GRAS Notice (GRN) No. 576
http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm

ORIGINAL SUBMISSION

Spherix

March 30, 2015

Office of Food Additive Safety
HFS-255
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

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FOOD ADDITIVE SAFETY

To Whom It May Concern:

Enclosed please find three copies of the dossier entitled "Generally Recognized As Safe (GRAS) Determination of a Combination of Oligofructose and Long-Chain Inulin in Exempt Term Infant Formulas" and the GRAS Expert Panel Consensus Statement. We have prepared these documents for Nutricia North America, Inc.

The data and information that serve as the basis for this GRAS determination is available for review and copying at reasonable times at the office of Claire L. Kruger, Ph.D., D.A.B.T., President, Spherix Consulting, Inc., 11900 Parklawn Drive, Suite 200, Rockville, MD 20852, Telephone: 301-897-0611; Facsimile: 301-897-2567; Email: clairek@chromadex.com, or will be sent to FDA upon request.

Should you have any questions or concerns, please contact me at the number listed above.

Sincerely,

(b) (6)

Claire L, Kruger, Ph.D., D.A.B.T.
President

Enclosures:

Three copies of the dossier entitled "Generally Recognized As Safe (GRAS) Determination of a Combination of Oligofructose and Long-Chain Inulin in Exempt Term Infant Formulas"

Three copies of the GRAS Panel Consensus Statement for the above-referenced GRAS Notification

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Rockville, MD 20852
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Generally Recognized As Safe Determination of a Combination of Oligofructose and Long-Chain Inulin in Exempt Term Infant Formulas

Prepared for:

Nutricia North America, Inc.
9900 Belward Campus Drive, Suite 100
Rockville, Maryland 20850
USA

Clinical trials were conducted by Nutricia Research BV, a corporate affiliate of Nutricia North America, Inc., both under joint control of Danone.

Prepared by:

Spherix Consulting, Inc.
11900 Parklawn Drive, Suite 200
Rockville, MD 20852
USA

January 29, 2015

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I. GRAS EXEMPTION CLAIM

A. NAME AND ADDRESS OF SPONSOR

Nutricia North America, Inc.
9900 Belward Campus Drive, Suite 100
Rockville, Maryland 20850
USA

B. COMMON OR USUAL NAME OF GRAS SUBSTANCE

The subject of this Generally Recognized As Safe (GRAS) determination is a combination of 9 parts oligofructose and 1 part long-chain inulin (lc-inulin), otherwise known as oligofructose/lc-inulin.

C. INTENDED USE

Nutricia North America, Inc. intends to add a combination of oligofructose/lc-inulin, in a 9:1 ratio of oligofructose:lc-inulin as a prebiotic in exempt powdered amino acid-based term infant formulas used for the dietary management of food allergies. The amount of the oligofructose/lc-inulin added will not exceed 6.8 g/L infant formula as consumed.

D. BASIS FOR GRAS DETERMINATION

This GRAS determination for the use of the oligofructose/lc-inulin in a powdered amino acid-based exempt term infant formula used for the dietary management of food allergies has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), as described under 21 CFR §170.30(b). To demonstrate that the oligofructose/lc-inulin is safe, and GRAS, under the intended conditions of use, the safety of the intake of oligofructose/lc-inulin has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to exempt infant formulas, and is based on generally available and accepted information.

The proposed use of oligofructose/lc-inulin as an ingredient in a powdered amino acid-based exempt term infant formula used for the dietary management of food allergies has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. Oligofructose (Orafti®P95) and lc-inulin (Orafti®HP) are oligomers and polymers of fructose, otherwise known as fructans, which are derived from chicory (*Cichorium intybus L.*) root inulin.

2. Oligofructose and lc-inulin are prebiotics, which, by definition, are selectively fermented food ingredients, that have a negligible systemic effects and that allow specific changes in the composition of and/or activity in the gastrointestinal microbiota, thus conferring benefits upon host health.

3. Orafti®P95, sold under the trade names Raftilose®, Raftilose®P95, and Beneo®P95, contains predominantly linear oligosaccharides three to six saccharides in length. Approximately 5% of the fructans in Orafti®P95 have a degree of polymerization (DP) more than six and 4% of the molecules have DP of two.

4. Orafti®HP, sold under the trade names, Raftiline®HP and Beneo®HP, contains primarily linear oligosaccharides and polysaccharides with degrees of polymerization (DP) ranging from 10 or 60. The average DP is greater than or equal to 23 and less than 1% of the fructans have chains being shorter than five.

5. Product specifications are met consistently ensuring a food-grade product and stability has been shown over its guaranteed shelf life of three years assuring that the oligofructose and lc-inulin are suitable for use in exempt term infant formulas.

6. Orafti®P95 and Orafti®HP reproducibly meet compositional standards and comply with limits on contaminants appropriate for food-grade ingredients.

7. All processes and processing aids used in the production have been the subject of the GRAS notifications GRN 392 (on Orafti®P95) and GRN 477 (on Orafti®HP) to which **FDA provided a "No Questions" response.** Moreover, the manufacturer represents that all processing aids either comply with the Food Chemicals Codex monograph, comply with the uses and limits stipulated in Chapter 21 of the United States Code of Federal Regulations, or are GRAS for their intended use.

8. The manufacturer also represents that all other food contact materials, such as plastics and packaging materials, comply with Regulation (EC) No 1935/2004, (EU) No 10/2011, 21 CFR 176.170 or 176.180 or equivalents thereof. Both Orafti®P95 and Orafti®HP have previously received a no questions letter from FDA (GRN 392; GRN 477).

9. Mature human milk contains 5.0 to 8.0 g oligosaccharides/L and although human milk oligosaccharides are unique in size, charge, composition and abundance, someoligosaccharides derived from other sources, have been shown to have similar effects as those in human milk.

10. Assuming that the infant formulas are the sole source of nutrition, the addition of 6.12 g oligofructose and 0.68 g lc-inulin/L to amino acid-based exempt term infant formulas as consumed will result in oligofructose intakes of 4.3 to 5.9 g/day and lc-inulin intakes of 0.5 to 0.7 g/day for one month-old and six month-old infants.

11. The combination of 6.12 g oligofructose and 0.68 g lc-inulin/L infant formula as consumed replicates the molecular size distribution of human milk oligosaccharides and maintains the osmolality of the formula in a range consistent with that recommended by the American Academy of Pediatrics.

12. Oligofructose, lc-inulin, and other prebiotics such as GOS have a long history of safe use.

    a. Oligofructose (Orafti®P95) was determined GRAS in 2011 for its use in term infant formula at a maximum level of 3 g/L formula as consumed and **subsequently received a "no questions" letter from the FDA in 2012 (GRN** 392).

    b. Lc-inulin (Orafti®HP) was determined GRAS in 2013 for its use in exempt and non-exempt term infant formulas at a level of 0.8 g/L formula as consumed (GRN477) **and received a "no questions" answer from FDA** in 2014.

    c. Infant formulas containing 8 g/L oligosaccharides, comprising a combination of 90% GOS (7.2 g/L) and 10% lc-inulin (0.8 g/L) have been marketed in Europe since 2000 with no reported adverse effects.

    d. Numerous clinical trials of oligofructose in infant formula at levels up to 4 g/L, given alone or in combination with other prebiotics, including lc-inulin documented that no increase in test-article-related adverse effects including anthropometric measures, clinical chemistries, serious adverse events or non-serious adverse symptoms such as flatulence or fussiness, or hydration status are produced from oligofructose ingestion.

13. Two clinical studies administering an amino acid-based exempt term infant formula containing 6.12 g/L oligofructose and 0.68 g lc-inulin/L as consumed to healthy and **cow's milk allergic infants showed that the formula was well tolerated. The test** formula used in these studies also contained 1.2 g/L acidic oligosaccharides plus *Bifidobacterium breve* M-16V (1.47×10^{10} colony forming units/L).

    a. When administered to healthy term infants for 16 wk, the addition of 8 g/L prebiotic (including the 6.8 g/L oligofructose/lc-inulin (9:1) and 1.2 g/L acidic oligosaccharide), as consumed, to a test infant formula supported growth similarly as did the control formula alone. For infants consuming the test formula, approximately ¼ of all stools assessed were rated as watery stools throughout the course of the study, but this did not appear to contribute to serious adverse events, or an increased dropout rate. In addition, there was no statistically significant difference in laboratory blood parameters such as sodium, potassium and chloride between study groups. Similar rates of watery or soft stools have been reported at similar time points in other studies administering oligosaccharides at comparable levels such as GOS/lc-inulin (9:1) at 8 g/L (van der Aa et al., 2010) and 8 g GOS/lc-inulin/L (Moro et al., 2006a). Adverse event rates (mild, moderate and severe) in the test and control formula groups were 63 and 88%, respectively; adverse events were mainly gastrointestinal-related disorders and infections. For both categories, adverse event rates were lower in the test formula group versus control. There was higher incidence of constipation in the control formula group (27%) versus test formula group (5%). Four severe adverse events were reported in the test group (3% of total adverse events in this group); four severe adverse events were also reported in the control group (2% of total adverse events in this group). A total of seven serious adverse events occurred in the study (two events in the test formula group and five events in the control formula group). There were no safety signals observed in the oligofructose/inulin formula group, versus control.

    b. When consumed by cow's **milk allergic infants and children, the formula** containing 8 g/L prebiotic (including the 6.8 g/L oligofructose/lc-inulin (9:1) combination and 1.2 g/L acidic oligosaccharide) was determined to be hypoallergenic as demonstrated by negative results in a double-blind, placebo-controlled food challenge (DBPCFC) plus a subsequent open challenge of seven d. There were no serious adverse events.

c. **When administered to cow's milk allergic** infants and children, both the control formula and test formula containing 8 g/L prebiotic (including the 6.8 g/L oligofructose/lc-inulin (9:1) combination and 1.2 g/L acidic oligosaccharide) significantly reduced atopic dermatitis severity, as well as other dermatological and respiratory symptoms related to atopy. In particular, redness/soreness was significantly reduced at 16 wk in a stool and gastrointestinal analysis subgroup. Fourteen subjects experienced events categorised as diarrhea in this study; 12 were in the test group and two were in the control group (p=0.004). Of the 12 subjects in the test group experiencing diarrhea, four experienced diarrhea prior to study formula ingestion and one treated with amoxicillin; therefore, the diarrhea in these subjects was likely not related to the test formula. Of the remaining seven subjects in the test group who experienced diarrhea, only one exhibited diarrhea immediately upon consumption of the test formula; the remaining six subjects had diarrhea occurring well after the date of the first study product intake. These cases indicate the diarrhea was unlikely to be related to the test product. None of cases required further action. Stool frequency, stool color, stool consistency and flatulence occurrence did not differ between groups at 16 wk, suggesting good tolerance. Overall adverse event rates (mild, moderate and severe) were not statistically significant different between groups; the adverse event rate was 79.6% and 67.9% in the test and control formula groups, respectively; the most frequent types of adverse events were GI system disorders and respiratory system disorders. Severe and moderate adverse event rates were not statistically different across the two groups. There was a statistically significant (p=0.01) difference between groups at 16 wk (change from baseline) for hemoglobin, with a mean value in the test group of 118 g/L and a mean value in the control group of 123 g/L. Mean levels of alkaline phosphatase were significantly increased (p=0.018) in the test group (292 U/L) versus the control group (246 U/L).As both of these results were within the normal reference ranges, these differences were not deemed clinically relevant. Although there was a decrease in the number of allergenic events reported and number of subjects reporting allergenic events during the trial, the decrease was apparent in both study groups.

Determination of the GRAS status of oligofructose/lc-inulin under the intended conditions of use has been made through the deliberations of Roger Clemens, Dr PH, CNS, FACN, FIFT, David Jenkins, MD, DSc, PhD, and Judith Jones, MD, PhD, FISPE. These individuals are qualified by scientific training and experience to evaluate the safety of infant formulas. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of oligofructose/lc-inulin and the human exposure to oligofructose/lc-inulin resulting from its intended use as an ingredient in a powdered amino acid-based exempt term infant formula used for the dietary management of food allergies and have concluded:

> *There is no evidence in the available information on oligofructose/lc-inulin that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when oligofructose/lc-inulin is used at levels that might reasonably be expected from the proposed applications. Oligofructose/lc-inulin is GRAS for use in a powdered amino acid-based exempt term infant formula used for the dietary management of food allergies as proposed by Nutricia North America, Inc.*

Therefore, oligofructose/lc-inulin is safe and GRAS at the proposed levels of addition to a powdered amino acid-based exempt term infant formula. Oligofructose/lc-inulin is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

E. AVAILABILITY OF INFORMATION

The data and information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Claire L. Kruger, Ph.D., D.A.B.T., President, Spherix Consulting, Inc., 11900 Parklawn Drive, Suite 200, Rockville, MD 20852, Telephone: 301-897-0611; Facsimile: 301-897-2567; Email: clairek@chromadex.com or be sent to FDA upon request.

F. SIGNATURE

Pursuant to the criteria provided in proposed 21 CFR § 170.36, Nutricia North America, Inc. hereby notifies the Food and Drug Administration that the use of the oligofructose/lc-inulin mix in foods under the intended conditions of use is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act, because Nutricia North America, Inc. has determined that such use is Generally Recognized As Safe through scientific procedures.

(b) (6)

Signature: Mr Stephen Peth
Vice President and Treasurer
Authorized Representative of Nutricia North America, Inc.

3/6/2015
Date: March 6th, 2015

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II. DESCRIPTION OF SUBSTANCE

A. PHYSICAL AND CHEMICAL PROPERTIES

The subject of this GRAS determination is a combination of 9 parts oligofructose (Chemical Abstract Service registry number (CAS#) 187112-48-7; chemical formula: $C_6H_{11}O_5(C_6H_{10}O_5)_nOH$) and 1 part long-chain inulin (lc-inulin; the CAS# for inulin is 9005-80-5; lc-inulin does not have a distinct CAS#; chemical formula: $C_6H_{11}O_5(C_6H_{10}O_5)_nOH$). Both oligofructose and lc-inulin are fructans, or polymers of fructose molecules, that may or may not have a terminal glucose molecule (Figure 1). The systematic name for all fructans is α-D-glucopyranoside-(1-2)-β-D-fructofuranosyl-[(1-2)-β-D-fructofuranosyl]$_n$, however α-D-glucopyranoside-(1-2) is not always present.

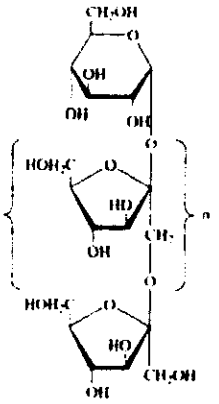

Figure 1. Chemical Structure of a Fructan with a Terminal Glucose Molecule

The bracketed fructose molecule indicates the presence of repetitive fructose molecules.
"n" indicated the number of repetitive fructose molecules.

1. Oligofructose

The oligofructose that is subject of this GRAS determination contains predominantly linear oligosaccharides, ranging 3 to 6 saccharides in length, and is sold by BENEO-Orafti (Beneo) under the trade name Orafti®P95. Orafti®P95 has been determined GRAS for use in infant formulas and received a "no questions" letter from the United States Food and Drug Administration (FDA) (GRN 392). Orafti®P95 has also been sold under the trade names Raftilose®, Raftilose®P95, and Beneo®P95.

Orafti®P95 is a colorless powder that has a slightly sweet taste, pH (30-50 Brix) of 5.0 to 7.0, and conductivity (28 Brix) of <250 μS/cm. It is soluble in water at >750 g/L. Approximately 5% of the fructans in Orafti®P95 have a degree of polymerization (DP) of more than 6, 4% have a DP of 2, less than 50% have a glucose endcap, and less than 2% are branched molecules.

2. Long-chain Inulin

The lc-inulin that is also subject of this GRAS determination contains primarily linear oligosaccharides and polysaccharides with DPs, ranging from 10 to 60 or more saccharides in length, and is also sold by Beneo under the trade name Orafti®HP. Orafti®HP has been determined GRAS for use in term infant formulas, toddler formulas, and medical foods and received a "no questions" letter from FDA (GRN 477). Orafti®HP has also been sold under the trade names, Raftiline®HP and Beneo®HP.

Orafti®HP is a colorless powder that has a neutral taste, pH (30-50 Brix) of 5.0 to 7.0, and conductivity (28 Brix) of <250 μS/cm. It is soluble in water at <5 g/L. The average DP of Orafti®HP is greater than or equal to 23, less than 1% of the fructans have chain-lengths shorter than 5, and less than 2% are branched molecules.

B. PRODUCTION PROCESS

1. Starting Materials

Orafti®P95 oligofructose and Orafti®HP lc-inulin are prepared from raw inulin extracted from chicory (*Cichorium intybus L.*) root, which are grown by individually sub-contracted farmers who receive free seeds from Beneo and grow only approved chicory varieties using approved agronomic practices and permitted pesticides.

2. Production of Orafti®P95 Oligofructose

The production of Orafti®P95 oligofructose takes place in three phases, the extraction of raw inulin, enzymatic hydrolysis of inulin, and refining of the raw oligofructose (Figure 2). Raw inulin is extracted by incubating washed and sliced chicory roots in hot water, removing the pulp, precipitating the non-inulin substances (i.e., minerals, some sugars and cell particles) with calcium hydroxide, filtering the raw juice/calcium hydroxide precipitate mix, and evaporating the filtrate. The raw inulin is then either crystallized or passed over a column to remove residual salts and sugars, and the resulting intermediates are treated with food-grade carbohydrase, which is derived from *Aspergillus niger*. The product, otherwise known as raw oligofructose or raw L95 is then refined in a series of evaporation, ultra-high temperature, filtering, and drying steps to reduce microbial load, reduce moisture content, and produce the finished product.

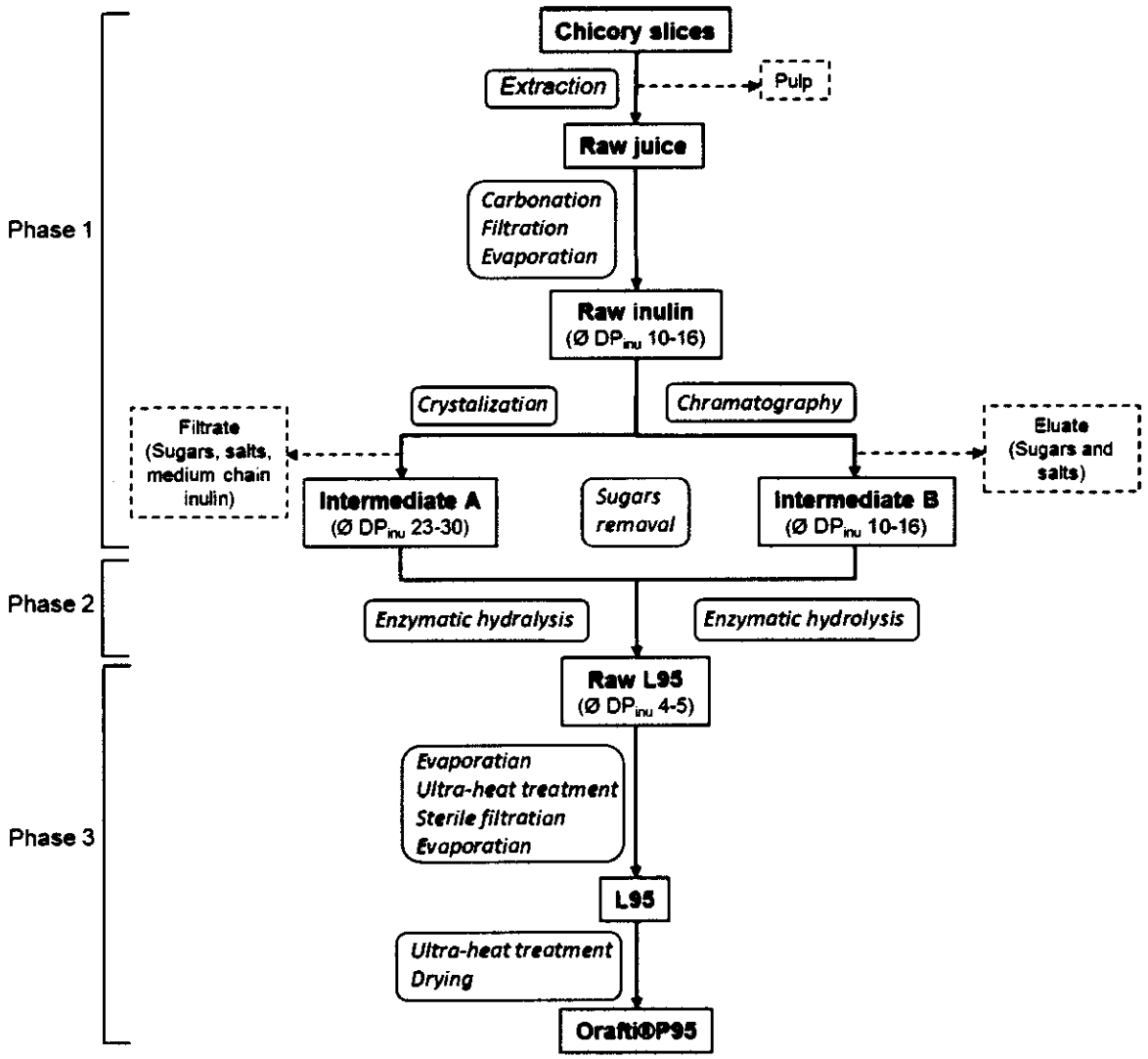

Figure 2. Production Process of Orafti®P95 Oligofructose
Phase 1: extraction of raw from Chicory slices; Phase 2: hydrolysis of raw inulin with carbohydrase; Phase 3: refining of raw L95.

3. Production of Orafti®HP Long-chain-inulin (lc-inulin)

The production of Orafti®HP also takes place in three phases (Figure 3). Raw inulin is extracted in a manner similar to that of Orafti®P95, however, the raw inulin is crystalized by cooling, which precipitates the less soluble long-chain fructans. The raw inulin/precipitate mix is filtered, and the precipitate, otherwise known as raw lc-inulin or raw HP, is collected for refining. The raw HP is refined by dissolving the precipitate in water, heating the solution at ultra-high temperatures, filtering, and evaporating. In some cases, the filtrate is also incubated with activated charcoal to remove any residual color.

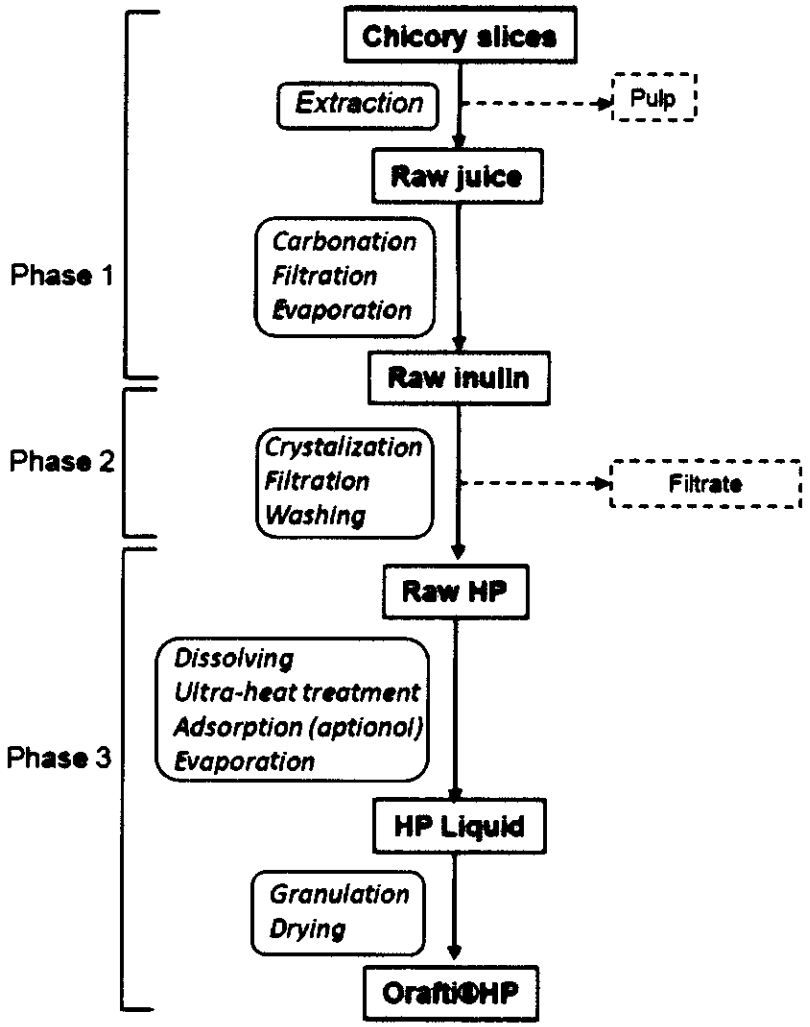

Figure 3. Production Process of Orafti®HP Long-chain Inulin
Phase 1: extraction of raw inulin from Chicory slices; Phase 2: removal of short chain fructooligosaccharide; Phase 3: refining of Raw HP.

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4. Compliance

All production sites of Orafti®P95 oligofructose and Orafti®HP lc-inulin are audited regularly and comply with International Organization for Standardization (ISO) 9001:2008, the International Featured Standard (IFS) Food, and a Hazard Analysis and Critical Point (HACCP) system. The carbohydrase used to hydrolyze the intermediates in the Orafti®P95 is derived from *Aspergillus niger* and is recognized by the Food and Drug Administration as GRAS in opinion letters (GRASP 3G0016; GRN 89). All processes used and processing aids, have been the subjects of GRAS notifications GRN 392 (on Orafti®P95) and GRN 477 (on Orafti®HP) to which FDA provided a "No Questions" response. Moreover, the manufacturer represents that all processing aids either comply with the Food Chemicals Codex monograph, comply with the uses and limits stipulated in Chapter 21 of the United States Code of Federal Regulations, or are GRAS for their intended use. The manufacturer also represents that all other food contact materials, such as plastics and packaging materials, comply with Regulation (EC) No 1935/2004, (EU) No 10/2011, 21 CFR 176.170 or 176.180 or equivalents thereof. Both Orafti®P95 and Orafti®HP, the subjects of this GRAS Notice, have previously received a no questions letter from FDA (GRN 392, 477, respectively).

C. FINISHED PRODUCT SPECIFICATIONS

1. Product Specifications and Batch Records for Orafti®P95

As cited in GRN 392, each batch of Orafti®P95 is evaluated by Beneo against specifications described in Table 1, 2 and 3 to ensure a consistent food-grade product. Data from 10 batches are also included in Tables 1, 2, and 3 to demonstrate control of the production process and compliance.

Table 1. Chemical Product Specifications and Batch Data for Orafti®P95 Oligofructose

Parameter	Method	Spec.	Batch Number (Production Date)									
			(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]
Dry matter	gravimetric	**≥ 95%**	93.9	94.3	94.6	95.6	96.2	96.2	95.5	96.0	96.8	96.5
Oligofructose **(DP ≥ 2 and ≤9)**	AOAC 997.08	**≥ 93.2%** dry matter	95.4	96.2	95.8	94.0	94.6	95.7	93.8	96.3	94.6	93.9
Glucose, fructose, and sucrose	AOAC 997.08	**≤ 6.8%** dry matter	4.6	3.8	4.2	6.0	5.4	4.3	6.1	3.7	5.5	6.1
Sulfated ash	ICUMSA GS3/4/7/8-11	< 0.2% dry matter	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Conductivity	ICUMSA GS2/3-17	< 250 µS/cm at 28 Brix)	7	5	5	33	21	9	27	4	15	17
Heavy Metals												
Lead	ICP- MS	**≤ 0.02** mg/kg	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
Arsenic	ICP- MS	**≤ 0.03** mg/kg	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03

Abbreviations:
Spec = Specification
AOAC = Association of Official Analytical Chemists
cfu = colony-forming units
ICUMSA = International Commission for Uniform Methods of Sugar Analysis
ICP-MS = inductively coupled plasma mass spectrometry
ISO = International Organization for Standardization
Neg = Negative

000021

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Table 2. Microbiological Product Specifications and Batch Data for Orafti®P95 Oligofructose

Parameter	Method	Spec.	Batch Number (Production Date)									
			(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]
Total mesophilic bacteria	ICUMSA GS2/3-41	≤ 1000 cfu/g	UD	UD	9	2	UD	UD	UD	UD	UD	1
Yeast	ICUMSA GS2/3-47	≤ 20 cfu/g	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
Mold	ICUMSA GS2/3-47	≤ 20 cfu/g	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
Enterobacteriaceae	ISO 21528-2	Neg./1 g	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Staphylococcus aureus	ISO 6888	Neg./0.1 g	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Salmonella	AOAC 2000.07	Neg./250 g	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

Abbreviations:
Spec = Specification
AOAC = Association of Official Analytical Chemists
cfu = colony-forming units
ICUMSA = International Commission for Uniform Methods of Sugar Analysis
ICP-MS = inductively coupled plasma mass spectrometry
ISO = International Organization for Standardization
UD = Undetectable
Neg = Negative

Table 3. *Cronobacter sakazakii* Product Specifications and Batch Data for Orafti®P95 Oligofructose Intended for Infant Formula Use

Parameter	Method	Spec.	Batch Number (Production Date)			
			(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]
Cronobacter sakazakii	ISO/TS 229640	Neg/300 g	Neg	Neg	Neg	Neg

Abbreviations:
Spec = Specification
ISO = International Organization for Standardization
UD = Undetectable
Neg = Negative

2. Product Specifications and Batch Records for Orafti®HP

Each batch of Orafti®HP is evaluated by Beneo against specifications described in Table 4 and 5 to ensure a consistent food-grade product. Data from 10 batches are included in Table 4 and 5 to demonstrate control of the production process and compliance.

3. Other Quality Control Parameters

Beneo also regularly analyzes raw inulin, Orafti®P95, and Orafti®HP for cadmium and mercury; sulfur dioxide, nitrate, and nitrite; polychlorinated biphenyls (PCB) and dioxin-like compounds (DLC); the mycotoxins aflatoxin B1, ochratoxin A, zearalenone, and desoxynivalenol; and ~450 pesticides for which maximum residue levels are set in EU Commission Regulation (EC) 149/2008. These analyses have found cadmium and mercury levels are < 0.01 mg/kg; sulfur dioxide < 5 mg/kg, nitrate < 50 mg/kg, nitrite < 0.5 mg/kg; aflatoxin B1 < 1 μg/kg (limit of detection (LOD)), ochratoxin A < 0.5 μg/kg (LOD), zearalenone < 10 μg/kg (LOD), desoxynivalenol < 0.1 μg/kg (LOD); and PCBs, DLCs, and pesticide levels are below the limits of detection. Orafti®P95, and Orafti®HP are also routinely monitored to verify that the products to do not contain unacceptable levels *Escherichia coli*, *Bacillus cereus*, and Clostridia. Lastly, magnesium, fluoride, and selenium levels in Orafti®P95, and Orafti®HP will monitored on a regular basis and not to exceed 1 ppm, 2 ppm, and 0.1 mg/kg, respectively

4. Phthalates

To verify that Orafti®HP lc-inulin does not contain unacceptable levels of phthalates, six batches were analyzed for dimethyphthalate, diethylphthalate, diisobutylphthalate, bis-(2-ethylhexyl) phthalate, benzylbutylphthalate, di-n-octylphthalate, di-isononylphthalate, and di-isodecylphthalate by gas chromatography-mass spectrometry. All batches contained less than 0.2 mg/kg, which was the limit of quantification for the assay.

D. STABILITY, OSMOLARITY, AND OSMOLALITY

1. Stability of Orafti®P95 Oligofructose

As cited in GRN 392, the thermal stability of Orafti®P95 oligofructose was tested under conditions simulating pasteurization, ultra-high temperature processing, and sterilization. Samples of Orafti®P95 were dissolved in water to form solutions with pH = 6.0. These samples were then exposed to temperatures of 95°C for 5 minutes, 140°C for 10 minutes, 115°C for 11 minutes, or 85°C for 15 minutes. No observable degradation occurred under any of these conditions.

Table 4. Product Specifications and Batch Data for Orafti®HP lc-inulin

Parameter	Method	Spec.	Batch Number (Production Date)									
			(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]
Inulin	AOAC 997.08	≥ 99.5% dry matter	99.8	99.9	99.9	99.8	99.9	99.9	99.7	99.8	99.9	99.7
Inulin (DP ≥ 5)	AOAC 997.08	≥ 99% dry matter	99.3	99.9	99.8	99.4	99.8	99.8	99.2	99.4	99.6	99.3
Average DP	AOAC 997.08	≥ 23	30	31	32	27	33	34	34	35	30	27
Glucose, fructose, and sucrose	AOAC 997.08	≤ 0.5% dry matter	0.2	0.1	0.1	0.2	0.1	0.1	0.3	0.2	0.1	0.3
Sulfated ash	ICUMSA GS3/4/7/8-11	< 0.2% dry matter	0.18	0.05	0.07	0.14	0.13	0.11	0.05	0.10	0.07	0.12
Conductivity	ICUMSA GS2/3-17	< 250 µS/cm at 28 Brix	182	54	77	242	133	137	84	150	136	156
Heavy Metals												
Lead	ICP-MS	≤ 0.02 mg/kg	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Arsenic	ICP-MS	≤ 0.03 mg/kg	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03
Microbiological Specifications												
Total mesophilic bacteria	ICUMSA GS2/3-41	≤ 1000 cfu/g	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
Yeast	ICUMSA GS2/3-47	≤ 20 cfu/g	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
Mold	ICUMSA GS2/3-47	≤ 20 cfu/g	UD.	UD	UD	UD	UD	UD	UD	UD	UD	UD
Enterobacteriaceae	ISO 21528-2	Neg./1 g	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Staphylococcus aureus	ISO 6888	Neg./0.1 g	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Salmonella spp.	AOAC 2000.07	Neg./250 g	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

Abbreviations:
Spec = Specification
AOAC = Association of Official Analytical Chemists
cfu = colony-forming units
ICUMSA = International Commission for Uniform Methods of Sugar Analysis
ICP-MS = inductively coupled plasma mass spectrometry
ISO = International Organization for Standardization
UD = Undetectable
Neg = Negative

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Table 5. *Cronobacter sakazakii* Product Specifications and Batch Data for Orafti®HP lc-inulin Intended for Infant Formula Use

Parameter	Method	Spec.	Batch Number (Production Date)			
			(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]	(b) (4) [redacted]
Cronobacter sakazakii	ISO/TS 229640	Neg/300 g	Neg	Neg	Neg	Neg

Abbreviations:
Spec = Specification
ISO = International Organization for Standardization
UD = Undetectable
Neg = Negative

000026

As cited in GRN 392, samples of Orafti®P95 were stored in the original packaging under warehouse conditions for 4 years, from 1997 to 2001, and % dry matter, pH, conductivity, and oligosaccharide and sugar content were evaluated (Table 6). Dry-matter content decreased slightly, possibly reflecting water absorption, and a small amount of oligosaccharide hydrolysis, reflected by a decrease in the oligosaccharide and a corresponding increase in free sugar content.

Table 6. Long-Term Stability of Orafti®P95 Oligofructose.

Parameter	Year of Analysis	
	1997	2001
Dry matter (%)	97.2	96.2
Oligosaccharide (% DM)	97.8	96.7
Sugars (% DM)	2.2	3.3
pH	5.1	5.1
Conductivity (µS/cm)	6.1	10.2

As cited in GRN 392, the stability of Orafti®P95 in infant formula was assessed in five batches of formula stored under accelerated conditions (37±2°C and 75% relative humidity; Table 7) and seven batches stored under ambient conditions (28±2°C and 70% relative humidity; Table 8) using AOAC 997.08. Although only two of the batches were stored under accelerated conditions and ambient conditions for a three year period, there was no evidence that the either condition significantly affected the amount of oligofructose. Increases in oligofructose content were likely due to analytical variability. Importantly, under the conditions of use, Orafti®P95 is stable over the shelf-life of the product.

Table 7. Stability of Orafti®P95 Oligofructose in Infant Formula under Accelerated Conditions (37±2°C and 75% relative humidity)

Batch	Oligofructose Content (g/L) by Month							
	0	3	6	9	12	18	24	36
I	1.40	-	1.35	-	-	1.47	1.51	1.55
2	2.65	-	2.45	-	-	2.82	2.84	2.88
3	3.23	3.18	-	-	-	-	-	-
4	3.25	3.12	-	-	-	-	-	
5	3.53	3.19	-	-	-	-	-	-

"-" denotes "not determined"

Table 8. Stability of Orafti®P95 Oligofructose in Infant Formula under Ambient Conditions (28±2°C and 70% relative humidity)

Batch	Oligofructose Content (g/L) by Month							
	0	3	6	9	12	18	24	36
1	1.40	-	1.30	-	-	1.40	1.50	1.51
2	2.65	-	2.48	-	-	2.78	2.78	2.88
3	2.92	2.67	2.29	-	2.41	2.79	-	-
4	3.23	3.14	-	-	-	-	-	-
5	3.25	3.30	-	-	-	-	-	-
6	3.53	3.05	-	-	-	-	-	-
7	5.16	4.52	3.99	3.99	4.26	4.39	-	-

"-" denotes "not determined"

2. Stability of Orafti®HP Long-chain-inulin

As cited in GRN 477, to evaluate the stability of Orafti®HP lc-inulin under acidic conditions, finished product was stored for a year at pH 3.5 and 4.0 and at 20°C or 25°C. At pH 4.0 there were no reductions in lc-inulin content at either temperature over 3 months, whereas there were 15% and 25% reductions in oligofructose content at one year when stored at 20 and 25°C, respectively. When the product was stored at pH 3.5, there was a 5% reduction in lc-inulin at both temperatures at 2 months, and 45% and 60% reductions at one year when stored at 20 and 25°C, respectively.

As cited in GRN 477, the thermal stability of Orafti®HP was assessed by dissolving samples of Orafti®HP in water with pH 5.5 or 3.5 and heating the solutions at 115°C for 15 minutes or 145°C for 10 seconds. When the samples were dissolved in water with a pH of 5.5, no hydrolysis occurred at either temperature. However, when they were dissolved in water with a pH of 3.5, 15% hydrolysis occurred when the samples were heated at 115 and 145°C.

As cited in GRN 477, long-term stability of Orafti®HP was determined by storing finished product in its original packaging for 4 years at 25°C with 60% relative humidity. No changes were observed. Importantly, under the conditions of use, Orafti®HP is stable over the shelf-life of the product.

Table 9. Long-Term Stability of Orafti®HP Oligofructose in Original Packing (25C and 60% relative humidity)[1]

Parameter	Batch					
	(b) (4) [redacted]		(b) (4) [redacted]		(b) (4) [redacted]	
	Year of Analysis		Year of Analysis		Year of Analysis	
	2001	2005	2001	2005	2001	2005
Dry matter (%)	96.8	96.1	96.7	96.3	97.1	97.2
pH	6.7	6.5	6.3	6.2	6.1	6.3
Conductivity (µS/cm)	116	125	123	146	167	184
Glucose, Fructose, Sucrose (g/100 g d.m.)	0	0.1	0.2	0.2	0.2	0.1
Fructans	100	99.9	99.8	99.8	99.8	99.9
Total mesophilic bacteria (CFU/g d.m.)	96	2	0	0	13	0
Yeast (CFU/g d.m.)	0	0	0	0	0	0
Molds (CFU/g d.m.)	0	2	0	0	0	1

d.m. = dry matter
CFU = colony forming units

3. Osmolarity and Osmolality of Infant Formulas Containing Orafti®P95 and Orafti®HP

The American Academy of Pediatrics (AAP) recommends that "formulas for normal infants have concentrations no greater than 400 mOsm/L" (Committee on Nutrition, 1976). To determine the osmolarity and osmolality of a powdered infant formula containing 6.8 g of 9:1 Orafti®P95 oligofructose/Orafti®HP lc-inulin mix/L, a formula containing the 9:1 oligofructose/lc-inulin mix was reconstituted at 14.1 g/100ml (100 calories/5 fl oz.) and the osmolarity and osmolality of the resulting solution was quantified using a freezing-point-depression osmometer (method NPD-DL-413, **"Determination of Osmalality in Oral Electrolyte Solution"). The osmolarity was 310 mOsm/L, which adheres to the recommendations of the** AAP. The osmolality of the formula containing the Orafti®P95 oligofructose/Orafti®HP lc-inulin mix was 360 mOsm/kg, which was similar to a formula without the two ingredients.

III. INTENDED EFFECT

Oligofructose and lc-inulin are prebiotics, which, by definition, are selectively fermented food ingredients that allow specific changes in the composition of and/or activity in the gastrointestinal microbiota, thus conferring benefits upon host health (Gibson et al., 2010). Human milk contains approximately 7 to 12 g/L of oligosaccharides, which are structurally complex and thought to have prebiotic effects, specifically promoting the growth of bifidobacteria and lactobacilli in the gastrointestinal tract of breast-fed infants. The intended effect of adding the 9:1 mix of oligofructose and lc-inulin to an exempt infant formula is to increase oligosaccharide and polysaccharide intake in formula-fed infants and promote the growth of beneficial bacterial, including, but not limited to bifidobacteria and lactobacillus.

IV. HISTORY OF USE AND INTENDED USES

A. HISTORICAL EXPOSURE TO OLIGOFRUCTOSE AND LONG-CHAIN INULIN

Oligofructose and lc-inulin have a long history of safe use. Orafti®P95 oligofructose was determined GRAS in 2011 for its use in infant formula at a maximum level of 3 g/L formula as consumed and subsequently received a **"no questions"** letter from the FDA in 2012 (GRN 392). Lc-inulin has been used for many years in infant formula in Europe and other markets worldwide as a component of a prebiotic combination with galacto-oligosaccharide (GOS). Orafti®HP lc-inulin was determined GRAS in 2013 for its use in exempt and non-exempt term infant formulas at a level of 0.8 g/L formula as consumed and received a **"no questions"** letter from FDA in 2014 (GRN 477).

B. INTENDED USES AND ESTIMATED DAILY INTAKES OF THE 9:1 OLIGOFRUCTOSE/LONG-CHAIN INULIN COMBINATION

1. Intended Uses

Nutricia North America, Inc. intends to add 6.8 g of the 9:1 oligofructose/lc-inulin mix/L to exempt powdered amino acid-based term infant formulas used for the dietary management of food allergies.

2. Estimated Daily Intakes of the 9:1 Oligofructose/Long-Chain Inulin Combination

The exempt powdered amino acid-based term infant formulas will contain 6.8 g/L of the 9:1 oligofructose/lc-inulin mix/L infant formula as consumed and result in approximately 6.12 g oligofructose and 0.68 g lc-inulin/L infant formula as consumed. Infant formulas in the US market typically provide 670 kcal/L (20 kcal/fl oz) (Martinez and Ballew, 2011). Assuming that the infant formulas are the sole source of nutrition, reconstituted at 141 g/L and a caloric density of 670 kcal/L, and the caloric requirements of one month-old and a six month-old infant are 472 kcal/day and 645 kcal/day, respectively (Institute of Medicine Panel on Macronutrients and Institute of Medicine Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005), resulting in an intake of 0.704 and 0.963 L formula/day for 1 and 6 month old infant. The addition of 6.12 g oligofructose and 0.68 g lc-inulin/L infant formula as consumed will result in oligofructose intakes of 4.3 to 5.9 g/day and lc-inulin intakes of 0.5 to 0.7 g/day for one month-old and six month-old infants, respectively.

V. METABOLIC FATE, PHYSIOLOGICAL EFFECTS AND SAFETY OF KEY COMPONENTS

A. INTRODUCTION

Breastfeeding is regarded as the gold standard for infant feeding. Human milk contains over 130 different oligosaccharides having a total concentration that can reach 20 g/L by the fourth day of life (Vandenplas, 2002; Kunz et al., 2000); however, levels decline with the duration of lactation and on average mature human milk contains from 5.0 – 8.0 g oligosaccharides/L (Kunz et al., 2000). Consistent with the potential for multiple nutritional and biological functions, the complex mixture of human milk oligosaccharides differ in size, charge and abundance. They are composed of both neutral and anionic species with building blocks of 5 monosaccharides: D-glucose, D-galactose, N-acetylglucosamine, L-fucose, and N-acetylneuraminic acid. The basic structure of human milk oligosaccharides include a lactose core at the reducing end. They are elongated with N-acetyllactosamine units. Greater structural diversity is conferred by extensive fucosylation and/or sialylation of the terminal positions. Currently, 200 species, consisting of mostly neutral and fucosylated oligosaccharides, have been identified in human milk (German et al., 2008). In addition, there are also high amounts of small molecular weight oligosaccharides (500-1500 Dalton) and low amounts of high molecular weight oligosaccharides (4000-5500 Dalton). Importantly, the majority of the human milk oligosaccharides pass through the upper gastrointestinal tract and serve as substrates for bacterial metabolism in the large intestine (Engfer et al., 2000). In addition, studies suggest that the gastrointestinal microbiota have differing fermentation activities based on the type of oligosaccharide and degree of polymerization (Boehm and Stahl, 2007). For example, *Bifidobacterium longum*, a predominant bifidobacteria species in the breast-fed infant colon, preferentially utilizes human milk oligosaccharides with a degree of polymerization ≤ 7 (Ninonuevo and Lebrilla, 2009).

Because of their complexity, it is unlikely to find natural sources that contain oligosaccharides identical to human milk oligosaccharides. Oligosaccharides, such as oligofructose, lc-inulin, and GOS have been identified, which, despite structural differences, are largely resistant to hydrolysis in the human upper intestine, not absorbed and have some function similar to human milk oligosaccharides infant formulas, that contain a combination of 90% galactooligosaccharide (7.2 g/L) and 10% lc-inulin (0.8 g/L) have been marketed in Europe since March 2000 under Danone brand names such as Nutrilon and Aptamil and Anamix Infant. However, unlike oligofructose and lc-inulin, which are derived from chicory root, GOS is

derived from cow's milk, which may not be suitable for addition to infant formula intended for the dietary management of infants with severe cow's milk allergy.

In the US, the use of oligofructose and lc-inulin as ingredients in infant formulas have been determined to be GRAS up to 3 g/L and 0.8 g/L, respectively (GRN 392, 477). GRAS Notifications 44, 118, 392 and 477 have addressed the safety and tolerance of β 2-1 fructans (inulin, oligofructose or FOS) for use in general foods (GRN 44, 118). The use of GOS in infant formulas has also been determined to be GRAS up to 7.2 g/L (GRN 236, 286, 334, 495).

B. LONG-CHAIN INULIN

Long-chain inulin (lc-inulin) has been determined to be GRAS for use in infant formulas (0.8 g/L), toddler formulas (1.2 g/L), and up to a maximum of 3.125 g/1000 kcal in medical foods (GRN 477). In addition, lc-inulin is a component of the prebiotic combination containing 90% GOS and 10% lc-inulin (maximum 8.0 g/L) that may be added to infant formula and has been commercially available in infant formulas in Europe since 2000.[1]

Consistent with their prebiotic effects, studies have reported that infants consuming infant formula containing 90% GOS and 10% lc-inulin combination have a gastrointestinal microbiota composition that is more similar to breast-fed children when compared with infants receiving the same formula without prebiotics (Fanaro et al., 2005; Knol et al., 2005a, b). Furthermore, some studies suggest that the ingestion of infant formulas containing 90% GOS and 10% lc-inulin may be associated with beneficial effects on development of atopy-related conditions such as IgE-positive atopic dermatitis and mild asthma (Moro et al., 2006a, b, c; van der Aa et al., 2010; van der Aa et al., 2011; van de Pol et al., 2010).

Importantly, the safety of these ingredients has been demonstrated in numerous prospective, randomized, double-blind, placebo controlled trials using concentrations up to 8 g/L, which are summarized in numerous GRAS Notices (GRN, 236, 286, 334, 392, 477). While occasional symptoms of intolerance such as crying or flatulence were reported, no serious adverse events attributable to the feeding have been observed. Infants consuming the prebiotic combination grew at the same rate as those consuming control formula and exhibited intestinal microbiota and stooling patterns more closely resembling those of human-milk-fed reference groups. The longest duration of GOS consumption was reported by Moro et al., (2006a), where 102 infants consumed an infant formula containing GOS at a concentration of 7.2 g day (2.2 g GOS/kg body weight) for a period of 6 months. No treatment related adverse events, tolerance

[1] Commission Directive 2006/141/EC

issues, or adverse effects on weight gain were observed. Furthermore, these subjects were followed up for an additional 18 months without evidence of adverse effects attributable to GOS consumption. To date, no reports of adverse effects on anthropometric indices of growth or effects on water balance leading to diarrhea have been reported (GRN 285).

Clinical studies supporting the intended use of the addition of the oligofructose + lc-inulin combination to infant formula at level not to exceed 6.8 g/L (ratio 9:1) are discussed in Section D.

C. OLIGOFRUCTOSE

Oligofructose is derived from chicory (inulin) and is a non-milk-derived alternative to GOS. Oligofructose and GOS are similar with respect to molecular size distribution. GOS products typically contain oligosaccharides with a terminal glucose molecule linked to 1 to 7 galactose units and predominantly a trisaccharide consisting of a terminal glucose molecule linked in series to two galactose molecules. Similarly, oligofructose is a mixture of saccharides composed of 1 to 7 fructose units linked in series to glucose. Importantly, combinations containing GOS or oligofructose and lc-inulin replicate the molecular size distribution of human milk oligosaccharides and maintain the osmolality of formulas that is recommended by the American Academy of Pediatrics (See Chapter 2; Committee on Nutrition, 1976).

Clinical studies and other data discussed in section D show that the use of oligofructose in combination with lc-inulin in infant formulas is as safe and well-tolerated as GOS:lc-inulin combinations.

D. CLINICAL STUDIES OF INFANT FORMULA CONTAINING 6.8 G/L OLIGOFRUCTOSE + LONG-CHAIN INULIN (9:1)

The safety and tolerance of oligofructose for use in infants as a replacement for GOS in formula at up to 90% of an 6.8 g/L combination of oligofructose + lc-inulin is shown in two pivotal clinical trials: 1) a clinical trial in healthy term infants; and 2) a clinical trial in infants and children with cow's milk allergy. (Harvey et al., 2012, 2014 reports the study in healthy **infants and hypoallergenicity in cow's milk allergic infants and children).**

1. Summary of Healthy Infant Study

a. Overview

Nutricia Research BV carried out a multicenter, 16 wk, double-blind, randomized, placebo-controlled study in healthy infants to evaluate the effect of an infant formula containing 8 g/L of a prebiotic combination of neutral oligosaccharides (85%; comprised of oligofructose:lc-inulin, 9:1) and acidic oligosaccharides (15%), plus *Bifidobacterium breve* M-16V at 1.47×10^9 CFU/100 mL (1.47×10^{10} CFU/L) on growth. The content of oligofructose was 6.12 g/L and the lc-inulin content was 0.68 g/L. One hundred fifteen healthy, full-term infants 0-15 days old subjects were enrolled and randomized to one of two study groups: 59 in the test group; and 56 in the control group. Exclusion criteria included infants less than 5 lb 8 oz at birth, or born at < 37 wk gestation, and those infants receiving breast milk at any time during the study period. There was no restriction on the type of formula consumed prior to enrollment and all systemic antibiotics and antifungal medications, bismuth-containing medications, any medications that neutralize or suppress gastric acid secretion, and herbal supplements were prohibited prior to entering the study. The study was conducted at 11 sites in the US. The enrolled infants received either a commercially available amino acid based infant formula (Neocate Infant DHA and ARA) or the test formula for 16 wk. The primary parameters measured during the study were weight, length and head circumference. Secondary parameters included stool characteristics, gastrointestinal symptoms, laboratory measurements (sodium, potassium, chloride, blood urea nitrogen, creatinine and CO_2), plus formula intake. Body weight, length, and head circumference were determined and recorded at each visit to the clinical sites. Clinical assessment and adverse events were also recorded. Volume of formula intake, stool characteristics (consistency, frequency, and color), and gastrointestinal symptoms (spitting up, vomiting, flatulence, and colic) were recorded in diaries by the parents/caregivers for the first 4 weeks of the study and for 1 week prior to each subsequent visit. Besides the added oligosaccharides and *B.breve* M-16V, the test formula also differed from the control formula by having an increased medium chain triglyceride (MCT) concentration (33 g MCT/100 g total fatty acids, versus 4 g MCT/100 g total fatty acids in control), and an alternative source of essential fatty acids (17.5% fat as low erucic acid rapeseed (canola) oil, versus soy oil). Both formulas were nutritionally complete amino acid-based infant formula and compiled with the maximum and minimum nutrient specifications for infant formula in 21CFR 107.100. Twenty-seven subjects dropped out of the test group and 18 subjects dropped out of the control group. There were 70 completers: 32 in the test group and 38 in the control group.

b. Methodology

Due to the varying ages of the subjects recruited to the study, anthropometric measurements were converted to the appropriate z-scores using National Center for Health Statistics/World Health Organization (NCHS/WHO) international reference population to allow for meaningful comparison of the ability of the products to promote growth. Because the primary outcome of the trial was growth, the statistical power had to be sufficient to allow for the detection of the smallest clinically meaningful difference in growth increments between the test and control groups. Based on reported differences in weight-gain between formula-fed and breast-fed infants, the American Academy of Pediatrics recommends that each group of a two group study contain 28 subjects of a specified sex to detect the smallest meaningful difference in infant growth increments of 3 g/day between the ages of 14 and 120 days (AAP, 1988). To meet this recommendation, Nutricia Research BV enrolled and randomized 115 healthy full-term infants to two study groups. The control group contained 56 infants (35 males (62.5%) and 21 females (37.5%)). The test group contained 59 infants (35 males (59.3%) and 24 females (40.7%)). For the statistical analysis, increases in length and head circumference were log transformed. Length and head circumference measurements were converted to z-scores as described for weight gain. Binary and categorical data were assessed using **Fisher's** Exact Test. Intention to treat (ITT) analyses were carried out using all subjects who were randomized and took at least one dose of the study product. Growth subgroup analyses were carried out without those subjects who did not solely consume the formula they were randomized to during the study. Subgroup analyses on stool characteristics and gastrointestinal (GI) symptoms were carried out without those subjects who took systemic antibiotics that could influence the stool and GI outcome parameters during the study.

c. Results: General

A total of 115 infants were enrolled in the study during the period of November 7, 2008-March 21, 2011. Seventy infants completed the study (60.9%), 32 (54.2%) in the test formula group and 38 (67.9%) in the control formula group. Baseline subject characteristics were similar across groups for the ITT population. The product exposure, measured in days, was comparable across groups and the amount of product consumed, measured in ounces per day, was comparable across groups at the time points measured (wk 2, 4, 8, 12 and overall). Forty-five infants (39.1%) dropped out of the study, 27 (45.8%) in the test formula group and 18 (32.1%) in the control formula group. Twelve subjects (44.4% of drop-outs) in the test formula group and 10 subjects (55.6% of drop-outs) in the control formula group withdrew from the study due to (serious) adverse events. Other reasons for withdrawal included being lost to follow up (2 test formula; 1 control formula), and withdrawal of informed consent (13 test formula; 7 control

formula). One subject was given an exemption from the protocol due to being 36 4/7 wk gestation (exclusion criteria stated that infants < 37 wk were to be excluded). Thirty-two subjects were excluded from the growth subgroup analysis because they did not solely consume the formula they were randomized to during the study. Three subjects took systemic antibiotics and so were excluded from the subgroup analysis of stool consistency and (GI) symptoms. Results from subgroup analyses are reported after the ITT analysis results below. These were the only reasons subjects were excluded from the two sets of subgroup analyses.

d. Results: Growth Parameters

At the end of the 16 wk study, the ratio (90% CI) in weight gain for the test formula/control formula groups was 0.98 (0.95, 1.01) (ITT) and 1.00 (0.97, 1.04) (growth subgroup) (Table 10). The ratio in length increase was 0.99 (0.98, 1.00) (ITT) and 0.99 (0.98, 1.01) (growth subgroup). The ratio in head circumference was 1.01 (1.00, 1.02) (ITT and growth subgroup).

Regarding the ITT population, the mean (± standard deviation) weight-to-age Z-scores for the test formula and control formula groups at baseline were -0.20 (0.81) and 0.03 (0.81), respectively. At 16 wk the means were -0.24 (1.13) and 0.20 (0.83), respectively. The mean (± standard deviation) length-to-age Z-scores for the test formula and control formula groups at baseline were 0.43 (1.10) and 0.56 (1.30), respectively. At 16 wk, the Z-scores were 0.70 (1.31) and 1.22 (1.03), respectively. The mean (± standard deviation) head circumference-to-age Z-scores for the test formula and control formula groups at baseline were 0.01 (1.22) and 0.15 (1.00), respectively. At 16 wk the means were 0.63 (1.08) and 0.53 (1.05), respectively (Table 10).

Regarding the growth subgroup of the ITT population, the mean (± standard deviation) weight-to-age Z-scores for the test formula and control formula groups at baseline were -0.29 (0.80) and 0.04 (0.85), respectively. At wk 16, the mean weight-to-age Z-scores for the test formula and control group were -0.09 (1.06) and 0.16 (0.91), respectively. The mean (± standard deviation) length-to-age Z-scores for the test formula and control formula groups at baseline were 0.35 (1.14) and 0.59 (1.32), respectively. At 16 wk, the Z-scores were 0.78 (1.35) and 1.16 (1.09), respectively. The mean (± standard deviation) head circumference-to-age Z-scores for the test formula and control formula groups at baseline were -0.15 (1.22) and 0.08 (1.09), respectively. At 16 wk, the Z-scores were 0.65 (1.11) and 0.52 (1.05), respectively. Subjects had similar mean weight, length and head circumference increase over the course of the study for both study formula.

e. Results: Tolerance Parameters

Numerous parameters assessed tolerance of the test and control formulas. For both the ITT population and stool and GI subgroups of the ITT population, stool consistency, frequency and color were measured, plus spitting-up, vomiting, flatulence and colic.

Regarding stool characteristics in the ITT population:

- The mean (± standard deviation) stool consistency at wk 2 for the test formula and control group was 1.88 (0.49) and 2.76 (0.70), respectively. Larger numbers indicate harder stools. At 16 wk, the stool consistency for the test formula and control group was 1.94 (0.64) and 2.62 (0.54), respectively.
- The mean (± standard deviation) stool frequency at wk 2 for the test formula and control group was 2.73 (1.25) and 2.59 (1.10) per day, respectively. Larger numbers indicate more frequent stooling. At wk 16, the stool frequency for the test formula and control group was 2.41 (0.98) and 2.25 (0.36), respectively.
- The mean (± standard deviation) stool color at wk 2 for the test formula and control group was 2.29 (0.58) and 2.10 (0.74), respectively. Stool color was defined using the following numerical descriptive scale: 1 = green; 2 = yellow; 3 = yellow/brown; 4 = dark brown; 5 = black. At wk 16, the stool color for the test formula and control group was 2.30 (0.66) and 2.07 (1.11), respectively.

Regarding occurrence of spitting-up, flatulence, vomiting or colic;

- The mean (± standard deviation) spitting-up score at wk 2 for the test formula and control group was 1.95 (0.64) and 1.92 (0.59), respectively. Spitting-up was defined using the following numerical descriptive scale: 1 = none; 2 = slight; 3 = moderate; 4 = severe. At wk 16, the spitting-up score for the test formula and control group was 1.65 (0.53) and 1.82 (0.61), respectively.
- The mean (± standard deviation) vomiting severity at wk 2 for the test formula and control group was 1.36 (0.79) and 1.36 (0.59), respectively. Vomiting severity was defined using the following numerical descriptive scale: 1 = none; 2 = slight; 3= moderate; 4 = severe. At wk 16, the vomiting severity for the test formula and control group was 1.05 (0.18) and 1.10 (0.38), respectively.
- The mean (± standard deviation) flatulence severity at wk 2 for the test formula and control group was 2.57 (0.78) and 2.08 (0.62), respectively. Flatulence severity was defined using the following numerical descriptive scale: 1 = none; 2 = slight; 3 =

moderate; 4 = severe; 5 = very severe. At wk 16 the flatulence severity for the test formula and control group was 2.14 (0.65) and 1.95 (0.62), respectively.

- The mean (± standard deviation) colic severity at wk 2 for the test formula and control group was 1.52 (0.75) and 1.30 (0.53), respectively. Colic severity was defined using the following numerical descriptive scale: 1 = none; 2 = slight; 3 = moderate; 4 = severe; 5 = very severe. At wk 16 the colic severity for the test formula and control group was 1.15 (0.42) and 1.15 (0.33), respectively.

Regarding the stool and GI subgroup of the ITT population (i.e. the subgroup which excluded those infants who took systemic antibiotics):

- The stool consistency characteristics are reported on a descriptive scale: watery; soft pudding like; soft formed; dry formed; and dry hard pellets (Table 11). At wk 2, the majority of stools assessed in the test formula group were rated as watery stools (26%), soft pudding-like stools (59%), or soft formed stools (12%); whereas the stools assessed in the control formula group were mainly rated as soft pudding-like stools (40%) and soft formed stools (38%). At wk 16, the stool consistency distribution was similar across each group: in the test formula group, stools were mainly rated as watery (27%), soft pudding like (53%), and soft formed (20%) stools; in the control group stools were mostly rated as soft pudding-like (46%) and soft formed (40 %);
- The stool frequency characteristics are reported on a descriptive scale: over 12 stools; 9-12 stools; 5-8 stools; 1-4 stools; and no stools per day. At wk 2, the most common categories recorded in the test formula group were: no stools (19% of the rated stools), 1-4 stools (65%), and 5-8 stools (15%); stool frequency in the control formula group was : no stools (11% of the rated stools), 1-4 stools (78%), and 5-8 stools (10%). At wk 16, the stool frequency distribution was comparable for both groups: in the test formula group, no stools or 1-4 stools (10 % and 85% of the assessments respectively) were mainly reported; in the control group also no stools or 1-4 stools (10 % and 90%, of the assessments respectively) were reported;
- The stool color characteristics are reported on a descriptive scale: black; dark brown; yellow/brown; yellow; and green. At wk 2, the majority of stools in the test formula group were rated as green (20%), yellow (33%) and yellow/brown (44%); the stools in the control group had a comparable distribution: green (35%), yellow (28%), and yellow/brown (28%). At wk 16, the stool color distribution of higher reporting of green stool for control subjects was maintained as follows: in the test formula group,

the majority of stools were rated as green (18%), yellow (36%) and yellow/brown (45%); whereas control subjects mainly rated stools as green (49%), yellow (12%), yellow/brown (21%) and dark brown (15%);

- Spitting-up was reported on a descriptive scale: severe; moderate; slight; and none. At wk 2, the majority of spitting-up events in the test formula group were rated as no spitting-up (31%), slight (54%), or moderate (13%) severe; the control group exhibited a similar pattern: no spitting-up (34% of the spitting-up events), slight (53%), or moderate (11%) severe. At wk 16, both groups had shifted to a more favorable profile: the test group exhibited mostly no spitting-up events (44% of the events) or slight (48%) spitting-up and the control subjects had mainly no spitting-up events (34% of the events) or slight (51%) spitting-up as well;

- The vomiting severity was reported on a descriptive scale: severe; moderate; slight; or none. At wk 2, the majority of the test group exhibited no vomiting (90% of the assessments recorded) and the control group had mainly no vomiting (80% of the recorded assessments) or slight (16%) severity. At wk 16, nearly all infants in the test group had no vomiting (96% of the reported examinations) and most of the control group also had no vomiting (94%);

- The flatulence severity was reported on a descriptive scale: very severe; severe; moderate; slight; and none. At wk 2, the majority of the subjects in the test formula group exhibited a range of flatulence severity: no flatulence (14% of the examinations), mild (30%), moderate (39%), and severe (16%); the majority of the control group exhibited a more favorable profile: no flatulence (18% of the assessments), mild (60%), and moderate (19%). At 16 wk, the profile of the test formula group had improved: most subjects had no flatulence (19% of the recorded examinations), mild (53%), or moderate (26%) flatulence; whereas the control group remained about the same: no flatulence (27% of the recorded assessments), mild (53%), and moderate (20%);

- The colic severity was reported on a descriptive scale: very severe; severe; moderate; slight; and none. At 2 wk, the test group exhibited mainly no colic (76% of the examinations) or mild (13%) and the control group was similar: none (82% of the assessments) and mild colic (12%). At 16 wk, all subjects in the test formula group had either no colic (90% of the recorded examinations) or mild colic (7%) and the control group had a similar profile: no colic (87% of the assessments) or mild colic (12%).

Table 10. Growth Parameter Outcomes, Expressed as Mean Z-scores (± standard deviation)

Formula and time point	Weight-to-age		Length-to-age		Head circumference-to-age	
	ITT population	Growth subgroup	ITT population	Growth subgroup	ITT population	Growth subgroup
Test formula						
0 wk	-0.20 (0.81)	-0.29 (0.80)	0.43 (1.10)	0.35 (1.14)	0.01 (1.22)	-0.15 (1.22)
16 wk	-0.24 (1.13)	-0.09 (1.06)	0.70 (1.31)	0.78 (1.35)	0.63 (1.08)	0.65 (1.11)
Control formula						
0 wk	0.03 (0.81)	0.04 (0.85)	0.56 (1.30)	0.59 (1.32)	0.15 (1.00)	0.08 (1.09)
16 wk	0.20 (0.83)	0.16 (0.91)	1.22 (1.03)	1.16 (1.09)	0.53 (1.05)	0.52 (1.05)

Table 11. Stool Parameter Outcomes Reported for the Stool and GI Subgroup[1]

Parameter	Event frequency ((# events/total events) ×100)				
Stool consistency	*Watery*	*Dry hard pellets*	*Dry formed*	*Soft formed*	*Soft pudding-like*
2 wk					
Test group	26	1	<1	12	59
Control group	5	8	9	38	40
16 wk					
Test group	27	0	0	20	53
Control group	3	<1	10	40	46
Stool frequency	*> 12 Stools*	*No stools*	*9-12 stools*	*1-4 stools*	*5-8 stools*
2 wk					
Test group	0	19	<1	65	15
Control group	0	11	1	78	10
16 wk					
Test group	0	10	0	85	4
Control group	0	10	0	90	0
Stool Color	*Black*	*Dark brown*	*Yellow/brown*	*Yellow*	*Green*
2 wk					
Test group	0	3	44	33	20
Control group	2	7	28	28	35
16 wk					
Test group	0	0	45	36	18
Control group	3	15	21	12	49

[1]Reported by parent/caregiver.

f. Discussion about Growth, Tolerance and Stool Parameters

Results demonstrate that the ability of the test formula to support normal growth was similar to the control formula when fed to healthy, term infants from birth to 16 weeks.

Tolerance of both test and control formula were comparable in relation to spitting-up, stool frequency, vomiting and colic.

Amino acid-based formulas are known to be associated with some changes in stool color (towards green) and consistency. Infants taking the test formula with added prebiotics had more stools reported as watery and soft pudding-like stools. Adverse events categorized as diarrhea were low (this is discussed further below) whereas infants taking the control formula had more reports of constipation. In absolute numbers a higher amount of stools were rated as watery stools in the test formula group versus the control group, which appeared to persist over the course of the 16 wk study. The incidence rate of watery stools in the test formula group was 26-27% (of all stools assessed), on a per subject basis, over the 16 wk study. This result has been seen with other oligosaccharides such as GOS and is also a common finding in human milk-fed infants. As noted in Section I, laboratory parameters were within normal range indicating that the higher amount of watery stools was not associated with any increased risk of dehydration.

Closa-Monasterolo et al (2013) also compared formulas with or without a combination of the lc-inulin/oligofructose ingredients (8 g/L 1:1 ratio) in 252 healthy term newborn infants in a double-blind, randomized, placebo-controlled study. In this study, breast-fed infants were also followed for comparison. Anthropometry, water balance, blood parameters, adverse events, stool frequency and characteristics and stool microbiota were assessed. In this study the test formula group had a significantly higher frequency of stool depositions than infants fed the control formula at all study time points. The stools were also softer in the test versus control group. Both stool deposition and stool consistency from the test group were more like those of the breast-fed group than the control formula stool. There were no differences between formulas in anthropometry and relevant adverse events, water balance or blood parameters.

Similarly, a previous study in healthy, term infants with normal birth weight that was carried out by Danone included a control formula group, a test formula group and a reference population of breast-fed infants (MIPS, 2009). These infants were fed a standard non-hydrolyzed cow's milk-based formula, or the formula plus GOS/lc-inulin (9:1) at 6.8 g/L and acidic oligosaccharides (pAOS) at 1.2 g/L, or breastmilk for 44-52 wk. This study tracked stool consistency on a descriptive scale of 1 to 5: 1 = watery, 2 = loose, 3 = soft, 4 = formed, 5 = hard. Figure 4 below illustrates the mean stool consistency per day over the course of the study for all

three groups. The breast-fed group exhibited the lowest mean stool consistency across all three groups until half way through the study. The test formula group had softer stool consistency versus the control group during the first half of the study as well. This effect of stool consistency was more similar to characteristics of healthy breast-fed infants than control formula. The results from this study suggest that the incidence of watery stools observed in the test formula group of the healthy infant study (Harvey et al., 2014) was comparable to the mean stool consistency observed in the GOS/lc-inulin/pAOS-supplemented formula group and the reference breast-fed infant group of the Danone MIPS (2009).

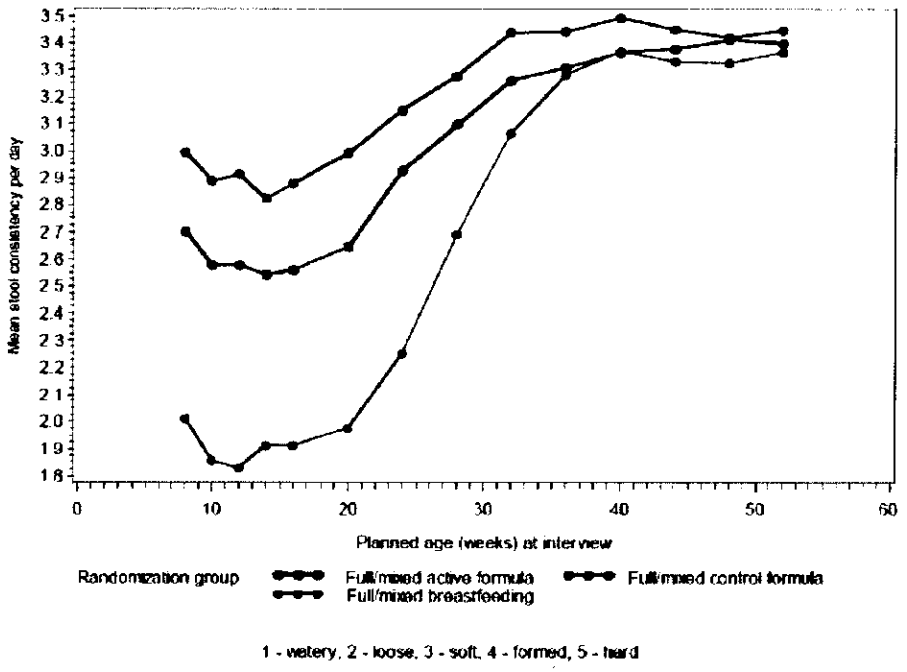

Figure 4. Mean Stool Consistency of Infants Consuming an Infant Formula Containing 6.8 g/L of a 9:1 Combination of GOS and lc-inulin, and 1.2 g/L Acidic Oligosaccharides pAOS

Infants were recruited during the first 8 weeks of life and fed either formula without GOS/inulin/pAOS (control formula), formula with GOS/lc-inulin/pAOS (active formula), or breast milk (breastfeeding) for 44 to 52 weeks. The stool consistency was monitored over time using a descriptive scale: 1 = watery, 2 = loose, 3 = soft, 4 = formed, 5 = hard (adopted from the Danone MIPS , 2009).

Another study in infants with atopic dermatitis aged < 7 mo and exclusively formula fed either an extensively hydrolyzed formula or the same formula plus *Bifidobacterium breve* M-16V at 1.3×10^{10} CFU/L and 8 g/L of GOS:lc-inulin (9:1) for 12 wk reported statistically

significant ($P = 0.05$) softer stool consistency in the test formula group versus control at 12 wk (van der Aa et al., 2010). Note that the GOS concentration administered by van der Aa et al. (2010) was higher than the oligofructose concentration administered by Harvey et al. (2014) to healthy infants and in the second study on **cow's** milk allergic infants (7.2 g/L vs. 6.12 g/L). The observation of soft and watery stools in infants consuming oligosaccharide-supplemented formula is consistent.

Softer stools occur for a period of time in infants consuming purified oligosaccharides via formula intake when compared with a control formula without these ingredients. These softer stools have been shown to be more similar to stools observed in breast-fed infants. A study by Moro et al. (2006a) that administered 0.8 g GOS/lc-inulin per 100 mL (8 g/L) or 8 g maltodextrin/L for 6 months reported that at the beginning of the study the percentage of infants having a stool consistency <2 (Score: 1 = watery, 2 = soft, 3 = seedy, 4 = formed, 5 = hard) was ~20 and 16%, respectively. At 3 months the incidence in the GOS/lc-inulin group was ~28% whereas the group receiving maltodextrin dropped to 0%. By 6 months the incidence of watery stool in the GOS/lc-inulin group dropped to ~8% and incidence in the group receiving maltodextrin was 0%. This result may suggest a physiological adjustment to the oligosaccharides used in the current study 8 g/L prebiotic combination (6.8 g of the oligofructose/lc-inulin/L and 1.2 g/L acidic oligosaccharide) and the Moro study (2006a; 8 g GOS/lc-inulin/L formula) develops in a subgroup of the infant population. Stool frequency was also slightly, but statistically significantly, increased in infants in the GOS/ lc-inulin study, versus the control formula. This effect was not observed in the current study. The Danone MIPS (2009) reported that stool consistency remained soft in breast-fed infants for 22-26 wk.

An increased occurrence of watery stools was noted at 16 wk in the test formula group (15.2% incidence on a per subject basis; Table 20) of the **cow's** milk allergic infant study in the stool and GI subgroup analysis. Results of the stool consistency frequency distribution were difficult to interpret as these were already statistically different at baseline, therefore average stool consistency score was used to assess if there were statistical differences between the groups (Manuscript in Preparation, see also section D.2.*e*). This study used the same test formula as was used in the healthy infant study performed by Harvey et al. (2014). Although 15.2% was not as high as the 26-27% incidence rate of watery stools that was reported for the same formula in the healthy infant study, the finding of increased watery stool incidence in the test formula group versus the control formula group is a consistent finding across the two studies. In the healthy infant study (Harvey et al., 2014), stool color at 16 wk in the test formula group was mainly yellow/brown, yellow and green. As discussed in the **cow's** milk allergic infant study, stool color frequency at 16 wk in the test formula group was mainly dark brown, yellow/brown, and green).

In the breast milk-fed infant reference group of the Danone MIPS (2009), at wk 16 the stools were mainly yellow; this was also true of the test formula group. Therefore, the stool color distribution in the test formula groups of both studies was similar, but not identical, to the stool color distribution observed in the breast-fed reference group of the Danone MIPS (2009). By the end of the Danone MIPS (44-52 wk), the stool color distribution in all three groups was mainly brown. It is known that amino acid based infant formula are typically associated with a change in stool color (green) versus stools from infants taking standard infant formula or human milk.

g. Adverse Events

A total of 86 subjects (74.8%) of the total study population) reported at least one adverse event during the study. Of these, 37 subjects were in the test formula group (63%) and 49 were in the control group (88%). Kaplan-Meier plots of the total dropout rate ($p = 0.12$; ITT population), and the dropout rate due to adverse and serious adverse events ($p = 0.54$; ITT population), measured as time (days) from randomization to withdrawal/completion, indicated that there were no statistically significant differences between the test formula and control formula groups in the healthy infant study.

Overall, the most frequent type of adverse events was classified as GI-related disorders (24 subjects, 41% of the test formula group; 34 subjects, 61% of the control formula group) plus infections/infestations (15 subjects, 25% of the test formula group; 23 subjects, 41% of the control formula group). Of the total number of adverse events occurring, 27% and 29% were determined to be related to the test formula and control formula, respectively. The remaining adverse events were determined to be unrelated to the formulas. Of the total adverse events, the percent that were classified as being severe was 3% and 2% in the test and control formula groups, respectively. There was a higher incidence of constipation in the control group (27%) versus test formula group (5%).

h. Serious Adverse Events

A total of 7 serious adverse events occurred during the study; 2 in two subjects in the test formula group (pneumonia and bronchiolitis) and 5 in five subjects in the control formula group (dehydration, non-RSV bronchiolitis, respiratory distress, respiratory syncytial virus and right partial skull fracture). Only 2 subjects dropped out of the study due to serious adverse events (1 in the test group; 1 in the control group). The incidence of dehydration in an infant taking the control formula occurred in association with gastroenteritis and was considered related due to this concomitant adverse event rather than the control product. The other six serious adverse events were determined to be unrelated to the formula received.

i. Laboratory Parameters

Specific laboratory blood parameters were measured at baseline and on the final visit (blood urea nitrogen, creatinine, sodium, potassium, chloride, and carbon dioxide) and there were no statistically significant differences between the test formula and control formula groups.

j. Conclusions

The Z-scores for various standard growth parameters observed at baseline and 16 wk indicated that the test formula and control formula groups both supported normal growth. An increase in Z-scores for weight-to-age, length-to-age, and head circumference-to-age was observed from 0 to 16 wk in both the ITT population and growth subgroup (Table 10). One exception was the ITT population of the test formula group, which had essentially the same mean Z-score at 0 and 16 wk for weight-to-age. However, the growth subgroup, (which excluded subjects not exclusively on the study product) showed significant improvement in mean Z-scores for weight-to-age from 0 to 16 wk. All other anthropometric parameters indicated an improvement over the course of the study from 0 to 16 wk for both the test formula and control formula.

Forty-five infants (39.1%) dropped out of the study, 27 (45.8%) in the test formula group and 18 (32.1%) in the control formula group. Of all the subjects in the test formula group who withdrew from the study (27), adverse events were cited as the reason for withdrawal for 12, or 44.4%. Of all the subjects in the control formula group who withdrew (18), adverse events were cited as the reason for withdrawal by 10, or 55.6%. A total of 86 subjects (74.8% of the total study population) reported at least one adverse event during the study. Of these, 37 subjects were in the test formula group (63%) and 49 were in the control group (88%). Overall, the most frequent type of adverse events was classified as GI-related disorders (24 subjects, 41% of the test formula group; 34 subjects, 61% of the control formula group) plus infections/infestations (15 subjects, 25% of the test formula group; 23 subjects, 41% of the control formula group). Of the total number of adverse events occurring, 27% and 29% were determined to be related to the test formula and control formula, respectively. Rates of adverse events and the type of adverse events observed did not differ significantly between the two groups. Overall, there was no significant safety signal observed that would differentiate the test formula from the control formula. Although infants consuming the test formula exhibited more watery stools versus the control group, there was a low incidence of diarrhea adverse events (three in the test group, two in the control group), suggesting that this was not a significant safety issue. Also, a similar study administering 8 g/L of GOS/lc-inulin to infants for up to 6 months (Moro et al., 2006a) has

reported a similar incidence of soft/watery stooling at 3 months; in this study, most incidents of watery stools resolved by 6 months.

2. Summary of Hypoallergenicity and Growth in Cow's Milk Allergic Infants Study

a. Overview

A two-phase multicenter study was conducted to evaluate the hypoallergenicity and growth effects of an infant formula in cow's milk allergic infants. Results of the hypoallergenicity phase are reported by Harvey et al. (2014). Subjects from birth to 3 yr old with documented allergy to cow's milk protein were eligible to participate in the study. Test formula contained 8 g/L of a prebiotic combination of neutral oligosaccharides (85%; comprised of oligofructose:lc-inulin, 9:1) and acidic oligosaccharides (15%), plus *Bifidobacterium breve* M-16V at 1.47×10^{10} CFU/L). The content of oligofructose was 6.12 g/L and the lc-inulin content was 0.68 g/L. Body weight, length, and head circumference were determined and recorded at each visit to the clinical sites. Volume of formula intake, stool characteristics (consistency, frequency, and color), and clinical symptoms were recorded in diaries by the parents/caregivers.

Phase I evaluated the hypoallergenicity of the test formula in infants aged from birth to 3 yr old with confirmed cow's milk allergy as described below. This phase enrolled 30 subjects who underwent a DBPCFC to both the control formula and the test formula after a 1 wk run in period followed by an additional minimum 6 d of ingestion of the study formula without a reaction. Subjects aged birth to 8 mo, who intended to continue in Phase II of the study after completing Phase I,were required to continue on the blinded formula they were first exposed to in the challenge and were required to ingest at least 50% of their daily energy requirements as study formula. Subjects aged 8 mo to 3 yr old were required to take unblinded test formula and were required to ingest a minimum of 8 fluid ounces of test formula per day. The total length of Phase 1 of the study was 2 wk.

Phase II evaluated growth in a minimum of 80 subjects aged birth to 8 mo old whose allergy to cow's milk was confirmed within the previous two months. Subjects were required to ingest at least 50% of their daily energy requirements as study formula. The total length of the study was 17 wk. One hundred and ten subjects consumed either test (n = 54) or control (n = 56) formula for 16 wk after a 1 wk run-in period. Subjects with other IgE and/or non-IgE mediated allergies to food in addition to milk protein were also eligible. Subjects aged up to 8 mo could continue in Phase II of the trial after completing Phase I. The primary endpoints evaluated included anthropometric measurements for growth (Phase II) and secondary endpoints included

the evaluation of the hypoallergenicity of the test formula (Phase I), tolerance (Phase II) and product intake (Phase I and II), plus effects on various allergic (SCORAD, allergic characteristics) and stool (consistency, frequency, color, flatulence) parameters. Biochemical and hematological parameters were also evaluated.

All subjects had a confirmed allergy to milk, as confirmed by one of the following criteria, within two months prior to the week -1 visit: 1) positive DBPCFC with cow milk; 2) a confirmed history of acute severe reaction after isolated accidental ingestion with a positive test result for cow's milk specific IgE antibody; 3) confirmed history of a reaction to cow milk protein with **cow's** milk-specific IgE of > 15 kU/L for children over 2 yr and >5 kU/L for children under 2-yr old; 4) confirmed history of a reaction to cow milk protein with a positive skin prick test with a resulting wheal diameter ≥ 3 mm; 5) no confirmed clinical history of a reaction to cow milk protein with a positive skin prick test with a wheal diameter ≥ 8 mm for children > 2 yr and ≥ 6 mm for children < 2 yr; 6) for children having an allergic eosinophilic gastroenteritis, documentation of eosinophilic infiltration and resolution of symptoms on a diet restricting **cow's** milk (and other foods), with reoccurrence after re-administration of **cow's** milk; 7) for infants ≤ 8 mo with a non-IgE mediated **cow's** milk allergy (including food protein-induced enterocolitis, food protein-induced proctitis, gastroesophageal reflux disease, food protein-induced enteropathy, etc.), documentation of symptoms suggestive of non-IgE-mediated **cow's** milk allergy with reoccurrence after re-administration of **cow's** milk.

Besides the abovementioned added oligosaccharides and *B. breve* M 16V, the test formula also differed from the control formula by having an increased medium chain triglyceride (MCT) concentration (33 g MCT/100 g total fatty acids, versus 4 g MCT/100 g total fatty acids in control), and an alternative source of essential fatty acids (17.5% fat as low erucic acid rapeseed (canola) oil, versus soy oil). Both formulas were nutritionally complete amino acid based infant formula and compiled with the maximum and minimum nutrient specifications for infant formula in 21CFR 107.100.

b. Methodology

All subjects randomized were included in the intention-to-treat (ITT) analysis set, which was the primary analysis set for all efficacy endpoints and safety analyses. The stool and GI subgroup included subjects who did not take systemic antibiotics that could influence the stool and GI outcome parameters during the study and was used to report an additional analysis on the stool characteristics and allergic characteristics. The ITT population consisted of 54 subjects in the test formula group and 56 subjects in the control formula group, while the stool and GI subgroup was comprised of 45 subjects from the test formula group and 37 subjects from the

control formula group. To confirm the hypoallergenicity of the test formula, based on AAP recommendations (2000) it was determined that it was necessary to recruit at least 29 consecutive subjects that would undergo a DBPCFC followed by an additional minimum 6 d of ingestion of the test product without a reaction. This number was considered sufficient to project with 95% confidence that 90% of cow's milk allergic children will not react to the test formula. It was decided that weight, length and head circumference measured at visits falling outside the set timing windows would not be included in the analysis. In the instance that study product intake exceeded 2.5 L in a day the data point would be excluded from the analysis. It was also decided that any data suggesting a decrease of > 1.2 cm in length and/or head circumference could not be trusted. Data from the previous visit and the concerning visit were not included in the analysis. One subject was randomized to receive one product but was given several cans of the other product before the error was realized and provided the correct product. The subject's data were analyzed according to the randomized (and finally provided) product that was assigned.

For all subjects, the exclusion criteria were: infants < 5 lb 8 oz at birth; infants < 37 wk gestation; infants with severe concurrent illness or major congenital malformations; suspected or documented systemic or congenital infections; unable to adhere to protocol instructions due to non-compliance of parent/caregiver; investigator's uncertainty about the willingness or ability of the parent/caregiver to comply with the protocol requirements; participation in any other studies involving investigational products concomitantly or within 28 d prior to entry into the study; an infant of any personnel connected with the study; infants whose parent/caregiver is younger than the legal age of consent. During Phase I, oral or injected steroids, antihistamines, beta-agonists, theophylline, and cromolyn sodium were prohibited prior to and during the DBPCFC. At the time of consent, a physician determined whether it was medically appropriate to temporarily discontinue these medications. During Phase II, physician-prescribed medications were allowed, but noted. Additional exclusion criteria for subjects aged from birth to 8 mo of age: dietary consumption of other sources of prebiotics or probiotics was prohibited 2 wk prior to inclusion in the study and during the study period; subjects must not have received systemic antibiotics in the previous 2 wk prior to entry into the study.

c. Results: General

For Phase I, 30 subjects were enrolled; 24 subjects completed 7 d of consumption of the study formula, 4 completed 6 d, 1 completed 5 d and 1 completed 4 d.

For Phase II, 110 subjects were enrolled; 54 were randomized to the test formula group and 56 were randomized to the control formula group. Baseline characteristics including: age,

weight, length, head circumference, sex and ethnicity were well balanced between test and control groups. There were 11 dropouts in the test formula group and 9 dropouts in the control formula group. Overall, 43 subjects in the test formula group and 47 subjects in the control formula group completed the study. Statistical power of at least 0.8 was confirmed both at beginning of the study and at the end of the study (based on the subjects that were randomized and on the subjects that completed the study). The ITT population in Phase II consisted of 54 subjects from the test formula group plus 56 subjects from the control group. Of these subjects, 45 constituted the stool and GI subgroup of the test formula group and 37 comprised the stool and GI subgroup of the control formula group. The main anthropometric parameters weight, length and head circumference, were all increased at wk 16 versus wk 0 across both groups (Table 12).

Use of both formulas resulted in similar weight gain for subjects after 16 weeks of use. At week 16 the difference (90% CI) in weight Z-scores (test group - control group) was 0.147 (-0.10; 0.39, p=0.32). These results were reflected in the earlier weeks of the study. Comparable results in length and head circumference increases were observed for both groups. At week 16 the difference (90% CI) in length Z-scores (test group - control group) was -0.299 (-0.69; 0.09, p=0.21) and difference in head circumference Z-scores (test group - control group) was 0.152 (-0.15; 0.45, p= 0.40). These results were also reflected in the earlier weeks of the study. In addition weight-for-age and length-for-age Z-scores did not differ between the test and control group.

Table 12. Growth Parameter Outcomes, Expressed as Mean Z-Scores (± Standard Deviation)

Formula and time point	Weight-to-age ITT population	Length-to-age ITT population	Head circumference-to-age ITT population
Test formula			
0 wk	-0.64 (0.86)	-0.25 (1.16)	0.02 (1.09)
16 wk	-0.04 (0.79)	0.38 (1.23)	0.40 (0.92)
Control formula			
0 wk	-0.56 (1.11)	0.01 (1.34)	0.20 (1.19)
16 wk	-0.06 (1.21)	0.70 (1.66)	0.55 (1.33)

d. Results: Hypoallergenicity and Allergic Characteristics

All of the 30 subjects who completed the DBPCFC in Phase I and started the open challenge (7 d continuing with same formula regimen) had negative responses in both parts of the study. There were no severe adverse events reported for the test formula group during the DBPCFC or extended 7 d feeding period.

In Phase II, mean SCORAD index results, which report on the extent, intensity and subjective symptoms of atopic dermatitis, were 16.58 and 15.93 at wk -1 for the test formula and control formula groups, respectively. By wk 16, mean SCORAD values had dropped to 5.17 and 7.76, respectively. Higher scores represent more severe atopic dermatitis.

Dermatological symptoms such as redness/soreness, dryness, crusting/flaking, and oozing were defined using the following numerical descriptive scale: 1 = none; 2 = slight; 3 = moderate; 4 = severe; 5 = very severe. Results for both the ITT population and the stool and GI subgroup are summarized in Tables 13 and 14, respectively. Regarding the ITT population results, dermatological symptoms improved over time in both groups. By wk 16, the test and control formula groups were not significantly different from one another, although improvement in redness/soreness was nearly significant ($P = 0.085$), in favor of the test formula group. In the stool and GI subgroup analysis, the test and control formula groups were reported to be statistically significantly different regarding redness/soreness at wk 16, in favor of the test formula group.

Respiratory symptoms such as coughing and wheezing were monitored, using the same numerical descriptive scale as described above, and results from both the ITT population and the stool and GI subgroup are summarized in Tables 15 and 16, respectively. Regarding the ITT population analysis, at wk -1 to wk 0, the test and control groups were not significantly different from one another for both the coughing and wheezing results ($P = 0.080$ and 0.055, respectively). Respiratory symptoms generally improved across both groups over time, although a few infants in both groups experienced severe or moderate coughing and/or wheezing at 16 wk. The stool and GI subgroup analyses also showed that there were no statistically significant differences between the formula groups for any of the respiratory parameters.

The test product was found to be hypoallergenic as demonstrated by the negative results of the DBPCFC and open challenge Phase I part of the study. In both product groups a decrease in the number of allergic characteristics reported was observed over the study period of Phase II.

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Table 13. Dermatological Outcomes Reported for the Phase II ITT Population[1]

Parameter	Percent of subjects experiencing symptoms ((# subjects[2]/total subjects) ×100)				
Redness/soreness	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	3.8	7.5	11.3	20.8	56.6
Control group	1.8	3.6	5.5	21.8	67.3
Wk 12 to wk 16					
Test group	0	0	0	15.8	84.2
Control group	2.2	0	11.1	8.9	77.8
Dryness	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	0	1.9	17.0	24.5	56.6
Control group	0	3.6	5.5	43.6	47.3
Wk 12 to wk 16					
Test group	0	0	2.6	26.3	71.1
Control group	0	4.4	4.4	11.1	80.0
Crusting/flaking	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	0	1.9	7.5	20.8	69.8
Control group	0	1.8	3.6	16.4	78.2
Wk 12 to wk 16					
Test group	0	0	0	5.3	94.7
Control group	0	0	8.9	6.7	84.4
Oozing	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	0	1.9	0	3.8	94.3
Control group	0	1.8	0	3.6	94.5
Wk 12 to wk 16					
Test group	0	0	0	0	100
Control group	0	0	2.2	6.7	91.1

[1]Reported by parent/caregiver.
[2]Number of subjects using this category as the most frequent category at each visit. If tied frequency then the most severe category is used.

Table 14. Dermatological Outcomes Reported for the Phase II Stool and GI Subgroup[1]

Parameter	Percent of subjects experiencing symptoms ((# subjects[2]/total subjects) ×100)				
Redness/soreness	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	4.5	6.8	11.4	20.5	56.8
Control group	2.8	5.6	2.8	22.2	66.7
Wk 12 to wk 16					
*Test group	0	0	0	18.2	81.8
*Control group	3.7	0	11.1	3.7	81.5
Dryness	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	0	2.3	15.9	25.0	56.8
Control group	0	5.6	2.8	44.4	47.2
Wk 12 to wk 16					
Test group	0	0	3.0	24.2	72.7
Control group	0	3.7	3.7	14.8	77.8
Crusting/flaking	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	0	2.3	9.1	22.7	65.9
Control group	0	2.8	2.8	16.7	77.8
Wk 12 to wk 16					
Test group	0	0	0	6.1	93.9
Control group	0	0	3.7	11.1	85.2
Oozing	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	0	2.3	0	2.3	95.5
Control group	0	2.8	0	5.6	91.7
Wk 12 to wk 16					
Test group	0	0	0	0	100
Control group	0	0	3.7	3.7	92.6

*Groups were statistically significantly different ($P < 0.05$).
[1]Reported by parent/caregiver.
[2]Number of subjects using this category as the most frequent category at each visit. If tied frequency then the most severe category is used.

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Table 15. Respiratory Outcomes Reported for the Phase II ITT Population[1]

Parameter	Percent of subjects experiencing symptoms ((# subjects[2]/total subjects) ×100)				
Coughing	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	0	0	5.7	26.4	67.9
Control group	0	0	1.8	45.5	52.7
Wk 12 to wk 16					
Test group	0	5.3	0	7.9	86.8
Control group	0	2.2	6.7	20.0	71.1
Wheezing	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	0	0	7.5	11.3	81.1
Control group	0	1.8	0	5.5	92.7
Wk 12 to wk 16					
Test group	0	0	0	5.3	94.7
Control group	0	0	2.2	2.2	95.6

[1]Reported by parent/caregiver.
[2]Number of subjects using this category as the most frequent category at each visit. If tied frequency then the most severe category is used.

Table 16. Respiratory Outcomes Reported for the Phase II Stool and GI Subgroup[1]

Parameter	Percent of subjects experiencing symptoms ((# subjects[2]/total subjects) ×100)				
Coughing	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	0	0	2.3	25.0	72.7
Control group	0	0	2.8	38.9	58.3
Wk 12 to wk 16					
Test group	0	3.0	0	9.1	87.9
Control group	0	0	7.4	22.2	70.4
Wheezing	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	0	0	6.8	11.4	81.8
Control group	0	2.8	0	5.6	91.7
Wk 12 to wk 16					
Test group	0	0	0	6.1	93.9
Control group	0	0	3.7	0	96.3

[1]Reported by parent/caregiver.
[2]Number of subjects using this category as the most frequent category at each visit. If tied frequency then the most severe category is used.

e. Results: Safety and Tolerance-Related Parameters

In Phase II, 9 subjects withdrew from the study early due to the occurrence of a (serious) adverse event; 6 were in the test formula group and 3 were in the control formula group. Gastrointestinal (GI) symptoms such as spitting-up and vomiting were recorded. Summaries of the GI-related results from both the ITT population and stool and GI subgroup appear in Tables 17 and 18, respectively. Regarding the ITT results, spitting-up improved across both groups over time. Vomiting remained the same. Regarding the stool and GI subgroup analysis, the formula groups were not significantly different for any of the gastrointestinal parameters.

Stool characteristics such as consistency, frequency, color and flatulence were reported. Stool parameter results from both the ITT population and the stool and GI subgroup are presented in Tables 19 and 20, respectively.

Regarding the stool consistency results, the distribution of the number of subjects over the categories was compared between the treatment groups using the Freeman Halton test. These analyses showed a statistically significant difference between test and control group at baseline (P=0.025). At later time points in the study stool consistency distribution was not different between groups.

To investigate if there was a difference in severity or water content between the groups, further post-hoc analyses were performed (unpublished data). These analyses revealed that there is no significant difference between the groups on average score of stool consistency when categorised as following:

1. Water content: 1) watery; 2) soft pudding-like; 3) soft formed; 4) dry formed; 5) dry hard pellets;

2. Severity: 1) soft pudding-like; 2) soft formed; 3) dry formed; 5) dry hard pellets; 5) watery.

Stool frequency, color and flatulence patterns did not significantly differ between groups at baseline and wk 16. Regarding the stool and GI subgroup analysis, the same statistically significant difference was observed between the groups at baseline for stool consistency; this difference was resolved by wk 16.

Table 17. Gastrointestinal Outcomes Reported for the Phase II ITT Population[1]

Parameter	Percent of subjects experiencing symptoms ((# subjects[2]/total subjects) ×100)				
Spitting-up	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	0	9.4	22.6	50.9	17.0
Control group	0	5.5	25.5	40.0	29.1
Wk 12 to wk 16					
Test group	0	0	10.5	34.2	55.3
Control group	0	0	15.6	28.9	55.6
Vomiting	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	0	0	7.5	11.3	81.1
Control group	0	0	1.8	9.1	89.1
Wk 12 to wk 16					
Test group	0	0	2.6	7.9	89.5
Control group	0	2.2	2.2	4.4	91.1

[1]Reported by parent/caregiver.
[2]Number of subjects using this category as the most frequent category at each visit. If tied frequency then the most severe category is used.

Table 18. Gastrointestinal Outcomes Reported for the Phase II Stool and GI Subgroup[1]

Parameter	Percent of subjects experiencing symptoms ((# subjects/total[2] subjects) ×100)				
Spitting-up	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	0	11.4	25.0	43.2	20.5
Control group	0	0	30.6	38.9	30.6
Wk 12 to wk 16					
Test group	0	0	12.1	33.3	54.5
Control group	0	0	18.5	25.9	55.6
Vomiting	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	0	0	9.1	11.4	79.5
Control group	0	0	0	5.6	94.4
Wk 12 to wk 16					
Test group	0	0	3.0	9.1	87.9
Control group	0	0	3.7	3.7	92.6

[1]Reported by parent/caregiver.
[2]Number of subjects using this category as the most frequent category at each visit. If tied frequency then the most severe category is used.

Table 19. Stool Parameter and Flatulence Outcomes Reported for the ITT Population[1]

Parameter	Percent of subjects experiencing symptoms ((# subjects[2]/total subjects) ×100)				
Stool consistency	*Watery*	*Dry hard pellets*	*Dry formed*	*Soft formed*	*Soft pudding-like*
Wk -1 to wk 0					
*Test group	3.8	3.8	5.7	34.0	52.8
*Control group	20.0	1.8	0	25.5	52.7
Wk 12 to wk 16					
Test group	13.2	0	2.6	52.6	31.6
Control group	4.4	2.2	15.6	46.7	31.1
Stool frequency	*> 12 Stools*	*No stools*	*9-12 stools*	*1-4 stools*	*5-8 stools*
Wk -1 to wk 0					
Test group	0	5.7	0	92.5	1.9
Control group	0	1.8	1.8	92.7	3.6
Wk 12 to wk 16					
Test group	0	7.9	0	89.5	2.6
Control group	0	6.7	0	88.9	4.4
Stool Color	*Black*	*Dark brown*	*Yellow/brown*	*Yellow*	*Green*
Wk -1 to wk 0					
Test group	13.2	17.0	15.1	20.8	34.0
Control group	10.9	20.0	23.6	7.3	38.2
Wk 12 to wk 16					
Test group	5.3	23.7	34.2	13.2	23.7
Control group	2.2	26.7	35.6	6.7	28.9
Flatulence	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	1.9	5.7	20.8	54.7	17.0
Control group	0	3.6	27.3	47.3	21.8
Wk 12 to wk 16					
Test group	0	2.6	18.4	39.5	39.5
Control group	0	0	6.7	44.4	48.9

* Groups were statistically significantly different ($P < 0.05$). A significant difference indicates that the distribution of the number of subjects over the different categories was different between treatment groups.
[1]Reported by parent/caregiver.
[2]Number of subjects using this category as the most frequent category at each visit. If tied frequency then the most severe category is used.

Table 20. Stool Parameter and Flatulence Outcomes Reported for the Stool and GI Subgroup[1]

Parameter	Percent of subjects experiencing symptoms ((# subjects[2]/total subjects) ×100)				
Stool consistency	*Watery (most severe)*	*Dry hard pellets*	*Dry formed*	*Soft formed*	*Soft pudding-like (least severe)*
Wk -1 to wk 0					
*Test group	4.5	2.3	4.53	38.6	50.0
*Control group	25.0	2.8	0	22.2	50.0
Wk 12 to wk 16					
Test group	15.2	0	0	51.5	33.3
Control group	7.4	0	22.2	37.0	33.3
Stool frequency	*> 12 Stools*	*No stools*	*9-12 stools*	*1-4 stools*	*5-8 stools*
Wk -1 to wk 0					
Test group	0	4.5	0	93.2	2.3
Control group	0	2.8	2.8	88.9	5.6
Wk 12 to wk 16					
Test group	0	6.1	0	90.9	3.0
Control group	0	7.4	0	88.9	3.7
Stool Color	*Black*	*Dark brown*	*Yellow/brown*	*Yellow*	*Green*
Wk -1 to wk 0					
Test group	13.6	13.6	13.6	20.5	38.6
Control group	11.1	13.9	25.0	11.1	38.9
Wk 12 to wk 16					
Test group	3.0	27.3	27.3	15.2	27.3
Control group	3.7	25.9	29.6	7.4	33.3
Flatulence	*Very severe*	*Severe*	*Moderate*	*Slight*	*None*
Wk -1 to wk 0					
Test group	2.3	4.5	20.5	52.3	20.5
Control group	0	2.8	33.3	50.0	13.9
Wk 12 to wk 16					
Test group	0	3.0	15.2	39.4	42.4
Control group	0	0	7.4	44.4	48.1

* Groups were statistically significantly different ($P < 0.05$). A significant difference indicates that the distribution of the number of subjects over the different categories was different between treatment groups.
[1]Reported by parent/caregiver.
[2]Number of subjects using this category as the most frequent category at each visit. If tied frequency then the most severe category is used.

f. Adverse Events

Regarding Phase I, there were no serious adverse events during the double blind, placebo-controlled food challenge or extended 7 d feeding period in the hypoallergenicity study for any of the 30 subjects receiving test formula.

Regarding Phase II, 81 subjects (73.6% of the total study population) reported at least one adverse event during the study: 43 (79.6%) in the test formula group and 38 (67.9%) in the control formula group. The most frequent types of adverse events experienced by the subjects were classified as GI system disorders (23 subjects in the test formula group, 42.6%; 18 subjects in the control formula group, 32.1%) and respiratory system disorders (29 subjects in the test formula group, 53.7%; 23 subjects in the control formula group, 41.1%). Reports of diarrhea were statistically significantly different ($P = 0.004$) between the groups: 12 subjects in the test formula group (22.2%) and 2 subjects in the control group (3.6%). Some of the events categorised as diarrhea were determined to be possibly (3) or probably (1) related to the formula. This is discussed further in Section V.D.2.*h*. More subjects in the control group reported infection (10 subjects, 17.9%), versus the test formula group (1 subject, 1.9%) ($P = 0.008$). Four subjects in the test group experienced yeast infections (7.4%), versus 0 in the control group ($P = 0.055$). Severe adverse events occurred at similar, and non-statistically significant, rates across groups: 5 subjects (9.3%) in the test formula group and 5 subjects (8.9%) in the control group. Moderate adverse events also occurred at similar, and non-statistically significant, rates across groups: 14 subjects (25.9%) in the test formula group and 19 (33.9%) in the control formula group. There was a statistically significant difference ($P = 0.045$) in mild adverse events across groups: 24 (44.4%) in the test formula group and 14 (25.0%) in the control formula group. As mentioned above, these tended to be gastrointestinal and/or related to infection incidence.

g. Laboratory Parameters

Hemoglobin, ferritin and total iron binding capacity were measured at wk -1 and wk 16. There was a statistically significant ($P = 0.010$) difference between groups at wk 16 (change from baseline) for hemoglobin, according to the ITT population analysis, with the test formula group having a lower mean value (118 g/L) versus the control formula group (123 g/L). The clinical significance, if any, of this finding is unclear, since values were within the normal reference ranges. The remaining parameters did not differ between groups at wk 16. Other biochemical parameters, such as plasma albumin, plasma prealbumin, blood urea nitrogen, potassium, calcium, white blood cell (WBC) count, red blood cell (RBC) count, hematocrit, mean corpuscular volume (MCV), platelet count, sodium, creatinine, and chloride were not significantly different between treatment groups. There was a statistically significant effect on

mean levels of alkaline phosphatase ($P = 0.018$) at 16 wk: 292 U/L in the test formula group versus 246 U/L in the control formula group. As all laboratory parameters were within the normal reference range, the differences were not deemed clinically significant.

h. Discussion of Dropout Rate and Diarrhea Incidence

The total dropout rate ($p = 0.59$; ITT population) was similar for the test formula and control formula groups in the cow's milk allergic infant study (data not shown). Therefore, there was no indication that the test formula caused a higher dropout rate, versus the control formula.

Nutricia Research BV has provided additional information about diarrhea onset time and concurrent systemic antibiotic use (Table 21) in the subjects exhibiting diarrhea in the cow's milk allergic infant study (14 total; 12 in the test group, 2 in the control group).

In the control formula group, one of the infants exhibiting diarrhea was reported to have taken Rocephin (Ceftriaxone) systemically; therefore, this subject was not considered further, as systemic antibiotic use may cause antibiotic-associated diarrhea.

In the test formula group, of the 12 infants exhibiting diarrhea, four subjects experienced diarrhea prior to intake of the product and one subject was reported to have taken amoxicillin systemically. Therefore, these five subjects were not considered further. Of the remaining 7 test group subjects, one (R1602) exhibited diarrhea immediately upon consumption of the test formula, suggesting that the diarrhea could be related to the test formula. This infant had received a flu vaccination 2 days prior to the event onset. It was noted that the diarrhea in this infant stopped after 18 days (self-limiting), and was prior to the subject being discontinued in the study due to wheat anaphylaxis. The remaining 6 subjects exhibited diarrhea onset long after the date of first study product intake, suggesting that the diarrhea in these subjects was unlikely related to the test formula. None of these events needed further action.

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Table 21. Dates of Diarrhea Onset and First Study Product Intake, Correlated with Antibiotic Use, in the Cow's Milk Allergic Infant Study

Subject	Study Product	Investigator Term	Visit 1 Date	Date First Study Product Intake	Adverse Event (AE) Start Date	AE Stop Date	Duration of AE (Days)	Severity	Relationship to Study Product	Additional Information	Antibiotic Use
R0213	NEO-SYN	Diarrhea	3/28/2009	4/9/2009	4/4/2009	4/22/2009	19	Mild	Possibly	Started before first study product intake	No antibiotics
R1701	NEO-SYN	Diarrhea	11/3/2009	11/15/2011	11/9/2011	11/10/2011	2	Mild	Possibly	Started before first study product intake	No antibiotics
R2204	NEO-SYN	Watery stools	9/29/2011	10/14/2011	10/7/2011	10/17/2011	11	Mild	Not related	Started before first study product intake	No antibiotics
R2302	NEO-SYN	Diarrhea	2/2/2012	2/9/2012	2/6/2012	2/9/2012	4	Mild	Unlikely	Started before first study product intake	No antibiotics
R0702	NEO-SYN	Diarrhea	9/15/2009	9/30/2009	10/20/2009	10/20/2009	1	Mild	Not related	-	No antibiotics
R0702	NEO-SYN	Diarrhea	9/15/2009	9/30/2009	12/17/2009	12/19/2009	3	Moderate	Not related	-	No antibiotics
R0704	NEO-SYN	Diarrhea	1/13/2010	1/20/2010	5/5/2010	After study end	Last 7 days of study	Mild	Unlikely	-	No antibiotics
R1601	NEO-SYN	Stool change: runny	10/5/2011	10/13/2011	12/22/2011	12/27/2011	6	Moderate	Possibly	-	No antibiotics
R1602	NEO-SYN	Watery stools	12/8/2011	12/16/2011	12/16/2011	1/2/2012	18	Moderate	Probably	-	No antibiotics
R2201	NEO-SYN	Diarrhea	7/21/2011	8/5/2011	10/21/2011	10/24/2011	4	Mild	Unlikely	-	No antibiotics

Table 21. Dates of Diarrhea Onset and First Study Product Intake, Correlated with Antibiotic Use, in the Cow's Milk Allergic Infant Study

Subject	Study Product	Investigator Term	Visit 1 Date	Date First Study Product Intake	Adverse Event (AE) Start Date	AE Stop Date	Duration of AE (Days)	Severity	Relationship to Study Product	Additional Information	Antibiotic Use
R2203	NEO-SYN	Diarrhea	7/27/2011	8/15/2011	8/16/2011	8/19/2011	4	Mild	Not related	-	Antibacterial for systemic use: amoxicillin
R2503	NEO-SYN	Diarrhea	1/6/2012	1/20/2012	2/26/2012	3/1/2012	5	Mild	Not related	-	No antibiotics
R2901	NEO-SYN	Watery stools	12/1/2011	12/8/2011	12/28/2011	1/9/2012	13	Mild	Unlikely	-	No antibiotics
R0703	NEO	Diarrhea	11/30/2009	12/12/2009	1/25/2010	1/27/2010	3	Mild	Not related	-	Antibacterial for systemic use: rocephin
R2902	NEO	Watery stools	12/7/2011	12/14/2011	12/19/2011	12/20/2011	2	Mild	Not related	-	No antibiotics
R2902	NEO	Decreased stool consistency	12/7/2011	12/14/2011	2/4/2012	2/6/2012	3	Mild	Not related	-	No antibiotics

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3. Discussion and Conclusions on Safety and Adverse Events Healthy Infants and Cow's Milk Allergic Infants

The test formula was determined to be hypoallergenic as demonstrated by the negative results of the DBPCFC plus open challenge (7 d) in infants and children with cow's milk allergy. There were no serious adverse events during this phase.

At 16 wk, in the allergic infants, SCORAD values as an indicator of atopic dermatitis severity, plus dermatological and respiratory symptoms related to allergy were reduced in both the test formula and control groups, versus 0 wk. In particular, redness/soreness was statistically significantly reduced at wk 16 in the stool and GI subgroup for the test formula group, versus control.

Gastrointestinal symptoms were reduced in cow's milk allergic infants in both groups at 16 wk versus 0 wk. Analysis of the distribution of subjects over various categories of stool consistency (Freeman Halton test) showed a statistically significant difference between test and control group at baseline (P=0.025) in relation to stool consistency. At later time points in the study stool consistency distribution was not different between groups. Stool frequency, stool color and flatulence occurrence pattems did not differ significantly between groups at 16 wk.

Occurrence rates of severe and moderate adverse events in the allergic infant study were not statistically significantly different between the formula groups. Most of the adverse events were gastrointestinal and/or related to respiratory system disorders. There were no significant safety signals observed in this study for the test formula and it supported adequate growth in infants who were allergic to cow's milk, relative to the control formula. In addition, there were similar decreases in the number of allergenic events reported and number of subjects reporting allergenic events during the trial.

In the healthy infant study, there were no statistically significant difference in weight gain, achieved length or achieved head circumference between the two formula groups (Harvey et al 2014). In the stool and GI subgroup of the healthy infant growth study, the incidence of watery stools in the test formula group was 26-27%, on a per subject basis, across the 16 weeks of the study. In the stool and GI subgroup of the cow's milk allergic infant study, the incidence of watery stools at 16 wk was 15.2% (per subject basis). Electrolyte laboratory parameters were within normal range in both studies, suggesting that dehydration was not present. Other studies administering prebiotics in infants have reported similar results (van der Aa et al., 2010; Moro et al., 2006a), with stool consistency firming-up by 6 months (Moro et al., 2006a); breast-fed infants continue to exhibit soft stools through 22-26 wk (Danone MIPS Study, 2009). Additional

evidence provided by Nutricia Research BV (Table 21) indicated that for reported cases of diarrhea, the onset in the cow's milk allergic infant study generally did *not* coincide with the date of first study product intake, suggesting that the diarrhea in these subjects was not related to the test formula.

In the healthy infant study, three subjects used systemic antibiotics; 1 in the test formula group and 2 in the control formula group. In the cow's milk allergic infant study, 28 subjects used systemic antibiotics; 9 in the test formula group and 19 in the control formula group. Therefore increased antibiotic usage was observed in the control compared with the test formula group. As noted above, the studies in healthy term infants and cow's milk allergy infants showed comparable results in growth parameters for both the test and control formula groups.

Regarding the detailed assessment of infant growth, as a conservative measure, Nutricia Research BV provided the Expert Panel with the individual growth charts for any infants who exhibited abnormalities in their growth patterns in both the healthy infant and cow's milk allergic infant studies. These patterns were compared to published World Health Organization (WHO) Child Growth Standards (Acta Pædiatrica, 2006), which is supported by the Center for Disease Control (CDC). For the healthy infant study, 19 charts were examined; 7 subjects consumed test formula and 12 subjects consumed control formula. Of these, one subject in the test formula group was reported to have had an upper respiratory infection that may have impacted growth. Three subjects in the control formula group were reported to have had upper respiratory infection/otitis media, influenza and upper respiratory infection/pneumonia that may have impacted growth.

For the cow's milk allergic infant study, 27 charts were examined; 10 subjects consumed test formula and 17 subjects consumed control formula. Of these, two subjects in the test formula group were reported to have had vomiting/diarrhea and dehydration/otitis media that may have impacted growth. Two subjects in the control formula group were reported to have had upper respiratory infections that may have impacted growth.

In both studies, the several growth charts that were initially rated as containing abnormalities were, after review by the Medical Monitor of Nutricia Research BV, considered normal even though the infant was for example a bit heavy or a bit tall (+2 SD).

Overall the panel concluded that the study subjects tended to track well relative to growth and length.

E. ADDITIONAL CLINICAL EVIDENCE OF SAFETY FOR THE ADDITION OF OLIGOFRUCTOSE TO INFANT FORMULA

Additional published clinical trials of infants and young children given oligofructose with and without other oligosaccharides document the safety, tolerance and prebiotic potential of this fructan. These studies are summarized in Table 22 Like human milk oligosaccharides and GOS, oligofructose is bifidogenic; infants given oligofructose-supplemented formula tend to develop a colonic microbiota that more closely resembles that found in breast-fed infants compared with infants fed the same formula without oligofructose. Additionally, infant formula containing oligofructose produces more frequent and softer stools than does control formula, more closely matching the stool characteristics of breast-fed infants (Closa-Monsterolo et al., 2013).

Nine of the clinical studies summarized in Table 22 were of Orafti®P95 oligofructose at levels up to 4 g/L. Oligofructose was given alone or in combination with other prebiotics, including lc-inulin. Importantly, no increase in test-article-related adverse effects including anthropometric measures, clinical chemistries, serious adverse events or non-serious adverse symptoms such as flatulence or fussiness, or hydration status were noted.

A study that highlights the gastrointestinal tolerance to oligofructose in combination with lc-inulin was conducted in children from 1- 15 years of age with a compromised gut who were given formula containing 3.5 g/L oligofructose and lc-inulin. The formula was well tolerated and stool consistency was normalized in this study of sensitive subjects with compromised gut function (Khoshoo et al., 2010).

As mentioned previously, a recent study by Closo-Monasterolo et al. (2013) investigated safety of an infant formula containing 0.8 g/100 ml of a prebiotic combination containing the same oligofructose and lc-inulin ingredients as proposed for use in this GRAS determination, but in a 1:1 ratio 50% oligofructose and 50% lc-inulin) in healthy infants. The trial reported results for adverse events, water balance, blood parameters, stool characteristics and gut microbiota. Results were compared with a control group receiving an infant formula without the added prebiotics. A reference group of healthy breast-fed infants were also included. There were no differences between groups in terms of anthropometry, test article related adverse events, water balance or blood parameters. This study corroborates that supplementation of these fructans at 8 g/L (1:1 ratio oligofructose/lc-inulin) in infant formula during the first 4 months of life is safe and well tolerated.

Three studies examined the effects of oligofructose in infants or children suffering from acute diarrhea (Agustina et al., 2007; Hoekstra et al., 2004; Vandenplas et al., 2011). Agustina et al. administered low lactose infant formula plus precooked rice (15 g/L) with (n=30) or without

(n=28) *Lactobacillus rhamnosus* (5×10^9 CFU/ L), lc-inulin (1.5 g/L), soy polysaccharides (2 g/L) and zinc + iron (4 + 6 g/L) for 7 d to infants aged 3-12 mo who had been suffering from acute diarrhea. The resulting intake of lc-inulin was 0.2 g/kg/d, or 1.6 g/d. The diet was well tolerated and the duration of diarrhea was significantly reduced in the group receiving the supplemented formula. Hoekstra et al. enrolled boys aged 1-36 mo who had acute infectious diarrhea, and administered oral rehydration solution with (n=70) or without (n=74) 10 g/L of a mixture of non-digestible carbohydrates that included soy polysaccharide (25%), alpha-cellulose (9%), gum arabic (19%), fructooligosaccharides (18.5%), inulin (21.5%), and resistant starch (7%). The concentrations of oligofructose and lc-inulin were 1.85 g/L and 2.15 g/L, respectively. The oral rehydration solution was administered until diarrhea ceased. The duration of diarrhea was similar in both treatment groups. Vandenplas et al. recruited 111 children aged 2-186 mo with acute diarrhea and administered oral rehydration solution with (n=57) or without (n=54) a probiotic capsule containing 20 mg oligofructose plus five probiotic strains (*Streptococcus thermophilus, Lactobacillus rhamnosus, L. acidophilus, Bifidobacterium lactis, B. infantis*) for 7 d. The intake of oligofructose was 20 mg/d. The median duration of diarrhea was decreased in the synbiotic group compared to the placebo group. At the doses tested, oligofructose and lc-inulin did not appear to exacerbate the pre-existing diarrhea.

There were six studies in which levels of *Clostridia* spp. were measured in fecal samples during administration of oligofructose (Bettler and Kullen, 2007; Bettler et al., 2006; Brunser et al., 2006b; Costalos et al., 2008; Waligora-Dupriet et al., 2007; Xia et al., 2012). One study, which resulted in two publications, fed healthy term infants (1-13 mo) formula without (n=48) or with (n=47) 3 g Orafti®P95/L for 8 wk (~2.3 g/d) and compared the fecal Clostridia counts to infant receiving breast milk (n=50). Although both formula-fed groups had higher fecal Clostridia counts than the breast-fed group, the lack of a difference in the fecal Clostridia counts between the two formula-fed groups indicated that Orafti®P95 did not promote the growth of Clostridia (Bettler and Kullen, 2007). Bettler et al. (2006) administered formula with (n=105) and without 1.5 g Orafti®P95/L plus *Bifidobacterium animalis* ssp. *lactis* strain BI-07 (n=105) for 28 d to healthy toddlers (12-34 mo), resulting in an average intake of 300-600 mg oligofructose/d. Oligofructose caused a small, but statistically significant reduction in fecal Clostridia counts at day 28 in both the symbiotic and probiotic groups compared to the control group. Brunser et al. (2006b) fed children 1-2 yr old follow-on formula without (n=23) or with 4.5 g of a combination of Orafti®P95 and Raftiline (lc-inulin) (7:3 ratio)/L (n=18) for 3 wk. Consumption of the formula containing the combination of fructooligosaccharides resulted in an intake of 0.25 g oligofructose/kg/d (1.6 g oligofructose /d) and 0.11 g lc-inulin/kg/d (0.7 g lc-inulin /d) and lower fecal Clostridia counts. Costalos et al. (2008) administered formula without (n=70) or with (n=70) 0.4% GOS:lc-inulin (9:1) for 6 wk to healthy term infants within 0-14 d after birth. Although long-chain inulin intake was not determined, the prebiotic group exhibited

lower fecal Clostridia counts than the control group. Waligora-Dupriet et al. (2007) to receive administered either 2g Orafti®P95 (n=10) or 2 g of maltodextrin/day (n=10) for 21 d to healthy term infants (7-19 mo). Fecal Clostridia counts declined in the oligofructose-treated group but did not decline in the maltodextrin-treated group. Xia et al. (2012) recruited healthy term infants aged 0-6 d to study the effect of fructoligosaccharides on the intestinal microbiota. The infants received either human breast milk (n=22), control formula (n=24) or formula with 2.0 (n=25) or 3.0 g (n=26) oligofructose/L for 4 wk. The intake of oligofructose was not reported. Although the formula-fed infants had higher counts of *Clostridium difficile* than the breast milk-fed infants, the differences between formula-fed groups were not statistically significant. Thus, these studies show that the administration of oligofructose to infants and children does not adversely affect fecal *Clostridium* spp. populations.

F. IN VITRO FERMENTATION OF GOS/LC-INULIN AND OLIGOFRUCTOSE/LC-INULIN

The pattern of gas fermentation and short chain fatty acid (SCFA) production of human milk oligosaccharides, GOS, oligofructose and the GOS/lc-inulin and oligofructose/lc-inulin combinations have been evaluated in *in vitro* models simulating the human gut. These studies provide evidence of similar fermentation profiles. Shen et al. (2011) investigated the changes in bacterial populations and SCFA production as a result of inoculating fecal microbiota from formula fed infants with human milk oligosaccharides from a single donor, from multiple donors, and an oligosaccharide (Raftilose) plus GOS combination (1:1). All three substrates significantly increased numbers of *Bifidobacteria, Bacteroides*, and bacteria aligning with the clostridial cluster XIVa. Neither the oligofructose:GOS mix nor the human milk oligosaccharides supported the growth of the Clostridium perfringens-histolyticum group. SCFA production corresponded to changes observed in bacterial populations. Oligofructose:GOS maintained the bacterial community profiles similarly to human milk oligosaccharides. Similarly, Bunesova et al. (2012) tested fecal bacteria from 33 infants for growth in media containing 2% GOS or 2% oligofructose (Raftilose). Bifidobacteria dominated, reaching counts of 8.77 and 8.74 log CFU/mL after cultivation on GOS or oligofructose, respectively, using fecal samples from vaginally born babies. Slightly lower stimulation of bifidobacteria was observed in fecal samples from children born by caesarean section. If bifidobacteria were not detected in fecal samples, they were also not present after cultivation with either prebiotic. There were no significant differences in vitro in bacterial growth with oligofructose compared with GOS.

In an internal report from 2010, Danone conducted a study comparing different prebiotic ingredients and combinations for gas production and (SCFA) production using a static fecal slurry model to simulate conditions in the human gut. Fresh fecal samples were collected from breast-fed infants aged 0-6 months. Fecal samples were pooled and divided in smaller portions

and mixed with glycerol (10%) in an anaerobic cabinet and were stored at -80°C. The negative control was a blank experiment performed without any substrate at pH 5.5. Prebiotics alone or as a mix were added at a total concentration of 200 mg fiber (DP>2) per 6 ml of fecal suspension. The prebiotic substrates included: GOS (Vivinal®GOS), lc-inulin (Orafti®HP) and oligofructose (Orafti®P95). Vivinal®GOS contains 60% fiber on dry matter, the other 40% are sugars lactose (20%), glucose (19%) and galactose (1%). Gas production with GOS/lc-inulin is more or less similar to that produced with the oligofructose/ lc-inulin combination (Figure 5).

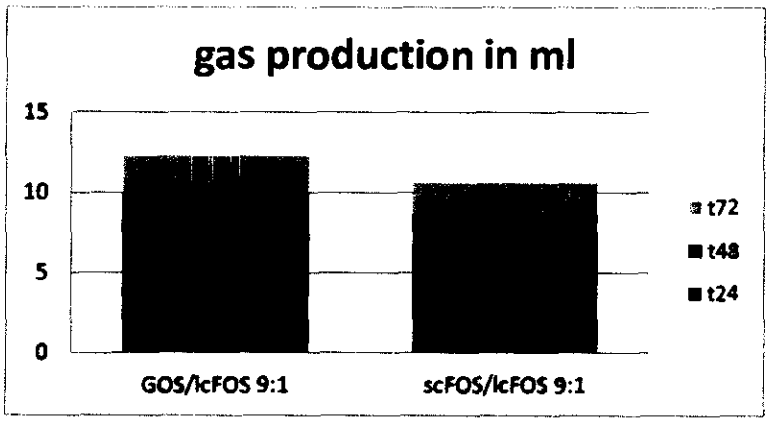

Figure 5. Gas Production in ml

b. Short Chain Fatty Acids (SCFA)

The fermentability of oligofructose/lc-inulin was demonstrated by the production of SCFA; oligofructose/lc-inulin produced more SCFA compared to GOS/lc-inulin (indicated by the scFOS/lcFOS in Figure 6). At 72 hours, luminal pH showed comparable values for the GOS/lcFOS (lc-inulin) and oligofructose/lc-inulin (scFOS/lc FOS) combinations.

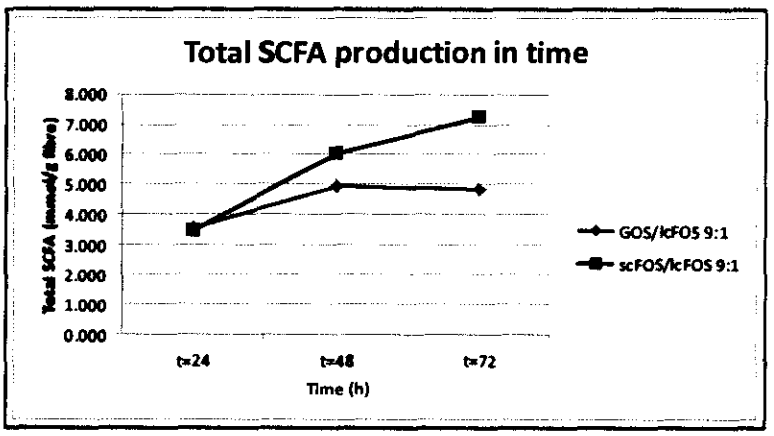

Figure 6. Total SCFA Production Over Time

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Table 22. Clinical Studies of Oligofructose in Infants and Children

Reference	Study Design	Subjects	Treatment and Numbers of Subjects	Intake and Duration	Results
Agustina et al. (2007) Aim: To assess the efficacy of special infant formula containing probiotics, prebiotics	Randomized double-blind, placebo-controlled	Infants aged 3-12 months, approximately 7.4 kg in weight, suffering from acute diarrhea.	Group 1 (Control Group): Received low lactose infant formula with precooked rice (15 g/L); n=28 Group 2: (Study Group): Received low lactose infant formula with precooked rice (15 g/L) and *L. Rhamonsus* LMG P-22799 (5x 10^9 CFU/L), lc-inulin (inulin not specified as to type; 1.5 g/L), soy polysaccharides (2 g/) and increased Zinc + Iron (4 and 6 g/L); n=30	Ad lib for 7 days (approx. 140 mL/kg/day) Intake of lc-inulin 0.2 g/kg/day or 1.6 g/day	• Early nutritional feeding during the acute phase of infantile diarrhoea was well tolerated. • Both groups showed similar growth. • Group 2 had improved energy intake and no increase in duration of illness compared to Group 1. • Duration of diarrhea was significantly reduced in Group 2. **Findings:** Study showed shortening of the duration of infantile diarrhea after oral rehydration by an infant formula, which includes inulin among other ingredients. However, no conclusions could be drawn about the contribution of each individual ingredient.

Table 22. Clinical Studies of Oligofructose in Infants and Children

Reference	Study Design	Subjects	Treatment and Numbers of Subjects	Intake and Duration	Results
Bettler and Kullen (2007; abstract) and Block et al., (2007; unpublished report) Aim: Evaluate the effects of oligofructose on bifidogenesis, incidence of adverse events, normal growth, fecal concentrations of other bacteria, level of fecal calprotectin, stool frequency and consistency, and anti-rotaviral activity of feces.	Prospective, randomized, double-blind, placebo-controlled, multi-center trail	Healthy term formula-fed and breast-fed infants aged 1-13 days	Group 1: Milk-based formula enriched with α-lactalbumin; n=48 Group 2: Milk-based formula enriched with α-lactalbumin with 3 g/L Orafti®P95; n=47 Group 3: Breast milk; n=50	~2.3 g/day for 8 weeks	• Differences in the incidence of and reasons for withdrawal and numbers of reported AEs and SAEs were not significant between the groups. Treatment-related adverse events were significantly less prevalent in the breast-fed reference group than in the formula-fed groups, but the 2 formula-fed groups did not differ from each other. • The breast-fed infants had significantly higher frequency of defecation and significantly softer stools than did the formula-fed infants, who did not differ significantly from each other. There were no differences between groups in body weight at any visit, nor in hydration status. • No difference was observed in bifidobacterial numbers between the 2 formula groups or between the formula groups and breast-fed reference group; all 3 groups showed increases in bifidobacteria from baseline to study completion. No differences between groups in numbers of bacteroides, but clostridia were consistently more numerous in the feces of the formula-fed infants than the breast-fed reference group until week 8, when there was no difference; the 2 formula groups did not differ from each other. Lactobacilli were higher in the oligofructose-fed group than in the control-formula group, which had significantly higher counts than did the breast-fed infants. No differences were seen between groups in fecal calprotectin concentration of anti-rotaviral activity. Findings: Formula acceptance and tolerance was similar for all three groups.

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Table 22. Clinical Studies of Oligofructose in Infants and Children

Reference	Study Design	Subjects	Treatment and Numbers of Subjects	Intake and Duration	Results
Bettler and Euler (2006) Aim: Evaluate growth and tolerance in infants fed formula supplemented with oligofructose	Randomized double-blind, placebo-controlled trial	Healthy term infants ≤ 14 days of age with baseline weight of approximately 3.5 kg.	Group 1: Cow's milk-based formula; n=66 Group 2: Cow's milk-based formula + 1.5 g Orafti®P95/L; n=72 Group 3: Cow's milk-based formula + 3.0 g Orafti®P95/L; n=74	Ad lib for 12 weeks Level of 1.5 or 3.0 g/L oligofructose; Intake was not reported.	• All formulas were safe and well tolerated based on anthropometric data, adverse event profiles and chemistry panel. Number of infants discontinuing formula due to adverse events was similar across formula groups. • The 3.0 g/L FOS group had fewer formula-related AEs, including those related to GI symptoms, than the other formula groups; in particular infants in this group experienced less constipation. The 1.5 g/L FOS group had slightly more AEs than the control group. The majority of AEs were milk-related and resolved without treatment or sequelae. **Findings:** Formula acceptance and tolerance was similar for all three groups.
Bettler et al. (2006) Aim: to assess the safety of addition of oligofructose *and B. animalis ssp. lactis* strain BI-07 in formula for healthy toddlers	Prospective, randomized, double-blind, placebo-controlled parallel-group trial	Healthy toddlers aged 12-34 months (mean = 22.1 months)	Group 1 (control group): Milk-based formula for toddlers; n=105 Group 2 (probiotic group): Milk-based formula for toddlers w/ 1 x 10^8CFU *Bifidobacterium lactis*/g (equiv. to 14 x 10^8CFU/L *B. lactis* strain BI-07); n=108 Group 3 (synbiotic group): Milk-based formula for toddlers w/ 1 x 10^8CFU *Bifidobacterium lactis* strain BI-07/g (equiv. to 14 x 10^8CFU/L *B. lactis* strain BI-07) and 1.5 g Orafti®P95/L; n=105	300-600 mg/ day for 28 days	• Both numbers of and reasons for withdrawal were similar in all feeding groups, none related to the study formulas. 186 toddlers reported at least one adverse event, but there were no differences between groups in the frequency, severity, or nature of the adverse events. • The amount of formula consumed was similar in all groups and gains in body weight did not differ between groups. By day 28 the proportion of toddlers with detectible fecal BI-07 had risen to 41% of those fed the probiotic formula and 50% of those receiving the synbiotic formula. By 2 weeks after feeding cessation, strain BI-07 was recovered in the feces of only 1 toddler. However, the groups did not differ significantly in fecal bifidobacteria, streptococci, or bacteroides counts. Relative to control, the probiotic and synbiotic groups had significantly increased counts of lactobacilli at day 7 and a small but statistically significant reduction in clostridia at day 28. **Findings:** Oligofructose in combination with probiotics was safe and well tolerated.

Table 22. Clinical Studies of Oligofructose in Infants and Children

Reference	Study Design	Subjects	Treatment and Numbers of Subjects	Intake and Duration	Results
Brunser et al. (2006a) Aim: To evaluate whether supplementation of formulas with lc-inulin or a probiotic, *Lactobacillus johnsonii* La1 (La1), could modulate the composition of the fecal microbiota of formula-fed infants, compared to BF infants.	Randomized, double-blind, placebo-controlled trial.	Healthy infants approximately 4 months old; approximately 6.5 kg in weight	Group 1 (Breast-fed): Breast milk; n=20 Group 2 (Control): Standard infant formula; n=23 Group 3 (Prebiotic): Standard infant formula + Raftilose P95 Oligofructose 2 g/L; n=18 Group 4 (Probiotic): Standard infant formula + $1.8*10^8$ La1 CFU/gram powder; n=20	13 wks average intake of 133, 126, 138 ml/kg bodyweight per day for control, prebiotic, and probiotic group, respectively. Intake of oligofructose of 0.25 g/kg/day or 1.6 g/day	• All formulas were well tolerated • Bifidobacterium is predominant genus in BF infants (20% of total faecal population vs. 4.6% in formula fed at enrollment) • No differences between the groups for weight, height, weight for height, weight for age and height for z-scores during the study (data not shown) • Oligofructose slightly increased bifidobacteria, which was not significant as absolute count, however was significant expressed as % of total population detected by FISH. • Only Probiotic group had increased faecal lactobacillus counts, similar to BF infants. **Findings:** The concentration of oligofructose used in this study showed little effect on the host microbiota but was well tolerated.
Brunser et al. (2006b) Aim: To determine the effects of milk formula with prebiotics on the microbiota of infants after antibiotic treatment.	Randomized, double-blind, controlled trial	Children 1-2 yrs of age after 1 wk amoxicillin treatment (for acute bronchitis); approximately 6.5 kg in weight	Group 1 (Control): Growing-up formula; n=66 Group 2 (Experimental): Growing-up formula + 4.5 g Raftilose P95 (oligofructose) and raftiline (lc-inulin) mix (70 : 30)/L); n=64	3 wk >500 mL/d Oligofructose 3.15 g/L, lc-inulin 1.35 g/L Intake of oligofructose of 0.25 g/kg/day or 1.6 g/day Intake of lc-inulin of 0.11 g/kg/day or 0.7 g/day	• No differences in drop outs were seen. Formulas were well tolerated; there was no deterioration of nutritional status and no differences in frequency of diaper rashes. GI symptoms were similar in both groups. No differences were observed in daily stool frequency and consistency. Higher counts of bifidobacteria were noted with prebiotic formula. **Finding:** Formula containing prebiotic oligosaccharides was well tolerated, led to normal somatic growth and suppresses the numbers of clostridia in faeces with a trend for higher percentage of stool bifidobacteria and lower percentage of *E. coli*.

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Table 22. Clinical Studies of Oligofructose in Infants and Children

Reference	Study Design	Subjects	Treatment and Numbers of Subjects	Intake and Duration	Results
Closa-Monasterolo et al. (2013)	Randomized double-blind, placebo-controlled, parallel trial	Healthy term infants randomized at birth for formula groups; breast-fed infants recruited.	Group 1 (control): Cow's milk-based infant formula; n=68 Group 2 (prebiotic): Cow's milk-based infant formula with 8 g oligofructose + lc inulin (50:50)/L; n=63 Group 3: Breastmilk; n=57	4 months Level of oligofructose: 4 g/L; range of intake 3.9 - 4.1 g/day Level of lc-inulin: 4 g/L; range of intake 3.9 - 4.1 g/day	• Microflora analysis showed a trend toward increased total counts of *Bifidobacterium* and significantly decreased *Bacteroides* and *Enterobacteriaceae* in the fecal flora of the prebiotic group compared to infants in the control group; flora was more similar to the breast-fed group. • Stools were more similar in frequency and consistency in the prebiotic group compare to those in the breast-fed group than in the control formula group. • No significant differences between formula groups in anthropometric measures, water balance parameters, blood parameters, formula intake and acceptance, or adverse events. **Finding:** Safety and efficacy of 8 g/L of oligofructose + lc-inulin in a 4 month feeding trial was demonstrated. No adverse effect on growth or hydration status was seen.
Costalos et al. (2008)	Randomized, double-blind, controlled trial	Healthy term infants within 0-14 days after birth; approximately 3 kg in weight	Group 1: Standard term formula; *n*=70 Group 2: Standard term formula + 0.4% prebiotics (GOS:lc-inulin 9:1); *n*=70	6 wk lc-inulin 0.4 g/L Intake of lc-inulin not estimated; formula intake not provided.	• Formula with prebiotics was well tolerated, and led to normal somatic growth • Stool softness and frequency were significantly higher in the prebiotics group • Prebiotics suppress the numbers of Clostridia in faeces with a trend for higher % of stool bifidobacteria and lower % *E.coli* **Finding:** Formula containing prebiotic oligosaccharides was well tolerated, lead to normal somatic growth and suppressed the numbers of clostridia in faeces with a trend for higher percentage of stool bifidobacteria and lower percentage of *E. coli*.

Table 22. Clinical Studies of Oligofructose in Infants and Children

Reference	Study Design	Subjects	Treatment and Numbers of Subjects	Intake and Duration	Results
Duggan et al. (2003) Aim: To study the effect of oligofructose on the prevalence of infant diarrhea	Prospective, randomized, double-blind, placebo-controlled trial.	Trial 1: Healthy infants aged 6-12 months (mean age = 8.8 months)	Group 1: Infant cereal; n=122 Group 2: Infant cereal supplemented with Orafti®P95 at a concentration of 550 mg/15 g cereal(1 serving); n=129	~675 mg/ day for 6 months	• Daily consumption of cereal did not differ between the groups. • There were no significant differences between the groups in the incidence or severity of diarrhea, the frequency with which diarrheal pathogens were isolated, constipation, blood chemistry, respiratory infections, side effects such as vomiting, or growth. **Findings:** Oligofructose did not provide a benefit in this population, but no adverse effects of treatment were noted.
		Trial 2: Healthy infants aged 6-12 months (mean age = 8.6 months)	Group 1: Infant cereal; n=154 Group 2: Infant cereal supplemented with Orafti®P95 at a concentration of 550 mg/15 g cereal(1 serving); n=164	~675 mg/ day for 6 months	• As in the first study, the reasons for study withdrawal (10 from the prebiotic group and 21 from the control group) did not differ. Also as in the first study, no differences were seen in any of the measured endpoints. **Findings:** Oligofructose did not provide a benefit in this population, but no adverse effects of treatment were noted.
ESPGHAN (2012) Aim: to determine the efficacy of a combination of oligofructose and lc-inulin for prevention of diarrhea and antibiotic associated diarrhea (AAD)	Randomized, double blind placebo controlled trail	Children ages 6 months to 14 years who were prescribed short term antibiotic therapy.	Group 1 (Placebo): maltodextrin given during the duration of antibiotic treatment; n=54 Group 2 (Prebiotic): Oligofructose + lc-inulin given during the duration of antibiotic treatment; n=51	Maximum of 5 g/day of oligofructose + lc-inulin	• No difference between prebiotic and placebo groups in the proportions of participants with diarrhea or AAD occurring during or up to 2 weeks after antibiotic therapy. • The combination of prebiotics was well tolerated, and no adverse events associated with the therapy were reported. **Findings:** Oligofructose + lc-inulin did not prevent diarrhea and AAD; however, frequency of diarrhea was low.

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Table 22. Clinical Studies of Oligofructose in Infants and Children

Reference	Study Design	Subjects	Treatment and Numbers of Subjects	Intake and Duration	Results
Euler et al. (2005) Aim: To determine the prebiotic effect of infant formula supplemented with oligofructose	Prospective, randomized, double-blind cross-over trial	Healthy term infants, 2 to 6 weeks of age	Group 1: Breastmilk; n=14 Group 2: Cow's milk-based infant formula with 1.5 Raftilose®P95 (oligofructose)/L; n=28 Group 3: Cow's milk-based infant formula with 3.0 g Raftilose®P95 (oligofructose)/L; n=30	1-week with 1-week washout preceding and following feeding. Intake not reported.	• At 7 days, mean counts of bifidobacteria were greater in the 1.5 g/L group than breast-fed or high dose group. Formula fed infants had higher counts of enterococci and bacteroides before oligofructose supplementation and these counts did not change after supplementation. • Oligofructose (3.0 g/L) resulted in more frequent and significantly softer stools. **Findings:** Supplementation of formula with up to 3.0 g/L oligofructose was safe but had minimal effect on fecal flora.
Firmansay et al. (2011) Aim: Growth and development of toddlers fed milk containing synbiotics	Randomized, double-blind, controlled clinical trial	12 months old healthy toddlers; approximately 8.8 kg in weight	Group 1 (Control): Cow's milk; n=142 Group 2 (Synbiotic): Cow's milk + *Bifidobacterium. longum* BL999 and *Lactobacillus. rhamnosus* LPR, a combination of oligo-fructose:lc-inulin (70 : 30), and long chain polyunsaturated fatty acids; n=148	12 months ≥400 mL/day Oligofructose 3.4272 g/L lc-inulin 1.4688 g/L Intake of oligofructose 1.37 g/day or 0.16 g/kg/day Intake of lc-inulin 0.59 g/day or 0.07 g/kg/day	• No treatment related safety issues were reported based on occurrence of SAEs and AEs, diary records, neurodevelopmental measures, interviews with parents and hematology results. No difference in drop outs was seen between groups • No difference in daily milk intake; weight gain was greater in synbiotics group between 12 and 16 months compared with control group. No differences were seen in length or head circumference. Compared with 12 months, there was a slight decrease in BMI in both groups at 16 months. No significant difference in stool frequency or any stool characteristics. • Increase in lactobacilli and enterococci counts between 12 months and 16 months in the synbiotics group **Findings:** Milk containing synbiotics and LCPUFA was well tolerated and promotes favourable gut colonization in healthy toddlers.

Table 22. Clinical Studies of Oligofructose in Infants and Children

Reference	Study Design	Subjects	Treatment and Numbers of Subjects	Intake and Duration	Results
Gerasimov et al. (2010) Aim: To assess the clinical efficacy and impact of *Lactobacillus acidophilus* DDS-1 , *Bifidobacterium lactis* UABLA-12 with fructo-oligosaccharide (FOS) on blood lymphocyte subsets	Randomized, double-blind, placebo-controlled trial	Children with atopic dermatitis aged 1-3 yrs; approximately 3.3 kg in weight.	Group 1 (Control): Rice maltodextrin powder (DDS®Junior UAS laboratories) twice a day for 8 wks; n=47 Group 2 (Probiotic): Rice maltodextrin powder with 5 x 10^{11}CFU *L. acidophilus* DDS-1 and *B. lactis* UABLA-12, FOS (50 mg non specified which FOS)/g twice a day for 8 wks; n=43	8 wks Intake of 100 mg FOS/day or 30 mg/kg/ day	• Status of atopic dermatitis as assessed percentage decrease in SCORAD improvement in probiotic group. • Use of topical steroids was lower in probiotic group **Findings:**: The administration of a probiotic combination containing *L. acidophilus* DSS-1, *B. lactis* UABLA-12, and FOS was associated with significant clinical improvement in children with atopic dermatitis.
Gotteland et al. (2005) Aim: To evaluate the effect of a probiotic, or synbiotics on *Helicobacter pylori* colonization in children	Randomized, open study	*H. pylori* positive asymptomatic children (8.4 +/- 1.64 y)	Group 1: Antibiotics; n=57 Group 2: Heat-killed and lyophilised *L. acidophilus* 10^9 twice daily; n=63 Group 3: Lyophilised *Saccharomyces boulardii* 25 mg + 5 g inulin (lc-inulin from Orafti) twice daily; n=62	8 wks Intake: lc-inulin of 10 g/d	• *H. pylori* eradication was successful in 12% of children in the *S. boulardii* + lc-inulin group. • Drop-outs were not significantly different among groups. **Findings:**: Probiotic plus lc-inulin treatment interfere with *H. pylori* in colonized individuals.

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Table 22. Clinical Studies of Oligofructose in Infants and Children

Reference	Study Design	Subjects	Treatment and Numbers of Subjects	Intake and Duration	Results
Hoekstra et al. (2004) Aim: To evaluate the efficacy and safety of a combination of non-digestible carbohydrates as an adjunct to oral rehydration therapy of acute infectious diarrhea	Randomized, double blind, placebo-controlled trial	Boys aged 1 to 36 months with diarrhea	Group 1 (Placebo): Oral rehydration solution (ORS); n=74 Group 2 (Fiber supplemented): ORS with A combination of non-digestible carbohydrates, including: 25% soy polysaccharide, 9% α-cellulose, 19% gum Arabic, 18.5% fructooligosaccharides, 21.5% inulin resistant, 7% starch; added at 10 g/L (Stimulance Multi Fibre Mix, Nutricia, Wageningen); n=70	ORS was given until diarrhea stopped •Level: oligofructose 1.85 g/L •inulin: 2.15 g/L Intake per day not reported.	• Duration of diarrhea, duration of hospital and risk of unscheduled iv rehydration stay was similar in both groups; no difference in significant adverse effects of fiber supplemented group compared to placebo. **Findings:** In boys with acute non-cholera diarrhoea with mild to moderate dehydration a combination of non-digestible carbohydrates was ineffective as an adjunct to oral rehydration therapy.
Holscher et al. (2012)	Randomized, double blind, placebo-controlled prospective trial	Healthy, term infants 2-8 weeks old	Group 1 (Control): Partially hydrolyzed whey control formula; n=47 (completed study); n=33 (eligible for stool analysis) Group 2 (Prebiotic): Partially hydrolyzed whey control formula with 4 g/L GOS: oligofructose (9:1); n=36 (completed study); n=33 (eligible for stool analysis) Group 3: Breast milk; n=40 (completed study); n=36 (eligible for stool analysis)	6 weeks Level: oligofructose 0.4 g/L Intake: 0.32 g oligofructose/day	• There was a higher attrition rate in the control formula group, but no differences in stool patterns, tolerance, growth or occurrence of adverse events among groups. Infants fed prebiotics had a higher absolute number and proportion of bifidobacteria than controls and did not differ from breastmilk fed infants. **Findings:** Prebiotic supplementation was well tolerated, increased abundance and proportion of bifidobacteria, and reduced fecal pH in health infants.

Table 22. Clinical Studies of Oligofructose in Infants and Children

Reference	Study Design	Subjects	Treatment and Numbers of Subjects	Intake and Duration	Results
Kapiki et al. (2007) Aim: To evaluate the bifidogenic effect of oligofructose in infant formula	Prospective, randomized, double-blind, placebo-controlled trial	Bottle-fed preterm infants less than 14 days old (mean age = 7.0 days) with gestational ages < 36 weeks (mean = 33.7 weeks), and admitted to a neonatal unit but other-wise healthy	Group 1 (Placebo): Standard preterm formula with 4 g/L maltodextrin; n=20 Group 2 (Prebiotic): Standard preterm formula with 4 g/L FOS (described as having been produced by partial enzymatic hydrolysis of chicory inulin, but no further information was provided); n=36	Intake was not reported; duration was 14 days Level: 4 g/L	• Infants in the placebo group gained significantly more weight and had significantly greater arm circumference, while those in the FOS group had greater body length (not significant). • Both formulas were well tolerated. Intake of the FOS-supplemented formula produced a significantly higher frequency of defecation and softer stools as well as significantly greater concentrations of fecal bifidobacteria and bacteroids and significantly lower numbers of *E. coli* and enterococci. **Findings:** Oligofructose was well tolerated and had a beneficial effect on microflora.
Khoshoo et al. (2010) Aim : to evaluate tolerance of a peptide-based formula with insoluble and prebiotic fiber in children with compromised gut function	Randomized, double-blind, cross-over trial	Children 1-15 years of age receiving 75-100 % of total calories from the formula orally or via feeding tube	Group 1 (Control): Partially hydrolyzed whey formula; n=14 Group 2: Partially hydrolyzed whey formula with 3.5 g oligofructose and inulin and 3.8 g insoluble fiber/L; n=14	2 weeks (2 weeks wash out) Levels: 3.5 g/L oligofructose and lc-inulin Intake of 3.5 g/day oligofructose + lc-inulin	• The fiber supplemented formula was well-tolerated and there were no drop outs; no differences in vomiting, abdominal pain, feeding intake, or weight gain compared to control. • Stool improved with fiber intake; stool consistency was softer (less hard stools) and the stool frequency was similar in both groups. • Children had a significantly lower proportion of stools characterized as watery when consuming fiber formula compared to control. **Findings:** The extremes of stool consistency were normalized with the fiber formula.

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Table 22. Clinical Studies of Oligofructose in Infants and Children

Reference	Study Design	Subjects	Treatment and Numbers of Subjects	Intake and Duration	Results
Lugonja et al. (2010) Aim: To compare the bifidogenic effects of breast milk and prebiotic-supplemented infant formula	Non-randomized, non-blinded, non-placebo-controlled study	Healthy infants aged 5 to 16 weeks (mean = 8.6 weeks)	Group 1 (Control): Breastmilk; n=10 Group 2: Term infant formula supplemented with a combination of inulin and oligofructose derived from chicory (no further information was provided) at a concentration of 4 g/L formula; n=11	Dose not reported; 28 days Level: 4 g/L of oligofructose + lc-inulin	• All infants exhibited normal rates of growth (length and weight) throughout the study; there was no difference between formula-fed and breast-fed infants. • There were no significant differences between groups in measures of intolerance, stool frequency, or stool consistency. • Counts of bifidobacteria increased significantly over the 28 days in both groups. Lactobacilli increased in both groups while aerobes, anaerobes, and fungi and yeasts decreased, but there were no significant differences between the formula and breast-fed groups. Total organic acids increased and pH decreased over time in both groups. • Most stools from infants in both groups were of normal consistency. The mean water content of the stools of infants receiving formula containing lc-inulin+oligofructose was 77.9%, non-significantly lower than the mean water content of breast-fed **infants'** stools (81.2%). **Findings:** 4 g/L oligofructose + lc-inulin was well tolerated and did not result in compromised water status.
Perez et al. (2010) Aim: To determine if probiotics maintain their immune-stimulating effects in children with a high exposure to microorganisms.	Randomized, double-blind, placebo-controlled trial	Children aged 9 months to 10 yrs who were vaccinated with DTP-Hib vaccine or 23-valent anti-pneumococcal vaccine.	Group 1 (Control): Low fat fermented milk; n=78 Group 2: Low-fat fermented milk with or without probiotics (*L. casei, L. acidophilus*) + oligofructose (950 mg/bottle) and inulin (240 mg/bottle); n=84	At least 4 months with a median of 124 days prior to vaccination and 34 days after vaccination. 1 bottle a day Intake levels: 950 mg oligofructose, 240 mg inulin per day	• No differences in immune parameters (serum levels of different immunoglobulins and Isoagglutinins) **Findings:** Prebiotic/probiotic supplementation of standard fermented milk had no benefit to children of low socio-economic status. The high natural rate of early microbial exposure in infants and children in this population may have accounted for the absence of an immune-stimulating effect by probiotics.

Table 22. Clinical Studies of Oligofructose in Infants and Children

Reference	Study Design	Subjects	Treatment and Numbers of Subjects	Intake and Duration	Results
Ripoll et al. (2011; Abstract) Aim: To assess the potential of oligofructose to increase the concentration of fecal Bifidobacteria	Randomized, double-blind, placebo-controlled trial	Formula-fed infants 4 months old	Group 1 (Placebo): Formula with 5 g/L maltodextrin; n= not provided Group 2 (Prebiotic): Formula with 5 g/L oligofructose; n=not provided	6 months >500 mL/d Intake 2.5 g oligofructose/day	• Increase in Bifidobacteria from 4-5 months of age was significantly greater in the oligofructose group • Growth and digestive tolerance were similar in control and prebiotic groups. **Findings:**: Oligofructose at 5 g/L in follow-on formula enhanced the growth of Bifidobacteria (especially in non-breast-fed infants) and was well tolerated.
Simakachom et al. (2011) Aim: To demonstrate the tolerance and safety of an enteral formula containing prebiotics/probiotics in critically ill children.	Randomized, double-blind, placebo-controlled trial	Critically ill patients between 1-3 yrs under mechanical ventilation needing enteral feeding	Group 1 (Control): Enteral formula; n=47 (ITT) Group 2 (Synbiotic): Enteral formula with 5 x 10^6 cfu *L. paracasei*/g, *B. longum* 2 x 10^6 cfu/g, 2.6 g/L oligofructose/ inulin mix, 2.8 g/L Acacia gum, and 43 mg/L DHA; n=47 (ITT)	7 days (investigated after 14 d) Intake of oligofructos/inulin mix not reported.	• Overall caloric intake and time to reach caloric goal were similar in groups • Abdominal distention, vomiting, and stool frequency were not affected by the supplementation with synbiotics • Synbiotic formula was safe and produced an increase in fecal bacterial groups previously reported to have beneficial effects. **Findings:** Enteral formula with synbiotics was well tolerated by children in intensive care units; it was safe and produced an increase in faecal bacterial groups of previously reported beneficial effects.
Vandenplas et al. (2011) Aim: To evaluate the additional benefit of Probiotical, a food supplement containing 5 probiotic strains and prebiotic fructo-oligosaccharides, above standard ORS treatment on the duration of acute diarrhea of likely infectious origin in primary health care in Belgian children.	Randomized, prospective placebo-controlled, un-blinded parallel trail	Children with acute diarrhea, median age 40 months (2-186 months)	Group 1: Hypo-osmolar oral rehydration solution (ORS) with placebo capsule (without symbiotics); n=54 Group 2: ORS rehydration with 1 Probiotical capsule containing 6.5 x 10^9 cfu *Streptococcus thermophilus*, 6.5 x 10^9 cfu *L. rhamnosus* and *L. acidophilus*, 6.5 x 10^9 cfu *B. infantis* and *B. lactis*, and 20 mg oligofructose; n=57	7 days Intake of oligofructose of 20 mg/day	• The number of children with normalised stool consistency was significant higher on day 2 and 3 in the Probiotical-supplemented group • In the placebo group more children received additional medication, 70 % in the placebo group versus 19 % in the Probiotical-supplemented group • There were no side effects reported by parents or physicians **Findings:**: Median duration of diarrhoea was shorter in synbiotic than in placebo group, associated with decreased prescription of additional medications.

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Table 22. Clinical Studies of Oligofructose in Infants and Children

Reference	Study Design	Subjects	Treatment and Numbers of Subjects	Intake and Duration	Results
Veereman-Wauters et al. (2011) Aim: To study physiological and bifidogenic effects of GOS, oligofructose, and long-chain inulin	Randomized, controlled trail	Healthy neonates	Group 1: Standard infant formula; n=21 Group 2: Standard infant formula with 8 g GOS/Orafti HP mix (90:10)/L; n=19 Group 3: Standard infant formula with 4 g Orafti Synergy1 (a 50:50 mix of Orafti HP and oligofructose) /L; n=21 Group 4: Standard infant formula with 8 g Orafti Synergy1 (a 50:50 mix of Orafti HP and oligofructose) /L; n=19 Group 5: Breast milk; n=29	28 days GOS:lc-inulin results in an intake level of 7.2 g/L GOS and 0.8 g/L lc-inulin Orafti Synergy1 results in a maximum intake of 4 g/L oligofructose, 4 g/L lc-inulin	• Length, weight and head circumference were comparable among groups. Regurgitation and vomiting scores were low and similar for all groups. • Stool frequency was similar in all groups, although breast-fed infants had the highest frequency. • Breast-fed group had the softest stools and children in the control group had the hardest stools. Stool consistency was comparable between the GOS:FOS and Orafti Synergy1 groups. • Total number of fecal bacteria increased in all prebiotic groups and resembled the breast-fed group • Bifidobacteria counts in the Orafti Synergy1 (8g/L) and GOS:FOS groups were comparable to the breast-fed group at day 14 and 28. **Findings:** Stool consistency and bacterial composition of infants taking the Orafti Synergy1 (8 g/L) or GOS:FOS – supplemented formula were closer to the breast-fed infants.
Waligora-Dupriet et al. (2007) Aim: study of tolerance and bifidogenic effects of oligofructose	Prospective, randomized, double-blind, placebo-controlled trial	Healthy term infants aged 7-19 months	Group 1 (Placebo): Maltodextrin; n=10 Group 2 (Oligofructose): Orafti®P95; n=10	2 g/day for 21 days	• Because common infections such as pharyngitis, otitis, and bronchitis and consequent use of antibiotics were frequent, only 10 infants in each group completed the study; all exclusions were due to antibiotic use. • Bifidobacteria populations increased while staphylococci and clostridia decreased in the Orafti•P95-supplemented group; the difference from the placebo group was statistically significant for clostridia but not for bifidobacteria or staphylococci. Other bacterial counts did not differ significantly • Flatulence, diarrhea, and vomiting were significantly less in the oligofructose group than in the placebo group. **Findings:** No intolerance due to oligofructose intake was noted.

Table 22. Clinical Studies of Oligofructose in Infants and Children

Reference	Study Design	Subjects	Treatment and Numbers of Subjects	Intake and Duration	Results
Xia et al. (2012) Aim: To study the effect of FOS on intestinal bacteria in term infants.	Randomized, double-blind, placebo-controlled trial	Healthy term infants 0 to 6 days of age	Group 1: Breast milk; n=22 Group 2: Cow's milk based formula; n=24 (ITT) Group 3: Cow's milk based formula with 2.0 g/L oligofructose; n=25 (ITT) Group 4: Cow's milk based formula with 3.0 g/L oligofructose; n=26 (ITT)	4 weeks Intake not reported.	• No difference in stool consistency and frequency or frequency of spit-up or vomit among groups. • Formula-fed infants had a greater abundance of *C. difficile* and *E. coli* than breast-fed infants, but had a similar abundance of *Bacteroids*, *Bifidobacterium* and *Lactobacillus*. Due to considerable variations within each group, differences in microbiota between groups were not statistically significant. **Findings:** The milk-based formula with or without oligofructose supported bifidobacterial and lactobacilli populations similarly to human milk, however did not suppress *E. coli* or *C. difficile* as effectively as human milk.
Yao et al. (2010; Abstract) Aim: Evaluated the effects of infant formula composition with and without oligofructose on stool characteristics and composition	Prospective, randomized, double-blind, parallel-group study	Healthy formula-fed term infants aged 7-14 days	Group 1 (Control): Standard formula Group 2: Standard formula with 40% sn-2 palmitate Group 3: Standard formula with 40% sn-2 palmitate and 3 g/L oligofructose Group 4: Standard formula with 40% sn-2 palmitate and 5 g/L oligofructose Group 5: Breast milk	~2.3 or ~3.8 g oligofructose /day for 8 weeks	• Withdrawals: 2 each from the breast milk group and the sn-2-supplemented group, 1 from the control group and 3.0-g/L oligofructose group, and 0 from the 5.0-g/L oligofructose group. • Infants receiving the sn-2-supplemented formulas with or without oligofructose had significantly less stool palmitate soaps and higher bifidobacteria counts than the control group and resembled the breast milk group; there was no difference in stool frequency. The sn-2-supplemented group also had significantly softer stools than did the control group, and the addition of oligofructose resulted in a further dose-dependent increase in stool softness; the 5.0 g oligofructose-supplemented group was not significantly different from the breast milk infants. • Physician-reported gastrointestinal events were few and not different among the 4 formula groups and the human-milk reference group; parental reports indicated no increase in the incidence of watery stools, gassiness, or other symptoms of intolerance with the addition of oligofructose.

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Table 22. Clinical Studies of Oligofructose in Infants and Children

Reference	Study Design	Subjects	Treatment and Numbers of Subjects	Intake and Duration	Results
					• Addition of up to 5.0 g oligofructose/L to formula had no effect on growth (weight, length, head circumference). **Findings:** Oligofructose up to 5 g/L was safe and well tolerated.
Zheng et al. (2006) Aim: Tolerance and effects of a fructo-oligosaccharide-containing enteral formula on faecal microbiota, nutritional status, biologic and immunologic outcomes of pediatric patients with cancer	Prospective, randomized, double-blind, placebo controlled trial	Hospitalized patients (1-12 y) diagnosed with cancer undergoing chemotherapy	Group 1: Enteral formula Group 2: Enteral formula with 2 g/L FOS (70:30 Raftilose:Raftiline)	13-30 days Average amount of FOS consumed was 60 mg/kg and 1.22 g/day	• Enteral formula containing FOS produced mild prebiotic effect without any gastrointestinal discomfort • Formulas were well-tolerated and accepted **Findings:** Oligofructose was well-tolerated.

Note: Underlined studies and text indicates use of Orafti®P95 oligofructose.

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EXPERT PANEL STATEMENT

We, the members of the Expert Panel, qualified by scientific training and experience to evaluate the safety of food and food ingredients, have performed a comprehensive and critical review of the available information and data on the safety and Generally Recognized As Safe (GRAS) status of a combination of oligofructose and long-chain inulin in exempt term infant formulas, and have determined oligofructose/lc-inulin to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), as described under 21 CFR §170.30(b). To demonstrate that oligofructose/lc-inulin is safe, and GRAS, under the intended conditions of use, the safety of the intake of oligofructose/lc-inulin has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to exempt infant formulas, and is based on generally available and accepted information.

The intended use of oligofructose/lc-inulin as an ingredient in a powdered amino acid-based exempt term infant formula used for the dietary management of food allergies has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. Oligofructose (Orafti®P95) and lc-inulin (Orafti®HP) are oligomers and polymers of fructose, otherwise known as fructans, which are derived from chicory (*Cichorium intybus L.*) root inulin.

2. Oligofructose and lc-inulin are prebiotics, which, by definition, are selectively fermented food ingredients that have a negligible systemic effects and allow specific changes in the composition of and/or activity in the gastrointestinal microbiota.

3. Orafti®P95, sold under the trade names Raftilose®, Raftilose®P95, and Beneo®P95, contains predominantly linear oligosaccharides three to six saccharides in length. Approximately 5% of the fructans in Orafti®P95 have a degree of polymerization (DP) more than six and 4% of the molecules have DP of two.

4. Orafti®HP, sold under the trade names, Raftiline®HP and Beneo®HP, contains primarily linear oligosaccharides and polysaccharides with degrees of

polymerization (DP) ranging from 10 or 60. The average DP is greater than or equal to 23 and less than 1% of the fructans have chains being shorter than five.

5. Product specifications are met consistently ensuring a food-grade product and stability has been shown over its guaranteed shelf life of three years assuring that the oligofructose and lc-inulin are suitable for use in exempt term infant formulas.

6. Orafti®P95 and Orafti®HP reproducibly meet compositional standards and comply with limits on contaminants appropriate for food-grade ingredients.

7. All processes and processing aids used in the production have been the subject of the GRAS notifications GRN 392 (on Orafti®P95) and GRN 477 (on Orafti®HP) to which FDA provided a "No Questions" response. Moreover, the manufacturer represents that all processing aids either comply with the Food Chemicals Codex monograph, comply with the uses and limits stipulated in Chapter 21 of the United States Code of Federal Regulations, or are GRAS for their intended use.

8. The manufacturer represents that all other food contact materials, such as plastics and packaging materials, comply with Regulation (EC) No 1935/2004, (EU) No 10/2011, 21 CFR 176.170 or 176.180 or equivalents thereof. Both Orafti®P95 and Orafti®HP have previously received a no questions letter from FDA (GRN 392; GRN 477).

9. Mature human milk contains 5.0 to 8.0 g oligosaccharides/L and although human milk oligosaccharides are unique in size, charge, composition and abundance, some oligosaccharides derived from other sources, have been shown to have similar effects as those in human milk.

10. Assuming that the infant formulas are the sole source of nutrition, the addition of 6.12 g oligofructose and 0.68 g lc-inulin/L to amino acid-based exempt term infant formulas as consumed will result in oligofructose intakes of 4.3 to 5.9 g/day and lc-inulin intakes of 0.5 to 0.7 g/day for one month-old and six month-old infants.

11. The combination of 6.12 g oligofructose and 0.68 g lc-inulin/L infant formula as consumed replicates the molecular size distribution of human milk oligosaccharides and maintains the osmolality of the formula in a range consistent with that recommended by the American Academy of Pediatrics.

12. Oligofructose, lc-inulin, and other prebiotics such as GOS have a long history of safe use.

    a. Oligofructose (Orafti®P95) was determined GRAS in 2011 for its use in term infant formula at a maximum level of 3 g/L formula as consumed and subsequently received a "no questions" letter from the FDA in 2012 (GRN 392).

    b. Lc-inulin (Orafti®HP) was determined GRAS in 2013 for its use in exempt and non-exempt term infant formulas at a level of 0.8 g/L formula as consumed (GRN477) and received a "no questions" answer from FDA in 2014.

    c. Infant formulas containing 8 g/L oligosaccharides, comprising a combination of 90% GOS (7.2 g/L) and 10% lc-inulin (0.8 g/L) have been marketed in Europe since 2000 with no reported adverse effects.

    d. Numerous clinical trials of oligofructose in infant formula at levels up to 4 g/L, given alone or in combination with other prebiotics, including lc-inulin documented that no increase in test-article-related adverse effects including anthropometric measures, clinical chemistries, serious adverse events or non-serious adverse symptoms such as flatulence or fussiness, or hydration status are produced from oligofructose ingestion.

13. Two clinical studies administering an amino acid-based exempt term infant formula containing 6.12 g/L oligofructose and 0.68 g lc-inulin/L as consumed to healthy and cow's milk allergic infants showed that the formula was well tolerated. The test formula used in these studies also contained 1.2 g/L acidic oligosaccharides plus *Bifidobacterium breve* M-16V (1.47×10^{10} colony forming units/L).

    a. When administered to term healthy infants for 16 wk, the addition of 8 g/L prebiotic (including the 6.8 g/L oligofructose/lc-inulin (9:1) and 1.2 g/L acidic oligosaccharide), as consumed, to a test infant formula supported growth similarly as did the control formula alone. For infants consuming the test formula, approximately ¼ of all stools assessed were rated as watery stools throughout the course of the study, but this did not appear to contribute to serious adverse events, or an increased dropout rate. In addition, there was no statistically significant difference in laboratory blood parameters such as sodium, potassium and chloride between study groups. Similar rates of watery

or soft stools have been reported at similar time points in other studies administering oligosaccharides at comparable levels such as GOS/lc-inulin (9:1) at 8 g/L (van der Aa et al., 2010) and 8 g GOS/lc-inulin/L (Moro et al., 2006a). Adverse event rates (mild, moderate and severe) in the test and control formula groups were 63 and 88%, respectively; adverse events were mainly gastrointestinal-related disorders and infections. For both categories, adverse event rates were lower in the test formula group versus control. There was higher incidence of constipation in the control formula group (27%) versus test formula group (5%). Four severe adverse events were reported in the test group (3% of total adverse events in this group); four severe adverse events were also reported in the control group (2% of total adverse events in this group). A total of seven serious adverse events occurred in the study (2 events in the test formula group and five events in the control formula group). There were no safety signals observed in the oligofructose/inulin formula group, versus control.

b. When consumed by cow's milk allergic infants and children, the formula containing 8 g/L prebiotic (including the 6.8 g/L oligofructose/lc-inulin (9:1) combination and 1.2 g/L acidic oligosaccharide) was determined to be hypoallergenic as demonstrated by negative results in a double-blind, placebo-controlled food challenge plus a subsequent open challenge of seven d. There were no serious adverse events.

c. When administered to cow's milk allergic infants and children, both the control formula and test formula containing 8 g/L prebiotic (including the 6.8 g/L oligofructose/lc-inulin (9:1) combination and 1.2 g/L acidic oligosaccharide) significantly reduced atopic dermatitis severity, as well as other dermatological and respiratory symptoms related to atopy. In particular, redness/soreness was significantly reduced at 16 wk in a stool and gastrointestinal analysis subgroup. Fourteen subjects experienced events categorised as diarrhea in this study; 12 were in the test group and two were in the control group (p=0.004). Of the 12 subjects in the test group experiencing diarrhea, four experienced diarrhea prior to study formula ingestion and one was treated with amoxicillin; therefore, the diarrhea in these subjects was likely not related to the test formula. Of the remaining seven subjects in the test group who experienced diarrhea, only one exhibited diarrhea immediately upon consumption of the test formula; the remaining six subjects had diarrhea

occurring well after the date of the first study product intake. These cases indicate the diarrhea was unlikely to be related to the test product. None of cases required further action. Stool frequency, stool color, stool consistency and flatulence occurrence did not differ between groups at 16 wk, suggesting good tolerance. Overall adverse event rates (mild, moderate and severe) were not statistically significant different between groups; the adverse event rate was 79.6% and 67.9% in the test and control formula groups, respectively; the most frequent types of adverse events were GI system disorders and respiratory system disorders. Severe and moderate adverse event rates were not statistically different across the two groups. There was a statistically significant (p=0.01) difference between groups at 16 wk (change from baseline) for hemoglobin, with a mean value in the test group of 118 g/L and a mean value in the control group of 123 g/L. Mean levels of alkaline phosphatase were significantly increased (p=0.018) in the test group (292 U/L) versus the control group (246 U/L). As both of these results were within the normal reference ranges, these differences were not deemed clinically relevant. Although there was a decrease in the number of allergenic events reported and number of subjects reporting allergenic events during the trial, the decrease was apparent in both study groups.

Determination of the GRAS status of oligofructose/lc-inulin under the intended conditions of use has been made through the deliberations of Roger Clemens, Dr PH, CNS, FACN, FIFT, David Jenkins, MD, DSc, PhD, and Judith Jones, MD, PhD, FISPE. These individuals are qualified by scientific training and experience to evaluate the safety of infant formulas. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of oligofructose/lc-inulin and the human exposure to oligofructose/lc-inulin resulting from its intended use as an ingredient in a powdered amino acid-based exempt term infant formula used for the dietary management of food allergies and have concluded:

There is no evidence in the available information on oligofructose/lc-inulin that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when oligofructose/lc-inulin is used at levels that might reasonably be expected from the proposed applications. Oligofructose/lc-inulin is GRAS for use in a powdered amino acid-based exempt term infant formula used for the dietary management of food allergies as proposed by Danone Trading Medical B.V.

Therefore, oligofructose/lc-inulin is safe and GRAS at the proposed levels of addition to a powdered amino acid-based exempt term infant formula. Oligofructose/lc-inulin is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

It is our opinion that other experts qualified by training and/or experience to evaluate the safety of food and food ingredients would concur with these conclusions.

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