



January 17, 2023

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 GRAS Filing Team:

Enclosed please find a CD containing "Generally Recognized As Safe Determination for the Use of VITAGOS™ IF Powder 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.

Sincerely,

Dietrich B. Conze, PhD
Managing Partner

Enclosure:

CD containing Form 3667, cover letter, GRAS Determination for the Use of VITAGOS™ IF Powder in Non-Exempt Term Infant Formula and Selected Conventional Foods, and all references

**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 Powder 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:**

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January 6, 2023

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CCP: Critical Control Points

CDC: United States Center for Disease Control

CEP: Contact Materials, Enzymes and Processing Aids

CFR: Code of Federal Regulations

DM: Dry Matter

DP: Degree of Polymerization

EDI: Estimated Daily Intake

EPA: United States Environmental Protection Agency

FCC: Food Chemical Codex

FDA: United States Food and Drug Administration

FFDCA: Federal Food, Drug, and Cosmetic Act

FNDDS: Food and Nutrition Database for Dietary Studies

FOB: Functional observational battery

FOIA: Freedom of Information Act

FOS: Fructo-oligosaccharides

FOSHU: Food for Specified Health Uses

FSANZ: Food Standards of Australia and New Zealand

FSSC: Food Safety System Certification

GLP: Good Laboratory Practices

GMO: Genetically Modified Organisms

GOS: Galacto-oligosaccharides

GRAS: Generally Recognized As Safe

GRN: GRAS Notification

GU: Glucose Unit

HACCP: Hazard analysis critical control point

HDPE: High Density Polyethylene

MEC: Mobile Examination Center

NCHS: National Center for Health Statistics

NHANES: National Health and Nutrition Examination Surveys

NOAEL: No Observed Adverse Effect Level

PSU: Primary Sampling Units

SCF: Scientific Committee on Food

USDA: United States Department of Agriculture

USP: United Stated Pharmacopeia

# 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 of Title 21 of the United States Code of Federal Regulations.

## 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 powder 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 powder 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 powder as an ingredient for the intended uses in foods and non-exempt, term, cow's milk-based 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 powder is a galacto-oligosaccharide (GOS)-containing ingredient manufactured using lactose and β-galactosidase derived from *Bacillus circulans* (*B. circulans* M3-1) with a production process that is the same as VITAGOS™ IF syrup, which is GRAS (GRAS Notice Pending). VITAGOS™ IF syrup is spray-dried to produce VITAGOS™ IF powder. VITAGOS™ IF powder 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 powder shows that VITAGOS™ IF powder is compositionally similar to Vivinal® GOS, which is GRAS (GRN 000236, 2008) and marketed globally for use in term, cow's milk-based infant formula and conventional foods and beverages.

3. All raw materials and processing aids used to produce VITAGOS™ IF powder comply with the 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 large intestine 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 non-exempt, term, cow's milk-based infant formula and

selected foods and beverages and received a "no questions" letter from the FDA in 2008 (GRN 000236). Since then, a total of nine GOS-containing ingredients have been determined GRAS for use in non-exempt, term, cow's milk-based infant formula and selected conventional foods at levels up to 7.8 g/L and 11 g/serving, respectively, and resulted in fourteen GRAS Notifications (GRN 000236, 2008; GRN 000285, 2009; GRN 000286, 2009; GRN 000334, 2010; GRN 000484, 2014; GRN 000489, 2014; GRN 000495, 2014; GRN 000518, 2014; GRN 000569, 2015; GRN 000620, 2016; GRN 000671, 2017; GRN 000721, 2017; GRN 000729, 2018; GRN 000896, 2020). 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. The notifier of GRN 000671 resubmitted as GRN 000721 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 fructooligosaccharides (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 (Anthony et al., 2006), which is compositionally similar to VITAGOS™ IF powder, supports the safety of VITAGOS™ IF powder. 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 ingredients administered for up to 90 days by gavage established NOAELs at the highest doses tested [1100 mg GOS/kg/day (Kobayashi et al. 2009); 2 g GOS/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 ingredients are not genotoxic.

   c. GOS-containing ingredients 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 ingredients 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 powder as an alternative for VITAGOS™, which is the subject of GRN 000721. Thus, the intended uses for VITAGOS™ IF powder include those specified in GRN 000721, as well as a variety of other uses that have been determined GRAS in GRN 000285, 000484, and 000489, which includes powdered, ready-to-feed, and concentrated powdered versions of non-exempt, term, cow's milk-based infant formula and selected conventional foods.

    a. The non-exempt, term, cow's milk-based infant formula will not exceed 7.8 g GOS/L reconstituted formula. This use level is higher than that proposed in GRN 000721 but is the same as that from GRN 000620 and GRN 000729. 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 powder 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 of 2.30 g/person/day (0.033 g/kg body weight/day) and 5.38 g/person/day (0.078 g/kg body weight/day), respectively. On an individual basis, the greatest mean and 90th percentile GOS EDIs occur in children ages 13-19 at 2.77 and 6.71 g/person/day. On a body weight basis, the greatest mean and 90th percentile GOS EDIs occur in infants ages 0 to 6 months at 0.23 and 0.39 g/kg body weight/day.

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

9. As established in GRN 000236, as well as other GOS Notices, clinical and toxicology studies of other compositionally similar GOS support the safety of the proposed intake of VITAGOS™ IF powder (GRN 000334, 2010; GRN 000484, 2014; GRN 000489, 2014; GRN 000495, 2014; GRN 000518, 2014; GRN 000569, 2015; GRN 000620, 2016; GRN 000671, 2017; GRN 000721, 2017; GRN 000729, 2018; GRN 000896, 2020).

Determination of the GRAS status of VITAGOS™ IF powder 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 powder and the human exposure to VITAGOS™ IF powder resulting from its intended use as an ingredient in powdered, ready-to-feed, and concentrated liquid versions of non-exempt, term, cow's milk-based infant formula and select conventional foods:

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

Therefore, VITAGOS™ IF powder is safe and GRAS at the proposed levels of addition to the intended foods. VITAGOS™ IF powder 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.

January 16, 2023

Signature Date

Authorized Representative of Vitalus Nutrition Inc.

# 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 Powder

## C. DESCRIPTION OF GALACTO-OLIGOSACCHARIDES

As stated in GRN 000495 (2014) and 000620 (2016), "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 ingredients, 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).

The subject of this Notice is a powdered form of VITAGOS™ IF, which is a GOS-containing powder synthesized from lactose using a β-galactosidase derived from *B. circulans* (*B. circulans* M3-1) that hydrolyzes the β (1-4) glycosidic bond between the galactose and glucose moieties of lactose and transgalactosylates the residual galactose with other molecules. VITAGOS™ IF powder contains a minimum of 65% GOS, among which DP3 GOS predominates. VITAGOS™ IF has a similar DP profile as other GOS synthesized from a β-galactosidase derived from *Bacillus circulans* such as Vivinal® GOS and King-Prebiotics® GOS which are the subjects of GRN 000236 (2008) and 000569 (2015), respectively (Table 1). The GOS that is the subject of GRN 000729 (2017) 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 000236)[a] | King-Prebiotics® (GRN 000569)[b] | VITAGOS™ IF Syrup*,[c] |
| 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
*Converted VITAGOS™ IF DP % to GOS fraction only by dividing by % of total GOS.
[a]Source of β-galactosidase: *B. circulans* ATCC 31382.
[b]Source of β-galactosidase: Strain not provided; Notifier considered the information to be proprietary.
[c]Source of β-galactosidase: *B. circulans* M3-1.

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 β (1-4) 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*, *Streptococcus thermophilus,* or a combination of β-galactosidases derived from *A. oryzae* and *S. thermophilus* contain predominantly oligosaccharide chains with β (1-6) 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 ingredient contains predominantly oligosaccharides with β (1-4) glycosidic bonds.

To identify the types of glycosidic linkages in the ingredient, the precursor to VITAGOS™ IF powder, VITAGOS™ IF syrup, was profiled by high-performance anion-exchange chromatography/pulsed amperometric detection (HPAEC-PAD) using a Dionex ICS-5000+ workstation equipped with a CarboPac PA-1 column (250 x 4 mm) and an ICS-5000 ED pulsed amperometric detector. The method used by Vitalus Nutrition is the method that was described by van Leeuwen et al. (2016), except for an extension of the reconditioning time. van Leeuwen et al. (2016) characterized the glycosidic linkages for Vivinal® GOS, which is manufactured using a β-galactosidase derived from *B. circulans* ATCC 31382 and the subject of GRN 000236 (2008). Twenty-two peaks were identified in VITAGOS™ IF syrup and, although

the retention times for each component were 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 were determined by van Leeuwen et al. (2016; Table 2), it is reasonable to conclude that the oligosaccharides in each peak in VITAGOS™ IF syrup contain the same 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 GOS present in VITAGOS™ IF syrup are essentially equivalent to the GOS present in Vivinal® GOS, and because VITAGOS™ IF powder is spray-dried VITAGOS™ IF syrup, the GOS present in VITAGOS™ IF powder are essentially equivalent to the GOS present in Vivinal® GOS.

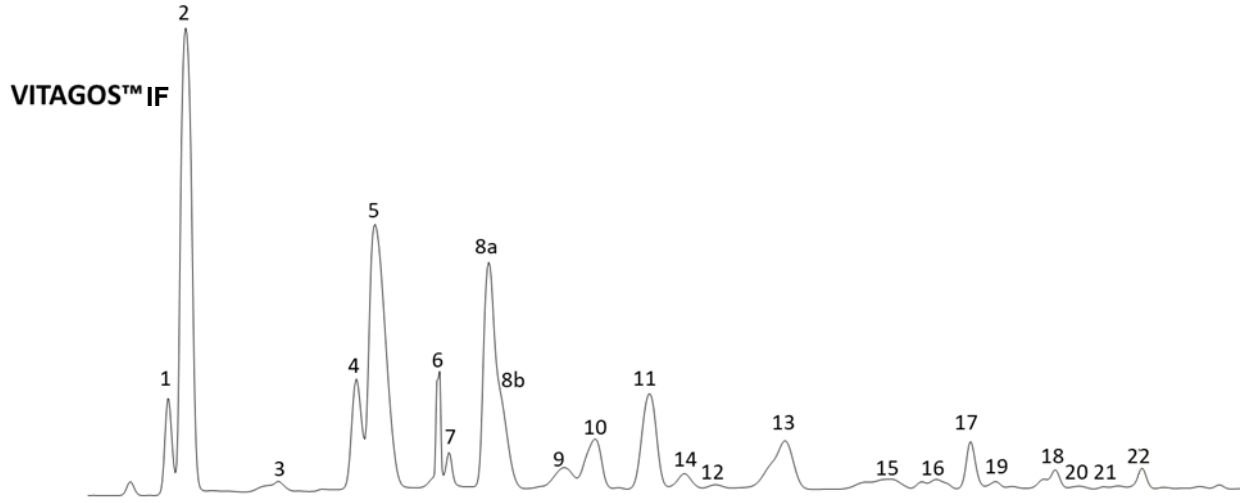


**Figure 1. High-Performance Anion-Exchange Chromatography/Pulsed Amperometric Detection (HPAEC-PAD) Profile of VITAGOS™ IF.**
Assigned peaks are numbered.

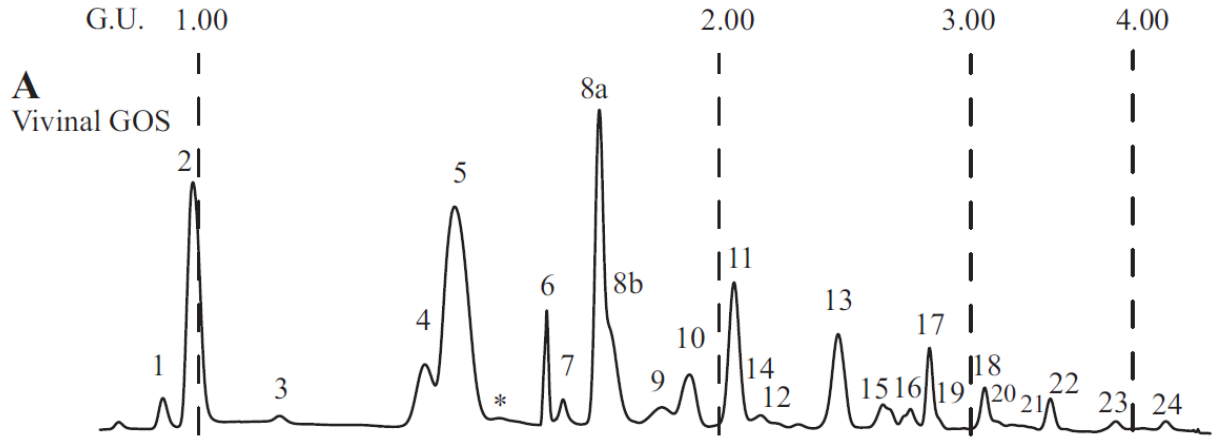


**Figure 2. HPAEC-PAD Profile of Vivinal® GOS.**
Adopted from van Leeuwen et al. (2016). Assigned peaks are numbered. "*" denotes lactulose generated from lactose during the industrial preparation of the batch. "G.U" denotes glucose unit values, which are based on elution times in relation to an external maltodextrin ladder.

**Table 2. Glycosidic Linkages of Oligosaccharides in Vivinal® GOS[a]**

| 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<br>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<br>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<br>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<br>Gal-β(1→4)-Gal-β(1→6)-[Gal-β(1→2)]-Glc<br>Gal-β(1→4)-Gal-β(1→4)-Gal-β(1→6)-Glc |
| 6 | Gal-β(1→6)-Gal-β(1→4)-Glc<br>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<br>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<br>Gal-β(1→4)-Gal-β(1→4)-Gal-β(1→4)-[Gal-β(1→6)]-Glc<br>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<br>Gal-β(1→4)-Gal-β(1→4)-[Gal-β(1→4)-Gal-β(1→2)]-Glc<br>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<br>Gal-β(1→4)-Gal-β(1→4)-Gal-β(1→2)-[Gal-β(1→6)]-Glc<br>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<br>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 | | |

[a]Adopted from van Leeuwen et al. (2016); bracketing denotes branching from the terminal saccharide.
[a]Manufactured with a β-galactosidase derived from *B. circulans* ATCC 31382.

## 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 a 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 ingredients containing GOS with different concentrations, varying chain lengths, and different β-glycosidic bonds (GRN 000489, 2014).

### 1. Compliance

VITAGOS™ IF powder is manufactured by Vitalus Nutrition Inc., located at 3911 Mt. Lehman Rd. Abbotsford, British Columbia, V2T 5W5, Canada and spray-dried into a powder by AmTech Ingredients under food-grade good manufacturing practice (GMP) conditions. Ingredients manufactured by Vitalus Nutrition Inc. including VITAGOS™ IF powder do not contain genetically modified organisms (GMOs) or ingredients derived from GMO-derived products. Both Vitalus Nutrition Inc. and AmTech have hazard analysis critical control point (HACCP) management systems in place and their manufacturing facilities have 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 powder are either stainless steel, aluminum, or otherwise 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 00090, 2002; GRN 000489, 2017; GRN 000620, 2016) (Table 3). The β-galactosidase enzyme (Lactazyme-B) manufactured by GenoFocus, Inc. (Korea) is obtained from a proprietary non-toxigenic, non-pathogenic, non-genetically modified strain of *B. circulans* and complies with FCC specifications. It is also used to produce the subject of GRN 000729 (2018), 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 used throughout the production process 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 §184.1033 |
| 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 powder, 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* M3-1 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 the 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 ingredient is concentrated by heating the ingredient to 66°C to 81°C with a brief introduction to a high temperature of 135°C at the beginning of the evaporation process to control the microbial load. The ingredient then passes through a screen to ensure a homogenous syrup and a metal detector to screen for metal contaminants and is packaged in cardboard containers lined with ultra-low density polyethylene food-grade liners under sanitary conditions, producing VITAGOS™ IF syrup. To spray-dry the ingredient, VITAGOS™ IF syrup is shipped in trucks that have been qualified for use by Vitalus under ambient conditions to AmTech Ingredients where it is mixed with water and steam, passed through a series of magnets and filters, spray-dried, milled, sifted, passed through a metal detector, packaged in containers lined with ultra-low density polyethylene food-grade liners under sanitary conditions, and stored under ambient conditions.

There are two critical control points (CCPs) in the manufacturing of VITAGOS™ IF powder. The first CCP occurs after the syrup passes through the filter screen and before the packaging step. The CCP entails monitoring the ingredient 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 ingredient is diverted, the metal is removed, and the diverted ingredient is discarded as waste. The second CCP is after the ingredient is spray-dried, milled, and sifted. The CCP entails passing the ingredient through another metal detector to ensure that no additional metal particles were introduced during the spray drying process. Additionally, the

quality of the ingredient is monitored throughout the manufacturing process with in-line testing for solids, conductivity, pH, color, and sugar profile.

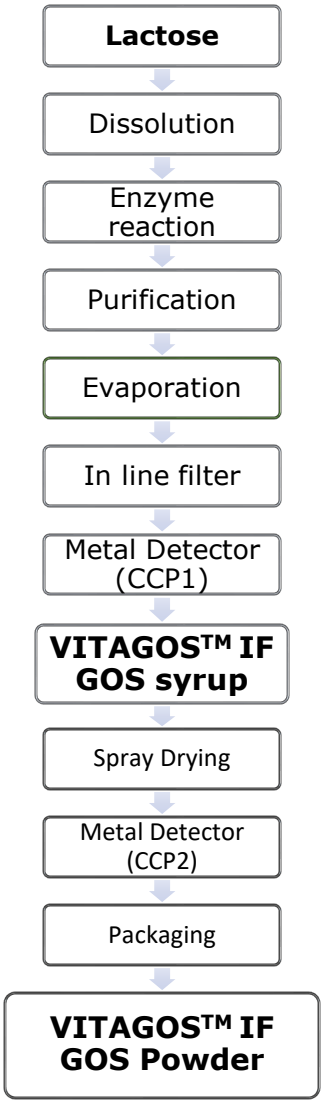


**Figure 3. Production Process for VITAGOS™ IF Powder**

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 syrup is concentrated by evaporation, heat-treated, filtered, passed through a metal detector and packaged in containers lined with food grade bags, and stored under ambient conditions. To produce VITAGOS™ IF powder, VITAGOS™ IF syrup is mixed with steam and water, passed through a series of magnets and filters, spray-dried, passed through a metal detector, and packaged in containers lined with polyethylene food-grade bags, and stored under ambient conditions. There are two critical control points (CCPs) in the production process, CCP1 and CCP2, and both entail passing the ingredient through a metal detector to ensure that the ingredient is devoid of metal particles introduced during manufacturing.

## E. FINISHED PRODUCT SPECIFICATIONS AND OTHER QUALITY ATTRIBUTES

### 1. Product Specifications

To ensure a consistent food-grade ingredient, each batch of VITAGOS™ IF powder is evaluated against an established set of product specifications (Table 4) using compendial or validated methods that are fit-for-use. Data from three batches demonstrate control of the production process and compliance with the product specifications.

**Table 4. VITAGOS™ IF Powder Product Specifications and Batch Data**

| Parameter | Specification | Method | Batch Number | | |
|---|---|---|---|---|---|
| | | | 1034564 | 1034592 | 1034607 |
| **Physical Characteristics** | | | | | |
| pH | 3.0-6.0 | TMS-QC 773 (pH meter) | 5.2 | 5.2 | 5.5 |
| Total Moisture (%) | ≤ 5.5 | Karl-Fisher | 3.7 | 3.7 | 3.8 |
| Dry Matter (Total %) | ≥ 94 | Vacuum Oven Solids (TMS-QC 2532) | 96.3 | 96.3 | 96.1 |
| Viscosity (cps) | 1000-5000 | TMS-QC 1392 (viscometer) | 2624 | 2316 | 1739 |
| **Chemical Composition** | | | | | |
| Galacto-oligosaccharides (% DM) | ≥ 65 | Mod. AOAC Method 2001.02 | 71.3 | 69 | 73 |
| Lactose (% DM) | ≤ 28 | Mod. AOAC Method 2001.02 | 18.9 | 18.1 | 18.7 |
| Glucose + Galactose (% DM) | ≤ 5.5 | TMS-QC 2535 | 4.9 | 4.5 | 4.5 |
| Nitrogen (%) | ≤ 0.032 | AOAC 991.20.E[2] | <0.02 | <0.02 | <0.02 |
| Sulfated Ash (% w/w) | ≤ 0.3 | USP / NF Current Version | <0.01 | <0.01 | <0.01 |
| Nitrate (mg/kg) | ≤ 50 | HPB LPFC-126[3] | 12.8 | 13.5 | 12.2 |
| Nitrite (mg/kg) | ≤ 2 | HPB LPFC-126 | 1.28 | 1.63 | 1.80 |
| **Microbiological Parameters[4]** | | | | | |
| Standard Plate Count (cfu/g) | < 3000 | MFHPB-18 | ND | ND | ND |
| Coliform (cfu/g) | < 10 | MFHPB-35 | ND | ND | ND |
| Enterobacteriaceae (cfu/ g) | < 10 | MFLP-09 | ND | ND | ND |
| *Escherichia coli* (cfu/g) | < 10 | MFHPB-34 | ND | ND | ND |
| Yeast and Mold (cfu/g) | < 100 | MFHPB-22 | ND | ND | 5 |
| *Staphylococcus aureus* (cfu/g) | < 10 | MFHPB-21 | ND | ND | ND |
| Salmonella (per 25g) | Negative | MFLP-29 | Neg | Neg | Neg |
| *Cronobacter sakazakii* (per 25g) | Negative | MFLP-27 | Neg | Neg | Neg |
| *Bacillus cereus* (cfu/g) | <100 | MFLP-42[5] | <10 | <10 | <10 |
| *Listeria monocytogenes.* (per 25g) | Negative | MFLP-28 | Neg | Neg | Neg |
| **Heavy Metals** | | | | | |
| Arsenic (ppm; w/w)[1] | < 0.4 | EPA 3050/6020, USP 730 | ND | ND | ND |
| Lead (ppm; w/w)[1] | ≤ 0.2 | EPA 3050/6020 USP 730 | 0.02 | ND | ND |
| Cadmium (ppm; w/w)[1] | < 0.06 | EPA 3050/6020 USP 730 | ND | ND | ND |
| Mercury (ppm; w/w)[1] | ND | EPA 3050/6020 USP 730 | 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.

[2]Limit of detection: Nitrogen = 0.02%

**Table 4. VITAGOS™ IF Powder Product Specifications and Batch Data**

| Parameter | Specification | Method | Batch Number | | |
|---|---|---|---|---|---|
| | | | 1034564 | 1034592 | 1034607 |

[3]Obtained from Compendium of Method for Chemical Analysis of Foods ( https://www.canada.ca/en/health-canada/services/food-nutrition/research-programs-analytical-methods/analytical-methods/chemical-compendium-analysis-foods.html)
[4]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.
[5]Limit of detection: <10 cfu/g.

## 2. Other Quality Attributes

### *a. Enzyme Deactivation*

To confirm that the production process deactivates the β-galactosidase used to manufacture the GOS, samples from 5 batches of VITAGOS™ IF syrup, the precursor to VITAGOS™ IF powder, were collected immediately after the reaction was terminated (0 hr) and then after the reaction was terminated and the ingredient was incubated for additional 24 hr under optimal reaction conditions (24 hr) (Table 5). The DP profile of the batches after the 24 hr incubation period was the same as the DP profile after the reaction was terminated, indicating that the production process parameters successfully deactivated the enzyme.

**Table 5. Lack of Enzymatic Activity in VITAGOS™ IF syrup**

| Saccharide | Time (hours) | |
|---|---|---|
| | 0[a] | 24[a] |
| DP5+ % | 6.2 ± 0.6 | 6.2 ± 0.18 |
| DP4 % | 10.82 ± 0.27 | 10.6 ± 0.18 |
| DP3 % | 22.9 ± 0.37 | 22.8 ± 0.17 |
| DP2 % | 20.6 ± 0.39 | 21.1 ± 0.50 |
| Lactose % | 18.86 ± 0.38 | 18.4 ± 0.56 |
| Glucose % | 19.22 ± 0.27 | 19.2 ± 0.11 |
| Galactose % | 1.44 ± 0.24 | 1.7 ± 0.16 |

DP = Degree of polymerization.
[a]n=5 batches of VITAGOS™ IF syrup.

## F. STABILITY OF VITAGOS™ IF POWDER

The stability of GOS-containing ingredients has been evaluated in numerous studies that have been summarized in previous GRAS Notices for other GOS-containing ingredients. The available data show that the ingredients are stable up to 2 years when stored under ambient conditions (US FDA, GRN 000236, 2008; US FDA, GRN 000285, 2009; US FDA, GRN 000286, 2009; US FDA, GRN 000334, 2010; US FDA, GRN 000484, 2014; US FDA, GRN 000489, 2014; US FDA, GRN 000495, 2014; US FDA, GRN 000518, 2014; US FDA, GRN 000569, 2015; US FDA, GRN 000620, 2016; US FDA, GRN 000721, 2017; US FDA, GRN 000729, 2018; US FDA, GRN 000896, 2020; US, FDA GRN 000671, Ceased). To confirm the stability of VITAGOS™ IF powder is similar to the stability of other GOS-containing ingredients, samples of three batches were stored in double-bagged sterile lab sample polyethylene bags under ambient conditions (18-25°C). Oligosaccharide content, microbiological content, and pH were determined at various time points and compared to the product specifications. Over an 8-month storage period, the amount of GOS, galactose, glucose, and lactose in VITAGOS™ IF powder was similar to the amount found in day zero VITAGOS™ IF powder samples and complied with the product specifications (Table 6); the distribution of GOS in DP2, DP3, DP4, and DP5 or greater was similar to VITAGOS™ IF powder at the beginning of the testing period; and the microbiological parameters and pH complied with the product specifications throughout the testing period (Table 7). Importantly, evaluating the stability of VITAGOS™ IF powder is an ongoing process and the stability of the ingredient will continue to be monitored to support the intended shelf-life.

**Table 6. Oligosaccharide Stability of VITAGOS™ IF Powder**

| Parameter | Specification* | Batches and Time (months) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1034564 | | | | 1034592 | | | | 1034607 | | | |
| | | 0 | 3 | 6 | 8 | 0 | 3 | 6 | 8 | 0 | 3 | 6 | 8 |
| Galacto-oligosaccharides (% DM) | ≥ 57 | 71.3 | 72.1 | 71.3 | 71.9 | 69.0 | 72.2 | 69.6 | 70.2 | 73.0 | 74.4 | 72.7 | 70.4 |
| Galactose + Glucose (% DM) | ≤ 5.5 | 4.9 | 4.9 | 5.1 | 5.1 | 4.5 | 4.7 | 4.7 | 4.9 | 4.5 | 4.7 | 4.6 | 4.9 |
| Lactose (% DM) | ≤ 28 | 22.1 | 23.1 | 22.8 | 21.8 | 22.0 | 22.6 | 22.0 | 21.9 | 21.1 | 22.9 | 24.3 | 21.4 |
| DP2 (% GOS) | ns | 25.9 | 24.9 | 25.5 | 25.8 | 26.4 | 25.5 | 26.6 | 26.3 | 27.0 | 25.2 | 24.1 | 26.4 |
| DP3 (% GOS) | ns | 28.0 | 28.0 | 27.8 | 28.0 | 28.0 | 28.1 | 27.9 | 27.9 | 28.3 | 28.1 | 28.1 | 28.0 |
| DP4 (% GOS) | ns | 12.4 | 12.8 | 12.3 | 12.6 | 12.4 | 12.9 | 12.2 | 12.5 | 12.5 | 12.4 | 12.3 | 12.6 |
| ≥DP5 (% GOS) | ns | 6.7 | 6.3 | 6.6 | 6.8 | 6.7 | 6.3 | 6.6 | 6.5 | 6.7 | 6.7 | 6.6 | 6.7 |

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 7. Microbial Stability of VITAGOS™ IF Powder**

| Parameter | Specification | Batches and Time (months) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1034564 | | | 1034592 | | | 1034607 | | |
| | | 3 | 6 | 8 | 3 | 6 | 8 | 3 | 6 | 8 |
| pH | 2.7-4.0 | 5.1 | 5.1 | 5.1 | 5.2 | 5.3 | 5.2 | 5.4 | 5.6 | 5.5 |
| Standard Plate Count | < 3000 cfu/g | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Enterobacteriaceae | < 10 cfu/g | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| *Escherichia coli* | <10 cfu/g | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Yeast | < 100 cfu/g | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Mold | <100 cfu/g | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| *Staphylococcus aureus* | < 10 cfu/g | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Salmonella | Negative/25g | 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

Galactooligosaccharides are synthetic non-digestible carbohydrates. Naturally-occurring non-digestible carbohydrates similar to GOS are components of the diet, found in foods such as vegetables, whole grains, fruits, cereal bran, flaked cereals, and flours (https://www.fda.gov/food/food-labeling-nutrition/questions-and-answers-dietary-fiber; accessed on December 15, 2022), as well as 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). Isolated or synthetic non-digestible carbohydrates include β-glucan soluble fiber, psyllium husk, cellulose, guar gum, pectin, locust bean gum, hydroxypropylmethylcellulose, mixed plant cell wall fibers, arabinoxylan, alginate, inulin and inulin-type fructans, high amylose starch, GOS, polydextrose, resistant maltodextrin/dextrin, cross-linked phosphorylated RS4, glucomannan, and gum Arabic are also found in foods. Importantly, isolated or synthetic GOS are used in a wide variety of products and, although they are structurally and compositionally less diverse than naturally occurring non-digestible carbohydrates, they contain glycosyl bonds that render them resistant to the digestive enzymes in the stomach and small intestine, and fermentable by the gastrointestinal microbiota present in the small and large intestine, similar to the naturally-occurring non-digestible carbohydrates (Wisker et al., 1985; Ohtsuka et al., 1990; Chonan et al., 2004) (Table 8).

GOS have a long history of safe use worldwide.

- In Japan, GOS have been commercially available since 1995 and are considered 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; United States Food and Drug Administration, Department of Human Health Services, 2018).
- In the United States, the first GOS ingredient was determined GRAS for use in non-exempt, term, cow's milk-based infant formula and selected conventional foods and

received a "no questions" letter from the FDA in 2008 (GRN 000236). Since then, a total of nine GOS-containing ingredients have been determined GRAS for use in non-exempt, term, cow's milk-based infant formula and selected conventional foods at levels up to 7.8 g/L and 33.4 g/serving, respectively, resulting in fourteen GRAS Notices (GRN 000236, 2008; GRN 000285, 2009; GRN 000286, 2009; GRN 000334, 2010; GRN 000484, 2014; GRN 000489, 2014; GRN 000495, 2014; GRN 000518, 2014; GRN 000569, 2015; GRN 000620, 2016; GRN 000671, 2017; GRN 000721, 2017; GRN 000729, 2018; GRN 000896, 2020). All GRAS Notifications have received "no questions" letters from the FDA, except for GRN 000671, which was ceased to be evaluated at the notifier's request. Importantly, the notifier of GRN 000671 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 (Scientific Committee on Food, 2003). GOS are currently approved for use in infant and follow-on formulas 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, 2003; EU 2016/127).

## C. INTENDED USE

Vitalus Nutrition Inc. intends to use VITAGOS™ IF powder as a substitute for VITAGOS™, which is GRAS for use in powdered, ready-to-feed, and concentrated liquid versions of cow's milk-based, non-exempt, term infant formula and conventional foods (GRN 000721, 2017). The use levels in infant formula will not exceed 7.8 g GOS/L reconstituted infant formula as specified in GRN 000620 (2016) and GRN 000729 (2018). The intended uses and use levels in conventional foods include the same uses specified in GRN 000721 (2017), as well as additional uses. A majority of the new uses have been determined GRAS for other GOS-containing ingredients (Table 8). Importantly, the subject of this GRAS Determination is not intended to be used in juices for which a standard of identity may preclude its use.

**Table 8. Intended Uses of VITAGOS™ IF Powder**

| Food Group | Proposed Food Uses | Maximum Use Level (g GOS/100 g Product) |
|---|---|---|
| Non-exempt, term, cow's milk-based infant formula | NA | 0.78[a,e] |
| Milk and milk products | Milk, milk substitute such as soy milk | 2[a] |
| | Milk drink | 4[a] |
| | Yogurt | 3.4[a] |
| | Milk based meal replacement | 2[a] |
| | White sauces, milk gravies, and cheese sauces | 1.25[a] |
| | Milk desserts, frozen like ice creams | 2[a] |
| | Pudding and custards including baby foods | 1.4[a] |
| | Cheese soups | 0.62[a] |
| 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[a] |
| | Dry soup bases | 0.82[b] |
| Nut beverages | Coconut beverages | 1.6[a] |
| Bakery products | Bread | 1[a] |
| | Brownies | 1[a] |
| | Cakes, heavy weight | 1[a] |
| | Cakes, medium weight | 1[a] |
| | Cakes, light weight | 1[a] |
| | Coffee cakes, crumb cakes, doughnuts, Danish, sweet rolls, sweet quick type breads, muffins, toaster pastries | 1[a] |
| | Cookies | 1[a] |
| | Crackers that are usually used as snacks | 1[a] |
| | French toast, pancakes | 1[a] |
| | Pies, cobblers, fruit crisps, turnovers, other pastries | 1[a] |
| | Waffles | 1[a] |
| | Grain-based bars with or without filling or coating, e.g., breakfast bars, granola bars, rice cereal bars | 1[a] |
| Cereals | Ready-to-eat cereals | 2[a] |
| | Ready-to-eat cereals (dry) for baby food | 4[a] |
| | Ready-to-serve cereals (wet) for baby food | 0.55[a] |
| Fruit and vegetable juices | Fruit juices (including citrus fruit juices) and nectars | 1.6[a] |

**Table 8. Intended Uses of VITAGOS™ IF Powder**

| Food Group | Proposed Food Uses | Maximum Use Level (g GOS/100 g Product) |
|---|---|---|
| | Vegetable juices | 1.6[a] |
| | Fruit juices, vegetable juices and juice mixtures baby food | 1.6[a] |
| Sugars and sweets | Jellies, jams, preserves | 25[a] |
| | Gum | 42.67[b] |
| | Boiled candy/hard candy | 8.53[b] |
| | Hard candy | 4.27[b] |
| | Candies | 4.27[b] |
| | Chocolate with filling | |
| | Fruit based bars/rolls candies | |
| | Chocolate bars | |
| | Soft candy | |
| Nonalcoholic beverages | Fruit drinks such as fruit juice drinks, fruit flavored drinks, sports drinks, etc. | 2[a] |
| | Non fruit beverages including energy drinks | 4.4[a] |
| | Beverage concentrate (powder) | 33.4[a,f] |
| | Isotonic electrolyte drinks | 0.83 |
| | Soft drinks/ carbonated beverages | 0.75[d] |
| | Fermented beverages | 0.83[c] |
| | Smoothies | 1.25[a] |
| Coffee & tea, hot chocolate | Coffee | 0.42[c] |
| | Coffee-based ready-to-drink beverage | |
| | Ice coffee | |
| | Coffee mixes | 0.42[c] |
| | Hot chocolate drink | 0.63[c] |
| | Hot chocolate powder | |
| Protein powder (Sports Nutrition)/reconstituted protein shakes | Sports nutrition drinks and meal replacement powder | 0.83[c] |
| Meal replacement bars | Protein bars | 0.75[b] |

NA = not applicable
[a]Use level is the same as the use level specified in GRN 000721.
[b]Use level is the same as the use level specified in GRN 000285.
[c]Use level is the same as the use level specified in GRN 000484.
[d]Use level is the same as the use level specified in GRN 000489.
[e]Maximum amount of GOS ingested is based on the caloric need of the infant (see Chapter III, Section D.1.)
[f]Prior to the dissolution of powder in water. When diluted in water, the resulting concentration of GOS will be 5 g/250 g serving.

## D. ESTIMATED DAILY INTAKE

### 1. Infant Formula

Powdered, ready-to-feed, and concentrated liquid versions of cow's milk-based, non-exempt, term infant formulas will contain 7.8 g GOS/L as consumed, as specified in GRN 000620 (2016) and GRN 000729 (2018). Therefore, the dietary intake of GOS among infant formula consumers is incorporated by reference from GRN 000620, pages 14-15, and GRN 000729, 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 90$^{th}$ 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 90$^{th}$ 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 mean and 90$^{th}$ percentile intakes of 3.0 and 7.1 g/day, respectively.

### 2. Assessment of VITAGOS™ IF Powder Use in Conventional Foods

The cumulative exposure to GOS from the use of VITAGOS™ IF powder in conventional foods was determined by calculating mean and 90th percentile estimated daily intakes (EDIs) from the intended uses using the food categories and maximum use levels specified in the original GRAS determination for VITAGOS™ IF syrup (Table 9), the proposed new food categories and use levels (Table 9), 2,272 unique food codes obtained from the Food and Nutrition Database for Dietary Studies (FNDDS) that represent the proposed food categories, and the food consumption data provided by the National Center for Health Statistics' (NCHS) 2017-2018 National Health and Nutrition Examination Surveys (NHANES) (Centers for Disease Control (CDC), 2018; United States Department of Agriculture and Agricultural Research Service, 2018).

### 3. Food Consumption Survey Data

#### *a. Survey Description*

The most recent NHANES data for the years 2017-2018 are available for public use. NHANES are conducted as a continuous, annual survey, and are released in 2-year cycles. In each cycle, approximately 10,000 people across the U.S. completed the health examination component of the survey. Any combination of consecutive years of data collection is a nationally representative sample of the U.S. population. It is well established that the length of a dietary survey affects the estimated consumption of individual users and that short-term surveys, such as

the typical 1-day dietary survey, overestimate consumption over longer time periods (Hayes and Kruger, 2014). Because two 24-hr dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) are available from the NHANES 2017-2018 survey, these data were used to generate estimates for the current intake analysis.

The NHANES provides the most appropriate data for evaluating food-use and food-consumption patterns in the United States, containing 2 years of data on individuals selected via stratified multistage probability sample of civilian non-institutionalized population of the U.S. NHANES survey data were collected from individuals and households via 24-hr dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person in the Mobile Examination Center (MEC), and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S. Small counties were combined to attain a minimum population size. These PSUs were segmented and households were chosen within each segment. One or more participants within a household were interviewed. Fifteen PSUs are visited each year. For example, in the 2009-2010 NHANES, there were 13,272 persons selected; of these 10,253 were considered respondents to the MEC examination and data collection. 9,754 of the MEC respondents provided complete dietary intakes for Day 1 and of those providing the Day 1 data, 8,405 provided complete dietary intakes for Day 2. The release data do not necessarily include all the questions asked in a section. Data items may have been removed due to confidentiality, quality, or other considerations. For this reason, it is possible that a dataset does not completely match all the questions asked in a questionnaire section. Each data file has been edited to include only those sample persons eligible for that particular section or component, so the numbers vary.

In addition to collecting information on the types and quantities of foods being consumed, the NHANES surveys collect socioeconomic, physiological, and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population.

Sample weights are incorporated with NHANES surveys to compensate for the potential under-representation of intakes from specific population groups because of sample variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (CDC, 2006; USDA, 2020).

*b. Statistical Methods*

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer in Octave© and used to generate estimates for the intake of VITAGOS™ IF powder by the U.S. population. Estimates for the daily intake of VITAGOS™ IF powder represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES data; these average amounts comprised the distribution from which mean and percentile intake estimates were produced. Mean and percentile estimates were generated incorporating sample weights to provide representative intakes for the entire U.S. population. "All-user" intake refers to the estimated intake of VITAGOS™ IF powder by those individuals consuming food products containing VITAGOS™ IF powder. Individuals were considered users if they consumed one or more food products containing VITAGOS™ IF powder on either Day 1 or Day 2 of the survey.

**4. Food Usage**

Eighty percent of the total U.S. population 2+ years was identified as consumers of VITAGOS™ IF powder (Table 9). The mean intakes of all VITAGOS™ IF powder consumers age 2+ from the intended uses are 2.30 g/person/day or 0.033 g/kg body weight/day. The heavy consumer ($90^{th}$ percentile) intakes are 5.38 g/person/day or 0.078 g/kg body weight/day.

Because VITAGOS™ IF powder is also intended to be an ingredient in non-exempt, term, cow's milk-based infant formula and baby foods, EDIs were calculated for infants aged 0-6 and 7-12 months, and toddlers aged 1-2 years. The mean EDIs for infants aged 0-6 and 7-12 months were approximately 1 g/person/day and 1.29 g/person day, or 0.23 g/kg body weight/day and 0.14 g/kg body weight/day, respectively. The heavy consumer ($90^{th}$ percentile) EDIs for infants aged 0-6 and 7-12 were 1.64 g/person/day and 3.1 g/person/day or 0.39 g/kg/day and 0.32 g/kg/day for infants aged 0-6 and 7-12 months, respectively. The mean EDIs for toddlers aged 1-2 years were 2.1 g/person/day or 0.17 g/kg/day. The heavy consumer ($90^{th}$ percentile) EDIs for toddlers aged 1-2 years were 4.88 g/person/day or 0.39 g/kg/day.

**Table 9. Estimated "All-user" Cumulative Daily Intake (EDI) of VITAGOS™ IF in Targeted Foods by Population Group (2017-2018 NHANES Data)**

| Population Group | N users | N population | % Users | Mean mass (kg) | Mean EDI (g) | 90th % EDI (g) | Mean EDI (g/kg) | 90th % EDI (g/kg) |
|---|---|---|---|---|---|---|---|---|
| Ages 0-0.5 years | 122 | 221 | 55.20 | 4.2544 | 0.98 | 1.64 | 0.230 | 0.386 |
| Ages 0.5-1 years | 162 | 175 | 92.57 | 9.4102 | 1.29 | 3.05 | 0.137 | 0.324 |
| Ages 1-2 years | 325 | 441 | 73.70 | 12.3865 | 2.10 | 4.88 | 0.169 | 0.394 |
| Ages 2-5 years | 547 | 785 | 69.68 | 16.8156 | 2.16 | 4.88 | 0.129 | 0.290 |
| Ages 6-12 years | 919 | 1271 | 72.31 | 37.5786 | 2.39 | 5.87 | 0.064 | 0.156 |
| Ages 13-19 years | 892 | 1038 | 85.93 | 68.6538 | 2.77 | 6.71 | 0.040 | 0.098 |
| Ages 20 years and up | 4654 | 5569 | 83.57 | 81.5814 | 2.21 | 5.14 | 0.027 | 0.063 |
| Ages 2 years and up | 7012 | 8663 | 80.94 | 69.4083 | **2.30** | **5.38** | **0.033** | **0.078** |

# 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 GRAS status of the use of VITAGOS™ IF powder in non-exempt, term, cow's milk-based infant formula and conventional foods is supported by a pivotal, publicly-available 90-day toxicology study conducted with Vivinal® GOS, which is manufactured using a β-galactosidase derived from the same species as VITAGOS™ IF powder, as well as numerous corroborative *in vitro*, toxicology, animal, and clinical studies, and the opinions of regulatory bodies worldwide regarding the safe use of GOS in infant formulas and selected conventional foods (US FDA, GRN 000236, 2008; US FDA, GRN 000285, 2009; US FDA, GRN 000286, 2009; US FDA, GRN 000334, 2010; US FDA, GRN 000484, 2014; US FDA, GRN 000489, 2014; US FDA, GRN 000495, 2014; US FDA, GRN 000518, 2014; US FDA, GRN 000569, 2015; US FDA, GRN 000620, 2016; US FDA, GRN 000721, 2017; US FDA, GRN 000729, 2018; US FDA, GRN 000896, 2020; US, FDA GRN 000671, Ceased; Scientific Committee on Food, 2001a; Scientific Committee on Food, 2001b; Food Standards Australia New Zealand, 2008). All GRAS Notifications have received "no questions" letters from the FDA, except for GRN 000671 which was ceased to be evaluated at the notifier's request. The notifier of GRN 000671 then resubmitted as the GRAS Determination as GRN 000721 and subsequently received a "no questions" letter. Additionally, the new data from toxicology and clinical studies published since the "no questions" letter for GRN 000729 do not contradict the GRAS status of the use of GOS in infant formula or conventional foods (Baek et al., 2021; Jain et al., 2019; Jain et al., 2020; Canfora et al., 2017; Vulevic et al., 2018; Wilms et al., 2021; Wilson e et al., 2021; Schoemaker et al., 2022). GOS are not mutagenic or genotoxic and do not produce toxicological effects. Additionally, clinical studies conducted with GOS show that the ingestion of GOS up to 8.55 g/L in infants and children and up to 15 g/day in adults is safe and well-tolerated. Taken together, the totality of the data supports Vitalus Nutrition Inc.'s conclusion that VITAGOS™ IF powder is GRAS for its use in cow's milk-based, non-exempt, term infant formulas and conventional foods.

## A. SAFETY OF GOS

The safety of VITAGOS™ IF powder is supported by its compositional equivalence to other GOS-containing ingredients manufactured with the same β-galactosidase, the general recognition of their metabolic fate, a pivotal 90-day toxicology study conducted with an equivalent GOS-containing ingredient (Anthony et al., 2006), numerous corroborative genotoxicology, sub-chronic toxicology, reproductive and developmental toxicology studies, and clinical studies conducted in infants and adults with GOS-containing ingredients manufactured with β-galactosidases derived from other strains of *B. circulans*, as well as other microbes, and the general recognition of the safe use of GOS in infant formulas and selected conventional

foods per authoritative opinions published by regulatory bodies worldwide (US FDA, GRN 000236, 2008; US FDA, GRN 000285, 2009; US FDA, GRN 000286, 2009; US FDA, GRN 000334, 2010; US FDA, GRN 000484, 2014; US FDA, GRN 000489, 2014; US FDA, GRN 000495, 2014; US FDA, GRN 000518, 2014; US FDA, GRN 000569, 2015; US FDA, GRN 000620, 2016; US FDA, GRN 000721, 2017; US FDA, GRN 000729, 2018; US FDA, GRN 000896, 2020; US, FDA GRN 000671, Ceased; Scientific Committee on Food, 2001a; Scientific Committee on Food, 2001b; Food Standards Australia New Zealand, 2008). In general, GOS are non-digestible carbohydrates that are not genotoxic, clastogenic, or mutagenic, and do not produce toxicological effects in preclinical models, and are well-tolerated in infants and adults.

### 1. Absorption, Distribution, Metabolism, and Excretion

VITAGOS™ IF powder contains not less than 65% GOS. The remaining components are lactose, glucose, and galactose, which are components of the diet. Importantly, the absorption, distribution, metabolism, and excretion of GOS is extensively summarized in the GRAS Notices for GOS containing ingredients (US FDA, GRN 000236, 2008; US FDA, GRN 000285, 2009; US FDA, GRN 000286, 2009; US FDA, GRN 000334, 2010; US FDA, GRN 000484, 2014; US FDA, GRN 000489, 2014; US FDA, GRN 000495, 2014; US FDA, GRN 000518, 2014; US FDA, GRN 000569, 2015; US FDA, GRN 000620, 2016; US FDA, GRN 000721, 2017; US FDA, GRN 000729, 2018; US FDA, GRN 000896, 2020) and by the Scientific Committee on Food (2001) and FSANZ (2008). Briefly, GOS are non-digestible oligosaccharides consisting of 1 to 7 galactose units linked via β (1-3), (1-4), or (1-6) glycosidic bonds to either a terminal glucose or galactose. Except for lactose, which is hydrolyzed by small intestinal brush border lactase, β-linked oligosaccharides 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) (US FDA, GRN 000620, 2016; 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, because the GOS present in VITAGOS™ IF powder are non-digestible oligosaccharides, the GOS are expected to be unabsorbed and fermented by the microbiota in the colon, producing short chain fatty acids, $CO_2$, and $H_2$ gas, similar to the GOS that are present in other GOS-containing ingredients that are GRAS.

## 2. Toxicology Studies

The toxicity of GOS-containing ingredients, such as VITAGOS™ IF powder, has been extensively tested in numerous genotoxicology, sub-chronic toxicology, and neonatal piglet tolerance studies, some of which are summarized in the GRAS Determination for VITAGOS™ IF syrup and incorporated by reference. Summaries of these studies are provided below. Importantly, GOS are not genotoxic, mutagenic, or clastogenic and because VITAGOS™ IF powder is manufactured using a β-galactosidase derived from *B. circulans*, which is the same enzyme used to manufacture Vivinal® GOS, the 90-day oral toxicology study conducted with Vivinal® GOS directly supports the safety of VITAGOS™ IF powder in infant formula and conventional foods. The NOAEL was determined to be the highest dose tested at 6.9 g Vivinal® GOS/kg-bw/day (Anthony et al., 2006). All other sub-chronic toxicology studies provide corroborative evidence that GOS do not produce toxicological effects in preclinical models.

### *a. Genotoxicity Studies*

The genotoxicity of GOS-containing ingredients has been evaluated in published and unpublished in vitro bacterial reverse mutation, micronucleus, and chromosomal aberration assays, and in vivo comet and micronucleus assays (Kobayashi et al., 2009; US FDA, GRN 000620, 2016), which are extensively summarized in GRNs 000334 and 000620 and therefore incorporated by reference. As summarized on pages 61-63 of GRN 000334, the published study conducted by Kobayashi et al. (2009) showed that GOS are not mutagenic, genotoxic, or clastogenic using a bacterial reverse mutation assay, chromosomal aberration assay, and an *in vivo* micronucleus study. As summarized on pages 20-21 of GRN 000620, the unpublished studies conducted by Narumi et al. (2014)**,** Verspeek-Rip (2015), and Verbaan (2015) also showed that GOS are not genotoxic using an *in vivo* comet assay, a bacterial reverse mutation assay and an *in vitro* micronucleus assay, respectively.

To identify new genotoxicity studies that have been published since GRN 000620, literature searches of Pubmed and Google Scholar were conducted using the search terms “galactooligosaccharides AND genotoxicity” and “galacto-oligosaccharides AND genotoxicity” on January 6, 2023. The titles and abstracts of the hits published since the publication of the “no questions” letter for GRN 000620 (July 21, 2016) were then reviewed to determine if the new studies tested the genotoxicity of only galactooligosaccharides. One new study was identified, Jain et al. (2019), and is summarized below.

To determine the mutagenic potential of Gossence™ (Tata Chemicals Ltd; 76.3% GOS DM), Jain et al. (2019) performed a bacterial reverse mutation assay. The initial assay was conducted using the test article at concentrations of 50, 159, 501, 1582, and 5000 µg/plate, in the

presence and absence of S9 activation, using the plate incorporation method. The growth of the bacterial background lawn and the 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 at 5000 µg/plate.

To determine the clastogenic potential of Gossence™, Jain et al. (2019) conducted an *in vitro* mammalian chromosomal aberration assay in HPBL cells. 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. Ethyl methane sulphonate (short and prolonged duration, -S9) and cyclophosphamide monohydrate (short duration, +S9) were the 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.

In summary, the new results published by Jain et al. (2019) are consistent with those published by Kobayashi et al. (2009) and summarized in GRN 000620, and support the conclusion that GOS and VITAGOS™ IF powder are not genotoxic, mutagenic, or clastogenic.

*b. Sub-chronic Rodent Toxicology Studies*

The safe use of VITAGOS™ IF powder is supported by the publicly available 90-day toxicology study conducted with Vivinal® GOS (Anthony et al., 2006), which is produced using a β-galactosidase derived from the same species as the β-galactosidase used to manufacture VITAGOS™ IF powder, extensively summarized on pages 38-41 of GRN 000236 (2008), and therefore incorporated by reference. In summary, Anthony et al. (2006) established a NOAEL for Vivinal® GOS at the highest dose tested, 2250 mg GOS/kg body weight/day, which is equivalent to 5000 mg Vivinal® GOS/kg body weight/day. It is also noteworthy that an unpublished, corroborative 90-day sub-chronic rat study using Vivinal® GOS was also described in GRN 000236 where Wistar rats were orally administered GOS at doses up to 6.9 g Vivinal® GOS/kg-bw/day. In this study, the NOAEL was determined to be the highest dose tested at 6.9 g Vivinal® GOS/kg-bw/day.

The safe use of VITAGOS™ IF powder is also supported by five other published and unpublished sub-chronic toxicology studies that were conducted with GOS-containing

ingredients manufactured with β-galactosidases that are different from the one used to manufacture VITAGOS™ IF powder (Kobayashi et al., 2009; Penard, 2015; Zhou et al., 2017; Jain et al., 2019; Jain et al., 2020). Three of these studies, Kobayashi et al. (2009), Penard (2015), and Zhou et al. (2017) have been summarized in previous GRAS Notices. Therefore, the summaries of these studies are incorporated by reference, and briefly summarized below.

As summarized on page 61 of GRN 000334 (2010), Kobayashi et al. (2009) administered a GOS-containing syrup (containing 55% GOS) manufactured by Yakult with β-galactosidases derived from *S. singularis* and *K. lactis* to Sprague Dawley rats. The rats were gavaged with water, 500, 1000, or 2000 mg/kg/day of the GOS-containing syrup for 90-days, resulting in a daily intake of 206.25, 412.5, and 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 the controls. The NOAEL was determined to be 2000 mg/kg/day for the GOS-containing ingredient, which is equivalent to 1100 mg GOS/kg/day.

As summarized on page 18 of GRN 000620 (2016), Penard (2015) administered a GOS-containing ingredient manufactured by Nestlé using a β-galactosidase derived from *Aspergillus oryzae* to Wistar rats at 0, 500, 1000, and 2000 mg GOS/kg bw/day for 30 days in an OECD 407-compliant study (note the study does not specify if the doses were GOS or the GOS-containing ingredient; the ingredient 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.

As summarized in GRN 000721 (2017), Zhou et al. (2017) administered 0, 1010, 2041, and 4082 mg VITAGOS™/kg bw/day (corresponding to 0, 500, 1000, or 2000 mg GOS/kg bw/day) to Sprague-Dawley rats for 90 days in an OECD 408-compliant study. VITAGOS™ is manufactured using β-galactosidases derived from *Aspergillus oryzae* and *Kluyveromyces lactis.* 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.

To identify new toxicology studies that have been published since the publication of the "no questions" letter for GRN 000620 (2016), literature searches of PubMed and Google Scholar were conducted using the search terms "galacto-oligosaccharide AND toxicity" on January 6, 2023. The titles and abstracts of the hits published since the original GRAS Determination (July 21, 2016) were then reviewed to identify new studies that tested the toxicity of only galactooligosaccharides. Three new studies were identified Jain et al. (2019), Jain et al. (2020), and Baek et al. (2021).

To evaluate the sub-chronic toxicity of Gossence™, Jain et al. (2019) conducted a 14-day dose-range-finding and an OECD Guideline 408-compliant 90-day oral toxicology study in adult Sprague Dawley male and female rats. The 14-day dose-range-finding study was performed to select the dose levels for the 90-day oral toxicology study. In the 14-day study, Gossence™ (76.28% GOS) was mixed with purified water and administered daily via gavage at 0, 1000, 2000, and 5000 mg GOS/kg/day (equivalent to 1347, 2694, and 6735 mg Gossence™/kg/day; n=6 rats/sex/group). A control group was administered purified water in parallel. Clinical signs, mortality, body weights, feed consumption, and feed conversion efficiency were monitored throughout the course of the 14-day treatment period. On day 14, blood from overnight-fasted rats was collected for hematology, coagulation, and clinical chemistry, urine was collected for urinalysis, and the animals were then euthanized necropsied and examined for gross pathological changes. Histopathology was performed on the rats in the vehicle- and 5000 mg/kg/day-treated groups, and the cecums were evaluated from all dose groups. During the study, there were no premature deaths, no morbidities, and no clinical signs of toxicity. Although the feed conversion efficiency was comparable among the groups, there was a dose-dependent decrease in feed consumption in the 2000- and 5000 mg/kg/day-treated groups and a non-significant decrease in overall body weight gain in the males of 5000 mg/kg/day-treated group compared to the vehicle-treated group. No test article-related changes were observed among the groups in the hematology, coagulation, clinical chemistry, and urinalysis parameters. At necropsy, the size of the cecum of 5/6 males and 4/6 females in the 5000 mg/kg/day-treated group and 2/6 males in the 2000 mg/kg/day-treated group was increased compared to the vehicle-treated group. Correspondingly, the mean absolute and relative cecum weights were increased in the males of the 2000- and 5000 mg/kg/day-treated groups and the females of the 5000 mg/kg/day-treated group. Mucosal epithelial hypertrophy was also observed in the 5000 mg/kg/day-treated group. Based on these results, the dose levels of 1000, 2000, and 5000 mg/kg/day were used in the subsequent 90-day oral toxicology study.

In the 90-day toxicology study, Gossence™ (76.28% GOS) was mixed with purified water and administered daily via gavage with either vehicle, 1000, 2000, and 5000 mg/kg/day GOS/kg/day (equivalent to 1347, 2694, and 6735 mg Gossence™/kg/day; n=10 rats/sex/group).

The study also included a recovery group at each dose level (n=6 rats/sex/group) that were treated in parallel with the main treatment groups for 90-days and did not receive vehicle or Gossence™ for an additional 28 days after the 90-day treatment period. Dose formulations were analyzed for 4'-galactosyllactose on day 1, and months 2 and 3 of the study using a validated high performance liquid chromatography (HPLC) assay. During the treatment period, morbidity, mortality, body weight, feed consumption, and feed conversion efficiency were monitored. Ophthalmological examinations were performed before and at the end of the treatment period. A functional observational battery was conducted and motor activity was assessed during the 13th week of dosing. Prior to euthanasia on day 91 for the treated groups and day 119 for the recovery groups, all animals were fasted overnight. Blood and urine were collected for clinical pathology analyses (hematology, coagulation, and clinical chemistry) and urinalysis, respectively. Then, all animals were euthanized and evaluated for gross pathological changes at necropsy. Organs were collected, weighed, and preserved. Organ weight ratios, as a percentage of body and brain weight, were calculated. All organs from all groups were preserved and histopathology was conducted on all organs from the control and 5000 mg/kg-treated groups plus the cecums from the 1000 and 2000 mg/kg-treated groups.

Stability and dose confirmation analyses showed that the formulations were stable in the vehicle for up to 7 days at 2 – 8°C and the amount of 4'-galactosyllactose in all doses at all time points tested was within the acceptable limits (% variation +/- 10% and %RSD <10%). No mortalities, clinical signs of morbidity or adverse ophthalmic findings were observed throughout the study. Although a slight, test article-related decrease in the mean final body weight of the males of the 5000 mg/kg-treated group compared to the males of the control group was observed, Gossence™ had no significant effect on either mean body weight or body weight gain. Importantly, the slight decrease in the body weight of the males of the 5000 mg/kg-treated group was determined to be non-adverse because the decrease was not statistically significant and returned to levels comparable to the control group by the end of the recovery period. Additionally, a significant reduction in feed consumption was observed in the males of the 5000 mg/kg-treated group beginning on day 40, resulting in a 14% decrease overall. The reduction was minimal and completely recovered by the end of the recovery period. There was also no effect of Gossence™ on feed efficiency, hematology, coagulation, clinical chemistry, or urinalysis parameters. Regarding the effect of Gossence™ on the cecum, the cecums of 6/10 males and 7/10 females in the 5000 mg/kg-treated group were enlarged at necropsy and corresponded with statistically-significant increases in absolute and relative cecum weights in the males of the 2000 and 5000 mg/kg-treated groups and the females of the 5000 mg/kg treated groups. Minimal mucosal hypertrophy also occurred in both males and females from the same treatment groups. All effects of Gossence™ on the cecum returned to normal after the recovery

period. Importantly, the authors considered the cecal effects to be non-adverse, resulting from compensatory/adaptive response to the increased ingestion of a non-digestible carbohydrate and its subsequent fermentation by the resident microbiota. Similar effects have been reported for other GOS-containing ingredients and other food ingredients such as modified starches, polyols, fibers, and lactose. Because all the significant findings were determined to be non-adverse, the no observed adverse effect level (NOAEL) for Gossence™ was determined to be at least 5000 mg GOS/kg/day, which is equivalent to 6735 mg Gossence™/kg/day.

In a subsequent study, Jain et al. 2020 evaluated the sub-chronic toxicity of Gossence™ (76.28% GOS) in juvenile Sprague Dawley rats. On post-natal day (PND) four, rat pups were allocated to four main groups and treated with daily doses of either vehicle, 1000, 2000, or 5000 mg GOS/kg/day (equivalent to 1347, 2694 or 6735 mg GOS) by gavage, respectively, for 7 weeks (n=12 rats/sex/group). Two additional groups were also included as recovery groups, which received daily doses of either vehicle or 5000 mg GOS/kg/day by gavage during the 7-week treatment period, followed by no treatment for 14-days (n=6 rats/sex/group). All dose formulations were analyzed for 4'-galactosyllactose on day 1 and day 46 using a validated HPLC assay. All treated pups were housed with their dam from birth until weaning on PND 21. At weaning, the pups were then transferred to new cages at two per sex per cage and the dams were euthanized. Over the course of the 7-week treatment period and the 14-day recovery period, all dams and pups were observed once daily for clinical signs of toxicity and twice daily for morbidity and mortality. Detailed clinical examinations were done from weaning day (PND 21) and at weekly intervals thereafter until necropsy. Individual body weights for all dams were recorded on days 1, 7, 14, and 21 of lactation period and for pups on day 1 of littering and twice weekly during the dosing and recovery period until euthanization. Terminal body weights (fasting body weight) were taken on the day of necropsy on PND 53 for the main group animals and PND 67 for the recovery group animals. Feed consumption was recorded twice weekly from the day of weaning (day21) until euthanization. All pups were observed for the onset of postnatal developmental landmarks such as hair growth, incisor eruption, ear opening, eye opening, balano-preputial separation, and vaginal opening. Ophthalmological examinations were performed on day 44 for the control and 5000 mg/kg/day-treated animals of the main group. Neurobehavioral examinations (functional observational battery (FOB)), such as home cage observations, handling observations, open-field observations, sensory observations, neuromuscular observations, and physiological observation (rectal temperature)) and motor activity were conducted on days 45 and 46 of the main groups. At the end of the dosing and recovery periods, blood and urine were collected from all animals after an overnight fast for hematology, coagulation, and biochemical evaluations, and urinalysis. All rats were then euthanized. Selected organs were collected from all the animals, and weighed and preserved for

histopathology, which was conducted on the rats from the vehicle and 5000 mg/kg/day-treated groups, and any rat found dead during the study. Gross changes from all rats were processed and evaluated microscopically.

Dose confirmation analyses showed that the amount of 4'-galactosyllactose in all doses was within acceptable limits of the nominal concentration (% recovery: 90-110%). The percent relative standard deviation was less than 10% GOS. During the study, there were no clinical signs of toxicity or mortality in the 1000 and 2000 mg/kg/day-treated groups. In the 5000 mg/kg/day-treated group, watery feces and yellow-colored stains in the urogenital region were observed in 13/34 animals (9 males and 4 females) and on day 15, two rats died (1 male and 1 female). The daily dose of 5000 mg/kg was then reduced to 3000 mg/kg from day 16 of the dosing phase and all remaining animals survived until the terminal euthanization. Although the exact cause of death in the two rats could not be determined via the gross and histomorphological evaluations, both the small and large intestines in the rats contained yellow liquid.

During the treatment period, a statistically-significant decrease in mean body weight was observed in the 5000 mg/kg/day main group on days 4 and 7 and a significant reduction in mean body weight gain was observed in the 5000 mg/kg/day recovery group on days 1-4 compared to the concurrent vehicle-treated group. However, after the 5000 mg/kg/day dose was reduced to 3000 mg/kg/day on day 16, no further decreases in body weight or body weight gain were observed. There were also no changes in mean body weight or body weight gain in the 1000 and 2000 mg/kg/day groups and mean feed consumption was comparable across all the groups. There were no Gossence™-related changes in hematology, coagulation, clinical chemistry, and urinalysis or in the postnatal developmental landmarks, functional observational battery, neuromuscular observations, motor activity, ophthalmological evaluations of the 5000/3000 mg/kg/day-treated group.

At necropsy, the cecums of the 5000/3000 mg/kg- and the 2000 mg/kg/day-treated groups were increased and correlated with increased cecal weights with the luminal contents, but there were no microscopic changes. The cecal enlargement completely recovered following the 14-day recovery period. Interestingly, the cecal weights of the 5000/3000 mg/kg- and the 2000 mg/kg/day-treated groups without the luminal contents were not affected, indicating that the increased weights were primarily due to the luminal contents. There were no other test item-related changes in any of the other organs that were collected, weighed, and preserved, and there were no gross changes and histological findings in any of the tissues that were examined, including the adrenal glands, aorta, axillary lymph nodes, biceps femoris, bone marrow, brain, cecum, colon, diaphragm, duodenum, epididymides, esophagus, eyes, femur bone with the joint, Harderian glands, heart, ileum with Peyer's patches, jejunum, kidneys, lacrimal glands, larynx,

liver, lungs, mandibular and mesenteric lymph nodes, mammary gland, optic nerve, ovaries, oviducts, pancreas, pharynx, pituitary, prostate (seminal vesicles and coagulating glands, quadriceps, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, testes, thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, ureters, uterus with cervix, and vagina. Because all the findings were considered non-adverse and the 5000 mg/kg/day dose was reduced to 3000 mg/kg/day, the NOAEL for GOS was determined to be 3000 mg/kg/day, which is equivalent to 4041 mg/kg/day of Gossence™.

Baek et al. (2021) conducted acute and subchronic (28-day) toxicity studies in Sprague-Dawley rats. In the acute study, rats were orally administered either physiological saline or 5000 mg/kg of GOS (n=5 male and 5 female rats/group) provided by Neocremar Co., Ltd, which was manufactured using a β-galactosidase derived from *B. circulans* and contained a GOS content not less than 70%. In the subchronic toxicity study, the rats were administered either physiological saline or 1000 mg/kg/day of GOS for 28 days (n=5 male and 5 female rats/group). Reverse transcriptase polymerase chain reaction (RT-PCR) analysis confirmed that the products did not contain milk-derived antigens. Although there was a statistically significant reduction in the total protein levels in the blood of GOS-treated group in the acute study, the authors deemed that they were not adverse because the reduced levels fell within the normal range. The reduction was also not seen in the GOS-treated rats in the 28-day study. Additionally, because there were no GOS-related effects on the clinical symptoms, weights, feed intakes, hematology, blood chemistry, relative organ weights, or gross pathologies in either study, the authors ascribed the NOAEL to 1000 mg GOS/kg/day.

Importantly, the results reported by Jain et al. (2019), Jain et al. (2020), and Baek et al. (2021) are consistent with those reported in other subchronic toxicology and non-toxicology studies conducted with GOS-containing ingredients (Anthony et al., 2006; Kobayashi et al., 2009; Kobayashi et al., 2014; Zhou et al., 2017; Lina et al., 1995 (summarized in GRN 000236, 2008); Penard et al., 2015 (summarized in GRN 000620, 2016)). Specifically, the NOAELs established by Jain et al. (2019), Jain et al. (2020), and Baek et al. (2021) lie within the range of NOAELs established by Anthony et al. (2006), Kobayashi et al. (2009), Kobayash et al. (2014), Zhou et al. (2017), Lina et al. (1995) and Penard et al. (2015) of least 825 mg GOS/kg/day to a maximum of 5000 mg/kg/day. Additionally, the mortalities reported by Jain et al. (2020) in neonatal rats were likely due to the administration of a very large bolus of undigestible fiber to the immature gastrointestinal system of the rat, resulting in physical intolerance, and cecal enlargement has also been reported in toxicology studies conducted with GOS-containing ingredients (Lina et al., 1995; Zhou et al., 2017), as well as a variety of non-toxicology studies conducted in rats and pigs. Importantly, cecal enlargement is an established physiological effect of GOS that is consistent with the transport of resistant sugars/carbohydrates to the large

intestine and widely recognized as being not toxicologically relevant to humans (Kawakami et al., 2005; Djouzi and Andrieux, 1997; Kikuchi-Hayakawa et al., 1997; Chonan and Watanuki, 1995; Chonan and Watanuki, 1996; Hayashi et al., 1991; Ohtsuka et al., 1990; Houdijk et al., 2002; World Health Organization, 1987). Thus, the new results reported by Jain et al. (2019), Jain et al. (2020), and Baek et al. (2021) do not contradict, but support the general recognition of GOS safety.

*c. Reproductive and Developmental Studies*

Additional toxicology studies that corroborate the safety of VITAGOS™ IF powder include a neonatal rodent toxicity study conducted in juvenile rats and a one-generation reproductive and developmental toxicity study conducted in rats (Kobayashi et al., 2014a; Kobayashi et al., 2014b). These studies are summarized in GRN 000620 (2016), pages 16-17, and their summaries are incorporated by reference. GOS did not exhibit developmental or reproductive toxicity at doses up to 853 mg GOS/kg bw/day in these studies.

In the study conducted by Kobayash et al. (2014), juvenile Sprague-Dawley rats were gavaged with 0, 500, 1000, or 2000 mg Oligomate 55N (56.9 % GOS)/kg/day, which is the GOS-containing syrup that is the subject of GRN 000334, for 42 days starting on post-natal day 4. The resulting daily intake of GOS was 213.4, 426.8, and 853.5 mg GOS/kg. GOS ingestion was reported to not affect the development of the animals and did not affect general condition, hematology, blood chemistry, or the outcome of the 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. (2014) evaluated the developmental and reproductive effects of the GOS-containing ingredient that was the subject of GRN 000334 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 ingestion did not produce toxicological effects on male or female parental animals and did not adversely affect reproduction from premating, copulation, implantation, or maintenance of pregnancy. The offspring were unaffected by the maternal consumption of GOS. Additionally, GOS ingestion had no effect on the number of pups born, the sex ratio of the litters, or the body weights of the pups. The growth and development of

the pups were also normal in all dose groups. The NOAEL for reproductive function of male and female parent animals was 2000 mg GOS per kg/day equivalent to 853.5 mg GOS/kg/day.

*d. Neonatal Piglet Studies*

The effects of GOS consumption on the intestinal microbiota have also been evaluated in a neonatal piglet study (Alizadeh et al., 2016). The study was initially summarized in GRN 000729 (2018), page 48, and the summary is incorporated by reference. In summary, 40 Landrace x Yorkshire piglets (24-48 hr 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.

To identify new tolerance studies that have been published since the publication of the "no questions" letter for GRN 000729 (2018), literature searches of PubMed and Google Scholar were conducted using the search terms "GOS piglet study" on January 6, 2023. The titles and abstracts of the hits published since the "no questions" letter for GRN 000729 were then reviewed to identify new studies that tested the tolerance of GOS alone, and not in combination with other test substances. One new study was identified, Tian et al. (2018), and is summarized below.

To evaluate the effect of GOS on growth and gastrointestinal function, Tian et al. (2018) orally administered either saline or a GOS-containing solution delivered at a rate of 1 g GOS/kg body weight/day to 60 Duroc x Landrace x Large weight neonatal piglets from day 1 to day 7 of life. A total of 6 litters (10 piglets/litter) were used and piglets from each litter were equally assigned to either the saline- or the GOS-treated group. The piglets were then weaned on day 21. One pig per group from each of the 6 litters was euthanized on days 8 and 21 of the study to evaluate the effect of GOS on the villus morphology. Although the study evaluated a variety of parameters that relate to the benefits of GOS ingestion, such as villus morphology, intestinal nutrient transporter, barrier function, and immune factor gene expression, D-lactic acid, diamine oxidase, and disaccharide activity, intestinal growth factor levels, and tight junction protein expression, the average body weight and health status of the piglets were monitored throughout the study. All piglets remained healthy during the treatment period and while there was no significant difference in the body weights of the piglets treated with GOS compared to the piglets that received saline, the average daily weight gain and crypt depth of the piglets that received GOS from day 1 to day 7 of life was significantly increased and decreased, respectively, on day 21. There were also a variety of significant effects on the parameters that relate to the benefits of GOS ingestion. Based on the totality of the evidence gathered in the study, the authors concluded

that GOS had a positive impact on piglet growth performance and enhance the functional development in the jejunum of suckling piglets.

### 3. Clinical Studies

Although no clinical trials have been conducted specifically with VITAGOS™ IF powder, the safety and tolerance of other GOS preparations in infant formula and conventional food have been evaluated in numerous published and unpublished clinical trials, which have been extensively summarized in GRAS Notices 000236 (FDA, 2008), 000285 (FDA, 2009), 000286 (FDA, 2009), 000334 (FDA, 2010), 000484 (FDA, 2014), 000489 (FDA, 2014), 000495 (FDA, 2014), 000518 (FDA 2014), 000620 (FDA, 2016), 000721 (FDA, 2017), and 000729 (FDA, 2018) and their summaries are incorporated by reference. All GRNs provided updates of the published studies to support the intended use of GOS in infant formulas and conventional foods, concluded that the newly available clinical data did not contradict GRAS status of GOS, and each GRAS Notice received "no questions" letters from FDA.

To identify new clinical studies that have been published since the publication of the "no questions" letter for GRN 000729 (2018), literature searches of PubMed and Google Scholar were conducted using the search terms "GOS clinical study/trial", "galacto-oligosaccharides clinical study/trial", "GOS study", and "galacto-oligosaccharides study". The titles and abstracts of the hits published since the "no questions" letter for GRN 000729 were reviewed. Only the studies that reported adverse events and/or safety parameters following GOS ingestion alone with no other test substances were retrieved and summarized. Five new studies were identified and are summarized in tabular format below (Table 10; Canfora et al., 2017; Vulevic et al., 2018; Wilms et al., 2021; Wilson et al., 2021; Schoemaker et al., 2022). All of the new studies were conducted in adults, and GOS ingestion ranged from 1.37 to 15 g GOS/day from 3 to 12 weeks. All of the new studies reported that the ingestion of GOS was well-tolerated.

**Table 10. Recent Studies of GOS Ingestion in Adults**

| Reference | Study Design and Population | Groups/Treatments (Numbers of Subjects) | Duration | Safety Parameters |
|---|---|---|---|---|
| Canfora et al. (2017) | Double-blind, placebo-controlled, randomized, parallel study<br><br>Overweight and obese men and postmenopausal women (45-70 years old; n=46) | Group 1: Placebo (n=22)<br><br>Group 2: 21.15 g Vivinal GOS powder/day (15 g GOS/day; n=24)*<br><br>* (69% GOS; FrieslandCampina Domo) | 12 weeks | • Two women dropped out of the study because of the use of antibiotics, one in the placebo-treated group and one in the GOS-treated group.<br>• No adverse events were reported and the participants reported no side effects of the GOS or placebo treatment (i.e., changes in stool frequency or gastrointestinal complaints).<br>• GOS ingestion had no effect on insulin sensitivity, body mass index (BMI), body weight, body fat percentage, body fat mass, lean mass, visceral adipose tissue mass, fasting plasma levels of glucose, insulin, glycerol, free fatty acid, triglycerides, leptin, peptide YY (PYY), glucagon-like peptide (GLP)-1, and the inflammatory markers interleukin (IL)-6, IL8, tumor necrosis factor (TNF)-α, and lipopolysaccharide-binding protein (LBP). |
| Vulevic et al. (2018) | Double-blind, placebo-controlled, crossover study<br><br>Men and women with gastrointestinal symptoms at least three times a month (18-65 years old; n=91) | Treatment 1: Placebo<br><br>Treatment 2: 2.75 g Bimuno GOS/day (1.37 g GOS/day)*<br><br>*Bimuno GOS (80% GOS; Clasado Biosciences Ltd.) | 7 weeks (2 1 week of screening, 2 weeks of treatment, 2 weeks of washout, 2 weeks of treatment) | • Eight subjects did not complete the trial due to the use of antibiotics, hospitalization due to a broken leg, relocation, and loss to follow-up.<br>• GOS was generally well-tolerated and appeared to have no effect on the number of bowel movements, consistency of stools, quality of life or the Hospital Anxiety and Depression scale. |

**Table 10. Recent Studies of GOS Ingestion in Adults**

| Reference | Study Design and Population | Groups/Treatments (Numbers of Subjects) | Duration | Safety Parameters |
|---|---|---|---|---|
| Wilms et al. (2021) | Double-blind, placebo-controlled, crossover study<br><br>Adult (25 – 50 years old; n=24) and elderly (70-85 years old; n=20) men and women without gastrointestinal symptoms | Treatment 1: Placebo<br><br>Treatment 2: 21.6g Biotis GOS powder/day (15 g GOS /day)*<br><br>* (69% GOS; FrieslandCampina Domo) | 4-week treatment period followed by a 4-6-week washout period | • Three adults dropped out due to either antibiotic use or stopping the treatment and one adult was non-compliant. Eight subjects did not complete the trial due to the use of antibiotics, hospitalization due to a broken leg, relocation, and loss to follow-up.<br>• Gastrointestinal symptoms were not significantly different between the GOS and placebo treatments both in the elderly and the adults. Additionally, average stool frequency, as well as average frequencies of hard stools and loose stools were not significantly different between GOS and placebo supplementation.<br>• Cytokine production (IL-1b, IL-6, IL-8, IL-10, TNF-α and interferon (IFN)-γ) in plasma after 24 h phytohemagglutinin-M (PHA) or lipopolysaccharide (LPS) whole blood stimulations and serum C-reactive protein (CRP) concentrations were not significantly different between the two treatments.<br>• Plasma malondialdehyde (MDA) and uric acid (UA) concentrations, as well as rolox equivalent antioxidant capacity (TEAC) values, did not differ significantly between elderly and adults after the GOS and placebo treatments. |
| Wilson et al. (2021) | Open label study<br><br>Adults (16–65 years old; n=18) with active ulcerative colitis | Treatment 2: 2.8 g Bimuno GOS/day (1.37 g GOS/day)*<br><br>*Bimuno GOS (80% GOS; Clasado Biosciences Ltd.) | 6 weeks | • Six subjects either dropped out or were excluded due to a protocol violation, the use of antibiotics, or unwillingness to complete the study.<br>• There was no effect of GOS on immune gene expression in venous blood, gastrointestinal symptom rating scale (GSRS) or simple clinical colitis activity index (SCCAI) score. |

**Table 10. Recent Studies of GOS Ingestion in Adults**

| Reference | Study Design and Population | Groups/Treatments (Numbers of Subjects) | Duration | Safety Parameters |
|---|---|---|---|---|
| Schoemaker et al. (2022) | Double-blind, placebo-controlled, randomized, parallel study<br><br>Healthy adults with self-reported constipation (18-59 years old; n=132) | Treatment 1: Placebo (n=43)<br><br>Treatment 2: 7.5 g Biotis GOS powder/day (5.5 g GOS /day; n= 44)*<br><br>Treatment 3: 15 g Biotis GOS powder/day (11 g GOS /day; n=45)*<br><br>* (FrieslandCampina Domo) | 3 weeks | • One hundred twenty-one subjects complied with the protocol. Reasons for exclusion were extensive weight gain, stomach flu, food poisoning, antibiotic use, missing baseline values, change in eating pattern, and a compliance of <90%.<br>• There were no differences between the groups in stool frequency or consistency.<br>• The study products were well-tolerated, and no serious adverse events were reported. |

## B. ALLERGENICITY

The allergenicity of GOS was extensively summarized in the GRAS Notices for GOS-containing ingredients and the summaries are therefore incorporated by reference (US FDA, GRN 000236, 2008; US FDA, GRN 000285, 2009; US FDA, GRN 000286, 2009; US FDA, GRN 000334, 2010; US FDA, GRN 000484, 2014; US FDA, GRN 000489, 2014; US FDA, GRN 000495, 2014; US FDA, GRN 000518, 2014; US FDA, GRN 000569, 2015; US FDA, GRN 000620, 2016; US FDA, GRN 000721, 2017; US FDA, GRN 000729, 2018; US FDA, GRN 000896, 2020). Briefly, GOS manufactured using β-galactosidases derived from *B. circulans* has been reported to provoke allergic reactions in sensitized subjects in Southeast Asia (Vo et al., 2012; Chiang et al., 2012). Since these initial case reports, a series of *in vitro* studies have indicated that: 1) GOS can directly crosslink basophil-bound IgE antibodies; 2) linear tetrasaccharides containing only β (1-4) linkages or branched tetrasaccharides with β (1-4) and (1-6) linkages can provoke GOS-induced allergenic reactions (Kaneko et al., 2014); and 3) the branched tetrasaccharide Galβ(1-4)Galβ(1-6)-[Galβ(1-4)]-Glc and the regioisomer do not induce basophil activation in subjects with confirmed GOS-allergy (Lee et al., 2022; Elferink et al., 2019). Thus, the identity of actual sensitizing and provoking allergen in the GOS-containing ingredients is still unclear. To determine if new case reports have been published since the original GRAS determination for VITAGOS™ IF, literature searches of PubMed and GoogleScholar were conducted using the search terms “galactooligosaccharides AND allergy”, “galacto-oligosaccharides AND allergy”, “galactooligosaccharides AND allergic”, “galacto-oligosaccharides AND allergic”. No new case reports or series were identified. Therefore, because VITAGOS™ IF is produced using the same enzyme that is used to produce the GOS that has provoked allergic reactions in Southeast Asia, which is the subject of GRN 000236 (2008), the likelihood of VITAGOS™ IF GOS in provoking allergic reactions in sensitized individuals will be the same as the subject of GRN 000236. Similar conclusions have been reached in GRN 000235 (FDA, 2009), 000495 (FDA, 2014), 000620 (FDA, 2016), and 000729 (FDA, 2018), which received “no questions” letters.

## C. SAFETY OF THE B-GALACTOSIDASE DERIVED FROM *BACILLUS 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 000729 (2018).

As stated on pages 54-55 of GRN 000729, "*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 000236), GTC Nutrition (GRN 000285 and GRN 000286), New Francisco Biotechnology Corporation (GRN 000518 and GRN 000569), for which all notifications received a "no questions" letter from FDA. Additionally, several enzymes derived from Bacillus species, such as amylase derived from* Bacillus lichenifomis*, 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

All information included in the following list of references is generally available.

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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 powder 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 powder 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 powder 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 powder is a galacto-oligosaccharide (GOS)-containing ingredient manufactured using lactose and β-galactosidase derived from *Bacillus circulans* (*B. circulans* M3-1) with a production process that is the same as VITAGOS™ IF syrup, which is GRAS (GRAS Notice Pending). VITAGOS™ IF syrup is spray-dried to produce VITAGOS™ IF powder. VITAGOS™ IF powder 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 powder shows that VITAGOS™ IF powder is compositionally similar to Vivinal® GOS, which is GRAS (GRN 000236, 2008) and currently marketed globally for use in non-exempt, term, cow's milk-based infant formula and conventional foods and beverages.
3. All raw materials and processing aids used to produce VITAGOS™ IF powder comply with the 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 large intestine 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 non-exempt, term, cow's milk-based infant formula and selected foods and beverages and received a "no questions" letter from the FDA in 2008 (GRN 000236). Since then, a total of nine GOS-containing ingredients have been determined GRAS for use in non-exempt, term, cow's milk-based infant formula and selected conventional foods at levels up to 7.8 g/L and 11 g/serving, respectively, and resulted in fourteen GRAS Notifications (GRN 000236, 2008; GRN 000285, 2009; GRN 000286, 2009; GRN 000334, 2010; GRN 000484, 2014; GRN 000489, 2014; GRN 000495, 2014; GRN 000518, 2014; GRN 000569, 2015; GRN 000620, 2016; GRN 000671, 2017; GRN 000721, 2017; GRN 000729, 2018; GRN 000896, 2020). 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 000671 resubmitted as GRN 000721 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 fructooligosaccharides (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 (Anthony et al., 2006), which is compositionally similar to VITAGOS™ IF powder, supports the safety of VITAGOS™ IF powder. 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 ingredients administered for up to 90 days by gavage established NOAELs at the highest doses tested [1100 mg GOS/kg/day (Kobayashi et al. 2009); 2 g GOS/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 ingredients are not genotoxic.
    c. GOS-containing ingredients 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 ingredients 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 powder as an alternative for VITAGOS™, which is the subject of GRN 000721. Thus, the intended uses for VITAGOS™ IF powder include those specified in GRN 000721 as well as a variety of other uses that have been determined GRAS in GRN 000285, 000484, and 000489, which includes powdered, ready-to-feed, and concentrated powdered versions of non-exempt, term, cow's milk-based infant formula and selected conventional foods.
    a. The non-exempt, term, cow's milk-based infant formula will not exceed 7.8 g GOS/L reconstituted formula. This use level is higher than that proposed in GRN 000721 but is the same as that from GRN 000620 and GRN 000729. 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 of 2.30 g/person/day (0.033 g/kg body weight/day) and 5.38 g/person/day (0.078 g/kg body weight/day), respectively. On an individual basis, the greatest mean and 90th percentile GOS EDIs occur in children ages 13-19 at 2.77 and 6.71 g/person/day. On a body weight basis, the greatest mean and 90th percentile GOS EDIs occur in infants ages 0 to 6 months at 0.23 and 0.39 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 powder from the intended uses will not increase the cumulative intake of GOS.

9. As established in GRN 000236 as well as other GOS Notices, clinical and toxicology studies of other compositionally similar GOS support the safety of the proposed intake of VITAGOS™ IF powder (GRN 000334, 2010; GRN 000484, 2014; GRN 000489, 2014; GRN 000495, 2014; GRN 000518, 2014; GRN 000569, 2015; GRN 000620, 2016; GRN 000671, 2017; GRN 000721, 2017; GRN 000729, 2018; GRN 000896, 2020).

Therefore, VITAGOS™ IF powder is safe and GRAS at the proposed level of addition to the intended infant formulas and general foods. VITAGOS™ IF powder 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 6, 2023

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

Signature 

Date:

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

Signature: 

Date: January 6, 2023

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

Signature: 

Date: January 6, 2023

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 |
|---|---|
| 001135 | February 14, 2023 |
| 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)*: _____

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<br>Adrian Wallace, MBA, LSSBB | Position or Title<br>Regulatory Affairs Specialist | | |
| | Organization *(if applicable)*<br>Vitalus Nutrition Inc. | | | |
| | Mailing Address *(number and street)*<br>3911 Mt. Lehman Rd. | | | |
| City<br>Abbotsford | State or Province<br>BC | Zip Code/Postal Code<br>V2T 5W5 | Country<br>Canada | |
| Telephone Number<br>604-856-3933 | Fax Number | E-Mail Address<br>awallace@vitalus.com | | |
| **1b. Agent or Attorney *(if applicable)*** | Name of Contact Person<br>Dietrich B. Conze,PhD | Position or Title<br>Managing Partner | | |
| | Organization *(if applicable)*<br>Spherix Consulting Group, Inc. | | | |
| | Mailing Address *(number and street)*<br>751 Rockville Pike, Unit 30-B | | | |
| City<br>Rockville | State or Province<br>Maryland | Zip Code/Postal Code<br>20852 | Country<br>United States of America | |
| Telephone Number<br>240-367-6089 | Fax Number | E-Mail Address<br>dconze@spherixgroup.com | | |

## SECTION C GENERAL ADMINISTRATIVE INFORMATION

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

Galacto-oligosaccharides, VITAGOS™ IF Powder

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*

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 powder 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 Powder
*(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 | 1/17/2023 |

## 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 Vitalus VITAGOS IF Powder 1-6-23 - Signed | Submission |
| 2 | Insert Clear All References | Submission |
| | Insert Clear | |
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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:** Dietrich Conze
**To:** Morissette, Rachel
**Cc:** Claire Kruger; Kathy Brailer
**Subject:** [EXTERNAL] Re: GRN 001135 request for CTE
**Date:** Tuesday, October 31, 2023 1:16:04 PM



Hi Rachel,

Thank you for the call yesterday. We discussed the issues with the Notice with our client and Vitalus has advised us to cease the evaluation. Please proceed with ceasing the evaluation. We would also greatly appreciate it if you could sending us the list of questions/issues that was generated during the review because they will help us identify and address the issues/questions in our future submission.

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 Oct 2, 2023, at 7:31 AM, Morissette, Rachel <Rachel.Morissette@fda.hhs.gov> wrote:
>
> Dear Dietz,
>
> We have completed our evaluation of GRN 001135 and are requesting that the notifier ask us to cease our evaluation of this notice due to the significant data gaps and corrections that would be necessary for us to reach a No Questions conclusions I'm providing the high-level issues we found, but this is not an exhaustive list of our questions for this notice. Namely, the two main areas of concern are the dietary exposure section and safety narrative, both of which would require significant revisions that go beyond the scope of an amendment. One of the main issues is that while the dietary exposure is presented as cumulative, there are missing data regarding background uses and uses in infant formula. The dietary exposure would need to be redone from our perspective. Regarding the safety section, the data and information used to support safety was not adequately discussed. For example, comparisons to

other GOS ingredients to support the compositional equivalence, and by extension safety, was missing, as well as lacking discussion on the clinical studies incorporated into the notice from a long list of prior GRNs.

If you would like to request a call to discuss further for a potential future resubmission, please let me know. Otherwise, we await the notifier's decision on how to proceed with this notice. We can offer to provide a list of our questions in detail after the notifier requests that we cease to evaluate.

Best regards,

*Rachel*

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**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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