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Spherix Consulting Group

January 24, 2022

Chris Kampmeyer, M.S.
Regulatory Review Scientist
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
CPK-2 Building, Room 2092
5001 Campus Drive, HFS-225
College Park, MD 20740

Dear Mr. Kampmeyer:

Enclosed please find a CD containing "Generally Recognized As Safe Determination for the Use of VITAGOS™ IF in Non-Exempt Term Infant Formula and Selected Conventional Foods", Form 3667, and all corresponding references. The data and information that serve as the basis for this GRAS notification is available for review and copying at reasonable times at the office of Dietrich Conze, PhD, Managing Partner, Spherix Consulting Group, Inc., 751 Rockville Pike, Unit 30-B, Rockville, MD 20852, Telephone: 240-367-6089; Email: dconze@spherixgroup.com, or will be sent to FDA upon request.

Please note that this GRAS Notice was previously submitted and went through the pre-filing review, and has now been updated based on our discussion with you on January 18, 2022. We thank you for taking the time to review this submission and ask that it be processed for filing as soon as possible. Should you have additional questions, please let us know.

Sincerely,

Dietrich B. Conze, PhD
Managing Partner

Spherix Consulting Group, Inc.
751 Rockville Pike, Unit 30-B, Rockville, MD 20852
301-557-0375; info@spherixgroup.com

Generally Recognized As Safe Determination for the Use of VITAGOS™ IF in Non-Exempt Term Infant Formula and Selected Conventional Foods

Prepared for:

Vitalus Nutrition Inc.
3911 Mt. Lehman Rd.
Abbotsford, BC, Canada V2T 5W5

Prepared by:

Spherix Consulting Group, Inc.
751 Rockville Pike, Unit 30-B
Rockville, MD 20852

January 24, 2022

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I. SIGNED STATEMENT OF THE CONCLUSION OF GENERALLY RECOGNIZED AS SAFE (GRAS) AND CERTIFICATION OF CONFORMITY TO 21 CFR §170.205-170.260

A. SUBMISSION OF GRAS NOTICE

Vitalus Nutrition Inc. is hereby submitting a GRAS notice in accordance with subpart E of part 170.

B. NAME AND ADDRESS OF THE SPONSOR

Vitalus Nutrition Inc.
3911 Mt. Lehman Rd.
Abbotsford, BC, Canada V2T 5W5

C. COMMON OR USUAL NAME

Galacto-oligosaccharides (GOS), also known as oligogalactosyllactose, oligogalactose, oligolactose, transgalactosylated oligosaccharide, and transgalacto-oligosaccharide.

D. TRADE SECRET OR CONFIDENTIAL INFORMATION

This notification does not contain any trade secret or confidential information.

E. INTENDED USE

VITAGOS™ IF will be added to powdered, ready-to-feed, and concentrated liquid versions of cow milk-based non-exempt term infant formulas, and selected conventional foods.

F. BASIS FOR GRAS DETERMINATION

This GRAS determination for the use of GOS for the intended uses specified above has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), as described under 21 CFR §170.30(b). The safety of the intake of VITAGOS™ IF has been determined to be GRAS by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food and is based on generally available and accepted information.

The proposed use of VITAGOS™ IF as an ingredient for the intended uses in foods and infant formulas has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. VITAGOS™ IF is a galacto-oligosaccharide (GOS)-containing product manufactured using lactose and β-galactosidase derived from *B. circulans* (*B. circulans* M3-1) with a production process similar to that of VITAGOS™, which is the subject of GRN 721. VITAGOS™ IF does not contain genetically modified organisms (GMOs) or ingredients derived from GMO-derived products.

2. A comparison of the manufacturing processes and product specifications for VITAGOS™ IF shows that VITAGOS™ IF is compositionally similar to Vivinal® GOS, which is GRAS (GRN 236) and currently marketed globally for use in infant formulas and conventional foods and beverages.

3. All raw materials and processing aids used to produce VITAGOS™ IF comply with appropriate US federal regulations.

4. GOS are non-digestible oligosaccharides consisting of 1 to 7 galactose units linked via β(1→2), β(1→3), β(1→4), or β(1→6) glycosidic bonds to either a terminal glucose or galactose.

    a. GOS pass through the upper gastrointestinal tract to the colon where they are metabolized by the resident microbiota into short-chain fatty acids, carbon dioxide, methane, and hydrogen, which are the same metabolites as those produced by the microbiota following the ingestion of other foods and are either absorbed, exhaled, or excreted.

    b. Oligosaccharides present in food include those that are naturally occurring in human milk and colostrum, bovine colostrum, and fermented milk products or enzymatically produced, which are then added to the food during formulation and processing.

    c. Enzymatically produced GOS have a long history of use worldwide.

        i. In Japan, GOS have been commercially available since 1995 and are considered as Food for Specified Health Uses (FOSHU).

        ii. In the United States, the first GOS ingredient was determined GRAS for use in term infant formula and selected foods and beverages and received a “no questions” letter from the FDA in 2008 (GRN 236). Since then, a total of nine GOS-containing ingredients have been determined GRAS for use in infant formulas and selected conventional foods at levels up to 7.8 g/L and 11 g/serving, respectively, and resulted in fourteen GRAS Notifications ((GRN 236; GRN 285; GRN 286; GRN 334; GRN 484; GRN 489; GRN 495; GRN 518; GRN 569;

GRN 620; GRN 671; GRN 721; GRN 729; GRN 896). All GRAS Notifications have received "no questions" letters from the FDA, except for GRN 671 which was ceased to be evaluated at the notifier's request. However, the notifier of GRN 671 resubmitted as GRN 721 and subsequently received a "no questions" letter.

iii. In the European Union, the safety of GOS was reviewed by the Scientific Committee on Food (SCF) in 2003, and GOS are approved for use in infant and follow-on formulas and in combination with fructo-oligosaccharides (FOS) at levels up to 8 g (90% GOS and 10 % FOS)/L (7.2 g GOS and 0.8 g FOS/L) (EU 2016/127).

iv. In Australia and New Zealand, the safety of GOS was reviewed by the Food Standards of Australia and New Zealand (FSANZ) in 2008 and GOS are permitted in infant and follow-on formulas at levels up to 290 mg/100 kJ, or approximately 8 g/L (Australia New Zealand Food Standards Code – Standard 2.9.1-7).

5. A pivotal toxicology study of Vivinal® GOS, which is compositionally similar to VITAGOS™ IF (Anthony et al., 2006), supports the safety of VITAGOS™ IF. This study established a no observed adverse effect level (NOAEL) of 2.25 g GOS/kg body weight/day (5g of Vivinal® GOS- the highest dose tested).

   a. Other GOS-containing products administered for up to 90 days by gavage established NOAELs at the highest doses tested [825 mg GOS/kg/day (Kobayashi et al. 2009); 2 g/kg/day (Zhou et al. 2017); 2 g GOS/kg/day (Penard 2015); 5 g GOS/kg/day (Jain et al. 2019)].

   b. GOS-containing products are not genotoxic.

   c. GOS-containing products are not reproductive or developmental toxicants.

6. GOS has been the subject of numerous clinical investigations in infants, children, and adults. It has been shown to be safe and well tolerated at levels that support the intended uses.

7. Although GOS-containing products have been reported to elicit allergic reactions in a limited number of sensitized individuals living in Southeast Asia, GOS preparations have been widely consumed in Southeast Asia as well as globally for over a decade by adults, children, and infants, which suggests that the risk of GOS allergenicity to GOS-containing foods is negligible.

8. Vitalus Nutrition Inc. intends to use VITAGOS™ IF as an alternative for VITAGOS™, which was the subject of GRN 721. Thus, the intended uses for VITAGOS™ IF will be identical to those specified in GRN 721, which includes powdered, ready-to-feed, and concentrated liquid versions of milk-based non-exempt term infant formulas and selected conventional foods.

    a. The infant formulas will not exceed 7.8 g GOS/L reconstituted infant formula. This use level is higher than that proposed in GRN 721 but is the same as that from GRN 620 and GRN 729. This will result in a mean and 90th percentile estimated daily intake (EDI) of GOS for infants 0-6 months of age of 6.4 and 9.2 g/day, respectively. For infants, 7-12 months of age, the mean and 90th percentile intakes of GOS are 5.6 and 8.6 g/day.

    b. The addition of VITAGOS™ IF to selected foods, beverages, and beverage concentrates results in a mean and 90th percentile EDIs for the total U.S. population from the ingestion of all GOS-containing foods are 12.2 g/person/day (0.28 g/kg body weight/day) and 25.3 g/person/day (0.7 g/kg body weight/day), respectively. On an individual basis, the greatest mean and 90th percentile GOS EDIs occur in children and male teenagers at 18.1 and 33.0 g/person/day. On a body weight basis, the greatest mean and 90th percentile GOS EDIs occur in infants at 1.44 and 2.42 g/kg body weight/day.

    c. Because the use and use levels of VITAGOS™ IF are substitutive for existing uses and use levels of GOS, the dietary exposure to VITAGOS™ IF from the intended uses will not increase the cumulative intake of GOS.

9. As established in GRN 236 as well as other GOS Notifications, clinical and toxicology studies of other compositionally similar GOS support the safety of the proposed intake of VITAGOS™ IF (GRN 334; GRN 484; GRN 489; GRN 495; GRN 518; GRN 569; GRN 620; GRN 671; GRN 721; GRN 729; GRN 896).

Determination of the GRAS status of VITAGOS™ IF under the intended conditions of use has been made through the deliberations of Roger Clemens, DrPH, CNS, CFS, FACN, FIFT, A. Wallace Hayes, Ph.D., DABT, FATS, ERT, CNS, FACN, and Thomas Sox Ph.D., JD. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of VITAGOS™ IF and the human exposure to VITAGOS™ IF resulting from its intended use as an ingredient in powdered, ready-to-feed and concentrated liquid versions of milk-based non-exempt term infant formulas and select conventional foods:

There is no evidence in the available information on VITAGOS™ IF that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when VITAGOS™ IF is used at levels that might reasonably be expected from the proposed applications of VITAGOS™ IF for use in powdered, ready-to-feed and concentrated liquid versions of milk-based non-exempt term infant formulas, and selected conventional foods as proposed by Vitalus Nutrition Inc.

Therefore, VITAGOS™ IF is safe and GRAS at the proposed levels of addition to the intended foods. VITAGOS™ IF is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

G. PREMARKET APPROVAL

The notified substance is not subject to the premarket approval requirements of the FD&C Act based on our conclusion that the substance is GRAS under the conditions of intended use.

H. AVAILABILITY OF INFORMATION

The data and information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Claire L. Kruger, Ph.D., DABT, Managing Partner, Spherix Consulting Group, Inc., at 751 Rockville Pike, Unit 30-B, Rockville, MD 20852. Telephone: 301-775-9476; Email: ckruger@spherixgroup.com, or be sent to FDA upon request.

I. FREEDOM OF INFORMATION ACT (FOIA)

Parts 2 through 7 of this notification do not contain data or information that is exempt from disclosure under the FOIA.

J. INFORMATION INCLUDED IN THE GRAS NOTIFICATION

To the best of our knowledge, the information contained in this GRAS notification is complete, representative, and balanced. It contains both favorable and unfavorable information, known to Vitalus Nutrition and pertinent to the evaluation of the safety and GRAS status of the use of this substance.

24 January 2022

Nisha Kaushik
Signature of Authorized Representative of
Vitalus Nutrition Inc.

Date

II. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT OF THE NOTIFIED SUBSTANCE

A. COMMON OR USUAL NAME

Galacto-oligosaccharides (GOS), also known as oligogalactosyllactose, oligogalactose, oligolactose, transgalactosylated oligosaccharide, and transgalacto-oligosaccharide.

B. TRADE NAME

VITAGOS™ IF

C. DESCRIPTION OF GALACTO-OLIGOSACCHARIDES

As stated in GRN 495 and 620, "there is no globally-adopted definition of galacto-oligosaccharides". Substances produced from lactose with the help of a microbial β-galactosidase and contain one glucosyl moiety and 1-7 galactosyl moieties, and disaccharides comprising at least one unit of galactose (Scientific Committee on Food, 2001, Tzortzis and Vulevic, 2009; FSANZ, 2008; Figure 1) are defined as galacto-oligosaccharides. These chains are usually linked via β-glycosidic bonds with β (1→2), β (1→3), β (1→4), or β (1→6) anomeric configurations depending on the type of β-galactosidase used during manufacturing and manufacturing conditions (reviewed in Torres et al., 2010). Although tri- to hexa-saccharides with 2 to 5 galactose units (degree of polymerization (DP) of 3 to 6) tend to be the main components of GOS-containing products, disaccharides (DP2) consisting of galactose and glucose with β-glycoside bonds different from lactose are also present and are considered to be GOS because they have similar physiological characteristics as longer chain GOS oligo-saccharides, namely GOS components of DP≥3 (Sangwan et al., 2011; Sako et al., 1999).

VITAGOS™ IF is a GOS-containing syrup synthesized from lactose using a β-galactosidase derived from *B. circulans* (*B. circulans* M3-1), which hydrolyzes the β1-4 glycosidic bond between the galactose and glucose moieties of lactose and transgalactosylates the residual galactose with other molecules. VITAGOS™ IF contains a minimum of 57% GOS, among which DP3 GOS predominate. VITAGOS™ IF has a similar DP profile as other GOS synthesized from a β-galactosidase derived from *B. circulans* such as Vivinal® GOS and King-Prebiotics® GOS which are the subjects of GRN 236 and 569, respectively (Table 1). The GOS that is the subject of GRN 729 is also produced using a β-galactosidase derived from *B. circulans*, but the DP composition was not provided.

Table 1. Degree of Polymerization Comparison of GOS Synthesized From β-galactosidase Derived From *B. circulans*

Saccharide (DP)	Relative Amount (% DM)		
	Vivinal GOS (GRN 236)	King-Prebiotics® (GRN 569)	VITAGOS™ IF*
Disaccharides (DP2)	33	23	34
Trisaccharides (DP3)	39	42	38
Tetrasaccharides (DP4)	18	21	18
Pentasaccharides and higher oligomers (DP≥5)	10	14	10

DM = dry matter; DP = Degree of Polymerization; GOS = Galacto-oligosaccharides
GOS in GRN 729 is also produced using a β-galactosidase derived From *B. circulans* but DP composition was not provided.
*Converted VITAGOS™ IF DP % to GOS fraction only by dividing by % of total GOS

Compositional studies have shown that the type of β-glycosidic bonds present in GOS is dependent on the type of β-galactosidases used during production (Yanahira et al., 1995; Greenberg and Mahoney, 1983; Martinez-Villaluenga et al., 2008; Rodriguez-Colinas et al., 2011; Rodriguez-Colinas et al., 2012; Rodriguez-Colinas et al., 2014; Urrutia et al., 2013; Kaneko et al., 2014). Specifically, GOS manufactured with β-galactosidase derived from *B. circulans, Sporobolomyces singularis,* or a combination of *S. singularis* and *Kluveromyces lactis* contain predominantly oligosaccharides with 1-4 β-glycosidic bonds (Rodriguez-Colinas et al., 2011; Rodriguez-Colinas et al., 2012; Kaneko et al., 2014; Yanahira et al., 1995) whereas GOS manufactured with β-galactosidases derived from *K. lactis*, *Aspergillus oryzae*, *S thermophilus,* or a combination of β-galactosidases derived from *A. oryzae* and *Streptococcus thermophilus* contain predominantly oligosaccharide chains with 1-6 β-glycosidic bonds (Greenberg and Mahoney, 1983; Martinez-Villaluenga et al., 2008; Rodriguez-Colinas et al., 2011; Rodriguez-Colinas et al., 2012; Rodriguez-Colinas et al., 2014; Kaneko et al., 2014; Urrutia et al., 2013). Because VITAGOS™ IF is manufactured with a β-galactosidase derived from *B. circulans*, it is likely that the product contains oligosaccharides with 1-4 β-glycosidic bonds.

To identify the types of glycosidic linkages in the product, VITAGOS™ IF was profiled on a Dionex ICS-5000+ workstation equipped with a CarboPac PA-1 column (250 x 4 mm) and an ICS-5000 ED pulsed amperometric detector, using the gradient described by van Leeuwen et al. (2016). Except for an extension of reconditioning time, the method was the same as was described by van Leeuwen et al. (2016), who characterized the glycosidic linkages for Vivinal® GOS, which is the subject of GRN 236. Twenty-two peaks were identified in VITAGOS™ IF and although the retention times for each component are different due to the differences in the equipment (ICS-5000 vs ICS-3000) and fractionation program, the profile was similar to the profile for Vivinal® GOS (Figures 1 and 2). Additionally, because the glycosidic linkages of the oligosaccharides in each peak of Vivinal® GOS was determined by van Leeuwen et al. (2016; Table 2), it is therefore likely that the oligosaccharides in each peak in VITAGOS™ IF contain

similar glycosidic linkages as Vivinal® GOS, as evidenced by the predominance of such peaks as 5, 11, 13, and 17 containing β1-4 linkages (Figures 1 and 2; Table 2). Therefore, the data presented demonstrate the compositional equivalence of VITAGOS™ IF to the Vivinal® GOS, the subject of GRN 236.

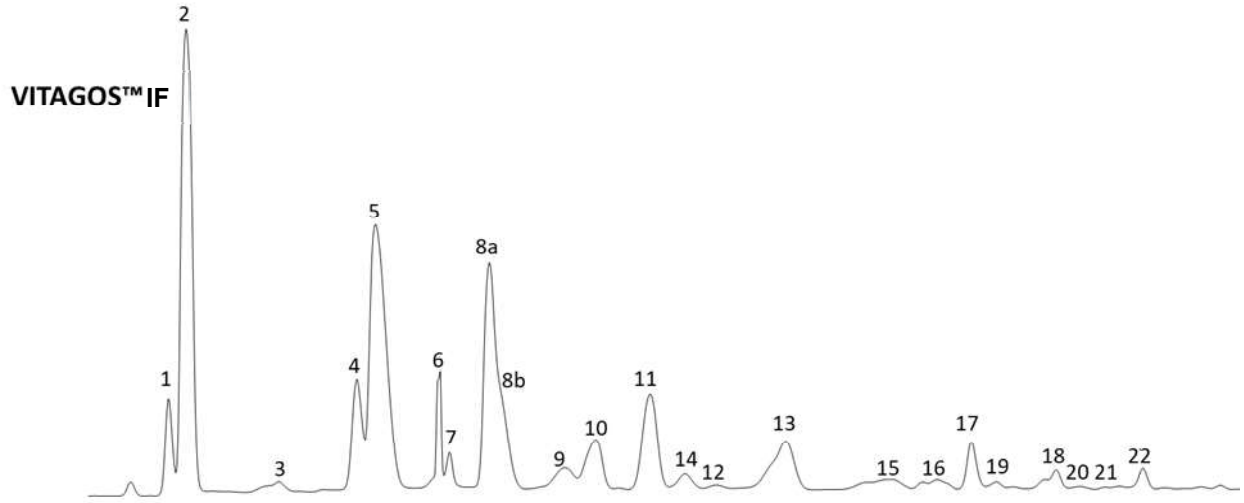

Figure 1. VITAGOS™ IF profiled on Dionex ICS-5000

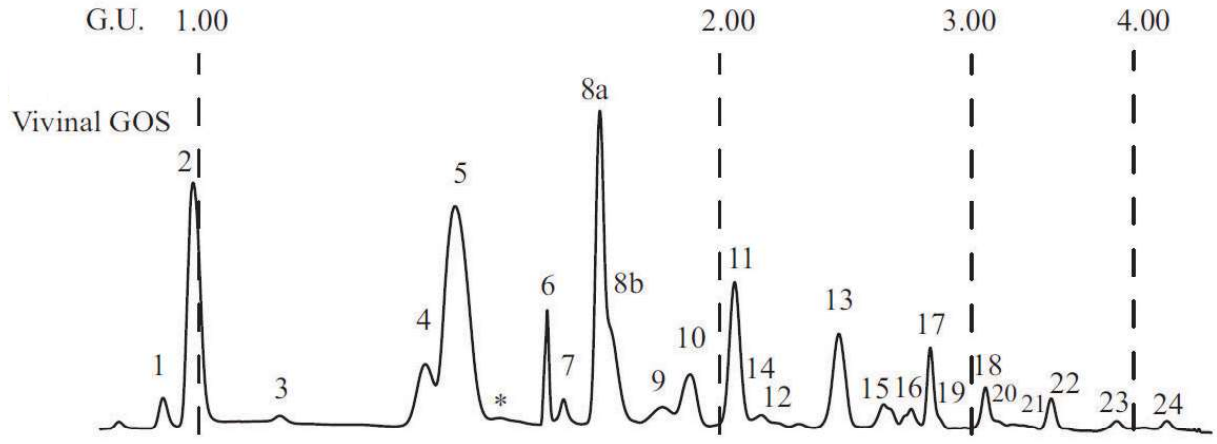

Figure 2. Vivinal® GOS from Van Leeuwen et al. (2016).
Glucose unit (G.U) values are based on elution times in relation to an external malto-oligosaccharide ladder.

Table 2. Glycosidic Linkages of Oligosaccharides in Vivinal® GOS			
Peak #	Structure	Peak #	Structure
1	Galactose	12	Gal-β(1→3)-Gal-β(1→4)-Glc
2	Glucose	13	Gal-β(1→4)-Gal-β(1→2)-Glc Gal-β(1→4)-Gal-β(1→3)-Glc
3	Gal-β(1→6)-Gal	14	Gal-β(1→4)-Gal-β(1→3)-[Gal-β(1→2)]-Glc Gal-β(1→4)-Gal-β(1→4)-[Gal-β(1→6)]-Glc
4	Gal-β(1→6)-Glc	15	Gal-β(1→4)-Gal-β(1→2)-[Gal-β(1→4)]-Glc Gal-β(1→4)-Gal-β(1→4)-[Gal-β(1→2)]-Glc
5	Gal-β(1→4)-Glc	16	Gal-β(1→4)-Gal-β(1→2)-[Gal-β(1→6)]-Glc Gal-β(1→4)-Gal-β(1→6)-[Gal-β(1→2)]-Glc Gal-β(1→4)-Gal-β(1→4)-Gal-β(1→6)-Glc
6	Gal-β(1→6)-Gal-β(1→4)-Glc Gal-β(1→6)-[Gal-β(1→4)]-Glc	17	Gal-β(1→4)-Gal-β(1→4)-Gal-β(1→4)-Glc
7	Gal-β(1→4)-Gal	18	Gal-β(1→4)-Gal-β(1→4)-Gal-β(1→2)-Glc Gal-β(1→4)-Gal-β(1→4)-Gal-β(1→3)-Glc
8a	Gal-β(1→2)-Glc	19	Gal-β(1→4)-Gal-β(1→4)-Gal-β(1→6)-[Gal-β(1→4)]-Glc Gal-β(1→4)-Gal-β(1→4)-Gal-β(1→4)-[Gal-β(1→6)]-Glc Gal-β(1→4)-Gal-β(1→6)-[Gal-β(1→4)-Gal-β(1→4)]-Glc
8b	Gal-β(1→3)-Glc	20	Gal-β(1→4)-Gal-β(1→4)-Gal-β(1→4)-[Gal-β(1→2)]-Glc Gal-β(1→4)-Gal-β(1→4)-[Gal-β(1→4)-Gal-β(1→2)]-Glc Gal-β(1→4)-Gal-β(1→4)-Gal-β(1→2)-[Gal-β(1→4)]-Glc
9	Gal-β(1→4)-[Gal-β(1→2)]-Glc	21	Gal-β(1→4)-Gal-β(1→4)-Gal-β(1→6)-[Gal-β(1→2)]-Glc Gal-β(1→4)-Gal-β(1→4)-Gal-β(1→2)-[Gal-β(1→6)]-Glc Gal-β(1→4)-Gal-β(1→6)-[Gal-β(1→4)-Gal-β(1→2)]-Glc
10	Gal-β(1→6)-[Gal-β(1→2)]-Glc Gal-β(1→6)-[Gal-β(1→3)]-Glc	22	Gal-β(1→4)-Gal-β(1→4)-Gal-β(1→4)-Gal-β(1→4)-Glc
11	Gal-β(1→4)-Gal-β(1→4)-Glc		
Source: van Leeuwen et al. (2016)			

D. PRODUCTION PROCESS

VITAGOS™ IF is manufactured using a standardized procedure that has been widely reviewed in the scientific literature (Sangwan et al., 2011; Torres et al., 2010). It consists of three basic steps: preparation of concentrated solution of lactose; treatment of the lactose solution with β-galactosidase to produce galacto-oligosaccharides (GOS); and termination of the enzymatic reaction with heat. The β-galactosidase performs two functions; the hydrolysis of lactose to the monosaccharides glucose and galactose, and the transgalactosylation of lactose, producing GOS. Importantly, the concentration, chain length, and type of β-glycosidic bonds of GOS are determined by the rate of hydrolysis, degree of transgalactosylation, and source of the β-galactosidase, and manipulation of these factors during production results in products containing GOS with different concentrations, varying chain lengths, and different β-glycosidic bonds (GRN 489).

1. Compliance

VITAGOS™ IF is manufactured by Vitalus Nutrition Inc., located at 3911 Mt. Lehman Rd. Abbotsford, British Columbia, V2T 5W5, Canada under food-grade conditions. Products manufactured by Vitalus Nutrition Inc. including VITAGOS™ IF do not contain genetically modified organisms (GMOs) or ingredients derived from GMO-derived products. Vitalus Nutrition Inc. has a hazard analysis critical control point (HACCP) management system in place and their manufacturing facility has been audited and determined to be compliant with the Food Safety System Certification (FSSC) 22000 standards by a third party. All food contact surfaces used in manufacturing VITAGOS™ IF are either stainless steel, aluminum, or suitable for use in the production of food ingredients. The whey used to produce the lactose is free of antibiotics and all raw materials and processing aids are either Food Chemical Codex (FCC) grade, comply with conditions of use stipulated in Parts 168, 173, 177, 182, and 184 of Title 21 of the United States Code of Federal Regulations or have been determined GRAS (GRN 90; GRN 489; GRN 620) (Table 3). The β-galactosidase enzyme (Lactaenzyme-B) manufactured by GenoFocus, Inc. (Korea) is obtained from a proprietary non-toxigenic, non-pathogenic, non-genetically modified strain of *B. circulans*, complies with FCC specifications, is used to produce the subject of GRN 729, which received a "no questions" letter in 2018, and is considered safe for use in the manufacture of GOS by the European Food Safety Authority (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), 2019). All ingredients and processing aids also comply with European Union and Codex requirements, and, because current Canadian legislation prohibits the use of bovine growth hormones in dairy cattle, are free of recombinant bovine somatotropic and growth hormones.

Table 3. Regulatory Compliance of Ingredients and Processing Aids	
Material	**Regulatory Status**
Lactose	21 CFR §168.122
β-Galactosidase (derived from *B. circulans* M3-1)	GRAS
Ion Exchange Resins	21 CFR §173.25
Citric Acid	21 CFR §182.1033
Hydrochloric Acid	21 CFR §182.1057
Sodium Hydroxide	21 CFR §184.1763
Tubing Materials	21 CFR 177.2600, 21 CFR 177.2490, 21 CFR 177.1550, 21 CFR 177.1520
Packaging materials	21 CFR §177.1520
CFR = Code of Federal Regulations; GRAS = Generally Recognized As Safe.	

2. Manufacturing Process

"To produce VITAGOS™ IF, food-grade lactose is dissolved in softened municipal drinking water and heated to a temperature greater than 88°C under agitation (Figure 3). The temperature and pH of the solution are then adjusted to optimum conditions for transgalactosylation and a β-galactosidase derived from *B. circulans* is added. The solution is then agitated for a set period of time to convert the lactose to GOS. The enzyme is deactivated by adjusting pH. The GOS are then purified by removing the enzyme residues and minerals using filtration and adsorption to several resins and ion exchange. The resulting product is concentrated using evaporation by heating the product at temperatures ranging from 66°C to 81°C with a brief introduction to a high temperature of 135°C at the beginning of the evaporation process. The product then passes through a screen to ensure a homogenous syrup and a metal detector before packaging. The product is packaged in containers lined with ultra-low density polyethylene food-grade liners under sanitary conditions.

There is one critical control point (CCP) in the manufacturing of VITAGOS™ IF, which occurs after the product passes through the filter screen and before the packaging step. The CCP entails monitoring the product for the presence of metal particles using the metal detector, which is verified for the detection of ferrous, nonferrous, and stainless steel. If metal is detected, the product is diverted, the metal is removed, and the diverted product is discarded as waste. Additionally, the quality of the product is monitored throughout the manufacturing process with in-line testing for solids, conductivity, pH, color, and sugar profile."

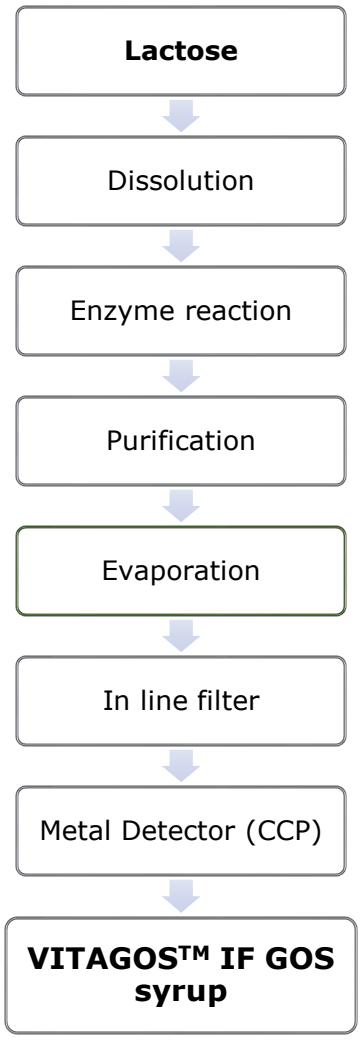

Figure 3. Production Process for VITAGOS™ IF

Lactose is dissolved in water and mixed with β-galactosidase from *B. circulans* M3-1. The enzyme is then deactivated and GOS are purified by filtration and adsorption with resins. VITAGOS™ IF is concentrated by evaporation, heat-treated, packaged in containers lined with food grade bags, and stored under ambient conditions. When compliance with the product specifications is met, VITAGOS™ IF is distributed to customers.

a. Enzyme Deactivation

To demonstrate control of the production process and validate enzyme deactivation by pH adjustment, samples were collected to determine the oligosaccharide profile after termination of the reaction (0 hour) and incubated under optimal conditions for 24 hours before a second determination of the oligosaccharide profile (24 hours) (Table 4). Even under optimal conditions, the DP profile of the batches was similar after incubation with deactivated enzyme for 24 hrs. Thus, the production process parameters successfully deactivate the enzyme.

Table 4. Termination of Enzymatic Reaction of β-galactosidases Derived from *B. circulans* M3-1										
Batch number	20217		20223		20225		20230		20232	
Time (hours)	0	24	0	24	0	24	0	24	0	24
DP5+ %	5.3	6.0	6.2	6.3	6.2	6.3	7.0	6.5	6.3	6.2
DP4 %	10.6	10.6	10.6	10.6	10.7	10.7	11.2	10.9	11.0	11.0
DP3 %	23.5	23.1	22.8	22.7	22.9	22.8	22.5	22.7	22.8	22.9
DP2 %	20.9	20.4	21.1	21.5	20.3	20.8	20.2	20.2	20.5	20.6
Lactose	19.2	19.3	18.4	17.8	19.3	18.7	18.6	18.9	18.8	18.4
Glucose %	18.8	19.2	19.2	19.4	19.2	19.3	19.4	19.5	19.5	19.3
Galactose %	1.7	1.4	1.7	1.7	1.3	1.6	1.2	1.3	1.3	1.6
DP = Degree of polymerization.										

E. FINISHED PRODUCT SPECIFICATIONS AND OTHER QUALITY ATTRIBUTES

1. Product Specifications

To ensure a consistent food-grade product, each batch of VITAGOS™ IF is evaluated against an established set of product specifications (Table 5) using validated methods that are fit-for-use. Data from five batches demonstrate control of the production process and compliance with the product specifications (Table 5).

Table 5. VITAGOS™ IF Product Specifications and Batch Data							
Parameter	Specification	Method	Batch Number				
			20217	20223	20225	20230	20232
Physical Characteristics							
Appearance	Clear to slight yellow	TMS-QC 773	Complies	Complies	Complies	Complies	Complies
pH	2.7 – 4.5	TMS-QC 773 (pH meter)	3.3	3.6	3.8	3.7	3.4
Viscosity (cPs @ 26ºC)	1000 - 5000 cPs	TMS-QC 1392 (viscometer)	2624	2316	1739	1257	2353
Dry Matter (Total %)	74-78	Vacuum Oven Solids (TMS-QC 2532)	75.7%	76%	75.7%	77.3%	77.2%
Chemical Composition							
Galacto-oligosaccharides (% DM)	≥ 57	Mod. AOAC Method 2001.02	58.8%	59.6%	58.2%	58.6%	58.2%
Lactose (% DM)	≤ 28	Mod. AOAC Method 2001.02	18.9%	18.1%	18.7%	18.5%	18.6%
Glucose (% DM)	≤ 22	TMS-QC 2535	18.9%	19.1%	19.0%	19.3%	19.4%
Galactose (% DM)	≤ 5	TMS-QC 2535	2.1%	2.2%	2.0%	1.9%	1.8%
Nitrogen (%)	≤ 0.032%	AOAC 991.20.E	< 0.02%	<0.02%	< 0.02%	< 0.02%	< 0.02%
Sulfated Ash (% w/w)	≤ 0.3	USP / NF Current Version	0.10%	<0.01%	0.03%	<0.01%	<0.01%
Microbiological Parameters							
Standard Plate Count (cfu/g)	< 3000	MFHPB-18	ND	ND	ND	ND	ND
Coliform (cfu/g)	< 10	MFHPB-35	ND	ND	ND	ND	ND
Enterobacteriaceae (cfu/ g)	< 10	MFLP-09	ND	ND	ND	ND	ND
Escherichia coli (cfu/g)	< 10	MFHPB-34	ND	ND	ND	ND	ND
Yeast and Mold (cfu/g)	< 100	MFHPB-22	ND	ND	ND	ND	ND
Staphylococcus aureus (cfu/g)	< 10	MFHPB-21	ND	ND	ND	ND	ND
Salmonella (per 25g)	Negative	MFLP-29	ND	ND	ND	ND	ND
Heavy Metals							
Arsenic (ppm; w/w)[1]	< 0.4	EPA 3050/6020, USP 730	ND	ND	ND	ND	ND
Lead (ppm; w/w)[1]	< 0.2	EPA 3050/6020 USP 730	ND	ND	ND	ND	ND
Cadmium (ppm; w/w)[1]	< 0.06	EPA 3050/6020 USP 730	ND	ND	ND	ND	ND
Mercury (ppm; w/w)[1]	< 0.005	EPA 3050/6020 USP 730	ND	ND	ND	ND	ND

AOAC = Association of Analytical Communities; cPs = centipoises; cfu = colony forming units; DM = dry matter; EPA = United States Environmental Protection Agency; MFHPB = Methods for the Microbiological Analysis of Foods; MFLP = Laboratory Procedures for the Microbiological Analysis of Foods; ND = not detected; NF = National Formulary; ppm = parts per million; TMS-QC = Internal Test methods referencing system; USP = United Stated Pharmacopeia; w/w = weight/weight

[1]Limit of detection: Arsenic = 0.01 ppm; lead = 0.01 ppm; cadmium = 0.001 ppm; mercury = 0.005 ppm.

Limit of detection: Nitrogen = 0.02%

All microbial methods obtained from the Compendium of Analytical Methods prepared by the Evaluation Division Bureau of Microbiological Hazards, Food Directorate, Health Products and Food Branch, Health Canada (http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index-eng.php).

All internal methods have been validated.

2. Other Quality Attributes

a. Pathogenic Bacteria

To confirm the absence of *Cronobacter sakazakii* and *Bacillus cereus*, Vitalus Nutrition Inc. analyzed five batches of VITAGOS™ IF using validated microbiological techniques that are fit-for-use (Table 6). *C. sakazakii* and *B. cereus* were undetectable in each batch. Importantly, Vitalus Nutrition Inc. monitors VITAGOS™ IF for the presence of these pathogenic bacteria on a quarterly basis.

Table 6. Lack of Pathogenic Bacteria in VITAGOS™ IF

Bacteria	Method	LOD	Batch Number				
			20217	20223	20225	20230	20232
Cronobacter sakazakii	MFLP-42†	<10 cfu/g	ND	ND	ND	ND	ND
Bacillus cereus	MFLP-27†	Neg./25 g	ND	ND	ND	ND	ND

LOD = limit of detection; MFLP = Laboratory Procedures for the Microbiological Analysis of Foods; ND = not detected; Neg = negative

†Obtained from the Compendium of Analytical Methods prepared by the Evaluation Division Bureau of Microbiological Hazards, Food Directorate, Health Products, and Food Branch, Health Canada (http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index-eng.php).

b. Protein Allergens

VITAGOS™ IF is manufactured on a production line that processes only milk products. No other potentially allergenic substances are used. A composite sample of the five batches in Table 5 was analyzed for casein and casein was below the level of detection (Table 7).

Table 7. Casein Testing in VITAGOS™ IF

Sample	Casein detected
VITAGOS™ IF Syrup composite sample	<2.6 ppm

Analysis performed at Kendrick Laboratories, Inc. (Madison, WI) by casein protein detection electrophoresis (internally validated sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)). The sample was run with and without spiked amounts of casein.
LOD = 2.6 ppm, determined from known amounts of casein spiked into samples

c. Minerals

The mineral content of 5 batches of VITAGOS™ IF was determined using the Environmental Protection Agency (EPA) 3050/6020, United States Pharmacopeia (USP) 730 validated method (Table 8).

Table 8. Minerals in VITAGOS™ IF					
	Batch Number				
Minerals[1]	**20217**	**20223**	**20225**	**20230**	**20232**
Aluminum (ppm)	0.2	<0.2	<0.2	0.3	0.2
Antimony (ppm)	0.01	<0.01	<0.01	<0.01	<0.01
Barium (ppm)	0.14	<0.02	<0.02	<0.02	<0.02
Beryllium (ppm)	<0.01	<0.01	<0.01	<0.01	<0.01
Bismuth (ppm)	<0.02	<0.02	<0.02	<0.02	<0.02
Boron (ppm)	<0.2	<0.2	<0.2	<0.2	1.2
Calcium (ppm)	17	5	3	2	5
Chromium (ppm)	0.03	0.02	0.02	0.03	0.23
Cobalt (ppm)	<0.01	<0.01	<0.01	<0.01	<0.01
Copper (ppm)	<0.01	<0.01	0.02	<0.01	<0.01
Iron (ppm)	<0.5	<0.5	<0.5	<0.5	1.1
Lithium (ppm)	<0.01	<0.01	<0.01	<0.01	<0.01
Magnesium (ppm)	<0.1	<0.1	<0.1	<0.1	<0.1
Manganese (ppm)	2.95	0.74	0.39	0.22	0.45
Molybdenum (ppm)	0.04	<0.02	<0.02	<0.02	<0.02
Nickel (ppm)	<0.01	<0.01	<0.01	<0.01	<0.01
Phosphorus (ppm)	<0.02	<0.02	0.07	<0.02	<0.02
Potassium (ppm)	3	3	3	4	5
Selenium (ppm)	0.8	1	<0.5	<0.05	1
Silver (ppm)	<0.1	<0.1	<0.1	<0.1	<0.1
Strontium (ppm)	<0.02	<0.02	<0.02	<0.02	<0.02
Sodium (ppm)	11	38	40	42	38
Thallium (ppm)	0.06	0.01	<0.01	<0.01	<0.01
Thorium (ppm)	<0.01	<0.01	<0.01	<0.01	<0.01
Tin (ppm)	<0.1	<0.1	<0.1	<0.1	<0.1
Titanium (ppm)	<0.01	<0.01	<0.01	<0.01	<0.01
Uranium (ppm)	<0.02	0.07	0.03	<0.02	<0.03
Vanadium (ppm)	<0.01	<0.01	<0.01	<0.01	<0.01
Zinc (ppm)	<0.01	<0.01	<0.01	<0.01	<0.01
Zirconium (ppm)	1.39	0.20	0.23	0.11	0.81

ppm = parts per million;
[1]Determined using Environmental Protection Agency (EPA) 3050/6020 United States Pharmacopeia (USP) 730.

F. STABILITY OF VITAGOS™

VITAGOS™ IF is stable for at least 8 months. Samples of five batches of VITAGOS™ IF were stored in high-density polyethylene (HDPE) bottles under ambient conditions (18-25°C). Oligosaccharide content, microbiological content, and pH were determined at various time points and compared to the acceptance limits stipulated in the product specifications. Over 8 months, the amount of GOS, galactose, glucose, and lactose in VITAGOS™ IF was similar to day zero VITAGOS™ IF samples and complied with the product specifications (Table 9). The distribution of GOS in DP2, DP3, DP4, and DP5 or greater was also similar to VITAGOS™ IF at the beginning of the testing period. Microbiological content and pH also complied with the product specifications throughout the testing period (Table 10). Importantly, determining the stability of VITAGOS™ IF is an ongoing process and will continue to be monitored to support the intended shelf-life of the finished product.

Table 9. Oligosaccharide Stability of VITAGOS™ IF

Parameter	Specification*	Batches and Time (months)														
		20217			20223			20225			20230			20232		
		0	6	8	0	6	8	0	6	8	0	6	8	0	6	8
Galacto-oligosaccharides (% DM)	≥ 57	58.8	57.3	58.3	59.6	58.8	57.8	58.2	57.6	58.9	58.6	56.9	59.3	58.2	59.8	58.9
Galactose (% DM)	≤ 5	2.1	2.4	2.0	2.2	2.6	2.1	2.0	2.3	2.0	1.9	2.2	1.9	1.8	2.2	1.8
Glucose (% DM)	≤ 22	18.9	18.5	19.1	19.1	18.7	19.3	19.0	18.6	19.0	19.3	18.8	19.2	19.4	18.8	19.3
Lactose (% DM)	≤ 28	18.9	18.7	18.4	18.1	18.0	17.6	18.7	18.0	18.5	18.5	18.1	18.2	18.6	18.4	18.0
DP2 (% GOS)	ns	20.6	21.3	21.0	21.0	21.9	21.6	20.5	22.0	20.9	20.4	21.7	21.2	20.4	21.3	21.3
DP3 (% GOS)	ns	23.0	23.4	22.9	22.7	23.2	22.8	22.8	23.4	22.9	22.6	23.2	22.8	22.8	23.3	22.9
DP4 (% GOS)	ns	10.6	10.4	10.6	10.6	10.3	10.5	10.7	10.4	10.6	10.9	10.4	10.5	10.9	10.5	10.6
≥DP5 (% GOS)	ns	5.9	5.3	6.1	6.2	5.3	6.1	6.3	5.3	6.2	6.4	5.6	6.2	6.2	5.6	6.2

DP = degree of polymerization; DM = dry matter; ns = no specification.
* Determined by HPLC-RID and HPAEC-PAD, which has been validated by Vitalus Nutrition, Inc.

Table 10. Microbial Stability of VITAGOS™ IF

Parameter	Specification	Batches and Time (months)														
		20217			20223			20225			20230			20232		
		0	6	8	0	6	8	0	6	8	0	6	8	0	6	8
pH	2.7-4.0	3.3	3.3	3.3	3.6	3.3	3.2	3.8	3.4	3.4	3.7	3.4	3.5	3.4	3.3	3.3
Standard Plate Count	< 3000 cfu/g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Enterobacteriaceae	< 10 cfu/g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Escherichia coli	<10 cfu/g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Yeast	< 100 cfu/g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mold	<100 cfu/g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Staphylococcus aureus	< 10 cfu/g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella	Negative/25g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND = not detected

III. DIETARY EXPOSURE

A. INTENDED EFFECT

The intended effect of adding GOS to powdered, ready-to-feed, and concentrated liquid versions of cow's milk-based non-exempt term infant formulas and general foods is to increase oligosaccharide intake in formula-fed infants and the general population.

B. HISTORY OF USE

GOS present in food include those that are either naturally occurring or synthetic forms added to food during formulation and processing. Naturally occurring GOS are present in human milk and colostrum, bovine colostrum, and fermented milk products (Kunz et al., 2000; Coppa et al., 1991; Coppa et al., 1997; Toba et al., 1982; Saito et al., 1987). Synthetic GOS are found in a wide variety of products (Table 6). The levels of naturally occurring GOS range from 5 – 15 g/L, 8.5 g/L, and 0.03 – 0.09% in human milk, bovine colostrum, and fermented milk products, respectively (Kunz et al., 2000; Coppa et al., 1991; Coppa et al., 1997; Saito et al., 1987; Toba et al., 1982). It is important to note that, although synthetic GOS are structurally and compositionally less diverse than naturally occurring GOS, both types contain glycosidic bonds, which render them resistant to the digestive enzymes in the stomach and small intestine, and fermentable by the gastrointestinal microbiota present in the small intestine and colon (Wisker et al., 1985; Ohtsuka et al., 1990; Chonan et al., 2004).

GOS have a long history of safe use worldwide.

- In Japan, GOS have been commercially available since 1995 and are considered as Food for Specified Health Uses (FOSHU).

- Health Canada's Food Directorate and the US FDA have reviewed and accepted GOS as an approved dietary fiber (Health Canada, 2017; GRN 721, 2018).

- In the United States, the first GOS product was determined GRAS for use in term infant formula and selected conventional foods and received a "no questions" letter from the FDA in 2008 (GRN 236). Since then, a total of nine GOS-containing ingredients have been determined GRAS for use in infant formulas and selected conventional foods at levels up to 7.8 g/L and 33.4 g/serving, respectively, resulting in fourteen GRAS Notifications (GRN 236; GRN 285; GRN 286; GRN 334; GRN 484; GRN 489; GRN 495; GRN 518; GRN 569; GRN

620; GRN 671; GRN 721; GRN 729; GRN 896). All GRAS Notifications have received "no questions" letters from the FDA, except for GRN 671, which was ceased to be evaluated at the notifier's request. Importantly, the notifier of GRN 671 resubmitted their GRAS determination as GRN 721 and subsequently received a "no questions" letter.

- In the European Union, the safety of GOS was reviewed by the Scientific Committee on Food (SCF) in 2003. GOS are currently approved for use in infant and follow-on formulas GOS in combination with fructo-oligosaccharides (FOS) at levels up to 8 g (90% GOS and 10% FOS)/L (7.2 g GOS and 0.8 g FOS/L) (Scientific Committee on Food; EU 2016/127).

C. INTENDED USE

Vitalus Nutrition Inc. intends to use VITAGOS™ IF in powdered, ready-to-feed, and concentrated liquid versions of milk-based non-exempt term infant formula and other general foods as a substitute for the GOS product that is the subject of GRN 721. The infant formulas will not exceed 7.8 g GOS/L reconstituted infant formula as notified in GRN 620 and GRN 729 and received a "no questions" letter. The conventional foods intended uses and use levels are the same as GRN 721 and will not exceed 33.4 g GOS/100 g product (Table 11).

Table 11. Intended Uses of VITAGOS™ IF

Food Group	Proposed Food Uses[2]	Maximum Use Level (g GOS/100 g Product)[1]
Milk and milk products	Milk, milk substitute such as soy milk	2
	Milk drink	4
	Yogurt	3.4
	Milk based meal replacement	2
	Infant formula	NA[3]
	White sauces, milk gravies, and cheese sauces	1.25
	Milk desserts, frozen like ice creams	2
	Pudding and custards including baby foods	1.4
	Cheese soups	0.62
Soups	Egg soups; soups with legumes as major ingredient; soups with grain products as major ingredient; potato soups; deep-yellow vegetable soups; tomato soups; other vegetable soups	0.62
Nut beverages	Coconut beverages	1.6
Bakery products	Bread	1

Table 11. Intended Uses of VITAGOS™ IF		
Food Group	**Proposed Food Uses[2]**	**Maximum Use Level (g GOS/100 g Product)[1]**
	Brownies	1
	Cakes, heavy weight	1
	Cakes, medium weight	1
	Cakes, light weight	1
	Coffee cakes, crumb cakes, doughnuts, Danish, sweet rolls, sweet quick type breads, muffins, toaster pastries	1
	Cookies	1
	Crackers that are usually used as snacks	1
	French toast, pancakes	1
	Pies, cobblers, fruit crisps, turnovers, other pastries	1
	Waffles	1
	Grain-based bars with or without filling or coating, e.g., breakfast bars, granola bars, rice cereal bars	1
Cereals	Ready-to-eat cereals	2
	Ready-to-eat cereals (dry) for baby food	4
	Ready-to-serve cereals (wet) for baby food	0.55
Fruit and vegetable juices	Fruit juices (including citrus fruit juices) and nectars	1.6
	Vegetable juices	1.6
	Fruit juices, vegetable juices and juice mixtures baby food	1.6
Sugars and sweets	Jellies, jams, preserves	25
Nonalcoholic beverages	Fruit drinks such as fruit juice drinks, fruit flavored drinks, sports drinks, etc.	2
	Non fruit beverages including energy drinks	4.4
	Beverage concentrate (powder)	33.4*

NA = not applicable
[1]Use levels are consistent with those specified in GRN 721.
[2]Food groups were obtained from the Food and Nutrition Database for Dietary Studies (FNDDS).
[3]Maximum amount of GOS ingested is based on the caloric need of the infant (see Chapter IV, Section C.1.)
*After dissolution of powder in water.

D. ESTIMATED DAILY INTAKE

1. Infant Formula

The powdered, ready-to-feed, and concentrated liquid versions of cow milk-based non-exempt term infant formulas will contain 7.8 g GOS/L as consumed, which was determined GRAS in GRN 620 and GRN 729 and received a "no questions" letter. Therefore, the dietary intake of GOS among infant formula consumers will be incorporated by reference from GRN 620 pages 14-15 and GRN 729 pages 32-34. In summary, based on the United States Department of Health and Human Service's 2003-2004 National Health and Nutrition Examination Survey NHANES survey data, the mean and 90th percentile intakes of GOS for infants 0-6 months of age are 6.4 and 9.2 g/day, respectively. For infants, 7-12 months of age, the mean and 90th percentile intakes of GOS are 5.6 and 8.6 g/day. Furthermore, only 3.7% of toddlers aged 1-2 years were estimated to consume GOS from infant formula with 90th percentile intakes of 3.0 and 7.1 g/day.

2. Conventional Food Uses

Since Vitalus Nutrition Inc. intends to use VITAGOS™ IF as a substitute for other GOS preparations per the intended uses and use levels specified in GRN 721, the exposure to GOS from the ingestion of VITAGOS™ IF will not increase compared to those resulting from other GOS preparations. Therefore, the GOS EDI for selected conventional food uses are summarized in GRN 721 pages 17 -19 and are incorporated by reference. The same EDI calculations were incorporated by reference in GRN 729 and received a "no questions" letter from FDA in 2018. In summary, the GOS EDIs were calculated using food consumption data reported in the 2003-2004 NHANES, and the mean and 90th percentile GOS EDIs for the total U.S. population from the ingestion of all GOS-containing foods are 12.2 g/person/day (0.28 g/kg body weight/day) and 25.3 g/person/day (0.7 g/kg body weight/day), respectively. On an individual basis, the greatest mean and 90th percentile GOS EDIs occur in children and male teenagers at 18.1 and 33.0 g/person/day. On a body weight basis, the greatest mean and 90th percentile GOS EDIs occur in infants at 1.44 and 2.42 g/kg body weight/day. Importantly, intake in infants included exposures from both infant formula and conventional food uses.

IV. SELF-LIMITING LEVELS OF USE

This part does not apply.

V. COMMON USE IN FOOD BEFORE 1958

This part does not apply.

VI. NARRATIVE ON THE CONCLUSION OF GRAS STATUS

The safety and GRAS status of GOS has been extensively reviewed and established. In the United States, a total of nine GOS-containing ingredients have been determined GRAS for use in infant formulas and selected conventional foods at levels up to 7.8 g/L and 33.4 g/serving, respectively, and resulted in fourteen GRAS Notifications (GRN 236; GRN 285; GRN 286; GRN 334; GRN 484; GRN 489; GRN 495; GRN 518; GRN 569; GRN 620; GRN 671; GRN 721; GRN 729; GRN 896). All GRAS Notifications have received "no questions" letters from the FDA, except for GRN 671 which was ceased to be evaluated at the notifier's request, and GRN 896 which is still pending. Importantly, the notifier of GRN 671 resubmitted as GRN 721 and subsequently received a "no questions" letter. Therefore, there is general scientific consensus that GOS-containing products are GRAS.

Because VITAGOS™ IF is manufactured with a β-galactosidase derived from *B. circulans*, which is the same enzyme used to produce Vivinal® GOS and has a similar GOS profile as Vivinal® GOS, the safety of VITAGOS™ IF is supported by the publicly available 90-day toxicology study conducted with Vivinal® GOS (Anthony et al., 2006). In the pivotal 90-day sub-chronic rat toxicology study of Vivinal® GOS, the NOAEL was the highest dose administered at 2.25 g GOS/kg body weight/day (5 g Vivinal® GOS/kg body weight/day). The safety of VITAGOS™ IF is also supported by the GRAS determinations of other GOS ingredients synthesized from a β-galactosidase derived from *B. circulans* (GRN 236; GRN 569; GRN 729) which have received a "no questions" letter. Numerous corroborative *in vitro*, toxicology, animal, and clinical studies, and opinions of regulatory bodies throughout the world on the use of GOS in infant formulas and selected conventional foods (GRN 236; GRN 285; GRN 286; GRN 334; GRN 484; GRN 489; GRN 495; GRN 518; GRN 569; GRN 620; GRN 671; GRN 721; GRN 729; GRN 896; Scientific Committee on Food, 2001; FSANZ, 2008) also support the safety of GOS and are incorporated by reference. Additionally, an updated literature search for publicly available toxicology and clinical studies supporting the safety of GOS reveal 15 additional studies that contain no new information that would draw into question the GRAS status of the use of GOS in infant formula and conventional foods. In summary, corroborative studies reinforce that GOS are non-mutagenic and non-genotoxic. GOS are also not toxic in subchronic studies and does not produce adverse test-article related effects in juvenile rats at doses up to 3 g GOS/kg bw/day except for increased weights of cecum. The cecal enlargement was not correlated with histopathologic changes and was completely reversed following a 14-day recovery period. Cecal enlargement is a common finding for non-digestible substances administered in rodent toxicology tests (Whitely et al., 1996; Oku et al., 1997; Zhou et al., 2017). The NOAEL was at the highest dose tested. Clinical studies of GOS at up to 8.55 g/L in infants

and children and doses of GOS up to 15 g/day in adults show that it is safe and well-tolerated. Taken together, the totality of the data supports Vitalus Nutrition Inc.'s conclusion that VITAGOS™ IF is GRAS for its use in cow's milk-based non-exempt term infant formulas and conventional foods.

A. SAFETY OF GOS

Because VITAGOS™ IF is manufactured with a β-galactosidase derived from *B. circulans*, which is the same enzyme used to produce Vivinal® GOS and has a similar GOS profile as Vivinal® GOS, the safety of VITAGOS™ IF is supported by the publicly available 90-day toxicology study conducted with Vivinal® GOS (Anthony et al., 2006). In the pivotal 90-day sub-chronic rat toxicology study of Vivinal® GOS, the NOAEL was the highest dose administered at 2.25 g GOS/kg body weight/day (5 g Vivinal® GOS/kg body weight/day) (Anthony et al., 2006). Numerous corroborative *in vitro*, toxicology, and clinical studies support the safety of GOS. Authoritative opinions from regulatory bodies throughout the world confirm the general recognition of the safety of the use of GOS in infant formulas and selected conventional foods (GRN 236; GRN 285; GRN 286; GRN 334; GRN 484; GRN 489; GRN 495; GRN 518; GRN 569; GRN 620; GRN 671; GRN 721; GRN 729; GRN 896; Scientific Committee on Food, 2001; FSANZ, 2008). Additionally, an updated literature search for publicly available toxicology and clinical studies was conducted and identified 15 additional studies which do not change the conclusion that GOS are safe and GRAS.

1. GOS Comparison

The GRAS status of VITAGOS™ IF is supported by the equivalence of other GOS ingredients synthesized from a β-galactosidase derived from *B. circulans* (GRN 236, 569, and 729). VITAGOS™ IF contains a minimum of 57% GOS, among which DP3 GOS predominate and has a similar DP profile to other GOS synthesized from a β-galactosidase derived from *B. circulans* such as Vivinal® GOS and King-Prebiotics® GOS (Table 1; GRN 236, 569). GOS in GRN 729 is also produced using a β-galactosidase derived from *B. circulans* but DP composition was not provided. As depicted in Figures 1 and 2, and Table 2, VITAGOS™ IF consists of a similar GOS profile to Vivinal® GOS, composed of mainly β1-4 glycosidic linkage species. Additionally, the specifications for VITAGOS™ IF are very similar to the GOS produced in a similar manner (Table 12; GRN 236; GRN 569; GRN 729). The subjects of GRN 569 and 729 do not present their own safety packages but rely on the pivotal data presented in GRN 236. Therefore, the toxicology data on Vivinal® GOS presented in GRN 236 is pivotal and supports the safety of VITAGOS™ IF.

Table 12. VITAGOS™ IF GOS and Other GOS Syrups Synthesized from β-galactosidase from *B. circulans* Specification Comparison

Parameter	Vivinal GOS (GRN 236)	King-Prebiotics® (GRN 569)	Nature's GOS-CL (GRN 729)	VITAGOS™ IF
Appearance	-	Off white light yellow	Yellow	Clear to slight yellow
pH	3.2-3.8	2.8 – 3.8	3.2 – 3.8	2.7 – 4.5
Viscosity (cPs @ 26ºC)	1000 – 5000 cPs	-	1000 – 5000 cPs	1000 - 5000 cPs
Dry Matter (Total %)	74 - 76	≥ 74	74 – 76	74 – 78
Galacto-oligosaccharides (% DM)	≥ 57	≥ 57	≥ 57	≥ 57
Lactose (% DM)	≤ 23	≤ 23	≤ 23	≤ 28
Glucose (% DM)	≤ 22	≤ 22	≤ 22	≤ 22
Galactose (% DM)	≥ 0.8	≥ 0.8	≥ 0.8	≤ 5
Nitrogen (%)	≤ 0.016	≤ 0.032%	< 0.1%	≤ 0.032%
Sulfated Ash (% w/w)	≤ 0.3	≤ 0.3	≤ 0.3	≤ 0.3
Protein (% DM)	-	-	≤ 0.3	-
Standard Plate Count (cfu/g)	< 3000	≤ 50	< 3000	< 3000
Coliform (cfu/g)	-	-	Neg/25g	< 10
Enterobacteriaceae (cfu/ g)	Absent in 1g	-	-	< 10
Escherichia coli (cfu/g)	Absent in 5g	< 3 MPN/g	< 10	< 10
Yeast and Mold (cfu/g)	< 50	< 20	< 50	< 100
Staphylococcus aureus (cfu/g)	Absent in 1g	Neg/25g	Neg/75g	< 10
Salmonella (per 25g)	Absent in 25g	Neg/25g	Neg/75g	Negative
Arsenic (ppm; w/w)	< 0.4	< 0.05	< 0.1	< 0.4
Lead (ppm; w/w)	< 0.2	< 0.02	< 1.0	< 0.2
Cadmium (ppm; w/w)	< 0.06	< 0.1	< 0.06	< 0.06
Mercury (ppm; w/w)	< 0.5	<0.01	< 0.1	< 0.005

2. Absorption, Distribution, Metabolism, and Excretion

GOS including VITAGOS™ IF are non-digestible oligosaccharides consisting of 1 to 7 galactose units linked via 1-3, 1-4, or 1-6 β-glycosidic bonds to either a terminal glucose or galactose. The absorption, distribution, metabolism, and excretion of GOS and their metabolites have been extensively reviewed in GRNs 236, 286, and 334, and by the Scientific Committee on Food (2001) and FSANZ (2008). It is generally recognized that with the exception of lactose, which is hydrolyzed by small intestinal brush border lactase, beta-linked sugars are not digested by human pancreatic or intestinal enzymes (Ohtsuka et al., 1990; Wisker et al., 1985; Chonan et al., 2004). GOS are not absorbed and are transported intact to the large intestine where they are subjected to fermentation by the indigenous microbiota. Although *in vitro* studies have reported slight differences in the efficiency by which particular bacterial species metabolize GOS, they are ultimately hydrolyzed to glucose and galactose, which are subsequently metabolized by the anaerobic microflora by the Embden-Meyerhof-Parnas pathway resulting in the production of short chain fatty acids, CO_2 and H_2 gas (common and innocuous dietary metabolites) (cited in GRN 620;. Ohtsuka et al., 1991; Suarez et al., 1999; Smiricky-Tjardes et al., 2003). Importantly, short-chain fatty acids, carbon dioxide, methane and hydrogen are the same metabolites as those

produced by the microbiota following the ingestion of other foods and are either absorbed, exhaled, or excreted (reviewed in Slavin, 2013). Therefore, VITAGOS™ IF is expected to be unabsorbed followed by fermentation by the microflora in the colon, producing short chain fatty acids, CO_2 and H_2 gas.

3. Toxicology Studies

a. Genotoxicity Studies

The genotoxicity of GOS-containing products is extensively reviewed in GRNs 334 and 620 and the subjects of this GRNs have been determined not to be genotoxic. As summarized in GRN 334 on pages 61-63, Kobayashi et al. (2009) showed that GOS are not mutagenic, genotoxic, or clastogenic using a bacterial reverse mutation, a chromosomal aberration assay, and an *in vivo* micronucleus study. As summarized in GRN 620 pages 20-21, Narumi et al. (2014) showed that GOS are not genotoxic using an *in vivo* comet assay. In addition, two non-publicly available bacterial reverse mutation assays and an *in vitro* micronucleus assay were reviewed in GRN 620 and, importantly, corroborate the lack of genotoxicity reported by Kobayashi et al. (2009) and Narumi et al (2014).

b. Sub-chronic Rodent Toxicology Studies

The safe use of VITAGOS™ IF is supported by the publicly available 90-day toxicology study conducted with Vivinal® GOS, which is extensively summarized on pages 38-41 of GRN 236 and therefore incorporated by reference (Anthony et al., 2006). In summary, in the pivotal 90-day sub-chronic rat toxicology study of Vivinal® GOS, the NOAEL was the highest dose administered at 2.25 g GOS/kg body weight/day (5 g Vivinal® GOS/kg body weight/day) (Anthony et al., 2006). In GRN 236, an unpublished, corroborative 90-day subchronic rat study using Vivinal® GOS was described where Wistar rats were orally administered GOS at doses up to 6900 mg Vivinal® GOS/kg-bw/day. The NOAEL for GOS was determined to be at the highest dose tested at 6900 mg Vivinal® GOS/kg-bw/day.

The safe use of VITAGOS™ IF is supported by other sub-chronic toxicology studies of GOS which are summarized in GRN 721 pages 23-33 and GRN 729 pages 41-44 and are incorporated by reference (Kobayashi et al. 2009; Penard 2015, unpublished; Zhou et al., 2017). In summary, GOS was non-toxigenic up to a dose of 2000 mg GOS/kg bw/day and the NOAEL for each study was the highest dose tested.

Briefly, the 90-day toxicology conducted by Kobayashi et al. (2009) using a GOS product manufactured with β-galactosidases derived from *S. singularis* and *K. lactis* utilized male and female Sprague Dawley rats gavaged with water, 500, 1000, or 2000 mg/kg/day of a syrup containing approximately 55% GOS (% dry matter). This resulted in a daily intake of 206.25, 412.5, 825 mg GOS/kg. There were no GOS-related changes in clinical signs, body

weight, water intake, feed intake, urinalysis, ophthalmology, hematology, blood chemistry, organ weights or cecum weight, gross pathology, or histopathology. The relative and absolute weight of the cecum was higher in the 2000 mg/kg/day male group compared to control. The NOAEL was set to 2000 mg/kg/day for the GOS-containing product equivalent to 825 mg GOS/kg/day.

An unpublished repeat dose toxicity study by Penard (2015), was conducted on Nestlé GOS by daily oral gavage to male and female Wistar rats (10 rats per sex per group). The rats were administered doses of 0, 500, 1000, and 2000 mg/kg bw/day of GOS for 30 days in accordance with OECD 407 (Study does not specify if the doses were GOS or GOS containing product; the product is 46% GOS minimum). There were no deaths and no significant differences in hematology, coagulation, clinical chemistries, or urinalysis between groups. The Nestlé GOS was well-tolerated and the NOAEL was determined to be 2,000 mg/kg bw/day, the highest dose tested.

A subchronic oral toxicity study of VITAGOS™ in Sprague-Dawley rats was conducted in accordance with OECD protocol 408 (Zhou et al., 2017). For 90 days, the rats were given 0, 2010, 2041, and 4082 mg GOS syrup/kg bw/day (corresponding to 0, 500, 1000, or 2000 mg GOS/kg bw/day) by oral gavage. There were no test article-related toxicologically relevant findings in body weight, hematology, clinical chemistries, urinalysis, organ weights, or histopathology. While significant increases in cecum weights treated with the highest dose of 2000 were associated with mucosal hyperplasia; no changes were seen at lower doses and these findings were consistent with previously seen effects by poorly digestible substances. The NOAEL for VITAGOS™ was established to be 4082 mg GOS syrup/kg bw/day or 2000 mg GOS/kg bw/day, the highest dose tested.

c. Reproductive and Developmental Studies

Additional toxicology studies that corroborate the safety of VITAGOS™ IF include a neonatal rodent toxicity study conducted in juvenile rats and a one-generation reproductive and developmental toxicity study are summarized in GRN 721 pages 33-34 and are incorporated by reference (Kobayashi et al., 2014a,b). In summary, GOS did not exhibit developmental or reproductive toxicity at doses up to 853 mg GOS/kg bw/day.

In the study by Kobayashi et al. (2014a), juvenile Sprague-Dawley rats were administered the GOS product that was the subject of GRN 334 by gavage for 42 days starting on post-natal day 4 at 0, 500, 1000, or 2000 mg/kg/day of a syrup containing approximately 56.9 % GOS (% dry matter), resulting in a daily intake of 213.4, 426.8, and 853.5 mg GOS/kg. GOS consumption was reported to have no effect on the development of the animals and did not affect general condition, hematology, blood chemistry, or the outcome of any functional examinations. No abnormalities in any of the groups were observed during the macroscopic examination, assessment of organ weights, or histopathology of the reproductive organs. The NOAEL for

Oligomate GOS in juvenile Sprague-Dawley rats was 2,000 mg/kg/day equivalent to 853.5 mg GOS/kg/day (Kobayashi et al., 2014a).

Kobayashi et al. (2014b) evaluated the developmental and reproductive effects of the GOS product that was the subject of GRN 334 in male and female parental rats, pregnant females, and their offspring. Male and female Sprague-Dawley rats (24 per sex per group) were administered GOS by gavage at doses of 0, 500, 1,000, or 2,000 mg/kg/day of a syrup containing approximately 56.9 % GOS (% dry matter), resulting in a daily intake of 213.4, 426.8, and 853.5 mg GOS/kg/day. Males were dosed 10 weeks prior to mating and 3 weeks thereafter; females were dosed 2 weeks before mating and GOS administration continued through pregnancy to day 20 of lactation. GOS consumption did not produce any toxicological effects on male or female parental animals and did not adversely affect reproduction/development from premating, copulation, implantation, or maintenance of pregnancy. The offspring were unaffected by the maternal consumption of GOS. No effects were observed on the number of live births, sex ratio, and external observation at the time of birth, body weight, pup survival, or external differentiation during lactation. The NOAEL for reproductive function of male and female parent animals was 2,000 mg GOS per kg/day equivalent to 853.5 mg GOS/kg/day (Kobayashi et al., 2014b).

d. Neonatal piglet Studies

A neonatal piglet study investigating the effects of GOS consumption on the intestinal microbiota was described in GRN 729 page 48 and incorporated by reference (Alizadeh et al., 2016). In summary, forty Landrace x Yorkshire piglets (24-48 post-partum) were given milk diets consisting of milk replacer alone or milk replacer supplemented with 0.8% GOS to mimic the amount of GOS added to infant formula, (Vivinal® GOS syrup, 75% GOS) for up to 26 days. While chemistry, hematology and pathology safety endpoints were not evaluated, it was noted that the piglets remained healthy, did not have significant changes in body weight, and did not exhibit diarrhea during the testing period.

e. New Studies

An updated literature search was conducted on August 6th, 2021 using the search terms "GOS toxicology", "galacto-oligosaccharides toxicology", "GOS animal studies", "galacto-oligosaccharides animal studies", "GOS genotoxicity", "galacto-oligosaccharides genotoxicity", "GOS piglet study", "galacto-oligosaccharides toxicology piglet study" and related terms in the Pubmed and Google Scholar databases and uncovered two additional genotoxicity studies, a 14-day range finding study, a subchronic 90-day rat toxicology study, and a juvenile rat toxicity study since the filing of GRN 729 (Jain et al., 2019; Jain et al 2020). These corroborative studies confirm the non-mutagenicity (at doses up to 5000 µg/plate) and non-toxigenicity (at doses up to

3000 mg GOS/kg bw/day) of GOS and are summarized below. The animal toxicity studies are also tabulated in Table 13.

Jain et al. (2019) performed a bacterial reverse mutation assay on Gossence™ (Tata Chemicals Ltd; 76.3% GOS DM). The initial assay was conducted using the test article at concentrations of 50, 159, 501, 501, and 5000 µg/plate, in the presence and absence of S9, using the plate incorporation method. The growth of the bacterial background lawn and mean number of revertant colonies were comparable to the control plates in both presence and absence of S9. There was no two-fold increase in the mean number of revertants of test strains TA98, TA100, WP2 uvrA or three-fold increase in test strains TA1535 and TA1537. A confirmatory assay was conducted using the test article at concentrations of 0, 99, 265, 699, 1869, and 5000 µg/plate in the presence and absence of S9, using the pre-incubation method. Results of the confirmation assay showed that the mean number of revertant colonies were similar to the initial assay. In conclusion, GOS was not cytotoxic or mutagenic up to 5000 µg/plate.

The clastogenic potential of Gossence™ was assessed using an *in vitro* mammalian chromosomal aberration assay in HPBL cells (Jain et al., 2019). The preliminary cytotoxicity data obtained for the test item concentrations from 0.0312 mg/mL to 5.0 mg/ mL were <8%. Concentrations studied were 0, 1.25, 2.5, and 5.0 mg/mL along with ethyl methane sulphonate (short and prolonged duration, -S9) and cyclophosphamide monohydrate (short duration, +S9) were employed as clastogenic positive controls. There were no statistically significant increases in the number of percent aberrant metaphase for GOS when compared to the control group. In conclusion, GOS was not clastogenic in the *in vitro* mammalian chromosomal aberration assay.

Jain et al. (2019) also performed an OECD-compliant 14-day dose range finding study and an OECD-compliant 90-day subchronic oral toxicity study in Sprague-Dawley rats on Gossence™ (Tata Chemicals Ltd; 76.3% GOS DM). For 14 days, 6 male rats and 6 female rats per group were administered 0, 1000, 2000, and 5000 mg GOS/kg bw/day (Gossence™; Tata Chemicals Ltd; 76.3% GOS DM) (equivalent to 1347, 2694, and 6735 mg/kg/day of Gossence™, respectively) by oral gavage. The control group was administered purified water. No clinical signs of toxicity were observed in any animal. A slight decrease (13%) in body weight gain in males at the 5000 mg/kg/day dose was observed, which correlated with a statistically significant reduction in overall food consumption (14%). No test-article related changes in hematology, coagulation, clinical chemistries, and urinalysis were observed at the completion of the study. There was a test-article related increase in cecum weight observed at higher doses (2000 and 5000 mg/kg) which correlated with mucosal epithelial hypertrophy at 5000 mg/kg.

In the 90-day subchronic toxicity study, doses were selected on the basis of the 14-day study, and rats were administered 0, 1000, 2000, and 5000 mg GOS/kg bw/day (equivalent to

1347, 2694, and 6735 mg/kg/day of Gossence™, respectively) by gavage with two additional 28-day recovery groups (control and high dose). There were 10 male and 10 female rats per the experimental group and 6 male and 6 female rats in the recovery groups (Jain et al., 2019). No mortalities or test-article related adverse findings in clinical signs, or ophthalmic findings at any dose level were observed. The overall food consumption (days 1–90) was comparable with the control group up to dose levels of 2000 mg/kg/day in males and up to dose levels of 5000 mg/kg/day in females. A statistically significant reduction (14%) in overall food consumption was observed in male rats at the 5000 mg/kg dose as compared to the control group but was considered minimal and consumption completely recovered during the recovery period. Food efficiency was comparable in test groups to the controls. No treatment-related changes were observed in neurological/functional examination and motor activity parameters carried out at the end of the dosing period for main groups. There were no test item-related changes in hematology, coagulation, clinical chemistries, or urinalysis. At necropsy, increased size of the cecum was observed in 6/10 males and 7/10 females at 5,000 mg/kg/day. Test item–related significantly increased absolute and relative weights (relative to body weight and brain weight) of cecum (with content) was observed in males at ≥ 2000 mg/kg/day and females at 5,000 mg/kg/day. Cecal weights (without content) were significantly increased only at 5,000 mg/kg/day in both male and female rats which correlated with minimal mucosal hypertrophy. No test item–related histopathology microscopic changes were observed in the cecum at lower dose levels. Organ weight, gross, and histopathology changes were not observed in the cecum of rats in the recovery group, indicating complete recovery from these changes, which is a common finding in non-digestible substances-administered toxicity tests (Whitely et al., 1996; Oku et al., 1997; Zhou et al., 2017) The NOAEL for GOS was established to be 5000 mg GOS/kg bw/day (equivalent to 6735 mg Gossence™/kg bw/day).

A juvenile toxicity study of Gossence™ (compliant with ICH 2009 and OECD Principles) in juvenile Sprague-Dawley rats 3 days post-partum was conducted for 49 days (Jain et al., 2020). The pregnant female rats were allowed to litter naturally. The day of delivery was designated as day "0" of lactation (postpartum). Each litter was observed as soon as possible after delivery for the number of pups born, sex of each pup, and presence of any gross abnormalities. Dams (F0 generation dams) were used to maintain F1 generation pups and hence were not considered as a part of the test system. On day 3 of the postpartum, the size of each litter was adjusted to four pups/sex/litter for selection of main group animals and three pups/sex/litter for selection of recovery group animals by removing extra pups randomly. The extra pups that are not assigned to the study were culled on day 3 of the postpartum (i.e. on the day of litter standardization/ randomization). No pup was cross-fostered during litter standardization. On postnatal day 3, a total of 120 pups (60 males and 60 females) from 16 dams were assigned to 4 main groups and 2 recovery groups. Pups were weaned on day 21. Juvenile rats were administered by gavage 0, 1000, 2000, 5000/3000 mg GOS/kg bw/day (Gossence™;

Tata Chemicals Ltd; 76.3% GOS DM; equivalent to 1347, 2694, and 6735/4041 mg/kg/day of GOS, respectively) from day 4 to 52. The high dose was reduced to 3000 mg/kg/day from day 16 due to mortality in 2 animals at 5000 mg/kg/day. There were 12 males and 12 females each for the main group and 6 males and 6 females each for the recovery control and recovery-highest dose group. The recovery period was 14 days.

There were 2 deaths that occurred on day 15 resulting in the decrease of the highest dose to 3000 mg/kg/day. While the cause of death could not be determined, both animals contained yellowish liquid in their intestines. After the reduction of the highest dose, there were no further deaths or clinical signs of toxicity in any of the groups. There were no test article-related changes in ophthalmoscopic observations. A statistically significant decrease in mean body weights at the 5000 mg/kg/day dose was observed in both sexes but after reduction of the highest dose, no further decrease in weight was observed with 3000 mg/kg/day dose group. There were no test article-related changes to the acquisition of different rat postnatal developmental landmarks such as hair growth, incisor eruption, ear opening, eye opening, balanopreputial separation, and vaginal opening during postnatal growth or in the percentage of pups acquiring these parameters on a particular day when compared to the vehicle control group. There were no test article-related changes observed in functional observational battery tests, neuromuscular observations, and motor activity parameters compared to control. There were no test-article related changes in hematology, coagulation, clinical chemistries, and urinalysis. An increase in cecum weights was increased in the 5000/3000 mg/kg/day dose group but did not correlate with histopathologic changes. The cecum enlargement completely recovered after the 14 day recovery period and is a common finding for non-digestible substances administered in rodent toxicology tests (Whitely et al., 1996; Oku et al., 1997; Zhou et al., 2017). There were no other test article-related changes in organ weights, gross changes, or histopathological findings. The NOAEL for this juvenile rat study was established to be 3000 mg GOS/kg/day. Based on the results of this study, the authors concluded that Gossence (GOS) has no effect on the growth and development of juvenile rats of ages day 4 to 7 weeks post birth.

Table 13. Recent Animal Toxicity Studies of GOS				
Reference	**Study Design and Population**	**Groups (Numbers of Subjects)**	**Duration**	**Safety Parameters**
Jain et al., 2019	14-day range finding study in Sprague-Dawley rats (OECD-compliant)	Oral gavage administration of 0, 1000, 2000, and 5000 mg GOS/kg bw/day (Gossence™; Tata Chemicals Ltd; 76.3% GOS DM) (equivalent to 1347, 2694, and 6735 mg/kg/day of Gossence™, respectively) Control group was administered purified water. 6 males and 6 females per group.	14 days	• Observations revealed no clinical signs of toxicity to any animal. • Slight decrease (13%) in body weight gain in males at the 5000 mg/kg/day dose, which correlated to statistically significant reduction in overall food consumption (14%) • No test-article related changes in hematology, coagulation, clinical chemistries, and urinalysis. • Increased cecum weight was observed at higher doses (2000 and 5000 mg/kg) which correlated with mucosal epithelial hyperplasia at 5000 mg/kg.
	Subchronic, repeat dose oral toxicity study in Sprague-Dawley rats (OECD-compliant)	Oral gavage administration of 0, 1000, 2000, and 5000 mg GOS/kg bw/day (Gossence™; Tata Chemicals Ltd; 76.3% GOS DM) (equivalent to 1347, 2694, and 6735 mg/kg/day of Gossence™, respectively) Control group was administered purified water. 10 males and 10 females per main group. 6 males and 6 females per recovery group.	90 days	• No mortalities, clinical signs, or ophthalmic findings at any dose level were observed. • A statistically significant reduction (14%) in food consumption was observed in male rats at the 5000 mg/kg dose as compared to the control group but was considered minimal and consumption completely recovered during the recovery period. • There were no test-item related changes in hematology, coagulation, clinical chemistries, or urinalysis. • Cecal weights in both male and female rats significantly increased correlating with minimal mucosal hypertrophy. However, organ weight and histopathology changes were not observed in the recovery group, indicating recovery. • The NOAEL for GOS was established to be 5000 mg GOS/kg bw/day (equivalent to 6735 mg Gossence™/kg bw/day)

Table 13. Recent Animal Toxicity Studies of GOS				
Reference	**Study Design and Population**	**Groups (Numbers of Subjects)**	**Duration**	**Safety Parameters**
Jain et al., 2020	Juvenile toxicity study in Sprague-Dawley rats (OECD-compliant; age day 3 post-partum)	Oral gavage by intubation administration of 0, 1000, 2000, 5000/3000* mg GOS/kg bw/day (Gossence™; Tata Chemicals Ltd; 76.3% GOS DM) *High dose reduced to 3000 mg/kg/day from day 16 due to mortality in 2 animals at 5000 mg/kg/day 12 males and 12 females each for the main group 6 males and 6 females each for the recovery control and highest dose group	49 days	• 2 deaths occurred at day 15 resulting in decrease of highest dose to 3000 mg/kg/day (cause of death could not be determined but both animals contained yellowish liquid in intestines) • No further deaths or clinical signs in any of the groups • No test article-related changes in ophthalmoscopic observations. • Statistically significant decrease in mean body weights at the 5000 mg/kg/day dose was observed in both sexes. After reduction of highest dose, no further decrease in weight was observed with 3000 mg/kg/day dose group. • There were no test article-related changes to the acquisition of different developmental landmarks such as hair growth, ear opening, etc. • There were no test article-related changes observed in functional observational battery tests, neuromuscular observations, and motor activity parameters compared to control. • There were no test-article related changes in hematology, coagulation, clinical chemistries, and urinalysis. • Cecum weights were increased in the 5000/3000 mg/kg/day dose group but did not correlate with light microscopic changes. Cecum enlargement completely recovered after the 14 day recovery period. • There were no other test article-related changes in organ weights, gross changes, or histopathological findings. • The NOAEL for this developmental juvenile rat study was established to be 3000 mg GOS/kg/day.
bw, body weight; DM, dry matter; NOAEL, no observed adverse effect level; OECD, Organization for Economic Co-operation and Development				

f. Clinical Studies

i. Infants and Children

No clinical trials have been conducted with VITAGOS™ IF in infants and young children, but other GOS preparations have been clinically evaluated. Although some of the studies are unpublished, all have been extensively summarized in GRAS Notifications 236, 285, 286, 334, 484, 489, 495, 518, and 620, 721, and 729. In general, GOS are well tolerated. The clinical trials using Vivinal® GOS and other sources of GOS in infants and young children cited in GRNs 721 pages 36-39 and 729 pages 49-52 are incorporated by reference (Bozensky et al., 2015; Civardi et al., 2015; Matsuki et al., 2016; Pontes et al., 2016). In summary, the four studies cited in GRNs 721 and 729 ranged in duration from 14 days to 6 months and included doses of 3g GOS/L to 8.5g GOS/L per serving of formula. Test articles were reported to be well tolerated in all studies.

A literature search was conducted on August 6th, 2021 using the search terms "GOS clinical study/trial", "galacto-oligosaccharides clinical study/trial", "GOS study", and "galacto-oligosaccharides study" in the Pubmed and Google Scholar databases and uncovered six additional clinical studies in infants and children as of the filing of GRN 729 (Paganini et al., 2017; Chua et al., 2017; Xinias et al., 2018; Kosuwon et al., 2018; Rodriguez-Herrera et al., 2019; Béghin et al., 2021). In summary, the studies ranged in duration from 1 month to 6 months with doses from 0.5 g/L to 8.55 g/L. In all of the studies, GOS was well-tolerated in infants and children. These studies are tabulated in Table 14 and summarized below.

In a randomized, double-blind, controlled study in healthy Kenyan infants at risk for iron deficiency anemia (age 6.5-9.5 months), Paganini et al. (2017) studied the effect of iron supplementation plus GOS (FrieslandCampina Vivinal® GOS) had on Kenyan infants at risk for iron deficiency anemia. At an intended use level of 7.5 g GOS/L, intake over four months resulted in no negative safety effects, and no serious adverse events were reported.

Chua et al. (2017) utilized a randomized, double-blind study to test the effects of a formula supplemented with 8 g/L scGOS/lcFOS and a formula supplemented with scGOS/lcFOS plus *B. breve* M-16V on the gut microbiome of cesarean-born infants for 16 weeks. While adverse events were monitored, no difference between groups and control were reported and test articles containing GOS were well tolerated.

In a controlled, open study in exclusively formula fed infants (3-13 weeks), Xinias et al. (2018) enrolled sixty infants with functional constipation (defined as less than 3 bowel movements per week with hard and tight stools) and forty-five of them ingested infant formula

with partial whey hydrolysate, *B. lactis*, and 5 g/L GOS plus 8 mg magnesium for one month. Results showed that the infant formula with GOS was well tolerated as there was no change in defecation frequency in either group.

Kosuwon et al. (2018) utilized a randomized, double-blind, placebo-controlled study to study the impact of young child formula supplemented with 9.5 g/L scGOS/lcFOS (9:1) and *B. breve* M-16V on the development of fecal microbiota in healthy toddlers (1-3 years old) for 12 weeks. There was no statistically significant difference between adverse events reported between the test and control group and no difference between stool frequencies indicating the test article was well tolerated.

In a prospective, double-blind, randomized, controlled study, Rodriguez-Herrera et al. (2019), studied the tolerance and safety of a partially fermented infant formula supplement with scGOS/lcFOS in healthy infants for 17 weeks. Infants received either control formula or formula consisting of 30% fermented formula with *B. breve* C50 and *S. thermophilus* 065 and scGOS/lcFOS (9:1; 0.8 g/L). As compared to the control formula, the experimental formula was well tolerated, supported adequate infant growth, and did not result in any significant differences in adverse events.

Lastly, Béghin et al. (2021) utilized a prospective, randomized, double-blind, controlled study to study the safety of formula containing the bioactive components from formula fermentation (FERM), formula supplemented with scGOS/lcFOS, and formula supplemented with both FERM and scGOS/lcFOS in healthy infants for 6 months. Formula was fermented with *B. breve* C50 and *S. thermophilus* 065 and contained a 9:1 ratio of scGOS/lcFOS. The formulas were well-tolerated as most adverse events reported were considered mild and unrelated to the study formulas, and the number of adverse events did not statistically differ between formula groups and control.

ii. Adults

No clinical trials have been conducted with VITAGOS™ IF in adults, however, other GOS preparations have been evaluated. Although some of the studies are unpublished, all have been extensively summarized in GRAS Notifications 236, 285, 286, 334, 484, 489, 495, 518, and 620, 721, and 729. In general, GOS are well tolerated in adults. The clinical trials using Vivinal® GOS and other sources of GOS in adults cited in GRN 721 (pg. 40) and GRN 729 (pg. 49-50) are incorporated by reference (Davis et al., 2010; Walton et al., 2012; Whisner et al., 2013; Vulevic et al., 2013; Ladirat et al., 2014). In summary, in the five clinical studies cited in GRN 721 and 729, GOS consumption ranged from 12 days to 16 weeks and included doses ranging from 2.5 g to 10 g GOS/day. Two of those studies were conducted on Vivinal® GOS, in

which adults consumed 5g GOS twice daily (Walton et al., 2012; Whisner et al., 2013). While there was one case of a withdrawal due to diarrhea, no other serious adverse events or dropouts due to GOS consumption in any of the studies were reported.

There have been four additional published clinical trials in adults since the filing of GRN 729 based on a literature search conducted on August 6th, 2021 (Krumbeck et al., 2018; Dall'Oglio et al., 2018; Schaafsma et al., 2021; Wilms et al., 2021). In summary, GOS consumption ranged from 3 weeks to 3 months and included doses ranging from 0.5 g to 15 g GOS/day. In all of the studies, GOS was well-tolerated in adults. These studies are tabulated in Table 15 and summarized below.

Krumbeck et al. (2018) utilized a double-blind, randomized, placebo-controlled trial to study the effect of a microorganism (*B. adolescentis* IVS-1 or *B. animalis* BB-12), 5 g/day Vivinal® GOS or combination supplement on gut microbiota in obese adults for 3 weeks. Results show no difference in complete blood counts and metabolic panels between any group and no serious adverse events were reported suggesting the test articles are safe and tolerated.

In an open, prospective, proof-of-concept trial, Dall'Oglio et al., (2018) evaluated the effect of 3-month oral supplementation of FOS and GOS in women with adult acne. Twelve women (mean age 35 years old) consumed one sachet daily containing 100 mg FOS and 500 mg GOS. No serious adverse events were reported and the product was well tolerated.

Schaafsma et al. (2021) utilized a randomized, placebo-controlled, cross-over study to study the effect of whey-protein and GOS in healthy adults with moderate sleep disturbances. Seventy adults (age 30-50) consumed a 150 mL dairy-based product (DP) with 5.3 g GOS or placebo for 21 days before switching after a washout period. There were no differences in tolerability, specifically flatulence, nauseous, and bloated feeling, and no statistically significant differences in adverse events between the products.

In a randomized, placebo-controlled, cross-over study in prefrail elderly and healthy adults, Wilms et al. (2021) studied the effect of GOS supplementation for 4 weeks. Participants consumed 3 sachets of powder a day which amounted to 21.6 g/day of maltodextrin (placebo) or Biotis™ GOS (15.0 g/day pure GOS). There were no significant differences in tolerance of the product compared to placebo. No serious adverse events were reported. Taken together, all clinical studies indicate that GOS are well tolerated and safe for consumption in infants, young children, and adults.

Table 14. Recent Studies of GOS Ingestion in Infants and Young Children

Reference	Study Design and Population	Groups (Numbers of Subjects)	Duration	Safety Parameters
Béghin et al., 2021	Prospective, randomized, double-blind, controlled, multi-center study in healthy infants (37-42 weeks of age	Group 1 (Control): Cow's milk-based, iso-caloric formula, n=70 Group 2: base formula supplemented with bioactive components from formula fermented with *B. breve* C50 and *S. thermophilus* 065 (FERM), n=70 Group 3: base formula supplemented with 9:1 scGOS/lcFOS, n=70 Group 3=4: base formula supplemented with FERM and scGOS/lcFOS, n=70	6 months	Withdrawals: • 83 infants dropped out of the study but there was no significant difference in drop-out rates between groups Adverse Events: • There were no statistically significant differences in adverse events reported in the supplemented groups as compared to the control group. • The majority of AEs were mild and unrelated to the test articles. Tolerance: • All formulas were well-tolerated Growth Parameters: • No significant differences in anthropometric measurements between any of the groups
Chua et al., 2017	Randomized, double-blind, multicenter center in Cesarean delivered infants	Group 1 (Control): Nonhydrolyzed cow's milk based formula; n=50 Group 2: Same formula supplemented with 0.8g/100mL short-chain GOS/long-chain FOS; n=51 Group 3: Formula supplemented with scGOS/lcFOS and *B. breve* M-16V (7.5x10^8 cfu/100mL); n=52	16 weeks	Adverse Events: • Only 1 adverse event related to study in control and scGOS/lcFOS groups (irritability and constipation of mild severity, respectively) Tolerance: • There were no significant differences between the groups

Table 14. Recent Studies of GOS Ingestion in Infants and Young Children

Reference	Study Design and Population	Groups (Numbers of Subjects)	Duration	Safety Parameters
Kosuwon et al., 2018	Randomized, double-blind, placebo-controlled study in healthy toddlers (1-3 years old)	Group 1 (Control): Young child formula, n=64 Group 2: Young child formula with 0.95 g/dl of GOS/FOS (9:1) + 1.8×10^7 cfu/g *B. breve* M-16V, n=65	12 weeks	Withdrawals: • 5 subjects withdrew in Group 1 • 3 subjects withdrew in Group 2 • Reasons for withdrawal were not reported. Adverse Events: • 246 AEs reported in the study, 122 in Group 1 and 124 in Group 2. These were not significantly different between the groups. • Gastrointestinal disorders were commonly reported, of which 33 were probably related to the study product: o 19 in Group 1 (12 constipation, 3 diarrhea, 3 flatulence, 1 vomiting o 15 in Group 2 (6 constipation, 8 diarrhea, and 1 viral gastroenteritis) Tolerance: • No difference found in stool frequency between the groups..
Paganini et al., 2017	Randomized, double-blind, controlled study in healthy Kenyan infants at risk for iron deficiency anemia (age 6.5-9.5 months)	Group 1 (Control): Micronutrient powder without iron and without GOS; n=51 Group 2 (Fe Group): Micronutrient powder with 2.5mg FeFum + 2.5mg NaFeEDTA; n=52 Group 3 (FeGOS Group): Micronutrient powder with 2.5mg FeFum + 2.5mg NaFeEDTA + 10.5g of 75% GOS (Vivinal® GOS 75; FrieslandCampina)	4 months	Withdrawals: • 10 subjects withdrew from the trial due to relocation or consent withdrawal (3 subjects each from Control and Fe Groups and 4 subjects from the FeGOS Group) Adverse Events: • Adverse events were monitored but no serious adverse events were reported

Table 14. Recent Studies of GOS Ingestion in Infants and Young Children

Reference	Study Design and Population	Groups (Numbers of Subjects)	Duration	Safety Parameters
Rodriguez-Herrera et al., 2019	Randomized, double-blind, multi-center, placebo-controlled study in healthy infants (37-42 weeks of age)	Group 1 (Control): Cow's milk-based, iso-caloric formula, n=94 Group 2: base formula supplemented with 30% fermented formula (fermented with *B. breve* C50 and *S. thermophilus* 065) and scGOS/lcFOS (9:1; 0.8 g/L), n=105	17 weeks	Withdrawals: • 48 infants dropped out but there was no statistically significant difference in drop-out rate between groups Adverse Events: • No clinically relevant differences in adverse events were observed • GI-related symptoms most reported AE but no difference between groups was observed Tolerance: • Both formulas were well tolerated Growth Parameters: • Both formulas supported adequate infant growth
Xinias et al., 2018	Controlled, open study in exclusively formula fed infants (3-13 weeks) with functional constipation with no organic cause	Group 1 (Control): Reassurance with no other intervention; n=25 Group 2: Study formula consisting of partial whey hydrolysate, *B. lactis*, and 0.5g/100mL GOS supplemented with 8mg magnesium; n=40	1 month	Withdrawals: • No subjects withdrew Adverse Events: • Adverse events were not monitored or reported Tolerance: • No change in defecation frequency in either group

Table 15. Recent Studies of GOS Ingestion in Adults

Reference	Study Design and Population	Groups (Numbers of Subjects)	Duration	Safety Parameters
Dall'Oglio et al., 2018	Open, prospective, proof-of-concept trial in women with adult acne (avg age 35 years of age)	Participants consumed one sachet daily containing 100 mg FOS and 500 mg GOS n=12	3 months	Withdrawals: • There were no withdrawals from the study Adverse Events: • No serious adverse events were reported Tolerance: • Product was well-tolerated
Krumbeck et al., 2018	Double blind, placebo-controlled randomized trial in obese adults (18-65 years of age)	Study investigating the effect of a microorganism (IVS-1 or BB-12), GOS (Vivinal® GOS) or combination supplement. Group 1 (control): lactose (7 g/day) Group 2: *B. adolescentis* IVS-1 (1×10^9 CFU/day) Group 3: *B. animalis* BB-12 (1×10^9 CFU/day) Group 4: GOS (5 g/day) Group 5: IVS-1 + GOS Group 6: BB-12 + GOS n≈20 per group	3 weeks	Withdrawals: • 7 subjects were lost to follow-up • 13 subjects did not follow the protocol • There were no differences in withdrawal rates between the groups Adverse events: • Adverse events were monitored and no serious adverse events were reported Tolerance: • Test articles were generally well tolerated with minimal reported side effects. Other parameters: • No difference in complete blood counts or metabolic panels between any test article group • Effects on gastrointestinal symptoms: The GOS group had significantly harder stools when compared to the BB-12 +GOS group. The "severity of passing gas" was significantly reduced in the BB-12 +GOS group, and severity of hard stools increased in the GOS group. • IVS-1 and all GOS-containing groups increased fecal Bifidobacterium counts. • IVS-1, IVS-1 + GOS and GOS had a beneficial effect on colonic permeability. There was no synergistic effect.

Table 15. Recent Studies of GOS Ingestion in Adults

Reference	Study Design and Population	Groups (Numbers of Subjects)	Duration	Safety Parameters
Schaafsma et al., 2021	Randomized, placebo-controlled, cross-over study in healthy adults (ages 30-50) with moderate sleep disturbances. Seventy adults (age 30-50). There were no differences in tolerability, specifically flatulence, nausea, and bloated feeling, and no statistically significant differences in adverse events between the products.	Participants consumed a 150 mL dairy-based product (DP) with 5.3 g GOS or placebo for 21 days before switching after a 3 week washout period n=70	3 weeks	Withdrawal: • Only 1 participant withdrew from study due to unrelated reason Adverse Events: • There were no significant differences in the number of adverse events reported between placebo and test article groups. Tolerance: • The test article and placebo did not show difference in tolerability specifically, flatulence, nauseous, and bloated feeling.
Wilms et al., 2021	Randomized, placebo-controlled, cross-over study in prefrail elderly (ages 70-85) and healthy adults (ages 25-50)	Participants consumed 3 sachets of powder a day which amounted to 21.6 g/day of maltodextrin (placebo) or Biotis™ GOS (15.0 g/day pure GOS). n=20 elderly and 24 adults (44 total)	4 weeks	Withdrawals: • 3 adults withdrew from the study. Adverse Events: • No serious adverse events were reported. Tolerance: There were no significant differences in tolerance of the product compared to placebo.

B. ALLERGENICITY

The allergenicity of GOS has been extensively summarized in GRN 729, 721, 620, and 236. As summarized in GRN 729 pages 53-54, a small percentage of GOS-mediated allergic reactions have been reported in Southeast Asia, specifically by the products manufactured with a β-galactosidase derived from *Bacillus circulans* (Vivinal® GOS) and *Aspergillus oryzae* (Chiang et al., 2012; Kaneko et al., 2014; Soh et al. 2015). No other GOS-mediated allergic reactions have been reported. To date the cause of the allergy to GOS has not been identified, however, some in vitro studies have suggested that the allergenicity of GOS may be due to the presence of linear and branched tetrasaccharides with the structures of Gal-β(1→4)-Gal-β(1→4)-Gal-β(1→3)-Glc or Gal-β(1→4)-(Gal-β(1→4)-Gal-β(1→6))-Glc, also known as "4-PX" whereas others have reported that 4-PX is not the causal allergen for the observed GOS allergy in Southeast Asia (Kaneko et al., 2014; Huang et al., 2016). Importantly, GOS preparations have been widely consumed in Southeast Asia, as well as globally for over a decade by adults, children, and infants, with only a limited number of reported allergenic reactions. Because VITAGOS™ IF is produced from a β-galactosidase derived from *B. circulans*, which is the same enzyme used to produce Vivinal® GOS, which is the subject of GRN 236, it is expected that the likelihood of VITAGOS™ IF GOS to provoke an allergic reaction in sensitized individuals will be the same as Vivinal® GOS, which is relatively low. Similar conclusions have been reached in GRN 235, 495, 620, and 729, which received "no questions" letters.

C. SAFETY OF THE B-GALACTOSIDASE DERIVED FROM *B. CIRCULANS* M3-1

The subject of this GRAS Determination is produced from food grade lactose via a transgalactosylation reaction catalyzed by a β-galactosidase enzyme obtained from the natural, non-GMO, non-pathogenic, bacterium *B. circulans*. The Bacillus genus is a group of gram positive, rod-shaped bacteria that contain a large number of bacterial strains that have been used industrially in the preparation of a number of enzymes that are utilized in food production (Schallmey et al., 2004). Importantly, the same enzyme is used to produce the subject of GRN 729.

As stated on pages 54-55 of GRN 729, "*the β-galactosidase enzyme preparation*" used by the notifier "*in the preparation of its GOS products is derived from* B. circulans*, is well characterized, reproducibly meets compositional and activity standards, and complies with limits on contaminants appropriate for food grade ingredients. Unpublished safety studies have shown that the β-galactosidase is obtained from a nonpathogenic and non-toxigenic microorganism. Additional steps employed in enzyme preparations and use of the enzyme further supports the safety. The enzyme is isolated using standard procedures for the enzymatic reaction with lactose.*

The constituents from the enzyme preparation are unlikely to become part of the product. The manufacture of GOS involves extensive purification steps that are likely to remove potential metabolic impurities and/or toxin(s) produced during fermentation.

The use of GOS produced from lactose with β-galactosidase derived from B. circulans *in various foods and in infant formula has been determined to be GRAS by the following companies: Friesland Foods Domo (GRN 236), GTC Nutrition (GRN 285 and GRN 286), New Francisco Biotechnology Corporation (GRN 518 and GRN 569), for which all notifications received a "no questions" letter from FDA. Additionally, several enzymes derived from Bacillus species, such as amylase derived from* Bacillus lichenifonnis*, pullulanase from* Bacillus subtilis *and* B. licheniformis*; and pectate lyase from* B. subtillis *are considered GRAS. Furthermore, carbohydrase and protease enzymes derived from* Bacillus subtilis *are affirmed as GRAS for use as direct food ingredients, and ct-acetolactate decarboxylase from recombinant* B. subtilis *is currently regulated by the FDA as a secondary direct food additive permitted for use in food for human consumption. In the European Union, as per Commission Directive 2003/95/EC, cycloglycosyltransferase enzyme derived from* B. circulans *is approved in the production of 13 cyclodextrin*."

In addition, EFSA considers the β-galactosidase derived from *B. circulans* M3-1 as safe for use in the production of GOS (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), 2019).

VII. REFERENCES

A. REFERENCES

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B. EXPERT PANEL STATEMENT

We, the members of the Expert Panel, qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food, have performed a comprehensive and critical review of available information and data on the safety and Generally Recognized As Safe (GRAS) status of VITAGOS™ IF GOS in infant formulas and selected general foods has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), as described under 21 CFR §170.30(b). The safety of the intake of VITAGOS™ IF GOS in infant formulas and selected general foods has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food and is based on generally available and accepted information.

The use of VITAGOS™ IF GOS as an ingredient for the intended use in infant formulas and selected general foods has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. VITAGOS™ IF is a galacto-oligosaccharide (GOS)-containing product manufactured using lactose and β-galactosidase derived from *B. circulans* (*B. circulans* M3-1) with a production process similar to that of VITAGOS™, which is the subject of GRN 721. VITAGOS™ IF does not contain genetically modified organisms (GMOs) or ingredients derived from GMO-derived products.

2. A comparison of the manufacturing processes and product specifications for VITAGOS™ IF shows that VITAGOS™ IF is compositionally similar to Vivinal® GOS, which is GRAS (GRN 236) and currently marketed globally for use in infant formulas and conventional foods and beverages.

3. All raw materials and processing aids used to produce VITAGOS™ IF comply with appropriate US federal regulations.

4. GOS are non-digestible oligosaccharides consisting of 1 to 7 galactose units linked via β(1→2), β(1→3), β(1→4), or β(1→6) glycosidic bonds to either a terminal glucose or galactose.

    a. GOS pass through the upper gastrointestinal tract to the colon where they are metabolized by the resident microbiota into short-chain fatty acids, carbon dioxide, methane, and hydrogen, which are the same metabolites as those

produced by the microbiota following the ingestion of other foods and are either absorbed, exhaled, or excreted.

b. Oligosaccharides present in food include those that are naturally occurring in human milk and colostrum, bovine colostrum, and fermented milk products or enzymatically produced, which are then added to the food during formulation and processing.

c. Enzymatically produced GOS have a long history of use worldwide.

   i. In Japan, GOS have been commercially available since 1995 and are considered as Food for Specified Health Uses (FOSHU).

   ii. In the United States, the first GOS ingredient was determined GRAS for use in term infant formula and selected foods and beverages and received a "no questions" letter from the FDA in 2008 (GRN 236). Since then, a total of nine GOS-containing ingredients have been determined GRAS for use in infant formulas and selected conventional foods at levels up to 7.8 g/L and 11 g/serving, respectively, and resulted in fourteen GRAS Notifications (GRN 236; GRN 285; GRN 286; GRN 334; GRN 484; GRN 489; GRN 495; GRN 518; GRN 569; GRN 620; GRN 671; GRN 721; GRN 729; GRN 896). All GRAS Notifications have received "no questions" letters from the FDA, except for GRN 671 which was ceased to be evaluated at notifier's request. However, the notifier of GRN 671 resubmitted as GRN 721 and subsequently received a "no questions" letter.

   iii. In the European Union, the safety of GOS was reviewed by the Scientific Committee on Food (SCF) in 2003 and is approved for use in infant and follow-on formulas and in combination with fructo-oligosaccharides (FOS) at levels up to 8 g (90% GOS and 10 % FOS)/L (7.2 g GOS and 0.8 g FOS/L) (EU 2016/127).

   iv. In Australia and New Zealand, the safety of GOS was reviewed by the Food Standards of Australia and New Zealand (FSANZ) in 2008 and is permitted in infant and follow-on formulas at levels up to 290 mg/100 kJ, or approximately 8 g/L (Australia New Zealand Food Standards Code – Standard 2.9.1-7).

5. A pivotal toxicology study of Vivinal® GOS, which is compositionally similar to VITAGOS™ IF (Anthony et al., 2006), supports the safety of VITAGOS™ IF. This study established a no observed adverse effect level (NOAEL) of 2.25 g GOS/kg body weight/day (5g of Vivinal® GOS- the highest dose tested).

    a. Other GOS-containing products administered for up to 90 days by gavage established NOAELs at the highest doses tested [825 mg GOS/kg/day (Kobayashi et al. 2009); 2 g/kg/day (Zhou et al. 2017); 2 g GOS/kg/day (Penard 2015); 5 g GOS/kg/day (Jain et al. 2019)].

    b. GOS-containing products are not genotoxic.

    c. GOS-containing products are not reproductive or developmental toxicants.

6. GOS has been the subject of numerous clinical investigations in infants, children and adults. It has been shown to be safe and well tolerated at levels that support the intended uses.

7. Although GOS-containing products have been reported to elicit allergic reactions in a limited number of sensitized individuals living in Southeast Asia, GOS preparations have been widely consumed in Southeast Asia as well as globally for over a decade by adults, children, and infants, which suggests that the risk of GOS allergenicity to GOS-containing foods is negligible.

8. Vitalus Nutrition Inc. intends to use VITAGOS™ IF as an alternative for VITAGOS™, which was the subject of GRN 721. Thus, the intended uses for VITAGOS™ IF will be identical to those specified in GRN 721, which includes powdered, ready-to-feed, and concentrated liquid versions of milk-based non-exempt term infant formulas and selected conventional foods.

    a. The infant formulas will not exceed 7.8 g GOS/L reconstituted infant formula. This use level is higher than that proposed in GRN 721 but is the same as that from GRN 620 and GRN 729. This will result in a mean and 90th percentile estimated daily intake (EDI) of GOS for infants 0-6 months of age of 6.4 and 9.2 g/day, respectively. For infants 7-12 months of age, the mean and 90th percentile intakes of GOS are 5.6 and 8.6 g/day.

    b. The addition of VITAGOS™ IF to selected foods, beverages, and beverage concentrates results in a mean and 90th percentile EDIs for the total U.S. population from the ingestion of all GOS-containing foods are 12.2 g/person/day (0.28 g/kg body weight/day) and 25.3 g/person/day (0.7 g/kg body weight/day), respectively. On an individual basis, the greatest mean and

90th percentile GOS EDIs occur in children and male teenagers at 18.1 and 33.0 g/person/day. On a body weight basis, the greatest mean and 90th percentile GOS EDIs occur in infants at 1.44 and 2.42 g/kg body weight/day.

c. Because the use and use levels of VITAGOS™ IF are substitutive for existing uses and use levels of GOS, the dietary exposure to VITAGOS™ IF from the intended uses will not increase the cumulative intake of GOS.

9. As established in GRN 236, as well as other GOS Notifications, clinical and toxicology studies of other compositionally similar GOS support the safety of the proposed intake of VITAGOS™ IF (GRN 334; GRN 484; GRN 489; GRN 495; GRN 518; GRN 569; GRN 620; GRN 671; GRN 721; GRN 729; GRN 896).

Therefore, VITAGOS™ IF GOS is safe and GRAS at the proposed level of addition to the intended infant formulas and general foods. VITAGOS™ IF GOS is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

Roger Clemens, DrPH, CNS, FACN, FIFT
GRAS Expert Panel Member
School of Pharmacy
University of Southern California

Signature: 

Date: January 24, 2022

A. Wallace Hayes, PhD, DABT, FATS, ERT
GRAS Expert Panel Member
University of South Florida College of Public Health

Signature: 

Date: January 24, 2022

Thomas E. Sox, PhD, JD
GRAS Expert Panel Member
Principal, Pondview Consulting LLC

Signature: 

Date: January 24, 2022

Claire Kruger, PhD, DABT
Scientific Advisor to the Panel
Spherix Consulting Group, Inc.

Signature: 

Date: January 24, 2022

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: August 24, 2021

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

SECTION B INFORMATION ABOUT THE NOTIFIER

1a. Notifier	Name of Contact Person: Nisha Kaushik	Position or Title: Regulatory & Scientific Affairs Manager		
	Organization *(if applicable)*: Vitalus Nutrition Inc.			
	Mailing Address *(number and street)*: 3911 Mt. Lehman Rd.			
City: Abbotsford	State or Province: BC	Zip Code/Postal Code: V2T 5W5	Country: Canada	
Telephone Number: 604 857 9080 ext. 442	Fax Number:	E-Mail Address: nkaushik@vitalus.com		
1b. Agent or Attorney *(if applicable)*	Name of Contact Person: Dietrich B. Conze,PhD	Position or Title: Managing Partner		
	Organization *(if applicable)*: Spherix Consulting Group, Inc.			
	Mailing Address *(number and street)*: 751 Rockville Pike, Unit 30-B			
City: Rockville	State or Province: Maryland	Zip Code/Postal Code: 20852	Country: United States of America	
Telephone Number: 240-367-6089	Fax Number:	E-Mail Address: dconze@spherixgroup.com		

SECTION C GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

Lactase, β-galactosidase (IUB Number: 3.2.1.23) (GODO-FAL)

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway
☒ Electronic files on physical media
☐ Paper

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☒ Yes *(Proceed to Item 5)* ☐ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☒ a) GRAS Notice No. GRN 620, 721, 729
☐ b) GRAS Affirmation Petition No. GRP _____
☐ c) Food Additive Petition No. FAP _____
☐ d) Food Master File No. FMF _____
☒ e) Other or Additional *(describe or enter information as above)* *GRNs 236, 285, 286, 334, 484, 489, 495, 518, 569, 671, 896; SCF, 2001, FSANZ, 2008*

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*
☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission
☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission
☐ Yes, a redacted copy of part(s) of the submission
☐ No

SECTION D INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

VITAGOS™ IF will be added to powdered, ready-to-feed, and concentrated liquid versions of cow milk-based non-exempt term infant formulas, and selected conventional foods.

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?
(Check one)

☐ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E PARTS 2 7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☐ Yes ☒ No

Did you include this other information in the list of attachments?

☐ Yes ☐ No

SECTION F SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Vitalus Nutrition Inc.
(name of notifier)

has concluded that the intended use*(s)* of Galacto-oligosaccharides, VITAGOS™ IF
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Vitalus Nutrition Inc. agrees to make the data and information that are the basis for the
(name of notifier)
conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

3911 Mt. Lehman Rd, Abbotsford, BC, Canada V2T 5W5
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
	Dietrich B. Conze, Managing Partner	

SECTION G LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
1	Insert Clear VITAGOS IF GRAS Dossier 1-24-22 to FDA	Submission
2	Insert Clear All References	Submission
	Insert Clear	
	Insert Clear	
	Insert Clear	
	Insert Clear	
	Insert Clear	
	Insert Clear	
	Insert Clear	

Add Continuation Page

OMB Statement: Public reporting burden for this collection of information is estimated to average 170 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, PRAStaff@fda.hhs.gov. (Please do NOT return the form to this address). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

From:	kbrailer@spherixgroup.com
To:	Morissette, Rachel; "Dietrich Conze"
Cc:	"Claire Kruger"
Subject:	RE: [EXTERNAL] Re: questions for GRN 001076
Date:	Thursday, December 22, 2022 8:54:44 AM
Attachments:	image001.png image002.png image003.png image004.png image005.png image006.png Form 3667-VITAGOS GRAS 1-24-22 - Corrected.pdf Response to FDA on GRN1076 12-22-22.pdf

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Dear Rachel,

Attached please find our response to your request for additional information on GRN 001076. Please confirm receipt.

Happy Holidays!

Kathy Brailer
Director of Administrative Services
Spherix Consulting Group, Inc.
751 Rockville Pike, Unit 30-B
Rockville, MD 20852
+1-301-557-0375
kbrailer@spherixgroup.com
www.spherixgroup.com

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Thursday, December 8, 2022 8:35 AM
To: Dietrich Conze <dconze@spherixgroup.com>
Cc: Claire Kruger <ckruger@spherixgroup.com>; Kathy Brailer <kbrailer@spherixgroup.com>
Subject: RE: [EXTERNAL] Re: questions for GRN 001076

Hi Dietz,

That will be fine. I'll look for responses on or before Jan. 5, 2023.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

From: Dietrich Conze <dconze@spherixgroup.com>
Sent: Thursday, December 8, 2022 8:08 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Cc: Claire Kruger <ckruger@spherixgroup.com>; Kathy Brailer <kbrailer@spherixgroup.com>
Subject: [EXTERNAL] Re: questions for GRN 001076

Hi Rachel,

We'd like an extension for sending in our responses to January 5th, 2023 to give us some time to correspond with our client and accommodate upcoming vacation schedules over the Holidays. Is this acceptable?

Regards,
Dietz

Dietrich Conze, PhD
Managing Partner
Spherix Consulting Group
751 Rockville Pike, Unit 30-B
Rockville, MD 20852

Tel: 240-367-6089
Fax: 301-230-2188
dconze@spherixgroup.com

On Dec 7, 2022, at 1:35 PM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:

Dear Dietz,

Please see attached our questions for GRN 001076. Let me know if you have any questions. Please also confirm receipt of this email.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

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<image007.gif>

<2022-12-07 Questions for notifier GRN 001076.pdf>

Spherix Consulting Group

December 22, 2022

Rachel Morissette, Ph.D.
Regulatory Review Scientist/ Biologist
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive, HFS-225
College Park, MD 20740

RE: Questions Regarding GRN 001076

Dear Dr. Morissette:

Below are our responses to your requests for additional information regarding GRN 001076 as stated in your email on December 7, 2022. Your requests are in italicized text and our responses are in plain text below.

Regulatory:

1. *In Form 3667, Vitalus lists different notified substances throughout the form. Specifically, page 2 of the form lists the notified substance as "Lactase,β-galactosidase(IUBNumber:3.2.1.23)(GODO-FAL)", while page 3 shows "Galacto-oligosaccharides, VITAGOS™ IF". Please provide a new Form 3667 with the correct notified substance.*

   A corrected Form 3667 is attached.

2. *Vitalus states that GOS is intended for use in fruit and vegetable juices. Please provide a statement that the GOS will not be used in juices for which a standard of identity may preclude its use.*

   The subject of GRN 001076 is not intended to be used in juices for which a standard of identity may preclude its use.

3. *In Table 3 on page 11 of the notice, we note that the regulation for "citric acid" is listed as 21 CFR 182.1033, which does not exist. The regulation for citric acid is 21 CFR 184.1033.*

   Thank you for the clarification.

751 Rockville P ke, Unit 30-B
Rockville, MD 20852
301-557-0375; info@spherixgroup.com
http://spherixgroup.html

Chemistry:

4. *In Table 11 on page 21 of the notice, the maximum intended use level for "Beverage concentrate (powder)" is listed as 33.4 g/100 g with a footnote stating that this corresponds to the level resulting after dissolution of the powder in water. Please clarify whether this use level was intended to be on the basis of the beverage concentrate prior to dissolution in water. For example, Footnote 1 of Table 11 states that the use levels are consistent with GRN 000721, which also listed a use level of 33.4 g/100 g and noted that the level was consistent with GRN 000334. The use level for beverage concentrate powder in GRN 000334 was 5 g/250 g serving after dissolution in water.*

   The use level of 33.4 g/100 g is for the beverage concentrate prior to its dissolution in water. When diluted in water, the resulting concentration of GOS will be 5 g/250 g serving, as stated in GRN 000334.

5. *In section E.2.c on pages 15-16 of the notice, the results of the five batch analyses of GOS for the mineral content are provided. It is not clear why these data were provided. Has Vitalus established specifications that limit the concentrations of these minerals and concluded that the levels found in their GOS ingredient do not present a safety concern for the intended uses?*

   The mineral content was provided in the Notice because Vitalus' customers use this data as a guide when formulating their products. Additionally, Vitalus has not established specifications that limit the concentrations of these minerals because their levels in the GOS ingredient do not present a safety concern for the intended uses.

6. *On page 10 of the notice, the β-galactosidase enzyme manufactured by GenoFocus, Inc. is identified as "Lactaenzyme-B". Please confirm that "Lactazyme-B" is the correct name for this enzyme product.*

   The correct name of the enzyme is Lactazyme-B.

Microbiology:

7. *Table 5 on page 14 of the notice does not include limits for Cronobacter sakazakii, Listeria monocytogenes, or Bacillus cereus. We request that you provide specifications for C. sakazakii, L. monocytogenes, and B. cereus, as the intended uses of GOS include powdered infant formula. For each specification, please provide a limit, sample size, method of detection, and the results of at least three non- consecutive batch analyses demonstrating that the product can meet the specifications.*

   The product specifications, sample sizes, methods of detection, and results for *C. sakazakii, L. monocytogenes, and B. cereus* analyses from 5 non-consecutive batches are provided in Table 1. The levels of all these microbes complied with the product specifications.

Table 1. Pathogenic Bacteria in VITAGOS™ IF Syrup

Parameter	Test Method	Specification	Detection Level	Lot Number				
				20217	20223	20225	20230	20232
Cronobacter sakazakii	MFLP-27	Negative in 25g	Negative in 25g	NEG	NEG	NEG	NEG	NEG
Bacillus cereus	MFLP-42	<100 cfu/g	<10 cfu/g	<10	<10	<10	<10	<10
Listeria monocytogenes	MFLP-28	Negative in 25g	Negative in 25g	NEG	NEG	NEG	NEG	NEG

NEG = Negative; MFHPB = Methods for the Microbiological Analysis of Foods; MFLP = Laboratory Procedures for the Microbiological Analysis of Foods; cfu = colony forming units; g = gram

Toxicology:

8. *On page 19 of the notice, Vitalus states:*

   "Naturally occurring GOS are present in human milk and colostrum, bovine colostrum, and fermented milk products..."

   While human milk oligosaccharides are structurally and potentially functionally related to GOS, we are not aware of any publications that indicate GOS (i.e., multimers of galactose) is present in human milk. Pease clarify Vitalus' statement.

   Galactooligosaccharides are synthetic non-digestible carbohydrates that mimic naturally occurring non-digestible carbohydrates present in human milk and colostrum, bovine colostrum, fermented milk products, and other foods.

9. *Vitalus states that an updated literature was conducted through August 2021 (we received the notice in January 2022). Please provide a brief statement that a more recent updated literature search identified no new published data that would be considered counter to Vitalus' GRAS conclusion. We note an additional published report not cited in the notice that may be relevant to Vitalus' safety narrative: Baek et al. Prev. Nutr. Food Sci. (2021) 26:315.*

   A more recent updated literature search was conducted on December 15, 2022 and did not identify any new published data that contradicts Vitalus's GRAS conclusion for the subject of GRN 001076. Additionally, the acute and subchronic studies conducted by Baek et al. (2021) further support Vitalus' GRAS conclusion because there were no GOS-related adverse effects in the clinical symptoms, weight, food intake, hematology, blood chemistry, relative organ weight, or gross pathology. As a result, Baek et al. (2021) ascribed the no observed adverse effect level (NOAEL) to the highest dose tested, 1000 mg/kg/day, which is consistent with the other toxicology studies summarized in the GRAS Notice.

Should you need any additional information, please feel free to contact me at 240-367-6089 or dconze@spherixgroup.com.

Sincerely,

Dietrich B. Conze, Ph.D.
Managing Partner

Reference:

Baek Y, Ahn Y, Shin J, Suh HJ, Jo K. Evaluation of Safety through Acute and Subacute Tests of Galacto-Oligosaccharide (GOS). Prev Nutr Food Sci. 2021 Sep 30;26(3):315-320. doi: 10.3746/pnf.2021.26.3.315. PMID: 34737992; PMCID: PMC8531431.

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: August 24, 2021

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier	Name of Contact Person Nisha Kaushik	Position or Title Regulatory & Scientific Affairs Manager		
	Organization *(if applicable)* Vitalus Nutrition Inc.			
	Mailing Address *(number and street)* 3911 Mt. Lehman Rd.			
City Abbotsford	State or Province BC	Zip Code/Postal Code V2T 5W5	Country Canada	
Telephone Number 604 857 9080 ext. 442	Fax Number	E-Mail Address nkaushik@vitalus.com		
1b. Agent or Attorney *(if applicable)*	Name of Contact Person Dietrich B. Conze,PhD	Position or Title Managing Partner		
	Organization *(if applicable)* Spherix Consulting Group, Inc.			
	Mailing Address *(number and street)* 751 Rockville Pike, Unit 30-B			
City Rockville	State or Province Maryland	Zip Code/Postal Code 20852	Country United States of America	
Telephone Number 240-367-6089	Fax Number	E-Mail Address dconze@spherixgroup.com		

SECTION C GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

Galacto-oligosaccharides, VITAGOS™ IF

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☒ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☒ Yes *(Proceed to Item 5)* ☐ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☒ a) GRAS Notice No. GRN 620, 721, 729

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☒ e) Other or Additional *(describe or enter information as above)* *GRNs 236, 285, 286, 334, 484, 489, 495, 518, 569, 671, 896; SCF, 2001, FSANZ, 2008*

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

VITAGOS™ IF will be added to powdered, ready-to-feed, and concentrated liquid versions of cow milk-based non-exempt term infant formulas, and selected conventional foods.

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☐ Yes ☒ No

Did you include this other information in the list of attachments?

☐ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Vitalus Nutrition Inc.
(name of notifier)

has concluded that the intended use*(s)* of Galacto-oligosaccharides, VITAGOS™ IF
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Vitalus Nutrition Inc. agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.
(name of notifier)

3911 Mt. Lehman Rd, Abbotsford, BC, Canada V2T 5W5
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
	Dietrich B. Conze, Managing Partner	1/24/2022

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
1	Insert Clear VITAGOS IF GRAS Dossier 1-24-22 to FDA	Submission
2	Insert Clear All References	Submission
	Insert Clear	
	Insert Clear	
	Insert Clear	
	Insert Clear	
	Insert Clear	
	Insert Clear	
	Insert Clear	

Add Continuation Page

OMB Statement: Public reporting burden for this collection of information is estimated to average 170 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, PRAStaff@fda.hhs.gov. (Please do NOT return the form to this address). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

From: kbrailer@spherixgroup.com
To: Morissette, Rachel
Cc: "Claire Kruger"; "Dietrich Conze"
Subject: [EXTERNAL] RE: questions for GRN 001076
Date: Thursday, January 5, 2023 1:39:31 PM
Attachments: image001.png
image002.png
image003.png
image004.png
image005.png
image006.png

Hi Rachel,

Claire tried responded, but got a bounce back. I'm copying here message below. Please let us know if you receive this.

Thanks,

Kathy

Dear Rachel,

Our response to your question of 12/22/22 follows:

FDA question:

Jain et al. (2020) states that while 5000 mg/kg bw/d was well tolerated by adult rats, "… 5000 mg/kg [bw]/day dose of Gossence exceeded the maximum tolerated dose in juvenile rats." This suggests that younger animals may have lesser tolerability of GOS at high levels. Please provide a short narrative describing why this new information does not negatively impact your GRAS conclusion.

Spherix Consulting Group response:

The publication by Jain et al. (2020) is preceded by and corroborated by the findings from other published rodent toxicology studies that evaluated the safety of GOS administered by gavage. In the previous studies conducted by Kobayashi et al. (2009), Kobayash et al. (2014), Zhou et al. (2017) and Anthony et al. (2006), NOAELs of 825, 1,130, 2,000 and 2,250 mg GOS/kg/day were established, respectively. Similar to the results reported in these earlier studies, Jain et al. (2020) reported a NOAEL of 3,000 mg GOS/kg/day in juvenile rats (dosing from day 4 to day 52 after birth), which is similar to that reported by Kobayash et al. (2014) for neonatal rats (dosing from day 4 to day 45 after birth; 1,130 mg/kg/day). In all these studies, a common finding was cecal enlargement for both adult and juvenile rats.

Jain et al. (2020) administered GOS at 1000, 2000, and 5000/3000 mg/kg/day. At the 5000 mg/kg/day dose, clinical signs of watery feces and yellow color stains at urogenital region were observed in 13 of 34 animals (9 males and 4 females) from days 15 to 17 (PND 18–20) of the dosing phase. Two deaths occurred (1 male and 1 female) on day 15 of the dosing phase (PND 18). Hence, for the high-dose group, the dose was reduced from 5000 mg/kg/day to 3000 mg/kg/day from day 16 of the dosing phase. No further deaths or clinical signs were noticed in animals at 3000 mg/kg/day from day 18 of dosing phase to until terminal euthanization. Gross observation at necropsy in two dead animals was characterized by the presence of yellowish liquid contents in the intestine (both in small and in large intestine). Exact cause of death could not be determined based on gross and histomorphological evaluations. At necropsy, Jain et al. (2020) reported that GOS, like other dietary fibers, produced increases in the size of the cecum at 5000/3000 mg/kg/day (male 6/12, female 2/12) and 2000 mg/kg/day (male 2/12, female 1/12). This was the only gross

observation at 3000 and 2000 mg/kg/day and correlated with the increased weights of ceca with contents. Because no correlating microscopic changes were found, the increased cecum weights were likely due to increased cecal contents. Additionally, the cecal enlargement completely returned to normal following 14-day recovery period, indicating that the change was reversible. As a result, these changes were considered to be non-adverse, compensatory, and adaptive responses to the administration of GOS.

The cecum enlargement is consistent with other reports for the same class of compounds because the cecum is an area of bacterial fermentation and growth. Cecal hypertrophy can occur due to trophic effects of short fatty acids which are produced during bacterial fermentation after an excessive amount of non-absorbed carbohydrate and dietary fiber enter the cecum and colon (Zhou et al. 2017).

An important factor to consider in the study design from the publication from Jain et al. (2020), is that the study utilized administration of GOS at much higher levels than previously studied. Spherix Consulting Group corresponded with the authors of the publication, and they confirmed that "the intent was to study the effect of GOS at 1000, 2000, or 5000/3000 mg/kg/day, hence considering GOS purity at 76.28%, animals were fed with 1347, 2694, and 6735/4041 mg/kg/day of Gossence, respectively. We used a potency correction factor of 1.347 (considering Gossence purity as 76.28% and moisture content as 2.66%)."

Therefore, the amount of GOS that was delivered into the cecum at the highest original dose was twice that used by previous researchers in adult rats and almost five times that used by Kobayash et al. (2014) in their study of neonatal rats. The NOAEL in adult rats from their GOS formulation was 5,000 mg/kg/day (Jain et al. 2019), which is higher than the NOAEL of 3,000 mg/kg/day in their study of juvenile animals.

It is important to note that administering a very large bolus of undigestible fiber into a neonatal rat at levels that result in an unphysiological perturbation produces a physical intolerance in this model. In addition, the gastrointestinal system of the rat differs from the human at birth. The rat gastrointestinal system includes a cecum, and the stomach, small intestine and large intestine are morphologically and functionally immature at birth in rodents compared with the human. The small intestine in rats is morphologically and functionally immature for the first 2 weeks of life. Functional equivalence between the rat and human is only achieved shortly before weaning (Schmitt et al. 2022). Thus, dosing of neonatal rats beginning at day 4 is not a good model for a human neonate in terms of gastrointestinal function. It is very likely that the highest dose of GOS utilized in the Jain et al. (2020) study may have been physically challenging for the juvenile animals; clinical signs of watery feces, yellow color stains at urogenital and yellowish liquid contents in the intestine indicate an adverse effect of a very high bolus dose of fiber in the immature gastrointestinal system. At doses below the highest dose, the target effect on the cecum was reversible, non-adverse, compensatory, adaptive and consistent with findings from other studies utilizing GOS. Jain et al. (2020) corroborated the findings and NOAEL established by previous publications which demonstrate similar tolerated doses of GOS in both adult and juvenile rats. Therefore, the information from Jain et al. (2020) does not negatively impact the conclusion of GRAS.

References:

Anthony JC, Merriman TN, Heimbach JT. 90-day oral (gavage) study in rats with galacto-oligosaccharides syrup. Food Chem Toxicol 44, 819–826, 2006.

Jain M, Kaur M, Tiwari DP, et al. Juvenile toxicity study of Gossence™ (galactooligosaccharides) in Sprague Dawley rats. Toxicology Research and Application, Volume 4: 1–13, 2020, DOI: 10.1177/2397847320913213.

Jain M, Narayanan S, Tiwari DP, et al. Safety assessment of Gossence™ (galactooligosaccharides): Genotoxicity and general toxicity studies in Sprague Dawley rats. Toxicology Research and Application, Volume 3: 1–14, 2019, DOI: 10.1177/2397847319860375.

Kobayashi T, Yasutake N, Uchida K, Ohyama W, Kaneko K, Onoue M. Safety of a novel galacto-oligosaccharide: Genotoxicity and repeated oral dose studies. Hum Exp Toxicol. 2009 Oct;28(10):619-30. doi: 10.1177/0960327109346789. Epub 2009 Sep 15. PMID: 19755435.

Kobayash T, Ishida S, Kaneko K, Onoue M. A 6-week oral gavage toxicity study of a novel galacto-oligosaccharide in juvenile rats. Hum Exp Toxicol. 2014 Jul;33(7):722-8. doi: 10.1177/0960327113506239. Epub 2013 Oct 9. PMID: 24107453.

Schmitt G, Barrow P. Considerations for and against dosing rodent pups before 7 days of age in juvenile toxicology studies. Reprod Toxicol. 2022 Sep;112:77-87. doi: 10.1016/j.reprotox.2022.06.010. Epub 2022 Jun 27. PMID: 35772686.

Zhou Y, Kruger C, Ravi GS, Santhosh Kumar DP, Vijayasarathi SK, Lavingia M, Chen X, Ambriz P. Safety Evaluation of Galacto-Oligosaccharides: Sub-Chronic Oral Toxicity Study in Sprague-Dawley Rats. Toxicology Research and Application, 2017, I, 1-12.

Best regards,
Claire

_____

Claire Kruger, Ph.D., D.A.B.T., CFS
Managing Partner
Spherix Consulting Group
751 Rockville Pike, Unit 30-B
Rockville, MD 20852
+1-301-775-9476

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Thursday, December 22, 2022 1:14 PM
To: kbrailer@spherixgroup.com; 'Dietrich Conze' <dconze@spherixgroup.com>
Cc: 'Claire Kruger' <ckruger@spherixgroup.com>
Subject: RE: [EXTERNAL] Re: questions for GRN 001076

Hi Kathy,

Tox had one follow-up question for GRN 1076.

- Jain et al. (2020) states that while 5000 mg/kg bw/d was well tolerated by adult rats, "… 5000 mg/kg [bw]/day dose of Gossence exceeded the maximum tolerated dose in juvenile rats." This suggests that younger animals may have lesser tolerability of GOS at high levels. Please provide a short narrative describing why this new information does not negatively impact your GRAS conclusion.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

From: kbrailer@spherixgroup.com <kbrailer@spherixgroup.com>
Sent: Thursday, December 22, 2022 8:54 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>; 'Dietrich Conze' <dconze@spherixgroup.com>
Cc: 'Claire Kruger' <ckruger@spherixgroup.com>
Subject: RE: [EXTERNAL] Re: questions for GRN 001076

Dear Rachel,

Attached please find our response to your request for additional information on GRN 001076. Please confirm receipt.

Happy Holidays!

Kathy Brailer
Director of Administrative Services
Spherix Consulting Group, Inc.
751 Rockville Pike, Unit 30-B
Rockville, MD 20852
+1-301-557-0375
kbrailer@spherixgroup.com
www.spherixgroup.com

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Thursday, December 8, 2022 8:35 AM
To: Dietrich Conze <dconze@spherixgroup.com>
Cc: Claire Kruger <ckruger@spherixgroup.com>; Kathy Brailer <kbrailer@spherixgroup.com>
Subject: RE: [EXTERNAL] Re: questions for GRN 001076

Hi Dietz,

That will be fine. I'll look for responses on or before Jan. 5, 2023.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

I GOT MY COVID-19 VACCINATION

From: Dietrich Conze <dconze@spherixgroup.com>
Sent: Thursday, December 8, 2022 8:08 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Cc: Claire Kruger <ckruger@spherixgroup.com>; Kathy Brailer <kbrailer@spherixgroup.com>
Subject: [EXTERNAL] Re: questions for GRN 001076

Hi Rachel,

We'd like an extension for sending in our responses to January 5th, 2023 to give us some time to correspond with our client and accommodate upcoming vacation schedules over the Holidays. Is this acceptable?

Regards,
Dietz

Dietrich Conze, PhD
Managing Partner
Spherix Consulting Group

751 Rockville Pike, Unit 30-B
Rockville, MD 20852

Tel: 240-367-6089
Fax: 301-230-2188
dconze@spherixgroup.com

On Dec 7, 2022, at 1:35 PM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:

Dear Dietz,

Please see attached our questions for GRN 001076. Let me know if you have any questions. Please also confirm receipt of this email.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

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<image007.gif>

<2022-12-07 Questions for notifier GRN 001076.pdf>