BioNeutra Inc.
(Edmonton, Alberta, Canada)

APPLICATION FOR THE APPROVAL OF ISOMALTO-OLIGOSACCHARIDE (IMO)

Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients

December 8th- 2008

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1.0 Administrative Data

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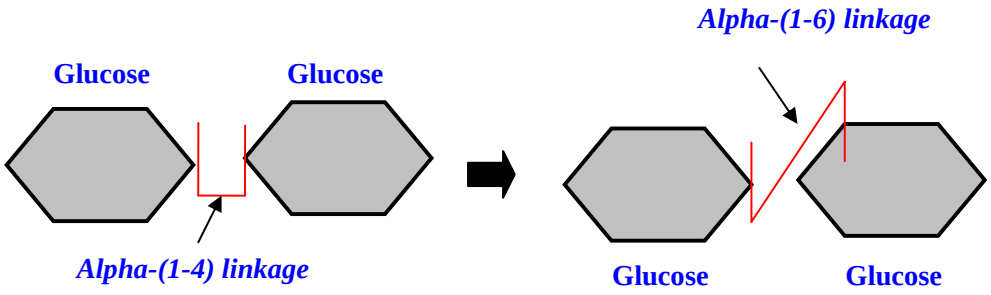
2.0 General Description of the Novel Food

The term "oligosaccharide" encompasses carbohydrates that are larger than simple di- or tri-saccharides, but smaller than polysaccharides (greater than 6 units). VitaSugar-IMO is a mixture of pure carbohydrates called isomalto-oligosaccharide (IMO). Isomalto-oligosaccharides (IMO), is a mixture of glucose oligomers with α-(1,6)-glucose linkages such as isomaltose, panose, isomaltotriose, isomaltopentose and higher branched oligosaccharides (Thomson Healthcare, 2001). IMO has been ingested by humans for hundreds of years as they are naturally found in honey, miso, sake and soy sauce.

BioNeutra produces VitaSugar-IMO *via* highly controlled enzyme-catalyzed process transforming starch molecules into functional health molecules of IMO.

Conversion of starch molecule into IMO by means of enzymatic catalysis is achieved by BioNeutra's unique patented technology, which convert starch molecules into useful IMO molecules. In order to convert these molecules into a functional and into a low-caloric prebiotic molecules, these α-1,4 glycosidic linkages are further enzymatically converted into more resistant α-1,6 glycosidic linkages, that consequently confers the property of "iso" linkages between the sugar moieties of oligosaccharides and thus in formation of Isomalto-oligosaccharide (IMO).



While human intestinal enzymes readily digest α-(1,4)-glycosidic bonds, α-(1,6)-linkages, particularly those linking longer polymers, are not easily hydrolyzed as they pass through the human gastrointestinal tract.

3.0 Identification of Essential Information Requirements

The structured schemes outlined for the assessment of a **Class 2.2** novel food ingredient, such as Isomalto-oligosaccharide, are listed below. The required questions are identified and discussed in detail in subsequent sections (Sections I through XIII).

I. Specification of the novel food

II. Effect of the production process applied to the novel food

III. History of the organism used as the source of the novel food

IX. Anticipated intake/extent of use of the novel food

XI. Nutritional information on the novel food

XII. Microbiological information on the novel food

XIII. Toxicological information on the novel food

I. Specifications of the Novel Food

Based on the SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to the specifications of the novel food:

- "…is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?"
- "Is the information representative of the novel food when produced on a commercial scale?"
- "Is there an appropriate specification (including species, taxon *etc*. for living organisms) to ensure that the novel food marketed is the same as that evaluated?"

These questions have been addressed collectively in Sections I.1 through I.7.

I.1 Common or Usual Name:

Isomalto-oligosaccharide (IMO)

I.2 Chemical Name:

Isomalto-oligosaccharide (IMO)

I.3 Trade Name:

VitSugar™ (IMO-Syrup); VitSugar™ (IMO-Powder)

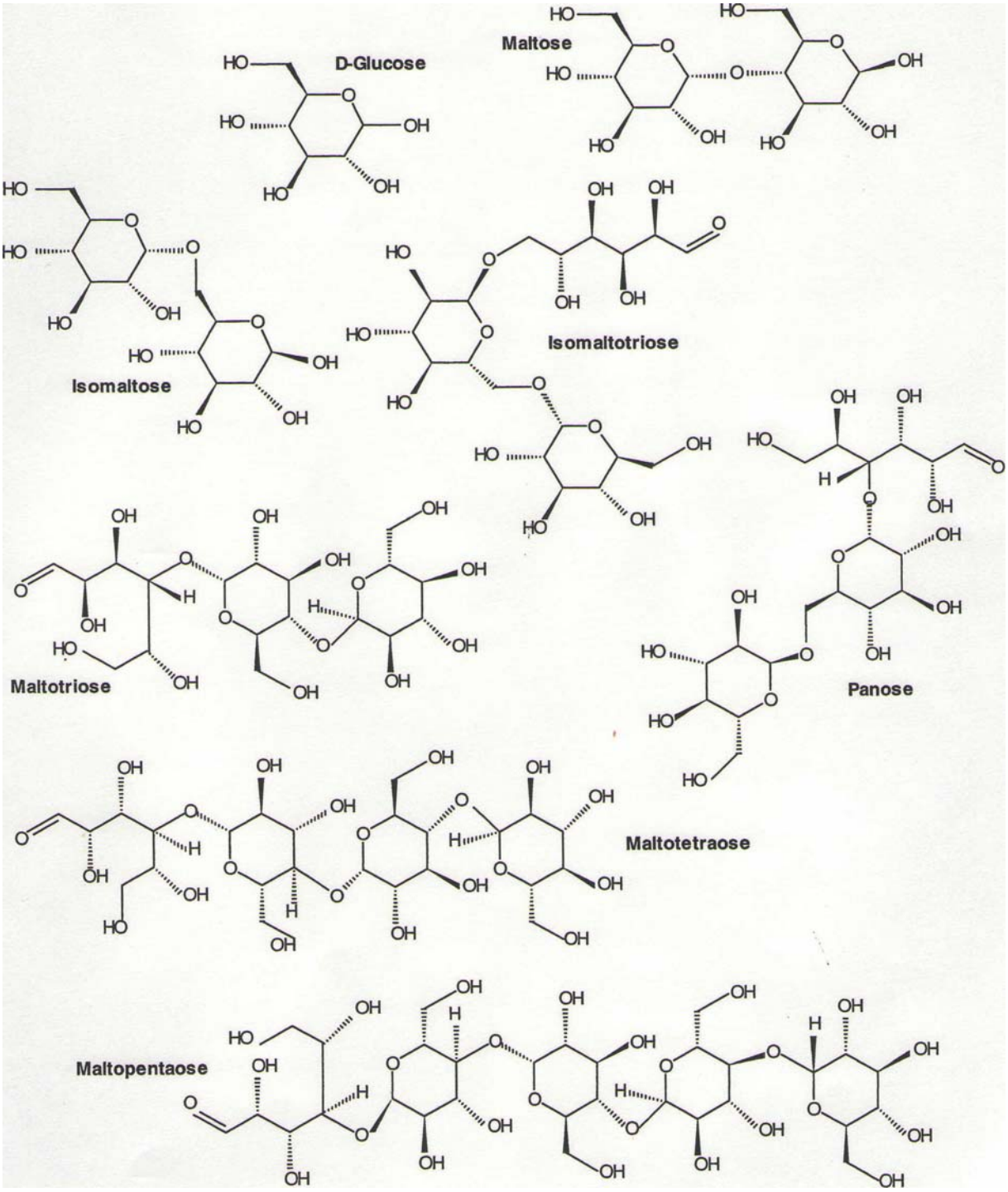
I.4 Molecular Formula & CAS Number

Description of the Saccharides in BioNeutra's Isomalto-Oligosaccharide (IMO) Products (VitaSugar-IMO)

Saccharides	CAS No.	Chemical Formula	Chemical Name
Monosaccharldes (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
Trisacchandes (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
Oligo- and polysaccharides (DP4 to DP9)			
Maltotetraose (DP4)	34612-38-9	$C_{24}H_{42}O_{21}$	O-α-D-glucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-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
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
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-a-D-glucopyranosyi-(1,4)-O-a-D-glucopyranosyl-(1,4)-Oα-D-glucopyranosyl-(1,4)-D-glucopyranose
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-gucopyranosyl-(1,4)-O-α-D-glucopyranosyl-(1,4)-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

I.5 Chemical Structure:

Structural Formulas of the Mono-, Di-, and Oligosaccharides (DP3 to DP5) Identified in BioNeutra's Isomalto-oligosaccharide (IMO) Products (VitaSugar);



I.6 Chemical and Physical Properties:

The term "oligosaccharide" encompasses carbohydrates that are larger than simple di- or tri-saccharides, but smaller than polysaccharides (greater than 6 units). Isomalto-oligosaccharides, specifically, are glucose oligomers with α-D-(1,6)-linkages, including among others isomaltose, panose, isomaltotetraose, isomaltopentaose, nigerose, kojibiose, and higher branched oligosaccharides (PDRNS, 2001). While human intestinal enzymes readily digest α-(1,4)-glycosidic bonds, α-(1,6)-linkages, particularly those linking longer polymers, are not easily hydrolyzed as they pass through the human gastrointestinal tract. The majority of oligosaccharides found in Vitasugar-IMO consist of 3 to 7 monosaccharide units linked together; however, disaccharides, as well as longer polysaccharides (up to 9 units) also are present. The disaccharide fraction of Vitasugar-IMO consists of the α-1→4 linked maltose and the α-1→6 linked isomaltose, while maltotriose, panose, and isomaltotriose make up the trisaccharide fraction. Maltotetraose, maltopentaose, maltohexaose, maltoheptaose, and small amounts of oligomers with 8 or more degrees of polymerization comprise the remaining oligomers in the product. Vitasugar-IMO syrup form is a transparent clear pale yellow colored liquid with no particulates and ~50% sweet as sucrose. Vitasugar-IMO Powder form is a white crystalline powder and ~50% sweet as sucrose.

I.7 Product Specifications and Analysis

I.7.1 Product Specifications

Chemical and physical specifications for the Vitasugar-IMO syrup and powder products are presented in Appendix A. On a dry basis, the powder and syrup formulations are prepared such that the content of isomaltose and larger oligosaccharides (DPS to DP9) is not less than 90%. A high-performance liquid chromatography method (HPLC) was designed to determine levels of individual oligosaccharides in the IMO mixture. All other parameters are measured using standard published methods. See Appendix B for details of the analytical methods.

I.7.2 Product Analysis

Several lots of the manufactured syrup and powder product were analyzed to verify that the manufacturing process produced consistent products within the product specifications. The chemical composition of the IMO products was determined by analyzing the glucose, isomaltose, and oligosaccharide (DPS to DP9) content using HPLC. A summary of the chemical and microbial product analysis for 3 non-consecutive lots (*i.e.*, Lot Nos. 6, 7, and 9) of VitaSugar-IMO syrup and 2 non-consecutive lots (*i.e.*, Lot Nos. 4 and 8) of VitaSugar-IMO powder are presented in Tables I.7.2-1 to I.7.2-4, respectively (Also see Appendix C).

Table I.7.2-1 Summary of the Product Analysis for VitaSugar-IMO (Syrup)

Specification Parameter	Specification	Lot Numbers		
		Lot No. 6	Lot No. 7	Lot No. 9
Dried solids (g/100 g)	> 75	75.76	75.6	76.0
Glucose (% dry basis)	≤ 5	Not detected	Not detected	Not detected
Isomaltose + DP3 to DP9 (% dry basis)	≥ 90	96.35	96.47	99.77
pH	4 to 6	5.07	5.15	5.24
Sulfated ash(g/100g)	≤ 0.3	0.081	0.057	0.095
Heavy metals				
Lead (mg/kg)	≤ 0.5	< 0.5	< 0.5	<0.5
Arsenic (mg/kg)	≤ 0.5	< 0.5	< 0.5	<0.5

Table I.7.2-2 Summary of the Product Analysis for VitaSugar-IMO (Powder)			
Specification Parameter	**Specification**	**Lot Numbers**	
		Lot No. 4	**Lot No. 8**
Solubility (water) (%)	> 99	100	100
Glucose (% dry basis)	≤ 5	Not detected	Not detected
Isomaltose + DPS to DP9 (% dry basis)	≥ 90	97.0	97.91
Moisture (%)	≤ 4	3.5	3.0
Sulfated ash(g/100g)	≤ 0.3	0.058	0.057
Heavy metals			
Lead (mg/kg)	≤ 0.5	<0.5	<0.5
Arsenic (mg/kg)	≤ 0.5	<0.5	<0.5

Table I.7.2-3 Summary of the Microbial Product Analysis for VitaSugar-IMO (Syrup)				
Specification Parameter	**Specification**	**Lot Numbers**		
		Lot No. 6	**Lot No. 7**	**Lot No. 9**
Total Aerobic Plate Count (CFU/g)	Less than 10,000	<1	<1	<10
Yeast	Less than 100	<10	<10	<10
Escherichia coli (MPN/g)	Less than 10	<2	<2	<2
Salmonella (CFU/g)	Absent	Absent	Absent	Absent

Table I.7.2-4 Summary of the Microbial Product Analysis for VitaSugar-IMO (Powder)			
Specification Parameter	**Specification**	**Lot Numbers**	
		Lot No. 4	**Lot No. 8**
Total Aerobic Plate Count (CFU/g)	Less than 10,000	<10	<10
Yeast	Less than 100	<10	<10
Escherichia coli (MPN/g)	Less than 10	<2	<2
Salmonella (CFU/g)	Absent	Absent	Absent

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the effect of the production process applied to the novel food:

- "Does the novel food undergo a production process?"
- "Is there a history of use of the production process for the food?"
- "Does the process result in a significant change in the composition or structure of the NF compared to its traditional counterpart?"

These questions have been addressed collectively in Sections II.1 through II.6

II.1 Manufacturing Process

IMO is produced in accordance with current good manufacturing practice (cGMP) *via* enzyme-catalyzed hydrolysis of starch from different cereal crops.

Following is the brief process steps involved in manufacturing of IMO:

Starch + Water → Starch slurry → Enzyme treatment →

Liquescent Starch → Enzyme treatment → Saccharification →

Decolouring & Filtration → Desaltation & Removal of Proteins →

Concentration → Drying final product → IMO (Isomaltooligosaccharide)

All starting materials are appropriate for use in food and meet the specifications of the Food Chemicals Codex, 5th Edition (FCC, 2003).

II.2 Raw Material, Biocatalyst Source, Chemicals/Reagent Specifications

II.2.1 Starch

Unmodified food-grade starch, meeting FCC specifications, is used as the starting material in the production of BioNeutra's IMO product. Moreover, starch is authorized by Food & Drug Regulation Canada to be used in food processing (Div. 16, Table V, item A.1 & A.2). (see Appendix D).

II.2.2 Enzyme's specifications *(Confidential)*

II.2.3 Yeast Specifications *(Confidential)*

II.2.4 Sodium Carbonate (Monohydrate)

Sodium carbonate (Na_2CO_3) (≥ 99.6% purity), consistent with FCC food-grade specifications, is used during the manufacturing process to control the pH of the reaction mixture. Sodium Carbonate is authorized by Food & Drug Regulation Canada to be used in food processing (Div. 16, Table XIII, item S.3A). In US, sodium carbonate is affirmed as GRAS for use a pH-adjusting agent levels not exceeding current good manufacturing practice (21 CFR § 184.1742) (see Appendix D).

II.2.5 Hydrochloric Acid

Hydrochloric acid (HCI) (35-37% purity) is used as a pH-adjusting agent during the manufacture of VitaSugar-IMO. Hydrochloric acid is authorized by Food & Drug Regulation Canada to be used in food processing (Div. 16, Table XIII, item H.1). In United States, it is affirmed as GRAS for use as a buffer and neutralizing agent in accordance with good manufacturing practice (21 CFR § 187.1057) (see Appendix D).

II.2.6 Sodium Hydroxide

High-purity sodium hydroxide (NaOH) (>96% purity), consistent with the food-grade specifications of the FCC, is used as a pH-control agent during the production of the IMO products. Sodium hydroxide, meeting the FCC specifications, authorized by Canadian Food & Regulation (Div. 16, Table XIII, item S.5), and in US is affirmed as GRAS for use in food as a pH-control agent at levels not to exceed current good manufacturing practice (21 CFR § 184.1763) (see Appendix D.

II.2.7 Activated Carbon Powder (1%)

Activated carbon powder is used during the manufacturing process as a purification and decolorizing agent. (see Appendix D)

II.2.8 Ion-exchange Resins

Ion exchange resins consisting of sulfonated polymers of styrene or styrene cross-linked with divinylbenzene (DVB) are employed during the purification stages to remove various impurities *(e.g.,* ash, weak organic acids, nitrogen compounds, anions, *etc.)* from the final product and as a means of demineralization. Sulfonated copolymers of styrene and DVB are permitted for use as ion exchange resins for the purification of food (21 CFR § 173.25) (see Appendix D).

II.3 Potential Impurities Resulting from the Production Process

II.3.1 General Considerations

Once yeast fermentation is completed, the yeast cells are removed from the culture broth by filtration. The filtrate is then subject to decolorized by treatment with activated carbon and heating up to near boiling temperature prior to second filtration. Final filtered solution is then passed through the series of Cation-exchange and Anion-exchange columns (two each). These purification steps, which are utilized to produce an uncontaminated product of high purity (~ 99 % purity). Any remaining residual ethanol generated during yeast fermentation is

completely evaporated off by the concentration step using triple-effect evaporation process. Final product is tested for chemical, metal and microbial analysis to confirm the purity.

II.3.2 Residual Biomass

It is unlikely that the yeast (*Saccharomyces cerevisiae*) would be present in the final product given the purification process. This is supported through the post-fermentation filtration step and subsequently rises in temperature near to boiling point. Final product is routinely tested for yeast and mold count which is found <1 X 10^2 CFU/g. Post fermentation product also passed through a series of Cation and Anion exchange columns to remove any protein or amino acid contaminants.

II.3.3 Residual Ethanol

Ethanol generated by yeast as a by-product during the manufacturing process of IMO is completely evaporated by subsequent elevated temperature at 90-98 ºC for 30 min (see section II.1). Final samples of IMO are routinely tested for the absence of residual ethanol (see Appendix E). For the alcohol sample analysis, the instrumental detection limit for the ethanol level was = 3-5 ppm (500-700 nRIU), setting attenuation to minimum.

II.3.4 Content of True Protein, Non-protein Nitrogenous Material

Final product (IMO) is passes through a series of ion-exchange columns (Cation & Anion) to remove any contaminated proteins, amino acids, metal ions or other charged molecules. Therefore, final concentrated product is completely devoid of any protein, amino acid, nucleic acids or fat (tested by: LabsMart Inc., protocol AOAC TKN).

II.4 Stability of Isomalto-oligosaccharide (IMO)

Our in-house studies to determination of shelf-life stability of VitaSugar-IMO revealed that IMO is very stable under acidic or conditions. Under high acidic conditions like pH 2.0 and high alkaline condition like pH 10, VitaSugar-IMO molecules was found to be > 99% stable particularly at pH 2.0 in term of breakdown of glucose molecules when incubated for up to one year at three given storage temperatures, *i.e*., at room temperature (25 ºC), at refrigerator temperature (4 ºC), and at high temperature (45 ºC) (see Appendix F).

The stability of VitaSugar-IMO was measured under conditions of high temperature or shear with varying pH levels. Changes in the carbohydrates composition was performed by using a HPLC method. Color changes were evaluated using a spectrophotometer. Two temperature/ time conditions was evaluated, 120°C for 30 minutes to represent a commercial sterility process (Retort) and 95°C for 1.5 hours to represent the worse case batch Pasteurization process. All test samples was a 10% solution (by weight) of VitaSugar-IMO with pH buffered water. The shear tests were conducted at room temperature (~22°C), 50°C and 95°C. All stability tests were done in triplicate. Data showed VitaSugar-IMO was stable in these conditions with slight degradation in free glucose under High Shearing conditions, however, keeping the overall molecular makeup intact (see Appendix F).

II.5 History of Use of Production Process

IMO have been produced in Asia for the past 15-20 years and are used in a variety of food applications. Most of the current use of IMO as a health food ingredient is in Asian countries, like Japan, China & Korea. According to Teruo (2003), the use of IMO is more prevalent in Japan than any other non-digestible oligosaccharide. In 2003, IMO demand in Japan was estimated about 11,000 tons. IMO has been used as a sweetener in Japan for many years. IMO syrup is effectively used for traditional fermented foods in Japan (Teruo 2003).

In Japan, China, Hong Kong, Korea and Taiwan, IMO has been recognized by regulatory agencies, and this food ingredient (IMO) is in market for many decades. Currently, IMO is being consumed by local populations in those countries by adding this product into a number of functional foods to exhibit health benefits, like prebiotic functions & overall improvement of digestive health.

The general manufacturing process for IMO described herein has already been in use by many companies in Japan and China. The precise control of enzymatic reactions to yield the desired degree of polymerization (DP), thus enhancing the quality of the product, is patent of BioNeutra, but the use of Starch as a raw material and use of α-amylases for breaking down the starch polysaccharides with subsequent use of Glucosidase enzyme to changeover the linkage is already been in practice for long time (see section III.2.4).

III. HISTORY OF THE ORGANISM USED AS THE SOURCE OF THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the history of the source organism:

- "Is the novel food obtained from a biological source (*i.e.*, a plant, animal or microorganism)?"

These questions have been addressed collectively in Sections III.1 and III.2.

III.1 Information about Starch Source and its Characterization

BioNeutra produces VitaSugar-IMO *via* highly controlled enzyme-catalyzed process transforming starch molecules into functional health molecules of IMO.
The unmodified food-grade starch used as a raw material meeting FCC specifications. The starch glucose polymers are sequentially broken down by set of enzymes and linkages between the glucose units (1→4) are enzymatically converted into (1→6) linked oligosaccharides.

III.2 Natural Occurrence of IMO in Diet

III.2.1 Traditional Product Exposure

Isomalto-oligosaccharides (IMO) have been ingested by humans for hundreds of years as they are naturally found in honey, miso, sake and soy sauce. IMO is a mixture of glucose oligomers with α1,6-glucosidic linkages such as isomaltose, panose, isomaltotriose, isomaltotetrose, isomaltopentose, and higher branched oligosaccharides (PDRNS, 2001).
Many of the natural products containing IMO have been traditionally consumed in Japan and other Asian countries for hundreds of years. Table III.2.1-1indicates the per capita consumption of IMO in Japan from three traditional food and beverage sources.

Table III.2.1-1: History of Traditional Exposure in Japan

Product	Annual Consumption of Product	% IMO in Product	Approximate Annual per Capita Consumption of IMO
Honey	300 g	1.0	3 g
Miso	4.6 kg	1.1	50 g
Sake	8 L	0.5	40 g

Japan's annual per capita consumption of honey is 300 grams (Tanaka, 1999). This is considerably lower than Canadian per capita consumption which is estimated at about 1000 grams per year (CFIA, 2004). The chemical composition of honey may vary with floral origin. Isomaltose is always present at a range of 0.5-1.5 % (European Commission, 2002).

Miso is soybean combined with rice and other ingredients and aged in cedar vats for up to three years. Its predecessor was known as "hisio," a seasoning made from fermenting soybeans, wheat, alcohol, salt and other ingredients. This fermented soybean paste was introduced into Japan around the 7th century.

Miso is often consumed daily in soups and used in sauces and marinades. Annual Miso production is about 600,000 MT per year in Japan (Noguchi, 2005). Given a population of 128.6 million, annual per capita consumption of Miso is thus estimated to be 4.6kg.

Isomaltose is produced during the miso fermentation process. Isomaltose is present in raw miso at 0.31% and increases to 1.11% by 5 days and 1.17% by day 50 (Hondo and Mochizuki, 1979).

Sake is a traditional Japanese alcoholic beverage made from rice. Annual per capita sake consumption has been declining in Japan (9.8L in 1985, 8.1 L in 2001) and is now down to 6.9L (Sake World, 2007). A study conducted on five commercial brands of sake showed varying levels of IMO including isomaltose, 2.4-4.1 mg/mL, panose 0.7-0.9 mg/mL, and isomaltotriose, 0.4-1.4 mg/mL (Hayakawa et al, 2000). On average, the sakes contained 5 mg IMO/mL.

Soy sauce is also reported to contain IMO (Tungland and Meyer, 2002). The data required to determine the per capita consumption of IMO from soy sauce in Japan was not available. Given the IMO consumption data from Table III.2.1-1, along with the knowledge that soy sauce would also make a contribution, it would be reasonable to estimate the annual consumption in Japan of IMO from traditional sources to be 100 grams.

III.2.2 Formulated Product Exposure

"Research on the production of oligosaccharides for foods were started around 1970-75 in Japan and several oligosaccharides were produced on an industrial scale from the early 1980s to the late 1990s" (Nakakuki, 2003).

IMO and other oligosaccharide ingredients have been approved by Japan and been on the FOSHU (Foods for Specified Health Use) ingredient list for more than 10 years. Over 50% of the FOSHU foods in Japan in 2002 incorporated oligosaccharides as the functional component (Nakakuki, 2003).

Oligosaccharides are purchased by food processors as an ingredient for a variety of functional foods. Table III.2.2-1 indicates the consumption of oligosaccharides in Japan (2002) based on the demand for the ingredients (Nakakuki, 2003).

Table III.2.2-1 Demand for Oligosaccharides in Japan, 2002

Oligosaccharide	Population (millions)	Demand (MT)	Annual Per Capital Consumption
Malto-oligosaccharide	128.6	15,000	120 g
Isomalto-oligosaccharide	128.6	15,000	120 g
Fructo-oligosaccharide	128.6	3,500	30 g

Table III.2.2-1 indicates that Japanese consumers are now consuming more IMO from formulated foods than traditional food sources.

Food manufacturers incorporating IMO into their products have the option of obtaining FOSHU certification. Not all foods containing IMOs are labelled FOSHU. Yamaguchi (2004) reported a list of 64 FOSHU products for gastrointestinal health that contain

oligosaccharides/prebiotics. The list contains numerous food types and products including: Soft drinks, frozen yogurt, confectionary products, sweeteners, cookies, coffee drink mixes, bread, tofu, chocolate, soup mixes and other beverages.

IX. ANTICIPATED INTAKE/EXTENT OF USE OF NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the effect of the production process applied to the novel food:

- "Is there information on the anticipated uses of the novel food based on its properties?"
- "Is there information to show anticipated intakes for groups predicted to be at risk?"
- "Will introduction of the novel food be restricted geographically?"
- "Will the novel food replace other foods in the diet?"
- "Are any of the replaced foods significant nutritional sources?"
- "Does the probable level of substitution have a nutritional significance for any population groups?"

These questions have been addressed collectively in Sections IX.1 through IX.5

IX.1 Intended Uses and Use Levels

IX.2. Estimated Daily IMO Intake from All Proposed Food Uses

IX.2.1 Natural Occurrence in the Diet

Isomalto-oligosaccharides are normal constituents of the human diet that occur naturally in a number of fermented foods, including rice miso, soy sauce, and sake. A detailed description is given under section III.2 of this document. BioNeutra intends to market VitaSugar-IMO (syrup and powder) as a general food ingredient (nutritive sweetener) with functional benefits of prebiotic and dietary fiber in conventional foods. Following are intake estimations first given for Canadian populations calculated based upon Stats Canada Survey (SCS), and subsequently given for E.U. populations based upon the National Diet and Nutrition Survey (NDNS) data.

IX.2.2 Estimated consumption of IMO from Proposed Food Uses in Canada

(based upon Stats Canada Survey)

The individual proposed food-uses and use-levels of VitaSugar-IMO are summarized in Table IX.2.2-1, in which available foods in the market are categorized in four groups.

Table IX.2.2-1: Summary of the Individual Proposed Food-uses, Use-levels, and Amount per Serving of VitaSugar-IMO in Canada

Food-Uses	Serving Size	Maximum IMO Amount per Serving (gm)
Grain Products		
Cereals or Cereals bars	30 g	7 g
Bakery products	60 g	10 g
Snack foods	30 g	2 g
Meats and Alternates		
Peanut butter	30 ml	3 g
Dairy and Alternates		
Flavored milk	250 ml	12 g
Flavored soy milk	250 ml	12 g
Yogurt	175 ml	17 g
Other Foods		
Beverages	250 ml	12 g
Condiments	15 ml	5 g
Toppings	25 ml	10 g
Candy (hard/soft)	10g/30g	10 g

Table IX.2.2-2 showed the quoted data from Stats Canada source about food availability per person per year (in retail weight) in the year 2006. Also note that, the data given in this table do not include the waste from food preparation, thus data are representative of highest level.

Table IX.2.2-2: Food available per person per year 2006 (in retail weight) *(Stats Canada)*

Food[1]	Total Intake (kg/person/yr)	% suitable for IMO inclusion	IMO Intake (kg/person/year)[1]
Dairy (yogurt)	23	30	7
Milk or Soy Beverage	59	5.7	3
Grains; -- Cereal and cereal bars[2] -- Bakery (cakes, cookies, muffins)[2]	61	Cereal and cereal bars = 6 Bakery = 24	4 16
Nuts/pulses[3]	8	16	1
Soft drinks; -- Energy Drinks [4] -- Other Drinks [4]	85	Energy Drinks = 3.7 Other Drinks = 3.5	Energy Drinks = 3 Other Drinks = 3

[1]Based on every possible brand of product incorporating IMO to its highest possible level

[2]Based on Stats Canada data showing 6% of grain products are cereals (Food Statistics-2006, Cat. no. 21-020), and Business Insights 2007 (*Growth Strategies in Bakery and Cereals by Helen Lewis*) data showing 24 % of products are bakery items such as cakes, cookies and muffin type products

[3]Based on Stats Canada data showing 32% of nuts/pulses are peanuts and USDA (USDA Economic Research Service, Oil Crops yearbook) indicating that 50% of consumption of peanuts is as peanut butter. IMO could be incorporated into peanut butter.

[4] Based on Business Insights 2008 (*Next generation soft drinks by Natasha Horton*) data showing 3.7% of soft drink category is energy drinks and 3.5 % are nutraceutical and sports drinks.

The recommended amount of food per serving from available categories of foods in Canada are given in Table IX.2.2-3 (simplified from Canada's Food Guide), for three groups of Canadian populations, *i.e*., children, teenage and adults. Also, included are the actual intakes of these categories according to Stats Canada survey (values given in parenthesis).

Table IX.2.2-3: Canada's Food Guide Servings; Recommended & Actual (in parenthesis)

Food Category	Children	Teenage	Adults
Beverages	4-6 (4)	7-8 (5)	7-10 (5)
Meat & Alternates	1-2 (2)	2-3 (3)	2-3 (2.5)
Nutritionally complete & fortified	2-4 (2.5)	3-4 (2.5)	2-3 (1.5)
Cereals Products	3-6 (6)	6-7 (6)	6-8 (6)

Table IX.2.2-4 presents an estimation of exposure of a specific population group, *e.g*., adults, to IMO in respect to individual food categories given in Table IX.2.2-1;

Table IX.2.2-4: Estimation of Exposure of Adults to IMO in Respective Individual Food Categories

Food Category	Consumption		IMO Amount Inclusive (gm)	Total IMO Intake per Food Category (gm)
	Sub-class	# of Servings per day		
Meat & Alternates	Peanut Butter	1	3	3
Dairy Products	Regular Milk	1	0	29
	Flavored Milk	1	12	
	Yogurt	1	17	
Grain Products	Bread	3	0	17
	Pasta	1	0	
	Cereal (30g)	1	7	
	Baked Good or Sports bar (60/40g)	1	10	
Other Foods	Sports Beverage (250ml)	1	12	26
	Snack	1	2	
	Condiments[1]	1	2	
	Candy	1	10	

Estimations of IMO Intake in Possible Upper Percentile Scenarios;

Scenario-I

Considering that if IMO is present in every brand of all the products included in the four major food groups (as given in Table IX.2.2-1), then based upon the estimations given in Table IX.2.2-2, total IMO intake (in term of kg/person/year) would be about 100g/day. The inclusion of IMO in all four major food groups in any single given time is highly unlikely.

Scenario-II

Based on the Canada's Food Guide, if a consumer bought all IMO-containing products in a single given time (from all four major food groups as showed in Table IX.2.2-3), then the estimated intake amount of IMO would be 75g/day. Again, this is highly unlikely for a consumer to buy all the food products containing IMO.

Based on Canadian market penetration, both above mentioned scenarios are highly unlikely, since the actual intake of IMO is expected to be approximately 10% of the maximum estimated percentile. Even in the case of maximum consumption, *i.e*., 75gm/day, the chances for sever gastric upset are the least, since the maximum permissible dose of IMO that does not cause

diarrhea is estimated >1.5 g/kg body wt. (*e.g.*, for an average person of 50 kg weight, the tolerable range of IMO would be ~75 gm/day) (Oku, T. & Nakamura, S., 2002).

IX.2.3 Estimated Consumption of IMO from Proposed Food Uses in the E.U.

Estimates for the intake of IMO in the E.U. were based on the proposed use-levels for IMO summarized in Table IX.2.3-1 and food consumption data collected as part of the United Kingdom (U.K.) Food Standards Agency's Dietary Survey Programmed (DSP).

Table IX.2.3-1 Summary of the individual Proposed Food-uses, use-Levels, and Amount per Serving of VitaSugar-IMO in the E.U.

Food Category	Proposed Food-Uses	Serving Size (grams)	Use-Level (%)	IMO per Serving (g/serving)
Beverages	Regular Soft Drinks	240	5	12
	Energy-Reduced Soft Drinks	240	6.5	15.6
	Energy Drinks	240	5.0	12
	Sports & Isotonic Drinks	240	6.5	15.6
	Fruit Juices	140	5	12
	Processed Vegetables and Vegetable Juices	100	5	12
Cereals Products	Cereals Bars	50	10	5
	Cookies, Biscuits	40	20	8
	Breakfast Cereal Bars	50	25	12.5
Sugar Confectionary	Hard Candies	10	97	9.7
	Soft Candies/Chocolate Bars	30	25	8.2
Nutritionally complete and fortified foods	Meal Replacement Bars	40	20	8
	Milk based Meal Replacement	40	20	8

Based upon the presented NDNS data, an estimated daily intake of IMO from all proposed food categories in the U.K. by population groups (as defined in NDNS programmed) are made and summarized in Table IX.2.3-2. The calculations are made based upon total intake of IMO (g/person/day) from all proposed food-uses in the E.U. by U.K. population group.

It has been indicated in 7-days survey that the percentage of users was high among all age groups evaluated in the current intake assessment; greater than 85.3% of the population groups consisted of users of those food products in which IMO is currently proposed for use (Table IX.2.3-1).

Young people had the greatest percentage of users at 99.6%. Of the individual groups, male teenagers were determined to have the greatest mean and 97.5th percentile all-user intakes of IMO on an absolute basis, at 33.5 and 86.7 g/person/day, respectively (Table IX.2.3-2). The lowest absolute all-user intake of IMO resulting from all proposed food uses was observed to occur in female adults with mean and 97.5th percentile intakes of 9.2 and 36.7 g/person/day, respectively.

Table IX.2.3-2 Summary of the Estimated Daily Intake of Isomalto-oligosaccharide from All Proposed Food Categories in the U.K. by Population Group (NDNS Data)

Population Groups	Age Group (years)	% Users	All-Person Consumption				All-Users Consumption			
			Mean (g)	Percentile (g)			Mean (g)	Percentile (g)		
				90th	95th	97.5th		90th	95th	97.5th
Children	1½ - 4½	98.3	15.3	29.5	35.3	38.3	14.2	21.6	26.8	28.3
Young People	4-10	99.6	26.7	44.8	51.8	62.1	26.7	44.8	51.8	62.1
Female Teenagers	11-18	99.3	24.8	45.5	53.7	63.3	24.9	45.5	53.9	63.3
Male Teenagers	11-18	99.5	33.4	59.5	69.2	86.7	33.5	39.5	69.2	86.7
Female Adults	16-64	88.1	8.1	19.3	25.8	34.3	9.2	20.7	26.5	36.7
Male Adults	16-64	85.3	9.0	22.5	33.1	40.8	10.6	24.4	35	41.5

On a body weight basis, young people (age 4-10) were identified as having the highest intakes of any population group, with mean and 97.5th percentile all-user IMO intakes of 0.9 and 2.5 g/kg body weight/day, respectively, while similar to the case observed for absolute intakes, female adults had the lowest 97.5th percentile intakes on a body weight basis, of 0.5 g/kg body weight/day. Male and female adults had equivalent mean all-user intakes of IMO, 0.1 g/kg body weight/day (Table IX.2.3-3).

Table IX.2.3-3 Summary of the Estimated Daily per Kilogram Body Weight Intake of Isomalto-oligosaccharide form All Proposed Food Categories in the U.K. by Population Group (NDNS Data)

Population Groups	Age Group (years)	% Users	All-Person Consumption				All-Users Consumption			
			Mean (g/kg)	Percentile (g/kg)			Mean (g/kg)	Percentile (g/kg)		
				90th	95th	97.5th		90th	95th	97.5th
Children	1½ - 4½	98.3	0.8	1.1	1.7	1.9	0.9	1.2	1.6	1.8
Young People	4-10	99.6	0.9	1.3	1.8	2.1	0.9	1.6	2.0	2.5
Female Teenagers	11-18	99.3	0.4	0.8	0.9	1.1	0.4	0.8	0.9	1.3
Male Teenagers	11-18	99.5	0.6	1.1	1.4	1.6	0.6	1.1	1.4	1.6
Female Adults	16-64	88.1	0.08	0.3	0.4	0.5	0.1	0.3	0.4	0.5
Male Adults	16-64	85.3	0.08	0.2	0.4	0.6	0.1	0.3	0.5	0.6

IX.3 Product's Labeling Information

See Appendix G for a sample copy of proposed label for IMO-Syrup and IMO-Powder form.

IX.4 Conclusion

In summary, on an all-user basis, the highest mean and 97.5th percentile intakes of IMO by the U.K. population from all proposed food-uses in the E.U., as observed in male teenagers, were estimated to be 33.5 g/person/day (0.6 g/kg body weight/day) and 86.7 g/person/day (1.6 g/kg body weight/day), respectively. On a body weight basis, young peoples (age 4-10) consumed the greatest amount of IMO, with mean and 97.5th percentile all-user intakes of 0.9 and 2.5 g/kg body weight/day, respectively.
Consumption data and information pertaining to the individual proposed food-uses for IMO were used to estimate the all-person and all-user IMO intakes of specific demographic groups in the U.K. population. This type of intake methodology is generally considered to be 'worst case scenario' as a result of several conservative assumptions made in the consumption estimates. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users.

As mentioned earlier, the inclusion of IMO in all proposed four major food groups in any single given time is highly unlikely as well as this is also highly unlikely for a consumer to buy all the food products containing IMO in one setting.

XI. NUTRITIONAL INFORMATION ON THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient nutritional information pertaining to the novel food:

- “Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?”
- "Is there information to show that the NF does not affect the bioavailability of nutrients from the diet or have any adverse physiological effects"?
- "Is there information to allow an assessment to be made of the nutritional impact of the introduction of the NF"?

These questions have been addressed collectively in Sections XI.1-XI.7

XI.1 Nutritional Equivalence to Existing Foods

Isomalto-oligosaccharide (IMO) is a poorly digestible carbohydrate which has resistance in digestion in human stomach and small intestine. However, in large intestine (colon), it could be partially breakdown by bacterial species (mostly *Bifidobacteria* and *Lactobacillus*), hence, act as prebiotic dietary fiber. Followings are two examples of poorly digestible and prebiotic short-chain carbohydrates, which are currently on market. IMO is closely related to FOS in term of functional benefits and energy values but superior in term of dosage and safety as per published scientific data.

XI.1.1 Fructo-oligosaccharide (FOS)

In 1998, the European Scientific Committee for Food (SCF) evaluated the safety of FOS for human consumption (SCF, 1998). FOS are a mixture of polymers of variable length comprised of fructose (usually 2 to 4) monomers linked *via a* ß-(2,1)-glycosidic bond and a terminal glucose unit attached *via* an α-(1,1)-glycosidic linkage. Since there are no enzymes in the body, which are capable of hydrolyzing the ß-(2,1)-glycosidic linkage between the fructose units in the oligomers, FOS largely escape digestion in the small intestine and are passed intact into the large intestine where they are subjected to fermentation by the microflora. The maximum non-effective dose for the induction of diarrhea as a result of FOS consumption in male and female human subjects was determined to be 0.3 and 0.4g/kg body weight (15 and 28g/person, respectively), respectively, whereas the dose level which causes diarrhea in 50% of healthy adults was estimated to be approximately 0.8g/kg body weight (48g/person). A daily dose level of 15g of FOS provided 3 times daily in equal amounts was associated with increased incidence of flatulence, bloating, and abdominal discomfort compared to subjects ingesting sucrose. Based on the human tolerance studies the SCF "concluded that although laxation may be observed at high intakes (30g/day) consumption of the order of 20g a day of FOS is unlikely to cause more undesirable laxative symptoms than isomalt, lactitol, maltitol, mannitol, sorbitol, and xylitol". Accordingly, the Committee had "no objections to the use of the FOS product provided the limitations due to its laxative actions [were] kept in mind".

Likewise, the U.S. FDA posed no questions in response to a GRAS notice submitted for FOS for use in food as a bulking agent resulting in estimated intake levels ranging between 3.1 to 12.8 g/person/day (U.S. FDA, 2000). It was noted that while a small portion of FOS is hydrolyzed to glucose and fructose, approximately 89% of FOS pass undigested into the colon where they are fermented by bacteria resulting in the production of gases and short-chain fatty acids.

Fructo-oligosaccharide (FOS), which has very similar physiological properties as that of Isomalto-oligosaccharide (IMO) has been given an energy value of 2 kcal/g, by the Bureau of Nutritional Sciences, Health Products and Food Branch, Health Canada (CFIA, Chapter 6, Section 6.4.2, Table 6-11)

XI.1.2 Pullulan

Pullulan is another poorly digestible carbohydrate, which consists primarily of repeating maltotriose units. Two 1→4 linked α-D-glucose units, followed by a 1→6 linked single α-D-glucose make up each maltotriose unit. Maltotetraose units and 1,3-branch points also are occasionally encountered within the polymer. Maltotetraose units also may be found at the terminal ends. A typical pullulan polymer contains between 300 and 12,000 glucose units. The U.S. FDA had no questions regarding the GRAS status of pullulan for use in food as a food ingredient (GRAS Notice No. GRN 000099) (U.S. FDA, 2002).

More recently, the European Food Safety Authority (EFSA) also has evaluated the safety of pullulan for use in various food applications (EFSA, 2004). *In vitro* digestion and fermentation assays conducted with pullulan demonstrated that a small fraction was liable to salivary and pancreatic amylase hydrolysis to produce smaller oligomers, which were ultimately further broken down by mucosal enzymes of the small intestine to glucose (less than 5%); however, the majority of pullulan passed unchanged into the lower gastrointestinal tract and was subject to fermentation (Okada *et al.,* 1990). Conversely, in another *in vitro* hydrolysis assay, incubation of pullulan with and a-amylase and amyloglucosidase enzyme system resulted in the hydrolysis of approximately 40% of the sample at 1 hour and 95 to 96% at 5 hours (Wolf *et al.,* 2003). In comparison, maltodextrin was almost completely hydrolyzed within the first 30 minutes. In human diabetic subjects, ingestion of 50g of pullulan resulted in peak glucose levels that were 50% lower than those obtained following consumption of equal amounts of maltodextrin. The time required to attain peak glucose levels did not differ significantly, but was slightly longer with pullulan. Furthermore, the incremental AUC with pullulan also was 50% reduced compared to maltodextrin. The exact amount of non-absorbable, but fermentable material versus that available for amylase hydrolysis, is dependent on the specific composition of the administered pullulan test material. In human tolerance studies, mild gastrointestinal disturbances were reported at dose levels of 10g per day and greater (EFSA, 2004).

In assessing the safety of pullulan, EFSA reviewed a number of animal toxicity studies. In a 62-week study, groups of Sprague-Dawley rats were administered 0,1, 5, or 10% pullulan in the diet (Kimoto *et al.,* 1997). Actual pullulan intakes were calculated to be 480, 2,320, and 4,450 mg/kg body weight/day in males, respectively, and 520, 2,630, and 5,080 mg/kg body weight/day in females, respectively. The study was originally designed to be 2 years in duration; however, as a result of high mortality rates at the mid- and high-dose levels, the study was terminated at 62 weeks. Results of urinalysis were unremarkable. None of the hematological, clinical chemistry, or organ weight changes, was dose-related and, thus, was not considered to be related to the administration of pullulan in the diet. The increase in absolute cecal weight in high-dose females was considered to be a physiological response to consumption of a poorly digested polysaccharide. Histopathology confirmed that early deaths were due to pneumonia and as such were not regarded as treatment-related. No other

microscopic variations were attributed to dietary administration of pullulan. Based on the results of this study, the no-observed-adverse-effect level (NOAEL) for pullulan was estimated to be 5,000 mg/kg body weight/day; however, the value of this study was noted to be somewhat limited as a result of the high infection and poor survival rates.

Two (2) further studies were conducted in which groups of Wistar rats were administered pullulan in the diet at concentrations of 0, 20, or 40% (approximately 0, 10, and 20 g/kg body weight/day, respectively) for periods of 4 or 9 weeks or 0, 5, or 10% (approximately 0, 2.5, and 5 g/kg body weight/day, respectively) for periods of 4 or 7 weeks (9 weeks for controls) (Oku *et al*, 1979). In the group receiving the highest concentration of pullulan in the diet *(i.e.,* 40%), several rats were reported to occasionally develop diarrhea or soft feces throughout the study. Body weight gain of pullulan-treated rats was reduced, in a dose-dependent manner, compared to controls. Organ examination revealed enlargements of the gastrointestinal organs *(i.e.,* stomach, small and large intestine, and cecum) of rats in the pullulan groups. As in the 62-week study, this was thought to be representative of a physiological adaptation. Based on this and other data, EFSA concluded that pullulan is similar to other poorly digested carbohydrates. Since the proposed use levels were below levels that are likely to cause abdominal fullness, the Panel determined that the expected pullulan intakes would not present any concern when used as a food additive at the proposed used levels; however, it was noted that the Panel might request additional data if higher levels of use or other uses were to be requested.

XI.1.3 Polyoles

Polyols and FOS and IMO display similar chemical properties. Table XI.1.3-1 is a summary of the characteristics of polyols available on the market.

Table XI.1.3-1 Characteristics of Polyols in the market

Product	Erythritol	Xylitol	Mannitol	Sorbitol	Maltitol	Isomalt	Lactitol
Caloric Value (Kcal/g)	4	5	6	6	12	12	12
Relative Sweetness	70%	100%	60%	60%	90%	50%	40%
Carbon n°	4	5	6	6	12	12	12
Molecular Weight	122	152	182	182	344	344	344
Melting Point (°C)	121	94	165	97	150	145-150	122
Heat of Solution (cal/g)	-43	-36.5	-28.5	-26	-18.9	-9.4	-13.9
Heat Stability	>160	>160	>160	>160	>160	>160	>150
Acid Stability (pH)	2-10	2-10	2-10	2-10	2-10	2-10	>3
Viscosity	Very low	Very low	Med low	Med	Med	High	Very low
Hygroscopicity	Very low	High	High	Med	Med	Low	Med
Solubility (% w/w at 25°)	37	64	20	70	60	25	57

***Source: Product Specification Sheet, Cargill Food and Pharma Specialties, 2004, source: AB Industry.**

The term "hydrogenated starch hydrolysates (HSH) " refers to a mixture of polyols produced by the partial hydrolysis and hydrogenation of hydrolysate from corn, wheat or potato. In the food industry, HSH consists of substantial quantities of hydrogenated oligo- and polysaccharides in addition to monometric and dimeric polyols such as sorbitol and maltitol. They do not have a specific polyol as the majority component. Developed in the 1960's by a Swedish company, HSH are used in confectionary products.

XI.1.4 Possible Adverse Physiological Effects

Our reviews have not found any reports that showed any severe adverse reactions with normal consumption of IMO by healthy individuals. In the United States, food manufacturers have started to incorporate IMO into food products in the past two years. Xyience is using BioNeutra's manufactured IMO with brand name as VitaSugar-IMO syrup in their breakfast energy bars called "XSTART XTREME BREAKFAST BAR" that comes in different flavours. Each "40"gm energy bar contains 10 grams of IMO. Xyience has not received any negative reports about their energy bars. The maximum permissible dose of IMO that does not cause diarrhea is estimated at 1.5 g/kg body weight, which is higher than for any other sugar substitute (Oku, 2002).

The GRAS (Generally Regarded As Safe) Expert Panel Committee (USA) affirmation document for VitaSugar-IMO placed the intake level for VitaSugar -IMO at 30g/day (see Appendix H). This level is expected to be well tolerated and, pending specific application area of use is open to future increases.

A dose of IMO higher then the 30 gm/day, there is a possibility of gastrointestinal symptoms (like increased flatulence, bloating or soft stool) or in extreme case diarrhea. Gastric upset is expected with a extremely high dose (*i.e*., about 4-5 times higher than the no-effect level for laxative effects in humans). Consumption of IMO within the recommended dosage (30-40 gm per day) is not expecting to pose any health-related concern.

XI.2 Nutritional Impact of the Novel Food

XI.2.1 Effect of Cecal Microflora

Prebiotics are defined as "non-digestible food ingredients that may beneficially affect the host by selectively stimulating the growth and/or activity of a limited number of bacteria in the colon" (PDRNS, 2001). Many oligosaccharides are not digested in the small intestine and instead are fermented by Bifidobacterium species in the colon, thus enhancing their proliferation. In this respect, fermentable oligosaccharides may be considered as prebiotics. Studies conducted to assess the digestibility of IMO mixtures indicate that the oligosaccharides are at least partially fermented by bacteria in the colon. Consequently, the un-hydrolyzed and, therefore, unabsorbed portion of an IMO mixture reaching the colon may stimulate the growth of bacterial subpopulations. Generally, however, this is regarded as a beneficial, rather than an adverse effect.

Kohmoto *et al.* (1988) conducted an *in vitro* study to assess the utilization of a mixture of isomalto-oligosaccharides (lsomalto-900®) and several other sugars *(i.e.,* isomaltose, isomaltotriose, panose, raffinose, and glucose) by human intestinal bacteria. The IMO product consisted of a mixture of glucose, maltose, and isomalto-oligosaccharides at concentrations of 1.8, 5.1, and 93.1%, respectively. Forty-nine (49) different strains of human intestinal bacteria were incubated with the various sugars and the bacterial utilization of these sugars was assessed by comparing the relative growth of the bacteria to the growth of bacteria on glucose. The results indicated that all tester strains of bifidobacteria (except *Bifidobacterium bifidum)* utilized isomaltose, isomaltotriose, panose, raffinose, and the IMO product. Different strains of the *Bacteroides* group *(e.g., B. fragilis, B. thetaiotaomicron,* and *B. distasonis)* also were able to utilize all the examined sugars. Sugars of the IMO mixture were utilized by *Enterococcus faecalis and Clostridum ramosum;* however, most other bacteria including *E. coli* were unable to utilize the sugars in the IMO mixture. In another *in vitro* assessment of the effect of different oligosaccharides on bacterial fermentation, a mixture of isomalto-oligosaccharides consisting in 91 % of lower-end oligosaccharides, 2% of glucose, and 7% of high molecular weight oligosaccharides significantly increased levels of bifidobacteria (Rycroft *et al.,* 2001),

Bacteroides levels also were significantly increased, but only after 5 hours of fermentation and not when re-evaluated at 24 hours. Changes in the levels of bacteria were accompanied by significantly elevated levels of short-chain fatty acid production, including lactate, acetate, and propionate. However, none of the sugar substrates induced significant increases in the production of butyrate. Of the different substances tested, fermentation of the IMO mixture generated the least amount of gas.

In addition to the *in vitro* assays, several feeding trials in laboratory animals, as well as human subjects were performed to evaluate the effect of mixtures of isomalto-oligosaccharides on the host microfloral composition. In male Wistar rats provided 3% of an IMO mixture in the drinking water (2.7-5.0 g IMO/kg body weight/day) for a period of 12 months, a significant increase was observed in the levels of intestinal lactobacilli following 3 months of treatment (Kaneko *et al.,* 1990). The authors also noted an IMO-induced stimulation of *Bifidobacterium* growth and a suppression of *Clostridium* growth. In another study, isomalto-oligosaccharides were administered to groups of 10 BABL/c mice at dose levels of 0, 0.75, 2.5, or 7.5g/kg body weight *via* gavages for a period of 7 days (Qing *et al.,* 2003). Analysis of fecal samples revealed increases in the levels of bifidobacteria and lactobacilli, and inhibition of *Clostridium perfriengenes* growth. Analysis of human fecal samples (15/sex) revealed similar results following 7 days of isomalto-oligosaccharide consumption at a dose level of 15g per day (Qing *et al.,* 2003).

In a group of 7 elderly males experiencing diarrhea, ingestion of up to 24g of an IM0 mixture containing 10 g of "active" components *(i.e.,* isomaltose, panose, isomaltotriose, isomaltotetraose, and dextrin) for a period of 30 days was associated with a significant increase in fecal bacterial mass in comparison to pre-treatment values (Chen *et al.,* 2001). In a separate study, fecal samples were collected from 6 healthy and 18 elderly individuals prior to (days -2 and 0), during (days 7 and 10), and after (day 24) consumption of 20g of an IMO product (lsomalto-900®) incorporated into a coffee or a mizuyokan jelly for 10 and 14 days, respectively (Kohmoto *et al.,* 1988). Isomalto-oligosaccharides comprised 13.5g of every 20 g of the IMO/product. Assessments of the fecal flora collected from healthy adult men indicated a significant increase in the total number of bifidobacteria (average increase from $10^{9.4}$ to 10^{10} bifidobacteria/g feces) and total number of bacteria (average increase from $10^{10.4}$ to $10^{10.6}$ total bacteria/g feces) after 7 and 10 days of consuming the IMO-containing jellies, and remained elevated when at follow-up (14 days after treatment discontinuation).

Additionally, significant increases in the number of bifidobacteria following ingestion of the IMO product were observed in 4 men in this group who exhibited low basal levels of bifidobacteria at study beginning. In the elderly group of patients, fecal flora assessments indicated an increase in bifidobacteria in 12 individuals, while fecal samples of the remaining 6 individuals showed no treatment-related effects. However, 2 of the 6 individuals in whom no changes were identified following treatment with the IMO product, suffered from liver cancer and cholelithiasis, respectively, and exhibited abnormal levels of intestinal flora manifested by

high basal levels of *C. perfringens* and low levels of bifidobacteria at baseline. Consequently, these patients were excluded from the data analyses. On average, an approximate 12-fold increase was observed in the number of bifidobacteria ($10^{8.3}$ to $10^{9.4}$ bifidobacteria/g feces) in the 12 subjects with changes in the microflora; however, in subjects with low basal bacterial counts, a 100-fold increase was observed at the end of the 14-day treatment period. Additionally, ingestion of the IMO mixture also induced a reduction in the average number of lactobacilli and enterobacteria (not statistically significant). At follow-up analysis, the total number of bifidobacteria approximated pre-treatment values ($10^{8.7}$ bifidobacteria/g feces).

In another study, a group of 7 subjects was provided 10g of an IMO preparation per day for 3 consecutive weeks, followed by a 1-week IMO-free interval (Kaneko *et al.,* 1993). The IMO f product was reinstated in the final week of the study (week 5; only 6 subjects). At the end of weeks 3 and 5, significant increases in levels of bifidobacteria, lactobacilli (week 5 only), and eubacteria (week 5 only) were observed in comparison to values recorded in the first week. Moreover, daily ingestion of the IMO product induced significant compositional changes in the fecal microflora, marked by an increase in bifidobacteria content and a reduction in the fraction of bacteroidaceae.

However, while other oligosaccharides such as FOS are reported to induce changes in the microflora at levels as low as 1g/day, Kohmoto *et al.* (1991) determined the minimum effective dose for mixtures of isomalto-oligosaccharides for improving the intestinal flora to be in the range of 8-10g/day In a two-stage administration test, 12 healthy adult males who consumed regular diets of Japanese food were divided into 2 groups of 6 (A and B). During the first administration test, group A and B subjects consumed daily 6.7 and 10.1 g of an IMO product (lsomalto-900®) suspended in a hot drink (4.3 and 6.5g of isomalto-oligosaccharides, respectively), respectively, for 10 consecutive days. In the second 10-day test, which was conducted 1 month following the first trial, group A and B subjects received 13.0 or 15.3 g of the IMO preparation per day, respectively (8.2 and 9.8g isomalto-oligosaccharides, respectively). A positive trend for an increase in the number of bifidobacteria was observed in group-A subjects consuming 8.2g of isomalto-oligosaccharides per day, while a significant increase in the number of fecal bifidobacterial cells was noted in group-B subjects following daily ingestion of 9.8g of isomalto-oligosaccharides in comparison to pre-treatment values. No significant changes in the fecal bifidobacteria content were observed following ingestion of the IMO mixture at the lower dose levels *(i.e.,* 4.3 or 6.5g). Fecal pH or water content remained unaffected throughout both test periods. Conversely, in another investigation, consumption of 10 g of a mixture of isomalto-oligosaccharides for a period of 14 days by 9 healthy adult males was I associated with a significant increase in the number of fecal bifidobacteria, accompanied by a significant decrease in fecal pH, but no changes in the fecal water content. Bouhnik *et al.* (2004) conducted a placebo-controlled double-blind study to assess the potential of several different non-digestible carbohydrates, including isomalto-oligosaccharides (Cerestar; composition not specified) to stimulate fecal bifidobacteria. The study was divided into 2

phases. Phase 1 was designed to determine whether the non-digestible carbohydrates provided to groups of 8 study participants at a dose level of 10 g/day induce any changes in the microflora of the gut, whereas phase 2 was a dose-response study, restricted to the evaluation of those non-digestible carbohydrates which showed a positive response in phase 1. The initial study consisted of a 15-day trial during which subjects consumed their habitual diets with only minor restrictions. From day 8 to 14, participants ingested 5 g of the non-digestible carbohydrates twice daily (total 10 g/day) or a placebo (50% sucrose and 50% of fully digestible maize-derived maltodextrin). In addition to a determination of potential bifidogenic effects, the study also included an evaluation of digestive tolerance and fecal pH. In comparison to the placebo values, consumption of isomalto-oligosaccharides increased levels of fecal bifidobacteria only slightly and not at levels of statistical significance and no differences were observed in the bifidobacterial count between treatment days 8 and 15. Likewise, no changes were observed in the fecal pH, total aerobe count, or levels of lactobacilli, *Bacteroides,* or enterobacteria. Since consumption of the isomalto-oligosaccharides was not associated with any bifidogenic properties in the preliminary stage of this 2-phase trial, the IMO mixture was not evaluated further in the second phase of the trial. Thus, the IMO-related effects on the microfloral composition appear to be somewhat attenuated, perhaps as a result of partial hydrolysis of the smaller components of the IMO mixtures occurring in the small intestine.

XI.2.2 Short-chain fatty acids (FSCA) Production

Non-digestible oligosaccharide, escaping digestion and absorption in the small intestine are fermented in the colon by intestinal bacteria. In addition to hydrogen, other products of bacterial fermentation include carbon dioxide (CO_2), methane (CH_4) water, tactic acid, and short-chain fatty acids such as acetate, propionate, and butyrate. Presently there is conflicting evidence regarding increased levels of butyrate production in the lower segments of the gastrointestinal tract. While a number of *in vitro* and *in vivo* studies have suggested that production of butyrate stimulates cell growth (Sakata, 1987; Gibson *et al.,* 1992), which may be pro-carcinogenic due to the increased probability of errors (mutations) occurring in the newly replicated DNA of colonocytes, human studies suggest that butyrate is protective and that it reduces colon cancer risk (Burn *et al.,* 1995; Cummings, 1995; Mathers, 1998; Topping and Clifton, 2001). Although the English-language abstract available for the 1-year rat study did not specifically identify the individual organs subjected to microscopic examination, it was noted that administration of the IMO mixture at a concentration of 3% in the drinking water was not associated with any histological alterations (Kaneko *et al.,* 1990).

In an *in vitro* comparative evaluation of the fermentability of various prebiotic oligosaccharides, 24-hour incubation of human fecal bacteria with isomalto-oligosaccharides was associated with statistically significant increases in lactate and acetate, but not propionate or butyrate production (Rycroft *et al.,* 2001). In a rat study (duration of study period not specified) primarily designed to assess potential effects of several different saccharides on

mineral absorption, a parallel investigation was conducted to determine whether ingestion of oligosaccharides possessed any effects on the pH in the cecum and concentrations of short-chain fatty acids *(i.e.,* acetate, propionate, butyrate, D-lactate, and L-lactate) (Ohta *et ai,* 1993). In comparison to a control group, no significant variations were observed in cecal pH and levels of acetate, propionate, butyrate, D-lactate, and L-lactate in rats administered 5% of an IMO mixture in the diet (approximately 2.5g/kg body weight/day) for an unspecified period of time. In comparison, ingestion of 5% dietary FOS resulted in significantly lower cecal pH and acetate and propionate concentrations, and was accompanied by significantly higher D-lactate and L-lactate concentrations compared to the control group, as well as rats receiving the IMO mix in the diet. Moreover, butyrate levels also were significantly greater in the FOS-treated group of rats compared to IMO-treated animals.

Likewise in humans, although significant increases were observed in fecal levels of acetate, propionate, and total short-chain fatty acids following daily consumption of up to 24 g of an IMO product of which 10 g consisted of isomaltose, panose, isomaltotriose, isomaltotetraose, and dextrin for a period of up to 30 days in comparison to pre-treatment values, post- and pre-treatment butyrate levels remained comparable (Chen *et al.,* 2001). In another human trial, Kaneko *et al* (1993) also observed a significant increase in total levels of fecal short-chain fatty acid production in a group of 7 male and female subjects consuming 10 g of an IMO product per day for a total of 4 weeks, with a 1-week IMO-free interval between weeks 3 and 5 (only 6 subjects in the 5th study week); however, increases in individual short-chain fatty acids, acetate and lactate, did not reach levels of statistical significance.

XI.2.3 Increased Bile Acid Secretion

It has been suggested that poorly absorbed oligosaccharides may increase fecal bile acid excretion, which may consequently act to improve serum cholesterol levels (Wang *et al.,* 2001). In a study in which male Sprague-Dawley rats were administered an IMO mixture in the diet at a concentration of 6% (approximately 3g/kg body weight/day) for a period of 5 weeks only a slight and not statistically significant increase in fecal bile acid excretion was observed in comparison to a control group (Sung *et al.,* 2004). Furthermore, no significant differences were observed in the excretion of neutral steroids *(i.e.,* cholesterol, coprostanol, and coprostanone), as well as triglycerides. Likewise, plasma total cholesterol and HDL-cholesterol levels of IMO-treated and control rats were comparable. In another study, no changes in plasma triglyceride and cholesterol levels were observed in normal male Sprague-Dawley rats administered an IMO preparation in the diet at a concentration of 12% (approximately 6g/kg body weight/day) for a period of 25 days or in diabetic Sprague-Dawley rats provided a diet containing an IMO product at a concentration of 10% (approximately 5g/kg body weight/day) for a total of 8 weeks *(i.e.,* 4 weeks prior to STZ induction and 4 weeks after) in comparison to corresponding controls (Ly *et al.,* 1999; Chai and Rhee, 2001).

Although bile acid secretion was not evaluated specifically in any of the above-reviewed human tolerance studies, hypocholesterolemic effects were observed following ingestion of IMO mixtures at relatively high-dose levels *(i.e.,* 30g/day (Wang *et al.,* 2001). Specifically, daily ingestion of 2 equal 15g doses of IMO syrup dissolved in water for a period of 28 days resulted in significant reductions in levels of serum triglyceride and total cholesterol and a significant increase in levels of HDL-cholesterol compared to pre-treatment values.

XI.2.4 Effect on Mineral Absorption

It has been reported that consumption of various dietary fibers may reduce mineral bioavailability, by binding the minerals and forming insoluble complexes, thereby resulting in decreased mineral absorption and increased fecal mineral excretion (Pilch, 1987). Although not entirely consistent, both animal and human studies conducted with non-digestible carbohydrates have demonstrated a positive effect on mineral absorption (Scholz-Ahrens *et al.,* 2001). Scholz-Ahrens *et al.* (2001) suggested that increased mineral solubility as a result of reduced colonic pH related to the microbial fermentation may be the underlying reason for the positive effect. It is also possible that other mechanisms such as enhanced expression of mineral binding proteins *(e.g.,* calbindin-D9k essential for calcium transport) or changes in the properties of the intestinal villi resulting from intake of non-digestible carbohydrates contribute to the apparent increase in mineral absorption in the presence of prebiotics. In hemodialysis patients, oral treatment with 30g of an IMO preparation resulted in a significant increase in hematocrit levels, which was considered by the authors of the study to be likely reflective of enhanced iron absorption (Wang *et al.,* 2001). It was suggested that ingestion of IMO mixtures may lead to an increase in the bifid microflora, resulting in increased production of lactate and acetate and consequently lowering of the gut pH. Since a reduction in the pH of the lower intestine has been previously noted to be conducive to iron absorption, IMO ingestion may thus indirectly contribute to enhanced iron absorption. Alternatively, it was also noted that bifidobacteria are known producers of Vitamin B complexes and thus the slow absorption of these vitamins may also have improved the anemia in the study subjects.

A single animal study was identified which was specifically performed to evaluate any possible variations in mineral absorption resulting from the oral administration of isomalto-oligosaccharides and other non-digestible sugars. Specifically, Ohta *et al.* (1993) assessed potential effects related to the ingestion of lactose, FOS, GOS, raffinose, and a mixture of isomalto-oligosaccharides (Isomalto 900-P®) on calcium, magnesium, and phosphorus absorption in weanling male Sprague-Dawley rats. Groups of 7 rats were fed either a control diet (consisting of 10% sucrose) or diets supplemented with 5% of the respective saccharide (each diet additionally consisting of 5% sucrose) (period of time was not identified in the English-language abstract). Mineral absorption in rats was evaluated as the difference between mineral intake and fecal output. In comparison to the control group, no significant differences were observed in the percent of calcium, magnesium, and phosphorus absorption of IMO-

treated rats. Conversely, rats fed the 5% FOS-supplemented diet reportedly exhibited a significant increase in mineral absorption. The absorption of magnesium also was observed to increase significantly in rats provided GOS and raffinose in the diet, while only raffinose-treated rats exhibited significantly elevated levels of calcium absorption in comparison to controls. However, a trend towards increased phosphate absorption was noted in rats of both the GOS-and raffinose-treatment groups. Furthermore, a significant positive correlation was noted between mineral absorption and L-lactate concentration in the cecum and thus, the authors postulated that the concentration of L-lactate in the rat cecum exhibited a direct effect on mineral absorption; however, administration of the IMO mixture in the diet did not significantly affect levels of L-lactate in the cecum.

XI.3 Evaluation of Nutritional Quality of IMO

XI.3.1 Animal Studies

The digestibility of an IMO mixture (Showa Sangyo Co. Ltd lsomalto-900P®; 33.4% isomaltose, 11.8% panose, 14.3% isomalto-DP3, 15.7% isomalto-DP4, and 10.3% other) was assessed *in vitro,* in model systems containing artificial gastric juice, rat intestinal mucosa enzymes, or human salivary or hog pancreatic α-amylase (Kaneko *et at.,* 1992). While no hydrolysis occurred in the presence of artificial gastric juice or α-amylase irrespective of its source, hydrolysis did occur in the system containing enzymes isolated from the rat intestinal mucosa. It was noted, however, that the hydrolysis ratio of the IMO product was markedly lower than that of maltose or isomaltose. Based on these results, the authors stated that IMO mixtures undergo partial hydrolysis, while the remaining (undigested) part of the oligosaccharide mixture passes unchanged into the lower intestine (Kaneko *et al.,* 1992).
In an *in situ* study comparing the digestibility of a mixture of isomalto-oligosaccharides to several other oligosaccharides using the rat jejunum loop method, the digestibility of the test material was shown to be related to the composition of the mixture (Kaneko *et al.,* 1995). Specifically, the digestibility of a typical mixture of isomalto-oligosaccharides (3.8% glucose, 4.5% maltose, 22.8% isomaltose, 11.6% panose, 16.7% isomaltose, 26.6% isomalto-DP4 to DP6, and 14% other DP2 to DP3), as well as a mixture enriched in isomaltose (IMO-2) and one with a greater proportion of highly polymerized isomalto-oligosaccharides (IMO-3) were assessed. In comparison to IMO-2, which consisted mainly of digestible saccharides, a slower luminal clearance was observed for the IMO mixture, but greater than that of IMO-3. IMO-2 demonstrated a slower clearance than maltose, but comparable to that of sucrose and maltotriose. The clearance for IMO-3, although smaller than that for the IMO mixture, was greater than the clearance of the non-digestible saccharides, maltitol, FOS, and raffinose. Furthermore, disappearance of hydrogenated IMO derivatives (H-IMO) (alcohols of isomalto-oligosaccharides) was significantly lower than that of the IMO mixture, but comparable to that of maltitol. In the case of IMO-2, the disaccharide fraction showed the fastest disappearance rate. Tri- and tetra-saccharide components of the IMO-3 mixture exhibited the fastest clearance rate. Disappearance of the longer oligosaccharides was accompanied by a gradual increase in

disaccharides, indicating that the tri- and tetra-saccharides were subjected to sequential degradation. Only a small reduction was observed in the levels of penta- and hexasaccharides. Overall, results of these studies support partial hydrolysis of IMO mixtures in the upper segments of the intestine, with the undigested portion passing into the lower intestine.

XI.3.2 Human Studies

A group of 6 healthy males (25 to 29 years of age) received an oral dose of 50 mg of a radio-labeled IMO preparation (^{13}C-IMO) and 25 g of an unlabelled IMO mixture in 150 ml of water while sedentary and again 2 weeks later prior to intermittent exercise (Kohmoto *et al.,* 1992). Breath samples were subsequently collected at various intervals for up to 480 and 720 minutes in the sedentary and exercise test, respectively. Additionally, blood samples were obtained 15 minutes prior to testing and at 30 to 60 minute intervals thereafter for up to 180 minutes (240 minutes in the exercise test) post-dosing. Excretion of isomaltose, isomaltotriose, and panose in the feces was monitored over a period of 3 days in 3 subjects. Isomaltose accounted for approximately 30% of the product's composition, whereas less than 30% of the mixture consisted of oligosaccharides with 4 or more degrees of polymerization. Peak levels of radioactivity expired as CO_2 were observed at 2 to 3 hours following consumption of the IMO mixtures in both tests. Given the relatively rapid appearance of the radioactive CO_2 in the breath samples, it is expected that the expired $^{13}CO_2$ levels resulted from the metabolism of absorbed glucose, rather than from colonic fermentation. Following ingestion of the IMO preparation in the sedentary state, a total of 513±15 μmol of $^{13}CO_2$ was expired in 8 hours, a recovery of 28.7±0.8%. In comparison, 60.9±3.2% of $^{13}CO_2$ (1,090±58 μmol) was collected over 12 hours when the IMO mixture was ingested before intermittent physical exercise which also induced a 2-fold increase in the breath volume compared to the sedentary state. The amount of recovered $^{13}CO_2$ indicates an energy value of approximately 70 to 80% for this IMO mixture relative to that for maltose for which CO_2 recovery was 34.5 and 88.4% in the sedentary state and during exercise as reported in another study (Tsuji *et al.,* 1990). At 30-minutes following ingestion of the oligosaccharides, significantly increased serum glucose and insulin levels were observed in subjects at rest, as well as during exercise. Significant reductions in levels of non-esterified fatty acids (NEFA) were observed during both test phases for the first 60 to 90 minutes (30 minutes in the sedentary test) after IMO ingestion. Analysis using HPLC did not reveal any remaining isomalto-oligosaccharides the fecal samples collected for 3 days after dosing.

Since microbial fermentation in the lower gastrointestinal tract is the only source of hydrogen gas (H_2) production in humans (Galloway *et al.,* 1966; Levitt, 1969; Muir *et al.,* 1995), excretion of breath hydrogen following consumption of IMO mixtures also can be used to assess the fermentability of the oligosaccharides (Galloway, 1966). In the study conducted by Kohmoto *et al.* (1992), breath hydrogen (H_2) expiration increased during periods of exercise, but remained constant for the duration of the sedentary trial. In total, the authors reported

evolution of 13.0±0.8 ml of hydrogen in the sedentary state and 15.2±1.0 mL in the exercise trial. In comparison, ingestion of an unspecified, but presumably comparable amount of the sugar alcohol, maltitol (4-O-α-glucopyranosyl-D-sorbitol), resulted in the expiration of 52.9±6.5 mL of hydrogen (Tsuji *et al.,* 1992 as cited in Kohmoto *et al.,* 1992), suggesting that about 25% of the ingested IMO mixture was fermentable compared to maltitol.

Another human trial was conducted with 9 males and 29 females, to determine breath hydrogen excretion as a measure of fermentability occurring in the large intestine following consumption of 10 to 20 g of FOS, galactosyl-sucrose, and an IMO preparation (Oku and Nakamura, 2003). The IMO product was reported to have a purity of greater than 90.8% (Showa Sangyo Co.) and consisted of a mixture of mono- (3.8 %), di- (40.4 %), tri- (29.2 %), and oligosaccharides (26.6 %). Trials were spaced apart 4 to 7 days and subjects were fasted for 12 hours prior to testing. Cookies and a soft drink consisting of fully digestible carbohydrates were provided to all subjects 2 hours before and 4 hours after ingestion of the test material to keep subjects from feeling hungry. The substances were dissolved in water and breath hydrogen was measured prior to ingestion of the solution and at 20-minute intervals for the first 2 hours (beginning at 40 minutes) and at 30-minute intervals thereafter until 7 hours after ingestion of the test material. Following administration of 10 g of each of the oligosaccharides, the area under the curve (AUC) values (for 420 minutes versus hydrogen excretion) were 9,768±3,253, 3,662±2,632, and 831±1,154 ppm for FOS, galactosyl-sucrose, and the IMO mixture, respectively, indicating that unlike the 2 other oligosaccharides, the IMO mixture was not subjected to extensive fermentation in the large intestine. Whereas only a marginal increase (1.4-times) was observed in hydrogen excretion following ingestion of 20 g of the IMO product in comparison to the lower dose level of 10 g, ingestion of 20 g of FOS or galactosyl-sucrose increased breath hydrogen excretion 1.7- to 2.7-times compared to that observed with the 10 g dose. Moreover, the differences in the amounts of expired hydrogen at 10 g compared to that following 20 g of FOS or galactosyl-sucrose consumption were statistically significant. Although consumption of 40 g of the IMO product resulted in approximately 2- and 3-fold increases in AUC values in comparison to when 20 and 10 g of the IMO mixture was ingested, respectively (statistical significance not reported), the AUC obtained when 40 g of the IMO mixture was ingested (2,440±20), was still markedly lower than the AUC values obtained with only 10 g of either FOS or galactosyl-sucrose. The authors noted that the quantity of breath hydrogen collected varied considerably from subject to subject; however, the pattern was similar among all study participants. Based on the limited breath hydrogen expiration and absence of any gastrointestinal discomfort following ingestion of up to 40g of IMO, the authors concluded that enzymes of the small intestine readily hydrolyzed the components of the ingested IMO mixture.

XI.3.3 Published Data on Nutrient Composition of IMO

Although the exact composition of the IMO preparations evaluated in the studies in support of the safety of VitaSugar-IMO slightly differed somewhat from the oligomer profiles of the VitaSugar-IMO products, these studies are considered to be nonetheless relevant to the overall assessment of the safety of VitaSugar-IMO. Firstly, since the production of IMO mixtures occurs *via* natural enzymatic processes, some compositional variability between different products is expected. Furthermore, variations in the composition among various products consisted primarily of differences in the percent distribution of specific oligomers. For example, some IMO products were characterized by a larger disaccharide fraction than the level of disaccharides found in VitaSugar-IMO. However, the BioNeutra's VitaSugar-IMO is manufactured using a patent technology consisting of a yeast-fermentation step for the removal of free glucose content generated during the enzymatic reaction (Saccharification step), compared to other reported test material used in studies which are purified by using membrane-filtration procedures for the removal of free glucose. Because of using a more efficient method for the removal of contaminant glucose from the mixture of other oligosaccharide, the overall health functional quality of VitaSugar-IMO is expected to be better then the published testing material.

Table XI.3.3-1 showed the composition of representative sample of Vitasugar-IMO after saccharification step (before yeast fermentation) and after yeast fermentation and presented in comparison to reported test materials used in different published studies. It should be noted that, an overall individual composition of Vitasugar-IMO at post-saccharification stage is in well comparison with that of various published studies (Table XI.3.3-1).

Table XI.3.3-1 Composition of Isomalto-oligosaccharide (IMO) Products[1] Tested in the Published Studies (in alphabetical order)

Test Mat.		DP1 (%)		DP2 (%)			DP3 (%)				DP4 (%)		DP5 (%)		DP6 (%)		Other (%)		Reference
		Glu	Fru	M	IsoM	O	P	M	IsoM	O	M	IsoM	M	IsoM	M	IsoM	IMO	Dex	
VitaSugar*	†Before Ferment	15-20	-	5-8	25-35	-	10-20	0.5-2	15-25	-	10-15		5-8		3-7		<10[2]	-	-
VitaSugar*	††After Ferment	0-4.5	-	5-6	10-15	-	20-25	2-4	25-30	-	15		7-9		4-5		<10[2]	-	-
IMO		20.9	0.5	15.4	12.0	-	29.1	3.9	2.6	-	3.2	9.9	-		-		-	2.5	Chen *et al.* (2001); Wang *et al.* (2001)
IMO		<0.2%		6.9			28.4	-	-	-	36.7		19.1		7.4		1.2[2]	-	Day and Chung (2004)
IMO-900P		-	-	38.0			25.2				23.7[3]						-	-	Kaneko *et al.* (1990)
IMO-900		-	-	52.5			25.4				15.2[3]						-	-	
IMO-900P®		-	-	-	34.4	-	12.2	-	14.7	-	-	16.2	-		-		10.6	-	Kaneko *et al.* (1992)
IMO-900®		3.8	-	4.5	22.8	13.1[4]	11.6	0.9	16.7	-	17.7		7.2		1.7[5]		-	-	Kaneko *et al.* (1995); Oku and Nakamura (2003)
IMO-2		0.4	-	2.1	64.3	22.3	4.7	-	5.7	-	0.5		-		-		-	-	Kaneko *et al.* (1995)
IMO-3		0.5	-	1.2	1.8	2.4	25.3	2.2	16.5	-	30.7		8.5		10.9[5]		-	-	
IMO-900®		1.8	-	5.1	48.8	3.7	6.9	-	16.9	1.6	15.2[3]						-	-	Kohmoto *et al.* (1988)
IMO-900®		4.1	-	10.5[6]	37.2	-	See DP3 IsoM	See DP2 M	26.8[7]	See DP3 IsoM	21.4[3]						-	-	Kohmoto *et al.* (1991)
IMO-900®		2.4	-	3.6	32.3	9.1[4]	12.3	-	14.8	-	15.5		6.9		3.3		-	-	Kohmoto *et al.* (1992)
^{13}C-IMO-900®		1.2	-	2.0	32.6	6.9	13.4	-	16.9	-	15.5		6.9		4.6		-	-	

Mat. = Material; DP = Degree of polymerization; Glu = Glucose; Fru = Fructose; M = Malto-; IsoM = Isomalto-; O = Other; P = Panose; IMO = Isomaltooligosaccharides; Dex. = Dextrin; IMO-2 = Disaccharide fraction from IMO; IMO-3 = Tri- and higher oligosaccharide fraction from IMO.
* Composition based on representative samples – Product specifications indicate glucose ≤5% and ≥90% isomaltose and DP3 to DP9.

[1] Expressed on a dry basis (%).
[2] DP7 and greater.
[3] DP4 and greater.
[4] Nigerose and kojibiose.
[5] DP6 and greater.
[6] Maltose and maltotriose combined.
[7] All tri-isomaltooligosaccharides

† Before Ferment = Composition of sample after Saccharification step.
†† After Ferment = Composition of sample after Yeast Fermentation step. **(Please see section XI.3.3 (page # 50) for explanation)**

XI.4 Impact of IMO on the Dietary Intake

IMO syrups could replace part or all of liquid sugar syrups to produce different sweetness profiles for beverages since they are about half as sweet as sucrose. They could also be added during beer production as non-fermentable sugar syrups to replace some of the fermentable sugars altering the residual sweetness and mouth-feel of the resulting beers.

The anti-cariogenic properties could be employed by using IMOs as replacements for sugars in many confectionery products. Dental caries are caused by insoluble glucan gums forming on the surface of teeth (plaque), and the formation of acids under this plaque which attacks the tooth enamel. Studies with animal models showed that IMO in place of sucrose reduces the amount of plaque formed and also reduces the amount of enamel attacking acids formed (Tsunehiro, 1997, Minami, 1989).

The reported higher moisture retaining (water-binding) capacity which would confer improved resistance to bacterial infection could be an advantage to industry. The reported anti-staling properties should be an advantage in the baking industries in developing products with slower staling rates. However it would appear that the major advantages and the major areas of use and interest are in the functional food area covering prebiotic products. In Japan there are a number of so called functional foods sold which have reported health benefits, some of which use IMO as ingredients. Prebiotics are non-digestible carbohydrates that pass through the small intestine undigested and are then fermented in the colon to produce a range of small chain fatty acids, specifically butyrate. It has been reported in clinical trials that IMO do not cause diarrhea when used in recommended doses. IMO are food sources that are preferentially chosen by probiotic bacteria (live beneficial bacteria) such as bifidobacteria in the gut that reportedly help modulate the gut microflora and improve the intestinal microbial balance.

Currently, IMO is being formulated by a number of companies in United States, particularly as a source of soluble fibre and prebiotic in a range of beverages. However, in E.U., the expected use of IMO by the general population will be as a nutritive sweetener with functionality of prebiotic and dietary fibre (a health sweetener with caloric value of ~1.5-2.0 calories per gm) (Roberfroid, 1999, Nakanishi 2006), mixing with a variety of other foods and beverages products for the purpose of sweetening. IMO will be used as a general food ingredient to be formulated with range of food products manufactured by beverage industries, diary industries and all kind sweets and dessert's making industries.

Followings are the list of published scientific papers supporting the IMO function as a Prebiotics, source of a Low Glycemic Index and improving overall digestive health in humans.

XI.4.1 IMO as a Prebiotic

Followings are the list of published scientific papers supporting the IMO function as a Prebiotics, source of a Low Glycemic Index and improving overall digestive health in humans.

- The effect of IMO on human fecal flora was studied. *Bifidobacteria* and the *Bacteroides* group from human intestine could utilize IMO but *E. coli* and other bacteria could not. After the administration of IMO (13.5g daily for 2 weeks) to 6 healthy adult men & 18 elderly individuals, the numbers of *Bifidobacteria* in the feces observed to be increased several fold. On average, an approximate 12 –fold increase was observed in the number of *Bifidobacteria* ($10^{8.3}$ to $10^{9.4}$ *Bifidobacteria*/g feces) in the 12 subjects (Kohmoto, T., 1988).
- IMO was administered to groups of 10BABL/c mice at dose levels up to 7.5g/kg body weight for a period of 7 days. Analysis of fecal samples revealed increases in the levels of *Bifidobacterai* and *Lactobacilli*, and inhibition of *Clostridim perfriengenes* growth. Analysis of human fecal samples (15/sex) revealed similar results following 7 days of IMO consumption at a dose of 15 g per day (Qing, G., 2003).
- In a group of 7 elderly males experiencing diarrhea, ingestion of up to 24 g of an IMO mixture for a period of 30 days was associated with a significant increase in fecal bacterial mass in comparison to pre-treatment values (Chen, H.-L., 2001).
- A group of 7 subjects was provided 10 g of an IMO preparation per day for 3 consecutive weeks followed by 1-week IMO-free interval. The IMO product was reinstated in the final week of the study (week 5). At the end of weeks 3 & week 5, significant increases in levels of *Bifidobacteria*, *Lactobacilli* and *Eubacteria* were observed in comparison to values recorded in the first week (Kaneko, T., 1993).
- In a dose-response studies of IMO for increasing fecal Bifidobacterium, the minimum effective dosage of IMO was found 10 gm/day for a period of 14 days consumed by 9 healthy individuals, and the result was a significant increase in the number of fecal *Bifidobacteria* (Kohmoto, T., 1991).
- The administration of IMO in amount from 5 to 20 g/day increased human intestinal *Bifidobacteria* in dose-dependent manner. An IMO intake of 10g/day and 5g/day produced a significant increase of *Bifidobacterial* number in feces and the ratio in fecal microflora within 12 days (Kaneko, T., 1994).

XI.4.2 IMO Exhibit Low Glycmeic Index (GI)

Twelve healthy adults were randomly divided into xylitol group and isomaltooligosaccharide group. Each group was orally administered 50g of xylitol or 50gm IMO & 50gm of glucose (as control). Blood glucose was analyzed at different intervals after oral intake of xylitol, IMO or glucose. This study repeated continuously for 3 days, and glycemic index were calculated. The glycemic index for IMO was 34.66 ±7.65 which represents a low GI (Sheng, G.E., 2006).

XI.4.3 IMO Relived Constipation & Lowered Blood Cholesterol

Seven older male subjects participated in this study that consisted of a 30-day control low fiber period followed b a 30-day IMO-supplemented (10gm) experimental period. Bowl functions such as defecation, enema use and bloating were monitored daily. Incorporation of IMO significantly increased the defecation frequency, wet stool output and dry stool weight by twofold, 70% & 55%, respectively. Consumption of IMO effectively improved bowel movement, stool output and microbial fermentation in the colon without any adverse effect observed in this study (Chen H-L., 2001).

This clinical trail study evaluates the therapeutic efficacy of IMO in the treatment of chronic server constipation and its effect on lipid profiles in 20 hemodialysis (HD) patients. After a 2-week basal period, these patients were received 30 g of IMO for a 4-week period. After the study period, those patients receiving IMO had reductions in levels of total cholesterol -17.6% , triglycerides -18.4%, and elevation of levels of HDL-C by +39.1%. Also, IMO induced a significant increase in number of bowel movements and hence improvement of constipation 76.3% + 30.9% of patients during the 4-week treatment. In conclusion; IMO once a day is effective in increasing bowel frequency and improving constipation in HD patients. In addition, IMO treatment was effective in lowering total cholesterol and triglycerides and in raising HDL-C in HD patients (Hsueh-Fang W., 2001).

XI.5 Effects of Storage & Further Processing

This topic was covered in detailed under "Stability of Isomalto-oligosaccharide"; section II.5

XI.6 Criteria for Nutrients Analysis

Final product is tested for the followings:

a) Carbohydrate Profiles including: Glucose, Maltose, Isomaltose, Maltotriose, Panose, Isomaltotriose (DP3), Isomaltotetraose (DP4), Isomaltopentaose (DP5), Isomaltohexaose (DP6), & Isomaltoheptaose (DP7)
b) Microbial Counts
c) Heavy metal contents, *i.e*., arsenic, cadmium, lead & total mercury
d) Alcohols
e) Gluten

Details about the test methodologies & tolerance limits are given in Table XI.6-1. List of proximal compositions, Nutrition Data, Physical properties & Microbiology are given in the product's specification sheet (Appendix A).

Table XI.6-1 Finished Product's Criteria.

Test Parameters	Test	Test Method	Tolerances
Identity (Finished Product)	Physical Description:	Visual & Tasting	Transparent, light golden, sticky liquid, light-sweet without aftertaste
	Qualitative Test; Carbohydrate Contents & Profiles	HPLC [1,2]	Complies to International Food Standards
Purity (Finished Product)	Microorganisms:		
	Yeast & Mold	See references [3]	< 1 X 10^2 CFU/g
	Total Aerobic Counts	See reference [4]	< 1 X 10^4 CFU/g
	Eschericia coli	See reference [5]	< 10 MPN/g
	Salmonella spp.	See references [6,7]	Absent
	Staphylococcus aureus	See references [8,9]	Absent
	Chemicals:		
	a) Heavy Metals:		
	Arsenic	See reference[10]	≤ 0.5 mg/kg
	Lead	See reference[11]	≤ 0.5 mg/kg
	Cadmium	See reference[12]	< 0.09 µg/kg b.w./day
	Mercury	See reference[13]	< 0.29 µg/kg b.w./day
	b) Other chemicals (Raw Material Stage)		
	Pesticides	See reference[14]	< 0.1 ppm
	Solvent impurities	See reference[15]	≤ 50 ppm
	Alcohol (EtOH)	HPLC-RI[16,1]	Negative
	c) Gluten	See reference[17]	Negative
Quantity/Potency (Finished Product)	Content of IMO; Glucose, DP2, DP3, DP4, DP5, DP6 & DP7	HPLC-RI[16,1]	Glucose = ≤ 5% Isomaltose & DP3 to DP9 = ≥ 90%, Total Carbohydrate = > 99.5%

References quoted in Table XI.6-1

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13. The United States Pharmacopeia; Limit Tests <261> Mercury (USP 28, NF 23); Jan 01, 2005
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16. Determination of Oligosaccharides Isomaltose, DP3 to DP7 by HPLC-RI; LabsMart (Edmonton) cGMP SOP protocol; Jan (2006)
17. RIDA Quick Gliadin Test Kit (r-biopharm)

XI.7 Energy Value of Novel Food

Suggested caloric value for IMO is 1.5-2.0 kcal/g which is based upon the following published studies;

a) All of the non-digestible oligosaccharides that are largely or completely fermented in the colon, be given a caloric value of 1.5 (6.3) kcal/g (kJ/g) (Roberfroid, 1999).

b) The standard tables of Food Composition in Japan and nutritional labeling standards explained the energy conversion factors for dietary fiber and poorly digestible saccharides (assigned as Group 2) like, mannitol, maltitol, isomaltitol, maltotriitol, lactitol, fructo-oligosaccharides, xylo-oligosaccharides (Isomalto-oligosaccharides under come in similar category). In table 2 of Energy conversion factors for food product components (correspond to nutritional labeling standards), the poorly digestible saccharides of group 2 is given energy conversion factor of 2 kcal/g compared to fully digestible carbohydrate (*e.g.*, glucose) of 4 kcal/g. (Nakanishi, 2004).

c) Fructo-oligosaccharide (FOS), which has very similar physiological properties as that of Isomalto-oligosaccharide (IMO) has been given an energy value of 2 kcal/g, by the Bureau of Nutritional Sciences, Health Products and Food Branch, Health Canada (CFIA, Chapter 6, Section 6.4.2, Table 6-11).

XII MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD

Based on the SCF guidelines, the following question must be addressed to ensure sufficient microbiological information on the novel food:

- "Is the presence of any microorganisms or their metabolites due to the novelty of the product/process?"

- "Is there information to show that the NF is unlikely to contain microorganisms and/or their metabolites of adverse public health significance?"

This question has been addressed collectively in Section XII.1

XII. MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD

Baker's derived active yeast *(Saccharomyces cerevisiae)* are used during the production of BioNeutra's IMO products to remove the monosaccharides (glucose) that form as a result of the enzymatic hydrolysis of the starch as the reactions proceed at various stages during the manufacturing process. The dried bakers active yeast used in the production of VitaSuga-IMO are food-grade and compliant with FCC specifications. Once the fermentation process is complete, yeast is separated from IMO product by filtration and undergoes several purification steps that result in a biomass-free final product. To ensure the absence of contaminating microorganisms, IMO is assayed for total aerobic plate count, yeast, mould, coliforms, *E. coli*, and *Salmonella* (see Bath Analysis Data; Appendix C).

XIII. TOXICOLOGICAL ASSESSMENT OF THE NOVEL FOOD

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient toxicological information pertaining to the novel food:

- "Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?"

- "Is there information from a range of toxicological studies appropriate to the novel food to show that the novel food is safe under anticipated conditions of preparation and use?"

- "Is there information which suggests that the novel food might pose an allergenic risk to humans?"

These questions have been addressed collectively in Sections XIII.1 through XIII.6

XIII. TOXICOLOGICAL ASSESSMENT OF THE NOVEL FOOD

XIII.1 Toxicological Evaluation of *Saccharomyces cerevisiae*

The dried bakers active yeast used in the production of VitaSugar-IMO are food-grade and compliant with FCC specifications. Moreover, it is authorized by Food & Drug Regulation Canada to be used in food processing (Div. 16, Table IV, item B.1).

Yeast used in the manufacture of BioNeutra's IMO products contain sorbitan monostearate which is an approved direct food additive for use as a re-hydration aid in the production of active dry yeast in an amount not to exceed 1 % by weight of the dry yeast (21 CFR § 172.842). BHA also is permitted for use in the U.S. as a food preservative in active dry yeast at concentrations of 1,000 ppm (21 CFR § 172.110).

XIII.2 Toxicological Evaluation of Isomalto-oligosaccharide (IMO)

XIII.2.1 Information to Establish Safety

The evaluation of the safety of BioNeutra's IMO products is primarily based on well-established metabolic profiles for the simple saccharide components of the mixture (*e.g.*, maltose, isomaltose), as well as animal and human studies assessing the digestion and elimination of the larger oligomers present in the IMO products. Additionally, in assessing the safety of BioNeutra's IMO products, results of several short- and long-term animal toxicity studies conducted with similar IMO mixtures also were reviewed. Although the exact composition of the IMO preparations evaluated in the studies discussed in support of the safety of BioNeutra's IMO products differed in the distribution of certain oligomers, these studies are considered to be nonetheless relevant to the overall assessment of the safety of BioNeutra's products and were acceptable by the GRAS Expert Panel for VitaSugar-IMO as a food ingredient (see section XIII.5) as well as by United States FDA authorities in filing Final GRAS Notification.

Firstly, since the production of IMO mixtures occurs via natural enzymatic processes, some compositional variability between different products is expected. Furthermore, variations in the composition among various products consisted primarily of differences in the percent distribution of specific oligomers. For example, some IMO products were characterized by a larger disaccharide fraction than the level of disaccharides found in BioNeutra's product. In other instances, BioNeutra's IMO products were identified to contain a greater proportion of isomalto-oligosaccharides compared to some of the test materials administered in the published studies. Essentially, malto-oligomers (1→4), as well as some of the smaller isomalto-oligomers (1 →6) such as isomaltose are digested by intestinal enzymes to glucose, which is taken up systemically and utilized in normal physiological pathways as a source of energy. Accordingly,

consumption of malto-oligosaccharides and smaller digestible isomalto-oligosaccharides is not expected to be associated with any adverse effects.

Conversely, the larger isomalto-oligosaccharides will pass essentially undigested through the gastrointestinal tract. The lack of absorption of the larger isomalto-oligosaccharides largely limits the potential for the occurrence of any systemic toxicity; however, the undigested oligosaccharides are utilized as a source of energy by the bacteria in the lower segments of the gastrointestinal tract. The bacterial fermentation results in the production of short-chain fatty acids and various gaseous by-products and, thus, ingestion of such products at high dose levels can lead to gastrointestinal discomforts (*e.g.*, increased flatulence and bloating). Consequently, results of studies performed with IMO products with an oligomer profile dominated by larger isomalto-oligosaccharides are appropriate for the evaluation of potential gastrointestinal disturbances that could result from the consumption and breakdown of the larger isomalto-oligosaccharides in the colon. Wherever provided, the specifications reported for a particular test material used in a study that is reviewed in this evaluation of IMO safety, were complied separately in Table XI.3.3-1.

Given the sequential hydrolysis of the smaller oligomers to glucose, a basic intermediate of carbohydrate metabolism, combined with a lack of systemic absorption of the larger isomalto-oligosaccharides, no toxicologically significant adverse effects related to the administration of the IMO mixtures are expected, which, as described in greater detail below, was confirmed by the animal feeding studies. However, since the "isomalto" constituents of the larger oligosaccharide fraction in the IMO products are expected to pass into the colon where they may be subject to extensive microbial fermentation, a number of human tolerance trials also are discussed. Furthermore, the non-digestible oligomers may affect the absorption of nutrients (*i.e.,* minerals), the composition of the microflora, with secondary effects on colonic short-chain fatty acid production, and enhance bile acid excretion. Accordingly, a number of studies preformed to assess specifically the nutritional implications of consuming isomalto-oligosaccharides also were identified.

XIII.2.2 Absorption, Distribution, Metabolism & Excretion (ADME)

Typically, mixtures of isomalto-oligosaccharides consist of both digestible and non-digestible saccharides. Following oral ingestion, the non-digestible oligosaccharides pass unabsorbed through the small intestine and are subject to microbial fermentation in the large intestine (Oku and Nakamura, 2003). As previously mentioned, microbial fermentation in the lower gastrointestinal tract results in the evolution of hydrogen (H_2), carbon dioxide (CO_2), and methane (CH_4). Approximately half of the hydrogen generated is eliminated with flatus; however, the remainder of the hydrogen is absorbed and expired. Conversely, the simple oligosaccharides, which also occur naturally in the diet, are subject to the enzymatic activity of maltase and isomaltase in the small intestine. Specifically, malto-oligosaccharides and smaller

isomalto-oligosaccharides are split at their non-reducing ends. Hydrolysis of these oligosaccharides results in the release of the monosaccharide, glucose, which is quickly absorbed from the gastrointestinal tract and utilized in well-characterized metabolic pathways as a source of energy. Glucose is an essential substrate for the synthesis of adenosine triphosphate (ATP), which provides energy for cellular functions via the pentose phosphate shunt pathways and the Krebs cycle (Glinsmann *et al*., 1986). Cellular metabolism of glucose produces approximately 38 mol ATP/mol of glucose and is equivalent to a caloric value of 3.9 kcal/g. The liver plays a major role in regulating blood glucose levels (Glinsmann *et al.,* 1986). Excess glucose is converted to glycogen, which is stored in the liver and skeletal muscle tissues, or to fat in a process called lipogenesis. Glycogen, as well as fat, provides a readily mobilizable source of energy for both liver and skeletal tissues during starvation or when increased energy needs arise. Blood glucose levels also are regulated through insulin and glucagon secretion by the pancreas (Glinsmann *et al.,* 1986).

Normally, digestion of carbohydrate polymers such as the starch polymers, amylase and amylopectin, begins in the mouth as salivary α-amylase is secreted and continues in the stomach with the release of pancreatic α-amylase. Both salivary and pancreatic α-amylase hydrolyze (α1,4)-linkages and, thus, reduce large polysaccharides into smaller oligomers. The smaller oligomers are ultimately broken down into monosaccharides that are subsequently absorbed. Specifically, a number of enzymes have been identified in the epithelial cells of the brush border of the intestinal mucosa, which are capable of hydrolyzing di- and larger oligosaccharides (Würsch, 1991). These enzymes include the sucrase-isomaltase complex, glycoamylase, and lactase. Sucrase-isomaltase is an intestinal enzyme that consists of 2 subunits with one cleaving (α1,4)-glycosidic linkages and the other the (α1,6)-linkages to release individual glucose molecules (Dahlqvist *et al*., 1963; Würsch, 1991; Heymann *et al.,* 1995; Oku and Nakamura, 2003). The sucrase fraction preferentially hydrolyzes α1,2-linkages and α1,4-linkages such as those of sucrose and maltose, respectively, as well as higher oligosaccharides such as maltotriose and maltopentaose (Würsch, 1991). Although unlike the 1→ 4 linked oligomers, 1→6 linked oligomers largely escape digestion in the mouth and stomach, α1,6-linkages of simple disaccharides such as isomaltose and palatinose (isomaltulose) are effectively hydrolyzed by the isomaltase moiety of the sucrase-isomaltase complex. Although the sucrase-isomaltase complex accounts for approximately 80 to 90% of total maltase activity, malto-oligosaccharides also are hydrolyzed by another intestinal enzyme, glucoamylase-maltase. Since there are two types of enzymes, which are capable of splitting malto-oligosaccharides, oligomers with α1,6-linkages such as isomaltose are less effectively digested in the gut in comparison to malto-oligosaccharides (Heymann *et al.,* 1995).
It is, therefore, expected that the fraction of BioNeutra's IMO products composed of isomaltose, maltose, and panose would be digested in the small intestine and absorbed as glucose following oral administration. Only the larger oligosaccharides with higher degrees of polymerization are expected to be more resistant to hydrolysis and, thus, pass into the lower gastrointestinal tract, where they would be fermented.

XIII.2.3 Acute Studies

In rats, an IMO mixture (IMO-900) consisting of di-, tri, and larger oligosaccharides *(i.e.,* 52.5, 25.4, and 15.2%, respectively) exhibited a very low order of acute oral toxicity with LD_{50} values estimated to be greater than 44 g/kg body weight (Kaneko *et al*., 1990). At the 44 g/kg body weight dose level, 2 of 6 rats died.

XIII.2.4 Subchronic and Chronic Studies

Groups of 8 male Sprague-Dawley rats (5 weeks old) were administered diets containing 0 (corn starch control) or 20% (approximately 20 g/kg body weight/day) of sucrose or preparations of isomalto-oligosaccharides (lsomalto-900P®; Showa Sangyo Co. Ltd.), maltose, or FOS, added at the expenses of starch, *ad libitum* for a period of 35 days (5 weeks) (Kaneko *et al.,* 1992; English-language abstract and tables). The IMO product comprised 33.4% isomaltose, 11.8% panose, 14.3% isomaltotriose, 15.7% isomaltotetraose, and 10.3% other isomalto-oligosaccharides. Rats were weighed periodically throughout the study period. At study termination, rats were killed and the liver, kidneys, stomach, small intestine, cecum, cecal contents, colon, and retro-abdominal adipose tissue removed and weighed. All rats were observed to gain weight during the feeding period; however, rats in the group receiving the IMO mixture exhibited significantly lower mean final body weights and mean body weight gain in comparison to rats treated with 20% sucrose-supplemented diets. Otherwise, no statistically significant differences in mean final body weights or weight gain were reported between IMO- treated rats and controls or rats administered FOS or maltose in the diet.

With the exception of the IMO group, rats administered FOS in the diet exhibited a significantly lower food intake compared to all other groups. Food intake of IMO rats was only slightly greater than the food intake of rats provided FOS in the diet and slightly lower than the intake of food of control or maltose-treated animals; however, sucrose-treated rats consumed significantly more food than IMO- or FOS-treated rats. Food utilization efficiency of IMO-treated rats was significantly lower only compared to controls and was about 96 to 97% that of the other test groups. Relative to the nutritional value provided by maltose, the body weight-to-food intake ratio of IMO-treated rats was suggestive of an 80% energy value for the IMO mixture. Relative liver weights of rats fed the IMO-supplemented diet were significantly lower in comparison to those fed the sucrose-supplemented diet; however, no significant difference in relative liver weights were observed between IMO-treated rats and those fed the control, maltose-, or FOS-supplemented diets. The study also included evaluation of a number of serum and lipid levels. Mean serum triglyceride levels were reported to be significantly lower in rats fed the IMO-supplemented diets in comparison to rats fed the control or sucrose-supplemented diets, but were significantly higher than serum triglyceride levels in rats fed the FOS-supplemented diets. Serum triglyceride levels of FOS-treated rats were significantly lower than levels reported in any of the other groups. Although several other statistically significant variations were observed in various lipid parameters between different test groups, serum

cholesterol, high-density lipoprotein (HDL)-cholesterol, phospholipids, and NEFA and liver cholesterol, triglyceride, and phospholipid levels of IMO-treated rats were similar to controls. Additionally, maltase and isomaltase activities of the jejunal mucosa obtained from rats in each of the treatment groups were assessed using *in vitro* digestion models (enzymes incubated with 1% maltose or isomaltose, respectively). Similar levels of enzymatic activity were observed in control rats and those provided the IMO mixture in the diet. Conversely, rats receiving fully digestible sucrose in the diet exhibited lower maltase and isomaltase activities compared to controls. Rats provided FOS in the diet also had decreased maltase activity and those receiving maltose exhibited reduced isomaltase activity. The potential toxicity of an IMO preparation also was evaluated in an unpublished 6-week feeding study in 2-month-old male Sprague-Dawley rats (Day and Chung, 2004). Groups of 5 to 6 rats were administered 0, 5, 10, or 20% of an IMO mix in the diet resulting in daily dose levels of approximately 0, 5,10, and 20 g IMO/kg body weight, respectively. Disaccharides accounted for 6.9% of the composition, whereas panose and larger oligosaccharides (up to DP 7) made up 28.4 and 64.4% of the IMO product, respectively. Food intake and body weight gain were recorded twice weekly throughout the 6-week treatment period. At the end of the study period, animals were necropsied, and several major organs *(i.e.,* heart, spleen, kidneys, lungs, and cecum) excised and weighed. Weight gain and food intake were comparable among all groups of rats; however, a positive trend was reported for increased food intake in the IMO-treated groups. Apart from increased cecal weights reported at the mid- and high-dose levels *(i.e.,* 10 and 20%, respectively), no other variations were observed in organ weights of test animals compared to controls. As suggested by the authors, increased cecal weights were likely reflective of an induction in the population of fermentation bacteria. In rats administered the I MO preparation in the diet, a dose-dependent reduction in abdominal fat also was reported.

Kaneko *et al.* (1990; English-language abstract and tables) performed a 1-year-long study in which male Wistar rats were administered an IMO product (IMO-900P; Showa Sangyo Co., Ltd.) in the drinking water at a concentration of 0 (control) or 3%. Based on the intake of drinking water, rats were estimated to consume between 2.7 to 5 g of the IMO mixture per kg body weight per day. Di-, tri-, and larger oligosaccharides comprised 38, 25.2, and 23.7% of the product's composition, respectively. Body weights of IMO-treated males remained comparable to those of control animals during the treatment period. With the exception of slight, but statistically significant reductions in hemoglobin and hematocrit levels and serum alanine aminotransferase (ALT) activity in males drinking IMO-supplemented water, no other differences in a series of standard hematological and biochemical parameters, including aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activity and total cholesterol and triglyceride levels, were noted between the 2 groups.

Blood urea nitrogen (BUN) levels were only reduced in IMO-treated males during the first month of the study period compared to controls. Since modulation of the immune system has been related to the ingestion of non-digestible carbohydrates (Macfarlane and Cummings,

1999; Swennen *et al.,* 2006), the study also included detailed analysis of white blood cell levels. Although no changes were observed in the absolute number of white blood cells of test rats relative to levels reported in the control group, significant variations in total and individual subgroups of lymphocytes were noted *(i.e.,* elevated levels of total lymphocytes, total T cells, B cells, and helper and suppressor T cells), but only in the first treatment month. At necropsy, neither the gross nor microscopic evaluation revealed any abnormalities in treated males. Administration of the IMO mixture in the drinking water also induced some changes in the intestinal microflora of rats *(i.e.,* significantly decreased levels of *Clostridium).*

Additionally, a few other non-traditional animal toxicity studies were performed, which included some toxicologically relevant endpoints. In a study conducted to assess potential effects of various oligosaccharides on blood lipid levels and intestinal physiology, adult male Sprague-Dawley rats received sponge cakes, with 40% of the standard sucrose content of the cakes replaced by an IMO preparation (composition not specified), mixed in the diet at 30% *(i.e.,* 12% IMO in the diet or approximately 6g/kg body weight/day) for a period of 25 days (Ly *et al.,* 1999). No significant differences were observed in the body weight gain, food intake, food utilization efficiency, levels of BUN, absolute and relative liver weight, length of the small intestine, relative cecal content weight (increase not significant), and relative weight of the cecal wall of test animals compared to the control group. Administration of the IMO preparation in the diet did, however, significantly affect the cecal content pH (decreased), dry fecal weight (increased), and fecal water content (increased). In a similar study, Sung *et al.* (2004) administered a mixture of isomalto-oligosaccharides (composition not specified) in a high-cholesterol diet at a concentration of 6% (approximately 3 g/kg body weight/day) to a group of 9 male Sprague-Dawley rats for a period of 5 weeks. In comparison to a control group of rats fed a similar basal diet without the IMO mixture, but with higher sucrose content, no significant variations were observed in body weight gain, food efficiency, and relative liver weight.

In another study, groups of 10 male Sprague-Dawley rats were provided an IMO product in the diet at concentrations of 0 or 10% (composition not specified) (approximately 10 g/kg body weight/day) for a period of 4 weeks following which diabetes was induced experimentally by streptozotocin (STZ) injection (Chai and Rhee, 2001). Subsequent to the induction of diabetes, rats continued on their respective diets for another 4-week period. In addition to the diabetic control group, a normal control group of rats was included in the study. No variations were observed in body weight, food intake, food utilization efficiency, or relative liver, kidney, and small intestine weights between IMO-treated and basal diet diabetic animals. An increase in the relative weight of the cecum was, however, observed in the IMO-test group in comparison to the diabetic control group. In comparison to the control group in which diabetes was induced, oral treatment with the IMO mixture was associated with a reduction in blood glucose levels; however, serum cholesterol and triglyceride levels of IMO-treated diabetic rats did not differ significantly from those of diabetic controls. Intestinal maltase, sucrase, and lactase activities

of rats administered the IMO product in the diet were comparable to the diabetic control group, while dietary treatment of rats with other oligosaccharides *(e.g.,* xylo-oligosaccharides and FOS) resulted in a reduction in enzyme activity.

XIII.2.5 Reproductive and Developmental Studies

No studies intended to specifically evaluate reproduction or development following treatment with mixtures of isomalto-oligosaccharides were identified. However, mixtures of oligosaccharides such as the IMO product manufactured by BioNeutra consist of both digestible and indigestible oligomers of glucose. Consequently, the digestible portion is expected to be hydrolyzed in the gut to glucose, which is subsequently absorbed, whereas the portion escaping digestion is expected to pass into the lower intestine where it is subjected to fermentation. Glucose is a normal constituent of the diet, which following absorption is utilized in well-characterized pathways of metabolism as a source of energy. The indigestible fraction will not be absorbed and as such is not expected to be associated with any systemic toxicity, including any adverse effects on reproduction or development.

XIII.2.6 Mutagenicity and Genotoxicity Studies

The potential genotoxicity/mutagenicity of an IMO product was evaluated in prokaryotic, as well as in mammalian *in vitro* test systems (Kaneko *et al.,* 1990). Evaluated *in vitro* in a standard battery of *Salmonella typhimurium (i.e.,* TA98, TA100, TA1535, and TA1537) and in *Escherichia* coli WP2uwA with and without metabolic activation, the IMO product did not induce significant increases in the number of revertant colonies at concentration of up to 10% per plate. Likewise, the IMO mixture failed to significantly increase the number of chromosome aberrations in Chinese hamster lung (CHL) cells at concentrations of up to 3% in either the absence or presence of a bioactivation system following a 24- or 48-hour incubation period.

XIII.3 Human Tolerance Studies

Although ingestion of non-digestible carbohydrates is generally associated with beneficial "prebiotic" effects, consumption of mixtures of indigestible isomalto-oligosaccharides at high dose levels may cause excessive gas production, an undesirable end-product of microbial fermentation, and other undesirable gastrointestinal symptoms. In an *in vitro* batch culture study comparing the fermentation properties of several different oligosaccharides *(i.e.,* lactulose, FOS, inulin, and xylo-, galacto-, soybean-, and isomalto-oligosaccharides), incubation of human fecal bacteria in the presence of isomalto- or galacto-oligosaccharides (GOS) for 24 hours was accompanied by the lowest evolution of gas, whereas inulin and lactulose produced the largest quantities of gas (Rycroft *et al.,* 2001).

In a trial investigating the digestibility of an IMO mixture, no gastrointestinal disturbances were observed following single-dose consumption of up to 40g of an IMO product dissolved in

water (Oku and Nakamura, 2003). In comparison, abdominal discomforts [*i.e.,* distension, borborygmus, and flatus (only with FOS)] were reported by all subjects following ingestion of 20g of FOS or galactosyl-sucrose. Although less severe, study participants also complained of gastrointestinal disturbances when provided 10g of FOS for oral consumption.
In a placebo-controlled double-blind study designed to assess the potential for bifidogenic properties related to the consumption of various non-digestible carbohydrates including , isomalto-oligosaccharides (Cerestar; composition not specified), an increase was observed in the severity of some gastrointestinal disturbances *(i.e.,* flatus, bloating, borborygmi, and abdominal pain) over the course of a 7-day treatment period during which subjects (n = 8) consumed daily 10g of the IMO product in 2 equal portions; however, no significant differences were observed among the different test groups *(i.e.,* placebo, FOS, GOS, soybean-oligosaccharides, resistant starch, lactulose, long-chain inulin, and IMO mixture) (Bouhnik *et al.,* 2004). Moreover, none of the subjects experienced diarrhea. Kohmoto *et al.* (1988) conducted a study to assess the potential effects of an IMO mixture on the composition of the human microflora in 6 healthy adult men (26 to 48 years of age) and 18 elderly persons (5 males and 13 females; 50 to 93 years of age) who consumed daily 20g of an IMO product (lsomalto-900®) incorporated in either a coffee jelly or a mizuyokan jelly in alternation every 3 days. Each 20 gram-dose of the IMO product contained 13.5g of isomalto-oligosaccharides. The group of healthy males received the IMO-containing jellies for a period of 10 days, whereas the elderly group was treated for a period of 14 consecutive days. None of the subjects enrolled in this study experienced diarrhea as a result of treatment with the IMO-supplemented food products. Conversely, an improvement in the fecal consistency was observed; however, increased flatulence was reported by 2 of the 24 participants in the first few days of the study, which subsided naturally as the study progressed. Analysis of fecal samples revealed increased levels of bifidobacteria.

A 4-week trial was conducted with a group of 20 (8 men and 12 women) hemodialysis patients (mean age 63.6 years; range 44 to 80 years) to evaluate the therapeutic efficacy of 30 g of an IMO mixture (King-Tech Chemical and Starch Co., Ltd.; 12% isomaltose, 29.1% panose, 2.6% isomaltotriose, 9.9% isomaltotetraose, 2.5% dextrin, and 43.9% monosaccharides and malto-oligosaccharides), provided twice daily in equal portions of 15 g, in the treatment of chronic severe constipation and its potentially beneficial effect on the lipidemic profile (Wang *et al.,* 2001). Although all study participants completed the trial, some mild gastrointestinal side effects were reported. Specifically, patients experienced diarrhea (5%), abdominal distension (10%), tormina (10.5%), borborgymi (6.1%), and abdominal spasms (4.5%). In comparison to the pre-treatment and follow-up period, the severity of all the gastrointestinal symptoms, with the exception of diarrhea, increased significantly with the ingestion of the IMO products; however, none of the subjects withdrew from the study as a result of the gastrointestinal symptoms. Treatment with the IMO preparation was associated with a significant increase in the number of bowel movements during the 4-weeks study period. Additionally, biochemistry and lipid parameters also were evaluated in this study. Consumption of the IMO product over

the 4-week treatment period was associated with decreased levels of total cholesterol and triglycerides, as well as increases in levels of hemoglobin and hematocrit, and HDL-cholesterol. No significant variations were observed between pre- and post-treatment blood glucose, BUN, creatinine, albumin, protein, calcium, and phosphorous values.

In a clinical investigation of the potential for isomalto-oligosaccharides to alter the intestinal microflora, no gastrointestinal disturbances were observed in a group of 31 healthy volunteers ingesting IMO or 15g of an IMO product per day (IMO-900P; composition in Japanese) for a period of 5 weeks (Kaneko *et al.,* 1993).

In another 5-week study evaluating the potentially beneficial effect of an IMO mixture on bowel and function in a group of 7 elderly men (mean age 75.2 years), no adverse effects were reported following consumption of up to 24 g of an IMO supplement (Chen *et al*., 2001). The dose level at which the IMO product was ingested was increased gradually from 8 to 24 g over the first 10 days of the study period. The supplement contained 42.7% of isomaltose, panose, isomaltotriose, isomaltotetraose, and dextrin *(i.e.,* "active" components), such that at the highest dose level subjects received 10g of "active" components. In comparison to baseline values, daily consumption of the IMO mixture was associated with significantly elevated levels of fecal acetate, propionate, and total short-chain fatty acids, but not butyrate. Increases in short-chain fatty acid levels were not accompanied by a decrease in fecal pH. Consumption of the IMO preparation also induced a close to 2-fold significant increase in fecal bacterial mass. With the exception of a small, but statistically significant reduction in mean serum sodium levels, no significant variations were observed in a number of other biochemical parameters *(i.e.,* glucose, total protein, albumin, calcium, phosphorus, and potassium), including no changes in several lipidemic indices *(i.e.,* triglycerides, total cholesterol, and HDL-cholesterol).

XIII.3.1 Consumption by Elderly people, Children & Infants

Scientific studies showed that, consumption of IMO (up to 30g/day) by elderly subjects was safe and did not exert any adverse effects (Wang, *et al.,* 2001). Although short-term safety and effects on the total number of bifidobacteria in stools have been repeatedly demonstrated, there are no data on long-term benefits in elderly populations.

Oligosaccharides are one of the main components of human milk, which contains, on average, 10 g/L of neutral oligosaccharides and 1g/L of acidic oligosaccharides (Boehm, *et al*., 2003). The composition of human milk oligosaccharides is very complex and more than 100 different oligosaccharide-like structures are known. They express an essentially bifidogenic effect and confer anti-infective properties to human milk.

Human milk or formula feeding in the neonatal period may have different effects on the colonization of the gastrointestinal tract, which is sterile at birth but becomes colonized during vaginal delivery with mainly the intestinal flora of the mother. Breast-fed infants show a predominance of bifidobacteria and/or lactobacilli in comparison to formula-fed infants, who develop an intestinal microflora richer in Enterobacteria and gram-negative organisms (Harmsen, *et al.,* 2000). The predominance of bifidobacteria in breast-fed infants is thought to contribute to a lower risk of enteric infections. Oligosaccharides, like IMO, might contribute to the natural defense against infections in two ways, that is, either directly, acting as receptor analogues to prevent attachment of enteropathogens on the epithelial surface and interacting with immune cells, or indirectly, altering the commensally gut microflora toward a more healthier composition (Vandenplas, *et al*., 2002, Boehm, *et al.,* 2003).

XIII.3.2 Review of Clinical Trials in Infants

To date, only few published clinical trials have evaluated prebiotic substances in dietetic products for term and preterm infants, respectively.

Moro *et al*., (2002) have tested a synergistic mixture of neutral galacto-oligosaccharides (GOS, derived from lactose) and long-chain fructo-oligosaccharides (FOS, derived from chicory). After 28 days of feeding, the term infants fed formula supplemented with the GOS/FOS mixture, at concentration of 0.4g/100 mL or 0.8g/100 mL, respectively, exhibited a dose-dependent stimulating effect on the growth of bifidobacteria and lactobacilli in the intestine. This combination resulted also in an increase of stool frequency and a reduction of stool consistency, closer to reference breast-fed infants. Boehm, *et al*., (2002) tested in preterm infants a mixture of 90% GOS and 10% FOS, with a distribution of molecules and a concentration of total oligosaccharides close to human milk, added to a standard preterm formula. The supplementation with the mixture of oligosaccharides resulted in a clearly bifidogenic effect, accompanied by more frequent softer stools. Furthermore, the Ca/P ratio in the urine was similar to that observed in breast-fed infants, suggesting also an influence of prebiotics on calcium absorption.

A study with term infants has evaluated the nutritional efficacy and bifidogenic characteristics of a new infant formula containing partially hydrolyzed whey proteins, modified fats with high ß-palmitate contents and prebiotics with starch (Schmelzle, *et al.,* 2003). According to the results, the new formula supported satisfactory growth, led to higher counts of bifidobacteria in the feces, and was well tolerated. A recent prospective study, suggesting that formula-fed infants with "minor" gastrointestinal symptoms (such as colic, regurgitation and constipation) improve within two weeks of feeding the same type of formula, still requires confirmation from randomized controlled trials (RCTs) (Savino, *et al*. 2003).

Other data from clinical trials, which have been communicated but are still not yet published, consider the bifidogenic effects of either prebiotic-enriched formulas or solid weaning foods with added prebiotic galacto-oligosaccharide (Knol, *et al*., 2002). Accordingly, a reduction of pathogens has been associated with the consumption of prebiotics. Other communications suggest that the GOS/FOS are detectable in stools in amounts similar to those displayed in infants given human milk oligosaccharides (Knol, *et al*., 2002, Knol, *et al*., 2003, Boehm, *et al.,* 2003, Scholtens, *et al*., 2003). Furthermore, the pattern of fecal short-chain fatty acids in infants fed the oligosaccharide mixture is similar to that of breast-fed infants but was significantly different from that of a group of infants fed with an un-supplemented formula (Bohem, *et al*., 2004).

In another study, the data of experimental research and clinical studies with a prebiotic mixture containing 90% short-chain galacto-oligosaccharides and 10% long-chain fructo-oligosacchrides were obtained (Günther, *et al.* 2005). The data demonstrate that, with this prebiotic mixture, the growth of bifidobacteria and *lactobacilli* can be stimulated, the fecal pH can be decreased, and the presence of pathogens can be reduced to levels similar to those of breastfed infants. Thus, prebiotic oligosaccharides such as the studied mixture provide beneficial effects for formula-fed infants (Günther, *et al.* 2005).

Although short-term safety and effects on the total number of bifidobacteria in stools have been repeatedly demonstrated, there are some data on long-term benefits and safety as well. In a study (Gigi, 2007), a mixture of long chain inulin (5-60 monomers) in combination with galactooligosaccharide (GOS) (2-7 monomers) has been added to infant formula in Europe in a a 10-90% ratio for over 5 years. Clinical studies have demonstrated that these prebiotic formulas have significant effects on flora composition, improve stool consistency, decrease intestinal permeability, and reduce the incidence of gastrointestinal (GI) and respiratory infection and atopic dermatitis. Oligofructose in weaning foods consumed by toddlers increases fecal Bifodobacteria counts and decreases fecal clostridia counts during consumption, leading to softer stools and fewer fever episodes and other GI symptoms.

XIII.3.3 Possible Adverse Affects

A dose of IMO higher then the 30 gm/day, there is a possibility of gastrointestinal symptoms (like increased flatulence, bloating or soft stool) or in extreme case diarrhea. Gastric upset is expected with an extremely high dose (*i.e*., about 4-5 times higher than the no-effect level for laxative effects in humans). Consumption of IMO within the recommended dosage (30-40 gm per day) is not expecting to pose any health-related concern.

XIII.4 Allergenicity Considerations

As described in detail in Section II.1, VitaSugar-IMO is subjected to extensive purifications during the manufacturing process, which minimizes the possibility of contamination of the final product with residual materials from the enzymes *(i.e.,* α-amylase, transglucosidase, and pullulanase) or the yeast *(Saccharomyces cerevisiae)* used during production. Specifically, the product is subject to passage through a series of ion exchange columns (Anion & Cation), as well as triple-effective evaporators as part of the final purification process.

XIII.4.1 Chemical Considerations

Several lots of the manufactured IMO-syrup and IMO-powder were analyzed to verify that the manufacturing process produced consistent products within the product specifications. The chemical composition of the IMO products was determined by analyzing the glucose, isomaltose, and oligosaccharides (DP3 to DP9) contents using HPLC. A summary of the chemical product analysis for 3 non-consecutive lots (*i.e*., Lot # 6, 7 & 9) of VitaSugar-IMO Syrup and 2 non-consecutive lots (*i.e*., lot # 4 & 8) of VitaSugar-IMO Powder are presented in Section I.7.2 (Table I.7.2-1 & I.7.2-2).

XIII.5 Safe for Consumption: (Expert Panel's Self-affirmed GRAS)

Last year on March 12th, 2007, BioNeutra received a self-affirmed GRAS (Generally Regarded As Safe) for Isomalto-oligosaccharide from the GRAS Expert Panel for VitaSugar-IMO as a food ingredient. The Expert Panel was consisted of the qualified scientific experts: Dr. Joseph Borzelleca (Medical College of Virginia), Dr. John Doull (University of Kansas Medical Center), and Dr. Robert Nicolosi (University of Massachusetts, Lowell) (see Appendix H).
The Panel, independently and collectively, critically examined a comprehensive package of publicly available scientific information and data compiled by Cantox Health Sciences International (Toronto) from the literature and other published sources through December of 2006. The data evaluated by the Panel included information pertaining to the method of manufacture and product specifications, analytical data, intended use levels in specified food products, and consumption estimates for IMO, as well as comprehensive literature on the safety of isomalto-oligosaccharides.

Following independent, critical evaluation of such data and information, the Panel unanimously concluded that under the conditions of intended use in traditional foods described herein, meeting appropriate food-grade specifications, and manufactured and used in accordance with current good manufacturing practice, isomalto-oligosaccharide is GRAS based on scientific procedures.

Based upon these findings, a formal Notification has been made to FDA for full GRAS, subsequent to the pre-notification meeting with the FDA on December 18th, 2007.
BioNeutra is expecting to receive a letter of no-objection from FDA in late 2008.

XIII.6 Overall Summary & Conclusion Pertaining to Safety

Summary of oral subchronic/chronic animal toxicity studies and clinical tolerance studies with Isomalto-oligosaccharide (IMO) products are presented in Table XIII.6-1 and XIII.6-2, respectively.

Table XIII.6-1 Summary of Oral Subchronic and chronic Animal Toxicity Studies with Isomalto-oligosaccharide (IMO) Products

Species (Strain, sex, No./group)	Duration	Concentrations (Dose levels)	Study-end Results[1]	Reference
Rat (Sprague-Dawley, male, 8/group)	35 days	0 (corn starch) or 20% in diet (~20 g/kg bw/day)	↓ in FUE and TG; No Δ in body weight, body weight gain, food intake, cecal contents, and relative organ weights (stomach, small intestine, cecum, colon, liver, kidney, retroabdominal adipose tissue); No Δ in serum and liver total Ch and PL, and serum HDL-Ch and NEFA.	Kaneko *et al*. (1992)
Rat (Sprague-Dawley; male; 5-6/group)	42 days	0 (Purina rat chow), 5, 10, or 20% in diet (~0, 5, 10, and 20 g/kg bw/day, respectively)	↑ in weight of cecum at 10 and 20%; ↓ (dose-dependent) in abdominal fat gain (normalized for food intake); No Δ in food intake, body weight gain, and absolute heart, spleen, kidneys, lungs, and brown and white adipose tissue weight.	Day and Chung (2004)
Rat (Wistar, males, 8/group)	365 days (1 year)	0 or 3% in drinking water (~0 and 3-5 g/kg bw/day, respectively)	No Δ in body weight gain and body weights, AST, ALP, LDH, Cre, BUN (↓ 1st month), UA, T-Ch, TG, WBC, and RBC; ↓ in serum Hb, Ht, and ALT; No gross or histopathological abnormalities. ↑ in *Lactobacillius* count and *Bifidobacterium* frequency of occurrence; ↓ *Clostridium*.	Kaneko *et al*. (1990)

No Δ = No variations between test and control animals; ALP = alkaline phosphatase; ALT = Alanine aminotransferase; AST = aspertate aminotransferase; BUN=Blood urea nitrogen; Ch=Cholesterol; Cre=Creatinine; FUE=Food utilization efficiency; Hb=Hemoglobin; HDL-Ch=High-density lipoprotein cholesterol; Ht=Hematocrit; LDH=Lactate dehydrogenase; NEFA=non-esterified fatty acids; PL=Phospholipids; RBC=Red Blood Cell count; TG=Triglycerides; UA=Uric acid; WBC=White Blood Count;
[1]Study-end results unless otherwise indicated; Results are provided for test animals relative to controls.

Table XIII.6-2 Summary of Clinical Tolerance Studies Conducted with Isomalto oligosaccharide (IMO) Products

Study Population and study design	Duration	Daily Dose Levels	Results	Reference
9 healthy males (~26 years old) and 29 females (~23 years old)	Single dose	10, 20, or 40 g	No GI disturbances.	Oku and Nakamura (2003)
81 healthy males and 119 females (~30 years old) (8 ingested IMO mix); double-blind placebo-controlled study	7-day run-in and 7-day treatment period	0 (placebo) or 10 g/day (2 equal portions)	↑ (slight) in excess flatus, bloating, borborygmi, and abdominal pains (all mild symptoms) *vs*. run-in period; however, No Δ in any of the GI symptoms *vs*. placebo control; None of the subjects experienced diarrhea.	Bouhnik *et al*. (2004)
6 healthy males (26-48 years old)	10 days	20 g/day	None of the subjects experienced diarrhea; only transient increase in flatulence in 2/24 subjects.	Kohmoto *et al*. (1988)
18 older subjects (5 males and 13 females; 50-93 years old)	14 days			
20 healthy females and 11 males (22 subjects w/ history of constipation) (~27 – 30 years old)	21 days (total)[2]	10 or 15 g	No GI disturbances. ↑ Defecation frequency in constipated subjects w/ 15 g IMO mix *vs*. 1st week.	Kaneko *et al*. (1993)
8 male and 12 female hemodialysis patients (~64 years old)	14-day run-in and 28-day treatment period	30 g/day (2 equal portions)	↑ in severity of distension (10%)[1], tormina (10.5%), borgorgymi (6.1%), spasms (4.5%) and in bowel movements; No Δ in diarrhea (5%). Clinical Chemistry ↑ in Hb, Ht, and HDL-Ch *vs*. run-in; ↓ in Tg, Ch; No Δ in glucose, albumin, total protein; BUN, Cre, Ca^{2+}, P, and LDL-Ch.	Wang *et al*. (2001)
7 elderly males w/ history of constipation (~75 years old)	30-day run-in and 30-day treatment period	↑ from 8 to 24 g (1st 10 days)	↑ in defecation frequency and wet and dry fecal weight per day and stool sample; no reports of GI disturbances. Clinical Chemistry ↑ in Na^{+}; No Δ glucose, total protein, albumin, TG, Ch, HDl-Ch, Ca^{2+}, P, and K *vs*. run-in.	Chen *et al*. (2001)

No Δ = No change; BUN = Blood urea nitrogen; Ca^{2++}=Calcium; Cre=Cretinine; Ch=Cholesterol; GI=Gastrointestinal; HB=Hemoglobin; HDL-Ch=High-density lipoprotein cholesterol; Ht=Hematocrite; LDL-Ch=Low density lipoprotein cholesterol; K=Potassium; Na^{+}=Sodium; P=Phosphorus; TG=Triglycerides.

[1] Percent in parentheses indicates percent of patients experiencing GI symptoms.

[2] 1st week run-in period; 2nd and 3rd week IMO mix ingestion; 4th week break; 5th week IMO mix ingestion.

VitaSugar-IMO consists of a mixture of malto- and isomalto-oligosaccharides and is proposed for use as an alternative sweetener in a variety of food products including baked goods and baking mixes, beverages and beverage bases, breakfast cereals, condiments and relishes, dairy product analogs, mayonnaise and mayonnaise-type dressings, salad dressings, frozen dairy deserts and mixes, gelatins, puddings, and fillings, gravies and sauces, hard and soft candies, jams and jellies, milk and milk products, meat and nut products, processed fruits and vegetables and fruit and vegetable juices, snack foods, sugar substitutes, sweet sauces, toppings, and syrups, and meal replacement bars and mixes. Based on the body weight-to-food intake ratio of rats treated with a mixture of isomalto-oligosaccharides for a period of 35 days and expiration of CO_2 by sedentary and active human subjects following consumption of an IMO preparation, the nutritive value of IMO preparations was estimated to be approximately 70 to 80% relative to that of maltose (2.7 to 3.3 kcal/g) (Kaneko *et al.,* 1992; Kohmoto *et al.,* 1992). VitaSugar-IMO is proposed for use at maximum levels of up to 15.6 g/serving. Based on 2006 production volume estimates of total refined sugar (sucrose), and assuming 2% replacement of it with VitaSugar-IMO, the *per capita* intake was estimated to be 1.38g VitaSugar-IMO/person/day; however, based on the anticipated production volume of VitaSugar-IMO by 2010, the *per capita* intake is estimated to be only 0.97g VitaSugar-IMO/person/day. Alternatively, assuming that a person will consume 2 servings of food per day to which VitaSugar-IMO has been added at levels of up to 15.6g/serving, a daily intake level of not more than 31.2g VitaSugar-IMO/person is estimated.

VitaSugar-IMO is produced *via* the enzymatic degradation of wheat or potato starch and subsequent removal of glucose by yeast, followed by extensive purification of the resulting mixture of isomalto-oligosaccharides and formulation to produce a powder or syrup product. Several down-stream purification steps are employed in the manufacturing of VitaSugar-IMO to minimize the potential for the occurrence of any residues of the biocatalyst or other processing aids used during the production process in the final products.

Isomalto-oligosaccharide preparations such as VitaSugar-IMO consist of a mixture of malto- and isomalto-oligosaccharides, as well as smaller saccharides including maltose, isomaltose, and panose. *In vitro* and *in vivo* animal and human studies evaluating the degradability demonstrate that isomalto-oligosaccharides are resistant to enzymatic hydrolysis in the upper gastrointestinal tract and remain unabsorbed following oral consumption. Consequently, there is no risk of systemic toxicity, and the effects, if any, are expected to be limited to those on the structure and function of the gastrointestinal tract. In the large intestine, the unabsorbed isomalto-oligosaccharides are subject to microfloral fermentation, resulting in the generation of short-chain fatty acids with the fermentation products subsequently absorbed and utilized in well-characterized biochemical pathways. Conversely, the smaller saccharides, such as the disaccharides maltose and isomaltose are subject to enzymatic hydrolysis. Furthermore, studies indicate that the malto-oligosaccharides also are subject to partial hydrolysis in the upper portion of the gastrointestinal tract. Glucose, produced as a result of the hydrolysis of the digestible saccharides, is absorbed and used by the body as a source of energy. Generally, in comparison to other oligosaccharides such as FOS or galactosyl-sucrose, the fermentability of IMO products in the colon is relatively limited in humans (Oku and Nakamura, 2003)

indicating that the majority of the products is hydrolyzed and absorbed as glucose following oral administration.

The results of the animal toxicity studies and human tolerance studies provide sufficient support that consumption of VitaSugar-IMO, at the levels associated with the intended uses (not greater than 30g/person/day), would not be expected to be associated with any adverse effects. In a short-term animal study with a limited number of toxicologically significant end-points in which several different digestible and non-digestible sugars were compared, 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 20g/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 observed 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 20g/kg body weight/day), no significant variations with the exception of increased cecal weights in mid- and high-dose test animals (10 and 20% or 10 and 20 g/kg body weight/day, respectively) were observed in comparison to untreated 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 single-dose level chronic toxicity study, male Wistar rats were provided 3% of an IMO product in drinking water, resulting in daily dose levels in the range of approximately 3 to 5g/kg body weight (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, which were conducted with physiologically normal, as well as diabetic Sprague-Dawley rats and were designed primarily to assesses the potential effects of IMO preparation on metabolic end-points and intestinal physiology, but also included at least determinations of body weights and body weight gain, food intake, and liver weights, differences observed between the rats receiving IMO mixtures in the diet at concentrations in the range of 6 to 12% (approximately 3 to 10g/kg body weight/day, respectively) and controls were largely limited to increased weights of the cecum or cecal contents (Ly *et al.,* 1999; Chai and Rhee, 2001; Sung *et al.,* 2004).

While no studies were identified which specifically assessed the potential effect of IMO consumption on reproduction or development, in light of the lack of systemic absorption of the larger isomalto-oligosaccharides which comprise VitaSugar-IMO and hydrolysis of the smaller saccharides to glucose, there is no reason to suspect any potential for systemic toxicity, as

confirmed by the results of the oral short- and long-term toxicity studies, including absence of any adverse effects on reproduction or development. 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).

By virtue of the disposition of IMO preparations *(i.e.,* hydrolysis of the smaller saccharides and lack of absorption of the larger isomalto-oligosaccharide components followed by microbial degradation), mixtures of isomalto-oligosaccharides are not expected to be associated with any systemic adverse effects in humans; however, given that a portion of the IMO preparation is undigested and, instead are fermented in the colon, consumption of isomalto-oligosaccharides may lead to some gastrointestinal discomforts. Several human studies ranging in duration from 7 to 30 days were conducted to assess 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). Conversely, in a number of other studies, increases were reported in the severity or incidence of various gastrointestinal symptoms *(e.g.* flatulence, abdominal pain and distension, bargorygmi) following consumption of 10 to 30 g of IMO preparations for 7 to 28 days; however, in none of these studies did the study subjects experience increased incidences or severity of diarrhea (Kohmoto et al., 1988; Chen *et al.,* 2001; Bouhnik *et al.,* 2004).

A few authors have reported a threshold value of 1.5g/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. Although the studies demonstrate some variability in the occurrence of gastrointestinal disturbances following ingestion of I MO preparations, such variations are expected in light of the compositional differences among the IMO mixtures. In 2 trials which also included evaluations of biochemistry, no significant variations were observed in several clinical chemistry parameters *(e.g.,* total protein, albumin, BUN, creatinine) 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 did, however, exhibited elevated hemoglobin and hematocrit values following ingestion of the IMO mixture, which as suggested by the study authors may have been at least in part due to enhanced iron absorption (Wang *et al.,* 2001).

Examined *in vitro,* mixtures of isomalto-oligosaccharides were shown to increase levels of bifidobacteria (Kohmoto *et al.,* 1988; Rycroft *et al.,* 2001), but only few other human bacterial species (Kohmoto *et al.,* 1988). In rats and mice, repeat administration of IMO mixtures in the

diet also was associated with increases in *Bifidobacterium* and *Lactobacillus* levels, paralleled by reductions in the growth of *Clostridium* species (Kaneko *et al.,* 1990; Qing *et al.,* 2003). Although results of some linear human trials *(i.e.,* comparison of pre- and post-treatment levels of colonic bacteria) have provided evidence to suggest that the consumption of IMO mixtures increased levels of bifidobacteria in the colon (Kohmoto *et al.,* 1988, 1991; Kaneko *et al.,* 1993; Chen *et al.,* 2001; Qing *et al.,* 2003), a clinical trial performed with a placebo group did not confirm these results (Bouhnik *et al.,* 2004).

Specifically, no statistically significant differences were observed in the fecal levels of bifidobacteria between subjects ingesting daily a total of 10g of an IMO mixture and the placebo group. Results of the study conducted by Bouhnik *et al.* (2004) suggest that the majority of the IMO mixture is efficiently hydrolyzed to glucose upon consumption. It should be noted, however, that the composition of the IMO product used in this study was not identified and, thus, the possibility that the mixture used in the placebo-controlled study differed from the IMO products used in the linear studies *(e.g.,* was characterized by a larger content of digestible material) cannot be excluded.

Consumption of non-digestible carbohydrates has been related to several nutritional effects, including increases in short-chain fatty acid production, enhanced bile acid excretion, and changes in mineral bioavailability. Levels of fecal short-chain fatty acid levels were measured in a few of the animal and human studies to determine whether ingestion of IMO products resulted in changes in short-chain fatty acid levels. While an increase was observed in lactate and acetate levels in an *in vitro* study in which fecal bacteria were incubated with isomalto-oligosaccharides, no variations were observed in propionate and butyrate levels (Rycroft *et al.,* 2001). Conversely, in a pre-clinical rat study, no changes were observed in levels of individual short-chain fatty acids or in the pH level of the cecum following administration of 5% of an IMO mixture in the diet (unspecified period of time) (Ohta *et al.,* 1993). In human trials, results were generally more comparable to those observed in the *in vitro* assays, with increases noted in acetate, propionate, and total short-chain fatty acid levels, but not in butyrate following daily ingestion of 10 to 24g of IMO-containing mixtures for a period of 4 to 5 weeks (Kaneko *et al.,* 1993; Chen *et al.,* 2001). Generally, the absence of a consistent effect related to the consumption of IMO mixtures on bacterial induction of the human microflora, as well as only minor variations in short-chain fatty acid levels, especially in comparison to other non-digestible carbohydrates such as FOS or galactosyl-sucrose, do not support extensive fermentation of the IMO mixture in the colon and suggest that most components of IMO preparations are in fact digested to glucose in the upper segments of the gastrointestinal tract.

Presence of increased amounts of undigested material in the colon has been also related to greater bile acid excretion in the stool; however, 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 observed between rats administered IMO mixtures in the diet and controls (Ly *et al.,* 1999; Chai and Rhee, 2001;

Sung *et al.,* 2004). In contrast, significant reductions were observed in serum triglyceride and total cholesterol levels, in association with increased HDL-cholesterol levels following daily consumption of 30 g of an IMO mixture compared to pre-treatment values in a human trial (Wang *et al.,* 2001). Furthermore, 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 ensuing changes in mineral absorption. In the only study in which absorption of several minerals 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).

4.0 EVALUATIONS AND CONCLUSIONS

Overall, when viewed in its entirety, the scientific evidence presented indicates that under conditions of intended use in foods, VitaSugar-IMO, a mixture of Isomalto-oligosaccharide, would not produce any adverse health effects. Vitasugar-IMO is produced by BioNeutra in accordance with current GMP and meets appropriate food grade specificity. Following oral consumption, the maltose-oligosaccharide fraction of the mixture, as well as the disaccharides are largely hydrolyzed in the gastrointestinal tract to glucose, which is subsequently absorbed and utilized by the body in well characterized metabolic pathways. The remaining undigested IMO pass through the gastrointestinal tract and are subjected to bacterial fermentation in the colon. Consequently, there is no risk of systemic toxicity related to the ingestion of IMO. Moreover, the safety of IMO mixtures in confirmed by a series of published animal toxicity studies, as well as several human tolerance studies reporting no adverse toxicological effects relevant to the conditions of intended use in foods.

In conclusion, there is a substantial body of evidence to support the safety of VitaSugar-IMO (Isomalto-oligosaccharide), a novel food ingredient based on its lack of prior history of use in the European Community. On the basis of the available toxicology data, nutritional evaluations, and appropriate food-grade specifications and manufacturing protocols in accordance with GMP, it is concluded that VitaSugar-IMO does not present a significant risk for human health at the intake, which would result from its intended uses in food.

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