pie, lemon meringue, individual size or tart
53410100 Cobbler, apple

**“Fruit prep” such as fruit filling in bars, cookies, yogurt and cakes**
[2´-FL] = 3.0 g/100 g

Mixed foods containing “Fruit prep” such as fruit filling in bars, cookies, yogurt and cakes
*Adjusted for content of 0.4 to 62.5%*
[2´-FL] = 0.01 to 1.88 g/100 g

53415100 Crisp, apple, apple dessert
53440700 Strudel, peach
53415500 Crisp, peach
53415300 Crisp, blueberry
53415400 Crisp, cherry
53440000 Strudel, apple
53440300 Strudel, berry
53415200 Fritter, banana
53440500 Strudel, cherry
53440800 Strudel, cheese and fruit
53450500 Turnover or dumpling, cherry
53451500 Turnover, guava
53415220 Fritter, berry
53452100 Pastry, fruit-filled
53104550 Cheesecake with fruit
53122080 Cake, shortcake, biscuit type, with fruit
53451000 Turnover or dumpling, peach
53450800 Turnover or dumpling, lemon
53450300 Turnover or dumpling, berry
53430200 Crepe, fruit filled
53123500 Cake, shortcake, with whipped topping and fruit, diet
53415120 Fritter, apple
53220030 Cookie, fig bar
53400300 Blintz, fruit-filled
53122070 Cake, shortcake, biscuit type, with whipped cream and fruit
53453150 Empanada, Mexican turnover, fruit-filled
53451750 Turnover, pumpkin
53453170 Empanada, Mexican turnover, pumpkin
53101250 Cake, angel food, with fruit and icing or filling
53123080 Cake, shortcake, sponge type, with fruit
55801010 Funnel cake with sugar and fruit
53450000 Turnover or dumpling, apple
53123070 Cake, shortcake, sponge type, with whipped cream and fruit

53220040 Cookie, fig bar, fat free
53118500 Cake, torte
53610170 Coffee cake, crumb or quick-bread type, with fruit
53113000 Cake, jelly roll
53220010 Cookie, fruit-filled bar, fat free
53102200 Cake or cupcake, applesauce, with icing or filling
53102700 Cake or cupcake, banana, with icing or filling
53114100 Cake or cupcake, lemon, with icing or filling
53116510 Cake or cupcake, pumpkin, with icing or filling
53233010 Cookie, oatmeal, with raisins
53237000 Cookie, raisin
53510100 Danish pastry, with fruit
53521140 Doughnut, jelly
53237010 Cookie, raisin sandwich, cream-filled
53224250 Cookie, lemon bar
53220000 Cookie, fruit-filled bar
53241600 Cookie, butter or sugar, with fruit and/or nuts
53233080 Cookie, oatmeal sandwich, with peanut butter and jelly filling

## Grain Products and Pastas

**Cereal bars, nutrition bars, & Meal Replacement Bars**
[2´-FL] = 3 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

**Non-exempt Term Infant Formulas**
[2´-FL] = 0.24 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 formulas**
[2´-FL] = 0.24 g/100 g

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)

11720430 Infant formula, NS as to form (Similac Expert Care for Diarrhea)
11720431 Infant formula, ready-to-feed (Similac Expert Care for Diarrhea)
11720620 Infant formula, NS as to form (Gerber Graduates Soy)

**Other Baby Foods for Infants and Young Children**

[2´-FL] = 1.2 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
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
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
57801000 Barley cereal, baby food, dry, instant
57803000 Mixed cereal, baby food, dry, instant
57804000 Oatmeal cereal, baby food, dry, instant
57805000 Rice cereal, baby food, dry, instant
57805080 Rice cereal with apples, baby food, dry, instant
57805090 Rice cereal with mixed fruits, baby food, dry, instant
57805100 Rice cereal with bananas, baby food, dry, instant
57805500 Brown rice cereal, baby food, dry, instant
57806000 Mixed cereal with bananas, baby food, dry, instant
57806050 Multigrain, whole grain cereal, baby food, dry, instant
57806100 Oatmeal cereal with bananas, baby food, dry, instant
57806200 Oatmeal cereal with fruit, baby food, dry, instant, toddler
57807010 Whole wheat cereal with apples, baby food, dry, instant
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
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
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
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 |

**Yogurt and juice beverages identified as "baby" drinks**

[2´-FL] = 1.0 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 |

**Baby crackers, pretzels, cookies, and snack items**
[2´-FL] = 5.7 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 |
| 67430500 | Yogurt and fruit snack, baby food |

## Jams and Jellies, commercial

**Jellies and jams, fruit preserves, and fruit butters**
[2´-FL] = 6.0 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 (whole milk, reduced-fat milk, low-fat milk, non-fat milk; including powdered milks, reconstituted)**
[2´-FL] = 0.12 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) |
| 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 |

<u>Mixed foods containing milk</u>
*Adjusted for milk content of 16.1 to 83.6%*
[2´-FL] = 0.019 to 0.10 g/100 g

| | |
|---|---|
| 92101910 | Coffee, Latte, decaffeinated |
| 92101911 | Coffee, Latte, decaffeinated, nonfat |
| 92101900 | Coffee, Latte |
| 92101901 | Coffee, Latte, nonfat |
| 92101917 | Coffee, Latte, decaffeinated, flavored |

| | |
|---|---|
| 92101918 | Coffee, Latte, decaffeinated, nonfat, flavored |
| 92101904 | Coffee, Latte, flavored |
| 92101905 | Coffee, Latte, nonfat, flavored |
| 92101950 | Coffee, Cafe Mocha |
| 92101965 | Coffee, Cafe Mocha, decaffeinated |
| 92101970 | Coffee, Cafe Mocha, decaffeinated, nonfat |
| 92101955 | Coffee, Cafe Mocha, nonfat |
| 92102510 | Coffee, Iced Latte, decaffeinated |
| 92102511 | Coffee, Iced Latte, decaffeinated, nonfat |
| 92102500 | Coffee, Iced Latte |
| 92102501 | Coffee, Iced Latte, nonfat |
| 92162000 | Coffee, Cappuccino, decaffeinated |
| 92162001 | Coffee, Cappuccino, decaffeinated, nonfat |
| 92161000 | Coffee, Cappuccino |
| 92161001 | Coffee, Cappuccino, nonfat |
| 92102513 | Coffee, Iced Latte, decaffeinated, flavored |
| 92102514 | Coffee, Iced Latte, decaffeinated, nonfat, flavored |
| 92102503 | Coffee, Iced Latte, flavored |
| 92102504 | Coffee, Iced Latte, nonfat, flavored |
| 92101851 | Coffee, cafe con leche, decaffeinated |
| 92101850 | Coffee, cafe con leche |
| 92306800 | Tea, hot, chai, with milk |
| 92102610 | Coffee, Iced Cafe Mocha, decaffeinated |
| 92102611 | Coffee, Iced Cafe Mocha, decaffeinated, nonfat |
| 92102600 | Coffee, Iced Cafe Mocha |
| 92102601 | Coffee, Iced Cafe Mocha, nonfat |
| 92101930 | Frozen coffee drink, decaffeinated |
| 92101931 | Frozen coffee drink, decaffeinated, nonfat |
| 92101920 | Frozen coffee drink |
| 92101921 | Frozen coffee drink, nonfat |
| 92102060 | Frozen mocha coffee drink, decaffeinated |
| 92102070 | Frozen mocha coffee drink, decaffeinated, nonfat |
| 92102000 | Frozen mocha coffee drink |
| 92102010 | Frozen mocha coffee drink, nonfat |
| 92101935 | Frozen coffee drink, decaffeinated, with whipped cream |
| 92101936 | Frozen coffee drink, decaffeinated, nonfat, with whipped cream |
| 92611100 | Oatmeal beverage with milk |
| 92101925 | Frozen coffee drink, with whipped cream |
| 92101926 | Frozen coffee drink, nonfat, with whipped cream |
| 92613010 | Cornmeal beverage |
| 92102090 | Frozen mocha coffee drink, decaffeinated, with whipped cream |
| 92102100 | Frozen mocha coffee drink, decaffeinated, nonfat, with whipped cream |
| 92102030 | Frozen mocha coffee drink, with whipped cream |
| 92102040 | Frozen mocha coffee drink, nonfat, with whipped cream |
| 92613510 | Cornmeal beverage with chocolate milk |

92101810 Coffee, macchiato
92101820 Coffee, macchiato, sweetened
92610030 Horchata beverage, made with milk

Foods adjusted for being present in dried form
*Reconstitution factor of 11*
[2´-FL] = 1.32 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

**Fermented & flavored milk, RTD & mixes**
[2´-FL] = 0.12 g/100 g

11115000 Buttermilk, fat free (skim)
11115100 Buttermilk, low fat (1%)
11115200 Buttermilk, reduced fat (2%)
11115300 Buttermilk, whole
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) |
| 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 |
| 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 |
| 11560000 | Chocolate milk drink |

<u>Foods adjusted for being present in dried form</u>
*Reconstitution factor of 14.34*
[2´-FL] = 1.72 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 |

**Dairy smoothies & meal replacement beverages**
[2´-FL] = 0.5 g/100 g

| | |
|---|---|
| 11551050 | Licuado or Batido |
| 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 |
| 78101100 | Fruit and vegetable smoothie, with dairy |
| 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) |
| 95104000 | Nutritional drink or shake, ready-to-drink, sugar free (Glucerna) |
| 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*
[2´-FL] = 3 to 5 g/100 g

| | |
|---|---|
| 95220000 | Nutritional powder mix, NFS |
| 95220010 | Nutritional powder mix, high protein, NFS |
| 95230000 | Nutritional powder mix, whey based, NFS |
| 95230020 | Nutritional powder mix, protein, light, NFS |
| 95230030 | Nutritional powder mix, protein, NFS |
| 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) |
| 95210020 | Nutritional powder mix, high protein (Slim Fast) |
| 95201200 | Nutritional powder mix (EAS Whey Protein Powder) |
| 95201500 | Nutritional powder mix, high protein (Herbalife) |
| 95201600 | Nutritional powder mix (Isopure) |
| 95201700 | Nutritional powder mix (Kellogg's Special K20 Protein Water) |
| 95210000 | Nutritional powder mix (Slim Fast) |
| 95210010 | Nutritional powder mix, sugar free (Slim Fast) |

**Yogurt**
[2´-FL] = 1.2 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 |

Mixed foods containing yogurt
*Adjusted for yogurt content of 93.2%*
[2´-FL] = 1.12 g/100 g

| | |
|---|---|
| 11446000 | Yogurt parfait, low fat, with fruit |

## Processed Fruits and Fruit Juices

**Fruit Juices, Drinks, Nectars**
[2´-FL] = 0.12 g/100 g

| | |
|---|---|
| 42403010 | Coconut water, unsweetened |

42404010 Coconut water, sweetened
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%
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

| | |
|---|---|
| 92307500 | Iced Tea / Lemonade juice drink |
| 92307510 | Iced Tea / Lemonade juice drink, light |
| 92307520 | Iced Tea / Lemonade juice drink, diet |
| 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) |
| 92612010 | Sugar cane beverage |

92801000 Wine, nonalcoholic
92802000 Wine, light, nonalcoholic
92803000 Nonalcoholic malt beverage
92804000 Shirley Temple
95342000 Fruit juice, acai blend

Foods adjusted for being present in dried form
*Reconstitution factor of 4 to 11*
[2´-FL] = 0.48 to 1.32 g/100 g

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
61210720 Orange juice, 100%, frozen, not reconstituted
92511000 Lemonade, frozen concentrate, not reconstituted

## Processed Vegetables and Vegetable Juices

**Vegetable juice**
[2´-FL] = 0.12 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

## Sweet sauces, Toppings, and Syrups

**Syrups used to flavor milk beverages**
[2´-FL] = 0.7 g/100 g

91301130 Strawberry drink syrup