ORIGINAL SUBMISSION

Clasado Inc
1620 Pinetree Drive
Pittsburgh, PA 15241
United States

Tel. +1 412-831-7220
Fax. +1 412-831-2274

Web.www.clasado.com

August 28, 2013

Dr. Moraima Ramos-Valle
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. Ramos-Valle:

Re: GRAS Exemption Claim for Galacto-Oligosaccharides

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 documents, are GRAS under specific conditions of use as a food ingredient, 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.

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

Sincerely,

(b) (6)

Michael Moore
Business Manager, North America
Clasado Inc.
Tel: 412-831-7220
Mike.Moore@clasado.us

Clasado
A New Approach to Health

Clasado Inc
1620 Pinetree Drive
Pittsburgh, PA 15241
United States

Tel. +1 412-831-7220
Fax. +1 412-831-2274

Web.www.clasado.com

August 28, 2013

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FOOD ADDITIVE SAFETY

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Office of Food Additive Safety (HFS-200)
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Food and Drug Administration
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Business Manager, North America
Clasado Inc.
Tel: 412-831-7220
Mike.Moore@clasado.us

August 28, 2013

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

Dear Dr. Ramos-Valle,

RE: Certification of Virus-Free Document – (GRAS Exemption Claim for Galacto-Oligosaccharides)

I hereby certify that the enclosed electronic files for the petition entitled, "GRAS Exemption Claim for Galacto-oligosaccharides" were scanned for viruses prior to submission and are thus certified as being virus-free using McAfee VirusScan 8.8.

Sincerely,

Judy Vowles
Manager, Word Processing

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August 28, 2013

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(b) (6)

Judy Vowles
Manager, Word Processing

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

July 3, 2013

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

July 3, 2013

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FOOD ADDITIVE SAFETY

GRAS Exemption Claim for Galacto-oligosaccharides

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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 use are Generally Recognized as Safe (GRAS).

Signed,

(b) (6)

3rd July 2013

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

Date

I.B Name and Address of Notifier

CEO
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 food and beverage products across multiple categories as described in Table I.D-1 at use levels providing up to 3.0 g of GOS per serving.

Table I.D-1 Summary of the Individual Proposed Food Uses and Use-Levels for Galacto-oligosaccharides in the U.S.[a]

Food Category	Proposed Food-Use	RACC[b] (g or mL)	GOS Level (g/serving)	GOS Use-Level (%)
Baked Goods and Baking Mixes	Breads (Wheat, Wholemeal, Other; excluding White)	50	1.28	2.56
	Brownies	40	1.28	3.20
	Cakes (including coffee cakes)	55 to 125	1.28	1.02 to 2.33
	Cookies	30	1.28	4.27
	Crackers, snack	30	1.28	4.27
	Muffins	55	1.28	2.33
	Pies, cobblers, fruit crisps, turnovers, other pastries	125	1.28	1.02
	Waffles & Pancakes	85	1.28	1.51
Beverages and Beverage Bases	Bottled and Flavored Waters	240	3.00	1.25
	Energy, Sport, and Isotonic Drinks	240	3.00	1.25
	Non-Milk Based Meal Replacements and Protein Beverages	240	3.00	1.25
Breakfast Cereals	Baby Cereals, dry	15	0.86	5.73
	Baby Cereals, ready-to-eat	110	1.28	1.16
	Ready-to-Eat Cereals	15 to 55	1.28	2.33 to 8.53
Coffee and Tea[c]	Coffee and Tea	240	1.50	0.625
Dairy Product Analogs[c]	Coffee Creamers and Whiteners (liquid)	15	0.80	5.33
	Coffee Creamers and Whiteners (powder)	2	0.80	40.00
	Soy Beverages	240	3.00	1.25
Frozen Dairy Desserts and Mixes	Frozen Dairy Desserts	125	3.00	2.40
	Ice Cream	125	1.28	1.02
Grain Products and Pastas	Cereal and Grain-Based Baby Snacks	7	1.00	14.29
	Grain-based bars (with or without fillings or coatings)	40	3.00	7.50
	Pasta	55	1.28	2.33
Milk and Milk Products	Flavored Milk and Milk Drinks	240	3.00	1.25
	Milk-Based Meal Replacements and Protein Beverages	240	3.00	1.25
	Yogurt	225	3.00	1.33
	Yogurt Drinks for babies/toddlers	120	3.00	2.50
Processed Fruits and Fruit Juices	Fruit Drinks and Smoothies	240	3.00	1.25
	Fruit Juices and Nectars	240	3.00	1.25
	Fruit Juices for babies/toddlers	120	3.00	2.50

Table I.D-1 Summary of the Individual Proposed Food Uses and Use-Levels for Galacto-oligosaccharides in the U.S.[a]

Food Category	Proposed Food-Use	RACC[b] (g or mL)	GOS Level (g/serving)	GOS Use-Level (%)
Processed Vegetables and Vegetable Juices	Vegetables Juices	240	3.00	1.25
	Vegetable Juices for babies/toddlers	120	3.00	2.50
Snacks	Novelty snacks	30	1.28	4.27
Sweet sauces	Syrup flavorings	30	3.00	10.00

[a] The individual food uses for Bimuno® Syrup will be the same as those for the powder; however use-levels will be adjusted to maintain a constant GOS level in each food use.
[b] RACC – Reference Amounts Customarily Consumed Per Eating Occasion (21 CFR §101.12). When a range of values is reported for a proposed food-use, particular foods within that food-use may differ with respect to their RACC (U.S. FDA, 2012).
[c] Bimuno is intended to be used in either single-serve coffee and tea products or in coffee creamers and whiteners.

2. Purpose for Which Substance is Used

Galacto-oligosaccharides manufactured by Clasado are intended for addition to food and beverage products described in Table I.D-1 as source of dietary GOS.

3. Description of the Population Expected to Consume the Substance

Galacto-oligosaccharides are expected to be consumed by members of the general United States (U.S.) population who may reasonably be expected to consume at least one food within the aforementioned food categories.

I.E. Basis for the GRAS Determination

Pursuant to Title 21, Section 170.30 of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2012), 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:

Business Manager, North America
Clasado Ltd.
1620 Pinetree Drive
Pittsburgh, PA 15241
USA

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, 2007). 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, 2001). 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 40%. This syrup also may be spray-dried with food grade drying-aids to form an off white powder.

Table II.A-1 Dimeric and Oligomeric Saccharides Present in Bimuno

Carbohydrate	Configuration of the Saccharide Linkages in Bimuno
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

Bimuno®

II.B. Method of Manufacture

1. Raw Materials and Processing-aids

All raw materials and processing-aids used for manufacture of Bimuno GOS, 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 GOS synthesis	FCC, 2012
BbgIV *beta*-galactosidase	Enzyme preparation catalyzing transgalactosylation reaction	Previously determined to be GRAS for exclusive use in the manufacture of GOS
Trisodium citrate	pH control	FCC, 2012 21 CFR § 184.1751 (U.S. FDA, 2012)
Tripotassium citrate	pH control	FCC, 2012 21 CFR § 184.1625 (U.S. FDA, 2012)
Maltodextrin	Drying-aid	FCC, 2012 21 CFR §184.1444 (U.S. FDA, 2012)
Calcium phosphate	Drying-aid	FCC, 2012 21 CFR §182.1217 (U.S. FDA, 2012)

2. Description of Manufacturing

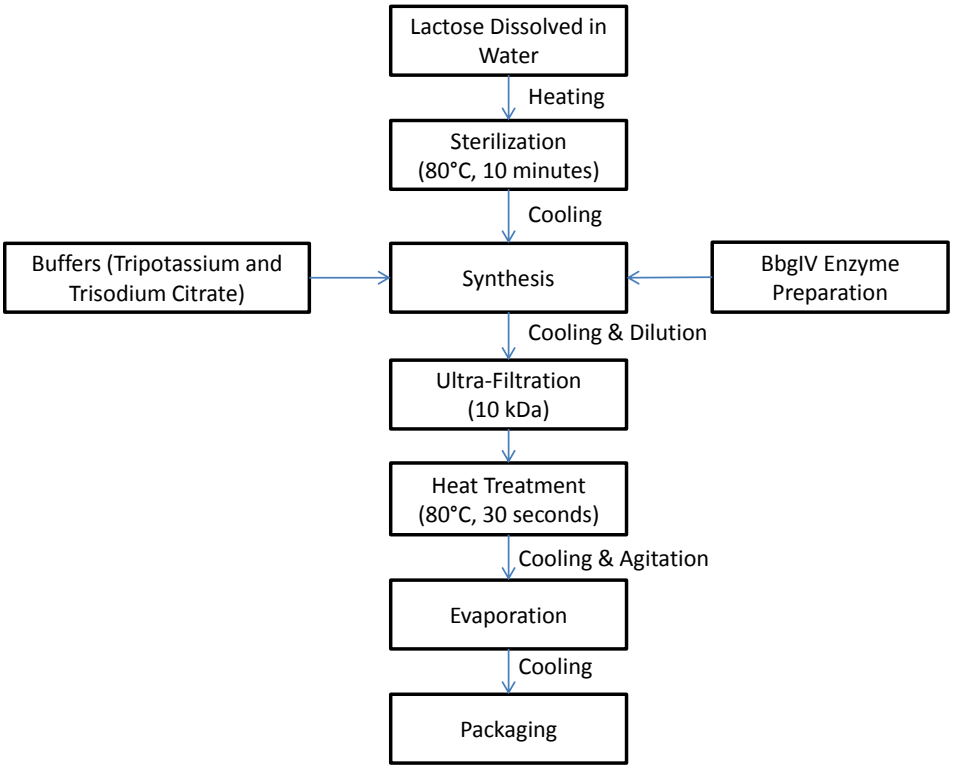
Bimuno GOS is produced from food grade lactose *via* the transgalactosylation activity of a *beta*-galactosidase enzyme preparation. The process is initiated by dissolution of the enzyme preparation and appropriate buffers in cold water for several hours. In a separate vessel food-grade lactose is dispersed 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 product is stored under ambient conditions and released following quality control checks (see Section 3.2.3 below). See Figure II.B-1 for a schematic overview of the manufacturing process.

For production of a spray-dried powder the syrup is subjected to nano-filtration to remove monosaccharides, and spray dried with permitted drying aids such as maltodextrin to result in a powder product. Calcium phosphate may be used as a free flowing agent.

Figure II.B-1 Schematic Overview of the Manufacturing Process for Bimuno Syrup



3. Quality Control

Clasado monitor a number of parameters in-line to ensure that the manufacturing process produces a consistent product. In addition, Bimuno GOS is analyzed to ensure conformance to compositional, chemical, physical, and microbiological limits prior to product release. A

comparison of the quality control checks in place in the syrup and powder manufacturing processes is outlined in Table II.B-2 below.

Table II.B-2 Quality Control Points

Test	GOS Syrup	GOS Powder
Near and In-line Monitoring		
Solids	Y	Y
Temperature	Y	Y
pH	Y	Y
Glucose	N	Y
Freezing point Depression	Y	NA
Scorched particles	Y	Y
Absence of enzymatic activity post purification	Y	Y
Turbidity	Y	Y
Final product moisture	Calibrated Refractometer and Karl Fischer Reference Method	N/A
Metal detection	Y	Y
Product Release		
Water activity	0.75	0.26
Chemical	Y	Y
Physical	Y	Y
Microbiological	Y	Y
Galacto-oligosaccharide content (DM)	49	50

DM = dry matter; N = not tested; NA = not applicable; Y = tested

II.C. Specifications and Product Analysis

1. Specifications

Food grade chemical and microbiological specifications for Bimuno GOS are presented in Table II.C-1 below.

Table II.C-1 Specifications for Bimuno GOS

Specification Parameter	Syrup	Spray Dried Powder	Method
Dry matter (%)	Minimum 70	Minimum 95	By calculation
Moisture (%)	Maximum 30	Maximum 5	AOAC 925.45.D.
Protein (%, on DM)	<0.3	1 to 1.5	AOAC 992.15.
Ash (%, on DM)	<1.5	1 to 1.5	ISO 936:1998
Galacto-oligosaccharide (%)	46 to 60 on DM	55 to 80 on DM	AOAC 2001.12
Lactose anhydrous (%)	<19 on DM	<25 on DM	AOAC 2001.12
Glucose anhydrous (%)	NS	NS	AOAC 2001.12
Galactose (%)	NS	NS	AOAC 2001.12
Total Monosaccharides (%)	>35 on DM	<15.3 on DM	AOAC 2001.12
Heavy Metal Specifications			
Lead (ppm)	<0.1	<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	<0.1	
Mercury (ppm)	<0.1	<0.1	
Microbiological Specifications			
Total viable count (CFU/g)	$<3.5*10^3$	$<1*10^3$	BS EN ISO 4833:2003
Salmonella spp. (per 25 g)	Not detected	Not detected	BS EN ISO 6579
Mesophilic spore count (spores/g)	$<1.5*10^3$	$<5*10^2$	BS EN ISO 6887-1; Betts *et al.*, 2004[1]
Thermophilic aerobic spore count (spores/2g)	NS	<200	External Lab In-house method
Mesophilic anaerobic spore count (spores/g)	$<1.5*10^3$	NS	BS EN ISO 6887-1: 1999; Betts *et al.*, 2004[1]
Staphylococcus aureus (CFU/g)	<10	<10	BS EN ISO 6888-1:1999
Enterobacteriaceae (CFU/g)	NS	NS	BS EN ISO 21528-2:2004.
Yeast (CFU/g)	NS	NS	BS ISO 21527-2:2008
Mold (CFU/g)	NS	NS	BS ISO 21527-2:2008
Yeasts and Molds	<10	$<10^2$	BS ISO 21527-2:2008
Escherichia coli (CFU/g)	<10	<10	BS ISO 4832:2006

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.)

2. Product Analysis

2.1 Chemical Analysis of Bimuno

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

Table II.C-2 Summary of the Chemical Product Analysis for 4 Lots of Bimuno Syrup

Specification Parameter	Specification	Manufacturing Lot			
		CKW1	CKW3	CKW13	CKW23
Dry matter (%)	Minimum 70	70.3	71.2	72.8	75.3
Moisture (%)	Maximum 30	29.7	28.8	27.2	24.7
Protein (% DM)	<0.3	<0.1	<0.1	<0.1	<0.1
Ash (% DM)	<1.5	0.26	0.25	0.26	0.24
Galacto-oligosaccharide (% DM)	46 to 60	55.7	49.4	48.1	50.5
Lactose anhydrous (% DM)	<19	3.5	4.1	3.7	3.0
Glucose anhydrous (% DM)	NS	28.7	32.6	31.8	36.8
Galactose (% DM)	NS	11.6	14.0	16.4	15.9
Total Monosaccharides (% DM)	>35	40.8	46.6	48.2	46.6
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 non-consecutive lots of Bimuno syrup demonstrating compliance with the product specifications is presented in Table II.C-3.

Table II.C-3 Summary of the Microbiological Product Analysis for 4 Lots of Bimuno Syrup

Specification Parameter	Specification	Manufacturing Lot			
		CKW1	CKW3	CKW13	CKW23
Total viable count (CFU/g)	$<3.5*10^3$	<5	<5	<5	35
Salmonella spp. (per 25 g)	Not detected	Not detected	Not detected	Not detected	Not detected
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

CFU = colony forming units

2.3 Additional Protein Analyses

Protein residues from the manufacturing are excluded from Bimuno galacto-oligosaccharides *via* ultra-filtration (10 kDa cut-off). Protein concentrations measurable within the ingredient are consistently below the specified limit of 0.3%. 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-6, these proteins were not detected at concentrations above the detection limits of the assays.

Table II.C-6 Summary of the Allergen Testing for 3 Lots of Bimuno

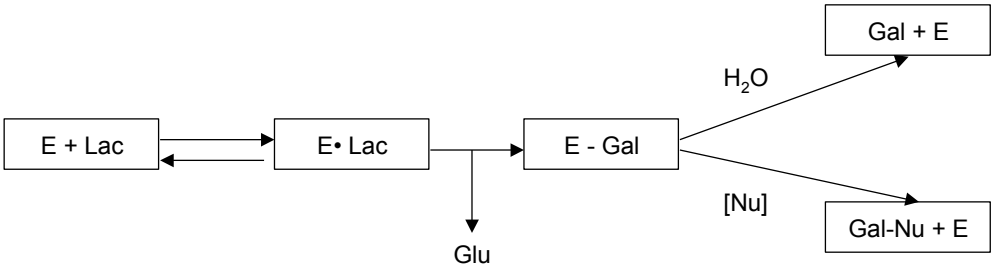
Specification Parameter	Specification	Manufacturing Lot		
		CKW3	CKW13	CKW23
beta-Lacto-globulin	ND	ND	0.9	ND
Casein	ND	ND	ND	ND

ND = not detected above detection limits 2.5mg/kg for casein and 0.5mg/kg for *beta*-lactoglobulin (dry weight basis)

3. Comparison of Bimuno Galacto-oligosaccharides with other GRAS Sources

All GRAS sources of galacto-oligosaccharide preparations that have been the subject of FDA Notifications (U.S. FDA, 2007, 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, slight bias towards a particular linkage and molecular weight distribution type may be observed between different preparations. Bimuno galacto-oligosaccharides also contain the galacto-oligosaccharide *alpha* (1,6) galactobiose, which is present within the preparation at usual concentrations of up to 7% of the total galacto-oligosaccharide content of the material. Quantitative determinations of the concentrations of *alpha*-galactobiose in other galacto-oligosaccharide preparations are not available. Galacto-oligosaccharides also contain glucose, galactose, and un-reacted lactose, and the residual monosaccharides can be partially removed using methods such as chromatographic processes or nano-filtration to produce higher-purity oligosaccharide products.

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



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A comparison of the starting materials, synthesis enzymes, and composition of Bimuno galacto-oligosaccharides with other galacto-oligosaccharide products that have been determined to be GRAS is provided below in Table II.C-5. Based on the information provided within Table II.C-5, it is demonstrated that Bimuno galacto-oligosaccharides are representative of galacto-oligosaccharide preparations that have previously been determined to be GRAS. A discussion of differences between various galacto-oligosaccharide preparations as they relate to potential differences in physiological effects and/or safety are discussed in Section IV.B.

Table II.C-5 Comparison of Bimuno and Other Sources of Galacto-oligosaccharides Determined to be GRAS

Parameter	Bimuno (Clasado)	Vivinal (Friesland Foods Domo)	Purimune (GTC Nutrition)	Oligomate 55N/55NP (Yakult)
Starting Material	Lactose	Lactose	Lactose	Lactose
Beta-Galactosidase Enzyme Origin	*Bifidobacterium bifidum*	*Bacillus circulans*	*Bacillus circulans*	*Sporobolomyces singularis*
Final Product Composition				
Galacto-oligosaccharides (% DM)	46 to 60	≥57	91.5	56.4
Lactose (% DM)	<19	≤23	8.0 to 8.5	12.7
Glucose (% DM)	NS	≤22	0 to 1	22.4
Galactose (% DM)	NS	≥0.8	0 to 0.5	8.6
Monosaccharides (% DM)	>35	19	0 to 1.5	31
Disaccharides[1] (% GOS)	36	33	17 to 23	26
Trisaccharides (% GOS)	40	39	15 to 21	61
Tetrasaccharides and greater (%GOS)	24	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, 2007, 2009a, 2010). NS = Not specified
[1] Exclusive of lactose

II.D Stability

1. Bulk Stability

1.1 Stability under Ambient and Accelerated Storage Conditions

Samples of Bimuno syrup (lot CKW3) were stored under ambient and accelerated storage conditions of 25°C/60% relative humidity (RH), 30°C/65% RH and 40°C/75% RH and assessed for conformance to the chemical, physical, and microbiological specifications. Results demonstrating the stability of the material over a period of 6 months are presented below in Tables II.D-1 through II.D-3.

Table II.D-1 Summary of the Chemical, Physical, and Microbiological Stability Results for Bimuno Lot CKW3 following Storage at 25°C and 60% Relative Humidity

Specification Parameter	Specification	Time-point of Assessment			
		T0 **0 Months**	**T1** **1 Month**	**T3** **3 Months**	**T6** **6 Months**
Chemical					
Moisture (%)	<30%	27.20	26.30	26.60	25.70
Galacto-oligosaccharide (%)	46 to 60 on DM	49.40	49.76	47.22	51.60
Total Monosaccharides (%)	>35 on DM	46.56	45.53	48.30	44.30
Lactose anhydrous (%)	<19 on DM	4.05	4.71	4.48	4.10
Physical					
Clarity	Clear	Clear	Clear	Clear	Clear
Color	Pale Yellow to Golden Brown	Yellow	Golden Yellow	Golden Yellow	Golden Yellow
Microbiological					
Total viable count (CFU/g)	$<3.5*10^3$	<10	NT	NT	NT
Salmonella spp. (per 25 g)	Not detected	ND	NT	NT	NT
Mesophilic spore count (spores/g)	$<1.5*10^3$	<10	NT	NT	NT
Mesophilic anaerobic spore count (spores/g)	$<1.5*10^3$	<10	NT	NT	NT
Yeasts and Molds (CFU/g)	<10	<10	<10	<10	<10
Escherichia coli (CFU/g)	<10	<10	<10	<10	<10

CFU = colony forming units; ND = not detected; NT = not tested

Table II.D-2 Summary of the Chemical, Physical, and Microbiological Stability Results for Bimuno Lot CKW3 following Storage at 30°C and 65% Relative Humidity

Specification Parameter	Specification	Time-point of Assessment			
		T0 0 Months	**T1 1 Month**	**T3 3 Months**	**T6 6 Months**
Chemical					
Moisture (%)	<30%	27.20	26.40	26.70	25.80
Galacto-oligosaccharide (%)	46 to 60 on DM	49.40	50.94	50.70	46.90
Total Monosaccharides (%)	>35 on DM	46.56	44.48	45.16	48.60
Lactose anhydrous (%)	<19 on DM	4.05	4.58	4.14	4.50
Physical					
Clarity	Clear	Clear	Clear	Clear	Clear
Color	Pale Yellow to Golden Brown	Yellow	Yellow	Golden Yellow	Golden Yellow
Microbiological					
Total viable count (CFU/g)	$<3.5*10^3$	<10	NT	NT	NT
Salmonella spp. (per 25 g)	Not detected	ND	NT	NT	NT
Mesophilic spore count (spores/g)	$<1.5*10^3$	<10	NT	NT	NT
Mesophilic anaerobic spore count (spores/g)	$<1.5*10^3$	<10	NT	NT	NT
Yeasts and Molds (CFU/g)	<10	<10	<10	<10	<10
Escherichia coli (CFU/g)	<10	<10	<10	<10	<10

CFU = colony forming units; ND = not detected; NT = not tested

Table II.D-3 Summary of the Chemical, Physical, and Microbiological Stability Results for Bimuno Lot CKW3 following Storage at 40°C and 75% Relative Humidity

Specification Parameter	Specification	Time-point of Assessment			
		T0 0 Months	**T1 1 Month**	**T3 3 Months**	**T6 6 Months**
Chemical					
Moisture (%)	<30%	27.20	26.10	26.70	26.40
Galacto-oligosaccharide (%)	46 to 60 on DM	49.40	48.43	49.50	51.80
Total Monosaccharides (%)	>35 on DM	46.56	46.47	46.25	44.30
Lactose anhydrous (%)	<19 on DM	4.05	5.10	4.25	3.90
Physical					
Clarity	Clear	Clear	Clear	Clear	Clear
Color	Pale Yellow to Golden Brown	Yellow	Golden Yellow/Amber	Golden Brown	Golden Brown
Microbiological					
Total viable count (CFU/g)	$<3.5*10^3$	<10	NT	NT	NT
Salmonella spp. (per 25 g)	Not detected	ND	NT	NT	NT

Table II.D-3 Summary of the Chemical, Physical, and Microbiological Stability Results for Bimuno Lot CKW3 following Storage at 40°C and 75% Relative Humidity

Specification Parameter	Specification	Time-point of Assessment			
		T0 0 Months	T1 1 Month	T3 3 Months	T6 6 Months
Mesophilic spore count (spores/g)	$<1.5*10^3$	<10	NT	NT	NT
Mesophilic anaerobic spore count (spores/g)	$<1.5*10^3$	<10	NT	NT	NT
Yeasts and Molds (CFU/g)	<10	<10	<10	<10	<10
Escherichia coli (CFU/g)	<10	<10	<10	<10	<10

CFU = colony forming units; ND = not detected; NT = not tested

As demonstrated in Tables II.D-1 through II.D-3, the galacto-oligosaccharide content is consistently stable (*i.e.*, 47 to 51% w/w DM) at both ambient and elevated temperatures and similar trends are observed for lactose and glucose. The noticeable change is the color of the syrup, which goes from yellow to golden yellow. The change in color can be attributed to the Maillard reaction, which is the reaction of the sugars with non-protein nitrogen residues in the syrup, and the change is more noticeable at higher temperature of 45°C/70% RH. The color change also is reflected in the taste of the syrup, which is reported to have a caramel taste. From a microbiological perspective, no change was observed in the *E. coli* and the Yeast/Mold counts.

To estimate the shelf-life of Bimuno syrup manufactured using BbgIV, the results from storage at 25°C/60% RH and 45°C/70% RH were extrapolated to 24 months. As shown in Figures II.D-1 and II.D-2 below, it was estimated that the galacto-oligosaccharide content would remain above the specification of 46% (DM) at 24 months.

Figure II.D-1 Extrapolation of GOS, Lactose, and Monosaccharide Content of Bimuno (Lot CKW3) following Storage at 25°C and 60% Relative Humidity

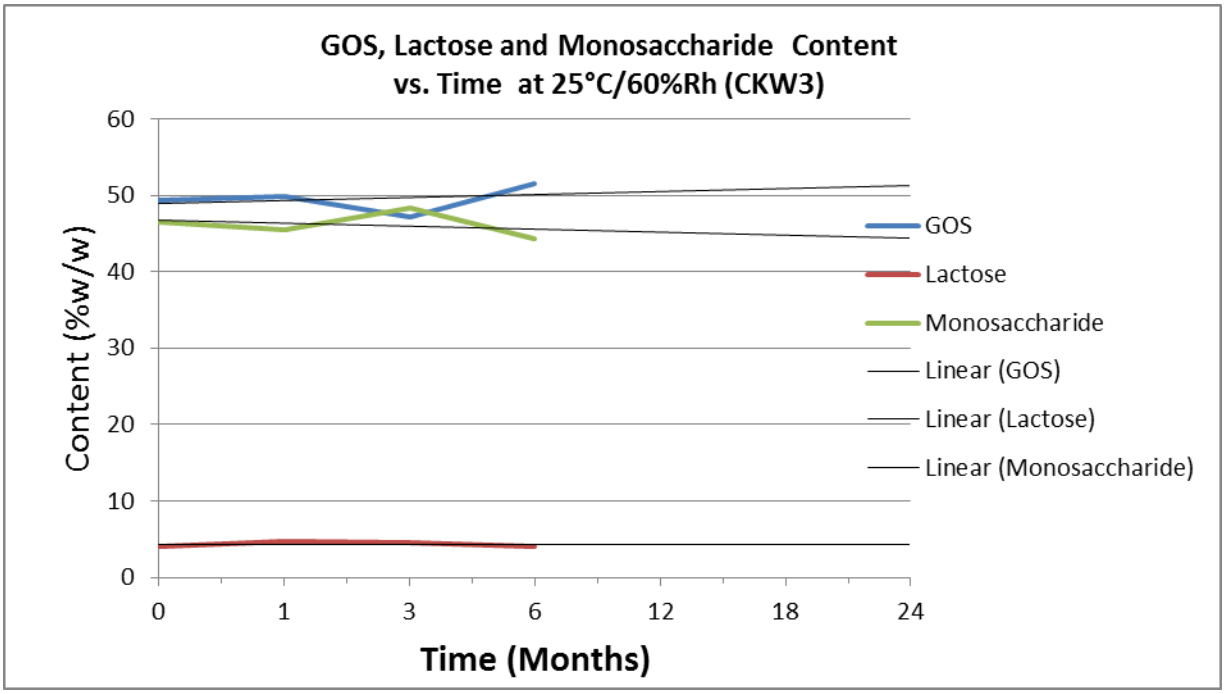
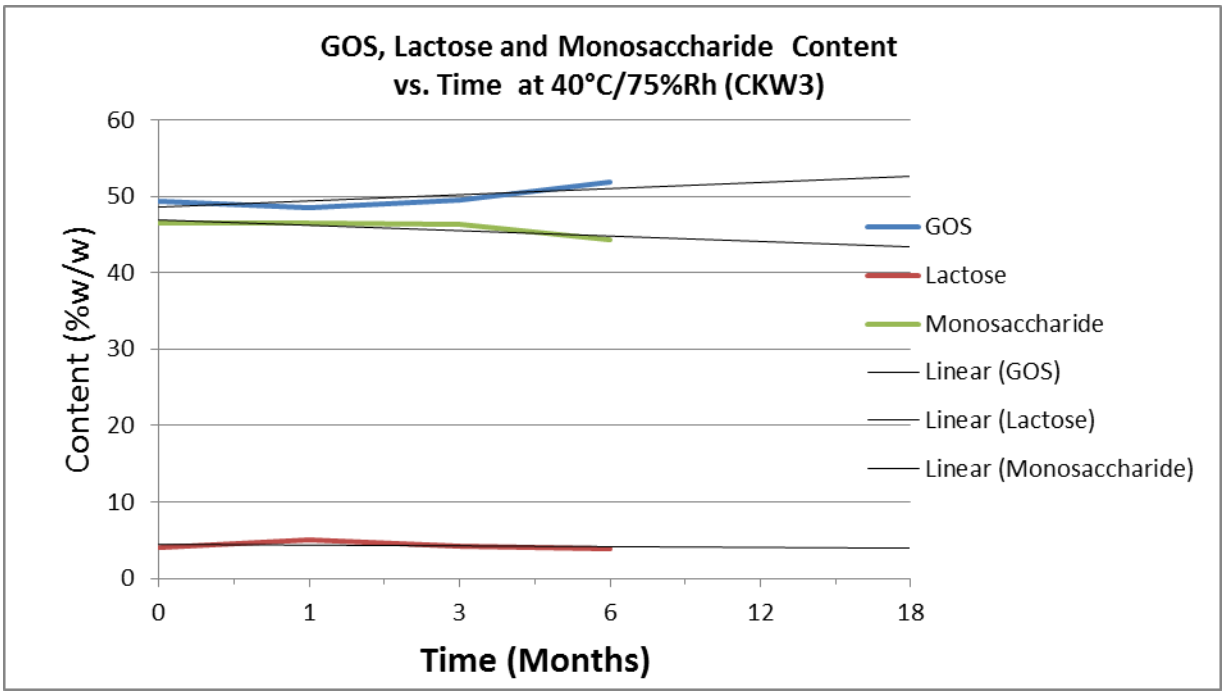


Figure II.D-2 Extrapolation of GOS, Lactose, and Monosaccharide Content of Bimuno (Lot CKW3) following Storage at 45°C and 70% Relative Humidity



In an ongoing study, samples of Bimuno syrup (lot CKW23) were stored under normal storage conditions of 25°C/60% RH and assessed for conformance to the chemical, physical, and microbiological specifications. The results from the first 6 months are presented below in Table II.D-4.

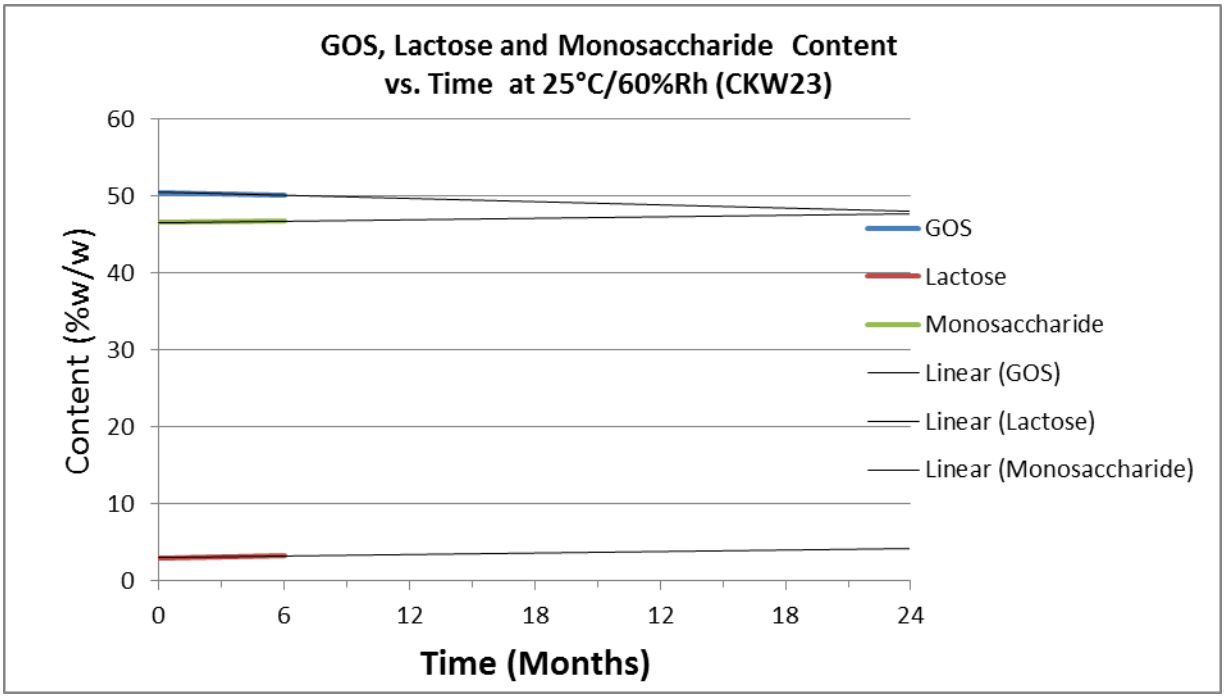
Table II.D-4 Summary of the Chemical, Physical, and Microbiological Stability Results for Bimuno Lot CKW23 following Storage at 25°C and 60% Relative Humidity

Specification Parameter	Specification	Timepoint of Assessment	
		T0 **0 Months**	**T6** **6 Months**
Chemical			
Moisture (%)	<30%	24.70	22.40
Galacto-oligosaccharide (%)	46 to 60 on DM	50.50	50.10
Total Monosaccharides (%)	>35 on DM	46.60	46.80
Lactose anhydrous (%)	<19 on DM	3.0	3.20
Physical			
Clarity	Clear	Clear	Clear
Color	Pale Yellow to Golden Brown	Yellow	Pale Yellow
Microbiological			
Total viable count (CFU/g)	$<3.5*10^3$	35	15
Salmonella spp. (per 25 g)	Not detected	Absent	Absent
Mesophilic spore count (spores/g)	$<1.5*10^3$	25	10
Mesophilic anaerobic spore count (spores/g)	$<1.5*10^3$	10	10
Yeasts and Molds (CFU/g)	<10	<10	<10
Escherichia coli (CFU/g)	<10	<10	<10

CFU = colony forming units

The galacto-oligosaccharide content is consistently stable at ambient temperature over the first 6 months of storage, and similar trends are observed for lactose and glucose. Extrapolating the results of Bimuno syrup from the first 6 months (see Figure II.D-3) yields an estimated 24-month shelf life. In addition, Bimuno syrup has been stored in bulk for >2 years under ambient conditions and has remained microbiologically and chemically stable.

Figure II.D-3 Extrapolation of GOS, Lactose, and Monosaccharide Content of Bimuno (Lot CKW23) following Storage at 25°C and 60% Relative Humidity



1.2 Stability in Beverages

Real-time stability of the syrup, powder, and Bimuno formulated in a juice matrix were analyzed for breakdown of galacto-oligosaccharides. Samples (10 g) of Bimuno powder and syrup were stored at 25 or 4°C in dry clean conditions, and at time intervals of 1, 2, 4, 6, 12, 18, and 24 months, the individual samples were analyzed (in triplicate) using high-performance liquid chromatography (HPLC) and HPAEC-PAD. The concentrations of glucose, galactose, lactose, and galacto-oligosaccharide contents were determined and compared against the content at time 0.

For stability testing in the fruit juice matrix (pH 4.5), polyethylene terephthalate (PET) bottles were filled with 0.5 L of fruit juice containing 1 g of Bimuno galacto-oligosaccharides and stored at room temperature for 6 months. At monthly intervals, individual bottles were opened and the galacto-oligosaccharide content determined using HPLC and HPAEC-PAD for galacto-oligosaccharide composition and compared against the content at time 0. Under the conditions of the study Bimuno galacto-oligosaccharides were stable over 24 months. The results are presented in Table II.D-5.

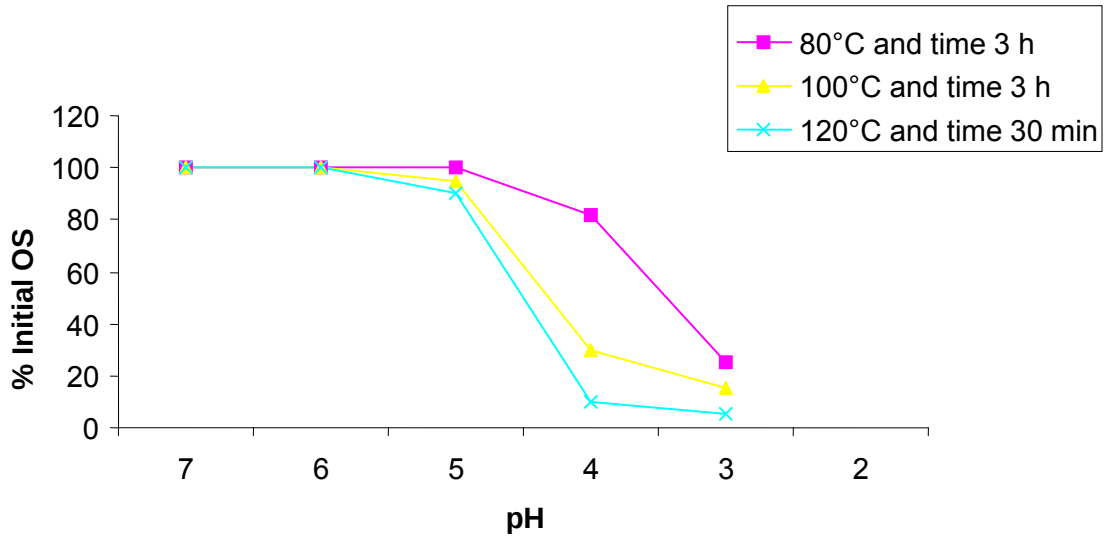
Table II.D-5 Stability of Galacto-oligosaccharides (GOS) Present in Bi^{2}muno® Under Intended Storage Conditions

Month	Amount of GOS (as % of initial GOS content)[a]		
	Syrup (at 4°C)[b]	Powder (at 25°C)[b]	In Fruit Juice Matrix (pH 4.5, at room temperature)[c]
0	100	100	100
1	95	97	96
2	98	95	103
4	94	101	97
6	102	92	98
12	96	97	NA
18	NA	96	NA
24	NA	95	NA

NA = not available
[a] Stability data for the syrup at 18 months will be available at the end of April, and stability data for the powder at 31 months will be available at the end of March.
[b] Results are the average of 3 samples.
[c] Results are from single samples.

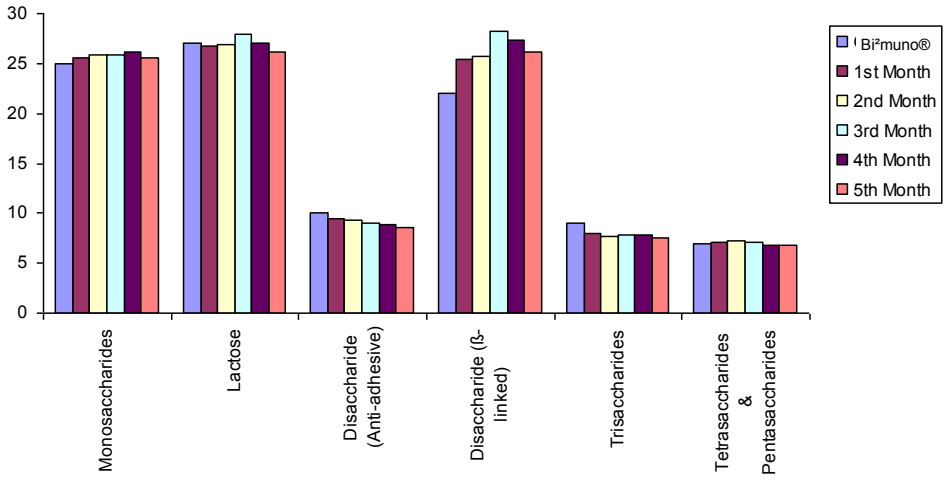
The stability of Bimuno in solution across a range of pH and temperature conditions was tested at a pH of 3, 4, 5, 6, and 7. Samples were dissolved in water and adjusted to the target pH value using 1M hydrochloric acid (HCl) or 1M sodium hydroxide (NaOH). Samples were then stored at 80 and 100°C for 3 hours or 120°C for 30 minutes. After the storage period, the oligosaccharide content of the samples was analyzed by HPLC and HPAEC-PAD and compared to the galacto-oligosaccharide content of a baseline control sample. At high temperatures (80 to 120°C), Bimuno remained relatively stable at pH 5 to 7. At high temperature and low pH hydrolysis of the galacto-oligosaccharide was observed. A representation of the results is presented in Figure II.D-4.

Figure IID-4 Stability of Bi^{2}muno® under Varied Temperature and pH Conditions



The stability of Bimuno galacto-oligosaccharides was also assessed in a fruit juice matrix at a pH of 4.5. Samples of Bimuno were tested over a period of 5 months and assessed for the oligosaccharide composition using HPAEC-PAD. There were no significant changes in the oligosaccharide composition of Bimuno over the 5-month period, as is demonstrated in Figure II.D-5 below.

Figure II.D-5 Stability of Bi²muno® Oligosaccharide Composition in Fruit Juice at pH 4.5



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 as described within Table I.D-1 is based on scientific procedures. Galacto-oligosaccharides, produced from food grade lactose using the activity of microbial *beta*-galactosidases, have been previously determined to be GRAS for use in a large number of food applications across multiple food and beverage categories. These GRAS determinations have been Notified to the United States Food and Drug Administration (FDA) without objection from the Agency (U.S. FDA, 2007, 2009a, 2010). Food uses within infant formula also have been determined to be GRAS (U.S. FDA, 2007, 2009b). Accordingly, published data and information supporting the safety of galacto-oligosaccharides for food and beverage applications have been critically reviewed by multiple qualified Experts and by the FDA. General recognition of the safety of galacto-oligosaccharides for use in food and beverage applications 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 (SCF, 2003; FSANZ, 2008). Galacto-oligosaccharides also are currently permitted for general food use without limitation on use in the marketplaces of the European Union and Australia New Zealand without the requirement for pre-market

approval as galacto-oligosaccharides are not subject to Novel Food regulations in these jurisdictions.

A discussion of estimated exposure to galacto-oligosaccharides following the introduction of Bimuno galacto-oligosaccharides to the U.S. marketplace under the intended conditions of use is presented in Section IV.A.3. As discussed (Section II.C.4), all GRAS sources of galacto-oligosaccharides currently available within the U.S. marketplace are produced in a similar manner, and Bimuno galacto-oligosaccharides are considered representative of galacto-oligosaccharide preparations that have previously been determined to be GRAS. Published studies relevant to the safety of food uses of galacto-oligosaccharide preparations, including information characterizing the metabolic fate, toxicity in animals, safety and tolerance in humans, effects on mineral balance, and allergenicity are therefore incorporated by reference to previous GRAS determinations (U.S. FDA, 2007, 2009a, 2010). To identify new data and information relevant to the safety of food uses of galacto-oligosaccharides (*i.e.*, those preparations produced from food grade lactose using the activity of a *beta*-galactosidase preparation) published since the previous GRAS determinations a comprehensive search of the published scientific literature was conducted through June 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[2] information, both favorable and unfavorable, identified during the literature search are summarized in Sections IV.B through IV.H below.

IV.A Consumption Estimates

1. Background Dietary Intake of Galacto-oligosaccharides

Natural sources of dietary galacto-oligosaccharides are largely limited to exposures to trace quantities from specialty dairy products, where *beta*-galactosidases are used to reduce the lactose content of the product to make it suitable for lactose sensitive individuals. Dietary exposure to galacto-oligosaccharides therefore is almost exclusively determined by introduced food sources.

Bimuno galacto-oligosaccharides contain the galacto-oligosaccharide *alpha*-(1,6)-galactobiose at a usual concentration of 3.5% (DW). Quantitative determinations of the concentrations of *alpha*-galactobiose in other galacto-oligosaccharide preparations are not available. a*lpha*-Galactobioses are widely distributed in plants and vegetables, such as soybeans, broccoli, asparagus, and whole grains (Saini, 1988) and the *alpha*-(1,6)-galactobiose linkage

[2] Studies deemed relevant to the safety evaluation of Bimuno galacto-oligosaccharides were those in which galacto-oligosaccharides were produced from lactose by the enzymatic activity of a *beta*-galactosidase enzyme preparation. Although Bimuno galacto-oligosaccharides are not proposed for infant formula uses, studies in infants administered galacto-oligosaccharides in combination with fructo-oligosaccharides also were considered.

also have been reported to occur as a component of some oligosaccharides within human milk from lactating women (Yamashita and Kobata, 1974). In soybeans, *alpha*-(1,6)-galactobiose is a component of the trisaccharide, raffinose, and the tetrasaccharide, stachyose (Taeufel *et al*., 1998). Estimates of *alpha*-galacto-oligosaccharide content of peas, beans, and lentils are reported to ranging from 5 to 8% (JECFA, 1998) and concentrations of 4 to 6% are reported for soybeans (LeBlanc *et al.*, 2004). Dietary exposure to *alpha*-galactobiose (*i.e*., stachyose and raffinose) is expected to occur among consumers of soy milk, and infants consuming soy based infant formula also are exposed to low levels of stachyose and raffinose within the formula (SMCN, 2003; Agostini *et al*., 2006).

2. Galacto-oligosaccharide Consumption from Current GRAS

As discussed, several galacto-oligosaccharide preparations have been determined to be GRAS for use in various conventional food and beverage products across multiple food categories in the United States (U.S. FDA, 2007, 2009a, 2010). A summary of these food uses and a summary of the corresponding estimated intakes that have been calculated for these food applications are presented in Table IV.A-1. The highest estimated intake of galacto-oligosaccharides from the current Notified food uses have been estimated for male teenagers, where potential daily exposures of up to 33 g per person per day have been estimated (U.S. FDA, 2010). Among infants and children, 90th percentile exposures of up to 30.4 g per day were estimated. These intakes are a reflection of the large number of potential food categories available for potential addition of galacto-oligosaccharides and the high use levels for some food applications (*e.g*., 7 g per serving for yogurt). Based on the cost of the ingredient and the intended nutritive food application, the introduction of galacto-oligosaccharides to all available food categories is unlikely to occur. Moreover, the 90th percentile exposures will over-estimate exposures as they are based on the assumption that a heavy user will consume the ingredient from all potential food applications to which galacto-oligosaccharides may be added at the highest use level on a daily basis, a situation that unlikely reflects real market exposures.

Table IV.A-1 Current GRAS Food Uses of Galacto-oligosaccharides Notified to the FDA

Company (GRAS Notice No.)	Food Uses	Use Level (g GOS Per Serving)	All-User 90th Percentile Exposure Estimates (g GOS/day)
GTC Nutrition (GRN 285; U.S. FDA, 2009a; GRN 286; U.S. FDA, 2009b)	baby, infant, and toddler foods	0.86 to 1.28	Infants: 9.8 Children: 14.2 Female Teenager: 14.6 Male Teenager: 20.2 Female Adult: 14.0 Male Adult: 17.8 Total Population 15.4
	certain beverages and beverage bases, dairy product analogs, milk products, bakery products, cereal and other grain products, desserts, dessert toppings and fillings, fruit and fruit juices, snacks, soups, and soft and hard candy	1.28	
Friesland Foods Domo (GRN 236; U.S. FDA, 2007)	bars, yogurt, frozen dairy desserts, fruit drinks and energy drinks, fitness water and thirst quenchers, fruit pie filling, fruit preparation, jelly and jam, baby juice, baby yogurt drink, baby dessert, baby snack, milk, milk drinks, syrup flavorings for milk, meal replacement drinks, meal replacement drinks for children, and milk substitutes	1 to 7.5	Infants: 11.2 to 12.5 Children: 16.4 to 18.8 Female Teenager: 16.7 Male Teenager: 18.9 Female Adult: 15.3 Male Adult: 17.7 Total Population: 16.8
Yakult Pharmaceutical Industry Co., Ltd. (GRN 334; U.S. FDA, 2010)	milk and milk substitutes such as soy milk; milk drink; yogurt; milk based meal replacement; white sauces, milk gravies and cheese sauces; milk desserts, frozen like ice creams; pudding and custards including baby foods; cheese soups, egg soups, soups with legumes as major ingredient, soups with grain products as major ingredient, potato soups, deep-yellow vegetable soups, tomato soups, and other vegetable soups; coconut beverages; bread; brownies; cakes (heavy weight); cakes (medium weight); cakes (light weight); coffee cakes, crumb cakes, doughnuts, Danish, sweet rolls, sweet quick type breads, muffins, and toaster pastries; cookies; Crackers that are usually used as snacks; French toast, pancakes; Pies, cobblers, fruit crisps, turnovers, other pastries; Waffles; Grain-based bars with or without filling or coating, e.g., breakfast bars, granola bars, rice cereal bars; Ready-to-eat cereals; Ready-to-eat cereals (dry) for baby food; Ready-to-serve cereals for baby food; Fruit juices (including citrus fruit juices) and nectars; Vegetable juices; Fruit juices, vegetable juices and juice mixtures baby food; Jellies, jams, preserves; Fruit drinks such as fruit juice drinks, fruit flavored drinks, sports drinks, etc; Non fruit beverages, including energy drinks; Beverage concentrated (powder)	0.3 to 11	Infants:26.8 Children: 30.4 Female Teenager: 24.2 Male Teenager: 33.0 Female Adult: 19.4 Male Adult: 24.8 Total Population: 25.3

GOS = galacto-oligosaccharides

3. Estimated Consumption of Galacto-oligosaccharides from Intended Food-Uses of Bimuno Galacto-oligosaccharides

Bimuno galacto-oligosaccharides are intended for food uses that have previously been determined to be GRAS as described with GRN No. 236, 285, and 334 (U.S. FDA, 2007, 2009a, 2010), at a use level of up to 3.0 g per serving. Since Bimuno galacto-oligosaccharides will serve as an alternative source of galacto-oligosaccharides to existing sources currently available on the marketplace, these food uses will not increase dietary exposure to galacto-oligosaccharides among the general population of U.S. consumers using these products.

Bimuno 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. Coffee and tea uses will be represented by pre-packaged ready to brew single serving products. Alternatively, the addition of Bimuno galacto-oligosaccharides to coffee and tea products may be achieved through the intended food use in pre-packaged single serving coffee creamer/whitener products intended exclusively for use with coffee and/or tea products at a use level providing up to 0.8 g of galacto-oligosaccharides per serving. The potential exposure to galacto-oligosaccharides from food uses in coffee and tea applications were estimated using data from the NHANES 2007 through 2010 surveys and Crème intake modeling software (CDC, 2011; USDA, 2012; Crème, 2013). Mean coffee consumption among all-users was estimated to be highest in male adults at 590 g per person per day. Total population all-user intake estimates were 519 g per person per day. Based on a 6 oz. serving of coffee and an intended use level of Bimuno galacto-oligosaccharides of 1.5 g per serving, intakes of galacto-oligosaccharides were estimated to be 5.0 g per person per day among adult males and total population all-users estimates were 4.4 g per person per day. These levels of intake are expected to be consistent with current food uses, which have been determined to be GRAS at use levels of between 5 and 7 g per serving for select food uses (*i.e.*, yogurt and fruit smoothies respectively under GRN 236) (U.S. FDA, 2007). It is expected that habitual users of galacto-oligosaccharide containing coffee products would intentionally seek out these products for their nutritional benefits. Uses in coffee and tea will likely serve as a replacement to consumption of other galacto-oligosaccharide containing foods, and cumulative intakes of multiple galacto-oligosaccharide products at the highest use levels on a chronic basis is unlikely to occur.

Table IV.A-2 Intake of Coffee with the U.S. Population (NHANES 2007-2010)

Population Group	Age Group (years)	Mean Daily Intake of Coffee Consumers Only (g/day)	Mean Serving of Coffee Consumers Only (No. of 6 oz servings/ day)	Mean GOS Intake of Coffee Consumers Only (g/day)
Children	3 to 11	83	0.5	0.7
Female Teenagers	12 to 19	215	1.2	1.8
Male Teenagers	12 to 19	252	1.4	2.1
Female Adults	20 and older	462	2.6	3.9
Male Adults	20 and older	590	3.3	5.0
Total Population	All ages	513	2.9	4.4

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 (Smiricky-Tjardes *et al.*, 2003; Suarez *et al.*, 1999). 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 and animals has been discussed previously (U.S. FDA, 2007, 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*. In humans, higher doses may result in flatulence, borborygmi, and abdominal discomfort. As discussed in Section II.C.4, Bimuno galacto-oligosaccharides are representative of other GRAS sources of galacto-oligosaccharides that have been previously determined to be GRAS. Since physiological changes within the gastro-intestinal tract are limited to the effects of microbial fermentation, a process that is largely indiscriminant to galacto-

oligosaccharide linkage and molecular weight, studies evaluating the toxicity of various galacto-oligosaccharide preparations in animals and tolerance in humans that have been evaluated to date are relevant to the safety evaluation of Bimuno galacto-oligosaccharides. This conclusion is supported by similar physiological and toxicological findings in rodents administered different galacto-oligosaccharide preparations (*e.g.*, Ohtsuka *et al.*, 1990; Anthony *et al.*, 2006; Kobayashi *et al.*, 2009), and observations from studies in humans administered Bimuno galacto-oligosaccharides are consistent with those reported for other GRAS preparations of galacto-oligosaccharides (Section IV.G). New information identified in the literature relevant to the metabolism of galacto-oligosaccharides was limited (Hernández-Hernández *et al.*, 2012), and were of limited usefulness to the safety evaluation of Bimuno galacto-oligosaccharides. For completeness a brief summary of this study is presented below.

Hernández-Hernández *et al.* (2012) evaluated the *in vivo* ileal digestibility of galacto-oligosaccharides manufactured from lactulose [GOS-Lu; degree of polymerization (DP) ≥2; 14% trisaccharides] and a commercial galacto-oligosaccharide preparation derived enzymatically from lactose (GOS-La; DP≥3; 35.1% trisaccharides). Male Wistar rats were fed diets containing 0 (control) or 1% of GOS-Lu or GOS-La (approximately equivalent to 1,893 mg/kg body weight/day based on reported food intake and body weight data) for a period of 14 days. Analysis of trisaccharide fractions in ileal samples using gas chromatography-mass spectrometry (GS-MC) revealed that GOS-La trisaccharides were partially digested, while GOS-Lu trisaccharides were more resistant to digestion. The digestibility rate of the trisaccharide fraction of GOS-Lu was reported to be 12.56±2.6%, while the digestibility of the trisaccharide fraction of GOS-La was much higher at a rate of 52.9±2.7%. The digestibility of the higher oligosaccharides was not assessed. Both GOS-Lu and GOS-La were absent from fecal matter, suggesting that the oligosaccharides were readily fermented by the intestinal microbiota within the large intestine. The results of this study are consistent with previous published information characterizing the metabolic fate of galacto-oligosaccharide preparations which demonstrate that orally administered galacto-oligosaccharides travel largely intact through the gastro-intestinal tract to the colon where they are subjected to complete fermentation by the resident microflora. Although the authors' report partial metabolism of GOS-La within the ileum, there is no published evidence demonstrating that galacto-oligosaccharides are susceptible to mammalian digestive enzymes, and the partial hydrolysis of GOS-La is likely attributed to fermentation by the established presence of commensal microorganisms within the small intestine of the rodent (Savage, 1969).

IV.E Toxicological Studies

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 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 of galacto-oligosaccharides. The threshold for this effect in humans has been reviewed previously (U.S. FDA, 2007, 2009a, 2010), and the significance of the endpoint to the safety of galacto-oligosaccharide preparations food uses of the ingredient is discussed by the Expert Panel (see Appendix A).

Animal studies identified during the updated literature search containing endpoints relevant to the safety evaluation are presented below in Sections IV.E.1 and IV.E.2. Findings from these studies are consistent with the conclusion that galacto-oligosaccharides are innocuous compounds that are of low toxic potential, even when administered at high dietary concentrations.

1. Other Animal Studies

Several repeated-dose studies investigating the effects of galacto-oligosaccharides in rats published subsequent to the previous galacto-oligosaccharide GRAS Notifications were identified in the literature (Dos Santos *et al.*, 2011; Weaver *et al.*, 2011; Hernández-Hernández *et al.*, 2012). While the majority of these studies were not specifically conducted to evaluate safety, some safety-related parameters (*e.g.*, food consumption, body weight, organ weights) were investigated. The results of these studies are discussed below and are summarized in Table IV.E.1-1.

Dos Santos *et al.* (2011) examined the effects of a galacto-oligosaccharide product on calcium absorption in a study wherein normal (sham-operated) and partially gastrectomized male Wistar rats were fed diets containing 50 g/kg diet of a galacto-oligosaccharides product (approximately 4,000 mg/kg body weight/day, equivalent to 2,000 mg GOS/kg body weight/day, based on reported food intake, body weight data, and the galacto-oligosaccharide content of the test article) or a basal control diet for 8 weeks. The galacto-oligosaccharide product was comprised of 50 to 55% of oligosaccharides, and was provided by Yakult Industry and Commerce. There were no significant differences in terminal body weights or food intake reported in sham-operated and partially gastrectomized rats fed galacto-oligosaccharides compared to their respective control groups. There were no significant differences in serum calcium concentrations reported between the sham-operated groups. Partial gastrectomy resulted in a

significant reduction in serum calcium concentrations compared to normal rats; however, galacto-oligosaccharide administration significantly increased serum calcium concentrations in partially gastrectomized rats compared to partially gastrectomized rats given the control diet.

The effects of galacto-oligosaccharides on mineral absorption and bone properties in rats were investigated by Weaver *et al.* (2011). In this study, male Sprague-Dawley rats were fed diets containing 0 (control), 2, 4, 6, or 8% galacto-oligosaccharides (equivalent to 0, 1,420, 2,825, 4,405, and 6,118 mg/kg body weight/day, respectively, based on reported galacto-oligosaccharide intake and body weight values) or a basal control diet for 8 weeks. The source of galacto-oligosaccharide was Vivinal® GOS, which contains approximately 90% galacto-oligosaccharides (supplied by Friesland Campina Domo). There were no significant differences in body weight gain, terminal body weights, food intake, or food efficiency ratio reported among groups. Absolute cecal wall and cecal content weights were reported to be significantly higher in groups administered galacto-oligosaccharides at dietary concentrations of 4% and greater (*i.e.,* ≥2,285 mg/kg body weight/day) compared to the control group. The same dose groups also exhibited a significant, dose-dependent decrease in cecal pH compared to the control group. The authors noted that the decreased cecal pH and increased cecal wall and content weights were a result of galacto-oligosaccharide fermentation in the colon (*i.e.,* a physiological adaptation).

Hernández-Hernández *et al.* (2012) compared the prebiotic effects of GOS-Lu (DP≥2) and GOS-La (DP≥3) in young rats. Male Wistar rats were fed diets containing 0 (control) or 1% of GOS-Lu or GOS-La (approximately equivalent to 1,893 mg/kg body weight/day based on reported food intake and body weight data) for a period of 14 days. There were no significant differences in food intake or body weight gain reported among groups. There were no significant differences in relative organ weights, including the stomach, small intestine, cecum, and colon, reported among groups, with the exception of significantly lower relative colon weights in the GOS-La group compared to the control group.

Table IV.E.1-1 Summary of Repeated Dose Studies on Galacto-oligosaccharides

Species (Strain)	Treatment	Test Article	Dose mg GOS/kg bw/d	Reported Effects [a,b]		Reference
Rat (Wistar) N=12 M/group	Oral (diet) 14 days	Galacto-oligosaccharides (derived from lactulose)	0 or 1,893[c]	General condition/survival	NR	Hernández-Hernández *et al.*, 2012
				Food and water intake	NSD in food intake Water intake NE	
				Body weight	NSD in body weight gain	
				Organ and tissue effects	NSD in relative stomach, small intestine, and cecum weights ↓ relative colon weight	
				Hematology, clinical chemistry, and urinalysis	NE	
				NOAEL	Not identified	
Rat (Wistar) N=12 M/group	Oral (diet) 14 days	Galacto-oligosaccharides Identity not characterized	0 or 1,893[c]	General condition/survival	NR	Hernández-Hernández *et al.*, 2012
				Food and water intake	NSD in food intake Water intake NE	
				Body weight	NSD in body weight gain	
				Organ and tissue effects	NSD in relative stomach, small intestine, cecum, and colon weights	
				Hematology, clinical chemistry, and urinalysis	NE	
				NOAEL	Not identified	
Rat (Wistar; gastroectomized or sham-operated) N=10 M/group	Oral (diet) 8 weeks	Galacto-oligosaccharides product (50 to 55% oligosaccharides)	0 or 2,000[c]	General condition/survival	NR	Dos Santos *et al.*, 2011
				Food and water intake	NSD in food intake	
				Body weight	NSD in body weight	
				Organ and tissue effects	NE	

Table IV.E.1-1 Summary of Repeated Dose Studies on Galacto-oligosaccharides

Species (Strain)	Treatment	Test Article	Dose mg GOS/kg bw/d	Reported Effects [a,b]		Reference
				Hematology, clinical chemistry, and urinalysis	NSD in serum calcium in sham-operated groups ↑ serum calcium in gastroectomized groups	
				NOAEL	Not identified	
Rat (Sprague-Dawley) N=15 M/group	Oral (diet) 8 weeks	Vivinal® galacto-oligosaccharides (~90% GOS)	0, 1,420, 2,825, 4,405, or 6,118[d]	General condition/survival	NR	Weaver *et al.*, 2011
				Food and water intake	NSD in total food intake or food efficiency ratio Water intake NE	
				Body weight	NSD in terminal body weights or body weight gain	
				Organ and tissue effects	Dose-dependent ↓in cecal pH [≥2,825] Dose-dependent ↑in absolute cecal wall weight and cecal content weight [≥2,825]	
				Hematology, clinical chemistry, and urinalysis	NE	
				NOAEL	Not identified	

F = female; M =male; NE = not evaluated; NOAEL = no-observed-adverse-effect level; NSD = no significant differences
[a] unless stated otherwise, all reported effects are statistically significantly different relative to control group(s)
[b] numbers in [ ] correspond to the dose(s) at which the reported effects were observed
[c] Based on reported food intake and body weight values.
[d] Based on reported galacto-oligosaccharide intake and body weight values

2. 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.F 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). 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 observed in humans administered 20 g of galacto-oligosaccharides per day for a duration of 4 days (van den Heuvel *et al.*, 2000). However, the effect of galacto-oligosaccharides on mineral absorption was not consistently reported across all studies (*e.g.*, van den Heuvel *et al*., 1998). The effects of galacto-oligosaccharide preparations on mineral absorption (*i.e.*, calcium and magnesium) have been discussed previously (U.S. FDA, 2007, 2009a, 2010), and a summary of this information as reviewed by the Expert Panel is presented in Appendix A. Studies identified during the updated literature search are discussed below. The results of these studies are consistent with those published previously, which suggest that galacto-oligosaccharides can produce improvements in calcium balance in rodent models.

In a study conducted to investigate the effects of a galacto-oligosaccharide product on calcium absorption, sham-operated and partially gastrectomized male Wistar rats were fed diets containing 50 g/kg diet of a galacto-oligosaccharide product (approximately 4,000 mg/kg body

weight/day, equivalent to 2,000 mg GOS/kg body weight/day) or a basal control diet for 8 weeks (Dos Santos *et al.*, 2011). The galacto-oligosaccharide product was comprised of 50 to 55% oligosaccharides, and was provided by Yakult Industry and Commerce. Parameters evaluated included serum calcium concentration, fecal calcium excretion, and bone calcium concentration. Calcium absorption was calculated. No significant differences in serum calcium concentrations were reported between the sham-operated groups. Partial gastrectomy resulted in a significant reduction in serum calcium concentrations compared to normal rats; however, serum calcium concentrations were significantly higher in partially gastrectomized rats administered galacto-oligosaccharides compared to their respective control group. Significantly lower fecal calcium concentrations were observed in both sham-operated and partially gastrectomized rats administered galacto-oligosaccharides compared to their respective control groups. Apparent calcium absorption was reported to be significantly higher in sham-operated and partially gastrectomized rats administered galacto-oligosaccharides compared to their respective control groups. Dietary administration of galacto-oligosaccharides also was reported to significantly increase bone calcium concentrations in sham-operated rats compared to the control group; however, no significant differences in bone calcium concentration were reported between the partially gastrectomized groups. The authors suggested that the lack of effect of galacto-oligosaccharides on bone calcium content in partially gastrectomized rats was likely due to the short study duration. Specifically, though partial gastrectomy reduces the apparent absorption of calcium in rats, the 8-week duration of the study may not have been long enough to result in a corresponding reduction in bone calcium. Based on their findings, the authors concluded that galacto-oligosaccharides may prevent osteopenia in partially gastrectomized rats.

The effects of galacto-oligosaccharides on mineral absorption and bone properties in rats were investigated by Weaver *et al.* (2011). In this study, male Sprague-Dawley rats were fed diets containing 0 (control), 2, 4, 6, or 8% galacto-oligosaccharides (equivalent to 0, 1,420, 2,825, 4,405, and 6,118 mg/kg body weight/day, respectively, based on reported galacto-oligosaccharide intake and body weight values) or a basal control diet for 8 weeks. The test article was Vivinal® GOS, which contains approximately 90% galacto-oligosaccharides (supplied by Friesland Campina Domo). Parameters assessed included fecal and urinary calcium and magnesium excretion, femur ^{45}Ca uptake, and urinary potassium and sodium excretion. Calcium and magnesium absorption and retention ratios were calculated. The calcium and magnesium absorption and retention ratios were higher in all galacto-oligosaccharides groups compared to the control group, with the effect achieving statistical significance at dietary levels of 2, 4, and 8% galacto-oligosaccharides for magnesium, and 6 and 8% galacto-oligosaccharides for calcium. A statistically significant increase in femur ^{45}Ca uptake was reported in the rats administered galacto-oligosaccharides (dose group not specified) compared to the control group. There were no significant differences in urinary potassium and sodium excretion reported among groups. Based on their findings, the authors concluded that dietary administration of galacto-oligosaccharides to growing rats had beneficial effects on calcium and magnesium absorption.

IV.G Human Studies

The results of studies conducted in 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) have demonstrated that consumption of Bimuno galacto-oligosaccharides 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 is well-tolerated. Numerous studies investigating the effects of other galacto-oligosaccharide preparations on prebiotic properties and mineral absorption in humans have been reviewed by the FDA previously (*i.e*., GRN 236, 285, 334) (U.S. FDA, 2007, 2009a, 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. Findings from human trials conducted using Bimuno galacto-oligosaccharides are consistent with those previously published and reviewed for other galacto-oligosaccharide preparations (*i.e*., GRN 236, 285, 334) (U.S. FDA, 2007, 2009a, 2010). 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 and others 1999)*." Similarly, the laxative threshold was determined to be 0.30 g galacto-oligosaccharides per kg body weight per day (20 g for a 60 kg individual), and the 50% effective dose (ED_{50}) for diarrhea was determined to be 0.6 g galacto-oligosaccharides per kg body weight per day (36 g 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). The data supporting these thresholds have been discussed previously (U.S. FDA, 2007, 2009a, 2010), and the significance of this information to the safety of galacto-oligosaccharides preparations intended for use in food was discussed by the Expert Panel (see Appendix A).

Studies identified during the updated literature search are discussed below. The results of these studies are consistent with those published previously, which demonstrate that galacto-oligosaccharides are well-tolerated and side effects are limited to gastrointestinal intolerance, an effect that would be self-limiting.

1. Studies Conducted in Adults

A number of human studies that were conducted to assess the effects of galacto-oligosaccharide supplementation subsequent to the previous galacto-oligosaccharides GRAS notifications were identified in the published literature. While the majority of these studies were not specifically conducted to evaluate safety, adverse events were assessed. Of the identified studies, 3 were conducted on Bimuno (galacto-oligosaccharide content of 48%) in healthy subjects (Drakoularakou *et al.*, 2010; Baran, 2012) and overweight subjects at risk for metabolic

syndrome (Vulevic *et al.*, 2013). Overall, Bimuno supplementation at a dose 5.5 g/day, providing approximately 2.6 g GOS/day, for periods of 3 to 12 weeks was reported to be well-tolerated by all subjects.

In addition, several clinical studies conducted to investigate the safety and efficacy of other galacto-oligosaccharide products (other than Bimuno) were identified (Davis *et al.,* 2010; Hughes *et al.*, 2011; Walton *et al.*, 2012). These studies were conducted in healthy elderly subjects, healthy, and academically stressed subjects. The test articles used included Vivinal GOS and Purimmune (supplied by Purimmune GTC Nutrition). Subjects ingested doses ranging from 2.3 to 9.2 g/day of galacto-oligosaccharides for periods ranging from 3 to 8 weeks with no treatment-related side effects reported. The overall absence of treatment-related adverse events further supports the safety of the intended use of Bimuno in conventional foods and beverages. The results of these studies relevant to safety are summarized in Table IV.G.1-1 below.

A clinical trial conducted to assess the effects of galacto-oligosaccharides on calcium absorption and gut microflora also was identified (Whisner *et al.*, 2012; abstract only). In this randomized, double-blind, crossover, study, healthy adolescent girls (ages 10 to 13 years) were given smoothie drinks containing 0, 2.5, or 5 g of GOS, twice daily for 3 weeks, separated by a 2-week washout period. Although information on adverse events or other safety-related parameters was not provided, the absence of reported adverse events supports the tolerability of GOS.

In addition to the human studies discussed above, 2 reviews investigating the safety and efficacy of galacto-oligosaccharides were identified in the literature (Niittynen *et al.,* 2007; Torres *et al.,* 2010). In their review of the effects of galacto-oligosaccharides on bowel function, Niittynen *et al.* (2007) reported that gastrointestinal symptoms, such as flatulence, which are a common side effect of increased fiber intake, may occur after galacto-oligosaccharide ingestion. Doses of 12 g of galacto-oligosaccharides or lower were reported to be generally well-tolerated, while increased flatulence was observed at a higher dose of 15 g/day in 1 study. As discussed above, Torres *et al.* (2010) reported that the only known adverse effect of galacto-oligosaccharides is transient osmotic diarrhea following consumption of large doses of galacto-oligosaccharides.

Table IV.G.1-1 Human Studies Conducted in Adults with Endpoints Related to Safety

Study Population	Study Design	Duration	Test Article	Dose of Test Article (Dose of GOS)	Results Relevant to Safety[a]		Reference
Bimuno							
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)	Bimuno[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	Bimuno[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.G.1-1 Human Studies Conducted in Adults with Endpoints Related to Safety

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	Bimuno[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	
Other Galacto-oligosaccharide Products							
18 subjects (5 F, 13 M; ages 19 to 50 years; healthy)	Non-randomized, single-blind, sequential treatments	3 weeks with no washout period	Purimune™ (galacto-oligosaccharide content of 91.8%; supplied by GTC Nutrition)	2.5, 5, or 10 g/d (providing 2.3, 4.6, and 9.2 g GOS/d, respectively)	Adverse event reporting	NSD in bowel movement, stool consistency, discomfort, flatulence, abdominal pain, or bloating	Davis *et al.*, 2010
39 subjects (ages 50 to 81 years; mean age of 58.9±5.9 years; healthy)	Randomized, double-blind, placebo-controlled, crossover	3 weeks with 3-week washout period	Vivinal® galacto-oligosaccharide (galacto-oligosaccharide content of 59%; supplied by Friesland Campina Domo)	8 g/d (providing 4.72 g GOS/d)	Adverse event reporting	NSD in stool consistency, intestinal bloating, abdominal discomfort, or flatulence severity and frequency	Walton *et al.*, 2012

Table IV.G.1-1 Human Studies Conducted in Adults with Endpoints Related to Safety

Study Population	Study Design	Duration	Test Article	Dose of Test Article (Dose of GOS)	Results Relevant to Safety[a]		Reference
427 subjects (mean age of 19.9 years; 207 M, 220 F; healthy, academically stressed)	Randomized, double-blind, placebo-controlled, parallel-arm	8 weeks	Purimune™ (supplied by GTC Nutrition) (galacto-oligosaccharide content of 86%)	2.5 (low-dose) or 5 (high-dose) g GOS/d	Adverse event reporting	1 subject in the high-dose group dropped out on the first day of the intervention with allergy-like symptoms ↓ symptoms scores for diarrhea, constipation, abdominal pain, and indigestion NSD in symptoms score for reflux syndrome NSD in number of stools per day	Hughes *et al.*, 2011

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] Bimuno was produced using permeabilized *B. bifidum* whole cells
[c] Study design included probiotic and symbiotic arms in addition to the Bimuno arm

Bimuno galacto-oligosaccharides contain small quantities of *alpha*-galactobiose at usual concentrations of 7% of the total galacto-oligosaccharide content of the ingredient. This galacto-oligosaccharide is a component of stachyose and the same linkage configuration also occurs in raffinose. The effect of soy-derived oligosaccharides (*i.e*., stachyose and raffinose) on prebiotic and digestive parameters has been reported by several authors (Benno *et al.*, 1987; Hayakawa *et al.*, 1990; Kapadia *et al.*, 1995). The effect of soy oligosaccharides on whole gut transit time, stool weight, and bowel movement frequency was investigated in 7 healthy subjects (Kapadia *et al.*, 1995). Volunteers consumed a diet supplemented with 150 g soy oligosaccharides for 4 to 7 days. Soy oligosaccharides had no effect on any of the variables measured and no adverse events were reported. A soybean oligosaccharide extract providing 3.0 g of *alpha*-galacto-oligosaccharides (2.3 g stachyose and 0.7 g raffinose) per day was consumed by healthy adults for 3 weeks with no adverse events reported (Hayakawa *et al.*, 1990). Supplementation with 15 g raffinose/day for 4 weeks induced diarrhea in 3 of 7 volunteers in another study (Benno *et al.*, 1987). A summary of the studies conducted using *alpha*-linked soy-derived galacto-oligosaccharides is provided in Table IV.G.1-2 below.

Table IV.G.1-2 Human Studies Conducted in Adults Using *alpha*-linked Galacto-oligosaccharides with Endpoints Related to Safety

Study Population	Study Design	Duration	Test Article	Dose of Test Article (Dose of GOS)	Results Relevant to Safety[a]		Reference
7 healthy subjects, age not reported	Randomized, cross-over dietary intervention	4 to 7 days	Soy oligosaccharide fiber (not further specified)	150 g soy oligosaccharide fiber (not further specified)	Whole gut transit time Mean daily stool wet weight Bowel movement frequency *In vitro* production of SCFA	↑ butyrate and total SCFA No adverse events reported.	Kapadia *et al.*, 1995 [abstract only]
6 healthy males aged 28 to 48 years	Cross-over dietary intervention; not randomized, not reported whether blinded or not	3 weeks	Stachyose and raffinose (Soybean oligosaccharide extract)	2.3 g stachyose and 0.7 g raffinose (Soybean oligosaccharide extract)	Fecal microflora Fecal moisture Fecal putrefactive products	↑ *Bifidobacterium* No adverse events reported	Hayakawa *et al.*, 1990
7 healthy adults aged 26 to 37 years (5 M, 2 F)	Dietary intervention, not blinded or randomized	4 weeks	Raffinose	15 g raffinose/day divided between 3 doses	Bacterial analysis of fecal microflora Fecal pH	↑ *Bifidobacterium* spp and % *Bifidobacterium* spp ↓ Bacteroidaceae and % Bacteroidaceae ↓ *Clostridium* spp and % *Clostridium* spp 3 volunteers developed diarrhea at onset of treatment	Benno *et al.*, 1987

F = female; M = male; SCFA = short chain fatty acids

2. Studies Conducted in Infants

Large daily intakes of galacto-oligosaccharides (*e.g.*, 7.2 g/day corresponding to 1.4 g/kg body weight for a 5 kg infant) are well tolerated by infants and the safety of galacto-oligosaccharides is supported by a large body of published studies that have been the subject of comprehensive and critical reviews (SCF, 2003; FSANZ, 2008 - P306; U.S. FDA, 2007, 2009b). New studies identified during the literature search are consistent with the conclusion that galacto-oligosaccharide preparations are safe and well tolerated (Table IV.G.2-1). The majority of these studies were conducted to assess the effect of prebiotic supplementation on fecal flora and stool characteristics; however, in many of the studies growth, as well as adverse events and tolerance to the formulas were assessed. In these studies, healthy-term infants were administered formulas supplemented with 2.4 to 5 g GOS/L for 12 to 18 weeks, or a mixture of galacto-oligosaccharides and FOS (9:1 ratio) at levels of 0.4 to 0.8 g/100 mL for 28 days to 6 months. No significant adverse effects on growth (as assessed by body weight, length, and head circumference) or side adverse effects attributable to galacto-oligosaccharides were reported in any study.

Table IV.G.2-1 Human Studies Conducted in Infants with Endpoints Related to Safety

Study Population	Study Design	Duration	Test Article	Dose	Results Relevant to Safety[a]		Reference
Studies Conducted Using GOS/FOS (9:1 ratio)							
76 infants (mean gestational age of 38.5 weeks; 33 M, 38 F, 5 NR; healthy)	Randomized, double-blind, placebo-controlled, parallel-arm	28 days	Short-chain GOS/ long-chain FOS (9:1 ratio) (supplied by Numico Research)[d]	5.04 g/day[e] Provided in infant formula at a level of 0.8 g/100 mL	Growth	NSD in body weight gain, length gain, or head circumference gain	Bisceglia *et al.,* 2009
					Adverse event reporting	No adverse events reported in any group ↑ stool frequency ↓ serum bilirubin levels from 72 hours of life and throughout intervention period	
40 infants (mean gestational age of 39 weeks; 22 M, 18 F; healthy)	Randomized, double-blind, placebo-controlled, parallel-arm	28 days	GOS/FOS (9:1 ratio) (Vivinal® galacto-oligosaccharides+ Orafti HP)	5.04 g/day[e] Provided in infant formula at a level of 0.8 g/100 mL	Growth	NSD in growth (length and body weight gain) during the intervention	Veereman-Wauters *et al.*, 2011
					Adverse event reporting	NSD in formula intake No serious adverse events reported throughout the study period NSD in number of withdrawn subjects NSD in crying behavior, regurgitation, vomiting, or stool frequency	
89 infants (gestational age of 37 to 42 weeks; sex NR; healthy)	Multi-centre, randomized, double-blind, placebo-controlled, parallel-arm	6 months	GOS/FOS (9:1 ratio) [Vivinal® galacto-oligosaccharides+ short-chain FOS (Beneo P95)]	2.88 g/d of galacto-oligosaccharides+ 0.32 g/d of FOS[g] Provided in a standard infant formula at a concentration of 4 g/L	Growth	NSD in body weight	Holscher *et al.*, 2012
					Adverse event reporting	NSD in tolerance or occurrence of adverse events NSD in stool frequency, consistency, color, or odor NSD in caregiver-perceived incidence of crying, fussing, parent-perceived "colic"/cramps, spitting up, vomiting, or flatulence frequency	

BMI = body mass index; F = female; FOS = fructo-oligosaccharides; HDL = high density lipoprotein; LDL = low density lipoprotein; M = male; NR = not reported; NSD = no significant difference

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

[b] The test formula was Frisolac Advanced manufactured by Friesland Nutrition. Therefore, it was assumed that the galacto-oligosaccharides used in the infant formula was also manufactured by Friesland.

[c] Based on reported formula intake (162±27 mL/kg body weight/day) and body weight value (3.3±0.42 kg).

[d] Study was sponsored by Numico Friedrichsdorf Germany; therefore, it was assumed that the galacto-oligosaccharide test article was supplied by Numico Research.

[e] Based on estimated daily formula intake of 630 mL for a 1 to 2-month-old infant (WHO, 2005).

[f] Based on estimated daily formula intake of 720 mL for a 2 to 4-month-old infant (WHO, 2005).

[g] Based on reported formula intake of 798±30 mL/day.

IV.H 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 another study, the results of 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, galacto-oligosaccharide fractions (2, 3, 4, 5, and 6 or more sugar units), and long-chain fructo-oligosaccharides indicated that galacto-oligosaccharides with a degree of polymerization of 3 or more were the allergen responsible for inducing anaphylactic reactions in 5 patients aged 5 to 38 years (median age of 6 years) in Singapore following the consumption of cow's milk formula supplemented with galacto-oligosaccharides (Chiang *et al.*, 2012). 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 Bimuno galacto-oligosaccharides to induce an allergic reaction is considered to be low.

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 should be noted that the *alpha*-galacto-oligosaccharides present in Bimuno are *alpha*-(1,6)-galactobiose, which are structurally different from the *alpha*-(1,3)-galactose to which IgE antibodies have been identified.

IV.I Expert Panel Evaluation

Clasado has determined that Bimuno galacto-oligosaccharides, as described herein, are GRAS for food and beverage products as described in Table I.D-1, 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 Michael W. Pariza, Ph.D. (University of Wisconsin), and Professor Steven L. Taylor, Ph.D. (University of Nebraska).

The Expert Panel, convened Clasado Inc., independently and critically evaluated all data and information presented herein. Additional data and information deemed relevant to the safety of food uses 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, 2007, 2009a, 2010). The Expert Panel concluded that Bimuno 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.J Conclusion

Based on the above data and information presented herein, Clasado Inc. has concluded that the intended food uses of galacto-oligosaccharides, as described in Table I.D-1, are GRAS based on scientific procedures. General recognition of Clasado Inc.'s GRAS determination is supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training, to evaluate the use of galacto-oligosaccharides in food, who similarly concluded that the intended uses of galacto-oligosaccharides as described herein are GRAS.

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.

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CFR Sections Referenced (Title 21—Food and Drugs)		
Part	**Section §**	**Section Title**
101—Food labeling	101.12	Reference amounts customarily consumed per eating occasion
170—Food additives	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

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Expert Panel Consensus Statement

Bimuno Galacto-Oligosaccharides are Generally Recognized as Safe (GRAS) for Use in Conventional Food and Beverage Products

June 17, 2013

Introduction

Clasado Incorporated (Clasado) has determined that Bimuno® galacto-oligosaccharide (GOS) ingredients are Generally Recognized as Safe (GRAS) for use in food and beverage products as described in Table A-1 at a use level of up to 3.0 g per serving. 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 Michael W. Pariza, Ph.D. (University of Wisconsin), 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 Clasado's Bimuno galacto-oligosaccharide ingredients in food as described in Table A-1. This information included available scientific data compiled from the literature and other published sources through April 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 conventional foods as described herein, Bimuno galacto-oligosaccharide ingredients, meeting appropriate food-grade specifications, and manufactured in accordance with current Good Manufacturing Practice (cGMP), are 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). Bimuno 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 Bimuno galacto-oligosaccharide syrup is specified at between 46 to 60% on a dry weight basis. The remaining composition of the ingredient consists of the monosaccharides glucose, and galactose, and the disaccharides lactose and galactobiose. Analytical data prepared by Clasado using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) as described in Association of Analytical Communities (AOAC) method 2001.02 demonstrate that Bimuno galacto-oligosaccharides contain a mixture of oligomers with a degree of polymerization ranging between 2 and 5. Bimuno galacto-oligosaccharides have 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 process. 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. Bimuno 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) 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 Bimuno galacto-oligosaccharides.

Clasado has developed food grade specifications for the ingredient. Bimuno galacto-oligosaccharide syrup contains at least 70% dry solids and galacto-oligosaccharides constitute between 46 and 60% of the ingredient on a dry weight basis. Seven percent (7%) of the total galacto-oligosaccharide content of the ingredient is represented by the galacto-oligosaccharide

galactobiose, and the remaining carbohydrates are represented by glucose, galactose and lactose. The ingredient is protein-free at a limit of detection of 0.1%. 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 non-consecutive lots of GOS and the findings confirmed compliance to the product specifications. The Expert Panel also reviewed other analytical data for multiple lots of Bimuno syrup demonstrating that the manufacturing conditions produce a reproducible galacto-oligosaccharide profile. Clasado has demonstrated that Bimuno galacto-oligosaccharides are stable under ambient and accelerated storage conditions for a period of at least 6 months.

Intended Uses and Estimated Exposure

Bimuno galacto-oligosaccharides are intended for use in food and beverage applications previously determined to be GRAS (U.S. FDA, 2008, 2009a, 2010) at use levels providing up to 3.0 g of galacto-oligosaccharides per serving (Table A1). In addition, Bimuno galacto-oligosaccharides also are intended for use in coffee and tea products. The use of Bimuno galacto-oligosaccharides in coffee and tea products will be achieved through the use of pre-packaged "ready-to-brew" single-serve cups, freeze-dried single serving packets, or *via* the use of single serving coffee creamers or sweetener products marketed exclusively for use with coffee or tea.

Estimated exposure to galacto-oligosaccharides from current GRAS Notified food uses have been previously estimated for the general U.S. population using individual proposed food uses and use levels in conjunction with survey data obtained from the National Health and Nutrition Examination Surveys (NHANES) (U.S. FDA, 2008, 2009a, 2010). Estimated mean intakes of galacto-oligosaccharides for all-user children (ages 3 to 11 years) range from 7.8 to 14.7 g per person. Corresponding intakes among heavy users (90th percentile) within this group range between 14.2 to 26.8 g. Among frequent consumers of foods potentially containing galacto-oligosaccharides, highest exposures have been estimated for teenage males where mean intakes of between 9.4 to 30.4 g have been estimated. Among 90th percentile users in this group, estimated intakes range between 18.9 to 30.4 g per person. Total population estimates range between 8.0 to 12.2 g per person for mean exposures and intakes of between 15.4 to 25.3 g may be expected among 90th percentile users. Bimuno galacto-oligosaccharides will serve as an alternative to existing sources of galacto-oligosaccharides sold in the U.S. marketplace. The ingredient is intended for addition to current GRAS Notified food uses of galacto-oligosaccharides at use levels that are equivalent to or less than those previously determined to be GRAS. Accordingly the introduction of Bimuno galacto-oligosaccharides under existing GRAS Notified food uses (*i.e.*, U.S. FDA, 2008, 2009a, 2010) will not increase exposures among users of these foods. The potential for additional consumption of galacto-oligosaccharides within the general U.S. population (exclusive of children who are not expected to consume coffee or tea in significant quantities), may however occur from the use of galacto-

oligosaccharides in coffee and tea applications. Estimates of coffee consumption were calculated using data from the NHANES 2007 through 2010 surveys and Crème intake modeling software. Mean coffee consumption among all-users was estimated to be highest in male adults at 590 g per person per day. Total population all-user intake estimates were 519 g per person per day. Based on a 6 oz. serving of coffee and an intended use level of Bimuno galacto-oligosaccharides of 1.5 g per serving, intakes of galacto-oligosaccharides were estimated to be 4.95 g per person per day among adult males and total population all-users estimates were 4.35 g per person per day. These levels of intake are expected to be consistent with current food uses, which have been determined to be GRAS at use levels of between 5 and 7 g per serving for select food uses (*i.e.*, yogurt and fruit smoothies respectively under GRN 236) (U.S. FDA, 2008). It is expected that habitual users of galacto-oligosaccharide containing coffee products would intentionally seek out these products for their nutritional benefits. Uses in coffee and tea will likely serve as a replacement to consumption of other galacto-oligosaccharide containing foods, and cumulative intakes of multiple galacto-oligosaccharide products at the highest use levels on a chronic basis is unlikely to occur. Adverse effects associated with consumption of galacto-oligosaccharides in excess would be limited to gastro-intestinal intolerance, an effect that is self-limiting.

Bimuno galacto-oligosaccharides also contain the monosaccharides glucose, galactose, and the disaccharides lactose and galactobiose at average concentrations of 32, 14, 4, and 3.5% respectively, on a dry matter basis. All of these constituents are common dietary ingredients and are expected to be consumed at quantitatively similar levels from the consumption of the background diet and/or through the consumption of GOS ingredients currently on the market. The safety of these components within Bimuno galacto-oligosaccharides was therefore supported by the quantitative similarities in their anticipated exposures from intended food uses of Bimuno GOS relative to current exposures to these common food ingredients in the diet. With respect to α-(1,6) galactobiose, Clasado cites references supporting that this oligomer is widely distributed in plants and vegetables, such as soybeans, broccoli, asparagus, and whole grains (Saini, 1988). This disaccharide as present in Bimuno is a component of stachyose and the same linkage configuration also occurs in raffinose. Various values have been reported for the content of *alpha*-oligosaccharides, also known as α-galactosides, in foods. The α-galactoside content in peas, beans, and lentils has been reported to range from 5 to 8% (FAO/WHO, 1998) and from 4 to 6% in soybeans (LeBlanc *et al.*, 2004).

Safety Assessment

The generally recognized as safe use of galacto-oligosaccharide preparations, produced from lactose using the enzymatic activity of microbial *beta*-galactosidases, in conventional food and beverage products have been previously evaluated by numerous qualified experts, and have been reviewed on multiple occasions by the United States Food and Drug Administration without objection from the Agency (U.S. FDA, 2008, 2009a,b, 2010). Authoritative opinions

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 Bimuno galacto-oligosaccharides to be representative of galacto-oligosaccharide preparations previously determined to be GRAS (*i.e*., GRN 236, 285, 334) (U.S. FDA, 2008, 2009a, 2010). Accordingly, the Expert Panel considered published safety information described within the GRAS Notified uses of galacto-oligosaccharides (*i.e*., GRN 236, 285, 334) (U.S. FDA, 2008, 2009a, 2010) to be applicable to the safety of the Bimuno galacto-oligosaccharides. 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, 285, 334) (U.S. FDA, 2008, 2009a, 2010). To identify new information, favorable and unfavorable, relevant to the intended use of galacto-oligosaccharides in food published since previous GRAS Notified uses, a comprehensive search of the published scientific literature was conducted from 2011 through May 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 Bimuno galacto-oligosaccharides is presented below.

Metabolic Fate

Galacto-oligosaccharides are not hydrolyzed by human digestive enzymes, are not absorbed intact in the small intestine, and therefore represent a source of dietary fiber that is transported unchanged through the gastrointestinal tract and subjected to fermentation by the indigenous microorganisms within the colon (Ohtsuka *et al.,* 1990, 1991; Chonan *et al.,* 2004). *In vitro* and *in vivo* studies have demonstrated that galacto-oligosaccharides promote the growth of bacteria, including *Bifidobacterium bifidum,* in the lower intestine (Tanaka *et al.,* 1983; Ishikawa *et al.,* 1995; Hopkins *et al.,* 1998; Smiricky-Tjardes *et al.,* 2003a; Matsumoto *et al.,* 2004; Tzortzis *et al.,* 2005; Hernot *et al.,* 2009). Bacterial fermentation of galacto-oligosaccharides in the colon was reported to produce short chain fatty acids (SCFAs), including butyrate, propionate, and acetate, and release small amounts of methane, hydrogen, and carbon dioxide in preclinical and clinical trials (Tanaka *et al.,* 1983; Ishikawa *et al.,* 1995; Suarez *et al.,* 1999; Smiricky-Tjardes *et al.,* 2003a,b; Chonan *et al.,* 2004). Feeding galacto-oligosaccharides to rats colonized with human fecal microflora was reported to cause a decrease in cecal pH, an increase in anaerobic bacteria, lactobacilli, and bifidobacteria, and an increase in hydrogen and SCFA production (Rowland and Tanaka, 1993; Kikuchi *et al.,* 1996). Ohtsuka *et al.* (1991) administered a purified *beta* (1-4) galacto-oligosaccharide preparation (DP=3) to male SD rats in the diet at a level of 5% for 2 weeks. Analysis of fecal matter on the final day did not identify any of the administered

compound and the authors concluded that the absence of the test article was indicative that *beta* (1-4) galacto-oligotrisaccharides were almost completely fermented by the microflora of the large intestine. Similarly, *alpha*-galacto-oligosaccharides within soybeans are completely fermented by the intestinal microflora, as was indicated by their apparent total (*i.e.*, 100%) digestion from the gastrointestinal tract (Smiricky-Tjardes *et al.,* 2003a).

Animal Toxicity Studies

The results of short-term rodent toxicity studies demonstrate that galacto-oligosaccharides are of low-toxicity. Ohtsuka *et al.* (1990) administered a galacto-oligosaccharide preparation (GOS) to adult male SD rats for 6 weeks and investigated the effects on growth, gastrointestinal function, and serum and liver lipid levels. Male SD rats (initial body weight of approximately 50 g) were divided into 5 groups (6 rats/group) and received a basal diet or the basal diet supplemented with 5% GOS (providing approximately 5,725 mg/kg body weight/day[1]), 10% 4'GL (providing approximately 10,892 mg/kg body weight/day[2]), 10% lactose, or 10% lactulose. Body weight increased in both groups receiving 4'GL-supplemented diets, although the increase was significant only in the higher-dose group. Food intake was significantly increased in both groups receiving GOS, but there were no significant differences in the food conversion ratio (food/gain). Diarrhea (not further defined) was reported to occur in the rats receiving 10% GOS and those receiving 10% lactulose, but the effect was transient, lasting only for a period of 2 weeks. A dose-related significant increase in relative cecum weight was reported for the rats receiving GOS when compared to those receiving the control diet. A non-dose related significant increase in relative colon weight also was reported for both groups receiving GOS compared to the control. There were no significant differences in the relative weights of the small intestine, liver, heart, kidney, or spleen and no significant differences in serum or liver lipids when the values from the rats receiving GOS were compared to those receiving the control diet. The no-observed-adverse-effects level (NOAEL) for male SD rats was 10,892 mg GOS/kg body weight/day, the highest dose tested.

The subchronic toxicity (90-day) of a galacto-oligosaccharide ingredient (Vivinal, Friesland Foods Domo, Netherlands) was evaluated using adult male and female Sprague-Dawley Crl:CD (SD) IGS BR rats (initial body weights of 229.3 ± 11.15 g for males and 160.7 ± 10.44 g for females) (Anthony *et al.*, 2006). Vivinal was provided as a syrup and had a galacto-oligosaccharide content of approximately 45%. Rats were divided into 4 groups (15/sex/group) and one of 4 treatments [reverse osmosis deionized (RODI) water control, fructo-oligosaccharide (FOS) reference control[3], or 2,500 or 5,000 mg Vivinal GOS/kg body weight/day] was administered once daily by gavage for a period of 89 to 91 days. The low and high doses provided 1,125 and 2,250 mg GOS/kg body weight/day, respectively. There were

[1] Calculated using body weight and food intake data.
[2] Calculated using body weight and food intake data.
[3] Dose volume was reported to be equivalent to the high-dose GOS group; however, dose level was not reported.

no adverse toxicological effects attributable to galacto-oligosaccharide administration. The authors determined a NOAEL of 2,250 mg of GOS/kg body weight/day, the highest dose tested.

In a subchronic toxicity study, adult male and female Crf:CD(SD) rats (10/sex/group; initial body weights ranging from 181.4 to 218.9 g for males and 131.9 to 156.8 g for females) were orally administered a galacto-oligosaccharide preparation at doses of 0 (control), 500 (low-dose), 1,000 (mid- dose), or 2,000 mg/kg body weight/day (providing approximately 0, 275, 550, and 1,100 mg/kg body weight/day of GOS, respectively) by gavage daily for 90 days (Kobayashi *et al.,* 2009[4]). Galacto-oligosaccharide administration was well-tolerated by the animals. High-dose males were reported to have higher absolute and relative cecum weights compared to their respective controls. The authors considered that the increase in cecal weights was a physiological adjustment to the dietary administration of indigestible carbohydrates, rather than indicative of toxicity. No toxicologically significant findings were observed at gross necropsy, and histopathological examination did not reveal any test article-related changes. Based on their findings, the authors determined the NOAEL for subchronic oral toxicity in rats to be 2,000 mg/kg body weight/day of the galacto-oligosaccharide product (equivalent to 1,100 mg/kg body weight/day of GOS), the highest dose tested.

The developmental toxicity of a galacto-oligosaccharide preparation was evaluated by Desbuards *et al.* (2012) using BALB/cj mice. Pregnant BALB/cj mice were fed a control diet (n=13) or a diet supplemented with a galacto-oligosaccharide product and inulin in a ratio of 9:1 (n=12) throughout gestation and lactation. Dams consumed an estimated 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. After weaning, the offspring were administered the same test diets as the dams for 48 days. No evidence of teratogenic or developmental toxicity related to consumption of the test article was reported. However, the Expert Panel noted that the study was not conducted in accordance with Good Laboratory Practice (GLP) and deviated from internationally accepted guidelines for developmental toxicity testing of chemicals and therefore was of limited value to the safety assessment of Bimuno galacto-oligosaccharides.

Based on observations from sub-chronic toxicity studies conducted, the Expert Panel concluded that galacto-oligosaccharide preparations produced from lactose by the enzymatic activity of *beta*-galactosidases are of low toxicity. The NOAELs determined following subchronic administration of galacto-oligosaccharides in the diet or *via* gavage are represented by the highest doses tested. The only effect attributable to subchronic consumption of galacto-oligosaccharides was cecal hyperplasia, an effect that is commonly observed in rodents administered high dietary levels of indigestible fiber or sugars, a finding that is not relevant to

[4] Previously published in Japanese as Kobayashi *et al.*, 2003:
Kobayashi T, Uchida K, Kaneko K, Mizutani T, Onoue M (2003). Ninety-day repeated oral dose toxicity study of GOS in rats. Yakuruto Kenkyujo Kenkyu Hokokushu (23):25-42.

humans (WHO, 1987). The only adverse findings from rodent toxicity studies considered relevant to humans was the potential for osmotic diarrhea, where transient diarrhea was observed in rats administered galacto-oligosaccharides at high dietary levels (*e.g*., 10% of the diet). The Expert Panel noted that the threshold for dietary galacto-oligosaccharides to induce osmotic diarrhea and gastro-intestinal intolerance in humans has been reported using human clinical investigations, and is discussed further below.

Mutagenicity/Genotoxicity Studies

Yasutake *et al.* (2003) evaluated the mutagenicity/genotoxicity of a galacto-oligosaccharide preparation in a bacterial reverse mutation test, a mouse micronucleus assay, and in Chinese hamster fibroblast cells (Yasutake *et al.*, 2003). All test results indicated that galacto-oligosaccharides were non-mutagenic and non-genotoxic. The mutagenicity/genotoxicity of a galacto-oligosaccharide preparation was examined in a battery of tests, including a bacterial reverse mutation assay (Ames test), an *in vitro* chromosomal aberration test in Chinese hamster lungs (CHL/IU) cells, and an *in vivo* mouse micronucleus assay using GLP and in accordance with Japanese Food Additive Guidelines (Kobayashi *et al.,* 2009). Galacto-oligosaccharides were not mutagenic/genotoxic under the conditions of these assays.

Tzortzis *et al.* (2005) investigated the *in vitro* toxicity of galacto-oligosaccharides on human colon adenocarcinoma HT29 cells. The galacto-oligosaccharide mixture was added at concentrations of 0.01, 0.1, 1, 10, or 100 mmol/L to serum standard growth medium and incubated for 24 and 48 hours with HT29 cells. No toxic effects were reported after 24 or 48 hours of incubation for all concentrations up to and including 10 mmol galacto-oligosaccharide/L. At 100 mmol galacto-oligosaccharide/L there was a reduction in cell viability that was statistically significant after both 24 and 48 hours ($P<0.001$) of incubation. These results demonstrate that the galacto-oligosaccharide mixture was not cytotoxic to representative cells of the gastrointestinal system when tested at levels similar to those that would be achieved by consumption of galacto-oligosaccharide-containing food products.

Human Studies

Studies evaluating the consumption of Bimuno galacto-oligosaccharides (galacto-oligosaccharide content of 39 to 48%) were critically reviewed by the Expert Panel, and included studies conducted in 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). Overall, Bimuno supplementation 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 was reported to be well-tolerated. In the study by Depeint *et al*. (2008) "*Two of the 30 volunteers reported abdominal discomfort and diarrhea when the 7-g B-GOS* [2.75 g GOS] *treatment was consumed, but overall both preparations were well tolerated.*" In a study on traveler's diarrhea, Drakoularakou *et al*. (2010)

reported that consumption of 5.5 g of Bimuno GOS providing 2.64 g of GOS per day resulted in statistically significant (P<0.05) decreases in the incidence of diarrhea (-36%), duration of diarrhea (-48%), and duration of abdominal pain (-43%) during the holiday treatment period relative to that observed in the placebo group. The Expert Panel concluded that findings from human trials conducted using Bimuno galacto-oligosaccharides are consistent with those previously published for other galacto-oligosaccharide preparations. A discussion of studies previously reviewed (*i.e*., GRN 236, 285, 334) (U.S. FDA, 2008, 2009a, 2010), and critically evaluated by the Expert Panel is presented below.

Numerous studies investigating the effects of galacto-oligosaccharides on prebiotic properties (Tanaka *et al.,* 1983, 1987; Shimoyama *et al.*, 1984; Ito *et al.,* 1990, 1993a,b; Tamai *et al.*, 1992, 1994; Iiu *et al.*, 1994; Ishikawa *et al.,* 1995; Bouhnik *et al.,* 1997; Deguchi *et al.,* 1997; Teuri and Korpela, 1998; Teuri *et al.*, 1998; Alles *et al.*, 1999; van Dokkum *et al.,* 1999; van den Heuvel *et al.,* 2000; Alander *et al.*, 2001; Satokari *et al.,* 2001; Scholtens *et al.,* 2003, 2006; Kanamori *et al.,* 2004; Matsumoto *et al.*, 2004; Sazawal *et al*., 2004; Bakker-Zierikzee *et al.*, 2005; Shadid *et al.*, 2007; Davis *et al.,* 2010; Hughes *et al.*, 2011; Walton *et al.*, 2012) and mineral absorption (van den Heuvel *et al.,* 2000; Whisner *et al.*, 2012) in humans have been published. These studies were designed to investigate efficacy related endpoints; however, information on tolerance and incidence of gastrointestinal side effects were reported in a number of these investigations. The doses administered ranged from 1 to 30 g galacto-oligosaccharides/day for up to 15 weeks. Side effects attributed to galacto-oligosaccharide consumption are typically associated with effects on gastro-intestinal intolerance. For example, instances of constipation and bloating in a pregnant woman following consumption of 2.73 g galacto-oligosaccharides and 0.29 g FOS/day for 15 weeks, and sensations of fullness, flatulence, and abdominal pain at 10 to 15 g galacto-oligosaccharides/day have been reported by others (Ito *et al.*, 1990; Deguchi *et al.*, 1997; Teuri *et al.*, 1998; Shadid *et al.*, 2007). One subject reported abdominal discomfort, which followed consumption of 18 g galacto-oligosaccharides/day (Alander *et al.*, 2001; Satokari *et al.,* 2001). The development of gastro-intestinal intolerance following consumption of galacto-oligosaccharide preparations is not always consistent at high doses and studies administering galacto-oligosaccharides at doses of 10 g or higher/day have been well tolerated by many individuals (Tanaka *et al.*, 1983, 1987; Ito *et al.*, 1993a; Iiu *et al.*, 1994; Ishikawa *et al.*, 1995; Bouhnik *et al.*, 1997; van Dokkum *et al.*, 1999). One study reported that 15 g galacto-oligosaccharides/day was well-tolerated by the subjects during a 3-week treatment period (van Dokkum *et al.*, 1999).

The long-term administration of a galacto-oligosaccharide preparation was evaluated in children. Sazawal *et al.* (2004) supplied galacto-oligosaccharides in sachets to be administered to children at doses of up to 2.4 g galacto-oligosaccharides/day for 12 months. The sachets also contained *Bifidobacterium lactis* HN019 and the general health of the children was recorded. Administration of galacto-oligosaccharides and *B. lactis* HN019 resulted in a decreased incidence of dysentery, decreased prevalence of severe illness days, decreased

number of days with fever, decreased prevalence of ear infections, and better growth at 6 months and 1 year.

Bimuno galacto-oligosaccharides contain small quantities of *alpha*-galactobiose at usual concentrations of 7% of the total galacto-oligosaccharide content of the ingredient. This galacto-oligosaccharide is a component of stachyose and the same linkage configuration also occurs in raffinose. The effect of soy-derived oligosaccharides (*i.e.*, stachyose and raffinose) on prebiotic and digestive parameters has been reported by several authors (Benno *et al.*, 1987; Hayakawa *et al.*, 1990; Kapadia *et al.*, 1995). The effect of soy oligosaccharides on whole gut transit time, stool weight, and bowel movement frequency was investigated in 7 healthy subjects (Kapadia *et al.*, 1995). Volunteers consumed a diet supplemented with 150 g soy oligosaccharides for 4 to 7 days. Soy oligosaccharides had no effect on any of the variables measured and no adverse events were reported. A soybean oligosaccharide extract providing 3.0 g of *alpha*-galacto-oligosaccharides (2.3 g stachyose and 0.7 g raffinose) per day was consumed by healthy adults for 3 weeks with no adverse events reported (Hayakawa *et al.*, 1990). Supplementation with 15 g raffinose/day for 4 weeks induced diarrhea in 3 of 7 volunteers in another study (Benno *et al.*, 1987).

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 and others 1999)*." Similarly, the laxative threshold and 50% effective dose (ED_{50}) for diarrhea was determined 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). Subjects were administered 5.2, 10.2, 20.5, 30.8, or 41.0 g of galacto-oligosaccharides once per day for a week. All subjects consumed all test doses for 1 week starting at the lowest level progressing to higher intakes each subsequent week. The threshold for osmotic diarrhea was 0.30 g galacto-oligosaccharides per kg body weight per day (20 g for a 60 kg individual). The ED_{50} was 0.6 g galacto-oligosaccharides per kg body weight per day (36 g for a 60 kg adult). Consumption of a beverage containing 15 g of galacto-oligosaccharides or placebo on a daily basis for a period of 2 weeks also was evaluated by the authors using a group of 20 healthy individuals. The only effect attributable to galacto-oligosaccharides reported by the authors was an increase in gas, no increases in the incidence of diarrhea relative to controls was observed.

The Expert Panel concluded that galacto-oligosaccharide preparations are well-tolerated up to daily intakes of 15 to 20 g. At high intakes (>10 g), sensitive individuals may experience gastro-intestinal intolerance, an effect that would be self-limiting and gastrointestinal health would return to normal following cessation of consumption of foods containing the ingredient. Previous evaluations have determined that galacto-oligosaccharides are GRAS at specified food uses

resulting in daily estimated intakes of up to 30.4 g per person per day among 90th percentile heavy consumers. Based on the highest use level of 3.0 g per serving, the Expert Panel noted that daily consumption of Bimuno galacto-oligosaccharides would be unlikely to exceed 15 to 20 g per day as a large number of daily servings would be required to achieve this level of intake. Therefore, the exposure to Bimuno galacto-oligosaccharides in the diet from intended uses was expected to be consistent with levels that have been demonstrated in human investigations to be well tolerated by adults and children.

Calcium Absorption

van den Heuvel *et al.* (2000) evaluated the effects of galacto-oligosaccharides administered orally for 9 days on calcium absorption. The study population was 12 post-menopausal women aged 55 to 65 years, and an escalating dose pattern was used, with an administered highest dose of 44 g Vivinal/day, providing 20 g galacto-oligosaccharides/day for 4 days. Calcium absorption in the women was reported to increase by 16% over the 9-day treatment period with Vivinal galacto-oligosaccharides without any adverse events. A clinical trial conducted to assess the effects of galacto-oligosaccharides on calcium absorption and gut microflora also was identified (Whisner *et al.*, 2012; abstract only). In this randomized, double-blind, crossover, study, 31 healthy adolescent girls (ages 10 to 13 years) were given smoothie drinks containing 0, 2.5, or 5 g of galacto-oligosaccharides, twice daily for 3 weeks, separated by a 2-week washout period. Preliminary results were reported to suggest that the consumption of galacto-oligosaccharides resulted in increased fractional calcium absorption. Results from animal studies also indicate that calcium absorption would be enhanced from the consumption of dietary galacto-oligosaccharides in both α- and β-linked anomeric configurations (Ohta *et al.*, 1993; Chonan and Watanuki, 1995, 1996; Chonan *et al.*, 1995, 2001; Yanahira *et al.*, 1997). This effect also has been reported to occur in response to consumption of lactose, other indigestible carbohydrates, and sugar alcohols (Chonan and Watanuki, 1995, 1996; Chonan *et al.*, 1995, 1996, 2001; Yanahira *et al.*, 1997), which are fermented in the cecum and large intestine. Fermentation of these substrates results in the production of volatile fatty acids and a lowered pH, which is hypothesized to result in improved solubility of calcium and magnesium, and hence improves their absorption. Alternatively, the results of a 21-day study of the effect of supplementation with 15 g galacto-oligosaccharides/day on calcium and iron absorption in 12 healthy males aged 20 to 30 years indicated that absorption of the minerals was not affected by treatment with galacto-oligosaccharides (van den Heuvel *et al.*, 1998). No adverse events were reported by the study authors. Post-menopausal women are known to have an increased requirement for calcium (Hudson, 2007). The results of van den Heuvel *et al.* (1998, 2000) indicate that dietary galacto-oligosaccharides may help maintain optimal calcium balance and only increase absorption of calcium in calcium-deficient populations. Pre-clinical data supports a role for galacto-oligosaccharides in maintaining calcium balance. When Chonan *et al.* (1996) fed male Wistar rats a diet containing excess phosphorus and calcium either with or without 5% galacto-oligosaccharides, the addition of galacto-oligosaccharides to the diet decreased the

concentration of calcium in the rats' hearts to levels that were equivalent to those in rats fed a standard diet. Dietary galacto-oligosaccharides also have been demonstrated to maintain sodium and potassium levels in homeostatic balance in rats by increasing fecal excretion and decreasing urinary excretion (Hayashi and Shimazaki, 1991; Hayashi *et al.*, 1991).

Based on the above information, the Expert Panel concluded that the intended uses of galacto-oligosaccharides as described herein would not adversely affect calcium balance.

Infants

Galacto-oligosaccharides also have been investigated for addition to infant formulas. Twenty-two studies were reviewed that investigated the effects of adding either galacto-oligosaccharides or galacto-oligosaccharide and fructo-oligosaccharide mixtures to infant formulas (Knol *et al.*, 2001, 2002, 2005; Rigo *et al.*, 2001; Boehm *et al.*, 2002, 2003; Moro *et al.*, 2002, 2003, 2005, 2006; Napoli *et al.*, 2003; Savino *et al.*, 2003; Schmelzle *et al.*, 2003; Ben *et al.,* 2004; Haarman and Knol, 2005, 2006; Rinne *et al.*, 2005; Alliet *et al.*, 2007; Kukkonen *et al.*, 2007; Ben *et al.*, 2008; Bisceglia *et al.,* 2009; Fanaro *et al.*, 2009; Veereman-Wauters *et al.*, 2011; Holscher *et al.*, 2012). The majority of these studies were conducted to assess the effect of supplementation on fecal flora and stool characteristics; however, many of the studies also included side effect parameters and tolerance to the formulas. Of the 22 identified studies, 2 were conducted using preterm infants (Boehm *et al.*, 2002, 2003), and the remainder were conducted with term infants or were not specified. Preterm infants were administered formula supplemented with 0.9 g galacto-oligosaccharides/100 mL for 28 days with no adverse events or differences in the incidence of side effects (crying, regurgitation, vomiting). Term infants were administered formula supplemented with 0.24 to 0.9 g galacto-oligosaccharides/100 mL for periods ranging from 14 days to 6 months. No adverse events were reported in any of the studies, and where measured, there were either no adverse effects (Rigo *et al.*, 2001; Moro *et al.*, 2002, 2003; Ben *et al.*, 2004; Knol *et al.*, 2005) or a beneficial effect (Savino *et al.*, 2003; Moro *et al.*, 2006) on the incidence of side effects. In one study, 91% of parents gave a positive judgment (*i.e.*, a score of greater than 6 out of 10 with respect to compliance and acceptability) of the study formula (Savino *et al.*, 2003).

Scholtens *et al.* (2003, 2006) investigated the effect of providing Vivinal galacto-oligosaccharides in weaning foods to infants aged 4 to 6 months. The target dose was 4.05 g galacto-oligosaccharides/day for 6 weeks and no adverse events were reported. No adverse events were repored during a 16-week trial in infants supplied formula supplemented with 0.54 g Vivinal galacto-oligosaccharides/100 mL (Bakker-Zierikzee *et al.*, 2005).

Although the intended uses of Bimuno galacto-oligosaccharides do not include infant formulas, the Expert Panel considered the large body of published information supporting the safe

consumption of galacto-oligosaccharides at high dietary levels (*e.g.*, 1.6 g/kg body weight[5]) in thispopulation group for extended periods of time and concluded that galacto-oligosaccharides are safe and well-tolerated. On a mg/kg basis, exposures in infants are anticipated to greatly exceed those occurring in adults from the intended food uses of Bimuno galacto-oligosaccharides.

Potential for Allergenicity

Consumption of products containing galacto-oligosaccharides 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 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*".

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.

[5] Based on typical use level of 0.8 g/100 mL formula and a 5 kg infant consuming 1,000 mL of infant formula per day.

Conclusion

We, the Expert Panel, have, independently and collectively, critically evaluated the data and information summarized above and conclude that the proposed uses in conventional food and beverage products of Bimuno galacto-oligosaccharides, meeting appropriate food grade specifications and produced consistent with current Good Manufacturing Practice (cGMP), are safe and suitable and Generally Recognized as Safe (GRAS) based on scientific procedures.

It is our opinion that other qualified scientific experts, critically evaluating the same information, would concur with these conclusions.

(b) (6)

29 June 2013

Professor Joseph F. Borzelleca, Ph.D.
Virginia Commonwealth University School of Medicine

Date

Professor Michael W. Pariza, Ph.D.
Food Research Institute
University of Wisconsin

Date

Professor Stephen L. Taylor, Ph.D.
Institute of Agriculture and Natural Resources
University of Nebraska

Date

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Professor Joseph F. Borzelleca, Ph.D.
Virginia Commonwealth University School of Medicine

Date

(b) (6)

Professor Michael W. Pariza, Ph.D.
Food Research Institute
University of Wisconsin

5 July 2013
Date

Professor Stephen L. Taylor, Ph.D.
Institute of Agriculture and Natural Resources
University of Nebraska

Date

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Professor Joseph F. Borzelleca, Ph.D.
Virginia Commonwealth University School of Medicine

Date

Professor Michael W. Pariza, Ph.D.
Food Research Institute
University of Wisconsin

Date

(b) (6)

Professor Stephen L. Taylor, Ph.D.
Institute of Agriculture and Natural Resources
University of Nebraska

28 June 2013
Date

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ATTACHMENT A1

Intended Food Uses of Bimuno Galacto-oligosaccharides

Table A1 Summary of the Individual Proposed Food Uses and Use-Levels for Galacto-oligosaccharides in the U.S.[a]

Food Category	Proposed Food-Use	RACC[b] (g or mL)	GOS Level (g/serving)	GOS Use-Level (%)
Baked Goods and Baking Mixes	Breads (Wheat, Wholemeal, Other; excluding White)	50	1.28	2.56
	Brownies	40	1.28	3.20
	Cakes (including coffee cakes)	55 to 125	1.28	1.02 to 2.33
	Cookies	30	1.28	4.27
	Crackers, snack	30	1.28	4.27
	Muffins	55	1.28	2.33
	Pies, cobblers, fruit crisps, turnovers, other pastries	125	1.28	1.02
	Waffles & Pancakes	85	1.28	1.51
Beverages and Beverage Bases	Bottled and Flavored Waters	240	3.00	1.25
	Energy, Sport, and Isotonic Drinks	240	3.00	1.25
	Non-Milk Based Meal Replacements and Protein Beverages	240	3.00	1.25
Breakfast Cereals	Baby Cereals, dry	15	0.86	5.73
	Baby Cereals, ready-to-eat	110	1.28	1.16
	Ready-to-Eat Cereals	15 to 55	1.28	2.33 to 8.53
Coffee and Tea[c]	Coffee and Tea	240	1.50	0.625
Dairy Product Analogs[c]	Coffee Creamers and Whiteners (liquid)	15	0.80	5.33
	Coffee Creamers and Whiteners (powder)	2	0.80	40.00
	Soy Beverages	240	3.00	1.25
Frozen Dairy Desserts and Mixes	Frozen Dairy Desserts	125	3.00	2.40
	Ice Cream	125	1.28	1.02
Grain Products and Pastas	Cereal and Grain-Based Baby Snacks	7	1.00	14.29
	Grain-based bars (with or without fillings or coatings)	40	3.00	7.50
	Pasta	55	1.28	2.33
Milk and Milk Products	Flavored Milk and Milk Drinks	240	3.00	1.25
	Milk-Based Meal Replacements and Protein Beverages	240	3.00	1.25
	Yogurt	225	3.00	1.33
	Yogurt Drinks for babies/toddlers	120	3.00	2.50
Processed Fruits and Fruit Juices	Fruit Drinks and Smoothies	240	3.00	1.25
	Fruit Juices and Nectars	240	3.00	1.25
	Fruit Juices for babies/toddlers	120	3.00	2.50

Table A1	**Summary of the Individual Proposed Food Uses and Use-Levels for Galacto-oligosaccharides in the U.S.[a]**			
Food Category	**Proposed Food-Use**	**RACC[b] (g or mL)**	**GOS Level (g/serving)**	**GOS Use-Level (%)**
Processed Vegetables and Vegetable Juices	Vegetables Juices	240	3.00	1.25
	Vegetable Juices for babies/toddlers	120	3.00	2.50
Snacks	Novelty snacks	30	1.28	4.27
Sweet sauces	Syrup flavorings	30	3.00	10.00

[a] The individual food uses for Bimuno® Syrup will be the same as those for the powder; however use-levels will be adjusted to maintain a constant GOS level in each food use.
[b] RACC – Reference Amounts Customarily Consumed Per Eating Occasion (21 CFR §101.12). When a range of values is reported for a proposed food-use, particular foods within that food-use may differ with respect to their RACC (U.S. FDA, 2012).
[c] Bimuno is intended to be used in either single-serve coffee and tea products or in coffee creamers and whiteners.

SUBMISSION END