ORIGINAL SUBMISSION

September 29, 2016

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

Dear Dr. Gaynor:

Re: GRAS Exemption Claim for VF-DP3-IMO

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 [BioNeutra North America Inc., 9608-25 Ave NW, Edmonton, Alberta, Canada, T6N 1J4], a Notice of the determination, on the basis of scientific procedures, that an isomalto-oligosaccharide (IMO) mixture comprised of saccharides with a degree of polymerization of 3 or greater (VF-DP3-IMO), produced by BioNeutra North America Inc., as defined in the enclosed documents, is GRAS under specific conditions of use as a food ingredient, and therefore, is 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 VF-DP3-IMO under the intended conditions of use, also are enclosed for review by the agency.

The enclosed electronic files for the Notice entitled, "Generally Recognized as Safe (GRAS) Notice for VF-DP3-IMO for Use in Conventional Foods" were scanned for viruses prior to submission and is thus certified as being virus-free using McAfee VirusScan 8.8.

Should you have any questions or concerns regarding this GRAS 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)

Jianhua Zhu, Ph.D.
President/CEO
BioNeutra North America Inc.

#674

GRN 000674

September 29, 2016

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
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5100 Paint Branch Parkway
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BioNeutra North America Inc.

#674

September 29, 2016

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

Dear Dr. Gaynor,

RE: Certification of Virus-Free Document – (Generally Recognized as Safe (GRAS) Notice for VF-DP3-IMO for Use in Conventional Foods)

I hereby certify that the enclosed electronic files for the petition entitled, "Generally Recognized as Safe (GRAS) Notice for VF-DP3-IMO for Use in Conventional Foods" were scanned for viruses prior to submission and is thus certified as being virus-free using McAfee VirusScan 8.8.

Sincerely,

(b) (6)

Robyn King
Word Processing
Intertek Scientific & Regulatory Consultancy

Generally Recognized as Safe (GRAS) Notice for VF-DP3-IMO for Use in Conventional Foods

Submitted to:

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

Submitted by:

BioNeutra North America Inc.
9608-25 Ave NW
Edmonton, Alberta, Canada
T6N 1J4

September 26, 2016

Generally Recognized as Safe (GRAS) Notice for VF-DP3-IMO for Use in Conventional Foods

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Appendices

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)]

As defined herein, an isomalto-oligosaccharide (IMO) mixture comprised of saccharides with a degree of polymerization of 3 or greater (VF-DP3-IMO) that has been developed by BioNeutra North America Inc. (BioNeutra) for use in foods has been determined to be Generally Recognized as Safe (GRAS), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, and on the consensus opinion of an independent panel of experts qualified by scientific training and expertise to evaluate the safety of VF-DP3-IMO under the conditions of intended use in food. This is an amendment to a previous GRAS notice for the addition of an IMO mixture to food (GRN 246). Therefore, the use of BioNeutra's made VF-DP3-IMO in food as described below is exempt from the requirement of premarket approval (Section 409 of the *Federal Food, Drug and Cosmetic Act*).

Signed,

(b) (6)

Jianhua Zhu, Ph.D.
President/CEO

Sept. 26, 2016
Date

I.B Name and Address of Notifier

Jianhua Zhu, Ph.D.
President/CEO
BioNeutra North America Inc.
9068-25 AVE
Edmonton, Alberta, T6N 1J4

I.C Common Name of the Notified Substance

VF-DP3-IMO

I.D Conditions of Intended Use in Food

The individual proposed food uses are identical to those which were proposed for use in the original GRAS notice for IMO (GRN 246). Since the monosaccharides (DP1) and disaccharides (DP2) will be largely removed from the new VF-DP3-IMO product, the relative concentration of

DP1 to DP2 is decreased and the concentration of DP3 to DP9 will therefore increase. The use levels of VP-DP3-IMO have been adjusted in order to provide the identical amounts of DP3 to DP9 in the food matrices as was provided within the original IMO product and are presented below in Table I.D-1.

Table I.D-1 Summary of the Individual Food-Uses, Use-Levels, and Amount per Serving of VF-DP3-IMO in the United States (U.S.)

Food-Uses	Serving Size (grams)[a]	Original IMO (Vitasugar™; DP1 to DP9)		VF-DP3-IMO (DP3 to DP9)	
		Maximum Use-Level (%)	Amount per Serving (grams)	Maximum Use-Level (%)	Amount per Serving (grams)
Baked Goods and Baking Mixes	60	25	15	21.92	13.15
Beverages and Beverage Bases	240	5	12	4.38	10.51
Breakfast Cereals	50	20	10	17.53	8.77
Condiments and Relishes	23	20	5	17.53	4.03
Dairy Product Analogs	240	5	12	4.38	10.51
Mayonnaise and Mayonnaise-type Dressings	23	30	7	26.30	6.05
Salad Dressings	30	30	9	26.30	7.89
Frozen Dairy Desserts and Mixes	100	10	10	8.77	8.77
Gelatins, Puddings, and Fillings	100	15	15	13.15	13.15
Gravies and Sauces	70	20	14	17.53	12.27
Hard Candies	10	100	10	87.66	8.77
Jams and Jellies	15	75	11	65.75	9.86
Meal Replacement Bars and Mixes	40	25	10	21.92	8.77
Meat Products	50	5	2.5	4.38	2.19
Milk and Milk Products	110	5	5.5	4.38	4.82
Nut Products	30	10	3	8.77	2.63
Processed Fruits and Fruit Juices	140	5	7	4.38	6.13
Snack Foods	30	5	1.5	4.38	1.31
Soft Candy	35	40	14	35.06	12.27
Sugar Substitutes	4	100	4	87.66	3.51
Sweet Sauces, Toppings, and Syrups	30	50	15	43.83	13.15
Processed Vegetables and Vegetable Juices	100	15	15	13.15	13.15
Soups and Soup Mixes	245	5	12	4.38	10.51

DP = degrees of polymerization
[a] Based on the Reference Amounts Customarily Consumed (RACC) Per Eating Occasion (21 CFR §101.12) (U.S. FDA, 2016a).

I.E Basis for the GRAS Determination

Pursuant to Title 21, Section 170.30 of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2016b), VF-DP3-IMO has been determined by BioNeutra to be GRAS on the basis of scientific procedures. This GRAS determination is based on data generally available in the public domain pertaining to the safety of IMOs, and on consensus among a panel of experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of VF-DP3-IMO as a component of food [see Appendix A, entitled, "**Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of VF-DP3-IMO an Isomalto-Oligosaccharide (IMO) Mixture for Use in Conventional Food**"]. The safety of VF-DP3-IMO is supported by a number of studies pertaining to the safety of IMOs that are available in the public domain.

The Expert Panel consisted of the following qualified scientific experts: Dr. Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Dr. John Doull (University of Kansas Medical Center), and Dr. Robert J. Nicolosi (University of Massachusetts, Lowell).

The Expert Panel, convened by BioNeutra, independently and critically evaluated all data and information presented herein, and concluded that VF-DP3-IMO was GRAS for use as an ingredient in food and beverage products as described in Table I.D-1 and in medical foods.

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:

BioNeutra North America Inc.
9068-25th AVE
Edmonton, Alberta, T6N 1J4

Attn:
Jianhua Zhu, Ph.D.
President/CEO
jzhu@bioneutra.ca
1-780- 466-1481 (ext. 111)

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

II. Detailed Information About the Identity of the Substance

II.A Identity

VF-DP3-IMO is in the form of a clear to light yellow syrup or a white spray-dried powder and is comprised of carbohydrates. The majority of saccharides present in BioNeutra's VF-DP3-IMO product are oligosaccharides with a degree of polymerization (DP) of 3 or greater, although a very small amount (≤5%) of disaccharides and glucose also are present. The saccharides identified in BioNeutra's VF-DP3-IMO product are presented below in Table II.A-1 along with the CAS registry number, molecular formula, and chemical name. Structural formulas of some of the identified mono-, di-, and oligo-saccharides (DP 3 to 5) are presented below in Figure II.A-1.

Table II.A-1 Carbohydrate Profile of BioNeutra's VF-DP3-IMO

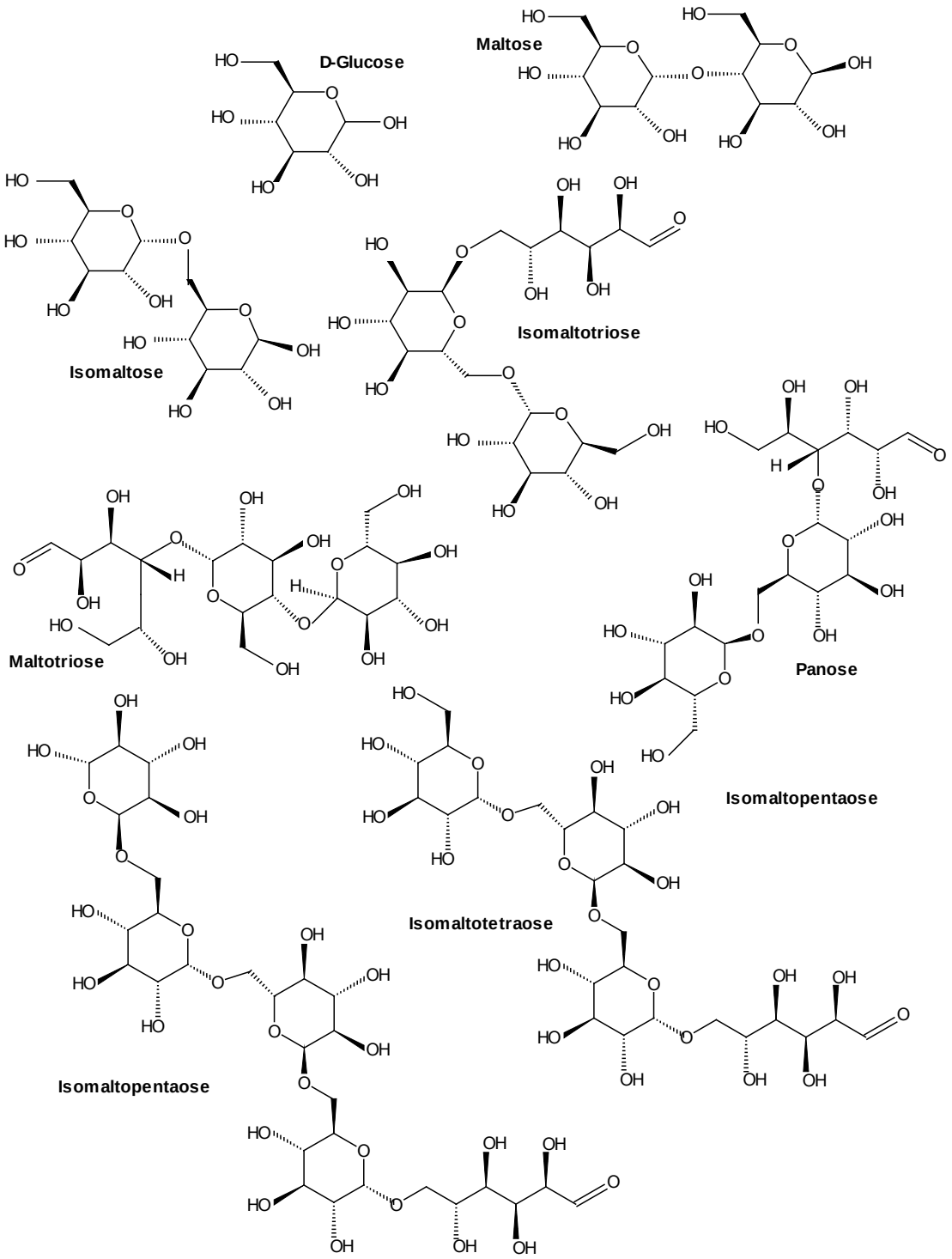
Common Name	CAS No.	Molecular Formula	Chemical Name
Monosaccharides (DP1)			
Glucose	50-99-7	$C_6H_{12}O_6$	D-Glucose
Disaccharides (DP2)			
Maltose	69-79-4	$C_{12}H_{22}O_{11}$	4-O-α-D-glucopyranosyl-D-glucose
Isomaltose	499-40-1	$C_{12}H_{22}O_{11}$	6-O-α-D-glucopyranosyl-D-glucose
Oligosaccharides (≥DP3)			
Maltotriose	1109-28-0	$C_{18}H_{32}O_{16}$	O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-D-glucose
Panose	33401-87-5	$C_{18}H_{32}O_{16}$	O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,4)-D-glucose
Isomaltotriose	3371-50-4	$C_{18}H_{32}O_{16}$	O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-D-glucose
Maltotetraose (DP4)	34612-38-9	$C_{24}H_{42}O_{21}$	O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-D-glucose
Isomaltotetraose (DP4)	35997-20-7	$C_{24}H_{42}O_{21}$	O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-D-glucose
Maltopentaose (DP5)	34620-6-3	$C_{30}H_{52}O_{26}$	O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-D-glucose
Isomaltopentaose (DP5)	6082-32-2	$C_{30}H_{52}O_{26}$	O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-D-glucose
Maltohexaose (DP6)	34620-77-4	$C_{36}H_{62}O_{31}$	O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-D-glucose
Isomaltohexaose (DP6)	6175-02-6	$C_{36}H_{62}O_{31}$	O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)- O-α-D-glucopyranosyl-(1,6)-D-glucose
Maltoheptaose (DP7)	1980-14-9	$C_{42}H_{72}O_{36}$	O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-D-glucose

Table II.A-1 Carbohydrate Profile of BioNeutra's VF-DP3-IMO

Common Name	CAS No.	Molecular Formula	Chemical Name
Isomaltoheptaose (DP7)	6513-12-8	$C_{42}H_{72}O_{36}$	O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-D-glucose
Maltooctaose (DP8)	6156-84-9	$C_{48}H_{82}O_{41}$	O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-D-glucose
Isomaltooctaose (DP8)	Not available	$C_{48}H_{82}O_{41}$	O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-D-glucose
Maltononaose (DP9)	6471-60-9	$C_{54}H_{92}O_{46}$	O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-D-glucose
Isomaltononaose (DP9)	Not available	$C_{54}H_{92}O_{46}$	O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-O-α-D-glucopyranosyl-(1,6)-D-glucose

DP = Degree of polymerization.

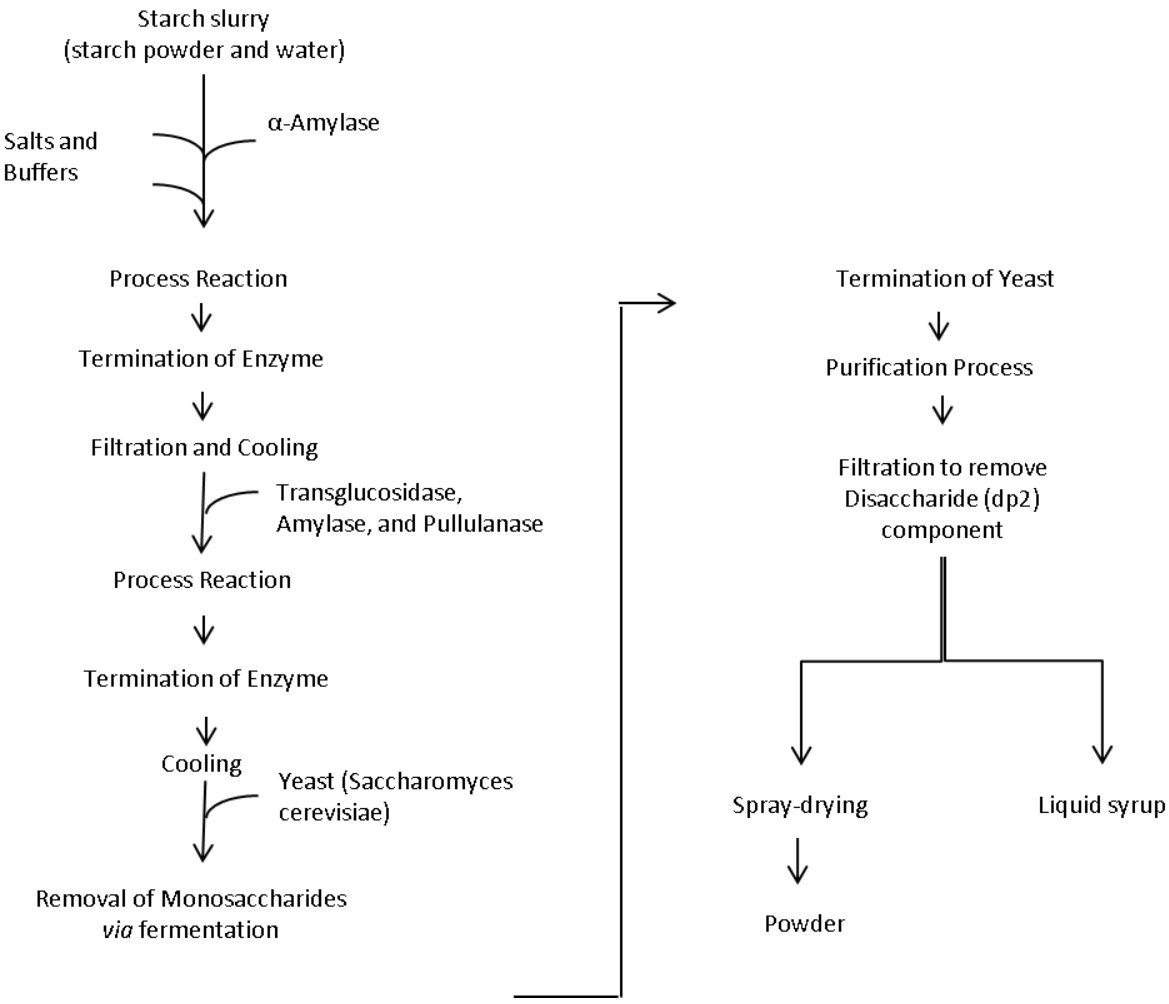
Figure II.A-1 Structural Formulas of the Mono-, Di-, and Oligosaccharides (DP3 to DP5) Identified in BioNeutra's VF-DP3-IMO



II.B Method of Manufacture

The method of manufacture was detailed in the previous GRAS Notice (GRN 246), with an additional manufacturing step now included for VF-DP3-IMO (U.S. FDA, 2009a). This additional manufacturing step will remove mono- and disaccharides (≤DP2 [*i.e.*, glucose, maltose, and isomaltose]) during the filtration process to reduce the level of absolute calories. This change results in an IMO product with a larger proportion of trisaccharides or saccharides of greater chain length (≥DP3) and only minor amounts of mono- and disaccharides (≤5%). Although the concentrations of ≥DP3 have increased within the ingredient, and the concentration of mono- and disaccharides have decreased, the IMO constituents produced using this modified manufacturing method are identical to those which were present in the previous version of the IMO product. This modified IMO product, VF-DP3-IMO, is produced consistent with current Good Manufacturing Practices (cGMP). A schematic overview of the manufacturing process of VF-DP3-IMO is presented below in Figure II.B-1.

Figure II.B-1 Schematic Overview of the Manufacturing Process for VF-DP3-IMO



II.C Specifications for Food Grade Material

The chemical, physical, and microbiological specifications for the VF-DP3-IMO syrup and powder products are presented in Table II.C-1. Although specifications for DP3 to DP9 (total oligo content) are set at ≥91% on a dry basis, in practice, the powder and syrup formulations are prepared such that the content of DP3 to DP9 is typically greater than 95%, based on analytical batch data. Analysis of nonconsecutive representative lots demonstrated compliance with final product chemical, physical, and microbiological specifications.

Table II.C-1 Specification for BioNeutra's VF-DP3-IMO

Parameter	Specification		Method of Analysis
	Syrup	Powder	
Physical			
Color (nm [max % transmittance])	A λ=520-575	N/A	Spectrophotometer/ visual
Taste	Light Sweet	Light Sweet	Physical
Appearance	Sticky syrup, no particulates	White powder, no particulates	Visual
Dried solids (g/100g)	75-77	N/A	Oven drying
Water Activity (a_w)	≤0.8	N/A	Water activity meter
pH	4 to 6	N/A	pH meter
Viscosity (*cps*)	3,100 to 3,800	N/A	Viscometer
Moisture Content	N/A	≤4%	Oven-drying method
Solubility	N/A	≥99%	Direct solubilization
Carbohydrate Content			
Glucose (% dry basis)	<2	<2	HPLC-RI
Maltose/Isomaltose (DP2) (% dry basis)	<7	<7	HPLC-RI
Total Oligo content (DP3 to DP9) (% dry basis)	≥91	≥91	HPLC-RI
Total Carbohydrates (% dry basis)	>99.5	>99.5	HPLC-RI
Heavy Metals			
Sulfated Ash (g/100g)	≤ 0.3	≤ 0.3	USP 281
Lead (mg/kg)	≤ 0.1	≤ 0.1	ICP-MS
Arsenic (mg/kg)	≤ 0.1	≤ 0.1	ICP-MS
Mercury (mg/kg)	≤ 0.1	≤ 0.1	ICP-MS
Microbial			
Total Aerobic Count (CFU/g)	$< 1 \times 10^3$	$< 1 \times 10^3$	USP 2021/2022
Yeast and mold (CFU/g)	$< 1 \times 10^2$	$< 1 \times 10^2$	USP 2021/2022
Escherichia coli (MPN/g)	$< 1 \times 10^1$	$< 1 \times 10^1$	USP 2021/2022
Salmonella (CFU/g)	$< 1 \times 10^1$	$< 1 \times 10^1$	USP 2021/2022
Staphylococcus aureus (CFU/g)	$< 1 \times 10^1$	$< 1 \times 10^1$	USP 2021/2022

CFU = colony forming units; DP = Degree of polymerization; HPLC-RI = High Performance Liquid Chromatography Refractive Index; ICP-MS = inductively coupled plasma mass spectrometry; N/A = Not available; USP = United States Pharmacopeia

II.D Stability

The stability of the original IMO product was previously assessed and detailed in GRAS Notice (GRN 246). The stability of VF-DP3-IMO is expected to be similar to the stability of IMO since it is a further purified form of IMO. The individual levels of glucose, DP2, DP3, DP4, and DP5 to 7 were measured during the stability studies. Since all components of the original IMO product were determined to be highly stable in these studies, VF-DP3-IMO also is expected to be highly stable.

II.E Physical or Technical Effect

VF-DP3-IMO is intended for use as a dietary fiber in conventional foods.

III. Self-Limiting Levels of Use

The use of VF-DP3-IMO in food will largely be limited based on its organoleptic properties.

IV. Detailed Summary of the Basis for GRAS Determination

The safety of the original IMO product (GRN 246) was based on scientific procedures, and included the nature of the metabolic fate of IMO demonstrating that there is no risk of systemic toxicity related to the ingestion of IMOs. The safety of IMO mixtures was confirmed by a series of published animal toxicity studies, and several human tolerance studies reporting no adverse toxicological effects relevant to the conditions of intended use in foods. Since use levels of VF-DP3-IMO will be adjusted in order to provide the identical amounts of DP3 to DP9 in the food matrices as was provided for the original IMO product, all safety data previously included in the original GRAS determination for IMO was deemed to be relevant to establish the safety of VF-DP3-IMO. Likewise, all safety data available subsequent to the original GRAS determination also is relevant to establish the safety of VF-DP3-IMO.

A summary of the consumption estimates of VF-DP3-IMO are presented below in Section IV.A, with a brief overview of the safety data included in the previous GRAS notice presented in Section IV.B and the newly available information presented in Section IV.C, which corroborates the original safety determination.

IV.A Consumption Estimates

The original exposure assessment of BioNeutra's IMO products was calculated based on 2 different approaches, as described in the original GRAS documentation. Briefly, the first approach was based on production volume estimates and expected market share of BioNeutra's IMO products as a percent of the total sweetener market, which was calculated to

be 0.15 g/person/day. For the second approach, intake estimates were determined based on the replacement of 2 servings per day of sucrose with BioNeutra's IMO products for a number of food categories, with the maximum intake calculated to be 30 g/day. Since the maximum use levels of VF-DP3-IMO product will provide identical maximum exposure levels of the DP3 to DP9 portion of the original IMO product (*i.e*., 26.3 g VF-DP3-IMO product/day providing 25 g DP3 to DP9/day; see Section I.D), estimated dietary consumption of VF-DP3-IMO is effectively the same as that which was described in the original GRAS notice, with a reduced level of the DP1 to DP2 component. As such, updated dietary intake estimates were not warranted.

IV.B Summary of Data Included in the GRAS Notice for the Original IMO Product

IV.B.1 Absorption, Distribution, Metabolism, and Elimination

In the GRAS notice for the original IMO product, available *in vitro* and *in vivo* animal and human studies demonstrated that following oral consumption, the maltose-oligosaccharide fraction of the mixture, as well as the isomalto-disaccharides are largely hydrolyzed in the gastrointestinal tract to glucose, which is subsequently absorbed and utilized by the body in well-characterized metabolic pathways (Glinsmann *et al*., 1986; Kaneko *et al*., 1992; Heymann *et al*., 1995). The remaining undigested IMOs travel through the gastrointestinal tract and are subjected to bacterial fermentation in the colon, resulting in the generation of short-chain fatty acids with the fermentation products subsequently absorbed and utilized in well-characterized biochemical pathways (Kaneko *et al*., 1992; Oku and Nakamura, 2003).

IV.B.2 Toxicological Studies and Human Studies

The results of the previously available animal toxicity studies and human tolerance studies provided sufficient support that consumption of IMO, at the levels associated with the original intended uses (*i.e*., not greater than 30 g/person/day), would not be expected to be associated with any adverse effects. In a short-term animal study, final body weights, body weight gain, and food intake, although not at levels of statistical significance, were slightly reduced in male Sprague-Dawley rats administered 20% of an IMO mixture in the diet (approximately 20 g/kg body weight/day) for a period of 35 days, whereas the decrease in food utilization efficiency reached levels of statistical significance in comparison to the basal diet control group (Kaneko *et al*., 1992). No significant differences were reported in the relative weights of a series of major organs including the liver in rats treated with the IMO mixture compared to the basal diet controls. In a 6-week study in which groups of male Sprague-Dawley rats were administered a mixture of IMOs in the diet at concentrations of up to 20% (approximately 20 g/kg body weight/day), no significant variations with the exception of increased cecal weights in mid- and high-dose groups (10 and 20% or 10 and 20 g/kg body weight/day, respectively) were reported compared to controls (Day and Chung, 2004). The study authors considered the increases in cecal weights as likely related to an increase in the colonic bacterial population. In a chronic

toxicity study, male Wistar rats were provided 3% of an IMO product in drinking water, resulting in daily doses of approximately 3 to 5 g/kg body weight, for 1 year (Kaneko *et al*., 1990). Significant variations in the hematology and clinical chemistry parameters at study completion were limited to decreases in levels of hemoglobin, hematocrit, and ALT in test animals compared to controls; however, neither the gross necropsy nor the histopathological examination revealed any abnormalities related to the administration of the IMO preparation. In a few other short-term studies (up to 30 days in duration) primarily evaluating metabolic end-points and intestinal physiology, safety-related effects were largely limited to increased weights of the cecum or cecal contents observed in Sprague-Dawley rats receiving IMO mixtures in the diet at concentrations of 6 to 12% (approximately 3 to 10 g/kg body weight/day, respectively) (Ly *et al*., 1999; Chai and Rhee, 2001; Sung *et al*., 2004). Examined *in vitro*, in bacterial and mammalian cells, IMO mixtures did not induce any mutagenic or genotoxic effects with or without metabolic activation (Kaneko *et al.*, 1990). While no studies were identified which specifically assessed the potential effect of IMO consumption on reproduction or development, the Expert Panel considered that due to the lack of systemic absorption of the larger IMOs which comprise the IMO product and hydrolysis of the smaller saccharides to glucose, there is no reason to suspect any potential reproduction or development toxicity.

Due to the metabolic fate of IMO preparations, mixtures of IMOs were not expected to be associated with any systemic adverse effects in humans; however, given that the majority of the IMO preparation is undigested and, fermented in the colon, consumption of IMOs may lead to some gastrointestinal discomforts. Several human studies were previously identified which range in duration from 7 to 30 days and assessed various indices related to the putative prebiotic properties of IMO preparations and also to evaluate their tolerability. In a 30-day study in which subjects consumed 10 to 15 g of an IMO mixture, no gastrointestinal symptoms were reported by study participants (Kaneko *et al*., 1993). In other studies, increases were reported in the severity or incidence of various gastrointestinal symptoms (*e.g*., flatulence, abdominal pain and distension, borborygmi) following consumption of 10 to 30 g of IMO preparations for 7 to 28 days; however, increased incidences or severity of diarrhea specifically was not experienced by the subjects in any of these studies (Kohmoto *et al*., 1988; Chen *et al*., 2001; Bouhnik *et al*., 2004). Some authors have reported a threshold value of 1.5 g/kg body weight or greater (approximately 90 g in the case of a 60 kg individual) for the induction of transient diarrhea resulting from the consumption of single bolus doses of IMOs (Oku and Okazaki, 1999; Oku and Nakamura, 2002). Moreover, the increase in flatulence reported by individuals in the study conducted by Kohmoto *et al.* (1988) was only temporary and subsided with treatment, suggesting that the microfloral population adapted to changes in the amount of undigested material passing into the colon. The variability in the occurrence of gastrointestinal disturbances following ingestion of IMO preparations may be due to compositional differences among the IMO mixtures. In 2 trials which included evaluation of clinical chemistry, no significant differences were reported in blood glucose, blood urea nitrogen (BUN), creatinine, albumin, total protein, calcium, phosphorous, and potassium values when elderly subjects or hemodialysis

patients were provided daily 24 or 30 g of an IMO preparation for 30 and 28 days, respectively (Chen *et al*., 2001; Wang *et al*., 2001). In comparison to pre-treatment values, the hemodialysis patients exhibited elevated hemoglobin and hematocrit values following ingestion of the IMO mixture which may have been in part due to enhanced iron absorption (Wang *et al*., 2001). The original IMO product was considered to be well tolerated at the proposed use level of 30 g/day. Since the intake levels of VF-DP3-IMO are equivalent to the exposure of the DP3 to DP9 component of the original IMO product (*i.e*., a maximum intake of 26.3 g/day), VF-DP3-IMO is expected to be well-tolerated.

It also is important to note that IMOs are normal constituents of the human diet that occur naturally in a number of fermented foods, including rice miso, soy sauce, and sake (Hondo and Mochizuki, 1979; Nishino *et al*., 1981; Nunokawa, 1981; Tungland and Meyer, 2002) as described in the original GRAS notice.

IV.B.3 Nutritional Considerations

The GRAS notice for the original IMO product also considered information related to nutritional considerations commonly associated with non-digestible carbohydrates, including increases in levels of bifidobacteria, short-chain fatty acid production, enhanced bile acid excretion, and changes in mineral bioavailability. This information was reviewed as part of the original GRAS dossier, and was re-considered it in terms of the safety of the new VF-DP3-IMO, recognizing that the digestible carbohydrate material has been largely removed. *In vitro*, animal, and human studies demonstrated varying results related to the effects of IMO on the levels of bifidobacteria in the colon; however, this may be related to the variable content of digestible and non-digestible material in the IMO products that were used in the studies (Kohmoto *et al*., 1988, 1991; Kaneko *et al*., 1990, 1993; Chen *et al*., 2001; Rycroft *et al*., 2001; Qing *et al*., 2003; Bouhnik *et al*., 2004). Increased levels of bifidobacteria in the colon are generally considered to be beneficial. Levels of fecal short-chain fatty acid levels were measured in some animal and human studies. Increases were reported in lactate and acetate levels in an *in vitro* study in which fecal bacteria were incubated with IMOs but no variations in propionate and butyrate were reported (Rycroft *et al*., 2001). In a study in male Sprague-Dawley rats, administration of 5% of an IMO mixture in the diet (unspecified period of time) did not result in changes in levels of individual short-chain fatty acids or in the pH level of the cecum (Ohta *et al*., 1993). In human trials, results were generally more comparable to those observed in the *in vitro* assays, with increases reported in acetate, propionate, and total short-chain fatty acid levels, but not in butyrate following daily ingestion of 10 to 24 g of IMO-containing mixtures for a period of 28 to 30 days (Kaneko *et al*., 1993; Chen *et al*., 2001). In rat studies in which fecal bile acid excretion was measured directly or plasma cholesterol levels were assessed as an indirect measure of changes in bile acid secretion, no changes were reported between rats administered IMO mixtures in the diet and controls (Kaneko *et al*., 1990, 1992; Ly *et al*., 1999; Chai and Rhee, 2001; Sung *et al*., 2004). Significant reductions in serum triglyceride and total cholesterol levels

and increased HDL-cholesterol levels following daily consumption of 30 g of an IMO mixture compared to pre-treatment values in a human trial were reported by Wang *et al*. (2001). Alterations in the colonic environment (*e.g*., decreases in pH levels) as a result of increased bacterial fermentation of non-digestible carbohydrates and secondary changes in short-chain fatty acid levels have been implicated in altered mineral absorption. In the only study in which absorption of calcium, magnesium, and phosphorus was assessed in rats provided diets supplemented with 5% of an IMO mixture, mineral absorption of IMO-treated rats did not differ from controls (Ohta *et al*., 1993).

IV.C Data Identified Subsequent to the GRAS Notice for the Original IMO Product

IV.C.1 Absorption, Distribution, Metabolism, and Elimination

Subsequent to the previous GRAS notice, BioNeutra has conducted a single-center, randomized, double-blind, placebo-controlled, parallel group study of their original IMO product with the main objective to examine the safety and tolerability, to assess the effects of IMO on the glycemic response, and nutritional considerations (BioNeutra, 2012 [unpublished]). The methods of the study are detailed in Section IV.C.3 (*i.e*., human studies section). Briefly, 60 healthy subjects (10/sex/group; 18 to 65 years) were assigned to receive dextrose powder at 36 g/day (placebo), or IMO powder at 36 g/day (low-dose) or 54 g/day (high-dose), dissolved in water, for a period of 4 weeks. Fecal samples were collected at baseline and at the end of the study and analyzed for the content of commensal bacterial species and short-chain fatty acids (SCFAs). A significant increase in the fecal counts of *Bifidobacteria* and *Lactobacillus* was reported in subjects of both the low- and high-dose groups, while the levels of *Clostridium* spp. were only minimally altered, compared to subjects receiving placebo (see also Section 6.3.3). This suggests that at least a portion of the IMOs was resistant to digestion in the upper gastrointestinal tract, and was capable of reaching the colon where it was fermented by the resident microflora. The levels of SCFAs (acetate, propionate and n-butyrate) in the feces did not significantly differ among intervention groups but this is difficult to interpret since the majority of SCFAs are known to be rapidly absorbed in the colon following their production (Topping and Clifton, 2001; Hijova and Chmelarova, 2007). Therefore, levels of SCFAs in the feces are not necessarily reflective of cecal and colonic fermentation, but rather, are indicative of the efficiency of SCFA absorption and/or the extent of ongoing fermentation in the distal colon (Scheppach *et al*., 1987; Eastwood, 2003).

It is important to note that although 2-hour dose challenge tests for the analysis of glucose and insulin were conducted in the BioNeutra (2012 [unpublished]) study, the glycemic response may differ slightly for the original IMO product when compared to the VF-DP3-IMO. Since VF-DP3-IMO contains approximately 5% disaccharides, and since the equivalent amount of DP3 to DP9 is recommended for the intended use (see Section 4.0), the level of exposure of disaccharides

is effectively reduced while the level of exposure of DP3 to DP9 (*i.e.*, the indigestible portion of the mixture) is effectively the same. The glycemic response may be decreased compared to that of the original IMO product. The effects on SCFA would be expected to be similar to that reported in BioNeutra (2012 [unpublished]) since the amount of non-digestible oligosaccharides capable of reaching the colon (*i.e.,* DP3 to DP9) would be similar.

IV.C.2 Toxicological Studies

Only one (1) new repeat-dose study was identified in the literature subsequent to the GRAS notice for the original IMO product, although the details of the study are limited as it is published in Japanese, with only a short English summary, and does not appear to have been conducted consistent with recognized guidelines for short-term studies (*e.g.*, OECD guidelines). Briefly, no effects on body weight gain or food intake were observed in male Sprague-Dawley rats administered an IMOs mixture at doses of up to 20% diet (equivalent to approximately 20 g/kg boy weight/day) for 6 weeks while a significant dose-dependent reduction in abdominal fat was reported (Day and Chung, 2007). A significant increase in the weight of the cecum likely related to an increase in the colonic bacterial population also was reported. These results provide corroborative evidence that IMOs do not pose any concerns for systemic toxicity.

IV.C.3 Human Studies

Subsequent to the GRAS notice for the original IMO product, BioNeutra conducted a single-center, randomized, double-blind, placebo-controlled, parallel group study to examine the safety and tolerability of their IMO preparation (BioNeutra, 2012 [unpublished]). Sixty (60) healthy subjects (10/sex/group; 18 to 65 years) were assigned to receive dextrose powder at 36 g/day (placebo), or IMO powder at 36 g/day (low-dose) or 54 g/day (high-dose) for a period of 4 weeks. Individual sachets of the test articles (*i.e.*, 12 g dextrose powder, 12 g IMO, or 18 g IMO) were dissolved into a full or half glass of water, and were taken with or without food 3 times daily. Prior to and during the randomization visit, subjects were instructed to complete a 3-day food record for any 2 weekdays and 1 weekend day, which was used to calculate caloric intake. Bowel habits and digestive symptoms were recorded in a diary just prior to and throughout the study. Fecal sample were collected at baseline and during the last week of the study for the analysis of *Bifodobacteria* spp., lactic acid bacteria, *Clostridium* spp., and SCFAs (*i.e.*, acetate, propionate, and n-butyrate). Body weights and vital signs were measured, fasting blood samples were collected, and 2-hour dose challenge tests were conducted at baseline and week 4.

There were no statistically significant differences between groups in body weight, vital signs, or standard hematology and clinical chemistry parameters. There were no significant changes reported in bowel habits (*i.e.*, ease of defecation, number of bowel movements per day) of subjects between groups; however, beneficial effects (*i.e.*, reduction in frequency of lumpy or hard stools) commonly observed with non-digestible carbohydrates were reported. In the high-

dose group, a significant decrease in the frequency and severity of nausea at week 1 and a significant decrease in the frequency of stomach discomfort at week 1 and 2 compared to controls was reported. A significant increase in the severity of the diarrhea in the high-dose group at week 1 was reported compared to baseline; however this was not significantly different from controls and this effect did not occur at weeks 2 through 4. The reported incidence of diarrhea occurring at any point in the study was higher in the high-dose group than in the controls (7 out of 19 *vs*. 2 out of 19) the difference was not statistically significant. No significant difference in the frequency of subjects reporting any adverse event and no serious adverse events were reported during the study. The authors determined that the results of the study suggest a threshold dose of 36 g/day based on the increased severity of diarrhea in the 54 g/day dose group. This is conservative since severity of diarrhea is subjective, only occurred during week 1, and was only significantly different from baseline and not from controls. This increase in severity of diarrhea in the 54 g/day dose group is unlikely to be related to the dose of IMO and the true threshold for tolerability of IMO may be 54 g/day under the conditions of this study.

An additional human study was identified in the literature which was primarily designed to assess the prebiotic properties of IMOs but contained some endpoints related to safety and tolerability. In this double-blind randomized, diet-controlled study, 13 male and female elderly subjects with constipation were administered IMOs according to the following regimen: 4-week placebo, two 4-week intervention periods (IO1 and IO2; duration between intervention periods not reported), and 4-week post-administration (Yen *et al*., 2011). During the first intervention period, the dose of IMOs was gradually increased from 11 g/day (5 g/day of "active component") to 22 g/day (10 g/day of "active component") over the first 7 days of treatment. The dose remained at 22 g/day for the remainder of the 2 intervention periods. All subjects were assigned a diet designed for each individual, from 1 month prior to the experiment to end of post treatment, using a 7-day cycle menu. Body weights were measured during each period (exact measurement schedule not reported) and blood samples were taken at the end of each 4-week period. Bowel function was assessed throughout the study period. No significant differences in body weight or levels of plasma albumin, glucose, triglyceride, high-density lipoprotein (HDL)-cholesterol, urea nitrogen, creatinine, or alanine aminotransferase (ALT) were reported. A significant increase in the number of spontaneous defecations at IO2, stool output at IO1 and IO2, and dry fecal mass at IO1 and IO2 were reported. This study further corroborates the safety of VF-DP3-IMO at current use levels.

IV.C.4 Nutritional Considerations

An additional animal study evaluating the nutritional considerations of the original IMO product was conducted subsequent to the previous GRAS notice. Five-week-old F344 rats (6/group; sex not reported) were administered diets containing BioNeutra's IMO product or inulin at 8 g/kg body weight/day, or a control basal diet for 6 weeks (Ketabi *et al.*, 2011). Stool samples were

collected from the animals at baseline, and at weeks 3 and 6 of the study. Rats fed the diets containing BioNeutra's IMO product had significantly higher total number of fecal bacteria and a significantly higher number of fecal *Lactobacilli* and *Bifidobacteria* compared to rats fed the control basal diet. Qualitative analysis suggested that rats administered the IMOs had increased biodiversity of *Lactobacillus* species compared to animals fed the control diet. Total levels of SCFAs, and fecal acetate concentrations, were significantly decreased in rats administered IMO compared to the control and inulin groups. The authors noted that studies conducted in humans reported the opposite effect, with increased SCFA concentrations observed after the consumption of IMO at similar levels. Approximately 95% of the SCFAs produced by intestinal microflora are rapidly absorbed in the colon, and IMO likely stimulates the SCFA production in the upper intestine of rats with only the unabsorbed SCFAs detected in the feces.

The human product specific study conducted subsequent to the previous GRAS determination, as described in Section ~~Section~~ IV.C.3, included measurements of the effects of IMO on cecal microflora and SCFA levels (BioNeutra, 2012 [unpublished]). A significant increase in the fecal counts of *Bifidobacteria* and *Lactobacillus* was reported in subjects of both the low- and high-dose groups, and the levels of *Clostridium* spp. were only minimally altered, compared to subjects receiving placebo. Although decreases in acetate levels and increases in propionate and butyrate levels were reported in subjects of the intervention groups, no significant differences were reported compared to placebo.

IV.C.5 Other Indigestible Saccharides

Since the previous GRAS determination for the original IMO product was notified to the FDA, a number of additional GRAS notifications for other indigestible saccharides have been submitted. Several notifications have been submitted for galacto-oligosaccharides (GOS) for which the FDA had no questions in response (GRN 000233, 236, 285, 286, 334, 484, 489, 495, 518, 569) (U.S. FDA, 2008, 2009b-d, 2010a, 2014a-d, 2015). The FDA also had no questions in response to GRAS notices submitted for xylooligosaccharides products (GRN 000343, 370, 458) (U.S. FDA, 2010b, 2011, 2013).

IV.D Summary and Basis for GRAS

BioNeutra's product, an IMO mixture, is GRAS for use in food (GRN 246). BioNeutra now proposes to modify the manufacturing process whereby monosaccharides and disaccharides (DP2 [*i.e.*, maltose and isomaltose]) are both removed during filtration. This change results in an IMO product with a larger proportion of trisaccharides or saccharides of greater chain length (≥DP3) and only minor amounts of mono- and disaccharides (≤5%). Although the concentrations of ≥DP3 have increased within the ingredient, and the concentration of mono- and disaccharides have decreased, the IMO constituents produced using this modified

manufacturing method are identical to that which are present in the previous version of the IMO product. The modified IMO product, VF-DP3-IMO, is produced consistent with cGMP and batch data demonstrate that the manufacturing process produces consistent products within the product specifications.

BioNeutra intends to market VF-DP3-IMO as a food ingredient for use as a fiber in conventional foods. The individual proposed food uses are identical to those proposed in the original GRAS notice. The use levels will be adjusted in order to provide the identical amounts of DP3 to DP9 in the food matrices as were provided for in the original IMO product. The use levels of the original IMO product resulted in a maximum exposure level of 25 g/day (*i.e.*, 82% of 30 g/day) based on 2 servings per day of the intended food uses. With the new VF-DP3-IMO product containing approximately 5% DP1 to DP2 and approximately 95% DP3 to DP9, a maximum intake of approximately 26.3 g/day would provide approximately 25 g/day of DP3 to DP9 (*i.e.*, 25 g/day ÷ 95% = 26.3 g/day). This maximum use level of 26.3 g/day (provided in 2 servings per day), will result in the same exposure of the DP3 to DP9 component in VF-DP3-IMO as per the original IMO product (*i.e.*, 25 g/day), with a reduced level of the DP1 and DP2 component. The estimated dietary consumption of the VF-DP3-IMO component is effectively the same as that which was described in the original GRAS documentation.

The safety of the original IMO product was assessed using scientific procedures, and was based on the nature of the metabolic fate of IMO demonstrating that there is no risk of systemic toxicity related to the ingestion of IMOs. The safety of IMO mixtures was confirmed by a series of published animal toxicity studies, and several human tolerance studies reporting no adverse toxicological effects relevant to the conditions of intended use in foods. Since use levels of VF-DP3-IMO will be adjusted in order to provide the identical amounts of DP3 to DP9 in the food matrices as was provided for the original IMO product, all safety data included in the original GRAS determination for IMO is relevant to establishing the safety of VF-DP3-IMO. All safety data available subsequent to the original GRAS determination also is relevant to establish the safety of VF-DP3-IMO.

Since the original GRAS determination was prepared, BioNeutra has conducted a new human study on their original IMO product to evaluate its safety and tolerability. Endpoints related to metabolic fate and nutritional considerations were included in the study. The highest dose of 54 g/day was considered to be tolerable under the conditions of this study. Additional publicly available literature included 2 repeat-dose studies conducted in rats evaluating toxicology and nutritional considerations, and a human efficacy study that contained some endpoints related to safety, tolerability, and nutritional considerations. The results of these studies further corroborate the safety of VF-DP3-IMO under the intended uses and use levels.

Based on the above data and information presented herein, BioNeutra has concluded that the intended food uses (*i.e.*, 26.3 g/day, providing approximately 25 g/day of DP3 to DP9) of BioNeutra's made VF-DP3-IMO, meeting appropriate food grade specifications and

manufactured consistent with current Good Manufacturing Practices (cGMP), is GRAS based on scientific procedures. General recognition of BioNeutra'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 VF-DP3-IMO in food, who similarly concluded that the intended uses of VF-DP3-IMO described herein are GRAS.

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Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of VF-DP3-IMO an Isomalto-Oligosaccharide (IMO) Mixture for Use in Conventional Food

February 25, 2016

At the request of BioNeutra North America Inc. (BioNeutra), an Expert Panel (the "Expert Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was re-convened, to conduct a critical and comprehensive re-evaluation of the available pertinent data and information concerning the intended use of VF-DP3-IMO. The original isomalto-oligosaccharide (IMO) product was previously considered Generally Recognized as Safe (GRAS) for use in a number of food categories at use levels resulting in a maximum intake of 30 g/day, provided in 2 servings (March 12, 2007). An additional manufacturing step has now been included which is intended to remove a large portion of the disaccharide (degree of polymerization of 2; DP2 [*i.e*., maltose and isomaltose]) content of the original IMO product resulting in an IMO with a larger proportion of trisaccharides or greater saccharides of greater chain length (≥DP3) and only minor amounts of mono- and disaccharides (≤5%). This product, VF-DP3-IMO, is intended to be used in foods in an equivalent 2 servings, resulting in an intake which is identical to the exposure of the DP3 to DP9 component of the original IMO product (*i.e*., a maximum intake of 26.3 g/day). The Expert Panel was asked to re-evaluate the information and determine whether the use of this additional filtration step would result in a product (to be used at the same exposure levels) that is likewise GRAS, based on scientific procedures. The Expert Panel consisted of the below-signed qualified scientific experts, who comprised the original Expert Panel: Dr. Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Dr. John Doull (University of Kansas Medical Center), and Dr. Robert J. Nicolosi (University of Massachusetts, Lowell). For purposes of the Expert Panel's evaluation, "safe" or "safety" means that there is a reasonable certainty of no harm under the intended conditions of use of the ingredient in foods, as stated in 21 CFR §170.3(i) (U.S. FDA, 2015).

The Expert Panel, independently and collectively, critically examined a comprehensive package of scientific information and data pertinent to VF-DP3-IMO compiled from the literature and other published sources through January 2016 and also included information pertaining to the method of manufacture, the product specifications, supporting analytical data, intended use levels, and a comprehensive assessment of the available scientific literature pertaining to the safety of IMO products. The data and information were presented to the Expert Panel in a dossier, "**Documentation Supporting the Generally Recognized as Safe (GRAS) Status of VF-DP3-IMO an Isomalto-Oligosaccharide (IMO) Mixture for use in Conventional Food"** dated January 7, 2016.

Following independent and collaborative critical evaluation of such data and information, the Expert Panel convened *via* teleconference on February 25, 2016. The Expert Panel unanimously concluded that the use in foods at levels resulting in a maximum intake of 26.3 g/day, provided in 2 servings, of VF-DP3-IMO meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice (cGMP), is GRAS based on scientific procedures.

A summary of the basis for the Expert Panel's conclusion appears below.

SUMMARY AND BASIS FOR GRAS

BioNeutra's product, VitaFiber™ an isomalto-oligosaccharide (IMO) product, is GRAS for use in food as an alternative sweetener (GRN 246; U.S. FDA, 2009). BioNeutra now proposes to modify the manufacturing process so as to remove mono- and disaccharides (≤DP2 [*i.e*., glucose, maltose, and isomaltose]) during the filtration process to reduce the level of absolute calories. This change results in an IMO product with a larger proportion of trisaccharides or saccharides of greater chain length (≥DP3) and only minor amounts of mono- and disaccharides (≤5%). Although the concentrations of ≥DP3 have increased within the ingredient, and the concentration of mono- and disaccharides have decreased, the IMO constituents produced using this modified manufacturing method are identical to those which were present in the previous version of the IMO product. The Expert Panel critically reviewed details of the manufacturing process. The modified IMO product, VF-DP3-IMO, is produced consistent with cGMP and batch data demonstrate that the manufacturing process produces consistent products within the product specifications. Stability studies demonstrate that VF-DP3-IMO can be expected to be highly stable.

The original GRAS determination of IMO was notified by BioNeutra to the FDA. Following their review, the FDA indicated that they had "*no questions*" in response to the notification, considering the intended conditions of use (GRN 246; U.S. FDA, 2009). Health Canada also indicated that they had no objections to the use of BioNeutra's IMO ingredient as a novel food ingredient (Health Canada, 2012). In July 2013, BioNeutra's IMO product was approved as a novel food ingredient that may be marketed for sale throughout the 28 Member States of the European Union (FSA, 2012, 2013). Isomalto-oligosaccharides are normal constituents of the human diet and occur naturally in a number of fermented foods, including rice miso, soy sauce, and sake (Hondo and Mochizuki, 1979; Nishino *et al*., 1981; Nunokawa, 1981; Tungland and Meyer, 2002).

BioNeutra intends to market the new VF-DP3-IMO product as a food ingredient for use as a fiber in conventional foods. The individual proposed food uses are identical to those which were proposed for use in the original GRAS determination, with the additional inclusion of the food category 'soups and soup mixes' at a maximum use level of 5%. The addition of the 'soups and

soup mixes' does not affect the estimated intakes of VF-DP3-IMO by the U.S. population to any appreciable extent as compared to its original uses. Since DP1 to DP2 will be largely removed from the new VF-DP3-IMO product, the relative concentration of DP1 to DP2 is decreased and the concentration of DP3 to DP9 will therefore increase. The use levels of VP-DP3-IMO have been adjusted in order to provide the identical amounts of DP3 to DP9 in the food matrices as was provided for the original IMO product. In the original GRAS documentation for IMO, the estimated daily intake amount was determined to be 30 g/day based on 2 servings per day of foods containing the maximum use level per serving. The original IMO product contained approximately 18 to 33.5% DP1 to DP2 and 66.5 to 82% DP3 to DP9; therefore, the estimated daily intake of 30 g/day would provide approximately 20 to 25 g/day of DP3 to DP9 (*i.e.,* 30 g/day x 66.5 to 82%). With the new VF-DP3-IMO product containing approximately 5% DP1 to DP2 and approximately 95% DP3 to DP9, an intake of approximately 26.3 g/day would provide approximately 25 g/day of DP3 to DP9 (*i.e*., 25 g/day ÷ 95% = 26.3 g/day). This maximum use level of 26.3 g/day, in turn, will result in the same exposure of the DP3 to DP9 component in VF-DP3-IMO as per the original IMO product, with a reduced level of the DP1 to DP2 component. Since the estimated dietary consumption of VF-DP3-IMO is effectively the same as that which was described in the original GRAS documentation, with a reduced level of the DP1 to DP2 component, updated dietary intake estimates were not warranted.

The safety of the original IMO product was based on scientific procedures, and included the nature of the metabolic fate of IMO demonstrating that there is no risk of systemic toxicity related to the ingestion of IMOs. The safety of IMO mixtures was confirmed by a series of published animal toxicity studies, and several human tolerance studies reporting no adverse toxicological effects relevant to the conditions of intended use in foods. Since use levels of VF-DP3-IMO will be adjusted in order to provide the identical amounts of DP3 to DP9 in the food matrices as was provided for the original IMO product, all safety data previously included in the original GRAS determination for IMO was deemed to be relevant to establish the safety of VF-DP3-IMO. Likewise, all safety data available subsequent to the original GRAS determination also is relevant to establish the safety of VF-DP3-IMO.

In the previous GRAS documentation, available i*n vitro* and *in vivo* animal and human studies demonstrated that following oral consumption, the maltose-oligosaccharide fraction of the mixture, as well as the isomalto-disaccharides are largely hydrolyzed in the gastrointestinal tract to glucose, which is subsequently absorbed and utilized by the body in well-characterized metabolic pathways (Glinsmann *et al*., 1986; Kaneko *et al*., 1992; Heymann *et al*., 1995). The remaining undigested isomalto-oligosaccharides travel through the gastrointestinal tract and are subjected to bacterial fermentation in the colon, resulting in the generation of short-chain fatty acids with the fermentation products subsequently absorbed and utilized in well-characterized biochemical pathways (Kaneko *et al*., 1992; Oku and Nakamura, 2003).

The results of the previously available animal toxicity studies and human tolerance studies provided sufficient support that consumption of IMO, at the levels associated with the original intended uses (*i.e*., not greater than 30 g/person/day), would not be expected to be associated with any adverse effects. In a short-term animal study, final body weights, body weight gain, and food intake, although not at levels of statistical significance, were slightly reduced in male Sprague-Dawley rats administered 20% of an IMO mixture in the diet (approximately 20 g/kg body weight/day) for a period of 35 days, whereas the decrease in food utilization efficiency reached levels of statistical significance in comparison to the basal diet control group (Kaneko *et al*., 1992). No significant differences were reported in the relative weights of a series of major organs including the liver in rats treated with the IMO mixture compared to the basal diet controls. In a 6-week study in which groups of male Sprague-Dawley rats were administered a mixture of isomalto-oligosaccharides in the diet at concentrations of up to 20% (approximately 20 g/kg body weight/day), no significant variations with the exception of increased cecal weights in mid- and high-dose groups (10 and 20% or 10 and 20 g/kg body weight/day, respectively) were reported compared to controls (Day and Chung, 2004). The study authors considered the increases in cecal weights as likely related to an increase in the colonic bacterial population. In a chronic toxicity study, male Wistar rats were provided 3% of an IMO product in drinking water, resulting in daily doses of approximately 3 to 5 g/kg body weight, for 1 year (Kaneko *et al*., 1990). Significant variations in the hematology and clinical chemistry parameters at study completion were limited to decreases in levels of hemoglobin, hematocrit, and ALT in test animals compared to controls; however, neither the gross necropsy nor the histopathological examination revealed any abnormalities related to the administration of the IMO preparation. In a few other short-term studies (up to 30 days in duration) primarily evaluating metabolic end-points and intestinal physiology, safety-related effects were largely limited to increased weights of the cecum or cecal contents observed in Sprague-Dawley rats receiving IMO mixtures in the diet at concentrations of 6 to 12% (approximately 3 to 10 g/kg body weight/day, respectively) (Ly *et al*., 1999; Chai and Rhee, 2001; Sung *et al*., 2004). Examined *in vitro*, in bacterial and mammalian cells, IMO mixtures did not induce any mutagenic or genotoxic effects with or without metabolic activation (Kaneko *et al.*, 1990). While no studies were identified which specifically assessed the potential effect of IMO consumption on reproduction or development, the Expert Panel considered that due to the lack of systemic absorption of the larger isomalto-oligosaccharides which comprise the IMO product and hydrolysis of the smaller saccharides to glucose, there is no reason to suspect any potential reproduction or development toxicity.

Due to the metabolic fate of IMO preparations, mixtures of isomalto-oligosaccharides were not expected by the Expert Panel to be associated with any systemic adverse effects in humans; however, given that the majority of the IMO preparation is undigested and, fermented in the colon, consumption of isomalto-oligosaccharides may lead to some gastrointestinal discomforts. Several human studies were previously identified which range in duration from 7 to 30 days and assessed various indices related to the putative prebiotic properties of IMO preparations and also to evaluate their tolerability. In a 30-day study in which subjects consumed 10 to 15 g of an

IMO mixture, no gastrointestinal symptoms were reported by study participants (Kaneko *et al*., 1993). In other studies, increases were reported in the severity or incidence of various gastrointestinal symptoms (*e.g*., flatulence, abdominal pain and distension, borborygmi) following consumption of 10 to 30 g of IMO preparations for 7 to 28 days; however, increased incidences or severity of diarrhea specifically was not experienced by the subjects in any of these studies (Kohmoto *et al*., 1988; Chen *et al*., 2001; Bouhnik *et al*., 2004). Some authors have reported a threshold value of 1.5 g/kg body weight or greater (approximately 90 g in the case of a 60 kg individual) for the induction of transient diarrhea resulting from the consumption of single bolus doses of isomalto-oligosaccharides (Oku and Okazaki, 1999; Oku and Nakamura, 2002). Moreover, the increase in flatulence reported by individuals in the study conducted by Kohmoto *et al.* (1988) was only temporary and subsided with treatment, suggesting that the microfloral population adapted to changes in the amount of undigested material passing into the colon. The variability in the occurrence of gastrointestinal disturbances following ingestion of IMO preparations may be due to compositional differences among the IMO mixtures. In 2 trials which included evaluation of clinical chemistry, no significant differences were reported in blood glucose, BUN, creatinine, albumin, total protein, calcium, phosphorous, and potassium values when elderly subjects or hemodialysis patients were provided daily 24 or 30 g of an IMO preparation for 30 and 28 days, respectively (Chen *et al*., 2001; Wang *et al*., 2001). In comparison to pre-treatment values, the hemodialysis patients exhibited elevated hemoglobin and hematocrit values following ingestion of the IMO mixture which may have been in part due to enhanced iron absorption (Wang *et al*., 2001). The original IMO product is well tolerated at the proposed use level of 30 g/day. Since the intake levels of VF-DP3-IMO are equivalent to the exposure of the DP3 to DP9 component of the original IMO product (*i.e*., a maximum intake of 26.3 g/day), VF-DP3-IMO is expected to be well-tolerated.

The Expert Panel noted that the original GRAS documentation also considered information related to nutritional considerations commonly associated with non-digestible carbohydrates including increases in levels of bifidobacteria, short-chain fatty acid production, enhanced bile acid excretion, and changes in mineral bioavailability. This information was reviewed as part of the original GRAS dossier, and the Expert Panel re-considered it in terms of the safety of the new VF-DP3-IMO, recognizing that the digestible carbohydrate material has been largely removed. *In vitro*, animal, and human studies demonstrated varying results related to the effects of IMO on the levels of bifidobacteria in the colon; however, this may be related to the variable content of digestible and non-digestible material in the IMO products that were used in the studies (Kohmoto *et al*., 1988, 1991; Kaneko *et al*., 1990, 1993; Chen *et al*., 2001; Rycroft *et al*., 2001; Qing *et al*., 2003; Bouhnik *et al*., 2004). Increased levels of bifidobacteria in the colon are generally considered to be beneficial. Levels of fecal short-chain fatty acid levels were measured in some animal and human studies. Increases were reported in lactate and acetate levels in an *in vitro* study in which fecal bacteria were incubated with isomalto-oligosaccharides but no variations in propionate and butyrate were reported (Rycroft *et al*., 2001). In a study in male Sprague-Dawley rats, administration of 5% of an IMO mixture in the diet (unspecified

period of time) did not result in changes in levels of individual short-chain fatty acids or in the pH level of the cecum (Ohta *et al*., 1993). In human trials, results were generally more comparable to those observed in the *in vitro* assays, with increases reported in acetate, propionate, and total short-chain fatty acid levels, but not in butyrate following daily ingestion of 10 to 24 g of IMO-containing mixtures for a period of 28 to 30 days (Kaneko *et al*., 1993; Chen *et al*., 2001). In rat studies in which fecal bile acid excretion was measured directly or plasma cholesterol levels were assessed as an indirect measure of changes in bile acid secretion, no changes were reported between rats administered IMO mixtures in the diet and controls (Kaneko *et al*., 1990, 1992; Ly *et al*., 1999; Chai and Rhee, 2001; Sung *et al*., 2004). Significant reductions in serum triglyceride and total cholesterol levels and increased HDL-cholesterol levels following daily consumption of 30 g of an IMO mixture compared to pre-treatment values in a human trial were reported by Wang *et al*. (2001). Alterations in the colonic environment (*e.g*., decreases in pH levels) as a result of increased bacterial fermentation of non-digestible carbohydrates and secondary changes in short-chain fatty acid levels have been implicated in altered mineral absorption. In the only study in which absorption of calcium, magnesium, and phosphorus was assessed in rats provided diets supplemented with 5% of an IMO mixture, mineral absorption of IMO-treated rats did not differ from controls (Ohta *et al*., 1993).

The animal studies identified in the literature subsequent to the original GRAS determination for IMO further corroborate the safety of VF-DP3-IMO under the intended uses and use levels. No systemic toxicity was reported in male Sprague-Dawley rats administered IMO in the diet for 6 weeks, with only a significant increase in cecal weights observed in the mid- and high-dose groups (10 and 20% or 10 and 20 g/kg body weight/day, respectively) compared to controls (Day and Chung, 2007). F344 rats (sex not reported) were administered IMO at a single dose of 8 g/kg body weight/day for 6 weeks and significant increases in fecal *Lactobacilli* and *Bifidobacteria* and significant decreases in fecal short-chain fatty acids (SCFAs) were reported. The authors concluded that IMO likely stimulates SCFA production in the upper intestine of rats, followed by rapid absorption (Ketabi *et al.*, 2011).

Subsequent to the previous GRAS determination, BioNeutra conducted a single-center, randomized, double-blind, placebo-controlled, parallel group study of their original IMO product with the main objectives to examine safety and tolerability, effects of IMO on the glycemic response, and other nutritional considerations(BioNeutra, 2012 [unpublished]). The results in this study corroborate the original determination that isomalto-oligosaccharides in general are resistant to enzymatic hydrolysis in the upper gastrointestinal tract and the unabsorbed isomalto-oligosaccharides are subject to microfloral fermentation in the colon, resulting in the generation of short-chain fatty acids with the fermentation products subsequently absorbed and utilized in well-characterized biochemical pathways. A significant increase in the fecal counts of *Bifidobacteria* and *Lactobacillus* and no significant differences in SCFA were reported compared to controls. A tolerability threshold based on unpleasant/adverse gastrointestinal symptoms of at least 36 g/day for 4 weeks was determined. Some subjects tolerated 54 g/day. Another

human study was identified that was designed primarily to assess the prebiotic properties of isomalto-oligosaccharides but contained some endpoints related to safety and tolerability (Yen *et al*., 2011). The administration of IMO at doses of up to 22 g/day did not result in significant effects on clinical chemistry and corroborates the safety of VF-DP3-IMO.

There appears to be no safety concerns from the use of BioNeutra's made VF-DP3-IMO.

CONCLUSION

We, the Expert Panel, have independently and collectively critically evaluated the information summarized above and conclude that intended uses in food presented in the dossier of BioNeutra's made VF-DP3-IMO, produced consistent with current Good Manufacturing Practices and meeting appropriate food grade specifications presented in the supporting dossier, **"Documentation Supporting the Generally Recognized as Safe (GRAS) Status of VF-DP3-IMO an Isomalto-Oligosaccharide (IMO) Mixture for Use in Conventional Food"**, dated January 7, 2016, is safe and is Generally Recognized As Safe (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with this conclusion.

(b) (6)

04 March 2016

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Bonnette, Richard

From: Jianhua Zhu <jianhua.zhu@bioneutra.ca>
Sent: Tuesday, October 11, 2016 5:13 PM
To: Bonnette, Richard
Subject: RE: Your submission to the FDA GRAS notification program

Dear Richard:

Thank you for your email and the question. I am sorry for my delayed response as the Thanksgiving holiday in Canada.

Yes, it was an oversight, please proceed on filing the notification in your early convenience. Please let me know if you have any question or concern.

With regards,

Jianhua

Jianhua Zhu, Ph.D.
President/CEO

BioNeutra North America Inc.
(A Subsidiary Company of BioNeutra Global Corporation (Toronto TSX-V: BGA)

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Phone: 780 466 1481 ext 111
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Cell: 780 995 9396
Email: jianhua.zhu@bioneutra.ca

From: Bonnette, Richard [mailto:Richard.Bonnette@fda.hhs.gov]
Sent: Friday, October 7, 2016 7:18 AM
To: Jianhua Zhu <jianhua.zhu@bioneutra.ca>
Subject: Your submission to the FDA GRAS notification program

Dear Dr. Zhu,
I have your submission regarding isomalto-oligosaccharide mixture on my desk and have looked it over as part of a standard pre-filing review. I have a question regarding the "confidential" notations on the GRAS panel report (Appendix A). Did you intend for this section to be confidential? Often we see these statements are accidentally left in, especially if a submission is used with multiple agencies/countries.

Broad claims (and even narrow ones) of confidentially in a submission raise questions about whether there can be consensus among experts regarding safety if there is no access to the information. If there are safety-relevant data and information in a submission that is considered confidential (new) 170.250 (2)(e) requires that there be a discussion in the narrative explaining how there can be a basis a conclusion of GRAS status if qualified experts do not have access to the confidential information. Also, we also typically make the entirety of submissions available online.

I suspect this is probably an oversight. If so, please let me know. I'll include your response in the record for this submission. No further action would be required by you before filing the submission as a GRAS notice.

Regards,
Richard Bonnette

Richard E. Bonnette, M.S.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
U.S. FDA, Center for Food Safety and Applied Nutrition

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SUBMISSION END