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ORIGINAL SUBMISSION

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GRAS Notification

Prepared for and Submitted to

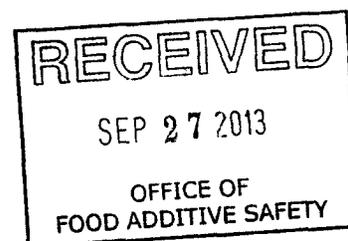
Office of Food Additive Safety (HFS-200),
Center for Food Safety and Applied Nutrition,
Food and Drug Administration,
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Table of Contents

List of Tables.....	4
1 GRAS Exemption Claim.....	5
2 GOS (galacto-oligosaccharide) Identity.....	6
2.1 Overview of GOS.....	6
2.2 CAS Registry Number.....	7
2.3 Chemical Name, Empirical Formula, Molecular Weight, Structural Formula.....	7
2.4 GOS and Degree of Polymerization.....	7
2.4.1 DP2 in a GOS.....	8
3 Floraid GOS Identity.....	8
3.1 Affirmation of AOAC 2001.02 Testing Procedure.....	8
3.2 Nutrient Composition of Floraid GOS Syrup.....	9
3.3 Nutrient Composition of Floraid GOS Powder.....	10
3.4 Inactivation of Enzyme.....	10
3.5 Dietary Fibre Content of Floraid GOS.....	10
3.6 Regulatory Dietary Fibre Status of GOS in Canada.....	11
4 Safety Evaluation of Floraid GOS by Health Canada.....	11
5 Sources of GOS.....	11
5.1 Naturally-occurring GOS in Food.....	11
5.1.1 Milk of Domesticated Animals.....	11
5.1.2 Bovine-milk based yoghurt.....	11
5.2 Manufacturing Procedure of Floraid® GOS Syrup.....	12
5.2.1 Manufacturer of Floraid GOS.....	12
5.2.2 Floraid GOS Production: Overview.....	12
5.2.3 Floraid GOS Production: Schematic.....	12
5.2.4 GMPs, Quality Control, Microbial Safety of Floraid® GOS.....	13
6 Self-limiting Factors.....	13
7 Digestion of GOS.....	14
7.1 Human Digestion of GOS.....	14
8 Purpose of GOS in Infant Formula.....	15
8.1 HMOs (Human Milk Oligosaccharides) as a Model Carbohydrate.....	15
8.2 Infant digestion of HMOs - In Vitro Investigation.....	16
8.3 GOS – Currently Next Best to HMOs.....	16
8.4 Infant digestion of GOS - In Vitro Investigation.....	16
9 Safety of GOS – Infants.....	17
10 Provision of Oligosaccharides (GOS:FOS) in Infant Formula.....	22
11 Selection of a Safe GOS Concentration in Infant Formula.....	22
11.1 Rejection of studies: 10g, 8g and 6g GOS:FOS per litre.....	22
11.1.1 Concentration of 10g GOS:FOS per litre.....	22
11.1.2 Concentration of 8g,6g GOS:FOS; 6g GOS:FOS:AOS per litre.....	23
11.2 Clinical Studies: 4g GOS:FOS per litre.....	27
11.3 Selection of GOS:FOS Concentration of 4g per litre.....	32
11.4 Safety Assessment Experts Qualified by Scientific Training and Experience.....	32
12 Safety of GOS - Adults.....	32
12.1 Safety Assessment Experts Qualified by Scientific Training and Experience.....	40
12.2 Selection of Level of use of GOS in Foods for Persons 2+.....	40
13 Intended Use and Proposed Level of Use of Floraid GOS.....	40
13.1 Infant Formula and Follow-on Foods.....	40
13.2 Foods for Persons Aged 2+.....	40

000121

14	Estimated Daily Intake GOS as a Food Supplement in Infant Formula and Toddler Foods.....	43
14.1	EDI GOS as Infant Formula Supplement.....	43
14.2	EDI GOS as a Baby, Infant and Toddler Food Supplement.....	45
15	EDI GOS as a Food Supplement, Persons Aged 2+.....	48
16	Comparison of Floraid GOS EDI to Previously reviews GOS GRNS.....	49
17	Conclusion – Determination of GRAS Exemption.....	52
18	References.....	54
	Appendix I Carbohydrate Characterization of Floraid GOS Syrup.....	60
	Appendix II Floraid Syrup Finished Product Specifications (Two C of A's).....	65
	Appendix III Carbohydrate Characterization of Floraid GOS Powder.....	67
	Appendix IV Dietary Fibre Content of Floraid GOS.....	71
	Appendix V Incubation Challenge Test_Protein Inert.....	72
	Appendix VI LONO Safety Assessment of GOS by Health Canada.....	73
	Appendix VII Naturally-occurring GOS.....	74
	Appendix VIII References <i>in vivo</i> clinical trials. Infants: GOS-only and GOS:FOS 9:1 supplemented Infant Formula; Adults: GOS.....	76
	Appendix IX Clinical Trials, Concentrations of 8g GOS:FOS, 6g GOS:FOS, 6g GOS:FOS:AOS.....	78
	Appendix X Comparison of Floraid GOS EDIs to the EDIs of GOS GRNs 334, 286, 285 and 236.....	79

List of Tables

Table 1	Nutrient Composition, Floraid GOS Syrup.....	9
Table 2	Nutrient Composition, Floraid GOS Powder.....	10
Table 3	Concentration of 4g GOS:FOS per litre Infant Formula.....	28
Table 4	Intended Level of Use and Proposed Use of GOS as a Food Ingredient in Infant Formula and Follow-on Foods.....	41
Table 5	Intended Level of Use and Proposed Use of GOS as a Food Ingredient in Foods for Persons Aged 2+.....	42
Table 6	<i>Per user 2-day average estimated daily intake of GOS from proposed use in term infant formula among infants and toddlers; WWEIA, NHanes 2007-2010.....</i>	44
Table 7	<i>Per user 2-day average estimated daily intake of GOS from proposed use in baby, infant, and toddler foods among non-nursing infants and toddlers age 6 to 35 months; WWEIA, NHanes 2007-2010.....</i>	46
Table 8	<i>Per user 2-day average estimated daily intake of GOS from proposed uses in dairy and beverages products by the US population 2 year and older; WWEIA, NHanes 2007-2010.....</i>	49
Table 9	Comparison with previous GRNs of EDI of GOS for combined uses in infant formula and baby food.....	50
Table 10	Comparison with previous GRNs of EDI of GOS for foods for persons aged 2+.....	51

000123

1 GRAS Exemption Claim

Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)] (U.S. FDA, 1997)

It is the intention of the notifier, International Dairy Ingredients Inc. (IDII), of Oakville, ON, Canada, to market Floraid™ GOS (galacto-oligosaccharide) in the USA, as a food supplement to infant formula; infant, baby and toddler foods; and select foods for persons age 2+. The notifier, IDII, has determined that Floraid GOS is Generally Recognized as Safe (GRAS), consistent with Section 201(s) of the Federal Food, Drug and Cosmetic Act.

This determination is based on scientific procedures as detailed herein and on the opinion of independent experts qualified by scientific training and expertise to evaluate the safety of GOS under the conditions of intended use in food. Therefore, the use of IDII's Floraid GOS in food, as described herein, is exempt from the requirement of premarket approval as specified by the Federal Food, Drug and Cosmetic Act, Section 409.

Also, please note that, prior to July 2013, the brand name of the subject GOS was "Promovita". A commercial entity has claimed prior and exclusive use of this brand name in the USA, thus, the subject GOS is newly branded as Floraid, for the USA marketplace. Accordingly, the Finished Product C of A's show the use of the name Promovita prior to legal challenge.

Should the FDA require additional information, or copies of documents cited herein, please do not hesitate to contact the undersigned.

Signed,

(b) (6)

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2 GOS (galacto-oligosaccharide) Identity

2.1 Overview of GOS

GOS (galacto-oligosaccharide) as a food ingredient has been commercialized since the 1990's. GOS syrup and GOS powder are each a mixture of the distinctive oligosaccharide (galacto-oligosaccharide) plus disaccharides, monosaccharides, and in the syrup format, water.

The manufacturing of GOS, a standardized procedure widely reviewed in the scientific literature, consists of three basic steps: the subjection of highly-refined lactose to a β -galactosidase (either fungal or enzymatic in origin); addition of water; application of heat to a specified temperature for a specified period of time. The β -galactosidase hydrolyzes the disaccharide lactose to the monosaccharides glucose and galactose, and alternatively catalyzes the transgalactosylation¹ of lactose to produce galacto-oligosaccharides, with a mixture of monosaccharides, disaccharides and water as co-products. The monosaccharides and disaccharides in the resultant GOS mixture have come to be known as the sugar profile or "sugar pattern".

GOS are chains of galactose units of varying lengths, usually with a terminal glucose molecule. Generally, the typical formula is [(galactose(gal))*n*-glucose(glu) where *n*=1-7], consisting of β -glycosidic links between the individual galactose-glucose molecules.

The chain length and concentration of GOS is determined by the rate of hydrolysis and degree of transgalactosylation, which, in turn, are a function of the β -galactosidase source, the substrate (lactose) concentration and the reaction factors of temperature and length of heat exposure.^{2,3} Manipulation and control of these three aspects of GOS production results in GOS of varying chain lengths and differing GOS concentration within the GOS/mono-disaccharide/water mixture.

Floraid™ GOS is available in two formats - a light-coloured syrup, and, a light-coloured powder. Floraid GOS is derived from subjection of refined lactose to a select enzyme, resulting in a mixture of high-quality GOS, water, and a blend of saccharides, identified herein. Details on the manufacturing of Floraid GOS are provided in herein.

¹ Occasionally, in the scientific literature, GOS is referred to as TOS or T-GOS, to signify the transgalactosylation process.

² Iliev I and Vasileva T. 2012. Study of the transgalactosylation activity of β -galactosidase from a new strain *Kluyveromyces lactis*. Journal of Bioscience and Biotechnology. 1(2):149-153.

³ Gosling A et al. 2010. Recent advances refining galacto-oligosaccharide production from lactose. Food Chemistry. 121(2):307-318.

2.2 CAS Registry Number

The CAS Registry Number 66455-21-8 for “oligosaccharides” includes β -oligosaccharides (beta-oligosaccharides), which is the classification for galacto-oligosaccharides.

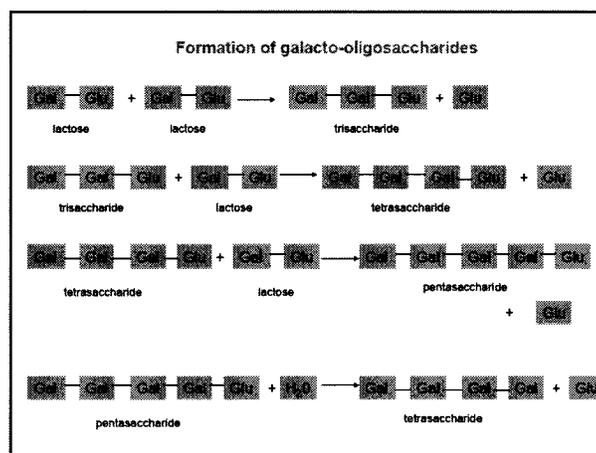
2.3 Chemical Name, Empirical Formula, Molecular Weight, Structural Formula

Not Applicable to Floraid GOS.

2.4 GOS and Degree of Polymerization

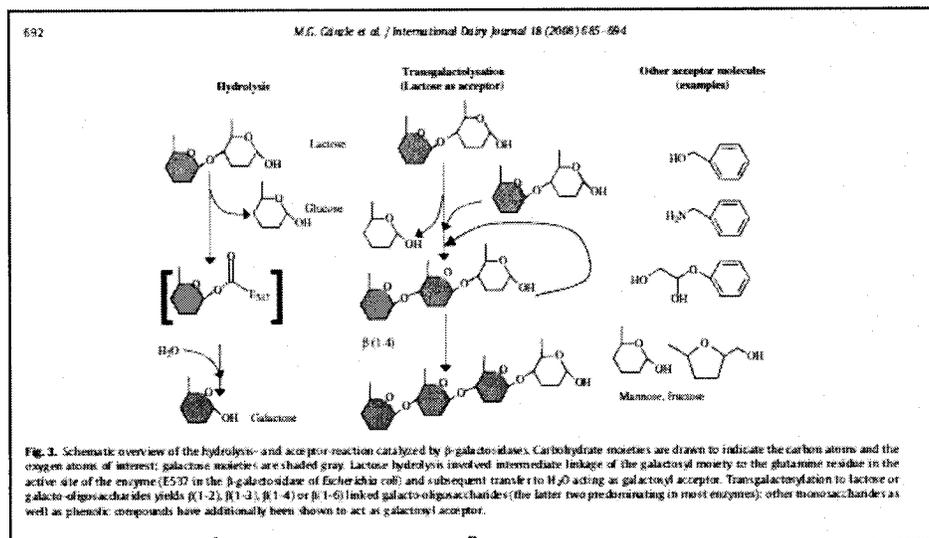
The varying length of oligosaccharide chains present in an oligosaccharide is referred to as the “degree of polymerization” or DP. The DP reflects the average chain length of the oligosaccharides in the GOS. An oligosaccharide having a DP3 or DP4 has an average of three or four monosaccharide linked units, respectively. A disaccharide has a DP2. The DP of a GOS can range from 2 to 8 monomeric linked units - a DP from 2 to 8. Later in this chapter, it will be demonstrated that, during the commercial production of GOS, the DP increases from lower to higher values. Figure 1, below, provides a simplified illustration of this sequential production of GOS of increasing chain length. Figure 2 illustrates the identical procedure from a chemical structure perspective.⁴

Figure 1. Schematic showing successive molecular formation of Floraid GOS



⁴ Ganzle, MG et al. 2008. Lactose: Crystallization, hydrolysis and value-added derivatives. International Dairy Journal. 18(7):685-694.

Figure 2. Schematic Overview of Galacto-oligosaccharide production, depicted through chemical structure



2.4.1 DP2 in a GOS

During the early stages of GOS production (detailed below) the prominence of glucose and galactose in the mixture results in a unique galactose-glucose combination (lactose) and as well, a unique galactose-galactose combination (single galactose unit with a galactose terminal end). These disaccharides, known as allo(α)-lactose (glu/gal), an isomer of lactose, and digalactan (gal/gal), respectively, have transgalactosylated linkages.^{5,6} These DP2 molecules are frequently referred to in the scientific literature as TD, or, transgalactosylated disaccharides. They are GOS, distinct and separate from conventional disaccharides such as the free lactose which is present in the carrier portion of a GOS product. Thus, even though the accepted definition of an oligosaccharide is that consisting of 3-10 monosaccharide units, it is customary and common for a GOS to contain disaccharides. Accordingly, units of GOS may have a DP as low as 2 to as high as 8.

3 Floraid GOS Identity

3.1 Affirmation of AOAC 2001.02 Testing Procedure

The concentration and characterization of GOS in Floraid GOS has been confirmed through the application of testing method AOAC 2001.02. Selection of this procedure to verify and quantify GOS in Floraid GOS and in prepared foods was based upon:

⁵ Splechna, B et al. 2006. Production of prebiotic galacto-oligosaccharides from lactose using α -galactosidases from *Lactobacillus reuteri*. *Journal of Agricultural and Food Chemistry*. 54(14):4999-5006.

⁶ Playne, M. and Crittenden, R. *Advanced Dairy Chemistry*. Volume 3: Lactose, Water, Salts and Minor Constituents. Springer Science+Business Media. "Galacto-oligosaccharides and Other Products Derived from Lactose". 2009. Page 134.

- Endorsement by the 26th Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses, July 2004, page 3:⁷
- Recommendation by the 31st Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses, November 2009, page 4.⁸
- Recommended by the certified laboratory retained to measure the GOS in Floraid GOS and in prepared foods.

3.2 Nutrient Composition of Floraid GOS Syrup

The composition of Floraid GOS complies with that described in the scientific literature.

Floraid GOS syrup has a DP of 3.2, an average 28% GOS concentration, and a sugar profile consisting of monosaccharides glucose, galactose and fructose, and, disaccharides lactose, maltose and sucrose, as shown in Table 1 below.

Table 1 Nutrient Composition, Floraid GOS Syrup

Calories per 100 g		294 kcal
Nutrient		Percentage (%)
Water		26.5
Total Carbohydrate		73.44 ⁹
Galacto-oligosaccharide ¹⁰ (% of syrup which is Dietary Fibre)		28.3 20.77
Glucose		21
Lactose		18
Galactose		8.5
Sucrose		0.8
Maltose		0.4
Fructose		0.2
Protein		<0.1
Fat		<0.1

A Certificate of Analysis of the carbohydrate content of Floraid GOS syrup, including the DP, is contained in Appendix I. A Certificate Analysis of the Finished Product Specifications of Floraid GOS syrup, is contained in Appendix II.¹¹

⁷ <ftp://ftp.fao.org/codex/ccnfsdu26/nf2603ae.pdf>

⁸ http://www.cclac.org/comites/doc_grupos/g_nfsdu/jun_2009/ccnfsdu31_df_MA_DRAFT_090505.doc

⁹ A portion of the lactose and galactose are also reported as GOS, thus, the summation of the individual carbohydrates is 77.3%, even though total carbohydrate concentration is 73.4%.

¹⁰ Includes α -lactose and β -galactan (transgalactosylated), the disaccharides which exhibit galacto-oligosaccharide characteristics.

¹¹ Prior to July 2013, the brand name of the subject GOS was "Promovita". A commercial entity has claimed prior and exclusive use of this brand name in the USA, thus, the subject GOS is newly branded as Floraid, for the USA marketplace. Accordingly, the Finished Product C of A's show the use of Promovita prior to legal challenge.

3.3 Nutrient Composition of Floraid GOS Powder

Floraid GOS powder has a DP of 3.2, an average 39% GOS concentration, and a sugar profile consisting of monosaccharides glucose, galactose and fructose, and, disaccharides lactose, maltose and sucrose, as shown in Table 2 below.

Table 2 Nutrient Composition, Floraid GOS Powder¹²

Calories per 100 g	400 kcal
Nutrient	Percentage (%)
Galacto-oligosaccharide	39.0
Lactose	28.6
Glucose	24.7
Galactose	9.33
Fructose	0.69
Sucrose	0.68
Maltose	0.15
Total	103.15

A Certificate of Analysis of the carbohydrate content of Floraid GOS powder, including the DP, is contained in Appendix III.

3.4 Inactivation of Enzyme

Floraid GOS was subjected to a challenge test through incubation for 4.5 hours at 55°C, to demonstrate total inactivation of the minimal-remaining amount of enzyme (See Table 1), as shown in Appendix V.

3.5 Dietary Fibre Content of Floraid GOS

Since Floraid GOS has a DP of 3.2, a large portion of the monomeric links measure as dietary fibre when subjected to nutrient analysis (AOAC 2009.01). As shown in Appendix IV, Floraid GOS syrup is 20.77% dietary fibre. As Floraid GOS syrup is 28.3% GOS, the GOS monomeric chains are 73.39% dietary fibre.

Clinical studies, both *in vivo* and *in vitro*, illustrate that consumption of GOS provides human physiological effects identical to those associated with dietary fibre, such as a bifidogenic effect and looser stool consistency.

¹² A portion of the lactose and galactose are also reported as trans-disaccharides in the GOS, thus, the percentage summation of the individual carbohydrates is 103.15%.

3.6 Regulatory Dietary Fibre Status of GOS in Canada

In April 2013, Health Canada published a list of novel fibres granted dietary fibre status in Canada¹³, one of which is GOS.¹⁴

4 Safety Evaluation of Floraid GOS by Health Canada

The Food Directorate of Health Canada evaluated the safety of Floraid GOS as a food ingredient and issued a Letter of No Objection in April 2012, a copy of which is provided in Appendix VI.

5 Sources of GOS

GOS naturally occurs in bovine milk, and to a greater degree, in yoghurt. As aforementioned, GOS can be commercially manufactured from lactose. In the following two sections, these sources of GOS will be described.

5.1 Naturally-occurring GOS in Food

5.1.1 Milk of Domesticated Animals

GOS of the composition Gal(β 1-3/6) Gal(β 1-4)Glc is present in a very low concentration in cow, sheep, goat and horse milks.¹⁵

5.1.2 Bovine-milk based yoghurt

The scientific literature indicates that GOS naturally occurs in yoghurt, in concentrations of 0.22% to 0.28%.^{16,17} Primary research and nutrient analysis (AOAC 2001.02) undertaken to prepare for the commercialization of Floraid GOS revealed a naturally-occurring GOS content of 0.4% in basic yoghurt. See Appendix VII.

¹³ Health Canada Food Directorate regards the description "dietary fibre" as both the name of an ingredient, and, an implied physiological claim. To that end, to be declared as a dietary fibre in the Nutrition Facts Table and Ingredient Statement in a labelled food, a novel fibre must undergo human clinical trials to demonstrate any one of four physiological effects associated with dietary fibre: laxation, serum glucose attenuation, serum cholesterol reduction, and end-expiratory breath hydrogen. Due to the agency's acquired familiarity with particular novel fibres, in April 2013, such novel fibres were granted generic regulatory fibre status when the composition thereof complies with that required of a dietary fibre (i.e. DP \geq 3, free of microbial hazards, etc.). One of the novel fibres granted generic dietary fibre status is GOS, as per the reference below.

¹⁴ Health Canada, Food Directorate. 2013. List of Dietary Fibres Permitted for Use in Foods Available for Sale in Canada. See also: <http://www.inspection.gc.ca/english/fssa/labeli/guide/ch6ae.shtml>

¹⁵ Boehm, G and Stahl B. 2007. Oligosaccharides from Milk. *Journal of Nutrition*. 137(3):847S-849S.

¹⁶ Lamoureaux L, Roy D, Gauthier SF. 2002. Production of oligosaccharides in yoghurt containing bifidobacteria and yoghurt Cultures. *Journal of Dairy Science*. 85(5):1058-1069.

¹⁷ Martinez-Villaluenga C et al. 2008. Study of galactooligosaccharide composition in commercial fermented milks. *Journal of Food Composition and Analysis*. 21(7):540-544.

5.2 Manufacturing Procedure of Floraid® GOS Syrup

5.2.1 Manufacturer of Floraid GOS

Floraid GOS is manufactured by Wright Agri Industries Limited, UK. Floraid GOS will be imported into the USA by International Dairy Ingredients Inc. of Oakville, ON, Canada.

5.2.2 Floraid GOS Production: Overview

The production of Floraid GOS complies with that described in the scientific literature as follows^{18,19}:

- i. Production begins with the substrate refined lactose, extracted from whey.
- ii. An enzyme derived from *Aspergillus oryzae* is added under controlled and optimum conditions.
- iii. The enzyme catalyzes the hydrolysis of the terminal non-reducing β -D-galactose residues in β -D-galactosides, transferring the terminal non-reducing β -D-galactose to a suitable acceptor.
- iv. At low lactose concentrations, water is a suitable acceptor and lactose is hydrolysed.
- v. At high lactose concentrations, lactose and other galacto-oligosaccharides are suitable acceptors and galactosyl(s):lactose molecules are formed.
- vi. An acceptable concentration of DP3+ units are formed and retained.
- vii. Both powder and liquid formats are produced.

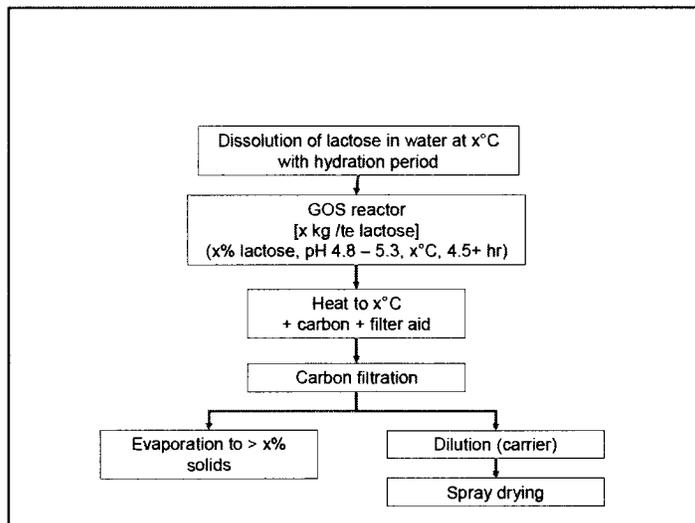
5.2.3 Floraid GOS Production: Schematic

A schematic overview of Floraid GOS production has been provided by the manufacturer of Floraid GOS in Figure 3.

¹⁸ Playne M, Crittenden R. 2009. Galacto-oligosaccharides and other products derived from lactose. Advanced Dairy Chemistry, Chapter 5. Volume 3: Lactose, Water, Salts and Minor Constituents. Springer Science Business Media.

¹⁹ Ibid. Gosling A et al. 2010.

Figure 3. Schematic and Detailed Description of Manufacturing Process of Floraid GOS²⁰



5.2.4 Good Manufacturing Procedures, Quality Control, Microbial Safety of Floraid® GOS

Procedures undertaken by Wright Agri Industries Limited to uphold the Good Manufacturing Procedures of Floraid GOS production are itemized below.²¹

- HACCP Pre-Requisite Program Details (Summary)
- HACCP Programme
- GOS Production Log
- Storage and Transportation Log
- GOS Handling and Safety Data
- Certificate/Certification of Food Safety Audit
- Pictographic Representation of Floraid GOS Storage Container & Facility

6 Self-limiting Factors

Food Formulation Constraints

The capacity of a food formulation to integrate an amount of GOS is limited. For example, only 1g GOS per cereal bar of 30g would be recommended, as 2g GOS per 30g cereal bar results in a product with unacceptable organoleptic properties.

Thermal Stability

Bench trials and analytical measurement indicate that food processing temperatures $\geq 110^{\circ}\text{C}$ result in disintegration of GOS.

²⁰ Floraid GOS supplier, Wright Agri Industries Limited UK.

²¹ Certifications of healthy and safety, as well as GMP compliance available upon request.

Yeast-leavened Foods

Yeast in a yeast-leavened food will utilize GOS as an energy source, resulting in significantly less GOS in the leavened and baked product than was added during the dough preparation stage.

7 Digestion of GOS

7.1 Human Digestion of GOS

Monosaccharides and Disaccharides in GOS Syrup and GOS Powder

The monosaccharides in Floraid GOS syrup and powder include glucose, galactose and fructose. The disaccharides in Floraid GOS syrup and powder include lactose, sucrose and maltose. Lactose consists of a β -(1 \rightarrow 4) glycosidic linkage of a β -glucose molecule to a β -galactose molecule. Allolactose – an isomer of lactose – consists of a β -(1 \rightarrow 6) glycosidic linkage of an α -glucose molecule to a β -galactose molecule. Sucrose consists of α (1 \rightarrow 4) linkage of a glucose molecule to a fructose molecule. Maltose consists of an α (1 \rightarrow 4) linkage of a glucose molecule to another glucose molecule.

Digestion of the Monosaccharides and Disaccharides in Floraid GOS Syrup and Powder

The monosaccharides and disaccharides enter the small intestine intact, where they are hydrolyzed by enzymes localized in, and secreted from, the intestinal brush border of the colon, so as to digest the monosaccharides and disaccharides as follows:

Disaccharides

Lactose \rightarrow glucose and galactose by the enzyme lactase (β -D galactosidase)

Sucrose \rightarrow glucose and fructose by the enzyme sucrase (α -D glucohydrolase)

Maltose \rightarrow glucose and glucose by the enzyme maltase (α -glucosidase)

Monosaccharides

Once the disaccharides are cleaved into glucose, galactose and fructose, these monosaccharides, along with those readily present in the Floraid GOS syrup and powder (also glucose, galactose and fructose) are transported via specific co-transporters across the luminal membrane and absorbed.²²

Digestion of the Galacto-oligosaccharides in Floraid GOS Syrup and Powder

The remaining components of Floraid GOS syrup and powder – small amounts of allolactose and galactan²³ and the galacto-oligosaccharides (GOS) – reach the small intestine intact. The enzyme β (1 \rightarrow 4) galactanase required to cleave the galactan, and the enzyme β (1 \rightarrow 4) galactosidase required to cleave the allolactose and hydrolyze the

²² R.A. Bowen. Professor, Department of Biomedical Sciences. Colorado State University. Fort Collins, CO http://www.vivo.colostate.edu/hbooks/pathophys/digestion/smallgut/absorb_sugars.html Retrieved June 30, 2013.

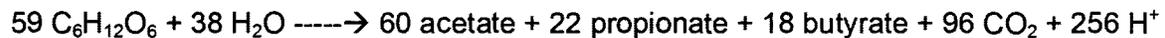
²³ Galactan also occurs as a polymer of the sugar galactose, in hemicellulose; allolactose is an isomer of lactose.

galacto-oligosaccharides, are not manufactured by the human body, nor provided by the small intestinal villi.

Hence, GOS or α -Glu-(1-4)-[β -Gal-(1-6)]_n (where n =1-7), having escaped digestion in the upper gastrointestinal tract, reach the proximal colon largely intact. GOS are fermented by selective colonic bacteria (mainly bifidobacteria and lactobacilli) to produce.^{24,25,26,27,28,29,30}

- short-chain fatty acids (SCFA), mostly acetate, propionate, and butyrate, as major end products of the microbial fermentation process in the colon. SCFA are rapidly absorbed by the colonic mucosa to result in further energy gain for the host. Up to 95% of the SCFA produced during GOS fermentation may be taken up and utilized by the host;
- gases: H₂, CO₂, CH₄, H₂S;
- reduced substances (electron sink products): lactate, pyruvate, ethanol, succinate, which directly influence the reduction and oxidation balance;
- bacterial cell mass

Cummings (1995) outlined an equation describing overall carbohydrate fermentation in the colon as follows:³¹



8 Purpose of GOS in Infant Formula

8.1 HMOs (Human Milk Oligosaccharides) as a Model Carbohydrate

One of the intended uses of Floraid GOS is addition to human milk substitute (infant formula), the purpose being an attempt to mimic, to the extent scientifically possible, the HMOs in human breast milk.

The concentration of HMOs in transition, mature human milk is (oligosaccharides/litre breast milk) 7–12 g³² or 8-12g³³ or 12-14g³⁴ making the oligosaccharide fraction a major

²⁴ Macfarlane GT and Macfarlane S. 2011. Fermentation in the human large intestine: Its physiologic consequences and the potential contribution of prebiotics. *J Clin Gastroenterol.* 45 (Suppl 3):S120-S127.

²⁵ Macfarlane GT, Steed H, and Macfarlane S. 2008. Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. *Journal of Applied Microbiology* 104(2):305-344.

²⁶ Macfarlane S and Macfarlane GT. 2003. Regulation of short-chain fatty acid production. *Proceedings of the Nutrition Society* 62:67-72.

²⁷ Roberfroid M, Gibson GR & Hoyles L. 2010. Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition.* 104(2):S1-S14.

²⁸ Cummings JH, Macfarlane GT and Englyst HN, 2001. Prebiotic digestion and fermentation. *American Journal of Clinical Nutrition.* 73(2):415S-20S.

²⁹ Rivero-Urgell M & Santamaria-Orleans A, 2001. Oligosaccharides: Application in infant foods. *Early Human Development* 65(Suppl):S43-S52.

³⁰ Dubert-Ferrandon A, Newburg DS & Walker A, 2008. Part 1 – Prebiotics: New medicine for the colon. *Nutrition Today* 43(6):245-249.

³¹ As quoted in Macfarlane GT and Macfarlane S. 2011.

³² Ibid. Boehm G and Stahl B. 2007.

³³ European Commission Health and Consumer Protection Directorate-General. 2003. Report of the Scientific Committee on Food on the Revision of Essential Requirements of Infant Formulae and Follow-on Formulae. SCF/CS/NUT/IF/65 Final.

component of human milk. They are important components of the defense system of human milk, having both prebiotic potential and direct interaction with the infant's immune cells.³⁵

HMOs are of a complexity which continues to challenge the most skilled and talented scientists.^{33, 36, 37, 38} German J.B. et al (2008) report that approximately 200 unique oligosaccharides, varying from 3 to 22 monosaccharide units, have been identified in human milk. In the authors' words: "The increasing complexity of oligosaccharides follows the general pattern of mammalian evolution, though the concentration and diversity of these structures in *Homo sapiens* are strikingly [*sic*]."³⁹

8.2 Infant digestion of HMOs - In Vitro Investigation

Engfer et al (2000)⁴⁰, using *in vitro* techniques, demonstrated that HMOs resist digestion in the upper gastrointestinal tract, reaching the large intestine where they serve as substrates for bacterial metabolism.

In a systematic review conducted by Coppa et al (2004)⁴¹, it was shown that the characterization of oligosaccharides in the feces of breast-fed infants and the identification of intestinal microflora using molecular analysis confirmed that HMOs are minimally digested in the small intestine, and reach the colon intact, where they selectively stimulate the development of bifidogenic flora.

8.3 GOS – Currently Next Best to HMOs

Boehm (2007) advises that, due to the unlikelihood of finding natural sources that contain oligosaccharides identical to HMOs, currently available oligosaccharides need be analyzed to identify those with structures and functions similar to those of HMOs. GOS, such as Floraid GOS, as well as FOS (derived from vegetable plants) are prime candidates, as both oligosaccharides have demonstrated non-digestion in the small intestine and fermentation by bifidobacteria in the colon, in both infants and adults.

8.4 Infant digestion of GOS - In Vitro Investigation

Boehm (2007) further reports that GOS, with a lactose-based chemical structure, shares similarities to the core molecules of HMOs, whereas there is no FOS present in human

³⁴ Coppa et al. 2004. The first prebiotics in humans: human milk oligosaccharides. *Journal of Clinical Gastroenterology*. 38 (Suppl 6):S80-3.

³⁵ Ibid. Boehm G and Stahl B. 2007.

³⁶ Ibid. Boehm G and Stahl B. 2007.

³⁷ Kunz C, Rudloff S, Baier W, Klein N, Strobel S. 2000. Oligosaccharides in human milk: Structural functional and metabolic aspects. *Annual Review of Nutrition*. 20:699–722.

³⁸ Boehm G and Stahl B. Oligosaccharides. 2003. In: Mattila-Sandholm T, editor. *Functional dairy products*. Cambridge: Woodhead Publishers. p. 203–43.

³⁹ German, JB et al. 2008. Human milk oligosaccharides: evolution, structures and bioselectivity as substrates for intestinal bacteria. *Nestlé Nutrition Workshop Series, Pediatric Program*. 62:205-18; discussion 218-22.

⁴⁰ Engfer et al. 2000. Human milk oligosaccharides are resistant to enzymatic hydrolysis in the upper gastrointestinal tract. *American Journal of Clinical Nutrition*. 71(6):1589-96.

⁴¹ Ibid. Coppa et al. 2004.

milk oligosaccharides. However, combining the low-molecular weight GOS with the high-molecular weight FOS in a ratio of 9:1 approximates the molecular weight of HMOs.^{42,43}

It was shown *in vitro* by Mikkelsen et al (2004)⁴⁴ after a GOS and FOS feeding trial on piglets that:

- (1) bacteria from the caecum and mid-colon of the piglets had the biggest capacity to degrade GOS and FOS;
- (2) bacteria from the distal small intestine to some extent fermented GOS and FOS;
- (3) bacteria from the stomach were nearly incapable of fermenting GOS and FOS.

Thus, drawing from the findings of Engfer (section 8.2, above) and Mikkelsen, it can be deduced that infant digestion of GOS is similar to infant digestion of HMOs. Furthermore, infant digestion of oligosaccharides is similar to the digestion of oligosaccharides by humans of older ages.

9 Safety of GOS – Infants

Several *in vivo* clinical trials conducted on infants have successfully demonstrated that GOS is safely consumed, and, due to its non-digestible, fermentable carbohydrates, will beneficially characterize the microbial profile of the colon. Three such studies have been selected for inclusion in this GRAS Notification, and summarized in the charts which follow, identified as:

Ashley et al (2012) at 4g GOS/litre; healthy infants 2-16 days old;
Fanaro et al (2009) at 5g GOS/litre; healthy infants 4-6 months of age; and
Ben et al (2008) at 2.4g GOS/litre; healthy infants, 1-3 months of age.

In all three studies, the GOS-supplemented infant formulas were well-tolerated and supported normal growth without adverse side effects. For convenience, complete reference description of these three studies is provided in Appendix VIII.

⁴² Ibid. European Commission. 2003.

⁴³ Moro et al. 2002. Dosage-related bifidogenic effects of galacto- and fructooligosaccharides in formula-fed term infants. *Journal of Pediatric Gastroenterology and Nutrition*. 34(3):291-5.

⁴⁴ Mikkelsen LL, Knudsen KEB, and Jensen BB, 2004. *In vitro* fermentation of fructo-oligosaccharides and transgalacto-oligosaccharides by adapted and unadapted bacterial populations from the gastrointestinal tract of piglets. *Animal Feed Science and Technology*. 116(3-4):225-238.

Summary of Intervention studies investigating the safety and physiological effects of GOS consumption							
Reference, Author, Year	Aim of Study	Design R (Randomized) NR (Non-randomized) C (Control group) SB (Single-blind) DB (Double-blind) P (Parallel) CO (Crossover)	Age range, Gender (M, F) No. recruited No. randomized No. in final sample	Daily intake; Frequency; GOS format; Route of intake; Duration of consumption;	Background Diet & Assessment Tool	Endpoints, Results & Statistics Changes in health effect Adverse effects	Relevant Authors' Conclusions
Legend	Aim of Study	Design R,NR,C,SB,DB P,CO	Sample Characteristics A, G, # Re, #Ra, # FS:	Treatment Characteristics Di, Frq; GOS Frm; Route; Dur.	Background Diet & Assessment Tool	Endpoint, Results & Statistics Changes in health effect Adverse effects	Relevant Authors' Conclusions

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Reference and Type of Evidence	Aim of Study	Trial Design R, NR, C, SB, DB, P, CO	Sample Characteristics A, G, # Re., #Ra., # FS:	Treatment Characteristics Di; Frq; GOS Fm; Route; Dur.	Background Diet & Assessment Tool	Endpoint Results & Statistics		Relevant Authors' Conclusions																																																																																		
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Reference Ashley et al, 2012	To evaluate growth and tolerance in healthy infants who received one of two investigational cow's milk-based formulas with adjustments in carbohydrate, fat, and calcium content and supplemented with a prebiotic blend of polydextrose (PDX) and GOS, or GOS alone.	R, C, DB, P	<ul style="list-style-type: none"> 426 infants recruited and randomized at 21 clinical sites in the US; 244 males and 182 females Healthy, 12 to 16-day old infants Singleton births at 37-42 weeks gestational age Birth weight \geq 2500g Solely formula-fed at least 24 h prior to randomization 287 in final sample; 167 males and 120 females 	Route: oral <ul style="list-style-type: none"> Blend of PDX + GOS (1:1 ratio) Dose 4g/L in investigational formula GOS Dose 4g/L in investigational formula Control Dose Infant formula Enfamil® LIPIL All study formulas were in powdered form. Identical mixing instructions were provided to yield a final product of 20 calories/fluid ounce. Duration: from 14 to 120 days of age (107 days) Background diet: none Assessment tool: a 24-h recall of diet, tolerance, and stool characteristics	Weight, length, and head circumference growth rates from 14 days to 30, 60, 90, and 120 days of age	<table border="1"> <thead> <tr> <th rowspan="2">Sex</th> <th rowspan="2">Day</th> <th rowspan="2">Group</th> <th colspan="3">Growth Rate</th> </tr> <tr> <th>Weight (g/day)</th> <th>Length (cm/day)</th> <th>Head cir. (cm/day)</th> </tr> </thead> <tbody> <tr> <td rowspan="6">M</td> <td rowspan="2">30</td> <td>GOS</td> <td>46.7± 1.6</td> <td>0.13± 0.009</td> <td>0.10± 0.005</td> </tr> <tr> <td>Contr.</td> <td>48.9± 1.5</td> <td>0.15± 0.009</td> <td>0.10± 0.005</td> </tr> <tr> <td rowspan="2">60</td> <td>GOS</td> <td>41.9± 1.2</td> <td>0.13± 0.004</td> <td>0.07± 0.002</td> </tr> <tr> <td>Contr.</td> <td>41.3± 1.1</td> <td>0.13± 0.004</td> <td>0.08± 0.002</td> </tr> <tr> <td rowspan="2">90</td> <td>GOS</td> <td>36.8± 1.1</td> <td>0.12± 0.003</td> <td>0.06± 0.002</td> </tr> <tr> <td>Contr.</td> <td>36.1± 1.0</td> <td>0.12± 0.003</td> <td>0.07± 0.002</td> </tr> <tr> <td rowspan="2">120</td> <td>GOS</td> <td>33.3± 0.9</td> <td>0.11± 0.002</td> <td>0.06± 0.001</td> </tr> <tr> <td>Contr.</td> <td>32.6± 0.8</td> <td>0.11± 0.002</td> <td>0.06± 0.001</td> </tr> <tr> <td rowspan="6">F</td> <td rowspan="2">30</td> <td>GOS</td> <td>34.6± 1.6*</td> <td>0.11± 0.012</td> <td>0.09± 0.007</td> </tr> <tr> <td>Contr.</td> <td>38.4± 1.5</td> <td>0.15± 0.012</td> <td>0.09± 0.006</td> </tr> <tr> <td rowspan="2">60</td> <td>GOS</td> <td>32.2± 1.3</td> <td>0.12± 0.006</td> <td>0.07± 0.003</td> </tr> <tr> <td>Contr.</td> <td>32.4± 1.2</td> <td>0.12± 0.005</td> <td>0.07± 0.003</td> </tr> <tr> <td rowspan="2">90</td> <td>GOS</td> <td>29.3± 1.0</td> <td>0.11± 0.003</td> <td>0.06± 0.002</td> </tr> <tr> <td>Contr.</td> <td>29.0± 1.0</td> <td>0.11± 0.003</td> <td>0.06± 0.002</td> </tr> <tr> <td rowspan="2">120</td> <td>GOS</td> <td>27.3± 0.9</td> <td>0.10± 0.003</td> <td>0.05± 0.002</td> </tr> <tr> <td>Contr.</td> <td>27.6± 0.9</td> <td>0.10± 0.003</td> <td>0.05± 0.002</td> </tr> </tbody> </table> <p>* Significantly lower than control, P<0.05, one-tailed test</p> <p>- There were no group differences in growth rate from 14 to 120 days of age</p> <p>- No group differences in overall study discontinuation were detected. Most common symptoms in participants discontinuing due to formula intolerance (13%) were fussiness, gas, and vomiting. Study discontinuation rates were as expected when compared to those reported in other large pediatric nutrition trials</p> <p>- No significant difference between GOS-treated and control groups in gassiness, fussiness, or study formula intake were detected during study weeks 1 or 2, or at 60, 90, and 120 days of age</p>	Sex	Day	Group	Growth Rate			Weight (g/day)	Length (cm/day)	Head cir. (cm/day)	M	30	GOS	46.7± 1.6	0.13± 0.009	0.10± 0.005	Contr.	48.9± 1.5	0.15± 0.009	0.10± 0.005	60	GOS	41.9± 1.2	0.13± 0.004	0.07± 0.002	Contr.	41.3± 1.1	0.13± 0.004	0.08± 0.002	90	GOS	36.8± 1.1	0.12± 0.003	0.06± 0.002	Contr.	36.1± 1.0	0.12± 0.003	0.07± 0.002	120	GOS	33.3± 0.9	0.11± 0.002	0.06± 0.001	Contr.	32.6± 0.8	0.11± 0.002	0.06± 0.001	F	30	GOS	34.6± 1.6*	0.11± 0.012	0.09± 0.007	Contr.	38.4± 1.5	0.15± 0.012	0.09± 0.006	60	GOS	32.2± 1.3	0.12± 0.006	0.07± 0.003	Contr.	32.4± 1.2	0.12± 0.005	0.07± 0.003	90	GOS	29.3± 1.0	0.11± 0.003	0.06± 0.002	Contr.	29.0± 1.0	0.11± 0.003	0.06± 0.002	120	GOS	27.3± 0.9	0.10± 0.003	0.05± 0.002	Contr.	27.6± 0.9	0.10± 0.003	0.05± 0.002	Investigational routine infant formulas supplemented with 4g/L of GOS were well-tolerated and supported normal growth.
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<p>Reference</p> <p>Fanaro et al, 2009</p>	<p>- To determine the bifidogenic effect of GOS in healthy infants in a follow-on formula</p> <p>- To assess GOS effects on stool characteristics, growth, and general well-being</p>	<p>R, C, DB, P</p>	<ul style="list-style-type: none"> • 172 healthy infants, exclusively formula-fed at enrollment (at 4-6 months), were recruited • 159 were randomized • 115 in final sample (53 males, 62 females) 	<p>Pre-study period: Standard infant formula for at least 14 days</p> <p>After randomization:</p> <ul style="list-style-type: none"> • GOS Dose 5g/L in a standard follow-on formula • Control Dose 5g/L maltodextrins in a standard follow-on formula <p>Duration: 18 weeks</p>	<p>Infants were evaluated at randomization (study day 1), 6 weeks later (study day 2), and finally 18 weeks after randomization (study day 3)</p> <p>Background diet: newly introduced solid foods</p> <p>Assessment tool: parent's diary to record daily milk volumes and solid foods introduced, characteristics of the stools, stool frequency and consistency, and clinical problems such as regurgitation, vomiting, and flatulence</p>	<p>Bifidobacteria counts in study population, expressed as [log₁₀ colony-forming units] (25%Q-75%Q)</p> <table border="1" data-bbox="1119 521 1692 769"> <thead> <tr> <th></th> <th>GOS</th> <th>Control</th> </tr> </thead> <tbody> <tr> <td>Study day 1 (week 0)</td> <td>8.90 (8.20-9.60)</td> <td>8.94 (8.32-9.68)</td> </tr> <tr> <td>Study day 2 (week 6)</td> <td>9.96 (9.21-10.53)</td> <td>9.64 (8.82-9.96)</td> </tr> <tr> <td>Study day 3 (week 18)</td> <td>9.86 (8.99-10.18)</td> <td>9.38 (8.35-9.90)</td> </tr> </tbody> </table> <p>At study day 2 and study day 3, the GOS group had a higher median number (CFU per gram of faeces) of bifidobacteria than did the control group.</p> <p>Adverse Effects: The incidence of crying, regurgitation, vomiting, and flatulence was not different between the groups</p>		GOS	Control	Study day 1 (week 0)	8.90 (8.20-9.60)	8.94 (8.32-9.68)	Study day 2 (week 6)	9.96 (9.21-10.53)	9.64 (8.82-9.96)	Study day 3 (week 18)	9.86 (8.99-10.18)	9.38 (8.35-9.90)	<p>The dietary treatment with low doses of GOS was well tolerated by all of the infants enrolled in the study and had no adverse effect on growth.</p> <p>No local or systemic side effects were recorded during the supplementation of GOS (5g/L) in a follow-on formula at weaning.</p> <p>In the present study, feeding a follow-on formula with GOS 5g/L is bifidogenic and safe during the feeding period.</p>
	GOS	Control																	
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<p>Reference</p> <p>Ben et al, 2008</p>	<p>To investigate the effect of a new infant formula supplemented with a low level (0.24g/100 ml) of GOS on intestinal micro-flora (Bifidobacteria, Lactobacilli, and E.coli) and fermentation characteristics in term infants, compared with human milk and a standard infant formula without GOS</p>	<p>R, C, SB, P</p>	<ul style="list-style-type: none"> • 371 term infants were approached. All started breast-feeding • Those who changed to formula-feeding within 4 wk after birth were randomized to either GOS or control group • 164 recruited for the 3-mo follow-up (87 males, 77 females) • 82 in final sample 	<p>Oral treatment:</p> <ul style="list-style-type: none"> • GOS (n=20) 0.24g/100mL of GOS in formula • Control (n=18) formula • Breast milk (n=15) • Combination (n=29) Breast milk and 0.24g/100mL of GOS in formula <p>Duration: 3 months</p> <p>Background diet: none</p> <p>Assessment tool: interviews with the mothers</p>		<p>Levels of Bifidobacteria at the end of 3-mo feeding period (mean \pm SD log₁₀ CFU/g wet faeces)</p> <table border="1" data-bbox="1163 500 1640 630"> <thead> <tr> <th>Group</th> <th>Bifidobacteria Count</th> </tr> </thead> <tbody> <tr> <td>GOS</td> <td>9.01 \pm 1.18</td> </tr> <tr> <td>Control</td> <td>8.16 \pm 0.99</td> </tr> <tr> <td>Breast milk</td> <td>9.25 \pm 0.93</td> </tr> <tr> <td>Breast milk + GOS</td> <td>8.97 \pm 0.85</td> </tr> </tbody> </table> <p>At the end of a 3-mo feeding period, the number of intestinal Bifidobacteria was significantly increased both in GOS and in breast-fed groups, compared with control. No difference was seen between the GOS group and the breast-fed group.</p> <p>Growth during study period (mean \pm SD)</p> <table border="1" data-bbox="1163 800 1665 954"> <thead> <tr> <th>Group</th> <th>Weight gain (g/d)</th> <th>Length gain (cm/wk)</th> </tr> </thead> <tbody> <tr> <td>GOS</td> <td>41.26 \pm 5.22</td> <td>0.95 \pm 0.11</td> </tr> <tr> <td>Control</td> <td>40.59 \pm 3.95</td> <td>0.96 \pm 0.11</td> </tr> <tr> <td>B-milk</td> <td>40.97 \pm 5.06</td> <td>0.93 \pm 0.10</td> </tr> <tr> <td>B-milk + GOS</td> <td>43.35 \pm 4.87</td> <td>1.01 \pm 0.11</td> </tr> </tbody> </table> <p>Weight gain and body height increase were similar among the groups.</p> <p>Scores of intensity of digestive symptoms (mean \pm SD)</p> <table border="1" data-bbox="1108 1076 1713 1255"> <thead> <tr> <th rowspan="2">Group</th> <th colspan="3">Score</th> </tr> <tr> <th>Crying</th> <th>Regurgitation</th> <th>Vomiting</th> </tr> </thead> <tbody> <tr> <td>GOS</td> <td>1.06 \pm 0.03</td> <td>1.34 \pm 0.55</td> <td>1.22 \pm 0.43</td> </tr> <tr> <td>Control</td> <td>1.05 \pm 0.03</td> <td>1.35 \pm 0.67</td> <td>1.25 \pm 0.38</td> </tr> <tr> <td>B-milk</td> <td>1.08 \pm 0.05</td> <td>1.41 \pm 0.58</td> <td>1.14 \pm 0.46</td> </tr> <tr> <td>B-milk + GOS</td> <td>1.04 \pm 0.02</td> <td>1.28 \pm 0.63</td> <td>1.18 \pm 0.34</td> </tr> </tbody> </table> <p>The GOS in formula did not influence the incidence of side effects (crying, regurgitation, vomiting).</p>	Group	Bifidobacteria Count	GOS	9.01 \pm 1.18	Control	8.16 \pm 0.99	Breast milk	9.25 \pm 0.93	Breast milk + GOS	8.97 \pm 0.85	Group	Weight gain (g/d)	Length gain (cm/wk)	GOS	41.26 \pm 5.22	0.95 \pm 0.11	Control	40.59 \pm 3.95	0.96 \pm 0.11	B-milk	40.97 \pm 5.06	0.93 \pm 0.10	B-milk + GOS	43.35 \pm 4.87	1.01 \pm 0.11	Group	Score			Crying	Regurgitation	Vomiting	GOS	1.06 \pm 0.03	1.34 \pm 0.55	1.22 \pm 0.43	Control	1.05 \pm 0.03	1.35 \pm 0.67	1.25 \pm 0.38	B-milk	1.08 \pm 0.05	1.41 \pm 0.58	1.14 \pm 0.46	B-milk + GOS	1.04 \pm 0.02	1.28 \pm 0.63	1.18 \pm 0.34	<p>Supplementation of GOS stimulates the growth of Bifidobacteria without affecting growth and the incidence of side effects.</p>
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10 Provision of Oligosaccharides (GOS:FOS) in Infant Formula

In several clinical trials, including those cited herein, it has been demonstrated that a GOS:FOS (9:1) blend, added to infant formula, stimulates the intestinal flora of formula-fed infants and results in looser fecal stools, wherein both effects are also observed in, and characteristic of, breast-fed infants. It follows, therefore, that the purpose of Floraid GOS in infant formula is to blend with FOS (fructo-oligosaccharide) in a ratio of GOS:FOS of 9:1, so as to provide formula-fed infants, to the extent currently scientifically possible, with oligosaccharides of a structure and concentration which mimics those available to breast-fed infants. As a result, infant clinical studies cited herein, as well as those profiled below, show increased bifidobacteria and looser stools, without adverse side effects, when fed a properly calibrated GOS:FOS supplemented infant formula.

As to the safety and acceptability of FOS in infant formula, we note GRN 392 for FOS (oligofructose), in which it is stated that an intention is to add the FOS to infant formula.

11 Selection of a Safe GOS Concentration in Infant Formula

A systematic scientific literature review of published, peer-reviewed human clinical trials in which infants were administered infant formula supplemented with GOS, or a GOS:FOS blend, has been, for the purpose of this GRAS notification, categorized by concentration of GOS per litre:

10g GOS:FOS per litre;
8g GOS:FOS per litre, or 8g GOS:FOS:AOS per litre;
6g GOS:FOS per litre; and
4g GOS:FOS per litre.

Subsequent evaluation of each study - which focused on the age of the infants, objective of the trial, length of feeding, and, outcomes and adverse effects - results in a recommendation of a concentration of 4g GOS per litre, or 4g GOS:FOS per litre infant formula for reasons elaborated on below.

11.1 Rejection of studies in which 10g, 8g and 6g GOS:FOS per litre were tested on infants

11.1.1 Concentration of 10g GOS:FOS per litre Infant Formula

Studies utilizing this concentration have, for the most part, been conducted on pre-term infants for a 28-day feeding trial. Reference is made to Knol (2005b), Boehm (2003) and Boehm (2002), in which the authors' respective conclusions are as follows:

Knol (2005b)

Double-blind study, GOS:FOS infant formula vs. control formula.

Adverse effects: GOS:FOS supplemented formula safely consumed with no significant difference in adverse effects (crying, regurgitating, inadequate weight gain) between the two groups.

Results: Supplementation of preterm infant formula by a 10g/litre concentration of GOS:FOS reduces the presence of clinically relevant pathogens in faecal flora, indicating that GOS:FOS might have the capacity to protect against enteral infections.

Boehm (2003) and Boehm (2002)

Double-blind study plus reference (human milk): GOS:FOS infant formula vs. control formula vs. human milk

Adverse effects: No effect of the different diets on the incidence of side effects (crying, regurgitation, vomiting) nor on weight gain and length gain.

Results: Supplementing preterm formula with a mixture of GOS:FOS at a concentration of 10g/litre stimulates the growth of bifidobacteria in the intestine and results in stool characteristics similar to those found in preterm infants fed human milk. Therefore, GOS:FOS may help to improve intestinal tolerance to enteral feeding in preterm infants.

These three studies are removed for further review for this GRAS notification due to the participants being pre-term infants, and thus, consumers outside the scope of intended uses.

11.1.2 Concentration of 8g and 6g GOS:FOS; 6g GOS:FOS:AOS per litre Infant Formula

Several studies using this concentration were conducted for therapeutic reasons on infants who have a risk for allergy due to parental/sibling history. The authors used GOS concentrations as a therapeutic agent, rather than as a food supplement. Such studies reviewed for this GRAS Notification are listed in Appendix IX, two of which are summarized in the charts below, solely to demonstrate long term post-trial safety (2 years) and long term consumption safety (6 months) at a concentration of 8g GOS:FOS/litre.

Since the proposed addition of Floraid GOS to infant formula is as a food supplement, studies at this concentration have been eliminated from further review for this GRAS Notification. Nevertheless, the formulas supplemented with 8g GOS:FOS per litre, and, 6g GOS:FOS per litre infant formula were well-tolerated, with few reported mild adverse side effects, not significantly different from the control group.

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Summary of Intervention studies investigating the safety and physiological effects of GOS consumption							
Reference, Author, Year	Aim of Study	Design R (Randomized) NR (Non-randomized) C (Control group) SB (Single-blind) DB (Double-blind) P (Parallel) CO (Crossover)	Age range, Gender (M, F) No. recruited No. randomized No. in final sample	Daily Intake; Frequency; GOS format; Route of intake; Duration of consumption;	Background Diet & Assessment Tool	Endpoints, Results & Statistics Changes in health effect Adverse effects	Relevant Authors' Conclusions
Legend	Aim of Study	Design R,NR,C,SB,DB ,P,CO	Sample Characteristics A; G; # Re; #Ra; # FS:	Treatment Characteristics DI; Frq; GOS Frm; Route; Dur.	Background Diet & Assessment Tool	Endpoint, Results & Statistics Changes in health effect Adverse effects	Relevant Authors' Conclusions

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Reference and Type of Evidence	Aim of Study	Trial Design R, NR, C, SB, D B, P, CO	Sample Characteristics A:, G:, # Re:, #Ra:, # FS:	Treatment Characteristics Di; Frq; GOS Frm; Route; Dur.	Background Diet & Assessment Tool	Endpoint Results & Statistics	Relevant Authors' Conclusions					
						Changes in health effect Adverse effects						
Reference Arslanoglu et al, 2008	To evaluate if the protective effects of GOS:FOS in the first 6 m of life (as detailed in Moro et al. (2006) were lasting beyond the intervention period	R, C, DB, P	152 healthy term infants with risk of atopy, previously studied by Moro et al. (2006) 134 completed the follow-up	<ul style="list-style-type: none"> • Blind follow-up for 2 years • GOS:FOS 8g/l: n=66 • Control : n=68 		Incidence of allergic manifestations (%)	<p>Early dietary intervention with GOS:FOS has a protective effect against both allergic manifestations and infections. This dual protection can be considered as a typical example of immunological programming. The observed protection lasting beyond the intervention period suggests that an immune modulating effect through the intestinal flora modification may be the principal mechanism of action.</p> <p>When mother's milk is not available, the supplementation of formulas with prebiotic oligosaccharides early in life may have promising clinical implications.</p>					
						<table border="1"> <thead> <tr> <th></th> <th>GOS:FOS</th> <th>Control</th> </tr> </thead> <tbody> <tr> <td>Atopic dermatitis</td> <td>13.6</td> <td>27.9</td> </tr> <tr> <td>Recurrent wheezing</td> <td>7.6</td> <td>20.6</td> </tr> <tr> <td>Allergic urticaria</td> <td>1.5</td> <td>10.3</td> </tr> </tbody> </table> <p>The GOS:FOS group had significantly lower incidence of allergic manifestations. The cumulative incidence of AD was successfully reduced by >50%, and recurrent wheezing episodes were reduced by two thirds in 2 y.</p> <p>The GOS:FOS group had significantly fewer episodes of physician-diagnosed overall and upper respiratory tract infections (P<0.01), fever episodes (P<0.00001), and fewer antibiotic prescriptions (P<0.05).</p> <p>Growth (expressed as mean body weight and length at 12, 18, and 24 m) was normal and similar in both groups.</p>			GOS:FOS	Control	Atopic dermatitis	13.6
	GOS:FOS	Control										
Atopic dermatitis	13.6	27.9										
Recurrent wheezing	7.6	20.6										
Allergic urticaria	1.5	10.3										

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Reference and Type of Evidence	Aim of Study	Trial Design R, NR, C, SB, D B, P, CO	Sample Characteristics A, G, # Ra, #Ra, # FS:	Treatment Characteristics Di; Frq; GOS Fm; Route; Dur.	Background Diet & Assessment Tool	Endpoint Results & Statistics	Relevant Authors' Conclusions																
						Changes in health effect Adverse effects																	
<p>Reference</p> <p>Moro et al, 2006</p>	<p>To investigate the effect of a prebiotic mixture of galacto- and long chain fructo-oligosaccharides on the incidence of atopic dermatitis (AD) during the first six months of life in formula-fed infants at high risk of atopy.</p>	R, C, DB, P	<p>259 infants at risk for atopy randomized: M (n=128) and F (n=131)</p> <p>206 in final sample: M (n=101) and F (n=105)</p> <p>Subgroup (n=98) provides samples for fecal flora analysis. Final sample for this subgroup: M (n=50) and F (n=44)</p>	<ul style="list-style-type: none"> Oral treatment; formulae were fed ad libitum GOS:FOS 8g/l: n=102 Control : n=104 <p>Duration : 6 m</p>		<p>During the 6-m study period, 10 infants (9.8%) in the GOS:FOS group and 24 infants (23.1%) in the control group developed AD. The severity of the dermatitis was not affected by diet.</p> <p>Bifidobacteria counts as CFU/g fresh stool, data presented as median (interquartile range)</p> <table border="1"> <thead> <tr> <th></th> <th>GOS:FOS</th> <th>Control</th> <th>p value</th> </tr> </thead> <tbody> <tr> <td>0 d</td> <td>8.17 (2.3)</td> <td>8.33 (2.4)</td> <td>ns</td> </tr> <tr> <td>3 m</td> <td>9.56 (0.9)</td> <td>8.30 (1.1)</td> <td><0.0001</td> </tr> <tr> <td>6 m</td> <td>10.28 (0.7)</td> <td>8.65 (1.2)</td> <td><0.0001</td> </tr> </tbody> </table> <p>Supplementation with GOS:FOS resulted in a significant increase in the number of bifidobacteria when compared with control.</p> <p>Stool characteristics (frequency and consistency) were significantly influenced by the diet. The GOS:FOS group produced softer, more frequent stools compared to control.</p> <p>Regarding acceptance and tolerance of the formulae, there were significantly lower reports of Regurgitation and crying in the GOS:FOS group, whereas there was no difference in the reported incidence of vomiting between the two groups.</p> <p>No adverse effects were observed during the entire study based on the diary record given by the parents and the results of the monthly examinations.</p>		GOS:FOS	Control	p value	0 d	8.17 (2.3)	8.33 (2.4)	ns	3 m	9.56 (0.9)	8.30 (1.1)	<0.0001	6 m	10.28 (0.7)	8.65 (1.2)	<0.0001	<p>Results show for the first time a significant beneficial effect of prebiotics on the development of atopic dermatitis in a high risk population of infants. Although the mechanism of this effect requires further investigation, it appears likely that oligosaccharides modulate postnatal immune development by altering bowel flora and have a potential role in primary allergy prevention during infancy.</p> <p>In this study GOS:FOS mixture was well tolerated and did not cause any adverse effects.</p>
	GOS:FOS	Control	p value																				
0 d	8.17 (2.3)	8.33 (2.4)	ns																				
3 m	9.56 (0.9)	8.30 (1.1)	<0.0001																				
6 m	10.28 (0.7)	8.65 (1.2)	<0.0001																				

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11.2 Clinical Studies: 4g GOS:FOS per litre Infant Formula

The majority of clinical studies in the domain of oligosaccharide-supplemented infant formula, consist of GOS paired with FOS in a ratio of 9:1 for reasons aforementioned. The results of these robustly-designed and well-conducted trials show this concentration to be safe, well-tolerated and beneficial to the infant in terms of looser stools.

Table 3 below lists the studies reviewed for this GRAS Notification using 4g GOS:FOS per litre infant formula.

Following Table 3, we have summarized three of these studies in charts, identified below, and selected due to:

- Horschler, 2012: recent study; thorough evaluation of infant feces;
- Bruzzese, 2009: large n, large age range of participants; long duration feeding trial;
- Moro, 2002: tested both 4g and 8g per litre infant formula, demonstrating that while 4g per litre is safe efficacious, dose dependent results demonstrate a more homogeneous pattern at 8g per litre infant formula.

Table 3 Concentration of 4g GOS:FOS per litre Infant Formula

<u>Author</u>	<u>Year</u>	<u>n</u>	<u>Participant Description</u>	<u>Duration</u>	<u>Significant Difference: Adverse Effects</u>	<u>Beneficial Effects Reported</u>
Holscher	2012	146	Healthy term infants	6w	no	GOS:FOS supplemented formula well-tolerated with increased bifidobacteria and reduced stool pH.
Bruzzese	2009	342	Healthy term infants, 15-120 d	12 m	no	Stool pattern of GOS:FOS treatment group generally characterized by softer but not diarrheic stools.
Costalos	2008	160	Healthy term infants	6w	no	GOS:FOS formula well-tolerated with higher stool frequency and softer stools than control group (standard formula). Higher proportion of bifidobacteria in the GOS:FOS group, but not significantly different from control.
Scholtens	2006	35	Infants aged 4-6m	6w	no	Addition of GOS:FOS to solid foods significantly increased fecal bifidobacteria versus control (Note: 4.5 g/day of GOS:FOS in solid weaning food)
Decsi	2005	97	Healthy term infants	12w	no	GOS:FOS supplemented infant formula significantly increased intestinal microflora compared to standard formula.
Moro	2003	115	Healthy term infants	28d	no	Four treatment arms: control, GOS:FOS 4g/l; GOS:FOS 8g/l and breast fed. Although effects are dose dependent, both 4g/l and 8g/l resulted in significant increase in bifidobacteria with stool characteristics similar to those of breast-fed infants.
Moro	2002	90	Healthy term infants	28d	no	Treatment arms identical to Moro 2003. Increased bifidobacteria and softer stools on GOS:FOS treatment arm. Dose dependent effects: results on 8g/l concentration similar to those of breast fed infants.

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Reference and Type of Evidence	Aim of Study	Trial Design R,NR,C,SB,D B, P,CO	Sample Characteristics A, G, # Re, #Ra, # FS:	Treatment Characteristics DI; Frq; GOS Frm; Route; Dur.	Back-ground Diet & Assessment Tool	Endpoint Results & Statistics Changes in health effect Adverse effects	Relevant Authors' Conclusions																																				
<p>Reference</p> <p>Holscher et al, 2012</p>	<p>To assess gastrointestinal tolerance and fecal microbiota, pH, and short-chain fatty acid (SCFA) concentrations of infants consuming formula with and without GOS:FOS</p>	<p>R, C, DB, P</p>	<p>146 healthy, full-term infants, 2-8 w were recruited</p> <p>89 was randomized, 50 was breast-fed reference</p> <p>102 in final sample</p>	<p>Oral treatment</p> <p>GOS:FOS 4g/l: n= 36 Control: n=33 Breast-fed: n=33</p> <p>Duration: 6 w</p>		<p>Absolute and relative abundance of <i>Bifidobacterium spp</i> in fecal sample (expressed as means ± SEM).</p> <table border="1" data-bbox="1134 479 1669 933"> <thead> <tr> <th></th> <th>GOS:FOS</th> <th>Control</th> <th>Breast-fed</th> </tr> </thead> <tbody> <tr> <td colspan="4">Time Absolute abundance (CFU/g wet stool)</td> </tr> <tr> <td>Week 0</td> <td>2.61 x 10⁹ ± 8.04 x 10⁸</td> <td>1.91 x 10⁹ ± 7.08 x 10⁸</td> <td>2.04 x 10⁹ ± 7.04 x 10⁸</td> </tr> <tr> <td>Week 3</td> <td>4.60 x 10⁹ ± 1.02 x 10⁹</td> <td>1.90 x 10⁹ ± 6.81 x 10⁸</td> <td>2.85 x 10⁹ ± 8.24 x 10⁸</td> </tr> <tr> <td>Week 6</td> <td>3.72 x 10⁹ ± 9.27 x 10⁸</td> <td>2.05 x 10⁹ ± 7.00 x 10⁸</td> <td>3.02 x 10⁹ ± 8.47 x 10⁸</td> </tr> <tr> <td colspan="4">Relative abundance (% of total)</td> </tr> <tr> <td>Week 0</td> <td>45.59±7.3 5</td> <td>36.70±6.6 8</td> <td>36.38±6.73 8</td> </tr> <tr> <td>Week 3</td> <td>49.46±7.1 2</td> <td>30.85±5.8 7</td> <td>48.77±7.33 7</td> </tr> <tr> <td>Week 6</td> <td>44.10±6.7 4</td> <td>32.62±8.0 9</td> <td>43.48±6.77 9</td> </tr> </tbody> </table> <p>Feces from the GOS:FOS group had a higher absolute number and proportion of bifidobacteria than control and did not differ from the breast-fed group.</p> <p>Feces from the GOS:FOS and control groups had higher concentrations of acetate, butyrate, propionate, and total SCFA than the breast-fed group; however, fecal pH was lower in the GOS:FOS and breast-fed groups than the control group.</p> <p>GOS:FOS did not affect caregiver-perceived incidence of crying, fussing, parent-perceived "colic"/cramps, spitting up, vomiting, and flatulence frequency. Nor did it alter body weight.</p>		GOS:FOS	Control	Breast-fed	Time Absolute abundance (CFU/g wet stool)				Week 0	2.61 x 10 ⁹ ± 8.04 x 10 ⁸	1.91 x 10 ⁹ ± 7.08 x 10 ⁸	2.04 x 10 ⁹ ± 7.04 x 10 ⁸	Week 3	4.60 x 10 ⁹ ± 1.02 x 10 ⁹	1.90 x 10 ⁹ ± 6.81 x 10 ⁸	2.85 x 10 ⁹ ± 8.24 x 10 ⁸	Week 6	3.72 x 10 ⁹ ± 9.27 x 10 ⁸	2.05 x 10 ⁹ ± 7.00 x 10 ⁸	3.02 x 10 ⁹ ± 8.47 x 10 ⁸	Relative abundance (% of total)				Week 0	45.59±7.3 5	36.70±6.6 8	36.38±6.73 8	Week 3	49.46±7.1 2	30.85±5.8 7	48.77±7.33 7	Week 6	44.10±6.7 4	32.62±8.0 9	43.48±6.77 9	<p>Infant formula containing GOS:FOS was well tolerated, increased abundance and proportion of bifidobacteria (to closely resemble those in the breast-fed group), and reduced fecal pH in healthy infants.</p> <p>GOS:FOS supplementation did not alter stool patterns, tolerance, or growth.</p>
	GOS:FOS	Control	Breast-fed																																								
Time Absolute abundance (CFU/g wet stool)																																											
Week 0	2.61 x 10 ⁹ ± 8.04 x 10 ⁸	1.91 x 10 ⁹ ± 7.08 x 10 ⁸	2.04 x 10 ⁹ ± 7.04 x 10 ⁸																																								
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Reference and Type of Evidence	Aim of Study	Trial Design R, NR, C, SB, D B, P, CO	Sample Characteristics A, G: # Ra, # Ra: # FS:	Treatment Characteristics DI; Frq; GOS Fm; Route; Dur.	Background Diet & Assessment Tool	Endpoint Results & Statistics Changes in health effect Adverse effects	Relevant Authors' Conclusions
Reference Bruzzese, 2009	To test the hypothesis that prebiotics reduce the incidence of intestinal and respiratory infections in healthy infants.	R, C, open, observational	342 healthy infants, aged 15-120 d were randomized 201 in final sample	Oral treatment GOS:FOS 4g/l: n=96 Control : n=105 Duration : 12 m		<p><u>Effects on intestinal infections:</u> Compared to control, the GOS:FOS group had: - significantly lower rate of diarrheal episode per child (0.12 ± 0.04 vs. 0.29 ± 0.05; $p=0.015$) - significantly lower number of children with at least 1 episode of acute diarrhea (10.4% vs. 23.8%; $p=0.01$)</p> <p><u>Effects on respiratory infections:</u> Compared to control, the GOS:FOS group had: - lower number of episodes of upper respiratory tract infections (URTI), but the difference was not significant ($p=0.4$) - similar number of children with at least 1 episode of URTI - lower number of children with recurrent URTI (defined as more than 3 episodes of URTI in 12 months), 17/60 vs. 29/65, and the difference was close to significance ($p=0.06$)</p> <p><u>Effects on antibiotic prescription:</u> GOS:FOS administration was associated with a lower number of antibiotic prescription. - The mean rate of antibiotic course prescribed for children fed with GOS:FOS was significantly lower compared to controls (1.03 ± 0.15 vs. 1.48 ± 0.16; $p=0.038$) - The percent of children receiving 2 or more antibiotic course/year was significantly lower in children receiving GOS:FOS (24/60 vs. 43/65; $p=0.004$)</p> <p><u>Effects on Growth:</u> Compared to control, the GOS:FOS group had: - increased mean body weight at 3 and 6 m - increased mean body length at 3.6, 9, and 12 m - similar mean head circumference at 3, 6, 9, and 12 m</p> <p><u>Safety and tolerance:</u> There was no report of major side effects. The stool pattern of children receiving GOS:FOS was generally characterized by softer but not diarrheic stools and in no case was the GOS:FOS formula withdrawn.</p>	<p>GOS:FOS administration reduced intestinal and, possibly, respiratory infections in healthy infants during the first year of age.</p> <p>GOS:FOS administration led to a transient increase in mean body weight and an increase in mean body length.</p> <p>GOS:FOS was well tolerated.</p>

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Reference and Type of Evidence	Aim of Study	Trial Design R, NR, C, SB, D B, P, CO	Sample Characteristics A, G, # Re., #Ra., # FS:	Treatment Characteristics DI; Frq; GOS Frm; Route; Dur.	Background Diet & Assessment Tool	Reference and Type of Evidence	Relevant Authors' Conclusions																								
Reference Moro et al, 2002	To analyze the bifidogenic effect of an experimental prebiotic oligosaccharides mixture consisting of GOS and FOS in 90 term infants.	R, C, P	90 term infants M and F	<ul style="list-style-type: none"> • Oral treatment • GOS:FOS 4g/l: n=30 • GOS:FOS 8g/l: n=27 • Control: n=33 Duration: 28 d		<p>Numbers of fecal bifidobacteria (log 10 of CFU/g wet faeces) expressed in median (interquartile range)</p> <table border="1"> <thead> <tr> <th></th> <th>Day 1</th> <th>Day 28</th> </tr> </thead> <tbody> <tr> <td>Control</td> <td>8.8 (6.1)</td> <td>7.2 (4.9)</td> </tr> <tr> <td>GOS:FOS 4g/l</td> <td>8.5 (1.9)</td> <td>9.3 (1.6)</td> </tr> <tr> <td>GOS:FOS 8g/l</td> <td>7.7 (6.1)</td> <td>9.7 (0.8)</td> </tr> </tbody> </table> <p>At the end of the study, the number of bifidobacteria in the stools was significantly higher in both groups fed GOS:FOS formula, compared to control. Moreover, there was also a significant difference between the group fed 4g/l GOS:FOS and the group fed 8g/l GOS:FOS</p> <p>Numbers of fecal lactobacilli (log 10 of CFU/g wet faeces) expressed in median (interquartile range)</p> <table border="1"> <thead> <tr> <th></th> <th>Day 1</th> <th>Day 28</th> </tr> </thead> <tbody> <tr> <td>Control</td> <td>3.4 (0.2)</td> <td>3.4 (1.8)</td> </tr> <tr> <td>GOS:FOS 4g/l</td> <td>3.3 (0.2)</td> <td>5.9 (1.5)</td> </tr> <tr> <td>GOS:FOS 8g/l</td> <td>3.4 (0.2)</td> <td>5.6 (2.1)</td> </tr> </tbody> </table> <p>At the end of the study, the number of lactobacilli in the stools was significantly higher in both groups fed GOS:FOS formula, compared to control. However, there was no significant difference between the group fed 4 g/l GOS:FOS and the group fed 8 g/l GOS:FOS. The different diets did not influence the incidence of crying, regurgitation, or vomiting. Weight gain and length increment were similar among the groups.</p>		Day 1	Day 28	Control	8.8 (6.1)	7.2 (4.9)	GOS:FOS 4g/l	8.5 (1.9)	9.3 (1.6)	GOS:FOS 8g/l	7.7 (6.1)	9.7 (0.8)		Day 1	Day 28	Control	3.4 (0.2)	3.4 (1.8)	GOS:FOS 4g/l	3.3 (0.2)	5.9 (1.5)	GOS:FOS 8g/l	3.4 (0.2)	5.6 (2.1)	<p>Supplementation of a formula for term infants with GOS:FOS stimulates the growth of bifidobacteria and lactobacilli in the intestine and results in softer stools with lower pH in a dose-dependent manner. A dosage of 4g/l results in significant effects, but the effects can be enhanced homogeneously to a level observed in breast-fed infants by increasing the dosage to 8g/l.</p> <p>Supplementation had no influence on the incidence of side effects (crying, regurgitation, vomiting) or growth.</p>
	Day 1	Day 28																													
Control	8.8 (6.1)	7.2 (4.9)																													
GOS:FOS 4g/l	8.5 (1.9)	9.3 (1.6)																													
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11.3 Selection of GOS:FOS Concentration of 4g per litre infant formula

Due to the demonstrated safety of GOS at 4g per litre infant formula, and, of a GOS:FOS concentration of 4g per litre infant formula, a concentration of 4g GOS per litre infant formula is selected as the upper limit for this GRAS Notification. This amount is 0.38% on a percentage basis. Accordingly, a concentration of 0.38% is proposed for for infant foods and follow-on foods as well.

11.4 Safety Assessment by Experts Qualified by Scientific Training and Experience

In fulfillment of GRAS notification requirements, the proposed GOS and GOS:FOS concentration of 4g per litre infant formula must acquire “general recognition of safety [...] determined in accordance with §170.30 (21 CFR 170.3(k)). The referenced section states that GRAS:

- “may be based only on the views of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food” (21 CFR 170.30(a)); and
- “requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food” (21 CFR 170.30(a)).

In this regard, we defer to the expert opinion of the European Commission Scientific Committee on Food, regarding the composition of Infant Formulae and Follow-on Formulae, who state⁴⁵:

- “In conclusion, the Committee reaffirms its previous statement that [it] has no major concerns on the inclusion of up to 0.8g/100 ml of a combination of 90% oligogalactosyl-lactose and 10% high molecular weight oligofructosyl-saccharose to infant formulae and follow-on formulae.”
- “Increased stool frequency and softer stool consistency may provide a relevant benefit in those subgroups of infants that suffer from hard stools and constipation.”

The proposed concentration of GOS and GOS:FOS in infant formula, in this GRAS Notification, is half that by said scientific committee of experts to be safe for infants.

12 Safety of GOS - Adults

Several clinical trials and *in vitro* studies have successfully demonstrated that GOS is safely consumed by adults, and, due to its non-digestible, fermentable carbohydrates, will beneficially characterize the microbial profile of the colon. Five such studies, identified below, have been identified for inclusion in this GRAS Notification, and summarized in the charts which follow, selected due to:

Walton (2011): 8g GOS/day; high-quality clinical trial; thorough fecal analysis; positive and clear results;

⁴⁵ Ibid. European Commission. 2003.

- Davis (2010): 2.5, 5 and 10g GOS/day; well-designed clinical trial; illustrates range of safe consumption; raises point of potential effect of food processing techniques (thermal stability) on amount of GOS in end product. Otherwise, well-controlled study.
- Depeint et al (2008): 3.6 and 7g GOS/day; high-quality clinical trial; illustrates range of safety and tolerance of two types of GOS;
- Bouhnik (2004): 2.5, 5, 7.5 and 10g GOS/day; high-quality clinical trial; illustrates range of safety of GOS
- Bouhnik et al (1997): 10g GOS/day; high-quality clinical trial; evaluation of the most often quoted upper daily limit of GOS; illustrates safety at this level of consumption.

Summary of Intervention studies investigating the safety and physiological effects of GOS consumption							
Reference, Author, Year	Aim of Study	Design R (Randomized) NR (Non-randomized) C (Control group) SB (Single-blind) DB (Double-blind) P (Parallel) CO (Crossover)	Age range, Gender (M, F) No. recruited No. randomized No. in final sample	Daily Intake; Frequency; GOS format; Route of intake; Duration of consumption;	Background Diet & Assessment Tool	Endpoints, Results & Statistics Changes in health effect Adverse effects	Relevant Authors' Conclusions
Legend for each Type of Evidence, if applicable	Aim of Study	Design R,NR,C,SB,DB , P,CO	Sample Characteristics A, G; # Re.; #Ra.; #FS;	Treatment Characteristics DI; Frq; GOS Frm; Route; Dur.	Background Diet & Assessment Tool	Endpoint, Results & Statistics Changes in health effect Adverse effects	Relevant Authors' Conclusions

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Reference and Type of Evidence	Aim of Study	Trial Design R, NR, C, SB, D B, P, CO	Sample Characteristics A, G, # Re; #Ra, # F3:	Treatment Characteristics Di; Frq; GOS Fm; Route; Dur.	Background Diet & Assessment Tool	Endpoint Results & Statistics Changes in health effect Adverse effects	Relevant Authors' Conclusions																								
Reference Walton et al, 2011	To determine if GOS could benefit a population of men and women of 50 years and above, through modulation of faecal microbiota and fermentation characteristics.	R, C, DB, CO	<ul style="list-style-type: none"> Thirty nine volunteers aged 50-81 years with BMI of 19.7-38.4 kg/m² were recruited M (n=18) and F (n=21) The results of two male participants who consumed antibiotics during the second treatment of the study were excluded 37 in final sample 	<p>Oral Treatment</p> <ul style="list-style-type: none"> GOS Dose 8g/d; 2 equal oral doses – preferably in the morning and evening + usual diet Control Dose 2x 250 ml/d orange juice + usual diet <p>The study consisted of 4-w run-in, 3-w intervention, and 3-w washout periods.</p> <p>Background diet: usual daily diet</p> <p>Assessment tool: 3-d detailed food diaries</p> <p>In vitro Treatment (as per Macfarlane & Gibson, 1997)</p> <p>Second baseline sample of freshly voided faeces from 3 randomly selected participants; subjected to 3-parallel, 3-stage continuous culture systems representing the proximal, transverse and distal regions of the colon. Samples were diluted; anaerobic conditions maintained; model dosed with 4 g GOS twice daily.</p>		<p>Mean ± SD of bifidobacteria count (log 10 CFU/g feces)</p> <table border="1"> <tr> <td>Pre-placebo</td> <td>8.87 ± 1.15</td> <td>Pre-GOS</td> <td>8.86 ± 0.82</td> </tr> <tr> <td>Post-placebo</td> <td>8.64 ± 0.95</td> <td>Post-GOS</td> <td>9.16^a ± 1.09</td> </tr> <tr> <td>Placebo washout</td> <td>8.82 ± 0.83</td> <td>GOS washout</td> <td>8.81 ± 1.06</td> </tr> </table> <p>^a Significantly different to control, P=0.024 – Tukey test.</p> <p>Adverse Effects: Subjects answered general questions concerning bowel habit and mood in daily diaries. No significant differences in stool consistency, intestinal bloating, abdominal discomfort, flatulence severity and frequency during GOS and control treatments. Markers of mood remained the same throughout the two treatment periods.</p> <p>SCFA (butyrate, mmol/l) profiles as determined by GC in <i>in vitro</i> continuous culture system using GOS as a substrate at 4g twice daily (mean ± SD; n=3 from three continuous culture systems - proximal, transverse and distal regions of the colon - three different baseline volunteer faecal samples provided the bacterial inoculum)</p> <table border="1"> <thead> <tr> <th></th> <th>Steady state 1</th> <th>Steady state 2</th> </tr> </thead> <tbody> <tr> <td>Vessel 1</td> <td>26.6 ± 3.0</td> <td>36.1^a ± 4.7</td> </tr> <tr> <td>Vessel 2</td> <td>27.4 ± 5.1</td> <td>39.0^a ± 5.2</td> </tr> <tr> <td>Vessel 3</td> <td>29.3 ± 6.1</td> <td>44.0^a ± 5.0</td> </tr> </tbody> </table> <p>^a Mean values were significantly different from steady state 1 (pre-treatment; P<0.05). Steady State 1 = steady state before treatment Steady State 2 = steady state following GOS treatment</p>	Pre-placebo	8.87 ± 1.15	Pre-GOS	8.86 ± 0.82	Post-placebo	8.64 ± 0.95	Post-GOS	9.16 ^a ± 1.09	Placebo washout	8.82 ± 0.83	GOS washout	8.81 ± 1.06		Steady state 1	Steady state 2	Vessel 1	26.6 ± 3.0	36.1 ^a ± 4.7	Vessel 2	27.4 ± 5.1	39.0 ^a ± 5.2	Vessel 3	29.3 ± 6.1	44.0 ^a ± 5.0	<p>GOS intake significantly increased bifidobacteria numbers <i>in vivo</i> and <i>in vitro</i>.</p> <p>Increased butyrate production and elevated bifidobacteria numbers may constitute beneficial modulation of the gut microbiota in a maturing population.</p> <p>A dosage of 4g GOS twice daily was very well tolerated, as there were no significant differences in stool consistency, intestinal bloating, abdomen discomfort, flatulence severity, and frequency during GOS and control treatments.</p>
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<p>Reference</p> <p>Davis et al, 2010</p>	<p>To determine the effect of different doses of GOS on the fecal microbiota of healthy adults, with a focus on bifidobacteria.</p>	<p>NR, C, SB, P</p>	<p>Healthy adults, but details were not provided</p> <p>Free-living</p> <p>21 recruited</p> <p>18 in final sample (dropouts unrelated to the experiment)</p> <p>19-50 yrs</p> <p>M (n=13) and F (n=5)</p>	<p>Oral Treatment</p> <ul style="list-style-type: none"> ● 6 g chocolate-flavoured chewable candies containing: (control) corn syrup, sugar, water, chocolate liquor, palm kernel oil, lecithin, and vanilla; (test) 1.25 g GOS ● <u>GOS Dose High</u> - 5.0g/d: 4 GOS chews and 4 control chews - 10.0g/d: 8 GOS chews ● <u>GOS Dose Low</u> 2.5g/d: 2 GOS chews and 6 control chews ● <u>Control Dose</u> Eight control chews ● 2-wk baseline period (no chews administered) ● 4 sequential testing periods during which chews were administered for <i>three weeks</i> with GOS dosages at levels of 0.0g, 2.5g, 5.0g, and 10.0g per day. ● Subjects were blinded in terms of the dose of GOS they received (test and control indistinguishable) ● Final 2-wk washout period (no chews) <p>● Usual diet</p> <p>● Assessment tool: N/A</p>		<p>Mean ± SD of bifidobacteria count (log₁₀ CFU/g feces)</p> <table border="1" data-bbox="1178 440 1654 651"> <tbody> <tr> <td>Baseline (n=18)</td> <td></td> <td>9.32± 0.79</td> </tr> <tr> <td>GOS 0.0g (n=18)</td> <td></td> <td>9.48± 0.73</td> </tr> <tr> <td>GOS 2.5g (n=18)</td> <td></td> <td>9.60± 0.80</td> </tr> <tr> <td>GOS 5.0g (n=18)</td> <td></td> <td>9.76± 0.48^a</td> </tr> <tr> <td>GOS 10.0g (n=18)</td> <td></td> <td>9.83± 0.56^b</td> </tr> <tr> <td>Washout (n=18)</td> <td></td> <td>9.42± 0.52</td> </tr> </tbody> </table> <p>^a Significantly different to 0.0g (control), p<0.05 ^b Significantly different to 0.0g (control), p<0.001</p> <p>Adverse Effects: Subjects recorded and rated adverse effects (bowel movement, stool consistency, discomfort, flatulence, abdominal pain, and bloating) in weekly symptoms diaries. No significant differences were detected for any of the symptoms between 0.0g GOS control dose and any of the GOS treatments. A significant symptom change was observed for flatulence (p<0.05), but only between the baseline and washout and the treatment periods. However, the increase in this score occurred not only for the GOS treatment, but even during the 0.0g GOS control period, suggesting that this outcome was due either to a placebo effect or was caused by another component of the chew.</p>	Baseline (n=18)		9.32± 0.79	GOS 0.0g (n=18)		9.48± 0.73	GOS 2.5g (n=18)		9.60± 0.80	GOS 5.0g (n=18)		9.76± 0.48 ^a	GOS 10.0g (n=18)		9.83± 0.56 ^b	Washout (n=18)		9.42± 0.52	<p>A high purity GOS, administered in a confectionery product at doses of 5g or higher, was bifidogenic, while a dose of 2.5g showed no significant effect. However, the results also showed that even when GOS was administered for many weeks and at high doses, there were still some individuals for which a bifidogenic response did not occur.</p> <p>GOS at doses 2.5, 5, and 10g/d are well tolerated by adults.</p> <p>Note: Formulation trials conducted in preparation for the commercialization of Floraid GOS indicates an upper limit of thermal stability of GOS of 110°C. Method of preparation of the chews was not elaborated on in this paper. We hypothesize that the amount of GOS added to the chews during product preparation may have been partially disintegrated due to thermal effects.</p>
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<p>Reference</p> <p>Depeint et al, 2008</p>	<p>To assess and compare the physiological effects of a novel GOS produced through the action of galactosidases originating from a probiotic Bifidobacterium bifidum strain, against a GOS produced through the action of industrial galactosidase, and, a placebo.</p>	<p>R, C, DB, CO</p>	<ul style="list-style-type: none"> Free-living 18-60 y; average age 34.4 + 6.7 y M (n=25) and F (n=34) 59 participated (number of volunteers recruited was not provided) 29 randomized in Phase 1 and 30 in Phase 2 59 in final sample (29 in Phase 1, 30 in Phase 2) 	<p>Oral Treatment</p> <p>Vegetable fat-filled milk powder (FFMP)</p> <p><u>GOS Dose High</u></p> <ul style="list-style-type: none"> Phase 1: 7g V-GOS (commercial GOS); Phase 2: 7g β-GOS (novel GOS) <p><u>GOS Dose Low</u></p> <ul style="list-style-type: none"> Phase 2: 3.6g β-GOS (novel GOS) <p><u>Control Dose</u></p> <ul style="list-style-type: none"> Phase 1: 0g V-GOS/d; 15g FFMP/d; Phase 2: 0g β-GOS (novel GOS)/d; 15g FFMP/d; 7g sucrose/d diluted with water (175 ml) and to be consumed at any time during the day 	<ul style="list-style-type: none"> Two 7-d interventions with a 7-d washout period in between Phase 1: 7-d treatment in one of 2 groups: <ol style="list-style-type: none"> Control Control + 7g V-GOS (commercial GOS) Phase 2: 7-d treatment in one of 3 groups: <ol style="list-style-type: none"> Control; 2. Control + 3.6g β-GOS (novel GOS); 3. Control + 7g β-GOS (novel GOS) Usual diet and fluid intake Assessment tool: N/A 	<p>Mean \pm SD of Proportions of counted Bifidobacterium: baseline and at end of intervention</p> <table border="1" data-bbox="1157 496 1629 849"> <thead> <tr> <th data-bbox="1157 496 1297 553">Phase 1 (n=29)</th> <th data-bbox="1297 496 1440 553">V-GOS 7g/d</th> <th data-bbox="1440 496 1549 553">Control</th> <th data-bbox="1549 496 1629 553"></th> </tr> </thead> <tbody> <tr> <td data-bbox="1157 553 1297 610">Baseline</td> <td data-bbox="1297 553 1440 610">5.00\pm 1.07</td> <td data-bbox="1440 553 1549 610">5.18\pm 1.13</td> <td data-bbox="1549 553 1629 610"></td> </tr> <tr> <td data-bbox="1157 610 1297 667">Intervention</td> <td data-bbox="1297 610 1440 667">5.98\pm 1.13^a</td> <td data-bbox="1440 610 1549 667">5.04\pm 1.46</td> <td data-bbox="1549 610 1629 667"></td> </tr> <tr> <th data-bbox="1157 667 1297 724">Phase 2 (n=30)</th> <th data-bbox="1297 667 1440 724">β-GOS 7g/d</th> <th data-bbox="1440 667 1549 724">β-GOS 3.6/d</th> <th data-bbox="1549 667 1629 724">Control</th> </tr> <tr> <td data-bbox="1157 724 1297 781">Baseline</td> <td data-bbox="1297 724 1440 781">4.05\pm 1.06</td> <td data-bbox="1440 724 1549 781">4.07\pm 0.92</td> <td data-bbox="1549 724 1629 781">4.54\pm 1.17</td> </tr> <tr> <td data-bbox="1157 781 1297 849">Intervention</td> <td data-bbox="1297 781 1440 849">6.74\pm 1.19^b</td> <td data-bbox="1440 781 1549 849">5.36\pm 1.12^a</td> <td data-bbox="1549 781 1629 849">4.02\pm 1.76</td> </tr> </tbody> </table> <p>^a Significantly different from beginning of treatment (paired t test), p<0.05.</p> <p>^b Significantly different from beginning of treatment (paired t test), p<0.001.</p> <p>Adverse Effects:</p> <p>In Phase 1, subjects did not report any adverse symptoms attributable to the consumption of either the FFMP or the V-GOS-supplemented treatment.</p> <p>In Phase 2, the subjects did not report any adverse symptoms when the 3.6g β-GOS treatment was consumed. Two of the 30 subjects reported abdominal discomfort and diarrhea when the 7g β-GOS treatment was consumed, but overall both preparations were well tolerated by the subjects.</p>	Phase 1 (n=29)	V-GOS 7g/d	Control		Baseline	5.00 \pm 1.07	5.18 \pm 1.13		Intervention	5.98 \pm 1.13 ^a	5.04 \pm 1.46		Phase 2 (n=30)	β -GOS 7g/d	β -GOS 3.6/d	Control	Baseline	4.05 \pm 1.06	4.07 \pm 0.92	4.54 \pm 1.17	Intervention	6.74 \pm 1.19 ^b	5.36 \pm 1.12 ^a	4.02 \pm 1.76	<p>Different galactosidases modify the physiological properties of each GOS. Enzymes originating from bifidobacterial species will increase the bifidogenic properties of the GOS.</p> <p>Overall, the novel GOS mixture was well tolerated during both the low- and high-dose treatments.</p>
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Reference Bouhnik et al, 2004	To determine the bifidogenic potential of different non-digestible carbohydrate (NDCHs) used in human diets.	R, C, DB, P	<ul style="list-style-type: none"> Healthy; BMI: 20-30; No history of gastrointestinal disease other than appendicitis; No antibiotics or laxatives taken during the 2 mo before the study; No other medication taken during the investigation period Free-living 18-54 y (mean age: 30 y) M (n=81) and F (n=119) 200 participated (number of volunteers recruited was not provided) 64 randomized in Phase 1 and 136 randomized in Phase 2 200 in final sample 	<p>Oral Treatment</p> <ul style="list-style-type: none"> GOS Dose High 10g/d; 2 equal oral doses - one after lunch and one after dinner - in each of Phase 1 and Phase 2 GOS Doses Low 2.5g/d, 5.0g/d, and 7.5g/d 2 equal oral doses - one after lunch and one after dinner - in each of Phase 1 and Phase 2 Control Dose 50% sucrose and 50% fully digestible waxy maize-derived maltodextrins <p>Each phase consisted of a 7-d stabilization/run-in period followed by a 7-d intervention:</p> <p>Phase 1: to evaluate the prebiotic effect of all selected NCDHs when consumed at a dose of 10g/d</p> <ul style="list-style-type: none"> 7-d treatment in one of 8 groups (total n=64): <ol style="list-style-type: none"> 10g/d GOS + usual diet (n=8) Control + usual diet (n=8) 3-8. Other NDCHs + usual diet (n=8 for each NCDH) <p>Phase 2: to evaluate possible dose-response prebiotic effects of the NCDHs that had been found during Phase 1 to be bifidogenic at a dose of 10g/d (total n=136)</p> <ul style="list-style-type: none"> 7-d treatment in one of 5 groups: <ul style="list-style-type: none"> GOS + usual diet (n=32) Control + usual diet (n=8) 3 other NDCHs + usual diet (n=32 for each NCDH) <p>Each group of 32 subjects was further divided randomly so that a subgroup of 8 subjects ingested daily doses of 2.5, 5.0, 7.5, or 10g/d. <ul style="list-style-type: none"> Usual daily diet Assessment tool: N/A </p>		<p>Mean \pm SD of fecal bifidobacteria counts (log CFU/g) on Day 1 (beginning of intervention), Day 8 and Day 15 (end of intervention)</p> <table border="1"> <thead> <tr> <th>Phase 1</th> <th>Day 8^a</th> <th>Day 15</th> </tr> </thead> <tbody> <tr> <td>GOS (10.0g/d) (n=8)</td> <td>9.74\pm 0.41</td> <td>10.12\pm 0.10^b</td> </tr> <tr> <td>Control (n=8)</td> <td>8.08\pm 0.59</td> <td>7.87\pm 0.60</td> </tr> <tr> <th>Phase 2</th> <th>Day 8^a</th> <th>Day 15</th> </tr> <tr> <td>GOS (2.5g/d) (n=8)</td> <td>9.31\pm 0.10</td> <td>12.32\pm 0.16^{c,d}</td> </tr> <tr> <td>GOS (5.0g/d) (n=8)</td> <td>10.19\pm 0.12</td> <td>12.37\pm 0.11^{c,d}</td> </tr> <tr> <td>GOS (7.5g/d) (n=8)</td> <td>9.59\pm 0.55</td> <td>12.40\pm 0.20^{c,d}</td> </tr> <tr> <td>GOS (10.0g/d) (n=8)</td> <td>9.40\pm 0.29</td> <td>12.43\pm 0.17^{c,d}</td> </tr> </tbody> </table> <p>^a Days 1-7 were a run-in period (no treatment); participants excluded GOS and fermented dairy products from their diet ^b Significantly different from control (t test after Bonferroni's adjustment for multiplicity), P=0.007 ^c Significantly different from Day 8 ^d No significant differences among doses tested</p> <p>Adverse Effects: Phase 1: Significant increases in excess flatus, bloating, borborygmi, and abdominal pain during the 7-d consumption of NDCHs; no significant differences among the 8 treatments (including placebo) was found with respect to changes in digestive symptoms. Phase 2: Significant increases in excess flatus, bloating, and abdominal pain were observed during the 7-d consumption of NDCHs; no significant differences among the 4 treatments tested with respect to change in digestive symptoms.</p> <p>Relevant Authors' Conclusions - GOS was bifidogenic at 10g/d when consumed for 7 d. - The 7-d treatment with different doses of GOS (2.5, 5, 7.5, and 10g/d) increased bifidobacteria counts but there was no dose-response relationship. - Adverse events in GOS treatment were comparable to those in other NDCH and were most likely caused by other components (sucrose and maltodextrins used in placebo)</p>	Phase 1	Day 8 ^a	Day 15	GOS (10.0g/d) (n=8)	9.74 \pm 0.41	10.12 \pm 0.10 ^b	Control (n=8)	8.08 \pm 0.59	7.87 \pm 0.60	Phase 2	Day 8 ^a	Day 15	GOS (2.5g/d) (n=8)	9.31 \pm 0.10	12.32 \pm 0.16 ^{c,d}	GOS (5.0g/d) (n=8)	10.19 \pm 0.12	12.37 \pm 0.11 ^{c,d}	GOS (7.5g/d) (n=8)	9.59 \pm 0.55	12.40 \pm 0.20 ^{c,d}	GOS (10.0g/d) (n=8)	9.40 \pm 0.29	12.43 \pm 0.17 ^{c,d}	
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Reference Bouhnik et al, 1997	To assess tolerance of GOS and the effects of their prolonged administration on bifidobacteria and fermentative activity of colonic flora.	NR, P	<ul style="list-style-type: none"> 8 healthy volunteers (4 males, 4 females, aged 20-32 y) recruited 8 in final sample 	<p>Oral Treatment</p> <ul style="list-style-type: none"> GOS Dose: 10g/d GOS powder in two 5g powder doses, taken morning and evening after meals Duration: 21 days Breath and feces were sampled on d1, 7, 14, and 21 Background diet: <ul style="list-style-type: none"> The evening before sampling, subjects ingested a residue-free meal, i.e. steak, rice, and rusk On the day of sampling, they fasted and at 0800 h were given 10g of GOS diluted in 100 mL of water; they continue to fast for 12h and did not receive the evening dose Assessment tool: a diary sheet on which the following symptoms were graded from 0 to 3: excess rectal gases, bloating, borborygmi, and abdominal pains. Stool frequency and consistency were also noted. 		<p style="text-align: center;">Bifidogenicity of GOS</p> <table border="1"> <thead> <tr> <th>Measurement Points</th> <th>Bifidobacteria concentration (log₁₀ CFU/g)</th> </tr> </thead> <tbody> <tr> <td>Day 1</td> <td>8.6 ± 0.6</td> </tr> <tr> <td>Day 7</td> <td>9.7 ± 0.5</td> </tr> <tr> <td>Day 14</td> <td>9.7 ± 0.6</td> </tr> <tr> <td>Day 21</td> <td>9.5 ± 0.6</td> </tr> </tbody> </table> <p>Bifidobacteria concentrations were significantly greater on d 7, 14, and 21 after GOS ingestion compared with d 1.</p> <p style="text-align: center;">Fecal data in healthy volunteers during ingestion of 10g GOS per day for 21 d (values are means ± SEM, n=8)</p> <table border="1"> <thead> <tr> <th></th> <th>Day 1</th> <th>Day 7</th> <th>Day 14</th> <th>Day 21</th> </tr> </thead> <tbody> <tr> <td>Stool weight (g/d)</td> <td>105 ± 25</td> <td>67 ± 14</td> <td>96 ± 19</td> <td>80 ± 15</td> </tr> <tr> <td>Fecal water (g/100g)</td> <td>74 ± 18</td> <td>73 ± 14</td> <td>73 ± 14</td> <td>78 ± 16</td> </tr> <tr> <td>Fecal pH</td> <td>6.8 ± 0.2</td> <td>6.6 ± 0.1</td> <td>6.6 ± 0.2</td> <td>6.8 ± 0.2</td> </tr> </tbody> </table> <p>Stool weight (24 h), the percentage of fecal water, and stool pH were not modified by GOS administration.</p> <p>Subjects did not experience any symptoms after ingesting GOS.</p> <p style="text-align: center;">Relevant Authors' Conclusions</p> <p>The addition to the diet of small amounts of GOS, which do not induce digestive symptoms, alters the concentrations of bifidobacteria and the intracolonic fermentation metabolism.</p>	Measurement Points	Bifidobacteria concentration (log ₁₀ CFU/g)	Day 1	8.6 ± 0.6	Day 7	9.7 ± 0.5	Day 14	9.7 ± 0.6	Day 21	9.5 ± 0.6		Day 1	Day 7	Day 14	Day 21	Stool weight (g/d)	105 ± 25	67 ± 14	96 ± 19	80 ± 15	Fecal water (g/100g)	74 ± 18	73 ± 14	73 ± 14	78 ± 16	Fecal pH	6.8 ± 0.2	6.6 ± 0.1	6.6 ± 0.2	6.8 ± 0.2	
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12.1 Safety Assessment by Experts Qualified by Scientific Training and Experience

The Natural Health Product Directorate (NHPD) of Health Canada assessed the safety of Floraid GOS consumption on a daily basis and ruled that a daily intake of 1 to 2 Tablespoons Floraid GOS syrup is adequate. The amount of 1 to 2 Tablespoons Floraid GOS syrup equates to a daily intake of 5.32g to 10.64g GOS per day.⁴⁶

12.2 Selection of Level of use of GOS in Foods for Persons 2+

The proposed level of use of GOS in foods for persons aged 2+ is 2.66 g per serving. This amount is slightly more than the minimum amount of GOS shown to be safe and efficacious as a non-digestible, fermentable carbohydrate in published clinical studies (2.5g per day), and, slightly more than half of the minimum daily intake (5.32g) determined to be safe and efficacious, as a non-digestible, fermentable carbohydrate, by Health Canada NHPD.

In addition, this amount of GOS slightly exceeds the minimum amount of dietary fibre per stated serving size, required to make a “good source of dietary fibre” claim in the USA marketplace, which is 2.5g dietary fibre per serving.⁴⁷

13 Intended Use and Proposed Level of Use of Floraid GOS

13.1 Infant Formula and Follow-on Foods

As shown in Table 4 below, the intention is to market Floraid GOS syrup and Floraid GOS powder such that the level of GOS is present at a maximum concentration of 0.38% in infant formula (4g GOS or 4g GOS:FOS per litre infant formula) and in toddler foods. This level of use is consistent with that cited in the literature, and as well, is consistent with industry practice to date in jurisdictions which permit addition of GOS to infant formula and toddler foods.

13.2 Foods for Persons Aged 2+

As explained in Section 12.2, the amount of GOS proposed per serving of food, is 2.66g. Table 5 identifies the foods intended to be supplemented with GOS, and as well, the intended concentration of GOS in these foods.

⁴⁶ Copy of Health Canada NHPD Floraid (Promovita) GOS safety evaluation available to the FDA on request.

⁴⁷ 21CFR101.54(c) and where applicable, 21CFR101.54(d)

Table 4

Intended Level of Use and Proposed Use of GOS as a Food Ingredient in Infant Formula and Follow-on Foods

Infant Formula			
Concentration (g GOS /litre) ¹			GOS
			4
g GOS per g IF (Infant Formula)			0.0038
Baby, Infant and Toddler Foods			
concentration (g GOS / g food)			0.0038
Category	Description & Serv Size (g)		GOS/serving
Baby and toddler, desserts, dinners, stews, soups and vegetables (ready-to-serve)	strained	60	0.2264
	junior	110	0.4151
	toddler vegetables	70	0.2642
	toddler all ex: desserts and vegetables	170	0.6415
Baby and toddler juice	-	125	0.4717
Baby and toddler biscuits (cookies, crackers)	baby	7	0.0264
	toddler	20	0.0755
Baby and toddler yoghurt drinks	-	125	0.4717
RTS pureed fruit mixtures	-	125	0.4717
Infant cereals	dry, instant, reconstitute to 110g	110	0.4151
	ready-to-serve	110	0.4151
¹ Specific gravity of IF is 1.06 g/ml, 1 litre weighs			1060
² Yoghurt contains naturally-occurring galacto-oligosaccharides, at an average concentration of 0.05% (1g per 175g serving). Thus, usage levels pertain only to added GOS.			

000160

Table 5**Intended Level of Use and Proposed Use of GOS as a Food Ingredient in Foods for Persons Aged 2+**

Foods for Persons Aged 2+				
Dairy Products				
Description	Serving Size	gram equivalent	g GOS per serving²	Concentration
Yoghurt ¹	175g	175	2.66	0.0152
Yoghurt drinks	250 ml	265	2.66	0.0100
Frozen yoghurt	125 ml	133	2.66	0.0200
Ice milk, custards, pudding	125 ml	133	2.66	0.0200
Dairy shake mixes, instant breakfasts, meal replacements	240 ml	254	2.66	0.0105
Beverages				
Carbonated and non-carbonated beverages, juice-based beverages, juice coolers, sweetened and/or flavoured water	240 ml	250	2.66	0.0106
RTD (Ready-to-drink) ice tea, iced coffee (flavoured and/or sweetened)	240 ml	250	2.66	0.0106
Sport and isotonic drinks - RTD and reconstituted powder	240 ml	250	2.66	0.0106
¹ Yoghurt contains naturally-occurring galacto-oligosaccharides, at an average concentration of 0.05% (1g per 175g serving). Usage levels pertain only to added GOS.				

14 Estimated Daily Intake GOS as a Food Supplement in Infant Formula and Toddler Foods

International Dairy Ingredients Inc. retained a team of nutrition scientists to conduct a systematic, scientifically-sound and robust Estimated Daily Intake (EDI) of Floraid GOS as per the intended uses and usage levels provided in Tables 4 and 5. The EDI of Floraid GOS is based on the What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Surveys (NHANES) 2007-2010. The sections below provide the resultant EDI of Floraid GOS.

Further, the findings were subjected to direct comparisons with estimated GOS intakes previously reported in GOS GRNs 334, 286, 285 and 236, where feasible. To that end, additional subgroups were evaluated in determining the EDI of Floraid GOS, so as to allow for appropriate comparisons to subgroups identified in these previous GRNs. The comparisons, with commentary, are provided in Appendix X.

14.1 EDI GOS as Infant Formula Supplement

As mentioned in section 11.3, the proposed concentration of Floraid GOS in infant formula as well as in baby food and toddler food, is derived from a systematic assessment of published clinical trials, which indicate that a concentration of 0.38% (4g GOS/litre infant formula) is a safe level of consumption, which results in a stool consistency similar to that of breast-fed infants.

As shown in Table 6, below, for infants less than 12 months of age, consumption of Floraid GOS supplemented infant formula results in a mean EDI of 3.2g/day and a 90th percentile EDI of 4.5g per day. Similarly, for infants and toddlers aged 6-35 months of age, the mean EDI is 2.8g/day and the 90th percentile EDI is 4.1g per day. These EDIs for Floraid GOS are safe levels of consumption.

Table 6

Per user 2-day average estimated daily intake of GOS from proposed use in term infant formula among infants and toddlers; WWEIA, NHANES 2007-1020

Infant Population	Total Sample Size ¹	Consumers ¹	% Users	Per User ² (g/day)	
				Mean	90th Percentile
Non-nursing infants, 0-11 months	531	495	91	3.2	4.5
0-5 months	241	241	100	3.4	4.8
6-11 months	290	254	84	2.9	4.1
Non-nursing toddlers, 12-23 months	432	23	5	--	--
Non-nursing toddlers, 24-35 months	470	5	1	--	--
Non-nursing infants and toddlers 6 -35 months	1192	282	18	2.8	4.1
All infants (nursing and non-nursing)					
0-6 months	434	355	79	3.1	4.6
7-12 months	353	249	63	2.6	3.9
All toddlers, 1-2 years	940	28	3	--	--

¹ Unweighted number of infants and toddlers; user estimates based on statistical weights provided by NCHS

² Assumed GOS use level of 0.0038 g GOS per g infant formula, which corresponds to 4 g GOS per L infant formula

-- Unweighted number of toddlers consuming infant formula is too small to reliably estimate the mean or 90th percentile of intake

000163

14.2 EDI GOS as a Baby, Infant and Toddler Food Supplement

As shown in Table 7, there is no reported intake of toddler food for children aged 24-35 months, which revises the scope of potential consumption to infants and toddlers 23 months of age and younger. Thus, the age group identified as 6-35 months equally represents the age group of consumers of 6-23 months.

Infants Aged 6-11 months

The highest EDI Floraid GOS, from the total of infant formula and baby food supplementation, occurs in the youngest of the three age groups surveyed - infants aged 6-11 months.

For infants aged 6-11 months, supplementation of baby food with Floraid GOS results in a mean EDI of 1.1g/day and a 90th percentile EDI of 2.2g per day. Inclusion of GOS intake from Floraid GOS supplemented infant formula, results in a mean EDI is 3.6g/day and a 90th percentile EDI of 5.2g per day. These EDIs for Floraid GOS are safe levels of consumption.

Toddlers Aged 12-23 months

The lowest EDI Floraid GOS, from the total of infant formula and baby food supplementation, occurs in the eldest of the three age groups surveyed - infants aged 12-23 months.

For infants aged 12-23 months, supplementation of baby food with Floraid GOS results in a mean EDI of 0.5g/day and a 90th percentile EDI of 1.1g per day. Inclusion of GOS intake from Floraid GOS supplemented infant formula, results in a mean EDI of 0.8g/day and a 90th percentile EDI of 2.3g per day. These EDIs for Floraid GOS are safe levels of consumption.

Infants and Toddlers, Aged 6-23/6-35 months

The composite EDI Floraid GOS, from the total of infant formula and baby food supplementation, for all infants and toddlers aged 6-23/6-35 months, is consistent with the findings reported above. The EDI Floraid GOS from proposed baby food uses for infants/toddlers aged 6-35 months, results in a mean of 0.8g/day and a 90th percentile of 1.8g/day, which is comparable to the EDI GOS from baby food uses for toddlers aged 12-23 months. Similarly, the EDI Floraid GOS from infant formula for infants/toddlers aged 6-35 months, results in a mean of 2.8/day and a 90th percentile of 4.1g/day, which is comparable to the EDI GOS from infant formula usage for infants aged 6-11 months.

The combined EDI Floraid GOS from supplemented infant formula and baby food results in a mean of 2.2g/day and a 90th percentile of 4.9g/day. These EDIs represent safe levels of consumption.

Table 7

Per user 2-day average estimated daily intake of GOS from proposed use in baby, infant, and toddler foods among non-nursing infants and toddlers age 6 to 35 months; WWEIA, NHANES 2007-2010⁴⁸

Non-nursing Infant & Toddler Population	Food Category	Total Sample Size ¹	Consumers ¹	% Users	Per User ² (g/day)	
					Mean	90th
6-35 months	Baby and toddler, desserts, dinners, stews, soups and vegetables	1192	241	19	0.5	1.0
	Baby and toddler juice	1192	161	10	0.4	0.8
	Baby and toddler biscuits (cookies, crackers)	1192	162	12	0.0	0.0
	Baby and toddler yogurt drinks	1192	3	1	—	--
	Ready-to-serve pureed fruit mixtures	1192	195	13	0.4	0.9
	Infant cereals	1192	259	18	0.4	0.8
	All proposed baby food uses	1192	416	30	0.8	1.8
Infant Formula	1192	282	18	2.8	4.1	
	Combined total (Infant formula + baby food uses)	1192	454	33	2.2	4.9
6-11 months	Baby and toddler, desserts, dinners, stews, soups and vegetables	290	172	65	0.5	1.1
	Baby and toddler juice	290	117	37	0.4	0.8
	Baby and toddler biscuits (cookies, crackers)	290	104	37	0.0	0.1
	Baby and toddler yogurt drinks	290	2	<0.5	—	--
	Ready-to-serve pureed fruit mixtures	290	159	55	0.4	0.9
	Infant cereals	290	197	70	0.4	0.8
	All proposed baby food uses	290	254	88	1.1	2.2
Infant Formula	290	254	84	2.9	4.1	
	Combined total (Infant formula + baby food uses)	290	275	94	3.6	5.2

⁴⁸ Table 7 continues on next page.

Non-nursing Infant & Toddler Population	Food Category	Total Sample Size ¹	Consumers ¹	% Users	Per User ² (g/day)	
					Mean	90th
12-23 months	Baby and toddler, desserts, dinners, stews, soups and vegetables	432	61	15	0.4	0.7
	Baby and toddler juice	432	38	6	0.5	1.0
	Baby and toddler biscuits (cookies, crackers)	432	53	12	0.0	0.1
	Baby and toddler yogurt drinks	432	0	NA	NA	NA
	Ready-to-serve pureed fruit mixtures	432	32	6	0.3	0.4
	Infant cereals	432	58	11	0.3	0.5
	All proposed baby food uses	432	135	29	0.5	1.1
Infant Formula	432	23	5	--	--	
Combined total (Infant formula + baby food uses)	432	148	32	0.8	2.3	
24-35 months	Baby and toddler, desserts, dinners, stews, soups and vegetables	470	8	2	--	--
	Baby and toddler juice	470	6	1	--	--
	Baby and toddler biscuits (cookies, crackers)	470	5	2	--	--
	Baby and toddler yogurt drinks	470	1	1	--	--
	Ready-to-serve pureed fruit mixtures	470	4	0	--	--
	Infant cereals	470	4	1	--	--
	All proposed baby food uses	470	27	8	--	--
Infant Formula	470	5	1	--	--	
Combined total (Infant formula + baby food uses)	470	31	9	--	--	

¹ Unweighted number of infants and toddlers age 6 to 35 months; user estimates based on statistical weights provided by the National Center for Health Statistics (NCHS)

² Assumed GOS use level of 0.38% in baby, infant, and toddler foods

-- Unweighted number of infants or toddlers consuming infant formula is too small to reliably estimate the mean or 90th percentile of intake

15 EDI GOS as a Food Supplement, Persons Aged 2+

As mentioned in section 12.2, the level of Floraid GOS supplementation of selected foods, for the age group 2+, is 2.66g per serving. This amount is half the established, minimum, safe and effective daily GOS intake, or 5.32g, as determined by a systematic review of published clinical trials involving GOS and healthy human subjects, wherein the upper daily limit is 10.64g.

As shown in Table 8 below, the mean EDI for all chosen food categories is within this stated range of daily consumption. These estimates represent safe levels of consumption.

The 90th percentile exceeds the upper limit of recommended daily intake of 10.64g/day only for the category of carbonated and non-carbonated beverages, juice-based beverages, juice coolers, sweetened and/or flavoured water. It is noted, however, that this 90th percentile estimate does not necessarily represent the average level of consumption.

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Table 8

Per user 2-day average estimated daily intake of GOS from proposed uses in dairy and beverage products by the US population 2 years and older; WWEIA, NHANES 2007-2010

Food Category	Total Sample ¹	Consumers ¹	% Users	Per User ² (g/day)	
				Mean	90th
Dairy products	15,032	3,866	28	1.90	3.65
Yoghurt and yoghurt drinks ³	15,032	1,880	13	1.59	2.89
Ice milk, frozen yoghurt, custards, pudding	15,032	1,577	12	1.45	2.77
Dairy shake mixes, instant breakfasts, meal replacements	15,032	910	7	2.25	4.30
Beverages	15,032	13,381	89	6.88	14.21
Carbonated and non-carbonated beverages, juice-based beverages, juice coolers, sweetened and/or flavoured water	15,032	12,814	85	6.07	12.83
RTD ice tea, ice coffee (flavoured and/or sweetened)	15,032	2,622	20	3.65	7.29
Sport and isotonic drinks, RTD and reconstituted powder	15,032	976	6	4.19	8.00
Dairy and beverage total	15,032	13,854	93	7.17	14.40

¹ Unweighted number of respondents; user estimates based on statistical weights provided by the National Center for Health Statistics (NCHS)

² Assumed GOS use level ranges from 1 – 2% in dairy and beverage products

³ Yoghurt drinks are included in the yoghurt group since NHANES additional description of food code 11422000 (Yogurt, vanilla, lemon, maple, or coffee flavor, lowfat milk) is inclusive of liquid yogurt, LeShake, Tuscan, Go-Gurt Portable Yogurt tube, and Yoplait Expresso Yogurt; GOS use level for this food code is assumed to be same as use level in yogurt, which is higher than proposed use in yoghurt drinks.

16 Comparison of Floraid GOS EDI to Previously reviews GOS GRNS

A comparison of the EDI of Floraid GOS to the EDI of previously reviewed and approved GOS GRNs is provided, as aforementioned, in Appendix X. Therein, it is illustrated that the EDI of Floraid GOS is considerably lower than previously submitted GOS EDIs, when comparing identical food uses and implementing the intended Floraid GOS concentration. A per summary Table 9 and Table 10, below, this lower EDI illustrates the safety of Floraid GOS consumption as per intended usage.

Table 9

Comparison with previous GRNs of EDI of GOS for combined uses in infant formula and baby food

Non-Nursing Infant Pop.	Mean Per User (g/day)				Per User 90 th Percentile (g/day)			
	Floraid	GRN 236 ¹	GRN 285 ²	GRN 334 ³	Floraid	GRN 236 ¹	GRN 285 ²	GRN 334 ³
6-11 months	3.6	6.1	--	7.9	5.2	10.1	--	11.8
12-23 months	0.8	5.3	--	--	2.3	11.2	--	--
6-35 months	2.2	--	5.7 (infants 0-2 yr)	14.7 (infants up to 1yr)	4.9	--	9.7 (infants 0-2 yr)	26.8 (infants up to 1 yr)

¹ GRN 236 Revised Table III-4, pg 189; ² GRN 285 Table IV.C-1, pg 25; ³ GRN 334 Table III-2, pg 25

--not reported

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Table 10**Comparison with previous GRNs of EDI of GOS for foods for persons aged 2+**

Food Categories	Use level (%)			
	Floraid	GRN 236 ¹	GRN 285 ²	GRN 334 ³
Dairy products +	1.0-2.0	3.3 - 4.3	0.48 - 0.57	0.61 - 3.8
Yogurt	1.5	3.3	0.57	3.3
Yogurt drinks	1.0	--	--	--
Frozen dairy	2.0	4.3	--	2.0
Dairy shake mixes, instant breakfast, meal replacements	1.05	2.0	0.48	--
Non-alcoholic beverages +	1.06	--	--	--
Beverage concentrate	1.06	--	--	2.0
Fruit drinks +	1.06	--	--	2.0
Vitamin/mineral fort. fruit drinks	1.06	2.1	--	--
Non fruit incl energy drinks +	1.06	1.3 - 2.1	0.53	4.4
Fitness water and thirst quenchers	1.06	1.3	0.53	2.0
Energy drinks	1.06	2.1	0.53	4.4
Fruit and veg juices	1.06	--	--	1.6
Nut beverages	--	--	--	1.6
RTD Non-milk based meal replacements and protein beverages	--	--	0.48	--
RTD Soy beverages	--	--	0.53	--
Soups	--	--	0.52	0.61
Bakery products +	--	--	1.02-4.27	1.0
Bars	--	12.5	--	1.0
Cereals, RTE	--	--	4.27	2.0
Sugars and sweets (jellies, jam, etc.)	--	25	--	25

+ Additional sub-categories of proposed foods not shown and may vary across GRN

¹GRN 236 (Freiland; Table 1, pg 4); ²GRN 285 (GTC; Table A1, pg76-78); ³GRN 334 (Yakult; Table III-I, Pg 22-23)

17 Conclusion – Determination of GRAS Exemption

Floraid GOS is a GOS manufactured in accordance with standard procedures published in the scientific literature. Available in both a viscous syrup and powder format, the composition of Floraid GOS, such as the DP of 3.2, monomeric chains of galacto-oligosaccharides, and a non-linked mixture of monosaccharides and disaccharides, also complies with the generally-accepted composition of GOS widely published in the scientific literature.

Certificates of Analyses, provided herein, confirm the aforesaid manufacturing procedure and composition.

The safety of Floraid GOS has been determined by the following scientific procedures:

- manufacture of Floraid GOS in a manner consistent with scientifically-validated and published procedures, in a safe and hygienic facility, following GMPs and adhering to HACCP protocol;
- nutrient analyses and microbial analyses conducted according to certified procedures, which validate a consistent nutrient profile and microbial-free product;
- acknowledgement of naturally-occurring GOS in commonly consumed foods such as yoghurt;
- published clinical trials in which a range of the targeted population groups - infants and persons aged 2+ - were subjected to GOS daily intakes of 2.5g to 10g per day;
- evaluation of these published clinical trials and selection of a safe level of intake for GOS-supplemented infant formula of 4g GOS or 4g GOS:FOS (ratio 9:1) per litre;
- incorporation of the safe level of Floraid GOS intake determined by Health Canada's Natural Health Product Directorate (NHPD) of 5.32g to 10.64g per day;
- based on these published documents, and direction from Health Canada's NHPD, selection of a level of Floraid GOS per serving representative of a safe intake, wherein such safety assessment acknowledges the dietary fibre attribute of Floraid GOS;
- exposure of the selected level of intended use (0.38% in infant formula [4g/litre] and in infant, baby and toddler foods, 2.66g per serving for foods for persons aged 2+) to scientific and rigorous Estimated Daily Intake (EDI) assessment;
- a resultant mean EDI Floraid GOS for non-nursing infants, from infant formula, of 3.2g, and 90th percentile EDI of 4.5g, well within previously EDI's GOS in infant formula
- a resultant combined mean EDI Floraid GOS for infants, babies and toddlers from consumption of selected foods of 3.6g, and a 90th percentile of 5.2g, also well within previously established EDI's GOS stemming from such foods;
- for persons aged 2+, a resultant mean EDI of 7.17g, which is within the aforementioned authorized range of safe daily GOS intake of 5.32g to 10.64g. It is noted that the 90th percentile of intake is higher than this

000171

- established range, however, the 90th percentile of intake represents a cell of outlier data, and as such, low likelihood of occurrence;
- regarding infant formula, the EFSA Expert Panel has approved a GOS concentration in infant formula of 8g/litre, which is twice the level proposed in this GRAS Notification;
 - last but not least, an EDI for infant formula use, infant, baby and toddler food use, and food for persons aged 2+, which is considerably lower than previously approved GOS GRNs.

Accordingly, it is respectfully put forth, that Floraid GOS, marketed as per intended uses cited herein, is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act, because we, the notifier, have determined that such use is GRAS.

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Appendix I Carbohydrate Characterization of Floraid GOS Syrup

3. Results

3.1 Dry matter content GOS syrup

The dry matter of the GOS syrups is measured in single applying the vacuum oven drying method. This method is specially developed for sugar-rich samples. The result is presented in table 2.

Table 2
Established dry matter content in the GOS syrup

Sample identification	Dry matter content (% w/w)
Sample 5367819	74.8

3.2 Sugar profile: mono- and disaccharide composition of the syrup

The mono- and disaccharide content in the syrup is quantified with HPAEC-PAD applying two different gradient elution profiles. One profile was applied for the separation and quantization of the monosaccharides galactose, glucose and fructose. The second profile results in a good separation of the disaccharides lactose, sucrose and maltose. However with this chromatographic profile the monosaccharides are not well separated anymore. The chromatograms are presented in figure 1.

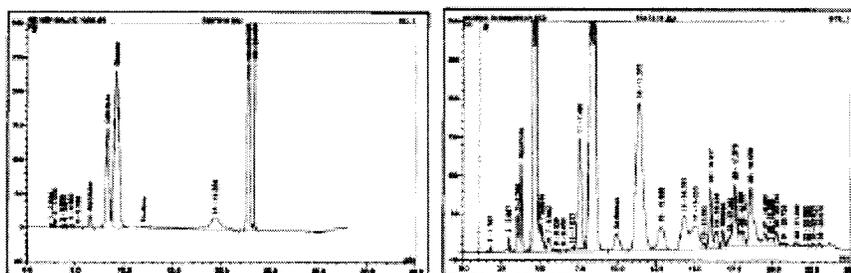


Figure 1
Chromatograms of the sugar profiles measured with two different gradients of the syrup sample.
Left: profile 1 for the separation of galactose and glucose
Right: profile 2 for the separation of glucose + galactose (coinciding peaks), fructose, lactose, sucrose and maltose

The galactose and glucose quantization applying elution profile 1 results in a good chromatogram (figure 1, left). No interference of the GOS oligomers is present. The chromatogram obtained by applying elution profile 2 results in much more complex chromatogram. A number of different GOS oligomeric peaks show up. The lactose peak (retention time 8.2 min) is well separated of the other (GOS) peaks in the chromatogram and can easily be quantified. Surprisingly now a peak shows up with the retention time of fructose (about 5 min) at the foot of the coinciding galactose-glucose peak. Applying elution profile 1 no significant amount of fructose could be quantified, thus this peak is an interfering GOS constituent. Surprisingly also a small peak at about the retention time of sucrose (retention time 10 min) and at about the retention time of maltose (retention time 16.8 min) was detected in the chromatograms. Quantization of these peaks resulted in sucrose and maltose contents of less than 1.0 % w/w. GOS is manufactured from lactose. Therefore it is very unlikely that maltose and sucrose will be present. It is expected that these peaks should be considered also as interfering GOS oligomeric constituents.

In the AOAC 2001.02 official method the free galactose, glucose, and lactose content in the syrup sample is quantified. It is known that the relative reproducibility standard deviation or variation coefficient (RSD_r) in the GOS analysis ranges from 5 – 11%. For that reason the AOAC 2001.02 GOS determinations have been done in duplicate at two different days. And thus also the content of free galactose, glucose and lactose have been measured in duplicate.

All the measured concentration of the sugars, expressed as % (w/w) in the product as received, are summarized in table 3.

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Table 3

The monosaccharide and disaccharide content in the GOS syrup, expressed as % (w/w) in the product as received

Sugar	Concentration % w/w			
	Sugar profile	Sample 5387819 Duplicates GOS analyses		Average
Galactose	8.8	8.4	8.5	8.6
Glucose	21.6	20.3	20.4	20.8
Fructose	(0.6)			
Lactose	18.6	17.7	17.7	18.0
Sucrose	(0.9)			
Maltose	(0.3)			
Total sugar	49.0	46.4	46.8	47.3

() = quantified as the specified sugar, but most likely an unknown GOS oligomer

3.3 GOS characterization by gel permeation chromatography

The separation mechanism of gel permeation chromatography is based on differences in the hydrodynamic volume of analytes in the sample. The small molecules elutes in the so-called total permeation and the large molecules in the total exclusion.

In the chromatogram the DP 1 – 8 oligomeric peaks are clearly detected. The GPC system is equipped with an RI detector. It is known that the RI detector has equal sensitivities for monosaccharides and oligomers. Based on a calibration with glucose standards, the different DP-peaks in the chromatogram were quantified and the results are presented in table 4.

The peaks in the GOS chromatogram in figure 2 are not pure constituents. The DP1 peak is a mixture of the monosaccharides galactose and glucose. And the DP2 peak is a mixture of lactose and GOS DP2 constituents.

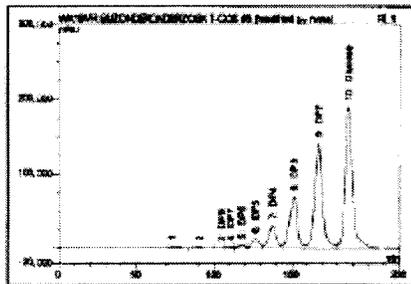


Figure 2
GPC separation of the sugars and the galacto-oligosaccharides in both GOS syrup

Table 4
Concentration of the different DP's in the GOS syrup, expressed as % (w/w) in the product as received

DP value peak	Sample 5387819	
	Concentration carbohydrates	Concentration GOS constituents ¹⁾
1	30.3	(0.0)
2	25.0	7.0
3	11.5	11.5
4	4.8	4.8
5	1.9	1.9
6	0.7	0.7
7	0.3	0.3
8	0.2	0.2
total	74.7	28.4

¹⁾ corrected for average concentration free monosaccharides and lactose in table 2

The calculated total carbohydrate content in the syrup (by adding DP1 up to DP8) is 74.7 % w/w. This figure is in very good agreement with the established dry matter contents of 74.6 % w/w (table 2). In principle we have a mass balance of 100% and no other constituents are present in the syrups than listed in table 4.

The average total monosaccharide concentrations, as measured with the two different HPAEC-PAD methods (table 2), are 29.4 % w/w. This result is in good agreement with the total monosaccharide concentration of 30.3 % w/w, as measured with the GPC. The difference of 0.9 % (w/w) is within the experimental error. The DP2 peak is a mixture of lactose (HPAEC-PAD result in table 2) and GOS DP2. The other peaks, DP3 and higher, are considered as being GOS peaks in which each DP peak contains different GOS isomers with identical monomeric units but different glycosidic linkages.

The average DP value (\overline{DP}) of the GOS constituents present in the syrup samples can be calculated with the figures in table 4 as follows:

$$\overline{DP} = \frac{\sum_{n=2-8} DP_n \times C_n}{\sum_{n=2-8} C_n}$$

This results in the following established average DP values of the GOS syrup:

$$\overline{DP}_{5387819} = \frac{2 \times 7.0 + 3 \times 11.5 + 4 \times 4.8 + 5 \times 1.9 + 6 \times 0.7 + 7 \times 0.3 + 8 \times 0.2}{7.0 + 11.5 + 4.8 + 1.9 + 0.7 + 0.3 + 0.2} = 3.22$$

This average DP value is important for the applied calculation method in the AOAC 2001.02 protocol [3] for the determination of the GOS concentration in a product. This quantitative analysis is based on the determination of the amount of galactose (galactose_{GOS}) which is set free during the enzymatic hydrolysis with β -galactosidase of the GOS present in the sample. The GOS concentration is then calculated with the formula:

$$\text{Concentration GOS} = \bar{k} \times \text{galactose}_{\text{GOS}}$$

in which \bar{k} is a correction factor the end standing glucose unit which is present in all GOS constituents. The factor \bar{k} is the ratio between the average molar mass of the GOS constituents including glucose, in formula $(180 + (n-1) \times 162)$ and the average molar mass of the is of the galactose which is set free enzymatically.

000181

$$k = \frac{180 + (n - 1) \times 162}{(n - 1) \times 180}$$

For the GOS constituents in the syrup samples this results in:

$$k_{\text{syrup}} = \frac{180 + (3.22 - 1) \times 162}{(3.22 - 1) \times 180} = 1.350$$

3.4 GOS determination following the AOAC 2001.02 official method

In the official AOAC 2001.02 method for quantifying the GOS content, firstly the content of free galactose and lactose are measured (HPAEC-PAD measurement). Then the GOS and lactose present in the sample are hydrolyzed enzymatically with β -galactosidase, followed by the HPAEC-PAD measurement of the total galactose content. The amount of galactose which is set free from the GOS is calculated by subtracting the free galactose content and the lactose bound galactose content from the total galactose content after the enzymatic hydrolysis. And at last the GOS content is calculated by multiplying the GOS originating galactose content with the above derived correction factor k.

As already mentioned above, regarding the relatively high RSD_R of the AOAC 2001.02 official analysis, the quantitative GOS analysis has been done in duplicate at two different days. The above established value of the correction factor k has been applied in calculating the GOS content of the syrup and the results are summarized in table 5 and compared with the by GPC established GOS contents in the syrup.

Table 5
GOS content measured according the AOAC 2001.02 and quantified by GPC

sample	GOS content (% w/w) in the syrup sample as is			GPC method
	AOAC 2001.02			
	Repetition 1	Repetition 2	average	
S387819	24.5	25.5	25.0	26.4

Within the experimental error, the results of both applied analytical methods for the determination of the GOS content in the syrup are in good agreement with each other.

The completeness of the enzymatic hydrolysis was verified by qualitative HPAEC-PAD analyses of the syrup sample before and after the enzymatic hydrolysis with the β -galactosidase including a blank enzymatic sample. The chromatograms are presented in figure 3.

In the fingerprint of the untreated GOS syrup sample the different GOS peaks are clearly present (figure 3, above). The peak at the retention time of about 5 min is the coinciding galactose/glucose peak and the peak at about 9.6 min retention time is the free lactose present in the sample. The chromatogram of the sample after the β -galactosidase hydrolysis (figure 3 middle) is, with the exception of the presence of the combined galactose/glucose peak, identical to the chromatogram of the enzymatic blank (figure 3, below). Based on these chromatograms, it is concluded that all GOS constituents present in the sample are fully hydrolyzed into their monosaccharide building blocks galactose and glucose.

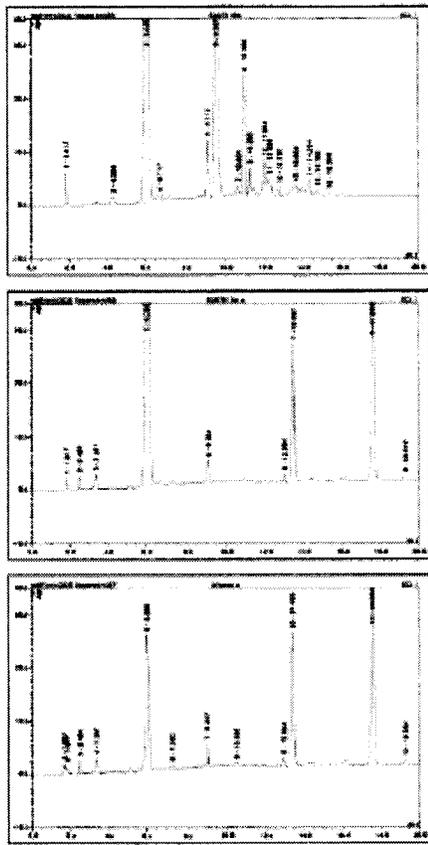


Figure 3
 HPAEC-PAD chromatograms (elution profile 4) to check for the completeness of the enzymatic hydrolysis of the GOS oligomers.
 Above: the GOS syrup sample before the enzymatic hydrolysis
 Middle: the GOS syrup sample after the enzymatic hydrolysis
 Below: the enzyme blank

Appendix II Floraid Syrup Finished Product Specifications (Two C of A's)



PROMOVITA GOS

Certificate of Analysis

Date of Manufacture	12 th Feb 2012
Best Before Date	12 th Feb 2013
Batch Number	L080212002

Chemical

Parameter	Specification	Results
Total Solids	≥70%	70.3%
Total GOS component	≥36.84% dm	conforms
: Glucose		18.7%
: Galactose		7.6%
: Lactose		31.2%
Protein	≤0.2% Max	0.1%
pH	3.1 – 3.8 units	3.0

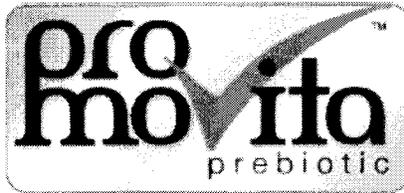
Microbiological

T.V.C	<100	<100
Enteroc's	<10	<10
Yeasts	50/g Max	<10
Moulds	50/g Max	<10
E.coli	Abs. in 5g	conforms
Staphylococci	Abs. in 1g	conforms
Salmonella	Abs. in 25g	conforms

Signed on behalf of WRIGHT AGRICULTURAL INDUSTRIES LIMITED

L J Wright

Wright Agri Industries Limited
The Old Smithy
Yeaton
Baschurch
Shropshire
SY4 3HY, England



PROMOVITA GOS

Certificate of Analysis

Date of Manufacture 27th Sept 2012
Best Before Date 27th Sept 2013
Batch Number L0270912002

Chemical

Parameter	Specification	Results
Total Solids	≥70%	70.7%
Total GOS component	≥36.69% dm	conforms
: Glucose		17.8%
: Galactose		7.1%
: Lactose		32%
Protein	≤0.2% Max	0.2%
pH	3.1 – 3.8 units	3.3

Microbiological

T.V.C	<100	<100
Enteroc's	<10	<10
Yeasts	50/g Max	<10
Moulds	50/g Max	<10
E.coli	Abs. in 5g	conforms
Staphylococci	Abs. in 1 g	conforms
Salmonella	Abs. in 25g	conforms

Signed on behalf of **WRIGHT AGRI INDUSTRIES LIMITED**

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Appendix III Carbohydrate Characterization of Floraid GOS Powder



Analytico Food

International dairy ingredient
T.a.v Mr. Douglas McEwen
1525 Cornwal Road - Unit 26
L6J 0B2 OAKVILLE

Voorlopig analysecertificaat

Certificaatnummer : 2013048776
 Rapportagedatum : 13-05-2013
 Startdatum : 19-04-2013
 Monster(s) ontvangen : 26-03-2013
 Datum monstername : Onbekend
 Klantnummer : 35405
 Klantnaam : International dairy ingredient
 Locatie :
 Uw projectnummer :
 Uw projectnaam :
 Projectcoördinator : Alle Jan Pool
 Contactpersoon : Mr. Douglas McEwen

Nr.	Monsterschrijving	Monsternametijd	EOL monsternummer
1	Sample T-gos powder		888-2013-ST-7510900

MTH	Analyse	Eenheid	1
		Analytico-nr	7510900
M1	Vocht (Karl Fischer)	% (m/m)	1.1
	Galactose (HPLC)	% (m/m)	9.33
	Glucose (HPLC)	% (m/m)	24.7
	Fructose (HPLC)	% (m/m)	0.69
	Lactose (HPLC)	% (m/m)	28.6
	Sacharose (HPLC)	% (m/m)	0.68
	Maltose (HPLC)	% (m/m)	0.15
	Transgalacto Oligosacchariden	% (m/m)	39.0

Zie de bijlage voor opmerkingen bij dit analysecertificaat.

* Indicateer waarde. | ** Zonder bevestiging.

De analyseresultaten hebben alleen betrekking op het monster. De meetonzekerheden van de toegepaste onderzoeksmethoden zijn opvraagbaar bij de afd. Projectcoördinatie.
 De analysemonsters worden tot 3 weken na de datum ontvangst bewaard.

Akkoord
S.L.M. Meersseman
Managing Director

(b) (6)

Pagina 1/1

Bijlage: B,C.Externe certifica(a)t(en)

Eurofins Food Testing Netherlands B.V.

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All offers and agreements are subject to our General
 Conditions directly available upon request.

1. Introduction

Eurofins was requested by International Dairy Ingredient to analyse a galacto-oligosaccharide (GOS) sample (Eurofins LIMS code 7510900; client description: Sample T-GOS powder) for total GOS content with AOAC method 2001.02 and GOS chain length distribution with gel permeation chromatography (GPC) and RI detection. The data were used to determine the average DP of the GOS material and the k conversion factor to be used in AOAC 2001.02

2. Methods

Methods of analysis

Total GOS and free lactose content (AOAC 2001.02)

HPAEC-PAD (high performance anion exchange chromatography) equipped with a PA-1 column was used for determination of the total GOS AOAC method 2001.02. This method also reveals the content of free galactose, glucose and lactose in the GOS samples.

Gel permeation chromatography

An HPLC equipped with a Biogel-P2 column (60 x 1.6 cm) and a RI detector was applied for the aqueous GPC separation. The separation was performed at elevated temperature (80 °C). The separation range of the Biogel-P2 stationary phase is according the manufacturer ranging from 100 – 1800 molar mass units (MMU).

3. Results

Total GOS and free lactose content (AOAC 2001-02)

The results for total GOS and free sugars content are shown in table 2. Data from both AOAC 2001.02 and sugar pattern analysis are shown for galactose, glucose and lactose.

Table 1 AOAC 2001-02 and sugar pattern results

Eurofins LIMS code	GOS (%m/m)	Free Galactose (%m/m)	Free Glucose (%m/m)	Free Lactose (%m/m)
7510900 (Gos assay)	39.0	9.3	23.9	29.1
7510900 sugar pattern		9.3	24.7	28.6

T-GOS content is calculated using an average chain length of 2.71 (n=1.71). The calculation factor k used is 1.4848.

Gel permeation chromatography

The GPC-RI chromatogram of the sample is shown in figure 1. In table 2 the oligosaccharide composition from monosaccharide (DP1, in case of GOS this is a mixed peak of glucose and galactose) up to degree of polymerization 8 (DP8) is shown.

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BNP Paribas S.A., Amsterdam, The Netherlands

Eurofins report: 2012-psa-ide-001

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Figure 1 GPC-RI chromatogram of a GOS sample (Eurofins LIMS 7510900).

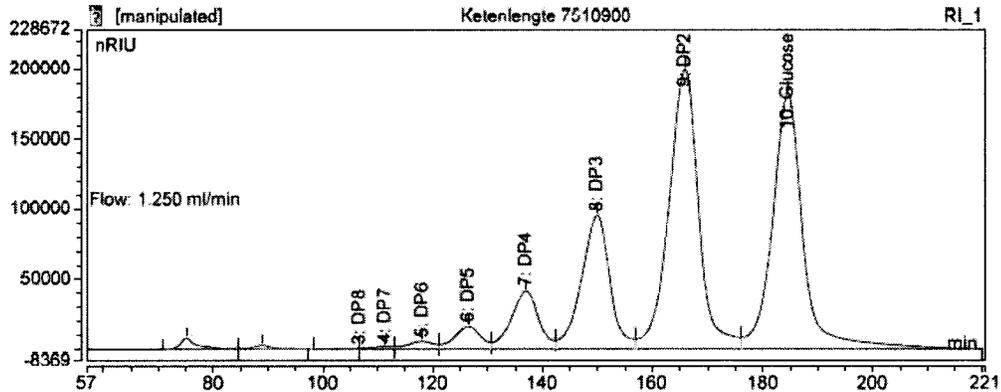


Table 2 DP1-DP8 distribution of the samples

Eurofins LIMS code	DP1 (%)	DP2 (%)	DP3 (%)	DP4 (%)	DP5 (%)	DP6 (%)	DP7 (%)	DP8 (%)
7510900	35.6	37.5	17.1	7.2	2.7	0.9	0.2	0.1

Since DP1 (glucose and galactose) and also lactose (eluting as part of DP2 in GPC) are not part of GOS, the content of DP2 in table 3 should be corrected for the amount of free lactose (table 2) in order to calculate the % of DP2 GOS. Using this % of DP2 GOS and the values from table 3 for DP3 up to DP8 (in pure GOS samples these should all be GOS components) the GOS composition can be calculated. These results are shown in table 3.

Table 3 GOS composition DP2 up to DP8 (% (w/w))

Eurofins LIMS code	DP2 (%)	DP3 (%)	DP4 (%)	DP5 (%)	DP6 (%)	DP7 (%)	DP8 (%)	TOTAL (%)
7510900	8.4	17.1	7.2	2.7	0.9	0.2	0.1	36.6

The total GOS content is not consistent with the data as found with AOAC 2001.02. The GOS content calculated from the Biogel distribution is lower because a wrong k factor (1.4848) is used in AOAC 2001.02. The data from table 3 were used to calculate the average DP and the k factor for this sample.

$$DP = \frac{\sum_{n=2-8} DP_n \times C_n}{\sum_{n=2-8} C_n}$$

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KvK No. 01070347
IBAN: NL05BNPA0227624827 BIC:BNPANL2A
BNP Paribas S.A., Amsterdam, The Netherlands

This results in the following established average DP values of the sample:

$$\overline{DP}_{7510900} = \frac{2 \times 8.4 + 3 \times 17.1 + 4 \times 7.2 + 5 \times 2.7 + 6 \times 0.9 + 7 \times 0.2 + 8 \times 0.1}{8.4 + 17.1 + 7.2 + 2.7 + 0.9 + 0.2 + 0.1} = 3.22$$

This average DP value is important for the applied calculation method in the AOAC 2001.02 protocol for the determination of the GOS concentration in a product. This quantitative analysis is based on the determination of the amount of galactose (galactose_{GOS}) which is set free during the enzymatic hydrolysis with β-galactosidase of the GOS present in the sample. The GOS concentration is then calculated with the formula:

$$\text{Concentration GOS} = k \times \text{galactose}_{\text{GOS}}$$

in which k is a correction factor the end standing glucose unit which is present in all GOS constituents. The factor k is the ratio between the average molar mass of the GOS constituents including glucose, in formula $(180 + (n-1) \times 162)$ and the average molar mass of the is of the galactose which is set free enzymatically:

For the GOS constituents in the sample this results in

$$k_{7510900} = \frac{180 + (3.22 - 1) \times 162}{(3.22 - 1) \times 180} = 1.350$$

Using this k factor in AOAC 2001.02 (instead of $k = 1.4848$) results in a GOS content of 35.5% in this sample, which is in fair agreement with the GOS content based on Biogel fractionation (36.6%).

Eurofins Food Testing Netherlands B.V.

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KvK No. 01070347
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BNP Paribas S.A., Amsterdam, The Netherlands

Eurofins report: 2012-psa-ide-001

000189

Appendix IV Dietary Fibre Content of Floraid GOS



Eurofins Scientific Inc.
Nutrition Analysis Center
2200 Rittenhouse Street, Suite 150
Des Moines, IA 50321
Tel. +1 515 265 1461
Fax. +1 515 266 5453

Eurofins Sample Code: 464-2011-03250252
Sample Description: GOS syrup
Client Sample Code: Control-Hot BJ0192
PO Number: 7112
Client Code: QD0003685

Reporting Date: 04/01/2011
Entry Date: 03/25/2011

Guelph Food Technology Centre
attn: Karen McPhee
88 McGilvray Street
Guelph, Ontario N1H 6J2
CANADA

Guelph Food Technology Centre
Attn: Karen McPhee
88 McGilvray Street
Guelph, Ontario N1H 6J2
CANADA

REPORT OF ANALYSIS



AR-11-QD-027250-01

Test	Result
Autoclave Import Samples	required
Insoluble dietary fiber (IDF)	0.32 %
Total dietary fiber (IDF + HMWSDF + LMWSDF)	20.77 %
High molecular wgt soluble dietary fiber (HMWSDF)	1.07 %
Low molecular wgt soluble dietary fiber (LMWSDF)	19.38 %

Method Reference

Autoclave Import Samples - Autoclave
Total dietary fiber HPLC (Includes Low MW Soluble Dietary Fiber) - AOAC 2009.01

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

David Gross, Support Services Manager

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Measurement of Uncertainty can be obtained upon request.

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Page 1 of 1

Analytical report: AR-11-QD-027250-01

000190

71

Appendix V Incubation Challenge Test_Protein Inert



Eurofins Scientific Inc.
Nutrition Analysis Center
2200 Rittenhouse Street, Suite 150
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Tel. +1 515 265 1461
Fax. +1 515 266 5453

Eurofins Sample Code: 464-2010-07120374
Sample Description: Syrup
Client Sample Code: Control
PO Number: 6784
Client Code: QD0003685

Reporting Date: 08/16/2010
Entry Date: 07/12/2010

Guelph Food Technology Centre
attn: Karen McPhee
88 McGilvray Street
Guelph, Ontario N1H 6J2
CANADA

Guelph Food Technology Centre
Attn: Karen McPhee
88 McGilvray Street
Guelph, Ontario N1H 6J2
CANADA

REPORT OF ANALYSIS



AR-10-QD-063295-02

This analytical report supersedes AR-10-QD-063295-01.

Test	Result
Autoclave Import Samples	required
T-GOS	28.3 % (w/w)

T-GOS content is calculated using an average chain length of 2.606. The calculation factor is 1.5227

Method Reference

Autoclave Import Samples - Autoclave
Galacto-oligosaccharides (t-GOS) - AOAC 2001.02, mod.

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

Kent Karsjens, Technical Manager

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000191

Appendix VI LONO Safety Assessment of GOS by Health Canada



Health
Canada

Santé
Canada

Health Products
and Food Branch

Direction générale des produits
de santé et des aliments

Bureau of Nutritional Sciences
Food Directorate
251 Sir Frederick Banting Driveway
Tunney's Pasture, A.L. 2203E
Ottawa, ON K1A 0K9

April 20, 2012

Douglas McEwen
President, International Dairy Ingredients Inc.
26-1525 Cornwall Road
Oakville, ON L6J 0B2

Dear Mr. McEwen:

This will refer to your request concerning the sale of Galacto-oligosaccharide (GOS) as a food ingredient. Officers of the Food Directorate, Health Products and Food Branch, have reviewed some requests regarding whether GOS is a novel food as defined in Division 28 of the *Food and Drug Regulations*.

To date our opinion has been that GOS is not a novel food, and we do not object to sale as a food ingredient. It is therefore not subject to pre-market notification under B.28.002 of the *Food and Drug Regulations*. It should be noted that this opinion is only in regard to the novelty of GOS and that it is the continuing responsibility of a manufacturer or importer to ensure that its product is in compliance with all applicable statutory and regulatory requirements. The sale of a food or food ingredient that poses a hazard to the health of consumers would contravene the provisions of the *Food and Drugs Act*.

Sincerely,

(b) (6)

Lynne Underhill, M.Sc.
Chief, Nutrition Premarket Assessment Division

Cc: Carol T. Culhane,
International Food Focus Ltd.

Cc: Luc Bourbonniere, Section Head,
Novel Food Section,
Bureau of Microbial Hazards,
Food Directorate, Health Canada

Canada

Appendix VII Naturally-occurring GOS



Eurofins Scientific Inc., Des Moines
3507 Delaware
Des Moines, IA 50313, US

Tel: +1 515 265 1461
Fax: +1 515 266 5453

Reporting Date: 10/16/2009
Entry Date: 10/08/2009

Eurofins Sample Code: 464-2009-10080419
Sample Description: Stirred Yogurt based Pre-fermentation 2 containers of same product
Client Sample Code: Yogurt Pre-fermentation
PO Number: credit card
Client Code: QD0003685
Guelph Food Technology Centre
attn: Karen McPhee
88 McGilvray Street
Guelph, Ontario N1H 6J2
CANADA

Guelph Food Technology Centre
attn: Karen McPhee
88 McGilvray Street
Guelph, Ontario N1H 6J2
CANADA



AR-09-QD-084507-01

REPORT OF ANALYSIS

Test	Result
Autoclave Import Samples T-GOS	required 0.4 % (w/w)

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Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

David Gross, Support Services Manager

Page 1 of 1

Analytical report: AR-09-QD-084507-01

000193



Eurofins Scientific Inc., Des Moines
3507 Delaware
Des Moines, IA 50313, US

Tel. +1 515 265 1461
Fax. +1 515 266 5453

Eurofins Sample Code: 464-2009-10080420
Sample Description: Stirred Yogurt 4 containers of same product
Client Sample Code: Yogurt
PO Number: credit card
Client Code: QD0003685

Reporting Date: 10/16/2009
Entry Date: 10/08/2009

Guelph Food Technology Centre
attn: Karen McPhee
88 McGilvray Street
Guelph, Ontario N1H 6J2
CANADA

Guelph Food Technology Centre
attn: Karen McPhee
88 McGilvray Street
Guelph, Ontario N1H 6J2
CANADA



AR-09-QD-084508-01

REPORT OF ANALYSIS

Test	Result
Autoclave Import Samples T-GOS	required 0.4 % (w/w)

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Measurement of Uncertainty can be obtained upon request.

Respectfully Submitted,
Eurofins Scientific Inc.

(b) (6)

David Gross, Support Services Manager

Appendix VIII References *in vivo* clinical trials. Infants: GOS-only and GOS:FOS 9:1 supplemented Infant Formula; Adults: GOS

Safety in Infants (GOS Only)

Ashley C, Johnston WH, Harris CL, et al. Growth and tolerance of infants fed formula supplemented with polydextrose (PDX) and/or galactooligosaccharides (GOS): double blind, randomized, control trial. *Nutrition Journal*. 2012;11:38

Fanaro S, Martin B, Bagna R, et al. Galacto-oligosaccharides are bifidogenic and safe at weaning: A double-blind randomized multicenter study. *Journal of Pediatric Gastroenterology and Nutrition*. 2009;48(1):82-88.

Ben XM, Li J, Feng ZT, et al. Low level of galacto-oligosaccharide in infant formula stimulates growth of intestinal *Bifidobacteria* and *Lactobacilli*. *World Journal of Gastroenterology*. 2008;14(42):6564-6568.

Safety in Infants (GOS:FOS in a 9:1 ratio)

Arslanoglu S, Moro GE, Boehm G, et al. Early neutral prebiotic oligosaccharide supplementation reduces the incidence of some allergic manifestations in the first 5 years of life. *Journal of Biological Regulators & Homeostatic Agents*. 2012;26(Suppl 3):49-59.

Holscher HD, Faust KL, Czerkies LA, et al. Effects of prebiotic-containing infant formula on gastrointestinal tolerance and fecal microbiota in a randomized controlled trial. *Journal of Parenteral and Enteral Nutrition*. 2012;36(Suppl 1):95S-105S.

Arslanoglu S, Moro GE, Schmitt J, et al. Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic manifestations and infections during the first two years of life. *The Journal of Nutrition*. 2008;138:1091-1095.

Moro G, Arslanoglu S, Stahl B, et al. A mixture of prebiotic oligosaccharides reduces the incidence of topic dermatitis during the first six months of age. *Archives of Disease in Childhood*. 2006;91(10):814-819.

Moro G, Minoli I, Mosca M, et al. Dosage related bifidogenic effects of galacto- and fructooligosaccharides in formula-fed term infants. *Journal of Pediatric Gastroenterology and Nutrition*. 2002;34:291-295.

Safety in Adults

Walton GE, van den Heuvel EG, Kusters MH, et al. A randomised crossover study investigating the effects of galacto-oligosaccharides on the faecal microbiota in men and women over 50 years of age. *British Journal of Nutrition*. 2012;107(10):1466-75.

Davis LMG, Martinez I, Walter J, et al. A dose dependent impact of prebiotic galactooligosaccharides on the intestinal microbiota of healthy adults. *International Journal of Food Microbiology*. 2010;144:285-292.

000195

Depeint F, Tzortzis G, Vulevic J, et al. Prebiotic evaluation of a novel galactooligosaccharide mixture produced by the enzymatic activity of Bifidobacterium bifidum NCIMB 41171, in healthy humans: a randomized, double-blind, crossover, placebo-controlled intervention study. American Journal of Clinical Nutrition. 2008; 87:785-791.

Bouhnik Y, Raskine L, Simoneau G, et al. The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans : A double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. American Journal of Clinical Nutrition. 2004;80:1658-64.

Bouhnik Y, Flourie B, D'Agay-Abensour L, et al. Administration of transgalactooligosaccharides increases fecal bifidobacteria and modifies colonic fermentation metabolism in healthy humans. The Journal of Nutrition. 1997;127:444-448.

Appendix IX Clinical Trials, Concentrations of 8g GOS:FOS, 6g GOS:FOS, 6g GOS:FOS:AOS

Concentration of 8g GOS:FOS per litre Infant Formula

<u>Author</u>	<u>Year</u>	<u>n</u>	<u>Participant Description</u>	<u>Duration</u>	<u>Reason for exclusion from GRAS Notification</u>
Arslanoglu	2012	92	Healthy term infants at risk of atopy	6 m	Therapeutic trial
Piemontese	2011	1130	Healthy term infants	12 m	Administered a blend which included AOS (acidic oligosaccharide) (6.8g GOS:FOS 9:1) + 1.2 g AOS).
Schouten	2011	74	Infants at risk of allergies	6m	Therapeutic trial
Salvini	2011	22	Healthy term	6m	n is small
Veereman-Wauters	2011	110	Healthy neonates	28d?	Appears to be an outlier as other trials at this concentration conducted for therapeutic purposes.
Van Hoffen	2009	84	At 3 m of age, infants vaccinated with Hexavac against DTP	6m	Therapeutic trial
Arslanoglu	2008	152	Healthy term infants with a parental history of atopy	6m	Therapeutic trial
Arslanoglu	2007	259	Healthy term infants with a parental history of atopy	6m	Therapeutic trial
Moro	2006	259	Healthy term infants with a parental history of atopy	6m	Therapeutic trial
Moro	2005	32	Healthy term infants	28d	n is low
Haarman	2005	10	Healthy term infants	6w	n is low
Knol	2005a	68	Healthy term infants	6w	n is low
Moro	2003	115	Healthy term infants	28 d	Both 4g/litre and 8g/litre tested. Acceptable results at 4g/litre, thus included at that concentration.
Schmelzle	2003	154	Healthy term infants	12w	Potential confounding variables as GOS:FOS not only difference between test and control formulas.
Moro	2002	90	Healthy term infants	28 d	Both 4g/litre and 8g/litre tested. Acceptable results at 4g/litre, thus included at that concentration.

Concentration of 6g GOS:FOS or 6g GOS:FOS:AOS per litre Infant Formula

<u>Author</u>	<u>Year</u>	<u>n</u>	<u>Participant Description</u>	<u>Feeding term</u>	<u>Reason for exclusion from GRAS Notification</u>
Vaisman	2010	104	Infants aged 9 to 24 m with acute diarrhea	12d	Therapeutic trial
Magne	2008	82	Healthy term infants	2m Included partial breast feeding	Inclusion of partial breast-feeding and inclusion of AOS in one treatment arm
Fanaro	2005	46	Healthy term infants	6w	Inclusion of AOS

000197

Appendix X Comparison of Floraid GOS EDIs to the EDIs of GOS GRNs 334, 286, 285 and 236

Note: The comparisons conducted by the nutrition scientists are provided below, verbatim, from the service provider's report. Thus, the Table #'s (i.e. 7, 8, etc.) are not in sequence as per the body of this GRAS Notification, but, are in sequence in the source document.

Previous GRNs, Infant Formula

Previous GRNs

The use of GOS in term infant formula has been the subject of three GRAS notices (GRN 334, 286 and 236). Based on the information provided to FDA by the submitters, the FDA had no questions regarding the submitters' conclusions that GOS is GRAS under the intended conditions of use. The GOS use levels in GRNs 334 and 286 are the same (7.2 g/L) and lower in GRN 236 (5.0 g/L). The EDIs reported in GRN 286 at the *per user* mean range from 5.2 g/day (infants 7-12 months) to 5.9 g/day (infants 0-6 months); and at the 90th percentile range from 7.9 (infants 7-12 months) to 8.5 g/day (infants 0-6 months). Also based on consumption data from NHANES 2003-2004, the GRN 236 reported a mean *per user* among non-breastfeeding infants of 4.8 g/day for infants 0-5 months and 4.0 g/day for infants 6-11 months; the 90th percentile estimates were 6.2 and 5.6 g/day, respectively (Friesland Foods Domo 2006). Although the GOS use level infant formula in GRN 334 also was 7.2 g/L, the EDIs reported in that notification include the proposed uses of GOS in foods, which include numerous baby foods, and consequently the estimated intakes are higher. In GRN 334, *per user* intakes at the mean are 6.9 g/day for infant 0-5 months and 7.9 g/day for infants 6-11 months; *per user* GOS intakes at the 90th percentile are 11.3 g/day for infants 0-5 months and 11.8 g/day for infants 6-11 months. GOS use level in term infant formula, intake estimates and the consumption database that were relied upon in these earlier GRAS notices are summarized in Table 3 below.

Table 3. Per user 2-day average estimated daily intake of GOS from uses in term infant formula (g/day) -- GRNs 334, 286 and 236

GRN (Submitter)	GOS Use Level in Formula	Population	Per User EDI (g/day)		NHANES Survey Year
			Mean	90 th	
236 (Friesland)	5.0 g/L ^a	Infants 0 - 5 months (non-nursing)	4.8	6.2	2003-2004
		Infants 6 -11 months (non-nursing)	4.0	5.6	
		Toddlers 12 - 23 months (non-nursing)	1.8	4.0	
286 (GTC Nutrition)	7.2 g/L	Infants 0 - 6 months	5.9	8.5	2003-2004
		Infants 7 -12 months	5.2	7.9	
		Toddlers 1 - 2 years	2.8	6.6	
334 (Yakult)	7.2 g/L ^{b,c}	Infants 0 - 5 months	6.9	11.3	2003-2004
		Infants 6 -11 months	7.9	11.8	
		Toddlers 12 - 23 months	19.4	27.8	
		All infants, 0 - 1 year	14.7	26.8	

^a The original notice in 2007 described the use level of 8.0 g/L and was amended to lower level of 5.0 g/L in 2008.

^b The EDIs include proposed uses of GOS in foods other than infant formula, including baby foods

^c FDA (2010) response letter for GRN 334 reports these values as intakes by infants under 1 year of age; the notification submitted by Yakult reports these values as intakes by infants 0-1 year. The estimated intakes of GOS by subpopulations of infants and toddlers in GRN 334, in combination with data reported in GRN 286, suggest that FDA erroneously cited the population group. The data here are presented as shown in GRN 334.

For the current proposed use of GOS in term infant formula at the use level of 4.0 g/L, the EDIs were derived based on the most current NHANES data (2007-2010). A direct comparison with the EDIs reported in GRN 334 is not possible as those estimates include food uses. However, the following comparison between the current EDIs with previous intake estimates from the use of GOS in infant formula in GRNs 236 and 286 can be summarized:

• *Comparison with GRN 236:*

GRN (Submitter)	GOS Use Level in Formula	Population	Per User EDI (g/day)		NHANES Survey Year
			Mean	90 th	
236 (Friesland)	5.0 g/L	Infants 0 - 5 months (non-nursing)	4.8	6.2	2003-2004
		Infants 6 -11 months (non-nursing)	4.0	5.6	
		Toddlers 12 - 23 months (non-nursing)	1.8	4.0	
Current GRAS	4.0 g/L	Infants 0 - 5 months (non-nursing)	3.4	4.8	2007-2010
		Infants 6 -11 months (non-nursing)	2.9	4.1	
		Toddlers 12 - 23 months (non-nursing)	--	--	

-- Unweighted number of toddlers consuming infant formula is too small to reliably estimate the mean or 90th percentile of intake

For the non-nursing infants 0 – 5 months, the mean *per user* (3.4 g/day) and 90th percentile (4.8 g/day) are well below those reported for non-nursing infants 0 – 5 months in GRN 236. For the non-nursing infants 6 – 11 months, the mean *per user* (2.9 g/day) and 90th percentile (4.1 g/day) are also well below those reported for non-nursing infants 6 – 11 months in GRN 236. This is to be expected as the GOS use level in GRN 236 is higher (5.0 g/L) than the proposed use of GOS in the current GRAS (4 g/L). For the non-nursing toddlers 12 – 23 months, the number of non-nursing toddlers with reported infant formula consumption in the NHANES 2007-2010 is too low to allow for estimating a reliable mean and 90th percentile.

• *Comparison with GRN 286:*

GRN (Submitter)	GOS Use Level in Formula	Population	Per User EDI (g/day)		NHANES Survey Year
			Mean	90 th	
286 (GTC Nutrition)	7.2 g/L	Infants 0 - 6 months	5.9	8.5	2003-2004
		Infants 7 - 12 months	5.2	7.9	
		Toddlers 1 - 2 years	2.8	6.6	
Current GRAS	4.0 g/L	Infants 0 - 6 months	3.5	4.8	2007-2010
		Infants 7 - 12 months	2.8	4.1	
		Toddlers 1 - 2 years	–	–	

– Unweighted number of toddlers consuming infant formula is too small to reliably estimate the mean or 90th percentile of intake

For all infants 0 – 6 months, the mean *per user* (3.5 g/day) and 90th percentile (4.8 g/day) are well below those reported for infants 0 – 6 months in GRN 286. For all infants 7-12 months, the mean *per user* (2.8 g/day) and 90th percentile (4.1 g/day) are also lower than those reported for infants 7 – 12 months in GRN 286. The number of toddlers 1 – 2 years with reported infant formula consumption in the NHANES 2007-2010 is too low to allow for estimating a reliable mean and 90th percentile.

Previous GRNs Baby, Infant and Toddler Foods

The use of GOS in baby, infant and toddler foods has been the subject of three GRAS notices (GRN 334, 285 and 236).

GOS intake estimates from the proposed use of GOS in various baby, toddler and infant foods (maximum use level of 0.38%) were developed for non-nursing infants and toddlers age 6 – 35 months and the following non-nursing sub-populations: 6 – 11 months, 12-23 months, and 24 – 35 months. The results are summarized in Table 4. GOS intake from infant formula uses (see

above) and the total GOS intake from all proposed uses combined (infant formula + baby, toddler and infant foods) were also generated for these non-nursing infant and toddler sub-populations. For the non-nursing infants and toddlers age 6 – 35 months, the total GOS *per user* intake from the combined uses (infant formula + baby, toddler and infant foods) is 2.2 g/day (mean per user) and 4.9 g/day (90th percentile), see Table 4.

Table 4. Per user 2-day average estimated daily intake of GOS from proposed use in baby, infant, and toddler foods among non-nursing infants and toddlers age 6 to 35 months; WWEIA, NHANES 2007-2010

Non-nursing Infant & Toddler Population	Food Category	Total Sample Size ¹	Consumers ¹	% Users	Per User ² (g/day)	
					Mean	90th
6-35 months	Baby and toddler, desserts, dinners, stews, soups and vegetables	1192	241	19	0.5	1.0
	Baby and toddler juice	1192	161	10	0.4	0.8
	Baby and toddler biscuits (cookies, crackers)	1192	162	12	0.0	0.0
	Baby and toddler yogurt drinks	1192	3	1	--	--
	Ready-to-serve pureed fruit mixtures	1192	195	13	0.4	0.9
	Infant cereals	1192	259	18	0.4	0.8
	All proposed baby food uses	1192	416	30	0.8	1.8
	Infant Formula	1192	282	18	2.8	4.1
Combined total (Infant formula + baby food uses)	1192	454	33	2.2	4.9	
6-11 months	Baby and toddler, desserts, dinners, stews, soups and vegetables	290	172	65	0.5	1.1
	Baby and toddler juice	290	117	37	0.4	0.8
	Baby and toddler biscuits (cookies, crackers)	290	104	37	0.0	0.1
	Baby and toddler yogurt drinks	290	2	<0.5	--	--
	Ready-to-serve pureed fruit mixtures	290	159	55	0.4	0.9
	Infant cereals	290	197	70	0.4	0.8
	All proposed baby food uses	290	254	88	1.1	2.2
	Infant Formula	290	254	84	2.9	4.1
Combined total (Infant formula + baby food uses)	290	275	94	3.6	5.2	

000201

Non-nursing Infant & Toddler Population	Food Category	Total Sample Size ¹	Consumers ¹	% Users	Per User ² (g/day)	
					Mean	90th
12-23 months	Baby and toddler, desserts, dinners, stews, soups and vegetables	432	61	15	0.4	0.7
	Baby and toddler juice	432	38	6	0.5	1.0
	Baby and toddler biscuits (cookies, crackers)	432	53	12	0.0	0.1
	Baby and toddler yogurt drinks	432	0	NA	NA	NA
	Ready-to-serve pureed fruit mixtures	432	32	6	0.3	0.4
	Infant cereals	432	58	11	0.3	0.5
	All proposed baby food uses	432	135	29	0.5	1.1
	Infant Formula	432	23	5	--	--
	Combined total (Infant formula + baby food uses)	432	148	32	0.8	2.3
24-35 months	Baby and toddler, desserts, dinners, stews, soups and vegetables	470	8	2	--	--
	Baby and toddler juice	470	6	1	--	--
	Baby and toddler biscuits (cookies, crackers)	470	5	2	--	--
	Baby and toddler yogurt drinks	470	1	1	--	--
	Ready-to-serve pureed fruit mixtures	470	4	0	--	--
	Infant cereals	470	4	1	--	--
	All proposed baby food uses	470	27	8	--	--
	Infant Formula	470	5	1	--	--
	Combined total (Infant formula + baby food uses)	470	31	9	--	--

¹ Unweighted number of infants and toddlers age 6 to 35 months; user estimates based on statistical weights provided by the National Center for Health Statistics (NCHS)

² Assumed GOS use level of 0.38% in baby, infant, and toddler foods

-- Unweighted number of infants or toddlers consuming infant formula is too small to reliably estimate the mean or 90th percentile of intake

For the nursing infants and toddlers age 6 – 35 months, the total un-weighted sample size is 112, with the following breakdown: n=74 for age 6 – 11 months, n=34 for age 12-23 months, and n=4 for age 24 – 35 months. Given the small sample sizes for the nursing infants and toddler sub-populations, reliable intake estimates cannot be derived. As such, only intake estimates for the overall nursing infants and toddlers age 6 – 35 months are generated; the *per user* mean and 90th percentile EDI of GOS from the combined proposed uses in infant formulas

and baby/toddler/infant foods are 1.1 g/day and 3.1 g/day, respectively (data not shown in Table 4). A data summary table was not generated in this report for nursing infants and toddlers age 6 – 35 month or subpopulations due to small sample sizes of the subpopulations.

Previous GRNs

Floraid’s GOS use levels at 0.38% in baby, toddler and infant foods are well below use levels in the previous GRNs. Table 5 provides a side-by-side comparison of Floraid’s GOS use levels in baby, toddler and infant foods and use levels in previous GRNs (236, 285 and 334).

Table 5. Comparison of GOS use levels in baby, toddler and infant foods

Baby, Toddler and Infant Foods	GOS Use Levels (%)			
	Floraid	GRN 236 ¹	GRN 285 ²	GRN 334 ³
Baby and toddler, desserts, dinners, stews, soups and vegetables	0.38	--	0.75 - 1.43	--
Dessert	0.38	2.7	0.78 - 1.43	1.4
Baby and toddler juice	0.38	2.5	0.68	--
Baby and toddler biscuits (cookies, crackers)	0.38	14	12.21	--
Baby and toddler yogurt drinks	0.38	2.4	--	--
Ready-to-serve pureed fruit mixtures	0.38	--	0.78 - 1.43	--
Infant cereals	0.38	--	0.78 - 6.41	0.5

¹GRN 236 (Friesland; Table 1, pg 4); ²GRN 285 (GTC; Table A1, pg76-78); ³GRN 334 (Yakult; Table III-I, Pg 22-23)

The intake assessment conducted in the previous GRNs (236, 285 and 334) were all based on data from NHANES 2003-2004. Friesland (GRN 236) estimated the mean GOS intake by infants 6 – 11 months of age as 6.1 g/day, and toddlers 12 – 23 months of age as 5.3 g/day. GTC (GRN 285) estimated the mean intake for infants and toddlers 0 – 2 year olds as 5.7 g/day and the 90th percentile as 9.8 g/day. Yakult (GRN 334) estimated the mean intake in infants up to 1 year of age as 14.7 g/day and the 90th percentile as 26.8 g/day. The EDIs from the current proposed uses of GOS for children 6 – 35 months are well below estimates reported in previous GRN, see comparative summary in Table 6.

Table 6. EDI of GOS for combined uses in infant formula and baby food – a comparison with previous GRNs

Non-Nursing Infant Pop.	Mean Per User (g/day)				Per User 90 th Percentile (g/day)			
	Floraid	GRN 236 ¹	GRN 285 ²	GRN 334 ³	Floraid	GRN 236 ¹	GRN 285 ²	GRN 334 ³
6-11 months	3.6	6.1	--	7.9	5.2	10.1	--	11.8
12-23 months	0.8	5.3	--	--	2.3	11.2	--	--
6-35 months	2.2	--	5.7 (infants 0-2 yr)	14.7 (infants up to 1yr)	4.9	--	9.7 (infants 0-2 yr)	26.8 (infants up to 1 yr)

¹ GRN 236 Revised Table III-4, pg 189; ² GRN 285 Table IV.C-1, pg 25; ³ GRN 334 Table III-2, pg 25
 --not reported

Previous GRNs US 2+ Food Uses

The use of GOS in foods for persons aged 2+ has been the subject of three GRAS notices (GRN 334, 285 and 236).

GOS intake estimates from the proposed use of GOS in the dairy products and beverages in the US 2+ population are summarized in Table 7. The *per user* EDI from the proposed GOS use in dairy products are 1.90 g/day (mean) and 3.65 g/day (90th percentile) and in beverages are 6.88 g/day (mean) and 14.21 g/day (90th percentile). The total *per user* EDI from the combined uses of GOS in dairy products and beverages are 7.17 g/day (mean) and 14.40 g/day (90th percentile), see Table 7.

Table 7. Per user 2-day average estimated daily intake of GOS from proposed uses in dairy and beverages products by the US population 2 year and older; WWEIA, NHANES 2007-2010

Food Category	Total Sample ¹	Consumers ¹	% Users	Per User ² (g/day)	
				Mean	90th
Dairy products	15,032	3,866	28	1.90	3.65
Yoghurt and yoghurt drinks ³	15,032	1,880	13	1.59	2.89
Ice milk, frozen yoghurt, custards, pudding	15,032	1,577	12	1.45	2.77
Dairy shake mixes, instant breakfasts, meal replacements	15,032	910	7	2.25	4.30
Beverages	15,032	13,381	89	6.88	14.21
Carbonated and non-carbonated beverages, juice-based beverages, juice coolers, sweetened and/or flavoured water	15,032	12,814	85	6.07	12.83
RTD ice tea, ice coffee (flavoured and/or sweetened)	15,032	2,622	20	3.65	7.29
Sport and isotonic drinks, RTD and reconstituted powder	15,032	976	6	4.19	8.00
Dairy and beverage total	15,032	13,854	93	7.17	14.40

¹ Unweighted number of respondents; user estimates based on statistical weights provided by the National Center for Health Statistics (NCHS)

² Assumed GOS use level ranges from 1 – 2% in dairy and beverage products

³ Yoghurt drinks are included in the yoghurt group since NHANES additional description of food code 11422000 (Yogurt, vanilla, lemon, maple, or coffee flavor, lowfat milk) is inclusive of liquid yogurt, LeShake, Tuscan, Go-Gurt Portable Yogurt tube, and Yoplait Express Yogurt; GOS use level for this food code is assumed to be same as use level in yogurt, which is higher than proposed use in yoghurt drinks, see Table 1.

Previous GRNs

Table 8 provides a side-by-side comparison of Floraid's GOS use levels in foods and use levels in previous GRNs (236, 285 and 334). The proposed GOS uses in foods in the current assessment are different from the uses in previous GRNs. GRN 236 and 334 both have higher use levels than those in the current assessment and GRN 285 generally has lower use levels but broader

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food uses than the current assessment. The proposed food uses and use levels in GRN 334 are the broadest and at the highest use levels.

Table 8. Comparison of GOS use levels in foods

Food Categories	Use level (%)			
	Floraid	GRN 236 ¹	GRN 285 ²	GRN 334 ³
Dairy products +	1.0-2.0	3.3 - 4.3	0.48 - 0.57	0.61 - 3.8
Yogurt	1.5	3.3	0.57	3.3
Yogurt drinks	1.0	--	--	--
Frozen dairy	2.0	4.3	--	2.0
Dairy shake mixes, instant breakfast, meal replacements	1.05	2.0	0.48	--
Non-alcoholic beverages +	1.06	--	--	--
Beverage concentrate	1.06	--	--	2.0
Fruit drinks +	1.06	--	--	2.0
Vitamin/mineral fort. fruit drinks	1.06	2.1	--	--
Non fruit incl energy drinks +	1.06	1.3 - 2.1	0.53	4.4
Fitness water and thirst quenchers	1.06	1.3	0.53	2.0
Energy drinks	1.06	2.1	0.53	4.4
Fruit and veg juices	1.06	--	--	1.6
Nut beverages	--	--	--	1.6
RTD Non-milk based meal replacements and protein beverages	--	--	0.48	--
RTD Soy beverages	--	--	0.53	--
Soups	--	--	0.52	0.61
Bakery products +	--	--	1.02-4.27	1.0
Bars	--	12.5	--	1.0
Cereals, RTE	--	--	4.27	2.0
Sugars and sweets (jellies, jam, etc.)	--	25		25

+ Additional sub-categories of proposed foods not shown and may vary across GRN

¹GRN 236 (Freisland; Table 1, pg 4); ²GRN 285 (GTC; Table A1, pg76-78); ³GRN 334 (Yakult; Table III-I, Pg 22-23)

Overall, in the current assessment, the EDIs for GOS for the US 2+ years (mean per user 7.17 g/day and 90th per user 14.40 g/day) are well below those provided in previous GRNs:

000206

- **GRN 000236** – Based on food intake data from NHANES 2003-2004, Friesland estimated the mean intake of their GOS ingredient as 8.0 g/day and 16.8 g/day at the 90th percentile for eaters 2+ years.
- **GRN 000285** – Based on food intake data from NHANES 2003-2004, GTC estimated the mean intake as 9.3 g/day the 90th percentile as 15.4 g/day.
- **GRN 000334** – Based on food intake data from NHANES 2003-2004, Yakult estimated the mean intake for the total population as 12.2 g/day and the 90th percentile as 25.3 g/day.

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Expert Opinion Letter

Evaluation of the Generally Recognized as Safe (GRAS) Status of Floraid™ GOS (Galacto-oligosaccharide)

I, the undersigned, a recognized expert, qualified by scientific training and relevant experience to evaluate the safety of food and food ingredients, was requested by International Dairy Ingredients Inc. (IDII) to assess the safe consumption of Floraid™ GOS (Galacto-oligosaccharide) as a food ingredient as per the Intended Uses detailed in the Floraid GOS GRAS Notification to the FDA, dated September 16, 2013.

IDII has established by scientific procedures that the consumption of Floraid GOS as defined in the Floraid GOS GRAS Notification is GRAS as a food ingredient for incorporation into select processed foods, at defined concentrations, for which no standard of identity has been determined and/or legislated.

The following summary provides the determination of safety and GRAS status:

- Floraid GOS is manufactured as per procedures published in the scientific literature;
- Certificate of Analyses have been provided which demonstrate a consistent nutrient profile and microbial-free product;
- Validation and acknowledgement that Floraid GOS has a DP of 3.2 and thus, results in human physiological effects characteristic of dietary fibre;
- Human clinical trials conducted on infants and older persons - the results of which have been published in the scientific literature - which demonstrate the safety of GOS, sometimes at levels higher than those proposed in this GRAS Notification;
- Incorporation of Floraid GOS into select foods at a concentration within the range of safety as determined by experts under the employ of federal governments in other jurisdictions;
- A robust Estimated Daily Intake, based on proposed levels of use in select foods, which result in mean and 90th percentile intake below that of previously reviewed and approved GOS GRAS Notifications;
- Extensive safety trials with GOS that supports its intended use described in this notification
- Acceptance in scientific literature that GOS is a dietary fiber
- Dietary fiber is a short fall nutrient and there is a need to add fiber throughout the lifecycle

It is my professional opinion that Floraid GOS is Generally Recognized as Safe (GRAS) and will be a useful food supplement in infant formula, infant, baby and toddler foods, and for persons age 2 and above.

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Dr. Joanne Slavin

Professor, University of Minnesota, Department of Food Science and Nutrition,

Date:

9-16-13

Contact information

Joanne Slavin, PhD, RD

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1334 Eckles Avenue

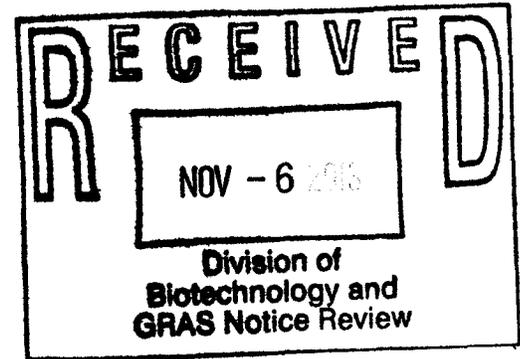
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Office of Food Additive Safety (HFS-200),
Center for Food Safety and Applied Nutrition,
Food and Drug Administration,
5100 Paint Branch Pkwy,
College Park, MD 20740,
USA

October 30, 2013.

To whom it may concern,

The information below is submitted in response to two questions posed by officials of the FDA's Office of Food Additive Safety regarding the Floraid GOS GRAS Notification submitted to the FDA, dated September 16, 2013.

1. Do and will any of the foods intended to incorporate Floraid GOS come under the jurisdiction of the USDA?

No. None of the GOS-supplemented food applications for persons aged 2+ (Table 5 in the Notification) include any foods under the jurisdiction of the USDA. As for the GOS-supplemented food applications intended for persons under age 2 (Table 4 in the Notification), none of these foods, including the possible USDA-pertinent applications of baby and toddler dinners, stews and soups, fall under the jurisdiction of the USDA. As for the determination of when a food falls under the jurisdiction of the USDA or the FDA, I defer to the USDA document¹ described in the footnote below, which provides, as a general rule of thumb, that foods containing less than 2% cooked meat or poultry are, as per exemption by the Secretary of Agriculture, regulated by the FDA, and not by the USDA. Further, the advisory stated in the aforementioned document is noted:

"...industry is strongly advised to seek clarification from FSIS in cases where the status of jurisdiction is in question."

Accordingly, should an intention arise to market a GOS-supplemented food which could possibly come under the jurisdiction of the USDA, the petitioner will notify the FDA in advance and request direction prior to commercialization of the potential USDA-applicable food.

¹USDA. August 2007. A GUIDE TO FEDERAL FOOD LABELING REQUIREMENTS FOR MEAT, POULTRY, AND EGG PRODUCTS. Page 9. http://www.fsis.usda.gov/wps/wcm/connect/f4af7c74-2b9f-4484-bb16-fd8f9820012d/Labeling_Requirements_Guide.pdf?MOD=AJPERES

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2. The exemption claim is void of a physical address at which the FDA may review and copy pertinent information. This is required in addition to a statement that additional information will be sent to the FDA upon request.

Any and all information pertinent to the Floraid GOS GRAS Notification that the FDA wishes to review and copy for their perusal is available at this address:

International Dairy Ingredients Inc.
c/o Mr. Doug McEwen, President,
26-1525 Cornwall Road,
Oakville, ON L6J 0B2 Canada
(1) 905-338-3600

Further, copies of any additional pertinent information the FDA wishes to receive and review will be promptly supplied, upon request.

I trust this addresses your concerns. Please do not hesitate to contact the undersigned with any further requests.

Sincerely,

Signed

(b) (6)



Carol T. Culhane, PHEc, MBA,
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SUBMISSION END

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