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Clasado
Delivering wellness through knowledge

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Division of
Biotechnology and
GRAS Notice Review

GRN 000495

Clasado Inc
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United States

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Web.www.clasado.com

December 18, 2013

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Dr. Gaynor:

Re: GRAS Exemption Claim for Galacto-Oligosaccharides (Infant Formula Use)

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized as Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)], I am submitting one hard copy and one electronic copy (on CD), as the notifier [Clasado Inc., 1620 Pinetree Drive Pittsburgh, PA, 15241, USA], a notice of the determination, on the basis of scientific procedures, that galacto-oligosaccharides, produced by Clasado Inc., as defined in the enclosed documentation, are GRAS under specific conditions of use as a food ingredient in infant formula and follow-on formula, and therefore, are exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act*. Information setting forth the basis for the GRAS determination, which includes detailed information on the notified substance and a summary of the basis for the GRAS determination, as well as a consensus opinion of an independent panel of experts in support of the safety of galacto-oligosaccharides under the intended conditions of use, also are enclosed for review by the agency.

The enclosed electronic files for the Notice entitled, "GRAS Exemption Claim for Galacto-oligosaccharides" were scanned for viruses prior to submission and is thus certified as being virus-free using McAfee VirusScan 8.8.

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

Sincerely,

(b) (6)

Michael Moore
Chief Operating Officer, Americas
Clasado Inc.
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Michael Moore
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Mike.Moore@Clasado.com

GRAS Exemption Claim for Galacto-oligosaccharides

Submitted to:

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD
U.S.A. 20740-3835

Submitted by:

Clasado Inc.
1620 Pinetree Drive
Pittsburgh, PA
15241
USA

October 10, 2013

GRAS Exemption Claim for Galacto-oligosaccharides

Table of Contents

Page

I. GRAS EXEMPTION CLAIM 3
- I.A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)] 3
- I.B. Name and Address of Notifier 3
- I.C. Common Name of the Notified Substance 3
- I.D. Conditions of Intended Use in Food 3
  - 1. Foods in which the Substance is to be Used 3
  - 2. Purpose for Which Substance is Used 4
  - 3. Description of the Population Expected to Consume the Substance 4
- I.E. Basis for the GRAS Determination 4
- I.F. Availability of Information 4

II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE NOTIFIED SUBSTANCE 5
- II.A. Identity 5
  - 1. Common or Usual Name 5
  - 2. Trade Name 6
- II.B. Method of Manufacture 6
  - 1. Raw Materials and Processing-aids 6
  - 2. Description of Manufacturing 6
- II.C. Specifications and Product Analysis 9
  - 1. Specifications 9
  - 2. Product Analysis 10
  - 3. Comparison of Clasado Galacto-oligosaccharides with Other GRAS Sources 11
- II.D. Stability 13

III. SELF-LIMITING LEVELS OF USE 13

IV. DETAILED SUMMARY OF THE BASIS FOR CLASADO'S GRAS DETERMINATION 14
- IV.A. Consumption Estimates 15
- IV.B. Absorption, Distribution, Metabolism and Excretion 15
- IV.C. Toxicological Studies 16
  - 1. Developmental and Reproductive Toxicity Studies 17
- IV.D. Human Studies 17
  - 1. Adults 17
  - 2. Studies Conducted in Infants 21
- IV.E. Nutrient Interactions 33
- IV.F. Allergy 33
- IV.G. Expert Panel Evaluation 34

IV.H Conclusions 35

V. REFERENCES 36

Appendix A Expert Panel Consensus Statement: Clasado Galacto-oligosaccharides are Generally Recognized as Safe (GRAS) for Use in Term Infant Formula and Follow-On Formula

Figures and Tables

Figure II.B-1 Schematic Overview of the Manufacturing Process for Clasado Galacto-Oligosaccharides 8
Figure II.C-1 Production of GOS from Lactose *via* Galactosidase.....12

Table II.B-1 Raw Materials and Processing-Aids..... 6
Table II.C-1 Specifications for Clasado Galacto-Oligosaccharides 9
Table II.C-2 Summary of the Chemical Product Analysis for 4 Lots of Clasado Galacto-Oligosaccharides 10
Table II.C-4 Summary of the Allergen Testing for 3 Lots of Clasado Galacto-Oligosaccharides 11
Table II.C-5 Comparison of Clasado Galacto-Oligosaccharide and Other GRAS Sources of Galacto-Oligosaccharides 13

I. GRAS EXEMPTION CLAIM

I.A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)]

Clasado Inc., hereby claim that the use of galacto-oligosaccharides, as described in Section I.D below, is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because we have determined that such uses are Generally Recognized as Safe (GRAS).

Signed,

Graham C. Waters CEO Clasado Inc. graham.waters@clasado.com	Date

I.B. Name and Address of Notifier

Clasado Inc.
1620 Pinetree Drive
Pittsburgh, PA 15241
USA

I.C. Common Name of the Notified Substance

Galacto-oligosaccharides

I.D. Conditions of Intended Use in Food

1. Foods in which the Substance is to be Used

Clasado intends to market their galacto-oligosaccharides (GOS) as an ingredient for addition to term infant formula and follow-on formula at a use level providing up to 7.2 g of galacto-oligosaccharides per L of the reconstituted or ready to consume product.

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Signed,

(b) (6)

October 22, 2013

Graham C. Waters
CEO
Clasado Inc.
graham.waters@clasado.com

Date

I.B. Name and Address of Notifier

Clasado Inc.
1620 Pinetree Drive
Pittsburgh, PA 15241
USA

I.C. Common Name of the Notified Substance

Galacto-oligosaccharides

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1. Foods in which the Substance is to be Used

Clasado intends to market their galacto-oligosaccharides (GOS) as an ingredient for addition to term infant formula and follow-on formula at a use level providing up to 7.2 g of galacto-oligosaccharides per L of the reconstituted or ready to consume product.

2. Purpose for Which Substance is Used

Galacto-oligosaccharide ingredients are intended for addition to term infant formula and follow-on formula as a dietary source of non-digestible oligosaccharides which are generally recognized to be a safe and suitable alternative to non-digestible human milk oligosaccharides that are present in high concentrations within human milk from lactating women (Oozeer *et al*., 2013).

3. Description of the Population Expected to Consume the Substance

Under their intended conditions of use Clasado galacto-oligosaccharides are expected to be consumed by term infants and toddlers within the general United States (U.S.) population who may reasonably be expected to consume infant formula and/or follow-on formula products.

I.E. Basis for the GRAS Determination

Pursuant to Title 21, Section 170.30 of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2013a), galacto-oligosaccharides have been determined to be GRAS on the basis of scientific procedures.

I.F. Availability of Information

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

Clasado Ltd.
1620 Pinetree Drive
Pittsburgh, PA 15241
USA

Attn:
Michael Moore
Chief Operating Officer, Americas
Mike.Moore@Clasado.com

Should the FDA have any questions or additional information requests regarding this notification, Clasado will supply these data and information.

II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE NOTIFIED SUBSTANCE

II.A. Identity

There is no globally-adopted definition of galacto-oligosaccharides; however, they have been defined as *"a mixture of those substances produced from lactose, comprising between 2 and 8 saccharide units, with one of these units being a terminal glucose and the remaining saccharide units being galactose and disaccharides comprising 2 units of galactose*" (Tzortzis and Vulevic, 2009). Food Standards Australia New Zealand (FSANZ) has defined galacto-oligosaccharides in the following manner: "*… The term 'galacto-oligosaccharides' (sometimes referred to as oligogalactosyl-lactose) is used consistently to describe those substances comprised of between two and eight saccharide units with one of these units being a terminal glucose and the remaining saccharide units being galactose*." (FSANZ, 2008). Similarly, the Scientific Committee on Food (SCF) provided the following characterization during their safety evaluation of the use of galacto-oligosaccharides in infant formula: "*Oligogalactose is produced from lactose with the help of a bacterial β-galactosidase, it contains one molecule of glucose and typically between 1 and 7 molecules of galactose.*" (SCF, 2001a,b). Galacto-oligosaccharides as manufactured by Clasado are synthesized from food grade lactose using the transgalactosidic activity of a microbial derived *beta*-galactosidase enzyme preparation which catalyzes the extension of lactose to form a mixture of *beta*-linked disaccharide, trisaccharide, tetrasaccharide and pentasaccharide chains in configurations depicted in Table II.A-1. The ingredient is manufactured as a syrup with a moisture content of up to 30%.

Table II.A-1 Composition of Clasado Galacto-Oligosaccharides

Disaccharides	Gal-β-(1,3)-Glc Gal-β-(1,3)-Gal Gal-β-(1,6)-Glc Gal-α-(1,6)-Gal
Trisaccharides	Gal-β-(1,3)-Gal-β-(1,6)-Gal Gal-β-(1,6)-Gal-β-(1,4)-Glc Gal-β-(1,6)-Gal-β-(1,6)-Gal Gal-β-(1,3)-Gal-β-(1,4)-Glc
Tetrasaccharides	Gal-β-(1,6)-Gal-β-(1,6)-Gal-β-(1,4)-Glc Gal-β-(1,3)-Gal-β-(1,6)-Gal-β-(1,6)-Gal Gal-β-(1,6)-Gal-β-(1,6)-Gal-β-(1,6)-Gal
Pentasaccharides	Gal-β-(1,6)-Gal-β-(1,6)-Gal-β-(1,6)-Gal-β-(1,4)-Glc Gal-β-(1,3)-Gal-β-(1,6)-Gal-β-(1,6)-Gal-β-(1,4)-Glc

Gal = galactose; Glc = glucose

1. Common or Usual Name

Galacto-oligosaccharides; GOS

2. Trade Name

Not applicable

II.B. Method of Manufacture

1. Raw Materials and Processing-aids

All raw materials and processing-aids used for manufacture of Clasado galacto-oligosaccharides, as described herein, are food grade ingredients[1] permitted by U.S. regulation, or previously determined to be GRAS, for their respective uses during manufacturing (Table II.B-1).

Table II.B-1 Raw Materials and Processing-Aids

Material	Purpose	Regulatory Status
Lactose	Substrate for galacto-oligosaccharide synthesis	FCC, 2012
beta-galactosidase	Enzyme preparation	Previously determined to be GRAS (U.S. FDA, 2013b)
Sodium citrate	pH control	FCC, 2012 21 CFR § 184.1751 (U.S. FDA, 2013a)
Potassium citrate	pH control	FCC, 2012 21 CFR § 184.1625 (U.S. FDA, 2013a)
Maltodextrin	Drying aid	FCC, 2012 21 CFR §184.1444 (U.S. FDA, 2013a)
Whey Protein Concentrate	Drying aid	FCC, 2012 21CFR§184.1979(c) (U.S. FDA, 2013a)
Calcium phosphate	Anti-caking aid	FCC, 2012 21 CFR §182.1217 (U.S. FDA, 2013a)

2. Description of Manufacturing

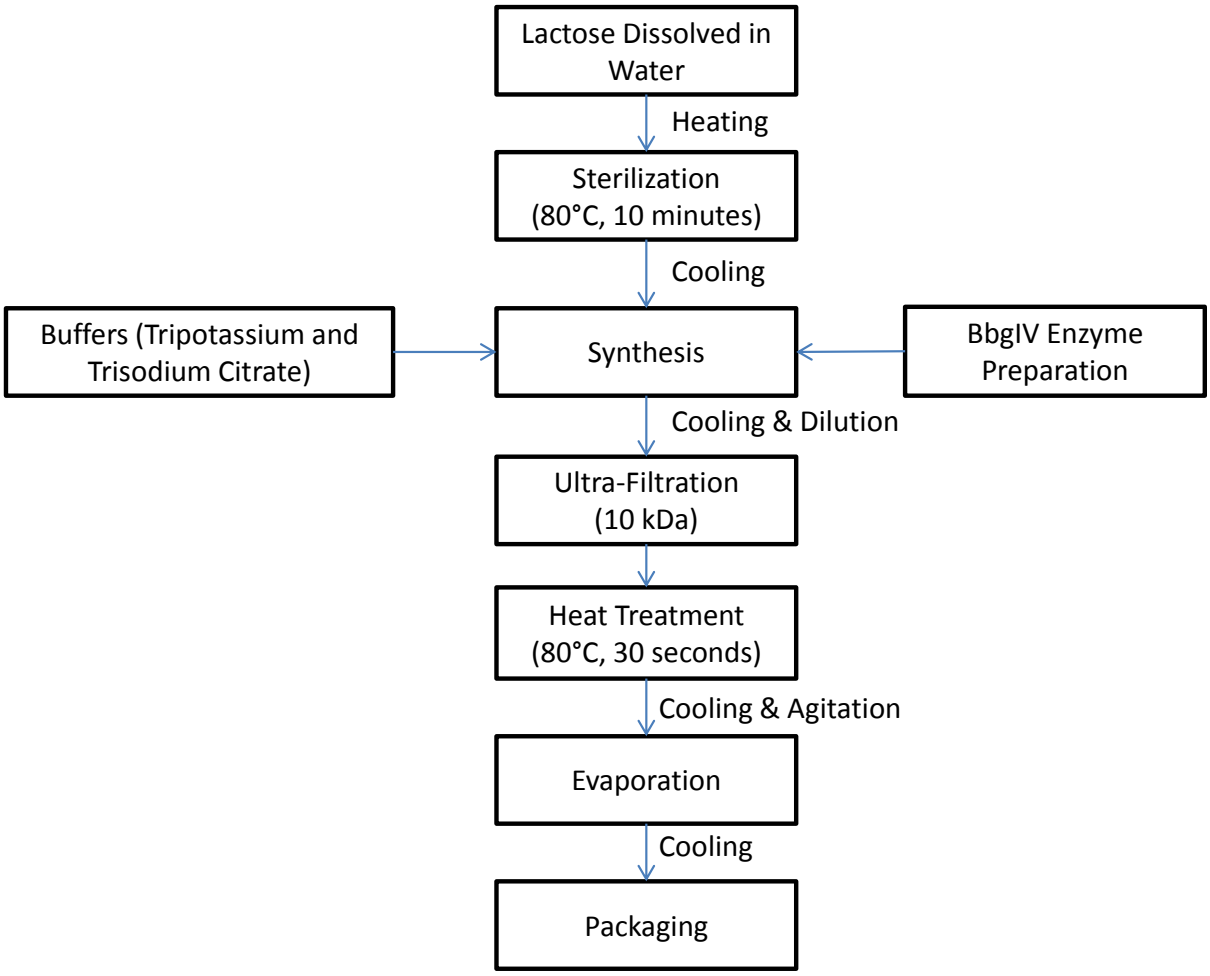
Clasado galacto-oligosaccharides are produced from food grade lactose *via* the transgalactosylation activity of a *beta*-galactosidase enzyme preparation. The process is initiated by dissolution of the enzyme preparation and appropriate buffers in cold water for several hours. In a separate vessel food-grade lactose is dispersed in slightly heated water using a Venturi mixer which is then heated to 80°C (176°F) until dissolution of the lactose is achieved. The solution is held at 80°C for 10 minutes to sterilize the solution. The buffer solution is then transferred to a stainless-steel reaction vessel, the solution cooled to appropriate synthesis temperatures followed by addition of the enzyme preparation. The solution is continually agitated for the duration of the synthesis process as lactose is polymerized to a mixture of GOS. The reaction is monitored using cryoscopy.

[1] Compliant with the specifications set forth in the Food Chemicals or equivalent international food or pharmacopeia standard (*e.g.*, JECFA, CODEX, EP).

Upon completion of the synthesis reaction, the mix is cooled, diluted in-line, and ultra-filtered using spiral wound 10 KDa membranes to remove the enzyme preparation, and residual milk proteins and peptides, the latter being minor components in food-grade sources of lactose. The mix is heat treated in bulk at 80°C (176°F) for 30 seconds for sterilization, and then cooled and further agitated prior to evaporation. After a second in-line heating step, the solution is evaporated to a concentration in excess of 70% total solids (Aw = 0.75), and then cooled, filtered (1 mm), and packed under hygienic conditions. The galacto-oligosaccharide syrup can be further refined *via* the use of nano-filtration to reduce the residual concentrations of monosaccharides (glucose and galactose) in the ingredient, and/or can be spray-dried with suitable drying aids to produce a powder formulation.

The finished product is stored under ambient conditions and released following quality control checks. See Figure II.B-1 for a schematic overview of the manufacturing process. Product specifications for the ingredient and corresponding batch analyses are presented below in Section II.C.

Figure II.B-1 Schematic Overview of the Manufacturing Process for Clasado Galacto-Oligosaccharides



II.C. Specifications and Product Analysis

1. Specifications

Food grade chemical and microbiological specifications for Clasado galacto-oligosaccharides are presented in Table II.C-1 below.

Table II.C-1 Specifications for Clasado Galacto-Oligosaccharides

Specification Parameter	Specification	Method
Dry matter (%)	Minimum 70	By calculation
Moisture (%)	Maximum 30	AOAC 925.45.D.
Protein (%, on DM)	< 0.3	AOAC 992.15.
Ash (%, on DM)	< 1.5	ISO 936:1998
Galacto-oligosaccharide (%)	≥57 on DM	AOAC 2001.02
Lactose anhydrous (%)	< 26 on DM	AOAC 2001.02
Glucose anhydrous (%)	< 23 on DM	AOAC 2001.02
Galactose anhydrous (%)	>0.8 on DM	AOAC 2001.02
Disaccharides (% GOS; lactose excluded)	> 30	AOAC 2001.02
Trisaccharides (% GOS)	>30	AOAC 2001.02
Tetrasaccharides (%GOS)	>5	AOAC 2001.02
Pentasaccharides and greater (% GOS)	>2	AOAC 2001.02
Heavy Metal Specifications		
Lead (ppm)	<0.1	ICP-MS UKAS Accredited Lab Method -based on NIOSH Method 73000, BS EN 13805:2002, BS EN 15763:2009, BS EN 13804:2002
Cadmium (ppm)	<0.1	
Mercury (ppm)	<0.1	
Microbiological Specifications		
Total viable count (CFU/g)	$<3.5*10^3$	BS EN ISO 4833:2003
Salmonella spp. (per 25 g)	Not detected	BS EN ISO 6579
Mesophilic spore count (spores/g)	$<1.5*10^3$	BS EN ISO 6887-1
Mesophilic anaerobic spore count (spores/g)	$<1.5*10^3$	BS EN ISO 6887-1: 1999
Staphylococcus aureus (CFU/g)	<10	BS EN ISO 6888-1:1999
Enterobacteriaceae (CFU/g)	NS	BS EN ISO 21528-2:2004.
Yeasts and Molds	<10	BS ISO 21527-2:2008
Escherichia coli (CFU/g)	<10	BS ISO 4832:2006
Cronobacter sakazakii	NS	FDA-BAM
Bacillus sp. (per 25 g)	NS	FDA-BAM

BS EN = British Standards European Norm; CFU = colony forming units; ISO = International Organization for Standardization (Betts RP, Oscroft CA, Baylis CL (2004). *A Code of Practice for Microbiology Laboratories Handling Food, Drink and Associated Samples, 3rd revised edition*. Gloucestershire, UK: Campden & Chorleywood Food

Table II.C-1 Specifications for Clasado Galacto-Oligosaccharides		
Specification Parameter	**Specification**	**Method**

Research Association.)

2. Product Analysis

2.1 Chemical Analysis of Clasado Galacto-Oligosaccharides

Analysis of 4 lots of Clasado galacto-oligosaccharides demonstrates that the manufacturing process produces a consistent product that meets specifications. A summary of the chemical analysis for the 4 lots of Clasado galacto-oligosaccharide syrup is presented in Table II.C-2.

Table II.C-2 Summary of the Chemical Product Analysis for 4 Lots of Clasado Galacto-Oligosaccharides

Specification Parameter	Specification	Manufacturing Lot			
		M800010	**M800011**	**M800012**	**M800013**
Dry matter (%)	Minimum 70	74	73.2	73.6	73
Moisture (%)	Maximum 30	26	26.8	26.4	27
Protein (% DM)	< 0.3	< 0.1	< 0.1	< 0.1	< 0.1
Ash (%, on DM)	< 1.5	0.26	0.25	0.26	0.24
Galacto-oligosaccharide (%)	≥57 on DM	64.3	63.8	62.6	66.1
Lactose anhydrous (%)	< 26 on DM	13	12	14	10.1
Glucose anhydrous (%)	< 23 on DM	21.5	22.5	22.1	21.8
Galactose anhydrous (%)	>0.8 on DM	1.5	1.7	1.3	2
Disaccharides (% GOS; lactose excluded)	> 30	41.5	39.7	40.5	43
Trisaccharides (% GOS)	>30	32	33.8	33.2	34
Tetrasaccharides (%GOS)	>5	17	18.5	18.1	16
Pentasaccharides and greater (% GOS)	>2	9.5	8	8.2	7
Heavy Metals					
Lead (ppm)	< 0.1	< 0.02	< 0.02	< 0.02	< 0.005
Cadmium (ppm)	< 0.1	< 0.01	< 0.01	< 0.01	< 0.001
Mercury (ppm)	< 0.1	< 0.01	< 0.01	< 0.01	< 0.003

DM = dry matter; NA = not applicable; NS = not specified; ppm = parts per million

2.2 Microbiological Analysis

Analysis of 4 lots of Clasado galacto-oligosaccharides demonstrating compliance with the product specifications is presented in Table IIC-3.

Table II.C-3 Summary of the Microbiological Product Analysis for 4 Lots of Clasado Galacto-Oligosaccharides

Specification Parameter	Specification	Manufacturing Lot			
		M800010	M800011	M800012	M800013
Total viable count (CFU/g)	$<3.5*10^3$	<5	<5	<5	35
Salmonella spp. (per 25 g)	ND	ND	ND	ND	ND
Mesophilic spore count (spores/g)	$<1.5*10^3$	<5	<5	<5	25
Mesophilic anaerobic spore count (spores/g)	$<1.5*10^3$	<5	<5	<5	10
Staphylococcus aureus (CFU/g)	<10	<10	<10	<10	<10
Enterobacteriaceae (CFU/g)	NS	<5	<5	<5	<5
Yeast (CFU/g)	NS	<10	<10	<10	<10
Mold (CFU/g)	NS	<10	<10	<10	<10
Yeasts and Molds (CFU/g)	<10	<10	<10	<10	<10
Escherichia coli (CFU/g)	<10	<10	<10	<10	<10
E. Sakazakii (CFU/g)	<50	<50	<50	<50	<50
Bacillus sp. (per 25g)	ND	ND	ND	ND	ND

CFU = colony forming units; ND = Not Detected; NS = Not specified

2.3 Additional Protein Analyses

Protein residues from the manufacturing are excluded from Clasado galacto-oligosaccharides *via* ultra-filtration (10 kDa cut-off). Protein concentrations measurable within the ingredient are limited to a specification of 0.3% (Kjeldhal). Additional sensitive analyses confirming the effectiveness of the filtration procedures for exclusion of protein in the finished ingredient are demonstrated by the result of ELISA analyses for residual levels of lactose derived *beta*-lactoglobulin and casein, common constituents of food grade lactose preparations. As shown in Table II.C-4, these proteins are typically absent from the finished product at detectable levels.

Table II.C-4 Summary of the Allergen Testing for 3 Lots of Clasado Galacto-Oligosaccharides

Specification Parameter	Specification	Manufacturing Lot		
		CKW3	CKW13	CKW23
beta-Lacto-globulin	ND	ND	0.9	ND
Casein	ND	ND	ND	ND

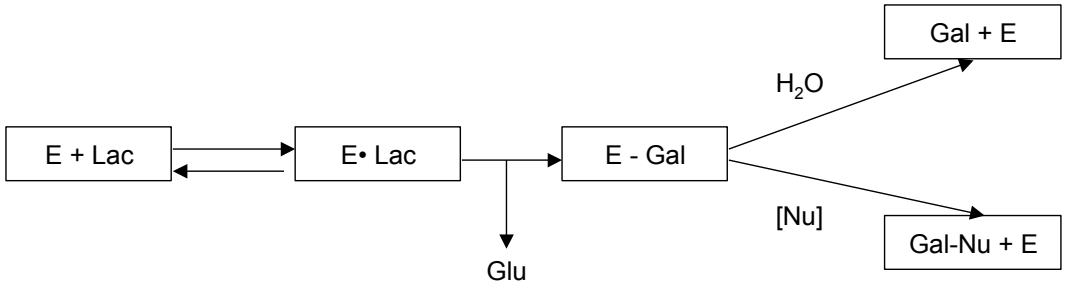
ND = not detected above detection limits 2.5 mg/kg for casein and 0.5mg/kg for *beta*-lactoglobulin (dry weight basis)
Data is representative of ingredient prior to nano-filtration

3. Comparison of Clasado Galacto-oligosaccharides with Other GRAS Sources

All GRAS sources of galacto-oligosaccharide preparations that have been the subject of the U.S. Food and Drug Administration (FDA) Notifications (U.S. FDA, 2008a, 2009a, 2010) are

produced in a similar manner using the activity of a bacterial derived *beta*-galactosidase on food grade lactose. These enzyme preparations, derived from non-pathogenic/non-toxicogenic micro-organisms, catalyze the reaction converting lactose into galacto-oligosaccharides following the general process outlined in Figure II.C-1. Although slight differences in the manufacturing conditions (*e.g.*, enzyme source organism, time, temperature, pH) are employed by each manufacturer, synthesis conditions typically yield mixtures consisting of oligosaccharides that contain between 2 and 8 sugar moieties (di-, tri-, tetra-, penta-, hexa-, hepta-, and octa-saccharides). Galacto-oligosaccharides produced by the activity of *beta*-galactosidases will contain *beta* linkages in 1→2, 1→3, 1→4, or 1→6 anomeric configurations. Depending on the source organism for which the *beta*-galactosidase enzyme is derived, a slight bias towards a particular linkage and molecular weight distribution type may be observed between different preparations. Galacto-oligosaccharides also contain glucose, galactose, and un-reacted lactose, and the residual monosaccharides can be partially removed using methods such as chromatographic separation or nano-filtration to produce higher-purity oligosaccharide products.

Figure II.C-1 Production of GOS from Lactose *via* Galactosidase



E = enzyme; Gal = galactose; Glu = glucose; Lac = lactose; Nu = nucleophile acceptor

A comparison of the starting materials, synthesis enzymes, and composition of Clasado galacto-oligosaccharides with other galacto-oligosaccharide products that have been determined to be GRAS is provided below. As shown in Table II.C-5, Clasado galacto-oligosaccharide preparations are chemically and compositionally representative of galacto-oligosaccharide preparations that have previously been determined to be GRAS.

Table II.C-5 Comparison of Clasado Galacto-Oligosaccharide and Other GRAS Sources of Galacto-Oligosaccharides

Parameter	Clasado	Vivinal (Friesland Foods Domo)	Purimune (GTC Nutrition)	Oligomate 55N/55NP (Yakult)
Starting Material	Lactose	Lactose	Lactose	Lactose
Enzyme Preparation	*Bifidobacterium bifidum*	*Bacillus circulans*	*Bacillus circulans*	*Sporobolomyces singularis*
Final Product Composition				
Configuration of linkages	β-(1-3) β-(1-4) β-(1-6) α-(1-6)	β-(1-2) β-(1-3) β-(1-4) β-(1-6)	β-(1-3) β-(1-4) β-(1-6)	β-(1-2) β-(1-3) β-(1-4) β-(1-6)
Galacto-oligosaccharides (% DM)	≥ 57	≥ 57	91.5	56.4
Lactose (% DM)	< 26	≤ 23	8.0 to 8.5	12.7
Glucose (% DM)	< 23	≤ 22	0 to 1	22.4
Galactose (% DM)	≥ 0.8	≥ 0.8	0 to 0.5	8.6
Disaccharides[1] (% GOS)	> 30	33	17 to 23	26
Trisaccharides (% GOS)	> 30	39	15 to 21	61
Tetrasaccharides and greater (%GOS)	> 5	28	27 to 32	13

Data were obtained from product specifications or analytical data from representative lots presented within GRN 236, 285, and 334 (U.S. FDA, 2008a, 2009b, 2010). NS = Not specified
[1] Exclusive of lactose

II.D. Stability

Galacto-oligosaccharides are highly stable in solution across a range of pH values and temperatures, and are considered suitable for applications in infant formula preparations (GRN 236, 286, 334) (U.S. FDA, 2008a, 2009a, 2010). The stability of Clasado galacto-oligosaccharide syrup has been evaluated under ambient and accelerated storage conditions for conformance to chemical, physical and microbiological specifications (U.S. FDA, 2013c). Clasado galacto-oligosaccharide syrup is stable for 6 months. Results provided from accelerated storage conditions suggest a shelf-life of 2 years.

III. SELF-LIMITING LEVELS OF USE

Self-limiting use levels are not known.

IV. DETAILED SUMMARY OF THE BASIS FOR CLASADO'S GRAS DETERMINATION

The determination that galacto-oligosaccharides, as manufactured by Clasado, are GRAS for food uses in infant formula and follow-on formula as described herein is based on scientific procedures. Multiple galacto-oligosaccharides preparations produced from lactose using food grade microbial derived *beta*-galactosidases, including Clasado galacto-oligosaccharides, have been previously determined to be GRAS, for use in food and beverage products across multiple categories (U.S. FDA, 2008, 2009a, 2010, 2013c). Food uses within infant formula and follow-on formula also have been determined to be GRAS (U.S. FDA, 2008, 2009b, 2010). Accordingly, publically available data and information supporting the GRAS use of galacto-oligosaccharide preparations within infant formula and follow-on formula have been critically reviewed by multiple qualified Experts and by the FDA. General recognition of the safety of galacto-oligosaccharides for use in infant formula is further established by opinions authorizing the safe use of galacto-oligosaccharides within infant formula preparations issued by the European Commission Scientific Committee on Foods and Food Standards Australia New Zealand[2] (SCF, 2001a, 2003; FSANZ, 2008).

As discussed (Section II.C.3), all GRAS sources of galacto-oligosaccharides currently available within the U.S. marketplace are produced in a similar manner from lactose using food grade *beta*-galactosidases. Clasado galacto-oligosaccharides were determined to be representative of galacto-oligosaccharide preparations that have previously been determined to be GRAS. Publically available data and information establishing general recognition of the safety of galacto-oligosaccharide preparations, including information characterizing the metabolic fate, toxicity in animals, safety and tolerance in healthy adults, safe and suitable use in term infants, effects on mineral balance, and allergenicity are therefore incorporated by reference to previous GRAS determinations (U.S. FDA, 2008, 2009b, 2010). To identify new data and information relevant to the safe use of galacto-oligosaccharides[3] in infant formula published since the previous GRAS determinations a comprehensive search of the published scientific literature was conducted through August 2013. The search was conducted using the electronic search tool, DIALOG, with several databases, including MEDLINE®, ToxFile, AGRICOLA, AGRIS, BIOSIS Toxline®, FOODLINE®: Science, Food Science and Technology Abstracts, CAB Abstracts, BIOSIS Previews®, NTIS, and EMBASE. Published studies, which included consideration of all relevant information, both favorable and unfavorable, identified during the literature searches are summarized below.

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[2] During their final opinion on use of GOS and FOS infant formula the Agency stated that "*based on the available evidence, FSANZ concludes that infant and follow-on formula containing up to 8 g/L of inulin derived substances and/or GOS, singularly or combined, in any ratio, are unlikely to pose a risk to infants*" (FSANZ, 2008).

[3] *I.e.*, those preparations produced from food grade lactose using the activity of a *beta*-galactosidase preparation

Published studies, which included consideration of all relevant information, both favorable and unfavorable, identified during the literature search are summarized in Sections IV.B through IV.H below.

IV.A. Consumption Estimates

Dietary exposures to galacto-oligosaccharides under the intended conditions of use as described in Section I.D., have been previously estimated (*e.g.*, GRN 236, 286, 334) (U.S. FDA, 2008, 2009b, 2010). Previous estimates conducted using survey data from the National Center for Health Statistics' (NCHS) 2003-2004 National Health and Nutrition Examination Survey (NHANES) (CDC, 2006; USDA, 2009) predict that 80% of the U.S. infant population is expected to consume galacto-oligosaccharides from these intended food uses (U.S. FDA, 2009a). Among infants aged 0 through 6 months, the mean and 90th percentile dietary intakes of galacto-oligosaccharides from the consumption of infant formula containing galacto-oligosaccharides at the maximum intend use level of 7.2 g/L was estimated to be 5.9 and 8.5 g per infant per day respectively. Among infants aged 7 through 12 months, dietary intakes of galacto-oligosaccharides was estimated to be 5.2 and 7.9 g per infant per day for mean and 90th percentile all-users respectively. Only 3.7% of toddlers aged 1 to 2 years were estimated to consume galacto-oligosaccharides from infant formula uses with estimated intakes of 2.8 and 6.6 g per child per day for 90th percentile consumers. Clasado galacto-oligosaccharides also are intended for addition to various general purpose food and beverages applications (U.S. FDA, 2013c). The potential for additive dietary exposure among infants and children consuming infant formula on other food sources of galacto-oligosaccharides has been previously determined not be a safety concern (GRN 286) (U.S. FDA, 2009b). In addition to food uses previously determined to be GRAS, Clasado galacto-oligosaccharides also are intended for addition to coffee and tea preparations at a use level providing up to 1.5 g of galacto-oligosaccharides per serving; however, infants and toddlers are not consumers of coffee and tea products.

Clasado concluded that the introduction of the company's galacto-oligosaccharides to the U.S. food supply, for use in general food products previously determined to be GRAS, and for use in term infant formula and follow-on formula as described herein will not increase dietary intake of galacto-oligosaccharides by infants and children above levels previously determined to be GRAS.

IV.B. Absorption, Distribution, Metabolism and Excretion

With the exception of lactose, which is susceptible to hydrolysis by human lactases, the linkages between sugar moieties within galacto-oligosaccharide oligomers are resistant to hydrolysis by human digestive enzymes (Wisker *et al.*, 1985; Ohtsuka *et al.*, 1990; Chonan *et al.*, 2004). All dietary galacto-oligosaccharides are therefore transported across the upper gastrointestinal tract to the colon and these oligomers, regardless of molecular weight, are not absorbed intact

(Ohtsuka *et al.*, 1991). Following transport to the colon, galacto-oligosaccharides become available for bacterial fermentation by the indigenous microflora of the gut resulting in the production of innocuous metabolites (*e.g.*, short-chain fatty acids, CO_2, H_2) that are common fermentation products of the normal diet (Suarez *et al.*, 1999; Smiricky-Tjardes *et al.*, 2003a,b). Since galacto-oligosaccharides are not digested by human enzymes and are not absorbed intact, all galacto-oligosaccharide preparations will produce similar physiological effects in humans and animals that are largely mediated by microbial fermentation. Although slight differences in the efficiency by which different microbial species metabolize galacto-oligosaccharides may occur (Ishikawa *et al.*, 1995; German *et al.*, 2008), fermentation by the intestinal microflora is indiscriminant to the oligosaccharide linkage and molecular weight, and bacterial metabolism of dietary galacto-oligosaccharides transported to the colon is expected to be largely complete (Ohtsuka *et al.*, 1991; Matsumoto *et al.*, 2004). Comprehensive discussions of the metabolism of galacto-oligosaccharides and the corresponding physiological effects on gastrointestinal physiology in humans and animals have been discussed previously (U.S. FDA, 2008a, 2009a, 2010). In general, the consumption of galacto-oligosaccharides by humans, rats, and pigs has been demonstrated to reduce the intestinal and fecal pH, improve fecal consistency, increase production of short-chain fatty acids and gas (*i.e.*, CO_2 and H_2), and produce increased growth of colonic and fecal populations of *Bifidobacteria* and *Lactobacilli* (Roberfroid *et al*., 2010).

IV.C. Toxicological Studies

Studies characterizing the toxicity and other effects of galacto-oligosaccharides in animals have been previously reviewed (U.S. FDA, 2008, 2009b, 2010). In brief, it is generally recognized that galacto-oligosaccharides are of low oral toxicity in rodents. Subchronic oral toxicity evaluations of galacto-oligosaccharides, produced from lactose using microbial *beta*-galactosidase preparations, demonstrate that galacto-oligosaccharide preparations are without toxicity up to the highest doses administered. For example, no adverse toxicological effects were observed in Sprague-Dawley Crl:CD (SD) IGS BR rats administered a galacto-oligosaccharide preparation *via* gavage for a period of 90 days (Anthony *et al.*, 2006). A no-observed-adverse-effect level (NOAEL) of 2,250 mg of GOS/kg body weight/day, the highest dose tested was determined by the authors. Similarly, a NOAEL of 2,000 mg GOS/kg body weight, the highest dose tested, was determined for Crj:CD (SD) IGS rats administered a galacto-oligosaccharide preparation *via* gavage for a period of 90 days (Kobayashi *et al*., 2009). The only effect attributable to oral administration of galacto-oligosaccharides in these studies is increased absolute and relative cecum weights compared to their respective controls. This effect is considered a physiological adjustment to the dietary administration of indigestible carbohydrates, an effect that is of no toxicological relevance to humans (WHO, 1987). At higher dietary concentrations (*i.e*., 10% in the diet), galacto-oligosaccharides are reported to produce transient diarrhea (Ohtsuka *et al*., 1990). Similar effects may occur in humans consuming large bolus quantities (*i.e*., ≥ 20 g) of galacto-oligosaccharides (Torres *et al*., 2010).

1. Developmental and Reproductive Toxicity Studies

Desbuards *et al.* (2012) evaluated the effects of perinatal galacto-oligosaccharides in pregnant mice and their offspring. The study was not conducted in accordance with Good Laboratory Practice and deviated from internationally accepted guidelines for the toxicity testing of chemicals. In this study, pregnant BALB/cj mice were fed a control diet (n=13) or a diet supplemented with a prebiotic mixture consisting of a galacto-oligosaccharide product and inulin (9:1) (n=12) throughout gestation and lactation. This was approximately equivalent to a dose of 1,620 mg GOS/kg body weight/day and 400 mg inulin/kg body weight/day, based on reported maternal food intake, body weight values, and the galacto-oligosaccharide content of the test article. The galacto-oligosaccharide product in the prebiotic mixture was supplied by Laiterie de Montaigu, and contained 45% GOS, 37% glucose, and 18% lactose. At weaning, male offspring were separated from their mothers, and weaned to the same test diet as their dams. The offspring were monitored and then killed at 48 days after weaning. No significant differences in maternal body weight gain or food intake during pregnancy were reported between groups. In addition, there were no significant differences in the number of offspring per dam. Male pups administered GOS/inulin exhibited significantly higher body weights at weaning, and at Days 2, 40, and 48 after weaning compared to the control group. Body length, colon length, and relative thigh muscle weights were reported to be significantly higher in male pups administered GOS/insulin compared to the control group. No other developmental or reproductive toxicological endpoints were examined.

IV.D. Human Studies

1. Adults

A large body of clinical data characterizing effects of various galacto-oligosaccharide preparations on tolerance, gastro-intestinal physiology, microflora balance and mineral absorption in healthy and unhealthy adult subjects and children have been reviewed previously (*i.e*., GRN 236, 285, 334) (U.S. FDA, 2008, 2009b, 2010). The doses administered in these studies ranged from 1 to 30 g galacto-oligosaccharides/day for up to 15 weeks. Side effects attributed to galacto-oligosaccharide consumption were typically limited to effects on gastro-intestinal intolerance. As reported by Torres *et al.* (2010), "*The only adverse effect of GOS known so far is transient osmotic diarrhea that occurs when an excess of GOS is consumed, similar to unabsorbed sugar alcohols or lactose (in symptomatic lactose-intolerant individuals). The amount of GOS that does not induce osmotic diarrhea has been estimated to be approximately 0.3 to 0.4 g/kg body weight, or about 20 g per human body (Sako et al., 1999)*." Similarly, the laxative threshold in adults was determined to be 0.30 g galacto-oligosaccharides per kg body weight per day (acute 20 g bolus intake for a 60 kg individual), and the 50% effective dose for diarrhea was determined to be 0.6 g galacto-oligosaccharides per kg body weight per day (acute 36 g bolus intake for a 60 kg adult) from a dose-response tolerance study

conducted in 24 healthy adult subjects (12 men and 12 women; age, 20 to 58 years; weight, 39.0 to 96.0 kg) by Kimura *et al*. (2004).

Clasado galacto-oligosaccharides have been safely administered to healthy subjects (Depeint *et al.*, 2008; Vulevic *et al.*, 2008; Drakoularakou *et al.*, 2010; Baran, 2012), overweight subjects at risk for metabolic syndrome (Vulevic *et al.*, 2013), and patients with irritable bowel syndrome (IBS) (Silk *et al.*, 2009) at intakes providing 1.37 to 2.75 g galacto-oligosaccharides/day, for periods of 7 days to 12 weeks (Table IV.D-1). Findings from human studies conducted using Clasado galacto-oligosaccharides are consistent with those reported for other galacto-oligosaccharide preparations (*i.e*., improved stool consistency, bifidogenic effects), and these studies further corroborate the determination that Clasado galacto-oligosaccharides are a safe and suitable alternative to existing sources of galacto-oligosaccharides on the U.S. marketplace.

Table IV.D-1 Human Studies Conducted in Adults Administered Clasado Galacto-Oligosaccharides

Study Population	Study Design	Duration	Test Article	Dose of Test Article (Dose of GOS)	Results Relevant to Safety[a]		Reference
Clasado Galacto-Oligosaccharides							
159 subjects (mean age of 38.5 years; 91 M, 68 F; healthy, visiting country at high risk for TD)	Randomized, double-blind, placebo-controlled, parallel-arm	7 days prior to travel, and throughout travel (14 to 60 days)	Clasado[b] (galacto-oligosaccharide content of 48%)	5.5 g/d (providing 2.6 g GOS/d)	Adverse event reporting	5 subjects dropped out due to mild abdominal pain, which occurred prior to travel (2 in galacto-oligosaccharides group, 3 in placebo group) NSD in incidence of abdominal pain, flatulence, or bloating NSD in stool consistency ↑ number of bowel movements during pre-travel period ↓ duration of diarrhea and abdominal pain ↑ quality of life score NSD in duration of fever, vomiting, anorexia, headache, or dizziness	Drakoularakou *et al.*, 2010
48 subjects (mean age of 49.9 years; 13 M, 35 F; healthy)	Randomized, double-blind, placebo-controlled, parallel	7 weeks followed by a 3-week washout period	Clasado[b] (galacto-oligosaccharide content of 48%)	5.5 g/d (providing 2.64 g GOS/d)	Adverse event reporting	1 subject experienced heartburn and withdrew from the study (group not specified[c]) 1 subject reported severe bloating and flatulence after 2nd week and requested to be excluded from study (group not specified[c]) NSD in stool number, stool consistency, bloating, abdominal pain, or frequency of flatulence	Baran, 2012

Table IV.D-1 Human Studies Conducted in Adults Administered Clasado Galacto-Oligosaccharides

Study Population	Study Design	Duration	Test Article	Dose of Test Article (Dose of GOS)	Results Relevant to Safety[a]		Reference
47 subjects (mean age of 44.6 years; 16 M, 29 F; overweight with 3 or more risk factors associated with metabolic syndrome)	Randomized, double-blind, placebo-controlled, crossover	12 weeks with 4-week washout period	Clasado[b] (galacto-oligosaccharide content of 48%)	5.5 g/d (providing 2.6 g GOS/d)	Plasma lipids	↑ TC, TC/HDL-cholesterol ratio, and TAG	Vulevic *et al.*, 2013
					Plasma CRP and insulin	↑ blood CRP and insulin	

CRP = C-reactive protein; F = female; FOS = fructo-oligosaccharides; HDL = high-density lipoprotein; IBS = irritable bowel syndrome; M = male; NSD = no significant differences; TAG = triacylglycerol; TC = total cholesterol; TD = travelers diarrhea

[a] unless stated otherwise, all reported effects are statistically significantly different relative to control group(s)

[b] Clasado was produced using permeabilized *B. bifidum* whole cells

[c] Study design included probiotic and symbiotic arms in addition to the galacto-oligosaccharide arm

2. Studies Conducted in Infants

The addition of galacto-oligosaccharides to infant formula is intended to provide a dietary source of oligosaccharides that are representative of oligosaccharides provided within human milk from lactating women (Veereman-Wauters, 2005; Oozeer *et al*., 2013). The consumption of galacto-oligosaccharide containing infant formulas has been extensively investigated in infants of all ages. To date over 50 studies characterizing the nutritional/physiological effects and/or safety of galacto-oligosaccharide containing infant formulas have been published within the literature, and have included studies conducted with premature infants, term infants, and infants with atopic disorders. The majority of studies reported within the literature have included studies in which galacto-oligosaccharides were administered in a 9:1 ratio with long-chain fructo-oligosaccharides; however, studies evaluating the singular administration of galacto-oligosaccharides, and the co-administration of galacto-oligosaccharides with acidic oligosaccharide preparations or polydextrose also are well represented within the literature. To date, the findings from these studies unanimously demonstrate that the addition of galacto-oligosaccharide preparations to infant formula at a use level of up to 7.2 g/L is safe and well-tolerated. These studies have been the subject of several comprehensive and critical reviews by various qualified scientific experts and scientific bodies (SCF, 2003; FSANZ, 2008; U.S. FDA, 2008, 2009b, 2010). These safety evaluations were conducted in accordance with recommendations of the Institute of Medicine, and the American Academy of Pediatrics (IOM, 2004; AAP, 1988), whereby studies used to support the safety of a new infant formula ingredient include the following endpoints within the study design: 1) inclusion of pre-term or term infants; 2) feeding initiated within the first two weeks of life; 3) feeding duration of at least 3 months; 3) inclusion of anthropometric safety evaluations and recruitment of sufficient study subjects to provide adequate statistical power to detect clinically meaningful changes in weight gain. Accordingly, the generally recognized as safe determination that the use of galacto-oligosaccharides in infant formula intended for use by term infants at a concentration of up to 7.2 g/L was largely based on pivotal findings by Schmelzle *et al*. (2003) and Moro *et al*. (2006). Findings from other investigators have provided strong corroborating evidence that the addition of galacto-oligosaccharide preparations to infant formula at use levels of up to 7.2 g/L is safe, well-tolerated, and does not adversely affect infant growth[4]. Although the nutritional significance of the addition of galacto-oligosaccharides to infant formula has yet to be definitively established (*e.g*., Macfarlane *et al.,* 2006), available data have tended to suggest that consumption of galacto-oligosaccharide supplemented infant formula produce the following beneficial physiological effects within gastro-intestinal tract: promotion of *Bifidobacteria* growth, increased production of short-chain fatty acids and corresponding reductions in stool pH, and overall changes in stool consistency that are similar to those observed in infants consuming human milk from lactating women (Veereman-Wauters, 2005; Roberfroid *et al*., 2010; Oozeer *et al*., 2013).

[4] These studies are comprehensively reviewed in GRN 236, 286, and 334

The updated search of the literature identified several new investigations of the effect of galacto-oligosaccharide consumption by infants. These studies have included the administration of galacto-oligosaccharides alone, or in combination with other oligosaccharides (*e.g*., long-chain fructo-oligosaccharides, polydextrose, acidic oligosaccharides) and/or probiotic ingredients (Table IV.D-2). The majority of these studies evaluated safety related endpoints including anthropometric indices of growth and monitoring of adverse events and gastrointestinal tolerance. Studies evaluating potential immunomodulatory effects in term-infants also have been published (Raes *et al*., 2010; van der Aa *et al*., 2012). Overall, newly available information characterizing the effect of dietary consumption of galacto-oligosaccharide supplemented infant formula are consistent with the historical literature, which have demonstrated that consumption of infant formula containing galacto-oligosaccharides at a concentration of up to 7.2 g/L is safe and well tolerated in term infants. No evidence of osmotic effects resulting in the development of diarrhea among infants consuming galacto-oligosaccharide supplemented formula's have been reported and overall the published literature continue to demonstrate that galacto-oligosaccharides produce physiological changes in stool characteristics that are representative of those observed in infants consuming human milk (*e.g*., soft stools, growth of bifidobacteria).

Two studies evaluating the immunomodulatory effects of galacto-oligosaccharides were recently reported in the literature (Raes *et al*., 2010; van der Aa *et al*., 2012). The study by Raes *et al.* (2010) was a double-blind, randomized, placebo-controlled intervention conducted with a group of 215 pregnant women and their infants recruited from clinical sites in Belgium. Infants participating in the trial were healthy term infants with normal birth weight. Eligible infants from mothers deciding to start feeding with infant formula, or after discontinuation of breast feeding, were randomized to receive a standard non-supplemented infant formula (n=62) or an infant formula supplemented with a mixture of 6 g/L of galacto-oligosaccharides (Vivanal; Borcula Domo; Netherlands) and long-chain fructo-oligosaccharides (Raftiline HP; Orafti Active; Belgium) in a 9:1 ratio (n=50). Test formulas were provided for a duration of 6 months. A breast-fed group of infants was included for reference (n=90). Demographic, clinical, and anthropometric data were obtained at birth and during regular visits at 8, 12, 16, and 26 weeks of age. Blood samples were obtained at 8 and 26 weeks of age for measurement of routine hematological parameters, serum immunoglobulins, plasma cytokines and flow cytometry immune-phenotyping. Upon completion of the study at 26 weeks the authors reported that groups did not differ with respect to gestational age, birth weight, birth length, head circumference, Apgar score or gender. Consumption of galacto-oligosaccharides did not influence measures of immune function. The authors concluded that this "…*study indicates that supplementation of infant formula with a mixture of prebiotic oligosaccharides did not change the basal level of the measured parameters of the developing immune system in healthy infants with a balanced immune system during the first 6 months of life in comparison to feeding a standard infant formula and in comparison to exclusive breastfeeding.*" Similar findings were reported by van der Aa *et al*. (2012) who evaluated the effect of an infant formula supplemented with a symbiotic mixture in a group of 90 infants (0 to 7 months of age) with atopic dermatitis.

Eligible infants from participating mothers were randomized to a control group administered standard infant formula or a symbiotic treatment group administered the same infant formula supplemented with galacto-oligosaccharides/fructo-oligosaccharides (8 g/L; 9:1 ratio) in combination with *Bifidobacterium breve* M-16V ($1.3x10^{10}$ CFU/L). Test formulas were provided for a duration of 12 weeks. Blood samples were obtained at baseline and week 12 for measurement of various atopic disease markers, *ex vivo* cytokine production, and circulating regulatory T cell percentage. Relative infants consuming the control formula no clinically relevant changes in various measure of the immune system function were observed among the infants consuming the symbiotic formula during the study.

Table IV.D-2 Administration of GOS to Infants

Reference and Study Design	Subjects[a]	Dose	Duration	Results[b]	
Studies Conducted Using GOS					
Maldonado *et al*., 2012 Randomized, double-blind, controlled	188 infants (term[c]; 6 months old; body weight NR; 94 M, 94 F; healthy)	***Treatment:*** 1. *Lactobacillus fermentum* CECT5716 and GOS (source NR) (4 g/L) 2. GOS (4 g/L)	6 months	Growth	NSD in weight, length, head circumference, and growth rate
				Adverse event reporting	No adverse effects No upper GI symptoms
				Infections	↓ Incidence rate of GI infections, URTI ↓ Total and recurrent respiratory infections ↓ Total number of infections NSD in incidence rates of otitis, UTI, and other infections NSD in antibiotic treatment and fever episodes
				Fecal microflora	↑ *Bifidobacteria* and *lactobacilli* NSD in other bacteria
				Stool effects	NSD in fecal IgA concentrations NSD in diarrhea NSD in SCFA in stool
Prasad *et al*., 2013; Sazawal *et al*., 2010 Randomized, controlled	613 children (1 to 3 years old; body weight NR; M and F; healthy) (128 children with severe anemia)	***Treatment:*** *Bifidobacterium lactis* HN019 and GOS 2.4 g/d ***Control:*** Standard formula All children with severe anemia (n=128) were given a therapeutic dose of iron for 3 months in addition to milk supplement.	12 months	Growth	↑ Weight gain
				Adverse event reporting	↓ Rate of diarrhea in children >24 months old NSD in rate of diarrhea in children 12 to 24 months old ↓ Rate of dysentery ↓ Incidence of pneumonia and ALRI (wide confidence interval) ↓ Days with severe illness ↓ Days with high fever ↓ Antibiotics usage
				Effects on anemia	Lower risk of being anemic and iron deficient NSD on iron status indicators (hemoglobin, zinc protoporphyrin, serum transferrin and ferritin)
				Fecal microflora	NSD in *bifidobacteria* NSD in cultivable *lactobacilli* NSD in total bacterial counts

Table IV.D-2 Administration of GOS to Infants

Reference and Study Design	Subjects[a]	Dose	Duration	Results[b]	
Studies Conducted Using GOS/FOS					
Vaisman *et al.*, 2010 Prospective, randomized, double-blind, placebo-controlled	42 children (9 to 24 months old; body weight NR; M and F; acute diarrhea)	***Treatment***: AOS with GOS/FOS (4.8 g/d; 9:1) taken with food ***Control***: Maltodextrin with food	12 days	Immune cell effects	NSD in absolute number or relative numbers of cytokines ↓ TNF-α when comparing subgroups of no relative change in cytokines *vs*. relative increase or decrease in cytokines
				Stool effects	NSD in stool cultures NSD in number of stools ↑ Stool consistency
Veereman-Wauters *et al.*, 2011 Randomized, double-blind, 2-center; placebo-controlled, parallel-arm	110 infants (term; average birth weight 3,480 g; 63 M, 47 F; healthy) (40 infants completed; 22 M, 18 F)	***Treatment:*** 1. GOS/FOS (Vivinal GOS + Orafti HP) (8 g/L; 9:1) 2. FOS (SYN 1) (dose 1) 3. FOS (SYN 1) (dose 2) ***Control:*** Standard formula (Breast-fed reference)	28 days	Growth	NSD in length and body weight gain
				Adverse event reporting	No serious adverse events reported NSD in number of withdrawn subjects NSD in formula intake NSD in crying behavior, regurgitation, or vomiting
				Fecal microflora	↑ *Bifidobacteria* in GOS/FOS and breast-fed groups NSD in *lactobacilli*
				Stool effects	NSD in stool frequency Softer stools in GOS/FOS and breast-fed groups
Westerbeek *et al.,* 2011a,b Randomized, double-blind, controlled trial	113 infants (pre-term; average 2.1 days old; gestational age <32 weeks; birth weight <1,500 g; 67 M, 46 F; healthy)	***Treatment***: AOS with GOS/FOS (Danone Research) (1.5 g/kg/d; 9:1) ***Control:*** Pre-term formula with maltodextrin	30 days	Intestinal permeability	NSD in lactulose or mannitol concentrations NSD between different host- and treatment-related factors on intestinal permeability
				Stool effects	NSD in stool frequency ↓ Stool viscosity at Day 30 ↓ Stool pH at Day 30 NSD in incidence of necrotizing enterocolitis

Table IV.D-2 Administration of GOS to Infants

Reference and Study Design	Subjects[a]	Dose	Duration	Results[b]	
van der Aa *et al*., 2010, 2011, 2012; de Kivit *et al*., 2012 Double-blind, placebo-controlled, multi-center trial	90 infants (term; <7 months old; birth weight NR; 59 M, 31 F; atopic dermatitis with SCORAD ≥15) (82 infants completed, 75 infants completed the1-year follow-up; 53 M, 22 F)	***Treatment:*** *B. breve* M-16V and GOS/FOS (Immunofortis®) (8 g/L; 9:1) ***Control:*** Standard formula	12 weeks 1-year follow-up for asthma-like symptoms and immune effects	Growth	NSD in growth or weight
				Adverse event reporting	NSD in adverse events NSD in renal and liver function NSD in diarrhea or gastroenteritis NSD in parent-reported bowel cramps, flatulence, and regurgitation
				Fecal microflora	↑ Percentage of *Bifidobacteria* Week 1 and 12 ↓ *Lactobaccili/enterococci* Week 1 ↓ Percentages of *C. lituseburense/C. histolyticum* and *E. rectal/C. coccoides* at 12 weeks
				Effects on atopic dermatitis	NSD in decrease of atopic dermatitis severity as determined by SCORAD score NSD in frequency of use of topical corticosteroids NSD in increase in total serum IgE NSD in decrease in serum eosinophilic granulocytes <u>Subgroup with IgE-associated atopic dermatitis:</u> NSD in improvement of atopic dermatitis at Week 4 and 8 Improvement in atopic dermatitis at Week 12
				Effects on asthma-like symptoms	Less frequent wheezing (≥ 3 episodes) and wheezing and/or noisy/rattly breathing apart from colds NSD in wheezing apart from colds ↓ Use of asthma medication ↓ New users of asthma medication NSD in change in IgE ↓ Percentage of children with elevated specific IgE against cat from baseline to Week 12 NSD in percentage of children with elevated specific IgE against dog or house dust mite from baseline to Week 12 <u>Subgroup with IgE-negative atopic dermatitis:</u> ↓ IgE at 1 year ↓ Change in IgE

Table IV.D-2 Administration of GOS to Infants

Reference and Study Design	Subjects[a]	Dose	Duration	Results[b]	
				Immune effects	NSD in plasma IL-5, IgG1, IgG4, CTAK and TARC ↓IL-4 production in unstimulated PBMCs at Week 12 NSD in production of IL-5, IL-6, IL-10, IL12p40/p70, IL-12p70, IL-13, IL-17, IFN-γ and TGF-β in unstimulated PBMCs NSD in cytokine production in allergen and anti-CD3/anti-CD28 stimulated PBMCs NSD in percentages of CD3+, CD4+CD8- , FoxP3+CD25+, or T regulatory cells ↑ Serum galectin-9 levels at 12 weeks <u>Subgroup with IgE-associated atopic dermatitis:</u> NSD in plasma IL-5, IgG1, IgG4, CTACK and TARC NSD in ex vivo cytokine production by unstimulated and anti-CD3/anti-CD28 stimulated PBMCs NSD in circulating percentage of T regulatory cells NSD in PBMCs stimulated with peanut and cow's milk allergen ↑ Change in IL-12p40/p70 in egg-stimulated PBMCs
				Stool effects	↓ Fecal pH ↑ L-lactate and D-lactate concentrations ↓ Percentages of butyric, isobutyric, and isovaleric acid NSD in percentages of acetic and propionic acid NSD in fecal frequency Softer fecal consistency ↓ Parent-reported constipation ↓ Episodes of dry stools ↓ Diaper dermatitis
Vivatvakin *et al*., 2010 Single-center, prospective, double-blind, randomized, parallel-group, controlled	224 infants (term; < 30 days old; birth weight 2,500 g to 4,500 g; 68 M, 76 F; healthy) (169 infants completed)	***Treatment***: LC-PUFA with GOS/FOS (4 g/L; 9:1) ***Control***: Standard formula (Breast-fed reference)	~3 months 18-month follow-up	Growth	NSD in mean body weights NSD in body length or head circumference
				Adverse event reporting	NSD in acceptability of the formula NSD in adverse events
				Fecal microflora	NSD in total bacterial count ↑*Bifidobacteria* (significance NR)
				GI effects	NSD in gastric emptying time
				Stool effects	↑ Soft stools

Table IV.D-2 Administration of GOS to Infants

Reference and Study Design	Subjects[a]	Dose	Duration	Results[b]	
					NSD in stool frequency NSD in stool color and odor
Raes *et al*., 2010 Randomized, double-blind, placebo-controlled, parallel-arm Note: results on serum lipids and fecal microflora available from previous kin publications before 2010 (Alliet *et al.*, 2007; Scholtens *et al*., 2008)	215 infants (term; 0 days old; birth weight >p5 and <p95[d]; 119 M, 96 F; healthy) (187 infants completed)	***Treatment***: GOS/FOS (Immunofortis®) (6 g/L; 9:1) ***Control***: Standard formula (Breast-fed reference)	6 months	Adverse event reporting	NSD in adverse events
				Immune cell effects	NSD in WBC count, lymphocyte numbers and CRP ↑ WBC count in breast-fed group at Week 26 NSD in total IgE, IgG, IgA or IgM ↑ IgG compared to standard formula only ↑ secretory IgA concentration in feces at Week 26 NSD in percentages of lymphocyte subsets between formula-fed groups ↑ Activated (CD38+) CD4+ T cells at Week 8 ↑ Activated (CD23+) B cells in breast-fed group compared to GOS/FOS or control at Week 8 ↑ Activated (CD25+) CD8+ T cells in breast-fed group compared to GOS/FOS ↑ Activated (CD25+) CD4+ T cells and NK cells in breast-fed compared to control ↓ Percentage of CD2+ cells, (CD38+) CD4+ and (CD38+) CD8+ T cells in the breast-fed group at Week 26 ↓ TNF-α in breast-fed compared to GOS/FOS at Week 8 ↓ IL5 in breast-fed compared to GOS/FOS at Week 26 NSD in cytokines between formula-fed groups
Holscher *et al.*, 2012 Multi-centre, randomized, double-blind, placebo-controlled, parallel-arm	123 infants (term; 2 to 8 weeks old; birth and body weight NR; M and F; healthy)	***Treatment***: GOS/FOS (Vivinal® GOS + Beneo P95 FOS) (4 g/L; 9:1) ***Control***: Standard formula (Breast-fed reference)	6 months	Growth	NSD in body weight
				Adverse event reporting	NSD in tolerance or occurrence of adverse events NSD in caregiver-perceived incidence of crying, fussing, parent-perceived "colic"/cramps, spitting up, vomiting, or flatulence frequency
				Fecal microflora	↑ *Bifidobacteria* in GOS/FOS and breast-fed groups NSD in *Bacteriodes/Prevotella, C. difficile*, or *lactobaccilli*
				Stool effects	NSD in stool frequency, consistency, color, or odor ↓ Fecal pH in GOS/FOS and breast-fed groups

Table IV.D-2 Administration of GOS to Infants

Reference and Study Design	Subjects[a]	Dose	Duration	Results[b]	
Salvini *et al*., 2011 Double-blind, randomized, placebo-controlled, explorative study	20 infants (term; average birth weight 3,320 g; 7 M, 13 F; healthy)	***Treatment***: GOS/FOS (source NR) (8 g/L; 9:1) ***Control***: Standard formula with maltodextrin	6 months 12-month follow-up	Growth	NSD in weight gain, length growth, or head circumference
				Adverse event reporting	No clinically relevant infections or antibiotic treatment required Well tolerated, no adverse events
				Immune effects	↑ T lymphocyte CD3+ titers ↑ IgE titers from 3 months NSD in anti-hepatitis B virus surface antibody titers
				Fecal microflora	↑ *Bifidobacteria* and *lactobacilli* after 3 months
				Stool effects	↓ Fecal pH
Modi *et al*., 2010 Multi-center, double-blind, prospective, randomized, controlled	154 infants (pre-term; gestational age <33 weeks; average birth weight 1,540 g; 98 M, 56 F, healthy)	***Treatment***: GOS/FOS (source NR) (8 g/L; 9:1) ***Control***: Standard pre-term formula	40 weeks or discharge, whichever occurred first	Growth	NSD in weight, length, or head circumference gain
				Adverse event reporting	NSD in mortalities 6 adverse events (5 not test article-related, 1 caused abdominal distension and tenderness) NSD in GI signs or indices of fluid balance
				Fecal microflora	NSD in microflora
				Stool effects	NSD in daily number of stools and stool characteristics
van Stuijvenberg *et al*., 2011 Randomized, double-blind, placebo-controlled, multi-center	830 infants (term; median age 37 days; median birth weight 3,345 g; 420 M, 410 F; healthy) (592 analyzed)	***Treatment***: AOS with GOS/FOS (Immunofortis®) (6.8 g/L; 9:1) ***Control***: Standard formula (Breast-fed reference)	1 year	Adverse event reporting	No serious adverse events
				Effects on fever	NSD in fever episodes at 1 year NSD in suspected causes of fever and symptoms (*i.e.* infections)

Table IV.D-2 Administration of GOS to Infants

Reference and Study Design	Subjects[a]	Dose	Duration	Results[b]	
Bocquet *et al.*, 2013 Multi-center, prospective, randomized, controlled, double-blind, parallel-group	439 infants (term; <42 days old; birth weight 2,500 g to 4,200 g; M and F; healthy) (321 infants analyzed)	***Treatment***: *B. lactis* and GOS/FOS (source NR) (4 g/L; 9:1) ***Control***: Formula with *B. lactis*	1 year (4 to 6 months starter formula, then follow-on formula)	Growth	NSD in daily volume of formula intake NSD in growth, except for length-for-age at 4 and 6 months and BMI-for-age at 12 months NSD in mean daily weight gain, or length and head circumference gain
				Adverse event reporting	No adverse events or serious adverse events attributed to the test article
				Infections	NSD in type of infection or annual rates of infection NSD in total number of infections or GI infections NSD in frequency of antibiotic use
				Stool effects	NSD in mean daily stool frequency ↑ Liquid stools before 10 months (except at 6 months) NSD in number of hard stools
Piemontese *et al*., 2011 Multi-center, randomized, double-blind, placebo-controlled	1130 infants (term; <8 weeks old; birth weight >p10 and <p90[e]; 554 M, 576 F; healthy) (858 infants completed)	***Treatment***: GOS/FOS (Immunofortis®) (8 g/L; 9:1) ***Control:*** Standard formula (Breast-fed reference)	1 year (6 months starter formula, 6 months follow-on formula)	Growth	NSD in mean body weight, length, or head circumference (lower compared to breast-fed) NSD in skin fold thicknesses and arm circumference (larger compared to breast-fed Week 8, smaller Week 52)
				Adverse event reporting	NSD in incidence of adverse events or serious adverse events NSD In occurrence of nappy rash NSD in number of spitting episodes within the first 24 weeks of age (lower compared to breast-fed) NSD in frequency of vomiting Occurrence of colic at 8 weeks higher than breast-fed NSD in incidence of any GI symptom between formula groups All significance NR
				Stool effects	NSD in stool frequency Stool consistency lower Weeks 8, 16, and 24 (similar to breastfed)

Table IV.D-2 Administration of GOS to Infants

Reference and Study Design	Subjects[a]	Dose	Duration	Results[b]	
Studies Conducted Using PDX/GOS					
Scalabrin *et al.*, 2012 Double-blind, randomized	289 infants (term; 21 to 30 days old; average body weight 4275 g; 138 M, 151 F; healthy) (230 infants completed)	***Treatment***: PDX/GOS (Litesse Two PDX + Vivinal GOS) (4 g/L;1:1) ***Control:*** Standard formula (Breast-fed reference)	60 days	Growth	Higher mean formula intake Day 16 to 30 NSD in weight growth rate from baseline to 30 or 60 days Mean weight growth rate at 60 days lower in breastfed group NSD in length or head circumference growth rates or achieved weight, length, or head circumference
				Adverse event reporting	NSD in number or type of adverse events NSD in gassiness (lower for breast-fed) or fussiness
				Fecal microflora	↓ *Bifidobacteria* in the breast-fed group at baseline NSD in bacterial counts at 30 days Trend towards higher counts in PDX/GOS group
				Stool effects	↑ Stool frequency in breast-fed group NSD in stool frequency between formula groups ↑ Stool consistency scores in breast-fed, and PDX/GOS groups ↑ Levels of fecal secretory IgA in breast-fed group
Ashley *et al*., 2012 Multi-center, double-blind, parallel-design, gender-stratified	287 infants (term; 12 to 16 days old; birth weight ≥2,500 g; 167 M, 120 F; healthy)	***Treatment:*** 1. PDX/GOS (Litesse Two PDX + Vivinal GOS) (4 g/L;1:1) 2. GOS (4 g/L) ***Control:*** Standard formula	106 days	Growth	↓ Growth rate Day 14 to 30 for M in PDX/GOS and F in GOS groups NSD in mean achieved weight, length, or head circumference
				Adverse event reporting	NSD in adverse events or reasons for study discontinuation
				GI effects	NSD in formula intake NSD in infant fussiness and gassiness
				Stool effects	↑ Stool frequency in PDX/GOS and GOS groups (Week 1 and 2, and at 60 days of age) NSD in stool frequency at 90 days ↑ Stool consistency (softer and formed stools) in PDX/GOS and GOS groups (Week 1 and 2) ↑ Stool consistency in PDX/GOS group from Week 2 to 120 days

Table IV.D-2 Administration of GOS to Infants

Reference and Study Design	Subjects[a]	Dose	Duration	Results[b]	
Ribeiro *et al.*, 2012 Randomized, double-blind, prospective	133 children (9 to 48 months old; body weight NR; 74 M, 59 F; healthy) (129 children completed)	***Treatment***: PDX/GOS (source NR) (~4.08 g/L; 1:1) ***Control***: Follow-on formula	108 days	Growth	NSD in formula intake NSD in body-weight-for-length/height measurements
				Adverse event reporting	NSD in number of episodes of diarrheal disease, defecation, ARI, or systemic antibiotic use Both formulas well tolerated NSD in incidence of adverse events
				Stool effects	Higher odds of having increased defecation

ARI = acute respiratory infection; ALRI = acute lower respiratory infection; AOS = acidic oligosaccharides; BMI = body mass index; CTAK = cutaneous T cell attracting chemokine; F = female; FOS = fructo-oligosaccharides; GI = gastrointestinal; GOS = galacto-oligosaccharides; IFN-γ = interferon- γ; LC-PUFA = long-chain-poly-unsaturated fatty acids; M = male; NR = not reported; NSD = no significant difference; PBMC = peripheral blood mononuclear cells; PDX = polydextrose; SCFA = short-chain fatty acids; SCORAD = SCORing Atopic Dermatitis; TARC = thymus activation-regulated chemokine; TGF-β = tumor growth factor-β; TNF-α = tumor necrosis factor-α; URTI = upper respiratory tract infections; UTI = urinary tract infections

[a] unless stated otherwise, number of subjects are reported on a per-protocol basis

[b] unless stated otherwise, all reported effects are statistically significantly different relative to control group(s)

[c] gestational age of 37 to 42 weeks

[d] normal birth weight between the 5th and 95th percentile

[e] normal birth weight between the 10th and 90th percentile

IV.E. Nutrient Interactions

Studies in rodents suggest the dietary consumption of galacto-oligosaccharide preparations may improve calcium absorption (*e.g.*, Ohta *et al.*, 1993; Chonan and Watanuki, 1995, 1996; Chonan *et al.*, 1995, 2001; Yanahira *et al.*, 1997). Although there are limited studies characterizing the effect of galacto-oligosaccharides on calcium absorption in humans, modest increases of calcium absorption (+16% relative to controls) have been reported in subjects consuming 20 g of galacto-oligosaccharides per day for a duration of 4 days (van den Heuvel *et al.*, 2000). The effects of galacto-oligosaccharides on mineral absorption have not been consistently reported across all studies (*e.g.*, van den Heuvel *et al*., 1998). The effects of galacto-oligosaccharides on mineral absorption are not nutritionally relevant and comprehensive evaluations of the literature on this subject have been previously discussed (U.S. FDA, 2008, 2009b, 2010). It is generally accepted that effects of galacto-oligosaccharide preparations on nutrient absorption are nutritionally beneficial effects.

IV.F. Allergy

Two articles were identified in which allergic reactions were reported following consumption of milk products supplemented with galacto-oligosaccharides (Chiang *et al.*, 2012; Vo *et al.*, 2012). Consumption of a fresh milk product newly-introduced to the market supplemented with galacto-oligosaccharides was implicated as the cause of allergic reactions consisting of acute onset of a skin rash and/or other allergic reactions in 17 cases, aged 2 to 15 years, in hospitals in Ho Chi Minh City in Vietnam between the 9th and 28th of October 2009 (Vo *et al.*, 2012). Despite the strength of the association (odds ratio of 34.0; 95% confidence interval: 3.9, 294.8) and the onset of symptoms following consumption of the milk product, the authors were unable to determine whether the galacto-oligosaccharides were the causative agent.

In Singapore, similar case reports of anaphylactic shock were reported in 5 patients aged 5 to 38 years (median age of 6 years) following the consumption of cow's milk formula supplemented with galacto-oligosaccharides (Chiang *et al.*, 2012). To identify the source of the allergen, skin prick tests and immunoglobulin-E mediated basophil activation tests were then conducted using the following test articles: 1) a commercial galacto-oligosaccharide supplemented cow's milk formulas, 2) a commercial cow's milk formula without galacto-oligosaccharides, 3) Vivinal galacto-oligosaccharides (Friesland, Netherlands), 4) low-protein Vivinal galacto-oligosaccharides (Friesland, Netherlands), 5) galacto-oligosaccharides fractionated by molecular weight (2, 3, 4, 5, and 6 or more sugar units), 6) and long-chain fructo-oligosaccharides. Test results suggested that galacto-oligosaccharides with a degree of polymerization of 3 or more were the allergen responsible for inducing anaphylactic reactions. Given the absence of reported allergic reactions to galacto-oligosaccharides in Japan and Europe, where the ingredients have been consumed for greater than 20 years, the authors suggested that "it is possible that a primary sensitizer might be an agent specific only to the Southeast Asian region". To date, the causative antigen(s) responsible for these cases have

not been identified. In addition, there is currently no scientific evidence demonstrating that simple carbohydrate molecules (*e.g.*, galacto-oligosaccharides) are capable of inducing anaphylactic responses in the absence of covalent linkage or strong association to a protein. Based on the long history of safe use of galacto-oligosaccharides in Japan and Europe, and the recent history of safe use of galacto-oligosaccharides in the U.S., the potential for galacto-oligosaccharide preparations to induce an allergic reaction is considered to be low.

IV.G. Expert Panel Evaluation

Clasado has determined that the company's galacto-oligosaccharides, as described herein, are GRAS for use in infant formula and follow-on formula as described in Section I.D, on the basis of scientific procedures. This GRAS determination is based on data generally available in the public domain pertaining to the safety of galacto-oligosaccharides, and on consensus among a panel of experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of galacto-oligosaccharides as a component of food. The Expert Panel consisted of the following qualified scientific experts: Professor Joseph F. Borzelleca, PhD. (Virginia Commonwealth University School of Medicine), Professor Paul B. Pecharz, MB, ChB, PhD, FRCP(C) (University of Toronto Hospital for Sick Children), and Professor Steven L. Taylor, Ph.D. (University of Nebraska).

The Expert Panel, convened by Clasado, independently and critically evaluated all data and information presented herein. Additional data and information deemed relevant to the safety of galacto-oligosaccharides as deemed relevant by the Expert Panel, including information presented within previous GRAS Notified uses of galacto-oligosaccharides also was critically evaluated by the Panel (*e.g.*, U.S. FDA, 2008, 2009b, 2010, 2013c). The Expert Panel concluded that Clasado galacto-oligosaccharides were GRAS for food uses as described in Table I.D-1., based on scientific procedures. A summary of data and information reviewed by the Expert Panel, and evaluation of such data as it pertains to the proposed GRAS uses of galacto-oligosaccharides is presented in Appendix A.

IV.H Conclusions

Based on the above data and information presented herein, Clasado Inc. has concluded following:

1. That Clasado galacto-oligosaccharides manufactured by Clasado as described herein is chemically and compositionally representative of galacto-oligosaccharide preparations that are marketed for use within infant formula in the United States and globally.

2. Newly published data and information continue to support a scientific conclusion that galacto-oligosaccharide preparations, manufactured from lactose using food grade *beta*-galactosidases, are generally recognized as safe and suitable for use in term infant formula and follow-on formula.

3. Clasado galacto-oligosaccharides, as described herein, meeting appropriate food grade specifications and manufactured in accordance with current Good Manufacturing Practice, is generally recognized as safe for use in term and follow-on formula at a use level providing galacto-oligosaccharides at a concentration of up to 7.2 g/L in the reconstituted and/or ready-to-serve formula, based on scientific procedures.

4. Food uses of Clasado Inc.'s galacto-oligosaccharides as described herein may therefore be marketed and sold for their intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the Code of Federal Regulations (U.S. FDA, 2013a).

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CFR Sections Referenced (Title 21—Food and Drugs)		
Part	**Section §**	**Section Title**
170—Food additives	170.3	Definitions
	170.30	Eligibility for classification as generally recognized as safe (GRAS)
182—Substances generally recognized as safe	182.1217	Calcium phosphate
184—Direct food substances affirmed as generally recognized as safe	184.1444	Maltodextrin
	184.1625	Potassium citrate
	184.1751	Sodium citrate
	184.1979	Whey

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Appendix A

Expert Panel Consensus Statement

Expert Panel Consensus Statement

Clasado Galacto-Oligosaccharides are Generally Recognized as Safe (GRAS) for Use in Infant Formula and Follow-on Formula

October 08, 2013

Introduction

Galacto-oligosaccharides (GOS) manufactured from food grade lactose using *beta*-galactosidase preparations are widely used within term infant formula products to provide a representative source of non-digestible oligosaccharides to human milk oligosaccharides that are present in high concentrations within human milk from lactating women (Oozeer *et al*., 2013). Multiple galacto-oligosaccharide ingredients have been determined to be generally recognized as safe for addition to term infant and follow-on infant formula at a use level of up to 7.2 g/L (GRN 236, 286, 334) (U.S. FDA, 2008, 2009, 2010). Similarly, Clasado Incorporated (Clasado) has determined that the company's galacto-oligosaccharides are Generally Recognized as Safe (GRAS) for use in infant formula and follow-on infant formula at a use level providing up to 7.2 g of GOS per L of reconstituted or ready to serve formula. Clasado convened a panel of experts (the "Expert Panel"), qualified by their relevant national and international experience and scientific training to conduct a critical and comprehensive evaluation of Clasado's GRAS determination. The Expert Panel consisted of the following individuals: Professor Joseph F. Borzelleca, PhD. (Virginia Commonwealth University School of Medicine), Professor Paul B. Pencharz, MB, ChB, PhD, FRCP(C) (University of Toronto, Hospital for Sick Children), and Professor Stephen L. Taylor, Ph.D. (University of Nebraska).

The Expert Panel, independently and collectively, critically examined a comprehensive package of data and information provided by Clasado, which included a summary of all available scientific data and information, favorable and unfavorable, relevant to the safety of the intended food uses of galacto-oligosaccharide ingredients in term infant formula and follow-on formula. This information included available scientific data compiled from the literature and other published sources through August 2013, and also included information pertaining to the method of manufacture, the product specifications, supporting analytical data, intended use levels in specified food products, consumption estimates from background and intended food uses, and a comprehensive assessment of the available scientific literature pertaining to the safety of galacto-oligosaccharides and other components. Following independent critical evaluation of such data and information, the Expert Panel unanimously concluded that under the conditions of intended use in term infant formula and follow-on formula as described herein, galacto-oligosaccharide ingredients as manufactured by Clasado, meeting appropriate food-grade specifications (Table A-1), and manufactured in accordance with current Good Manufacturing

Practice (cGMP), is GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion is provided below.

Chemical Identity, Manufacturing, and Specifications

There is no globally-adopted definition of galacto-oligosaccharides; however, they have been defined as *"a mixture of those substances produced from lactose, comprising between 2 and 8 saccharide units, with one of these units being a terminal glucose and the remaining saccharide units being galactose and disaccharides comprising 2 units of galactose*" (Tzortzis and Vulevic, 2009). Food Standards Australia New Zealand (FSANZ) has defined galacto-oligosaccharides in the following manner: "*… The term 'galacto-oligosaccharides' (sometimes referred to as oligogalactosyl-lactose) is used consistently to describe those substances comprised of between two and eight saccharide units with one of these units being a terminal glucose and the remaining saccharide units being galactose*." (FSANZ, 2008). Similarly, the Scientific Committee on Food (SCF) provided the following characterization during their safety evaluation of the use of galacto-oligosaccharides in infant formula: "*Oligogalactose is produced from lactose with the help of a bacterial β-galactosidase, it contains one molecule of glucose and typically between 1 and 7 molecules of galactose.*" (SCF, 2001a,b).

Clasado's galacto-oligosaccharides are produced *via* the transgalactosidic activity of a microbial derived *beta*-galactosidase enzyme preparation using food-grade lactose as the reaction substrate. The ingredient is manufactured as a clear slightly sweet golden yellow syrup or spray-dried powder. The galacto-oligosaccharide purity of Clasado's galacto-oligosaccharide syrup is ≥57% on a dry weight basis. The remaining composition of the ingredient consists of lactose and the monosaccharides glucose, and galactose. The ingredient contains a mixture of oligomers with a degree of polymerization ranging between 2 and 5. Clasado's ingredient has been characterized by Clasado to 100% purity and the Expert Panel concluded that the ingredient was adequately characterized.

The Expert Panel critically reviewed details of the manufacturing processes. Food grade lactose is mixed in a tri-blender with buffers (*e.g.,* tri-sodium citrate, tri-potassium citrate) and the solution heated to 80°C to ensure dissolution of the lactose and to pasteurize the mixture. The mixture is then cooled to appropriate temperature and the reaction is initiated by the addition of the enzyme preparation to the solution. The quantity of enzyme used for manufacture is pre-determined using pilot scale studies for each enzyme batch and the reaction conditions (time, temperature, and pH) are carefully monitored during manufacturing to ensure production of consistent product. Following enzymatic processing the solution is then cooled, diluted in-line with water, and ultra-filtered using spiral wound 10 kDa membranes to remove the enzyme preparation and other milk proteins and peptides typically present within lactose preparations. The filtered solution is heated to 80°C and evaporated to a concentration of 70% total solids (Water activity = 0.75). Following a final cooling step, the syrup is filtered through a 1 mm filter and packaged. Clasado's galacto-oligosaccharide syrup also may be spray-dried

with safe and suitable food grade drying aids permitted for use in food in the U.S. (*e.g.*, maltodextrin, whey protein concentrate), to produce a powdered product. All raw materials and processing-aids, including the enzyme preparation, are food grade ingredients previously determined to be GRAS and/or permitted by U.S. regulation for their intended uses as applied to the manufacture of Clasado's galacto-oligosaccharides.

Clasado has developed food grade specifications for the ingredient. The syrup contains at least 70% dry solids and galacto-oligosaccharides constitute at least 57% of the ingredient on a dry weight basis. The remaining carbohydrates are represented by lactose, glucose, and galactose within specified limits. The ingredient is protein-free at a limit of detection of 0.3% (Kjedhal). Ash constitutes <1.5% of the ingredient. Analyses for heavy metals and robust testing for microbial contamination are conducted on each batch of material. The Expert Panel reviewed batch analyses from 4 lots of the ingredient demonstrating compliance to the product specifications. The Expert Panel understands that Clasado galacto-oligosaccharides are stable under ambient and accelerated storage conditions for a period of at least 12 months, and the Expert Panel considers galacto-oligosaccharide syrups to be generally recognized as stable and suitable for use in infant formula preparations (GRN 236, 286, 334) (U.S. FDA, 2008, 2009, 2010).

Intended Uses and Estimated Exposure

Clasado's galacto-oligosaccharides are intended for use as a food ingredient within infant formula and follow-on formula products at concentrations not to exceed 0.72% (7.2 g of GOS per liter of infant formula) of the final reconstituted or ready-to-serve product. Clasado's galacto-oligosaccharides will serve as an alternative source of galacto-oligosaccharides to those currently available on the U.S. marketplace for use in infant formula, and the introduction of Clasado's galacto-oligosaccharides to the U.S. marketplace will not increase dietary exposure to galacto-oligosaccharides among the infant consumers. The Expert Panel also noted that concentrations of non-digestible oligosaccharides within milk from lactating women is reported to range between 8 to 12 g/L, and concentrations as high as 25 g/L in colostrums have been reported (Kunz *et al.*, 1999, 2000); these observations provide important teleological support for the safety and suitability of the intended use and use level of galacto-oligosaccharides in term infant formula as described herein.

Various galacto-oligosaccharide preparations, including galacto-oligosaccharides manufactured by Clasado, have been previously determined to be GRAS for a variety of conventional food uses, which may be consumed on occasion by infants (*e.g.*, cereals, baby food, yogurt). Potential additive dietary exposures to galacto-oligosaccharide from infant formula and other dietary sources have been considered previously (GRN 286) (U.S. FDA, 2009). It was concluded that consumption of these types of foods in young infants (*i.e.*, 0 to 4 months) is limited, and, among older infants dietary intake of galacto-oligosaccharides from other galacto-oligosaccharide containing foods is likely to partially replace dietary intakes from infant formula

consumption. The Expert Panel concluded that introduction of galacto-oligosaccharides, as manufactured by Clasado, to the U.S. food supply will not increase dietary intake of galacto-oligosaccharides by infants and children above levels previously determined to be GRAS.

As discussed, Clasado's galacto-oligosaccharides, like all galacto-oligosaccharide preparations, also contain lactose and the monosaccharide's glucose, and galactose. The Expert Panel considered dietary exposure of these constituents from the intended uses. All of these constituents are expected to be readily metabolized by term infants and each of these sugars is present at comparable levels to concentrations within GRAS sources of galacto-oligosaccharides currently used within infant formula and therefore are not a safety concern. The Expert Panel noted that galacto-oligosaccharides ingredients in general are not appropriate for use by infants with galactosemia, and identification of galactose within the product specification of the material is recommended.

Safety Assessment

The generally recognized as safe use of galacto-oligosaccharide preparations, produced from lactose using the enzymatic activity of food grade microbial *beta*-galactosidases, in term infant formula and follow-on formula has been previously evaluated by numerous qualified experts, and has been reviewed on multiple occasions by the United States Food and Drug Administration without objection from the Agency (U.S. FDA, 2008, 2009, 2010). Authoritative opinions/authorizations supporting the safety of galacto-oligosaccharide preparations intended for use in general food applications and infant formula have been issued by the European Commission Scientific Committee on Food, and Food Standards Australia New Zealand (SCF, 2003; FSANZ, 2008). Based on information provided by Clasado describing the method of manufacture and chemical composition of the ingredient, the Expert Panel considered Clasado's galacto-oligosaccharides to be representative of galacto-oligosaccharide preparations previously determined to be GRAS (*i.e*., GRN 236, 286, 334) (U.S. FDA, 2008, 2009, 2010). The Expert Panel recognized that subtle differences in the activity of a particular *beta*-galactosidase enzyme preparation and/or synthesis conditions employed by each manufacturer will produce galacto-oligosaccharide preparations with differences in molecular weight distribution and a bias towards the synthesis of a particular linkage types (*i.e*., *beta*-1,4, *beta*-1,3, *beta*-1,6) are often observed. However, all galacto-oligosaccharides are structurally similar, and at their basic molecular level are compositionally and chemically equivalent (*i.e*., composed of galactose and glucose). Based on available scientific evidence characterizing the physiological effects of various galacto-oligosaccharide preparations in adults, infants, and in various animal models, all galacto-oligosaccharide preparations (those produced from lactose using a food grade *beta*-galactosidase) can be considered functionally equivalent. Fermentation of galacto-oligosaccharides by indigenous bacteria within the gastro-intestinal tract is predominated by saccharolytic bacteria species (*e.g*., *Bifidobacteria, Lactobacillus*) that metabolize dietary carbohydrates *via* the Embden-Meyerhoff pathway. Thus, the fermentation

products of all galacto-oligosaccharides will be limited to the metabolic products of galactose and glucose metabolism, which include short chain fatty acids (mainly acetate, lactate, butyrate, and propionate), and gases (mainly hydrogen, carbon dioxide and methane) (Gibson and Roberfroid, 1995). Short chain fatty acids are common fermentation products of the normal diet and, following absorption, are metabolized by well established pathways: Butyrate is utilized by the colonic epithelium, propionate, L-lactate and acetate are metabolized by the liver. Acetate also is metabolized by the muscles, and other peripheral tissues (Gibson and Roberfroid, 1995).

Accordingly, based on the physiological/functional equivalence of galacto-oligosaccharide preparations, the Expert Panel considered published safety information described within the GRAS Notified uses of galacto-oligosaccharides (*i.e*., GRN 236, 286, 334) (U.S. FDA, 2008, 2009, 2010) to be applicable to the safety of the galacto-oligosaccharides manufactured by Clasado. Published information characterizing the metabolic fate, non-clinical toxicity, and safety and tolerance in healthy adults, children and infants were therefore incorporated by reference to previous GRAS determinations (*i.e*., GRN 236, 286, 334) (U.S. FDA, 2008, 2009, 2010). To identify new information, favorable and unfavorable, relevant to the intended use of galacto-oligosaccharides in infant formula published since previous GRAS Notified uses, a comprehensive search of the published scientific literature was conducted from 2011 through August 2013. The search was conducted using the electronic search tool, DIALOG, with several databases, including MEDLINE®, ToxFile, AGRICOLA, AGRIS, BIOSIS Toxline®, FOODLINE®: Science, Food Science and Technology Abstracts, CAB Abstracts, BIOSIS Previews®, NTIS, and EMBASE. A summary of the published data and information critically reviewed by the Expert Panel, and considered relevant to the safety of Clasado's galacto-oligosaccharides is presented below.

Absorption, Distribution, Metabolism and Excretion

With the exception of lactose, which is susceptible to hydrolysis by human lactases, the linkages between sugar moieties within galacto-oligosaccharide oligomers are resistant to hydrolysis by human digestive enzymes (Wisker *et al.*, 1985; Ohtsuka *et al.*, 1990; Chonan *et al.*, 2004). All dietary galacto-oligosaccharides, are therefore transported across the upper gastrointestinal tract to the colon and these oligomers, regardless of molecular weight, are not absorbed intact (Ohtsuka *et al.*, 1991). Following transport to the colon, galacto-oligosaccharides become available for bacterial fermentation by the indigenous microflora of the gut resulting in the production of innocuous metabolites (*e.g.*, short-chain fatty acids, CO_2, H_2) that are common fermentation products of the normal diet (Suarez *et al.*, 1999; Smiricky-Tjardes *et al.*, 2003a,b). Since galacto-oligosaccharides are not digested by human enzymes and are not absorbed intact, all galacto-oligosaccharide preparations will produce similar physiological effects in humans and animals that are largely mediated by microbial fermentation. Although slight differences in the efficiency by which different microbial species metabolize galacto-oligosaccharide preparations may occur (Ishikawa *et al.*, 1995; German *et al.*, 2008),

fermentation by the intestinal microflora is indiscriminant to the oligosaccharide linkage and molecular weight, and bacterial metabolism of dietary galacto-oligosaccharides transported to the colon is expected to be largely complete (Ohtsuka *et al.*, 1991; Matsumoto *et al.*, 2004). Comprehensive discussions of the metabolism of galacto-oligosaccharides, and the corresponding physiological effects on gastro-intestinal physiology with humans (adults and infants) and animals has been discussed previously (*i.e.*, GRN 236, 286, and 334) (U.S. FDA, 2008, 2009, 2010). In general, the consumption of galacto-oligosaccharides by humans, rats, and pigs has been demonstrated to reduce the intestinal and fecal pH, improve fecal consistency, increase production of short-chain fatty acids and gas (*i.e.*, CO_2 and H_2), and promote the growth of colonic *Bifidobacteria* and *Lactobacilli spp*. within the colon. These effects are generally recognized as desirable nutritional effects (Roberfroid *et al*., 2010).

Toxicological Studies

Galacto-oligosaccharides are of low oral toxicity in rodents. Subchronic oral toxicity evaluations of food grade galacto-oligosaccharide preparations demonstrate that these ingredients are without toxicity up to the highest doses administered. For example, no adverse toxicological effects were observed in Sprague-Dawley Crl:CD (SD) IGS BR rats administered a galacto-oligosaccharide preparation *via* gavage for a period of 90 days (Anthony *et al.*, 2006). A no-observed-adverse-effect level (NOAEL) of 2,250 mg of GOS/kg body weight/day, the highest dose tested was determined by the authors. Similarly, a NOAEL of 2,000 mg GOS/kg body weight, the highest dose tested, was determined for Crj:CD (SD) IGS rats administered a galacto-oligosaccharide preparation *via* gavage for a period of 90 days (Kobayashi *et al*., 2009). The only effect attributable to oral administration of galacto-oligosaccharides in these studies is increased absolute and relative cecum weights compared to their respective controls. This effect is considered a physiological adjustment to the dietary administration of indigestible carbohydrates, an effect that is of no toxicological relevance to humans (WHO, 1987). At higher dietary concentrations (*i.e*., 10% in the diet or ~5,000 mg/kg body weight), galacto-oligosaccharides have been reported to produce transient diarrhea in rats (Ohtsuka *et al*., 1990). Thresholds for galacto-oligosaccharide induced diarrhea in humans were reviewed by the Expert Panel and are discussed below.

Developmental and Reproductive Toxicity Studies

Desbuards *et al.* (2012) evaluated the effects of perinatal galacto-oligosaccharides in pregnant mice and their offspring. The study was not conducted in accordance with Good Laboratory Practice and deviated from internationally accepted guidelines for the toxicity testing of chemicals. In this study, pregnant BALB/cj mice were fed a control diet (n=13) or a diet supplemented with a prebiotic mixture consisting of a galacto-oligosaccharide product and inulin (9:1) (n=12) throughout gestation and lactation. This was approximately equivalent to a dose of 1,620 mg GOS/kg body weight/day and 400 mg inulin/kg body weight/day, based on reported

maternal food intake, body weight values, and the galacto-oligosaccharide content of the test article. The galacto-oligosaccharide product in the prebiotic mixture was supplied by Laiterie de Montaigu, and contained 45% GOS, 37% glucose, and 18% lactose. At weaning, male offspring were separated from their mothers, and weaned to the same test diet as their dams. The offspring were monitored and then killed at 48 days after weaning. No significant differences in maternal body weight gain or food intake during pregnancy were reported between groups. In addition, there were no significant differences in the number of offspring per dam. Male pups administered GOS/inulin exhibited significantly higher body weights at weaning, and at Days 2, 40, and 48 after weaning compared to the control group. Body length, colon length, and relative thigh muscle weights were reported to be significantly higher in male pups administered GOS/insulin compared to the control group. No other developmental or reproductive toxicological endpoints were examined. Although findings by the authors are consistent with the fact that galacto-oligosaccharide preparations are non-toxic in rodent studies, due to several limitations in the study design, the Expert Panel considered this study to be of limited use to the safety assessment.

Nutrient Interactions

Studies in rodents suggest that the dietary consumption of galacto-oligosaccharide preparations may improve calcium absorption (*e.g.*, Ohta *et al.*, 1993; Chonan and Watanuki, 1995, 1996; Chonan *et al.*, 1995, 2001; Yanahira *et al.*, 1997). Although there are limited studies characterizing the effect of galacto-oligosaccharides on calcium absorption in humans, modest increases of calcium absorption (+16% relative to controls) have been reported in subjects consuming 20 g of galacto-oligosaccharides per day for a duration of 4 days (van den Heuvel *et al.*, 2000). The effects of galacto-oligosaccharides on mineral absorption have not been consistently reported across all studies (*e.g.*, van den Heuvel *et al*., 1998), and although potential effects of galacto-oligosaccharides on mineral absorption are generally regarded as a beneficial effect, the nutritional relevance of these purported effects are unclear.

Human Studies

A large body of clinical data characterizing effects of various galacto-oligosaccharide preparations on tolerance, gastro-intestinal physiology, microflora balance and mineral absorption in healthy and unhealthy adult subjects and children have been reviewed previously (*i.e*., GRN 236, 286, 334) (U.S. FDA, 2008, 2009, 2010). The doses administered ranged from 1 to 30 g galacto-oligosaccharides/day for up to 15 weeks. Side effects attributed to galacto-oligosaccharide consumption were typically associated with effects on gastro-intestinal intolerance. The only adverse effect of excess galacto-oligosaccharide consumption by humans is transient osmotic diarrhea, an effect that is similar to that occurring from unabsorbed sugar alcohols or lactose (in symptomatic lactose-intolerant individuals), and the threshold for this

effect is reported to occur following acute/bolus dietary intakes in excess of 0.3 to 0.4 g/kg body weight or about 20 g for a 60 kg individual (Sako *et al*., 1999; Kimura *et al*. 2004).

The results of studies conducted using healthy subjects (Depeint *et al.*, 2008; Vulevic *et al.*, 2008; Drakoularakou *et al.*, 2010; Baran, 2012), overweight subjects at risk for metabolic syndrome (Vulevic *et al.*, 2013), and patients with irritable bowel syndrome (Silk *et al.*, 2009) have demonstrated that consumption of galacto-oligosaccharides manufactured by Clasado at doses ranging from 3.6 to 7 g/day, providing 1.37 to 2.75 g galacto-oligosaccharides/day, for periods of 7 days to 12 weeks also are well-tolerated. Findings from human trials conducted using Clasado's galacto-oligosaccharides are consistent with those reported for other galacto-oligosaccharide preparations (*i.e*., improved stool consistency, bifidogenic effects). Findings from these studies corroborate the functional equivalence and safety of Clasado's galacto-oligosaccharide ingredients to other galacto-oligosaccharide preparations.

Studies Conducted in Infants

The addition of galacto-oligosaccharides to infant formula is intended to provide a dietary source of oligosaccharides that are representative of non-digestible oligosaccharides present within human milk from lactating women (Veereman-Wauters, 2005; Oozeer *et al*., 2013). Clasado reported that to date, over 50 studies characterizing the safety, tolerance, and/or nutritional effects of galacto-oligosaccharides in premature infants, healthy term infants, and infants with atopic disorders have been published within the literature. Studies within the published literature have typically evaluated the administration of infant formula's containing galacto-oligosaccharides in a 9:1 ratio with long-chain fructo-oligosaccharides. Studies evaluating the singular administration of galacto-oligosaccharides, and the co-administration of galacto-oligosaccharides with other oligosaccharide ingredients (*e.g*., acidic oligosaccharides, polydextrose) also are well represented within the literature. To date, the findings from these studies unanimously demonstrate that the addition of galacto-oligosaccharide ingredients to infant formula at a use level of up to 7.2 g/L is safe and well-tolerated. These studies have been the subject of several comprehensive and critical reviews by various qualified scientific experts and scientific bodies (SCF, 2003; U.S. FDA, 2008, 2009, 2010; FSANZ, 2008).

The Expert Panel noted that the safety evaluation of a new infant formula ingredient/additive should be conducted in accordance with recommendations of the Institute of Medicine (IOM), and the American Academy of Pediatrics (AAP) (AAP, 1988; IOM, 2004), whereby studies used to establish the safety of a new infant formula ingredient should include the following study design: 1) inclusion of pre-term or term infants; 2) feeding initiated within the first 2 weeks of life; 3) feeding duration of at least 3 months; 3) inclusion of anthropometric safety evaluations and recruitment of sufficient study subjects to provide adequate statistical power to detect clinically meaningful changes in weight gain. Accordingly, the determination that the use of galacto-oligosaccharides in infant formula intended for use by term infants at a concentration of up to 7.2 g/L is generally recognized as safe was largely based on pivotal findings by Schmelzle

et al. (2003) and Moro *et al*. (2006). Findings from other investigators have provided strong corroborating evidence that the addition of galacto-oligosaccharide preparations to infant formula at use levels of up to 7.2 g/L is safe, well-tolerated, and does not adversely affect infant growth. Although the nutritional significance of the addition of galacto-oligosaccharides to infant formula has yet to be definitively established (*e.g*., Macfarlane *et al*., 2006), available data have tended to suggest that consumption of galacto-oligosaccharide supplemented infant formula produce the following beneficial physiological effects within gastro-intestinal tract: promotion of *Bifidobacteria* growth, increased production of short-chain fatty acids and corresponding reductions in stool pH, and overall changes in stool consistency that are similar to those observed in infants consuming human milk (Veereman-Wauters, 2005; Roberfroid *et al*., 2010; Oozeer *et al*., 2013). The direct effects of dietary galacto-oligosaccharides on promoting host-microbiotia symbiosis in infants (*e.g*., *Bifidogenic* effects) also may have indirect effects on other aspects of infant nutrition including the development of a healthy immune system (Oozeer *et al*., 2013).

The updated search of the literature conducted since previous GRAS determinations and authoritative opinions identified several new investigations of the effect of galacto-oligosaccharide consumption by infants[1]. These studies included the administration of galacto-oligosaccharides alone, or in combination with other oligosaccharides (*e.g*., long-chain fructo-oligosaccharides, polydextrose, acidic oligosaccharides) and/or probiotic ingredients. These studies were summarized by Clasado and the majority of these studies were determined to contain various safety related endpoints including anthropometric indices of growth and monitoring of adverse events and gastrointestinal tolerance. Published studies evaluating potential immunomodulatory effects in term-infants were identified (Raes *et al*., 2010; van der Aa *et al*., 2012). Overall, newly available information characterizing the effect of dietary consumption of galacto-oligosaccharide supplemented infant formula is consistent with the historical literature, which has demonstrated that consumption of infant formula containing galacto-oligosaccharides at a concentration of up to 7.2 g/L is safe and well-tolerated in term infants. No evidence of adverse osmotic effects resulting in the development of diarrhea among infants consuming galacto-oligosaccharide supplemented formula's has been reported and overall the published literature continues to demonstrate that galacto-oligosaccharides produce physiological changes in stool characteristics that are representative of those observed in infants consuming human milk (*e.g*., soft stools, growth of bifidobacteria).

[1] Modi *et al*., 2010; Raes *et al*., 2010; Sazawal *et al*., 2010; Vaisman *et al*., 2010; van der Aa *et al*., 2010, 2011, 2012; Vivatvakin *et al*., 2010; Piemontese *et al*., 2011; Salvini *et al*., 2011; van Stuijvenberg *et al*., 2011; Veerman-Wauters *et al*., 2011; Westerbeek *et al*., 2011a,b; Ashley *et al*., 2012;de Kivit *et al*., 2012; Holscher *et al*., 2012; Maldonado *et al*., 2012; Ribeiro *et al*., 2012; Scalabrin *et al*., 2012; Bocquet *et al*., 2013; Prasad *et al*., 2013.

Allergenicity

Consumption of products containing galacto-oligosaccharides in a milk based beverage has been associated with the acute onset of skin rash and/or other allergic reactions in 17 cases (aged 2 to 15 years) reporting to hospitals in Vietnam (Ho Chi Minh City) between the 9th and 28th of October 2009 (Vo *et al.*, 2012). Despite the strength of the association (odds ratio of 34.0; 95% confidence interval: 3.9, 294.8) and the onset of symptoms following consumption of the milk product, the authors were unable to determine whether the galacto-oligosaccharides were the causative agent. Similar cases of anaphylaxis have recently been reported in 5 patients (aged 5 to 38; median age 6 years) in Singapore following the consumption of cow's milk formula containing galacto-oligosaccharides (Chiang *et al.*, 2012). Skin prick tests and immunoglobulin-E mediated basophil activation tests conducted with 2 commercial galacto-oligosaccharide supplemented cow's milk formulas, commercial cow's milk formula without galacto-oligosaccharides, standard Vivinal galacto-oligosaccharides, low-protein galacto-oligosaccharides, and galacto-oligosaccharide high performance liquid chromatography fractions (2, 3, 4, 5, and 6 or more sugar units) suggested that a galacto-oligosaccharide with a degree of polymerization of 3 or more was the allergen responsible for inducing anaphylactic reactions. Given the absence of reported allergic reactions to galacto-oligosaccharides in Japan and Europe, where the ingredients have been consumed for greater than 20 years, the authors suggested that "*it is possible that a primary sensitizer might be an agent specific only to the southeast Asian region*".

It is also of interest that IgE antibodies to *alpha*-(1,3)-galactose (*alpha*-gal) have been identified in individuals experiencing serious anaphylactic reactions to the monoclonal antibody cetuximab, and to proteins within red meat and pork kidney (Commins and Platts-Mills, 2009; Morisset *et al*., 2012). The primary sensitizer in these cases has been suspected to be previous exposure to *alpha*-galactosylated proteins originating from arthropod bites (*e.g*., tick infections) (Commins and Platts-Mills, 2009). It was noted that *alpha*-galacto-oligosaccharides present in Clasado's galacto-oligosaccharides are *alpha*-(1,6)-galactobiose, which are structurally different from the *alpha*-(1,3)-galactose to which IgE antibodies have been identified.

Despite the above reports of anaphylaxis to food products containing galacto-oligosaccharides, the Expert Panel notes that, to date, the causative antigen(s) responsible for these cases have not been identified. In addition, there is currently no scientific evidence demonstrating that simple carbohydrate molecules (*e.g*., galacto-oligosaccharides) are capable of inducing anaphylactic responses in the absence of covalent linkage or strong association to a protein. Based on the current history of wide-spread and safe consumption of galacto-oligosaccharides by individuals of all ages and demographic backgrounds throughout North America, Europe and Japan, the Expert Panel concluded that the potential for galacto-oligosaccharides to produce allergic responses is low.

Conclusion

We, the Expert Panel, have, independently and collectively, critically evaluated the data and information summarized above and conclude that the intended use of Clasado's galacto-oligosaccharide ingredient meeting appropriate food grade specifications and manufactured in accordance with current Good Manufacturing Practice, is Generally Recognized as Safe (GRAS) for use in term and follow-on formula at a use level providing up to 7.2 g/L in the reconstituted and/or ready-to-serve formula.

This GRAS determination is based on scientific procedures, and it is our opinion that other qualified scientific experts, critically evaluating the same information, would concur with these conclusions.

Professor Joseph F. Borzelleca, Ph.D. Virginia Commonwealth University School of Medicine	Date
Professor Paul B. Pecharz, MB, ChB, PhD, FRCP(C) University of Toronto Hospital for Sick Children	Date
Professor Stephen L. Taylor, Ph.D. Institute of Agriculture and Natural Resources University of Nebraska	Date

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(b) (6)

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ATTACHMENT A1

Product Specification

Table A-1 Specifications for Clasado Galacto-Oligosaccharides		
Specification Parameter	**Specification**	**Method**
Dry matter (%)	Minimum 70	By calculation
Moisture (%)	Maximum 30	AOAC 925.45.D.
Protein (%, on DM)	< 0.3	AOAC 992.15.
Ash (%, on DM)	< 1.5	ISO 936:1998
Galacto-oligosaccharide (%)	≥57 on DM	AOAC 2001.02
Lactose anhydrous (%)	< 26 on DM	AOAC 2001.02
Glucose anhydrous (%)	< 23 on DM	AOAC 2001.02
Galactose anhydrous (%)	>0.8 on DM	AOAC 2001.02
Disaccharides (% GOS; lactose excluded)	> 30	AOAC 2001.02
Trisaccharides (% GOS)	>30	AOAC 2001.02
Tetrasaccharides (%GOS)	>5	AOAC 2001.02
Pentasaccharides and greater (% GOS)	>2	AOAC 2001.02
Heavy Metal Specifications		
Lead (ppm)	<0.1	ICP-MS UKAS Accredited Lab Method -based on NIOSH Method 73000, BS EN 13805:2002, BS EN 15763:2009, BS EN 13804:2002
Cadmium (ppm)	<0.1	
Mercury (ppm)	<0.1	
Microbiological Specifications		
Total viable count (CFU/g)	$<3.5*10^3$	BS EN ISO 4833:2003
Salmonella spp. (per 25 g)	Not detected	BS EN ISO 6579
Mesophilic spore count (spores/g)	$<1.5*10^3$	BS EN ISO 6887-1
Mesophilic anaerobic spore count (spores/g)	$<1.5*10^3$	BS EN ISO 6887-1: 1999
Staphylococcus aureus (CFU/g)	<10	BS EN ISO 6888-1:1999
Enterobacteriaceae (CFU/g)	NS	BS EN ISO 21528-2:2004.
Yeasts and Molds	<10	BS ISO 21527-2:2008
Escherichia coli (CFU/g)	<10	BS ISO 4832:2006
Cronobacter sakazakii	NS	FDA-BAM
Bacillus sp. (per 25 g)	NS	FDA-BAM
Other information		
Contains milk		

BS EN = British Standards European Norm; CFU = colony forming units; ISO = International Organization for Standardization (Betts RP, Oscroft CA, Baylis CL (2004). *A Code of Practice for Microbiology Laboratories Handling Food, Drink and Associated Samples, 3rd revised edition*. Gloucestershire, UK: Campden & Chorleywood Food Research Association.)

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