SCG
Spherix Consulting Group

June 4, 2021

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 is a GRAS Determination entitled "GRAS Determination for the Use of 6'-Sialyllactose Sodium Salt in Selected Conventional Foods and Enteral Tube Feeding Formulas". We believe that this GRAS Determination should be considered as a new notification because Chr. Hansen A/S intends to expand the use of its 6'-sialyllactose sodium salt ingredient to selected conventional foods and enteral tube feeding formulas.

We thank you for taking the time to review this GRAS Determination. Should you have additional questions, please let us know.

Sincerely,

Dietrich B. Conze, Ph.D.
Managing Partner

Enclosure: CD containing
- Form 3667
- Cover Letter
- GRAS Determination for the Use of 6'-Sialyllactose Sodium Salt in Selected Conventional Foods and Enteral Tube Feeding Formulas
- References

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GRAS Determination for the Use of 6'-Sialyllactose Sodium Salt in Selected Conventional Foods and Enteral Tube Feeding Formulas

Prepared for:

Chr. Hansen A/S[1]
9015 W Maple St.
West Allis, WI 53214

Prepared by:

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

May 18, 2021

[1] Jennewein Biotechnology GmbH is now Chr. Hansen HMO GmbH. The legal entity (including the same company identification number), manufacturing premises, manufacturing processes and quality systems and certifications remains the same.

All documentation bearing the name of Jennewein Biotechnologie GmbH is in the process of being updated to Chr. Hansen HMO GmbH/Chr. Hansen A/S as appropriate. This is however an ongoing process; Chr. Hansen assures that the documents released with the Jennewein Biotechnologie GmbH's name, remain valid until the full update is completed.

Likewise, updated certificates and commercial registrations will be issued by the relevant competent authorities in due course; meanwhile the current certificates and commercial registrations remain valid until further notice.

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LIST OF ABBREVIATIONS

2'-FL: 2'-Fucosyllactose

3-FL: 3-Fucosyllactose

3'-SL: 3'-Sialyllactose

6'-SL: 6'-Sialyllactose

Alb: Albumin

ALT: Alanine aminotransferase

araA: Arabinose isomerase

BMI: Body mass index

BW: Body weight

CBPI: Cytokinesis-block proliferation index

CFR: United States Code of Federal Regulations

CFU: Colony forming units

CHO: Chinese hamster ovary cells

CI: Confidence interval

COSY: Correlation spectroscopy

DSMZ: Deutsche Sammlung für Mikroorganismen und Zellkulturen

DW: Dry weight

EDI: Estimated daily intake

EFSA: European Food Safety Authority

EU: Endotoxin unit

F6PPK: Fructose-6-phosphate phosphoketolase

FCC: Food Chemicals Codex

FDA: United States Food and Drug Administration

FFDCA: Federal Food, Drug, and Cosmetic Act

FOIA: Freedom of information Act

FOS: Fructooligosaccharides

Fru-1,6-BP: Fructose-1,6-bisphosphate

Fru-6-P: Fructose-6-phosphate

FSSC: Food Safety System Certification

FUT: Fucosyltransferase

GI: Gastrointestinal

Glc-1-P: Glucose-1-phosphate

Glc-6-P: Glucose-6-phosphate

Gln-1-P: Glucosamine-1-phosphate

Gln-6-P: Glucosamine-6-phosphate

Glob: Gobulin

GluNAc-6-P: *N*-acetylglucosamine-6-phosphate

GMO: Genetically modified organism

GMP: Good manufacturing practices

GOS: Galactooligosaccharides

GRAS: Generally Recognized As Safe

GRN: GRAS Notification

HCD: Historical control data

HDL-C: High-density lipoprotein cholesterol

HMBC: $^{1}H^{13}C$-Heteronuclear multiple bond correlation

HMO: Human milk oligosaccharides

HPAEC-PAD: High performance anion exchange chromatography coupled with pulsed amperometric detection

HSQC: $^{1}H^{13}C$-Heteronuclear single quantum correlation

ICP-MS: Inductively coupled plasma mass spectrometry

IFNγ: Interferon gamma

LC-MS: Liquid chromatography coupled with mass spectrometry

LDL-C: Low-density lipoprotein cholesterol

LDPE: Low-density polyethylene

LNDFHI: Lacto-*N*-difucohexaose I

LNnT: Lacto-*N*-*neo*tetraose

LNT: Lacto-*N*-tetraose

LOD: Limit of detection

LOQ: Limit of quantitation

MCH: Mean corpuscular hemoglobin

MCV: Mean corpuscular volume

ND: Not detected

NHANES: National Health and Nutrition Examination Surveys

NIH: National Institutes of Health

NMR: Nuclear magnetic resonance

NOAEL: No Observed Adverse Effect Level

OECD: Organization for Economic Cooperation and Development

PCR: Polymerase chain reaction

Ph Eur: European Pharmacopoeia

pLNnH: para-lacto-*N*-*neo*hexaose

qPCR: Quantitative polymerase chain reaction

RI: Replicative index

TP: Total protein

UDP-Gal: UDP-galactose

UDP-Glc: UDP-glucose

UDP-GlcNAc: UDP-*N*-acetylglucosamine

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

Chr. Hansen A/S is hereby submitting a GRAS notice in accordance with subpart E of part 170.

B. NAME AND ADDRESS OF THE SPONSOR

Chr. Hansen A/S
9015 W Maple St.
West Allis, WI 53214

C. COMMON OR USUAL NAME

6'-Sialyllactose Sodium Salt (6'-SL)

D. TRADE SECRET OR CONFIDENTIAL INFORMATION

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

E. INTENDED USE

Chr. Hansen A/S intends to use 6'-SL as an ingredient in toddler formulas, foods for infants and young children, meal replacements drinks for adults, non-carbonated drinks, bars, oral electrolyte solutions, and enteral tube feeding formulas (Table 1).

Table 1. Intended Uses and Use Levels

Intended Uses	Intended Use Level (g/kg or g/L)
Toddler milks (Go and Grow by Similac®)	0.4
Milk-based meal replacement beverages for children (Pediasure®)	2.28
Cereals, prepared, ready-to-serve, for infants and young children	1.5
Cereals, dry instant, for infants and young children	1.5
Meal replacement drinks for adults (including dairy and non-dairy drinks for weight reduction); including formulas for pregnant women	2.28
Non-carbonated drinks (e.g. fitness water, thirst quenchers, sports and isotonic drinks)	0.7
Bars, including snack bars, meal-replacement bars, and breakfast bars	1.5
Enteral tube feeds used as sole source nutrition (Ensure®, Glucerna®, and Boost®)	3.8
Oral Electrolyte Solutions	0.15

F. BASIS FOR GRAS DETERMINATION

6'-Sialyllactose for the intended use and use level 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 6'-SL has been determined to be GRAS by demonstrating that the safety of the intended 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 6'-SL as an ingredient for the intended use in toddler formulas, foods for infants and young children, meal replacements drinks for adults, non-carbonated drinks, bars, oral electrolyte solutions, and enteral tube feeding formulas has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. The subject of this GRAS Determination is a spray-dried, powdered food ingredient that contains not less than 90% 6'-SL dry weight. The remaining components include carbohydrate by-products, ash, and moisture.
    a. 6'-Sialyllactose is a naturally occurring acidic oligosaccharide in human milk.
    b. Published studies showing that the amount of 6'-SL in breast milk ranges from 0.01 to 1.7 g/L.
    c. Human milk oligosaccharides, including 6'-SL, are resistant to the digestive enzymes in the gastrointestinal tract, poorly absorbed, and pass through the gastrointestinal tract where they are either fermented by the microbiota or excreted unchanged.
2. The subject of this GRAS Determination is the subject of GRN 922, which received a "no question" letter on April 23, 2021 for the use of 6'-SL in non-exempt term infant formula.
    a. The subject of this GRAS Determination is manufactured using a genetically engineered strain of *Escherichia coli* BL21(DE3) by Chr. Hansen A/S in a Food Safety System Certification (FSSC) 22000-, ISO 9001:2015-, GMP-, and International Featured Standards Food 6.1-compliant facility. Chr. Hansen A/S is an FDA-registered food facility.
    b. The genetically engineered strain of *E. coli* BL21(DE3) used by Chr. Hansen A/S is not toxigenic and not capable of DNA transfer to other organisms and has the same virulence profile as *E. coli* BL21(DE3).

c. All raw materials, processing aids, and food contact substances are GRAS and/or conform to the specifications stated in 21 CFR and/or the Food Chemicals Codex (FCC).

d. Fermentation by-products include lactose, sialic acid, and *N*-acetylglucosamine which are known human milk oligosaccharides; their presence in the finished ingredient is not of toxicological concern.

e. Process procedures and product specifications are in place to control the levels of residual impurities and carbohydrate by-products, as well as heavy metals, microbes, and production organism-derived DNA and possible endotoxin, ensuring a consistent, safe, food-grade finished ingredient.

f. The available stability studies indicate a shelf-life of one year when stored from the date of production under ambient conditions.

g. Use of the subject of this GRAS Determination in the intended selected conventional foods and enteral tube feeding formulas results in mean and 90th percentile estimated daily intakes (EDIs) of 0.258 and 0.706 g/day (0.004 and 0.010 g/kg bw/day) for consumers not less than 2 years-old.

h. Use of the subject of this GRAS Determination in selected conventional foods and enteral tube feeding formulas results in mean and 90th percentile cumulative estimated daily intakes (EDIs) of 0.208 and 0.42 g/day (0.003 and 0.006 g/kg bw/day) for consumers not less than 2 years-old.

i. Use of the subject of this GRAS Determination in oral electrolyte solutions results in an estimated daily intake of 0.15 – 0.3 g of 6'-SL (equivalent to 1.1 – 2.2 mg of 6'-SL /kg bw/day assuming a 13.5 kg toddler and 0.2 – 0.4 mg of 6'-SL /kg bw/day assuming a 70 kg adult). Because OESs are intended for short term use, intake of 6'-SL from OESs will not impact the cumulative 6'-SL intake resulting from the use of 6'-SL in select conventional foods and enteral tube feeding formulas.

3. Genotoxicology and subchronic toxicology studies published by Phipps et al. (2019) show that 6'-SL is not genotoxic and has a no observed adverse effect level (NOAEL) of 5 g/kg bw/day, which was the highest dose tested.

4. The safety of exposure to Chr. Hansen A/S's 6'-SL at its intended use level is supported by:

   a. Data demonstrating the qualitative and quantitative similarities between the subject of this GRAS Determination and the 6'-SL ingredient tested in the pivotal

genotoxicology and subchronic toxicology studies conducted by Phipps et al. (2019), which is also the subject of GRN 881;

b. The lack of genotoxicity and NOAEL for 6'-SL established in the 90-day subchronic dietary toxicology conducted by Phipps et al. (2019);

c. Published genotoxicology and 90-day subchronic toxicology and neonatal piglet studies conducted with 6'-SL or a mixture of HMOs containing the subject of the GRAS determination (Parschat et al., 2020; Monaco et al., 2020; Hanlon, 2020);

d. Clinical data showing the ingestion of HMOs are well tolerated in infants up to 1.0g/day and adults up to 20 g/day;

e. Clinical data showing that the use of other non-digestible carbohydrates in infants, adults, enteral tube feeding products, and oral electrolyte solutions is well tolerated up to 63 g/day;

f. The GRAS status of the subject of this GRAS Determination for use in infant formula (GRN 922);

g. The GRAS status of other 6'-SL products for use in selected conventional foods (GRN 881).

Therefore, 6'-SL is safe and GRAS at the proposed level of addition to the intended toddler formulas, foods for infants and young children, meal replacements drinks for adults, non-carbonated drinks, bars, oral electrolyte solutions, and enteral tube feeding formulas. 6'-Sialyllactose 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 Dietrich Conze, PhD, Managing Partner, Spherix Consulting Group, Inc., at 751 Rockville Pike, Unit 30-B, Rockville, MD 20852; Telephone: 240-367-6089; Email: dconze@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 Chr. Hansen A/S and pertinent to the evaluation of the safety and GRAS status of the use of this substance.

[redacted]
Signature of Authorized Representative of
Chr. Hansen A/S

26 May 2021
Date

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

A. COMMON OR USUAL NAME

6'-Sialyllactose sodium salt (6'-SL; CAS No. 35890-39-2)

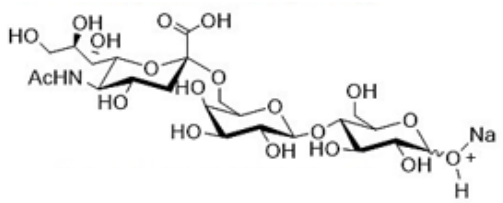
B. CHEMICAL NAME

N-acetyl α-neuraminic-(2→6)-β-D-galactose-(1→4)-D-glucose sodium salt

C. MOLECULAR FORMULA AND MASS

$C_{23}H_{39}NNaO_{19;}$ 633.55 g/mol

D. STRUCTURAL FORMULA



E. DESCRIPTION OF 6'-SL

Approximately 15%-20% of the naturally occurring oligosaccharides (HMOs) found in human milk (the total HMO fraction accounts for 10 to 15 g/L of human milk) are comprised of acidic oligosaccharides. These acidic oligosaccharides contain sialic acid (SA), an acidic sugar with a nine-carbon backbone, and are identified as sialylated HMOs (Bode, 2012). The most recognized sialylated HMOs are the two trisaccharide isomers, 3'- and 6'-sialyllactose, which are both formed as a result of lactose sialylation and account for a significant portion of the acidic HMOs. Both 3'- and 6'-sialyllactose consist of lactose at the reducing terminus and a SA residue at the non-reducing terminus via α2,3 or α2,6 bonding, respectively.

The subject of this GRAS Determination is a 6'-SL sodium salt that is the subject of GRAS Notice 922, which is GRAS for use in infant formula and received a "no questions" letter from FDA on April 23, 2021. The subject of this GRAS Determination is produced by Chr. Hansen A/S by fermentation using a genetically engineered strain of *Escherichia coli* BL21 (DE3). The 6'-SL sodium salt is then purified from the culture medium and spray-dried, producing a powdered finished product. The finished product contains not less than 90% 6'-SL and the structure of the 6'-SL present in the finished product produced by this process is consistent with 6'-SL found in breast milk as confirmed by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, double-quantum filtered $^{1}H^{1}H$-COSY NMR spectroscopy,

phase-sensitive $^{1}H^{13}C$-heteronuclear single quantum correlation (HSQC) NMR spectroscopy, phase-sensitive $^{1}H^{13}C$-heteronuclear multiple bond correlation (HMBC) NMR spectroscopy, and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Residual impurities include lactose and carbohydrate by-products. Chr. Hansen A/S intends to expand the intended use of its 6'-SL product to toddler formulas, foods for infants and young children, meal replacements drinks for adults, non-carbonated drinks, bars, oral electrolyte solutions, and enteral tube feeding formulas.

F. PRODUCTION PROCESS

Because the production process was extensively reviewed in GRN 922, the description of the production strain and manufacturing process are incorporated by reference (see pages 2 – 6 of GRN 922). The only difference is that cobalt is no longer added to the fermentation medium. Briefly, 6'-SL is produced by fermentation using the genetically engineered strain of *E. coli* BL21(DE3) *JBT-6SL* in a contained, sterile environment at the Chr. Hansen A/S production facility, which is Food Safety System Certification (FSSC) 22000 and ISO 9001:2015 compliant, and FDA-registered (Registration # 1303109037512). Following synthesis, 6'-SL is purified from the fermentation medium and the resulting concentrate is spray-dried into a powder. All other manufacturers involved in the 6'-SL manufacturing are Chr. Hansen A/S-qualified and either GMP-, ISO-, or International Featured Standards Food 6.1-compliant.

G. FINISHED PRODUCT SPECIFICATIONS

1. 6'-SL Product Specifications and Batch Data Compliance

To ensure a consistent food-grade product that is free of genetically modified ingredients, each batch of 6'-SL is evaluated against the same product specifications as those specified in GRN 922 using compendial or validated methods that are fit-for-use. Data from five non-consecutive batches of 6'-SL shows that the manufacturing process reproducibly produces a finished product that complies with the product specifications and removes the production organism from the finished product (Table 2).

H. STABILITY

The production strain and finished ingredient stability were extensively reviewed in GRN 922. Therefore, the summaries of the genomic and finished product stability are incorporated by reference (see pages 9 and 10 of GRN 922). Briefly, the production strain is not expected to lose its ability to produce a consistent finished product because it contains stably integrated genes and no plasmids or episomal vectors. The shelf-life of the finished ingredient is expected to be 1 year from the date of production when stored under ambient conditions.

Table 2. Product Specifications and Batch Data for 6'-Sialyllactose

Parameter	Method	Specification	6'-SL Powder Batch No.				
			11020039	11020049	11020059	11020069	11021019
Physical Parameters							
Appearance (Color)[4]	Visual	White to ivory Colored	Complies	Complies	Complies	Complies	Complies
Appearance (Form)[4]		spray-dried powder	Complies	Complies	Complies	Complies	Complies
Chemical Parameters							
6'-Sialyllactose[4]	HPAEC-PAD	≥ 90% DW	94.6	94.3	92.3	95.4	95.8
Sum of other carbohydrates[4]		≤ 10%	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
D-lactose[4]		≤ 5%	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Sialic acid[4]		≤ 10%	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
N-Acetylglucosamine[4]		≤ 5%	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Protein[4]	Nanoquant (modified Bradford)	≤ 100 µg/g	16.8	16.8	14.4	16.6	16.0
Ash[1]	ASU L 06.00-4	≤ 8.5%	5.7	3.8	6.4	6.6	5.7
Moisture[4]	KF titration	≤ 9.0%	7.7	7.8	7.6	8.0	8.2
Sodium[1]	PV-347 ICP-MS	≤ 4.2 %	3.0	3.1	3.5	3.1	3.2
Endotoxins[3]	Ph. Eur. 2.6.14	≤ 10 EU/mg	0.034	0.014	0.034	0.016	0.009
Aflatoxin M1[1]	DIN EN ISO 14501	≤ 0.025 µg/kg	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
GMO residues[2]	qPCR	≤ 0.01%	Negative	Negative	Negative	Negative	Negative
Heavy Metals							
Arsenic[1]	ASU L 00.00-135 – ICP-MS	≤ 0.2 mg/kg	ND	ND	ND	ND	ND
Cadmium[1]		≤ 0.1 mg/kg	ND	ND	ND	ND	ND
Lead[1]		≤ 0.02 mg/kg	ND	ND	ND	ND	ND
Mercury[1]		≤ 0.5 mg/kg	ND	ND	ND	ND	ND
Microbes							
Standard Plate Count[1]	ISO 4833-2	≤ 10000 cfu/g	< 10	< 10	< 10	< 10	< 10
Yeast and Molds[1]	ISO 21527-2	≤ 100 cfu/g	< 20	< 20	< 20	< 20	< 20
Coliform/*Enterobacteriaceae*[1]	ISO 21528-1	≤ 10 cfu/g	< 10	< 10	< 10	< 10	< 10
Salmonella[1]	ISO 6579	Absent/25 g	Absent	Absent	Absent	Absent	Absent
Cronobacter sakazakii spp.[1]	ISO/TS 22964	Absent/10 g	Absent	Absent	Absent	Absent	Absent

Abbreviations: DW, dry weight; cfu, colony forming units; STDEV, standard deviation; KF, Karl-Fischer; HPAEC-PAD, high performance anion exchange chromatography coupled with pulsed amperometric detection; qPCR, quantitative polymerase chain reaction; ICP-MS, Inductively coupled plasma mass spectrometry; EU, endotoxin unit; Ph Eur., European Pharmacopoeia; LOQ, limit of quantitation; ND, not detected.

[1]Determined by the Institut für Produktqualität GmbH, which is a DIN EN ISO/IEC 17025-accredited laboratory; Ash LOQ = 0.01 %. Arsenic limit of detection (LOD) = 0.05 mg/kg; Cadmium LOD = 0.01 mg/kg; Mercury LOD = 0.005 mg/kg; Lead LOD = 0.01 ppm; Aflatoxin M1 LOQ = 0.025 µg/kg.

[2]Determined by GeneCon International GmbH, which is a DIN EN ISO/IEC 17025-accredited laboratory. Limit of detection = 0.01% of the finished product.

[3]Determined by Mikrobiologisches Labor. Dr. Michael Lohmeyer GmbH, which is a DIN EN ISO/IEC 17025-accredited laboratory; limit of quantitation = 0.005 EU/mg.

[4]Determined by Chr. Hansen A/S using internally validated methods. Protein LOQ = 10 µg/g; carbohydrate by-products with a percent area greater than 0.5% (limit of quantitation) are considered.

III. DIETARY EXPOSURE

A. INTENDED EFFECT

The intended effect of adding 6'-SL to toddler formulas, foods for infants and young children, meal replacements drinks for adults, non-carbonated drinks, bars, oral electrolyte solutions, and enteral tube feeding formulas to increase 6'-SL intake.

B. HISTORY OF EXPOSURE

6'-Sialyllactose is a naturally occurring acidic oligosaccharide found in human milk and is also present at comparable levels in bovine, goat, and, to a lesser extent, donkey milk (Martín-Sosa et al., 2003; Claps et al., 2014; Licitra et al., 2019). Synthetic forms of 6'-SL have been approved for use in infant formula and conventional foods (GRN 881, 2020; GRN 922, 2021).

Acidic oligosaccharides make up 15-20% of all HMOs found in human milk (Bode, 2012). The concentration of 6'-SL in human milk has been analyzed in numerous studies and as summarized in GRN 922, ranges from 0.01 – 1.7 g/L (Asakuma et al., 2007; Austin et al., 2016; Austin et al., 2019; Azad et al., 2018; Bao et al., 2007; Coppa et al., 1999; Gabrielli et al., 2011; Goehring et al., 2014; Hong et al., 2014; Kunz et al., 1999; Kunz et al., 2017; Larsson et al., 2019; Leo et al., 2010; McGuire et al., 2017; McJarrow et al., 2019; Martín-Sosa et al., 2003; Nijman et al., 2018; Paganini et al., 2019; Samuel et al., 2019; Smilowitz et al., 2013; Spevacek et al., 2015; Sprenger et al., 2017; Sumiyoshi et al., 2003; Tonon et al., 2019; Thurl et al., 2010; Thurl et al., 2017; Van Niekerk et al., 2004; Wejryd et al., 2018). Unlike other HMOS, such as 2'-fucosyllactose (2'-FL), 3-fucosyllactose (3-FL), and lacto-*N*-tetraose (LNT), 6'-SL levels are not affected by Secretor status of the mother and remain relatively constant over the course of lactation. Additionally, a systematic review conducted by Thurl et al. (2017) reported means and 95% percentile confidence limits for the amount of 6'-SLin the milk of mothers of term infants of 0.35 g/L and 0.29 – 0.42 g/L and mothers of preterm infants of 0.60 g/L and 0.40 – 0.80 g/L.

In the United States, two synthetic forms of 6'-SL are used in non-exempt term infant formula at levels up to 0.4 g/L (GRN 881, 2020; GRN 922, 2021). One of the forms is also used in beverages and formula for young children (>12 months of age) up to 0.3 g/L, foods for infants and young children at levels up to 2.5 g/kg; yogurt up to 5 g/kg; buttermilk and fluid milk (flavored and unflavored) up to 0.5 g/L; meal replacement drinks up to 1 g/L; meal replacement bars up to10 g/kg; cereal and granola bars up to 5 g/kg; and soft drinks, fruit- based drinks, sports drinks, "energy drinks," and enhanced waters up to 0.5 g/L (GRN 881, 2020).

Thus, humans have been exposed to 6'-SL either through the ingestion of human milk, milk from other mammals, and/or products containing synthetic forms of 6'-SL.

C. INTENDED USE

6'-Sialyllactose is GRAS for use in non-exempt infant formula at 0.4 g/L and selected conventional foods at 0.3 to 10 g/kg (GRN 881, 2020; GRN 922, 2021). The 6'-SL ingredient manufactured by Chr. Hansen A/S is GRAS for use in only non-exempt term infant formula at 0.4 g/L (GRN 922, 2021). Chr. Hansen A/S intends to expand the use of the subject of GRN 922 to toddler formulas, foods for infants and young children, meal replacements drinks for adults, non-carbonated drinks, bars, oral electrolyte solutions, and enteral tube feeding formulas at levels ranging from 0.15 to 3.8 g/L (Table 3). Importantly, these expanded uses include new uses, substitutional uses for other forms of 6'-SL that are GRAS for use in infant formula and conventional foods, and increases in 6'-SL use levels in uses that have already been determined GRAS. Therefore, a cumulative estimated daily intake must be calculated using the maximum use level for all uses to determine if Chr. Hansen A/S's intended uses increase 6'-SL overall exposure.

Table 3. Comparison of Uses and Use levels That Are GRAS with the Intended Uses and Use Levels

Uses That Are GRAS[1]	Use Levels That are GRAS (g/kg or g/L)[1]	Intended Uses	Intended Use Level (g/kg or g/L)	Maximum Use Level Used for Cumulative EDI Calculations (g/kg or g/L)
Non-exempt infant formula	0.4	-	0.4	0.4
Toddler formula	0.3	Toddler milks (Go and Grow by Similac®)	0.4	0.4
-	-	Milk-based meal replacement beverages for children (Pediasure®)	2.28	2.28
Baby food	2.5	-		2.5
-	-	Cereals, prepared, ready-to-serve, for infants and young children	1.5	1.5
-	-	Cereals, dry instant, for infants and young children	1.5	1.5
Drinks for children	0.3	-	-	0.3
Meal replacement drinks (including dairy and non-dairy drinks for weight reduction)	1	Meal replacement drinks for adults (including dairy and non-dairy drinks for weight reduction); including formulas for pregnant women	2.28	2.28
Sports, Isotonic, and Energy Drinks	0.5	Non-carbonated drinks (e.g. fitness water, thirst quenchers, sports and isotonic drinks)	0.7	0.7
Meal replacement bars for Weight Loss	10		1.5	10

Table 3. Comparison of Uses and Use levels That Are GRAS with the Intended Uses and Use Levels

Uses That Are GRAS[1]	Use Levels That are GRAS (g/kg or g/L)[1]	Intended Uses	Intended Use Level (g/kg or g/L)	Maximum Use Level Used for Cumulative EDI Calculations (g/kg or g/L)
Bars, snack	5	Bars, including snack bars, meal-replacement bars, and breakfast bars	1.5	5
Unflavored, pasteurized milk	0.5	-	-	0.5
Buttermilk	0.5	-	-	0.5
Flavored Milk	0.5	-	-	0.5
Yogurt	5	-	-	5
-	-	Enteral tube feeds used as sole source nutrition (Ensure®, Glucerna®, and Boost®)	3.8	3.8
-	-	Oral Electrolyte Solutions	0.15	-[2]

[1]Obtained from GRN 881 and GRN 922.
[2]Not included in the cumulative estimated daily intake calculation because the products are intended for short-term use only.

D. ESTIMATED DAILY INTAKE

1. Estimated Daily Intake of 6'-SL from Oral Electrolyte Solutions

Oral electrolyte solutions (OESs), such as Pedialyte, are specially formulated to replenish fluids and minerals and recommended to be used under medical supervision to prevent dehydration caused by vomiting, diarrhea, exercise, travel, or heat exhaustion. Conditions of use state 1-2 L of OES, such as Pedialyte, may be needed per day to maintain proper hydration, however, a medical professional should be consulted if vomiting, fever, or diarrhea continues beyond 24 hr or if consumption needs are greater than 2 L/day. Due to its infrequent use and low number of users within the database (1 user), calculation of an EDI using the National Center for Health Statistics' (NCHS) 2015-2016 National Health and Nutrition Examination Surveys (NHANES) is not appropriate.

A conservative EDI can be calculated from the intended use of OES. Consumption of a maximum of 1-2 L of an OES per day at a use level of 0.15 g of 6'-SL/L would result in a daily intake of 0.15 – 0.3 g of 6'-SL (equivalent to 11.1 – 22.2 mg of 6'-SL /kg bw/day assuming a 13.5 kg toddler and 2.1 – 4.2 mg of 6'-SL /kg bw/day assuming a 70 kg adult). Because OESs are intended for short term use, intake of 6'-SL from OESs will not impact the cumulative 6'-SL intake resulting from the use of 6'-SL in select conventional foods and enteral tube feeding formulas.

2. Estimated Daily Intake of 6'-SL from Selected Conventional Foods and Enteral Tube Feeding Formula

Estimates for the intake of Chr. Hansen A/S's intended uses of 6'-SL were based on the food uses and Chr. Hansen A/S's use level in Table 2, in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) 2015-2016 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2018; USDA, 2018). Nutritional beverages such as Boost, Ensure, and Glucerna were used as surrogates for enteral and tube-feeding formulas. A total of 110 food codes representative of each approved use were chosen from the Food and Nutrition Database for Dietary Studies (FNDDS) for the corresponding biennial NHANES survey. Calculations from NHANES for the mean and 90th percentile intakes were performed for Chr. Hansen A/S's representative food uses of 6'-SL.

To determine the impact of Chr. Hansen A/S's intended uses on the cumulative estimated daily intake of 6'-SL from all uses that are GRAS, a cumulative estimated daily intake was calculated using the maximum use level for all uses that are GRAS with the food consumption data included in the National Center for Health Statistics' (NCHS) 2015-2016 National Health and Nutrition Examination Surveys (NHANES) (Table 3; CDC, 2018; USDA, 2018). A total of 638 food codes representative of each approved use were chosen from the Food and Nutrition Database for Dietary Studies (FNDDS) for the corresponding biennial NHANES survey. As described previously, nutritional beverages such as Boost, Ensure, and Glucerna were used as surrogates for enteral and tube-feeding formulas. Calculations from NHANES for the mean and 90th percentile intakes were performed for all representative food uses of 6'-SL.

3. Food Consumption Survey Data

a. Survey Description

The most recent NHANES data for the years 2015-2016 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 et al., 2014). Because two 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) are available from the NHANES 2015-2016 survey, these data were used to generate estimates for the current intake analysis.

The NHANES provide 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 a civilian non-institutionalized population of the U.S. NHANES survey data were collected from individuals and households via 24-hour 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. 9754 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 as a result of sample variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (CDC, 2006; USDA, 2012).

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 6'-SL by the U.S. population. Estimates for the daily intake of 6'-SL 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 6'-SL by those individuals consuming food products containing 6'-SL. Individuals were considered users if they consumed one or more food products containing 6'-SL on either Day 1 or Day 2 of the survey.

4. Food Usage

The estimated "all-user" total intakes of 6'-SL from Chr. Hansen A/S's intended uses only from 110 proposed food uses listed in NHANES in the U.S. by population group is described in Table 4. In summary, 9.38% of the total U.S. population 2+ years was identified as consumers of Chr. Hansen A/S's intended uses of 6'-SL in the 2015-2016 survey. The mean intakes by 6'-SL consumers age 2+ from Chr. Hansen A/S's intended food uses were estimated to be 0.285 g/person/day or 0.004 g/kg body weight/day. The heavy consumer (90^{th} percentile) intakes were estimated to be 0.706 g/person/day or 0.010 g/kg body weight/day. The highest consumers on a mean EDI by body weight basis were ages 13 months to 2 years at 0.007 g/kg body weight/day (0.088 g/day).

The cumulative estimated "all-user" total intakes of 6'-SL from 685 proposed food uses listed in NHANES in the U.S. by population group is described in Table 5. In summary, 62.0% of the total U.S. population 2+ years was identified as consumers of 6'-SL from the selected food uses in the 2015-2016 survey. The mean intakes by all 6'-SL consumers age 2+ from all 6'-SL food uses were estimated to be 0.208 g/person/day or 0.003 g/kg body weight/day. The heavy consumer (90^{th} percentile) intakes were estimated to be 0.42 g/person/day or 0.006 g/kg body weight/day. The highest consumers on a mean EDI by body weight basis were ages 13 months to 2 years at 0.012 g/kg body weight/day (0.147 g/day).

Importantly, a comparison of the mean and 90^{th} percentile EDIs of 6'-SL ages 2+ from Chr. Hansen A/S's food uses and all food uses shows that the EDI decreases from 0.285 and 0.706 to 0.208 to 0.42 g/day, which is likely due to dilution from a broader range of uses and an increased number of users (compare Tables 4 and 5, respectively). Thus, Chr. Hansen A/S's intended uses and use levels do not increase 6'-SL exposure.

Table 4. Estimated "All-user" Daily Intake (EDI) of 6'-SL from Chr. Hansen A/S's Food Uses by Population Group (2015-2016 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 0-6 months	49	197	24.87	7.00	0.013	0.023	0.002	0.003
ages 7-12 months	72	207	34.78	9.44	0.040	0.085	0.004	0.009
ages 13 months-2 years	44	535	8.22	12.56	0.088	0.141	0.007	0.011
ages 2-5 years	69	915	7.54	16.92	0.108	0.200	0.006	0.012
ages 6-12 years	146	1505	9.70	36.58	0.185	0.434	0.005	0.012
ages 13-19 years	145	1143	12.69	67.35	0.26	0.608	0.004	0.009
ages 20 years and up	513	5748	8.92	80.76	0.33	0.905	0.004	0.011
ages 2 years and up	873	9311	9.38	67.35	0.285	0.706	0.004	0.010

Table 5. Cumulative Estimated "All-user" Daily Intake (EDI) of 6'-SL in All Food Uses by Population Group (2015-2016 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 0-6 months	142	197	72.08	7.00	0.065	0.098	0.009	0.014
ages 7-12 months	169	207	81.64	9.44	0.102	0.266	0.011	0.028
ages 13 months-2 years	373	535	69.72	12.56	0.147	0.296	0.012	0.023
ages 2-5 years	566	915	61.86	16.92	0.153	0.313	0.009	0.019
ages 6-12 years	975	1505	64.78	36.58	0.159	0.266	0.004	0.007
ages 13-19 years	821	1143	71.83	67.35	0.202	0.369	0.003	0.005
ages 20 years and up	3415	5748	59.41	80.76	0.234	0.535	0.003	0.007
ages 2 years and up	5777	9311	62.04	67.35	0.208	0.42	0.003	0.006

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 subject of this GRAS determination is a synthetic form of 6'-SL, which is a non-digestible oligosaccharide found in human milk, also known as a human milk oligosaccharide (HMO). Acidic oligosaccharides, including 6'-SL, make up 15-20% of all HMOs found in human milk (Bode, 2012). As summarized in GRN 922, the average concentration of 6'-SL in human milk ranges from 0.1 - 0.8 g/L.

To obtain a thorough and comprehensive understanding of the safety of 6'-SL per the intended uses and use levels, searches of the published scientific literature were conducted using Pubmed. All articles published up to May 10, 2021 that evaluated the safety of 6'-SL in conventional foods, oral electrolytes solutions (OESs), and enteral tube feeding formulas were retrieved and reviewed. Consistent with the requirements of the GRAS standard, Chr. Hansen A/S considered the totality of publicly available data and information relevant to the safety of 6'-SL including the use of other HMOs in selected conventional foods and oral electrolyte solutions, and non-digestible carbohydrates in enteral tube feeding products. This document includes the entire results of these searches.

Currently, two synthetic 6'-SL products are GRAS (GRN 881, 2019; GRN 922, 2021). The subject of GRN 881 is manufactured by Glycom A/S using a genetically engineered strain of *E. coli* and is GRAS for use in non-exempt term infant formula and selected conventional foods. The subject of GRN 922, which is also the subject of this GRAS determination and is also produced using a genetically engineered strain of *E. coli* and is GRAS for use in non-exempt term infant formula. Importantly, as summarized in GRN 922, the subjects of GRN 881 and this GRAS determination are structurally identical to the 6'-SL in human milk, qualitatively comparable and quantitatively similar, and supported by a battery of published genotoxicology, subchronic toxicology, and neonatal piglet tolerance studies conducted with 6'-SL and mixtures containing 6'-SL. Publicly available clinical data also show that the ingestion of the HMOs, 2'-fucosyllactose, 3'-SL and 6'-SL, as well as other non-digestible carbohydrates is well tolerated in infants, children, and adults, including susceptible population groups that received enteral tube feeding formulas.

Because infants are considered a susceptible population group from a safety perspective and the subject of this GRAS determination is qualitatively comparable and quantitatively similar to the 6'-SL tested by Phipps et al. (2019) and the subject of GRN 881 (Scientific Committee on Food, 1998; GRN 833, 2019; GRN 923, 2020), there is reasonable certainty that the use of the subject of this GRAS determination per the intended uses will also be safe in children, adults, and enteral tube feeding formulas. Chr. Hansen A/S therefore concludes that the subject of this GRAS Determination is GRAS as an ingredient in toddler formulas, foods for infants and young children, meal replacements drinks for adults, non-carbonated drinks, bars, oral electrolyte solutions, and enteral tube feeding formulas at the intended use levels.

A. SAFETY OF THE PRODUCTION ORGANISM

The safety of the host organism *E. coli* BL21(DE3) and the production organism was thoroughly summarized in GRN 922. Therefore, the summaries of the safety of the host organism and the production strain are incorporated by reference (see pages 24 and 25 of GRN 922). Importantly, because *JBT-6SL* was engineered with genes with known functions, which do not confer toxicogenicity, virulence, or DNA, using site-specific homologous recombination or transposition, *JBT-6SL* is non-toxigenic, not capable of DNA transfer to other organisms, and has the same virulence profile as *E. coli* BL21(DE3). Therefore, based on the widespread use of *E. coli* BL21(DE3) as a host strain, the strategy used to genetically engineer *JBT-6SL* and its comprehensive characterization, use of *JBT-6SL* as the production strain is not expected to result in safety concerns.

B. ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

The ADME of human milk oligosaccharides (HMOs) and other non-digestible carbohydrates, such as galactooligosaccharides, have been extensively summarized in previous GRAS Determination and opinions published by worldwide authoritative bodies, including GRN 922, which summarizes the GRAS status of the use of Chr. Hansen A/S's 6'-SL in infant formula (GRN 484, 2014; GRN 546, 2015; GRN 547, 2014; GRN 571, 2015; GRN 650, 2016; GRN 659, 2016; GRN 735, 2018; GRN 749, 2018; GRN 766, 2018; GRN 815, 2019; GRN 833, 2019; GRN 919, 2020; GRN 921; 2020; GRN 922, 2021; GRN 923, 2020; EFSA Panel on Dietetic Products, 2015; EFSA Panel on Nutrition et al., 2019). As summarized on page 21 of GRN 922, HMOs, including 6'-SL, are highly resistant to the digestive enzymes of the gastrointestinal (GI) tract and poorly absorbed.

C. TOXICOLOGY

The pivotal toxicology studies that support the use of Chr. Hansen A/S's 6'-SL ingredient in conventional foods, infant meal replacers, and enteral formulas include a battery of genotoxicity and subchronic toxicity studies conducted using a 6'-SL-containing ingredient manufactured by Glycom A/S and published by Phipps et al. (2019). Additional genotoxicity and subchronic toxicity studies with a 6'-SL ingredient manufactured by GeneChem Inc. and published by Gurung et al. (2018), and a mixture containing 2'-fucosyllactose (2'-FL), 3-fucosyllactose (3-FL), lacto-*N*-tetraose (LNT), 3'-SL, and 6'-SL manufactured by Chr. Hansen A/S and published by Parschat et al. (2020) have also been conducted. All of these studies were extensively summarized in GRN 922 and, therefore, their summaries are incorporated by reference (see pages 22 – 38 of GRN 922). Briefly, Phipps et al. (2019) conducted an OECD-compliant bacterial reverse mutation assay, an OECD-compliant *in vitro* mammalian cell micronucleus test, and an OECD-compliant 90-day feeding toxicity study with a product containing 96.8% 6'-SL to support the GRAS status of the subject of GRN 881. Gurung et al.

(2018) conducted the corroborating FDA Redbook-compliant bacterial reverse mutation, chromosomal aberration, and *in vivo* micronucleus assays, and an acute oral toxicity and a 90-day dietary toxicity study with a product containing >98 % 6'-SL. Parschat et al. (2020) evaluated the genotoxicity and subchronic toxicity of Chr. Hansen A/S's 6'-SL in combination with 2'-FL, 3'-FL, LNT, and 3'-SL in an OECD-compliant bacterial reverse mutation assay, an OECD-compliant *in vitro* micronucleus assay, a seven-day pilot dietary toxicity study, and an OECD-compliant 90-day dietary toxicity study. 6'-Sialyllactose is not genotoxic and has a no observed adverse effect level (NOAEL) of 5000 mg/kg bw/day, which was the highest dose tested in the subchronic toxicology study. Similar results were reported by Gurung et al. (2018) and Parschat et al. (2020).

As summarized in GRN 922 (pages 22 and 23), the 6'-SL products used by Phipps et al. (2019) and the subject of this GRAS Determination are both manufactured by fermentation using genetically engineered strains of *E. coli* and contain similar amounts of 6'-SL (96.8 vs 94.5, respectively (average 6'-SL content from Table 2 vs. 6'-SL content reported by Phipps et al. (2019)). They also have comparable carbohydrate by-products and other impurities controlled by product specifications, such as protein, ash, and moisture. Because Chr. Hansen A/S's 6'-SL product is qualitatively comparable and quantitatively similar to the 6'-SL product tested by Phipps et al. (2019), the results of the genotoxicology and subchronic toxicology studies conducted by Phipps et al. are pivotal to supporting the safety of Chr. Hansen A/S's 6'-SL product. Thus, based on the results reported by Phipps et al. (2019), adverse effects from the ingestion of 6'-SL per the intended uses and use levels are not expected.

D. TOLERANCE STUDIES IN NEONATAL PIGLETS

Two published studies have evaluated the tolerance of 6'-SL in the neonatal piglet, which is an appropriate model for understanding the tolerance of food ingredients in infants (Litten-Brown et al., 2010). Monaco et al. (2020) evaluated the safety and tolerance of a 6'-SL sodium salt (> 98% purity) manufactured by enzymatic synthesis by GeneChem. Hanlon (2020) evaluated the safety and tolerance of a mixture of HMOs containing 2'-FL, 3'-FL, LNT, 3'-SL, and 6'-SL manufactured by Chr. Hansen A/S. The study conducted by Hanlon is extensively summarized in GRN 921 and its summary is therefore incorporated by reference.

In the study conducted by Monaco et al. (2020), forty-eight two day-old piglets were fed one of four diets containing varying amounts of a 6'-SL sodium salt manufactured by GeneChem for 21 days (n=12/group). The control diet was a commercially-available non-medicated sow-milk replacer formula (Advance Liqui-Wean, Milk Specialties Co., Dundee, IL, USA). The 6'-SL-containing diets were the control diet supplemented with 300, 600, or 1200 mg/L 6'-SL, equivalent to 289.9, 579.8, and 1159.7 mg/L 6'-SL, respectively, after correcting for sodium content. 6′-Sialyllactose was well-tolerated at all doses over the 21-day treatment period. The 6'-SL containing diets supported growth and development at levels comparable to those observed in

the piglets fed the control diet. Although minimal/mild and a few marked microscopic findings in the tissues were reported, the 6'-SL diets had no significant effect on serum chemistry, hematology and organ microscopic structure. Monaco et al., concluded that the addition of an enzymatically-synthesized 6'-SL to a milk replacer formula supported growth and clinical outcomes similar to the control formula in the neonatal piglet.

As summarized on pages 38 – 70 in GRN 922, Hanlon (2020) administered a mixture of HMOs containing 2'-FL, 3'-FL, LNT, 3'-SL, and 6'-SL to two-day-old Yorkshire crossbred piglets for 21 days. Thirty-six experimentally naïve domestic two-day-old Yorkshire crossbred piglets were assigned to one of three treatment groups (n=12/group). The treatment groups received either a control diet, a diet containing 5.75 g/L of HMO MIX 1, or a diet containing 8.0 g/L HMO MIX 1. The control diet was Land O'Lakes Specialty Milk Replacer and was used as the base diet for both HMO Mix 1 test diets. HMO MIX 1 was obtained from Chr. Hansen A/S (Rheinbreitbach, Germany) and contained 49.1% 2'-FL, 10.4% 3-FL, 19.9% LNT, 3.5% 3'-SL, and 4.2 % 6'-SL on a dry weight basis. The endpoints that were evaluated included mortality, clinical observations, body weight, feed consumption, feed efficiency, compound consumption, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), gross necropsy findings, organ weights, and histopathologic examinations. Except for one male piglet in the 8.0 g/L dosing group, which was euthanized on day 7 for humane reasons, all of the remaining animals survived until the scheduled study termination on day 22. The clinical and veterinary observations of the male piglet in the 8.0 g/L dosing group that was euthanized included yellow discolored feces, thin body condition, unkempt appearance, generalized muscle wasting, and lateral recumbency. Additionally, *E. coli* was detected in a fecal culture of the one male piglet that was euthanized. Based on the presence of *E. coli* in the feces and the constellation of observations, the unscheduled death/euthanasia of the one male in the 8.0 g/L treatment group was determined to be due to an underlying infection that was distributed evenly among the animals in all dosing groups, not HMO Mix 1-related, and did not affect the validity of the results. The clinical pathology values and macroscopic and microscopic findings in the remaining animals did not reveal a relationship to the HMO Mix 1 treatment and, although increased cecum weights in males and females at ≥5.75 g/L, increased colon weights in males at ≥5.75 g/L, and decreased rectum weights in males and females at 8.0 g/L were observed, the changes were considered not adverse as there were no microscopic correlates. Together these results indicate that daily dietary administration of HMO Mix 1 to neonatal piglets for 3 weeks at concentrations up to 8.0 g/L with calculated intakes of 3.6 and 3.7 g/kg/bw of the HMO Mix 1 (0.15 and 0.16 g 6'-SL/kg bw) in males and females, respectively, was well-tolerated, did not produce adverse effects on growth and development. Since the filing of GRN 922, this study was published by Hanlon (2020).

E. CORROBORATIVE ANIMAL STUDIES

The additional animal studies that corroborate the safety of 6'-SL were conducted by Jacobi et al. (2016), Obelitz-Ryom et al. (2018), Monaco et al. (2018), Wang et al. (2019) and Obelitz-Ryom et al. (2019). Although these studies focused on the effect of sialyllactose on brain and gut development, as well as effects on the microbiome, none reported adverse effects related to sialyllactose and 6'-SL supplementation. Only the endpoints relevant to the safety and tolerability of sialyllactose and 6'-SL supplementation are briefly summarized below.

Jacobi et al. (2016) fed day-old piglets diets containing 0, 2, or 4 g 6'-SL three times daily for 21 days to determine if different isomers of sialyllactose affect brain sialyllactose levels and modulate the microbiome. 6'-SL did not affect feed intake, growth or fecal consistency.

Obelitz-Ryom et al. (2018) fed preterm piglets intact unpasteurized Jersey cow's milk supplemented with either GOS or 4.5% sialyllactose (a 6:1 ratio of 3'-SL and 6'-SL) for 19 days and assessed gut development and colonization. No adverse events related to the experimental diet were reported in the study, and there were no differences in body weight gain between the treatment groups. There were no differences in serum biochemistry or phagocytic capacity of neutrophils observed between the two treatment groups.

Monaco et al. (2018) fed 2-day old male piglets increasing doses of sialyllactose (130, 380, or 760 mg sialyllactose/L milk replacer; 3' or 6' isomer was not specified) for 30 days to investigate the effect of sialyllactose on weight gain, gastrointestinal development, and microbiota composition. No differences were observed among the treatment groups in body weight gain over the test period. Although some differences were observed among treatment groups in hematology parameters, these differences were within the historical background range for this species and laboratory and were not considered treatment-related or adverse. There were no changes observed in clinical chemistry parameters among the treatment groups, except glutamate dehydrogenase. This difference was not dose dependent and was not considered treatment related or adverse.

Wang et al. (2019) performed a study using sow replacement milk supplemented with a combination of 7.6 g/kg 3'-SL and 1.9 g/kg 6'-SL to observe the effect that sialylated milk oligosaccharides had on neurotransmitters and brain metabolites in piglets. Neonatal piglets were fed sow replacement milk supplemented with sialylated oligosaccharides from 3 days to 38 days of age. The sialylated oligosaccharide intervention did not significantly influence body weight gain, brain weight gain, or weight gain in specific regions of the brain compared to controls.

Obelitz-Ryom et al. (2019) fed preterm piglets intact unpasteurized Jersey cow's milk supplemented with either lactose or 4.5% sialyllactose (a 6:1 ratio of 3'-SL and 6'-SL) for 19 days and assessed cognitive performance. No adverse events related to the experimental diet were reported in the study.

F. CLINICAL STUDIES

Additional support for the safe use of 6'-SL in toddler formulas, foods for infants and young children, meal replacements drinks for adults, non-carbonated drinks, bars, oral electrolyte solutions, and enteral tube feeding formulas at the intended use level is based on results of numerous clinical studies that evaluated the safety and tolerance of HMOs, such as 2'-FL, lacto-*N*-neotetraose (LNnT), 3'-SL and 6'-SL, as well as other non-digestible carbohydrates in infants, adults, sensitive populations consuming enteral tube feeding formulas and oral electrolytes solutions. In general, HMOs are well tolerated in infants up to 1 g/day, adults up to 20 g/day, and non-digestible carbohydrates are well tolerated in enteral tube feeding formulas up to 63 g/day and oral electrolyte solutions up to 50 g/L.

1. Clinical Studies with HMOs in Infants and Adults

6'-Sialyllactose is a non-digestible HMO that is GRAS for use in infant formula and conventional foods (GRN 881, 2020; GRN 922, 2021). Although no clinical studies have been conducted with 6'-SL specifically, numerous clinical studies have evaluated the tolerability of ingesting other HMOs, such as 2'-fucosyllactose (2'-FL), lacto-N-neotetraose (LNnT), the 6'-SL isomer 3'-SL, and a mixture of 3'-SL and 6'-SL in infants and adults, Storm et al.(2019), Marriage et al. (2015), Goehring et al. (2016), Puccio et al. (2017), Nowak-Wegrzyn et al. (2019), Kajzer et al. (2016), Alliet et al. (2016), Steenhout et al. (2016), Meli et al. (2014), Simeoni et al. (2016), Cooper et al. (2016), Radke et al. (2017), Elison et al. (2016), Rasko et al. (2000), Parente et al. (2003), Gurung et al. (2018), Riechmann et al. (2020), Iribarren et al. (2020), Palsson et al. (2020), and Ryan et al. (2021). Four of these clinical studies, Cooper et al. (2016), Meli et al. (2014), Radke et al. (2017), and Simeoni et al. (2016), evaluated the tolerability of ingesting 6'-SL in combination with 3'-SL, bovine milk oligosaccharides, galactooligosaccharides, and live microorganisms in infants. Three of these clinical studies, Parente et al. (2003), Rasko et al. (2000), and Gurung et al. (2018), evaluated the tolerability of ingesting 3'-SL in adults with *H. pylori* infections. The remaining thirteen studies were conducted in infants or adults with 2'-FL or LNnT alone or a combination of 2'FL and LNnT, Storm et al. (2019), Marriage et al. (2015), Goehring et al. (2016), Puccio et al. (2017), Nowak-Wegrzyn et al. (2019), Kajzer et al. (2016), Alliet et al. (2016), Steenhout et al. (2016), Elison et al. (2016), Riechmann et al. (2020), Iribarren et al. (2020), Palsson et al. (2020), and Ryan et al. (2021). Except for the studies conducted by Riechmann et al. (2020), Iribarren et al. (2020), Palsson et al. (2020), and Ryan et al. (2021), all of these studies have been extensively summarized in previous GRAS Notifications (GRN 546, 2015; GRN 571, 2015; GRN 571 Supplement, 2019; GRN 650, 2016; GRN 659, 2016; GRN 735, 2018; GRN 749, 2018; GRN 766, 2018; GRN 815, 2019; GRN 852, 2019; GRN 880, 2020; GRN 897, 2020; GRN 919, 2020; GRN 921, 2020; GRN 922, 2021). Therefore, their summaries are incorporated by reference and

the studies are briefly summarized in tabular format below along with the new studies published by Riechmann et al. (2020, Iribarren et al. (2020), Palsson et al. (2020), and Ryan et al. (2021) (Table 6 and 7).

In infants, Cooper et al. (2016), Meli et al. (2014), Radke et al. (2017) and Simeoni et al. (2016) administered a mixture of oligosaccharides containing 3'-SL, galactooligosaccharides, and 6'-SL up to a total of 10 g oligosaccharides/L (equivalent to approximately 10 g total oligosaccharides/day assuming that infants consume one liter of formula/day). Although the levels of 3'- SL and 6'-SL ingested in the studies were not provided in the publications, these studies collectively showed that the oligosaccharide mixture was well tolerated and had no adverse effect on growth and development (Table 6). Storm et al. (2019), Marriage et al. (2015), Goehring et al. (2016), Puccio et al. (2017), Nowak-Wegrzyn et al. (2019), and Riechmann et al. (2020) administered up to 1.0 g 2'-FL/L and 0.5 g LNnT/L (equivalent to approximately 1.0 g 2'-FL/day and 0.5 g LNnT/day assuming that infants consume one liter of formula per day) and reported similar effects (Table 6). Importantly, none of the studies reported serious adverse events related to the ingestion of the HMOs and the most common effects were occasional flatulence, abdominal distress, diarrhea and loose stools, which are not unexpected considering what is known to occur following the ingestion of diets containing high amounts of non-digestible carbohydrates (Eldridge et al., 2019).

In adults, Rasko et al. (2000), Parente et al. (2003), and Gurung et al. (2018) administered up to 20 g 3'-SL/day and showed that the HMO was well tolerated and as expected, the most common complaints were flatulence, abdominal distress, and abdominal pain (Table 7). Similar results were reported by Elison et al. (2016), Iribarren et al. (2020), Palsson et al. (2020), and Ryan et al. (2021) in healthy adults and adults with inflammatory bowel disease (IBS), ulcerative colitis, Crohn's disease, or celiac disease following the ingestion of up to 20 g/d of 2'-FL, LNnT, or a combination of 2'-FL and LNnT.

Taken together, the clinical studies conducted with 3'-SL 6'-SL, 2'-FL, and LNnT the publicly available studies provide corroborative clinical evidence that long-lasting, irreversible adverse effects resulting from the ingestion of HMOs, including 6'-SL, are not expected.

Table 6. Clinical Studies with Human Milk Oligosaccharides and Infants

Reference	Study Design and Population	Groups (Numbers of Subjects)	Duration	Safety Parameters	GRN Reference
3'-Sialyllactose and 6'-Sialyllactose					
Radke et al., 2017	Multicenter, randomized placebo-controlled, double-blind study Healthy term infants 0-14 days old	Group 1: Control formula; (n=207) Group 2: Test formula containing 5.8 ± 1.0 g BMOs*/100 g powdered formula (8 g/L in the reconstituted formula) and $1x10^7$ cfu/g *B. lactis* CNCM I-3446; (n=206) Group 3: Breastfed reference group; (n=63) *BMOs were generated from whey permeate and contained galactooligosaccharides and milk oligosaccharides, such as 3'- and 6'- sialyllactose; the concentrations of 3'- and 6'- sialyllactose are not known	6 months Follow-up at 12 months, no test formula 6-12 months	• A total of 58 infants (27 in each of the Test and the Control groups and four in the Breast-fed group) were excluded from the ITT analyses because they dropped out before the 1-mo visit. • The population that completed the entire study duration was 150 infants in the test formula group, 157 in the control formula group, and 49 in the breastfed group. • The proportion of infants with AEs related to infections was comparable among the formula groups. • No significant difference in diarrhea or febrile infections incidence among the groups at 6 and 12 months. • Test formula was well tolerated and no difference in anthropometric measures were observed among the groups. • The test formula group showed similar gut microbiota patterns, fecal IgA, and stool pH to breastfed infants and was significantly different than the control formula group.	GRN 766, pages 62-64
Cooper et al., 2017	Multicenter, randomized, placebo-controlled, double-blind study Healthy term infants born to HIV+ mothers	Group 1: Cesarean-delivered infants consuming standard formula; (n=101) Group 2: Cesarean-delivered infants and standard formula containing 5.8 ± 1.0 g BMOs*/100 g powder formula (8 g/L in the reconstituted formula) and $1x10^7$ cfu/g *B. lactis* CNCM I-3446; (n=92)	4 months	• Four hundred and thirty infants were randomized into the study. o Nine (2.1%) infants were lost to follow-up after randomization but before starting the study formulas. o Eight infants were found to be HIV infected, seven at the 4-week visit (v2) and one became positive at 6 months (v5). o Of the eight that were HIV infected, three infants died and one discontinued the study. o Over the course of the study, there were a total of 55, 57, 47, and 55 discontinuations in the vaginal starter formula containing BMOs and *B. lactis* CNCM I-3446, vaginal group starter formula, cesarean starter formula containing BMOs and *B. lactis* CNCM I-3446, and cesarean starter formula groups, respectively.	GRN 766, pages 62-64

Table 6. Clinical Studies with Human Milk Oligosaccharides and Infants

Reference	Study Design and Population	Groups (Numbers of Subjects)	Duration	Safety Parameters	GRN Reference
		Group 3: Vaginally delivered infants and standard formula; (n=113) Group 4: Vaginally delivered infants standard formula containing 5.8 ± 1.0 g BMOs/100 g powder formula (equivalent to 8 g/L in the reconstituted formula) and $1x10^7$ cfu/g *B. lactis* CNCM I-3446; (n=115) *BMOs were generated from whey permeate and contained galactooligosaccharides and milk oligosaccharides, such as 3'- and 6'- sialyllactose; the concentrations of 3'- and 6'- sialyllactose are not known		○ There were no significant differences in tolerability and adverse events between the groups in both delivery methods. • Test formula supplemented with BMOS lowered fecal pH and improved fecal microbiota counts in both delivery methods.	
Simeoni et al., 2016	Randomized, placebo-controlled, double-blind study Healthy 5-day old, term infants	Group 1: Standard formula; (n=37) Group 2: Standard formula plus 5.7±1.0 g/100 g bovine milk oligosaccharides (BMOs*; 8.0 g/L reconstituted formula) and $1x10^7$ cfu/g of *B. lactis* CNCM I-3446; (n=39) Group 3: Human milk; (n=37)	12 weeks	• No difference in compliance or tolerability was observed among the three groups. ○ 10 infants discontinued in the human milk/breastfed group (5 withdrew voluntarily and 5 for other reasons) ○ 7 infants discontinued in the standard formula group (2 withdrew due to GI symptoms, 4 withdrew voluntarily, and 2 were lost to follow-up ○ 7 infants discontinued in the standard formula with the BMOS and *B. lactis* CNCM I-3446 group (3 withdrew due to GI symptoms, 2 withdrew voluntarily, and 3 were lost to follow-up • There were no differences in anthropometric measures among the three groups. • There were no differences in the standard formula and standard formula with BMOS and *B. lactis* CNCM I-3446 groups in 'spitting up', vomiting, crying, colic, flatulence and irritability.	GRN 766, pages 62-64

Table 6. Clinical Studies with Human Milk Oligosaccharides and Infants

Reference	Study Design and Population	Groups (Numbers of Subjects)	Duration	Safety Parameters	GRN Reference
		*BMOs were generated from whey permeate and contained galactooligosaccharides and milk oligosaccharides, such as 3'- and 6'- sialyllactose; the concentrations of 3'- and 6'- sialyllactose are not known		• Infants from the standard formula with BMOS and *B. lactis* CNCM I-3446 group, but not the standard formula group, showed a proportion of yellowish versus greenish stools equivalent to the breast-fed infants. • Infants in the standard formula with BMOS and *B. lactis* CNCM I-3446 group showed more liquid stools than infants in the standard formula group; liquid stools were the dominant observation in the breast-fed infants.	
Meli et al., 2014	Randomized, double-blind, single-center study Healthy term infants (<14 days old)	Group 1: Standard formula; (n=84) Group 2: Standard formula plus 10 g bovine milk oligosaccharides (BMOs*/L); (n= 99) Group 3: Standard formula plus 10 g BMOs/L, 2×10^7 cfu/g *Bifidobacterium longum* ATCC BAA-999 (Bl999), and 2×10^7 cfu/g *Lactobacillus rhamnosus* CGMCC 1.3724 (LPR); (n=98) Group 4: Human milk; (n=39) *BMOs were generated from whey permeate and contained galactooligosaccharides and milk oligosaccharides, such as 3'- and 6'- sialyllactose; the concentrations of 3'- and 6'- sialyllactose are not known	4 months	• 90 infants from formula groups and 18 infants from breastfed groups withdrew o Higher rates of discontinuations were observed in the BMOS-supplemented formula groups (36.4% in Group 2; 34.7% in Group 3) compared with the standard formula-treated group (23.8%), although the differences did not reach statistical significance. o GI symptoms (i.e., regurgitation, vomiting, diarrhea, constipation, and abdominal pain characterized by prolonged crying) were the most common reason for study discontinuation in all three formula groups: 14.3% of infants in the standard formula-treated group, 17.2% in Group 2 and 13.3% in the Group 3 discontinued due to GI symptoms. • Weight gain and length and head circumference showed no significant differences between standard and BMOS-containing formula groups • BMOS groups had more frequent and less hard stools compared to control • No significant differences were observed between the standard and BMOS containing formula-treated groups in caregivers' reports of flatulence, vomiting, spitting up, crying, fussing, and colic.	GRN 766, pages 62-64

Table 6. Clinical Studies with Human Milk Oligosaccharides and Infants

Reference	Study Design and Population	Groups (Numbers of Subjects)	Duration	Safety Parameters	GRN Reference
			2'-Fucosyllactose		
Riechmann et al., 2020	Non-randomized, open-label, prospective study Healthy term infants 7 days to 2 months old	Group 1: Formula-fed infants (n=82) Group 2: Infants consuming formula and human milk; the formula contained 1.0g/L of 2'-FL, 0.5 g LNnT, and Lactobacillus reuteri (n=62) Group 3: Breast-fed infants (n=63)	8 weeks	• Sixteen subjects dropped out of Group 1 (six were excluded due to protocol deviations, three dropped out due adverse events (AEs), and seven were lost to follow-up). • Fourteen subjects dropped out of Group 2 (eight were excluded due to protocol deviations, 3 dropped out due to adverse events, and three were lost to follow-up. • Eighteen subjects dropped out of Group 3 (11 were excluded due to protocol deviations, one dropped out due to adverse events, and 6 were lost to follow-up. • There were no significant differences between any of the groups for any of the anthropometric measures. • Composite Infant Gastrointestinal Symptom Questionnaire (IGSQ) scores demonstrated low gastrointestinal distress in all feeding groups at all time points and there were no significant differences among feeding groups at baseline, 4, or 8 weeks. o There were no significant differences among the groups in the gassiness, fussiness, crying or spitting-up/vomiting domains of the IGSQ. o For the stooling domain, Group 2 were significantly different than Group 3 at baseline and 8 weeks. • A total of 49 subjects experienced 58 adverse events over the course of the study. There were 19 AEs in Group 1, 21 in Group 2, and 18 AEs in Group 3. The incidence was generally low and not significantly different among the groups o Three subjects experienced potentially product-related AEs, including two instances of cow's milk intolerance (one in Group 1 and one in Group 2) and one instance of irritability in Group 1. • Six serious adverse events occurred (four in Group 1 and 2 in Group 2), all of which were bronchiolitis. All were considered unrelated to the study feeding.	Not previously summarized
Nowak-Wegrzyn et al., 2019	Double-blind, placebo-controlled food challenges	Treatment #1: Whey-based extensively hydrolyzed formula Treatment #2: Whey-based extensively hydrolyzed	Not applicable	• Sixty-four children completed at least one DBPCFC. • Three children were excluded due to protocol deviations (n = 61). • There was one allergic reaction to the Test, and one to the Control formula. • Sixty-one of the 64 subjects completed the open-label home challenge phase with the Test formula	GRN 919, page 33

Table 6. Clinical Studies with Human Milk Oligosaccharides and Infants

Reference	Study Design and Population	Groups (Numbers of Subjects)	Duration	Safety Parameters	GRN Reference
	Children with cow milk protein allergy	formula containing 1.0 g/L 2'-FL and 0.5 g/L LNnT		○ One subject vomited on Day 1 of the home challenge but completed the home challenge without further problems. ○ One patient developed diarrhea on the last day of the challenge, which the site investigator attributed to gastroenteritis. ○ No significant gastrointestinal symptoms (flatulence, abnormal stool frequency/consistency, increased spitting-up, or vomiting) were reported. • No serious adverse events occurred during the entire study.	
Storm et al., 2019	Randomized, placebo-controlled double-blind study Healthy term infants 14 days old ±5 days.	Group 1: Formula containing Bifidobacterium animalis ssp lactis Bb12 (n=40) Group 2: Formula containing Bifidobacterium animalis ssp lactis Bb12 + 0.25 g/L 2'-FL (n=38)	6 weeks	• In the 2'-FL-treated group, one subject was lost to follow-up, one caregiver wished to withdraw, three subjects withdrew due to adverse events (AEs), and three subjects did not comply with feeding only the study formula. • In the control group, one subject was lost to follow-up, one caregiver wished to withdraw, three subjects withdrew due to adverse events, and two subjects did not comply with feeding only the study formula. • Infant gastrointestinal symptom questionnaire scores were similar in both groups at baseline and after 6 weeks of treatment. • Stool frequency and consistency did not differ between the groups over the course of treatment. • Significantly more stools were reported to be difficult to pass in the control group than in the test group ($p<0.05$), however, the number of infants with stools reported as difficult to pass was not different between the groups. • Crying, fussing duration, vomiting frequency, and the proportion of babies reported to have any spit up over the 2-day diary period were similar between the two groups. • Among the babies whose caregivers reported spit-up, significantly more were reported to have spit up >5 times/day in the 2'-FL group compared to the control group. • There were no serious AEs and the AEs were equally distributed among the two groups. • There were significantly more subjects that experienced infections and infestations in the control group than in the 2'-FL-treated group (n=9 vs n=3; p=5). • There were no effects of the 2'-FL-containing formula on anthropometric measures (body weight and lengths, and weight-for-age and length-for-age).	GRN 571 supplement, page 21

Table 6. Clinical Studies with Human Milk Oligosaccharides and Infants

Reference	Study Design and Population	Groups (Numbers of Subjects)	Duration	Safety Parameters	GRN Reference
Puccio et al., 2017	Prospective, randomized, placebo-controlled study Healthy, term infants 0 to 14 days old	Group 1: Formula (n=87) Group 2: Formula with 1.0 g/L 2'-FL and 0.5 g/L LNnT (n=88)	6 months (after 6 months, all infants were switched to a non-HMO containing formula)	• Twenty infants in control and 24 infants in the HMO containing formula withdrew before the primary outcome assessment at 4-months. The dropout rate was comparable between groups. The most common reason for discontinuation was an adverse event (n=11 in control; n=12 in test). Other reasons for discontinuation before 4 months included parent/guardian request (n=3 in control; n=6 in test); lost to follow-up/missing (n=5 in control; n=6 in test); and other (n=1 in control; n=40 in test). • There was no difference in weight gain, mean weight-for-age, length-for-age, head circumference-for-age, and BMI-for-age z scores between the groups. • Parent-reported infant behavioral patterns including restlessness/irritability and colic were similar in the HMO and control groups except for softer stool (P=0.021) and fewer nighttime wake-ups (P = 0.036) in the test group at 2 months. • Infants receiving the HMO-containing formula had significantly fewer parental reports (P = 0.004 – 0.047) of bronchitis through 4 (2.3% vs 12.6%), 6 (6.8% vs 21.8%), and 12 months (10.2% vs 27.6%); lower respiratory tract infection (adverse event cluster) through 12 months (19.3% vs 34.5%); antipyretics use through 4 months (15.9% vs 29.9%); and antibiotics use through 6 (34.1% vs 49.4%) and 12 months (42.0% vs 60.9%) compared to the infants receiving the control formula.	GRN 650, page 38
Goehring et al., 2016	Prospective, randomized, placebo-controlled study Healthy, term infants 5 days old	Group 1: Formula with GOS (n=39) Group 2: Formula with GOS + 0.2 g/L 2'-FL (n=37) Group 3: Formula with GOS + 1.0 g/L 2'-FL (n=37) Group 4: human milk (HM)(n=42)	16 weeks	• Note: This is a sub-study of the clinical study conducted by Marriage et al., 2015. The objective was to investigate the effects of feeding formulas supplemented with HMO 2'-FL on biomarkers of immune cell function. • Circulating plasma concentrations of inflammatory cytokines IL-1a, IL-1b, IL-6, and TNF-a and anti-inflammatory IL-1ra were significantly higher (82%, 72%, 76%, 58%, and 58%, respectively) in the group fed formula compared to the group receiving human milk ($p \leq 0.05$). • Both the groups receiving the formulas containing 2'-FL exhibited profiles that were significantly different from the formula group and not different from the human milk group or each other. There were no differences in plasma cytokines IFN-a2, IFN-g, IL-10, IP-10, or RANTES between any of the groups.	GRN 735, page 62

Table 6. Clinical Studies with Human Milk Oligosaccharides and Infants

Reference	Study Design and Population	Groups (Numbers of Subjects)	Duration	Safety Parameters	GRN Reference
Marriage et al., 2015	Prospective, randomized, placebo-controlled study Healthy, term infants 5 days old	Group 1: Formula with GOS (n=101) Group 2: Formula with GOS + 0.2 g/L 2'-FL (n=104) Group 3: Formula with GOS + 1.0 g/L 2'-FL (n=109) Group 4: human milk (HM)(n=106)	17 weeks	• 338 infants completed the study (84 in the control group, 81 in the group receiving the formula containing 0.2 g/L 2'-FL, 83 in the group receiving the formula containing 1.0 g/L 2'-FL, and 90 in the HM group); 304 of whom completed the study on the assigned feeding or HM (79 in the control group, 70 in the group receiving the formula containing 0.2 g/L 2'-FL, 72 in the group receiving the formula containing 1.0 g/L 2'-FL, and 83 in the HM group). The number of premature terminations was not statistically significant among the formula-fed groups. • Although the HM group gained significantly more weight than the group receiving 0.2 g/L 2'-FL from day 14 to 28 and the group receiving 1.0 g/L 2'-FL than the HM group from day 84 to 119, there were no significant differences (sex-specific or sex- combined) in mean weight, length, or head circumference among feeding groups during the study, and no significant differences among feeding groups in mean gains in these measures from day 14 to 119. • The mean number of stools/day was significantly higher for the HM group compared to all groups receiving the formulas for the three days before the study visits at day 28, 42, and 84. The mean number of stools/day was also significantly higher for the HM group compared to the control formula group for the three days before the study visits at day 119. • Although spitting-up or vomiting was significantly higher in the formula-fed groups compared to the HM group from enrollment to day 28, there were no differences after day 28. • Although the mean rank stool consistency was significantly higher for the group receiving 2'-FL from enrollment to day 28 and was significantly higher in the formula-treated groups than the HM group for the remainder of the study, there was no difference among the formula-treated groups over the course of the study. • There were no significant differences in the overall percentage of subjects experiencing adverse events or serious adverse events in the formula-treated groups. • The control formula and the 1 g/L 2'-FL groups had significantly more subjects with reported adverse events in the "infections and infestations" category compared with the 0.2 g/L group ($p<0.05$), but the types of adverse events were similar (upper respiratory tract symptoms; otitis media, viral infections, and oral candidiasis. The control formula-treated group also had a significantly higher percentage of subjects with eczema ($p<0.05$)	GRN 650, page 37

Table 6. Clinical Studies with Human Milk Oligosaccharides and Infants

Reference	Study Design and Population	Groups (Numbers of Subjects)	Duration	Safety Parameters	GRN Reference
Kajzer et al., 2016 (abstract)	Prospective, randomized, double-blind, placebo-controlled study Healthy term infants 0 and 8 days of age.	Group 1: Formula (n=42) Group 2: Formula with 0.2 g/L 2'-FL and 2 g/L scFOS (n=46) Group 3: human milk (HM)(n=43)	5 weeks	• Thirty-six (86%) subjects in the group receiving formula, 41 (89%) in the group receiving oligosaccharides and 42 (98%) in the group receiving human milk completed the study. • There was no difference in the mean rank stool consistency among the groups. • The average number of stools per day for the human milk group was significantly higher in the human milk group than both formula-fed groups. • There were no differences among groups for the average volume of study formula intake, number of study formula feedings/day, anthropometric data or percent feeding with spit-up/vomit. • Safety endpoints not reported.	GRN 571, page 21
Alliet et al., 2016 (abstract)	Randomized, placebo controlled, study Healthy term infants 0-14 days old	Group 1: Cow's milk-based infant formula (n=87) Group 2: Cow's milk-based infant formula w/ 1.0 g/L 2'-FL and 0.5 g/L LNnT (n=88) Group 3: Human milk	3 months	• 2'FL and LNnT shift the stool microbiota towards that observed in breastfed infants. • Safety endpoints not reported.	GRN 815, page 55
Steenhout et al., 2016 (abstract)	Randomized, placebo controlled, study Healthy term infants 0-14 days old	Group 1: Cow's milk-based infant formula (n=87) Group 2: Cow's milk-based infant formula w/ 1.0 g/L 2'-FL and 0.5 g/L LNnT (n=88) Group 3: Human milk	3 months	• 2'FL and LNnT shift the stool microbiota towards that observed in breastfed infants. • Safety endpoints not reported.	GRN 735, page 62

Table 7. Clinical Studies with Human Milk Oligosaccharides and Adults

Reference	Study Design and Population	Groups (Numbers of Subjects)	Duration	Safety Parameters	GRN Reference
			3'-Sialyllactose		
Gurung et al., 2018	Randomized, double-blind, placebo-controlled study Adults with *H. pylori* infection	Group 1: Placebo (n=17) Group 1: 12 g/day 3'-SL (n=24)	4 weeks	• There were no significant differences between pre- and post-dose gastrointestinal tolerance and clinical chemistry (serum biochemistry, hematology, and urine analysis) outcomes. • Pre- and post-dose urea breath test values were not significantly different within or between the 3'-SL and placebo groups. • Compliance and adverse events were similar between the groups.	GRN 880, pages 35, 36
Parente et al., 2003	Randomized, double-blind, placebo-controlled study Adults with *H. pylori* infection (dyspepsia)	Group 1: Placebo (n=21) Group 2: 10 g/day 3'-SL sodium salt (n=17) Group 3: 20 g/day 3'-SL sodium salt (n=22)	4 weeks	• Five patients were excluded from analysis due to protocol violation. • Adverse events recorded in 6 patients were halitosis, asthenia, epigastric pain, and headache. • One patient dropped out due to headache associated with epigastric pain. • No serious adverse events were observed. • *H. pylori* colonization documented by the ^{13}C-Urea Breath Test (UBT) decreased significantly (*p*-value not provided) in both treatment groups and placebo but was most likely due to regression toward mean effect.	GRN 766, pages 64-67
Rasko et al., 2000	Randomized, double-blind, placebo-controlled study Adults with *H. pylori* infection	Group 1: Placebo (n=6) Group 2: 4g 3'-SL (n=6) Group 3: 8g 3'-SL (n=7) Group 4: 20g 3'-SL (n=7)	56 days for Control and Groups 1 and 2 28 days for Group 3	• Oral supplementation of 3'-SL did not change Lewis antigen expression of *H. pylori* strains isolated from human gastric mucosa. • No adverse effects on safety or tolerance were reported.	GRN 766, pages 64-67

Table 7. Clinical Studies with Human Milk Oligosaccharides and Adults

Reference	Study Design and Population	Groups (Numbers of Subjects)	Duration	Safety Parameters	GRN Reference
2'-Fucosyllactose and/or Lacto-N-neotetraose					
Ryan et al., 2021	Open-label, single arm study Adults (21 – 75 years old) with a BMI of 19-40 kg/m2 and with previously diagnosed inflammatory bowel disease (IBS), ulcerative colitis, Crohn's disease, or celiac disease	Group 1: 4 g of 2'-FL in combination with micronutrients, macronutrients, amino acids, and isomalto-oligosaccharide (n=20)	6 weeks	• Twelve subjects completed the study. • Eight subjects withdrew from the study o Two dropped out/declined to participate o Three dropped out due to non-serious adverse events. They reported worsening of pre-existing gastrointestinal symptoms, gastrointestinal upset, and a non-study-related viral infection • Three were lost to follow-up.	Not previously reviewed
Palsson et al., 2020	Open-label, single arm study Adult male and female patients (18 and older) with IBS	Group 1: 5 g of 2'-FL/LNnT (4:1 ratio) (n=317)	12 weeks	• Thirteen subjects were discontinued after completing the baseline survey because they did not start the intervention. Therefore, 273 patients completed the study. o Eight subjects withdrew due to an adverse event. o Four subjects withdrew consent. o Nineteen subjects were lost to follow-up. • The authors reported that there were no incidents causing safety concerns and the patients generally reported that the intervention was well-tolerated o Forty-seven patients reported a total of 87 adverse events (AEs) in the study o Sixty-one of the AEs were related to the gastrointestinal tract. o The most common side effect was passing gas, followed by abdominal distension and pain. • One serious AE occurred (hospitalization due to colitis) but was determined to be unrelated to the intervention by the study's medical safety officer.	Not previously reviewed

Table 7. Clinical Studies with Human Milk Oligosaccharides and Adults

Reference	Study Design and Population	Groups (Numbers of Subjects)	Duration	Safety Parameters	GRN Reference
Iribarren et al., 2020	Parallel, double-blind, randomized, placebo-controlled study Adult male and female patients (18 – 64 years old) with inflammatory bowel syndrome (IBS).	Group 1: Placebo (n=21) Group 2: 5 g 2'-FL/LNnT (4:1 ratio) (n=20) Group 3: 10 g 2'-FL/LNnT (4:1 ratio) (n=20)	4 weeks of treatment followed by a 4-week washout	• Group 1: one patient discontinued intervention due to worsening of symptoms during the treatment period; one patient was lost to follow-up during the washout period. • Group 2: no patients left the study • Group 3: one patient discontinued intervention due to worsening of symptoms during the treatment period; one patient was lost to follow-up during the washout period. • There were no differences in overall gastrointestinal symptom severity among the groups at week four or week eight. • None of the treatments aggravated the IBS symptoms. • There were no significant differences among the groups in the individual domains of the Gastrointestinal Symptom Rating Scales (abdominal pain, bloating, constipation, diarrhea, and satiety). • Within the groups: o There was a decrease in the severity of bloating and diarrhea in Group 1 at week 4. o In Group 2 and 3, there was a decrease in bloating and abdominal pain at week 8, respectively. • There were no differences between groups or within the groups at week 4 or 8 regarding IBS symptom severity.	Not previously reviewed

Table 7. Clinical Studies with Human Milk Oligosaccharides and Adults

Reference	Study Design and Population	Groups (Numbers of Subjects)	Duration	Safety Parameters	GRN Reference
Elison et al., 2016	Randomized, placebo-controlled double-blind study Healthy male and female adults ages 18 to 60 years.	Group 1: 2g glucose (n=10) Group 2: 5 g 2'-FL (n=10) Group 3: 10 g 2'-FL (n=10) Group 4: 20 g 2'-FL (n=10) Group 5: 5 g LNnT (n=10) Group 6: 10 g LNnT (n=10) Group 7: 20 g LNnT (n=10) Group 8: 3.3 g 2'-FL; 1.7 g LNnT (n=10) Group 9: 6.7 g 2'-FL; 3.4 g LNnT (n=10) Group 10: 13.3 g 2'-FL; 6.7 g LNnT (n=10)	1-2 week run-in period followed by a 2 week treatment period	• All subjects were compliant and completed the study according to the protocol without any dropouts. • Fifty-six adverse events were reported by forty-four subjects. o All were judged as 'mild', and all subjects tolerated the investigational products throughout the trial period. o Adverse events were usually reported as a complex of multiple symptoms such as flatulence, bloating and constipation, and were primarily reported at the end of the 2-week intervention. o Most adverse events were reported by subjects taking the highest doses of 2′FL and LNnT. Gas/ flatulence was the most common adverse event reported, followed by stomach pain, diarrhea/loose stools and rumbling, but at lower frequencies. • No significant difference in bowel movement was observed compared to Group 1. • No change in clinical significance in any physical parameter including pulse rate and blood pressure was found during the 2-week intervention. • There was no difference in clinical chemistry or hematology among the groups at the end of the 2-week intervention period	GRN 735, page 61

2. Clinical Studies with Other Non-digestible Carbohydrates and Enteral Tube Feeding Formulas

Enteral tube feeding is indicated in any patient that has a functioning and accessible gastrointestinal tract and cannot meet their nutritional requirements by consuming food orally (reviewed in Wireko and Bowling, 2010). Enteral tube feeding is administered either as a bolus or continuously via nasogastric tubes, nasojejunal tubes, or gastrostomy and can be associated with issues with the tubes and their insertion, as well as adverse effects in the patient, such as diarrhea, constipation, nausea, and vomiting/aspiration/reflux, bloating, refeeding syndrome and various electrolyte disturbances (https://gi.org/topics/enteral-and-parenteral-nutrition/; accessed on February 11, 2021). As a result, enteral tube feeding is generally administered and managed in a medical setting. Importantly, the purpose of using non-digestible carbohydrates in enteral tube feeding formulas is to help alleviate alterations in bowel function and maintain the healthy balance of the microbiota.

Although no clinical studies have been conducted with enteral tube feeding formulas containing 6'-SL, published clinical studies administering other non-digestible, poorly absorbed carbohydrates in enteral tube feeding formulas are relevant to understanding the tolerance of 6'-SL as a non-digestible carbohydrate in enteral tube feeding formulas. As summarized in an amendment to GRN 897 to support the safe use of another HMO, 2'-FL, in enteral formulas, numerous published clinical studies have administered non-digestible carbohydrates, such as partially hydrolyzed guar gum (PHGG), galactomannan, fructooligosaccharides (from short-chain FOS to long-chain inulin), galactooligosaccharides (GOS), and GOS/FOS blends in enteral formulas to infants, children, healthy adults, bed-ridden elderly adults, and patients hospitalized for a variety of serious medical conditions (Akatsu et al., 2016; Alam et al., 2000; Alam et al., 2005; Armanian et al., 2016; Fussell et al., 1996; Garleb et al., 1996; Homann et al., 1994; Homann et al., 2004; Karakan et al., 2007; Khoshoo et al., 2010; Lampe et al., 1992; Meier et al., 1993; Modi et al., 2010; Nakao et al., 2002; Peters and Davidson, 1996; Rushdi et al., 2004; Simakachorn et al., 2011; Spapen et al., 2001; van den Berg et al., 2015; Zheng et al., 2006). Because these studies are extensively summarized in an amendment to GRN 897, their summaries are incorporated by reference and briefly summarized in tabular format below (Table 8). Collectively these studies show that the use of non-digestible carbohydrates in enteral tube feeding formulas at levels up to 63 g/day is well-tolerated.

The Institute of Medicine has also evaluated the potential adverse effects associated with overconsumption of non-digestible carbohydrates such as PHGG, FOS, and GOS, and concluded that although occasional adverse gastrointestinal symptoms can occur (flatulence, abdominal distress, and diarrhea), serious chronic adverse effects have not been observed. Additionally, due to the bulky nature of these substances, excess consumption is likely to be self-limiting and tolerable upper limit (UL) was not established (Eldridge et al., 2019).

Taken together, these data indicate that the risk of adverse effects from the judicious use of non-digestible carbohydrates, such as 6'-SL, in enteral formulas intended for patients with serious medical conditions is generally low and within the GRAS standard of reasonable certainty of no harm.

Table 8. Clinical Studies of Non-digestible Carbohydrates Administered Via Enteral Feeding[1]

Citation	Study Design	Treatments	Duration	Safety-Related Findings
Partially Hydrolyzed Guar Gum (PHGG)				
Lampe et al., 1992	Prospective, randomized, placebo-controlled, double-blind, crossover study 11 healthy men	1. Self-selected diet 2. Enteral formula containing no added fiber (maltodextrin) 3. Enteral formula containing 15 g PHGG/day 4. Enteral formula containing 15 g soy polysaccharide	18 days with a 10 day - washout between each diet period	• 12 subjects completed the study; one man did not comply with the diet protocol and his data were excluded from the analyses. No other adverse events were reported. • Compared to the enteral diet with no fiber, fecal wet and dry weights, frequency, stool weight, fecal consistency, fecal moisture, and fecal pH were not statistically different, whereas mean transit time and fecal nitrogen were significantly increased in the PHGG-treated group. • Compared to the enteral diet with no fiber, fecal wet and dry weights, fecal nitrogen, frequency, stool weight, fecal consistency, and fecal pH were not statistically different, whereas mean transit time was significantly decreased and fecal moisture was significantly increased in the soy polysaccharide-treated group. • Colonic fluid acetate, propionate, butyrate and total short chain fatty acids were not significantly different between the PHGG- and no fiber-treated groups • The authors concluded that "despite significant differences in mean transit time, few differences in other parameters of bowel function were observed when healthy subjects consumed enteral formula diets containing 0 g of fiber and 15 g of total dietary fiber as modified guar and soy."
Meier et al., 1993	Randomized, placebo-controlled crossover study 12 healthy men	1. Standardized normal diet 2. Liquid formula diet 3. Liquid formula diet supplemented with PHGG; intake 42 g PHGG/day	7 days with a 7-day washout between each diet	• Significantly increased colonic but not orocecal transit time compared with either a self-selected diet or the enteral formula without fiber. • PHGG did not affect on stool consistency or frequency.

Table 8. Clinical Studies of Non-digestible Carbohydrates Administered Via Enteral Feeding[1]

Citation	Study Design	Treatments	Duration	Safety-Related Findings
Homann et al., 1994	Prospective, randomized, double-blind, placebo-controlled trial 100 hospital patients (30 receiving total enteral nutrition and 70 receiving enteral supplementation)	1. Standard diet 2. Standard diet with 20 g PHGG/L of formula; intake of TPN patients = 24 g PHGG/day; intake of enteral supplementation patients = 20 g PHGG/day	Total enteral nutrition was given for a minimum of 5 days	• Patient receiving either total or supplemental enteral nutrition had reduced incidence of diarrhea, but increased flatulence when receiving the standard diet with PHGG compared to those receiving the standard diet alone. • In the patients receiving total enteral nutrition, four patients on the standard total enteral diet, but no patients on the standard diet with PHGG discontinued due to diarrhea. • In the supplemental feeding groups, four patients receiving the standard diet vs. two receiving the standard diet with PHGG discontinued gastrointestinal side effects. • The authors, therefore, reported that: o The total number of patient with gastrointestinal side effects that resulted in discontinuation of the enteral feeding dropped from eight to two in the standard diet vs the standard diet with PHGG o The total number of GI-side effects was not different in the two groups (17 in each group).
Fussell et al., 1996 (Abstract)	Prospective, randomized, double-blind, placebo-controlled study 57 tube-fed adults in 5 diagnostic categories: abdominal surgery/ trauma, cerebral trauma, head/neck surgery, multiple fractures, and vascular surgery	1. Fiber free tube feeding formula 2. Fiber free tube feeding formula w/14 g PHGG/L of formula	5-14 days	• Forty-four patients completed the protocol. • There was no effect of the fiber on daily diarrhea, nor on albumin, transthyretin, or flatulence. • The PHGG was generally well tolerated.

Table 8. Clinical Studies of Non-digestible Carbohydrates Administered Via Enteral Feeding[1]

Citation	Study Design	Treatments	Duration	Safety-Related Findings
Peters and Davidson, 1996	Prospective, randomized, double-blind cross-over study 12 enterally fed patients with Type 1 diabetes	1. Formula containing 29% fat, 55% carbohydrate, and PHGG 2. Formula containing 40% fat, 44% carbohydrate, and PHGG 3. Formula containing 50% fat, 33% carbohydrate, and soy polysaccharide 4. Ensure (53% carbohydrate and no fiber	1 day with a week in between treatments	• The 2 formulas containing PHGG (concentration not specified) were not effective in attenuating the postprandial glucose response. • No adverse effects were reported.
Spapen et al., 2001	Prospective, randomized, double-blind, placebo-controlled study 25 ICU patients (13 M, 12 F; mean age = 68.5±13.1 years) with severe sepsis and septic shock fed enterally	1. Control formula 2. Formula containing 22 g PHGG/L of formula	At least 6 days	• The group receiving PHGG supplementation exhibited a significantly reduced frequency of diarrhea and a reduction in the number of days with diarrhea • PHGG supplementation had no significant effect on sepsis-related mortality (1 death in the test group, 4 in the control) or duration of stay in the intensive care unit. • The authors concluded: o "Fiber treatment was well-tolerated" o "Total enteral nutrition supplemented with soluble fiber is beneficial in reducing the incidence of diarrhea in tube-fed full-resuscitated and mechanically ventilated septic patients."
Homann et al., 2004	Prospective, randomized, double-blind, placebo-controlled trial 100 medical and surgical patients (50 patients per group); 30 patients received total enteral nutrition and 70 patients received 1000 ml/day supplemental enteral nutrition	1. Standard diet 2. Standard diet with 20 g PHGG/L of formula; intake of TPN patients = 24 g PHGG/day; intake of enteral supplementation patients = 20 g PHGG/day	Total enteral nutrition was given for a minimum of 5 days	• The PHGG-supplemented formula significantly reduced the number of patients with diarrhea (6 vs. 15 on the fiber-free formula) and the number of days patients suffered from diarrhea (10.2 vs. 40.6 days). • The number of patients experiencing GI side effects was the same in both groups (n = 17 per group), although flatulence was reported in more patients in the PHGG group. • Enteral nutrition was discontinued due to GI side effects in 4 patients on the control/standard diet, but no patients on the PHGG-supplemented diet.

Table 8. Clinical Studies of Non-digestible Carbohydrates Administered Via Enteral Feeding[1]

Citation	Study Design	Treatments	Duration	Safety-Related Findings
Rushdi et al., 2004	Prospective, randomized, double-blind, controlled study 30 IBS patients (11 M, 9 F; aged 28-73 years with mean age = 57/5±13/8 years) on enteral nutrition with 3 or more liquid stools/day	1. Standard fiber-free feed 2. Enteral feed enriched with 222 g PHGG/L (22 to 37 g PHGG/day)	4 days	• 20 patients completed the protocol (n=10/group); the ten patients that did not complete the protocol because they switched to parenteral nutrition or oral diet, death, or leaving the ICU before completing the study. • Supplementation with PHGG significantly reduced the number of liquid stools. • There were no differences in the incidence or severity of gastrointestinal symptoms between the two groups. • The authors discussed tolerance issues extensively: "Throughout the course of this clinical trial, in the fiber- enriched feed group, only two patients complained of flatulence (20%). On the other hand, in the control group, four patients complained of flatulence (40%), two patients got vomiting (20%) and one case of constipation (10%) was reported. However, no statistical significance was found between both groups as regards incidence or severity of gastrointestinal symptoms. None of these symptoms was severe enough to necessitate therapeutic intervention."
Galactomannan				
Nakao et al., 2002	Open-label study 20 elderly bed-ridden males and females (10 M, 10 F, mean age = 79.3±5.1 years) receiving enteral feeding	A semi-digested formula containing galactomannan 7 g galactomannan/day during the first week; the dose was increased 7 g/day each week until they received 28 g galactomannan/ day for the fourth week	4 weeks	• No adverse effects were reported. • Serum diamine oxidase activity significantly increased following the treatment with the semidigested formula containing galactomannan. • The water content of the feces decreased, and the frequency of normal stools increased with the semidigested formula containing galactomannan. • The frequency of bowel movements, the number of aerobic bacteria, and the pH of feces decreased, while fecal SCFA, especially acetic and propionic acids, increased with the semidigested formula containing galactomannan. • All effects reversed after termination of the galactomannan supplementation. • There was no change in counts of total bacteria or anaerobes and no change in body weight, total serum protein, prealbumin, transferrin, retinol-binding protein, total cholesterol, triacylglycerol, iron, copper, or zinc.

Table 8. Clinical Studies of Non-digestible Carbohydrates Administered Via Enteral Feeding[1]

Citation	Study Design	Treatments	Duration	Safety-Related Findings
Fructooligosaccharides				
Karakan et al., 2007	Randomized, double-blind, placebo-controlled study 30 patients aged 46.1±14.0 years with severe acute pancreatitis requiring stoppage of oral feeding for 48 hr	1. Diet 2. Diet containing 0.7 g/soluble fiber and 0.8g/100 g insoluble fiber (24 g/day)	2 days	• Both enteral feeding solutions were well tolerated with no reported adverse effects. • The median duration of enteral feeding and the hospital stay was significantly shorter in the group receiving the fiber-containing diet. • The fiber-containing diet also significantly improved the pancreatitis severity scores. • The authors concluded that nasojejunal EN with fiber supplementation in severe AP improves hospital stay, duration of nutrition therapy, acute phase response and overall complications compared to standard EN therapy.
Khoshoo et al., 2010	Randomized, double-blind crossover study 14 children aged 1-15 years receiving 75- 100% of calories via feeding tube and were candidates for receiving a peptide-based enteral formula based on documented gastrointestinal dysfunction	1. Formula 2. Formula with 3.5 g FOS/L (approximately 3.5 g FOS/ day)	2 weeks with a 5-day washout period between treatment periods	• There were nine patients with neurological disorders; 3 patients with inflammatory bowel disease; and 2 patients with short bowel syndrome • There were no withdrawals. • There was no significant difference in the daily number of bowel movements between children receiving either the fiber or control formulas when evaluating the three diagnoses groups combined or the short bowel syndrome group alone. • The children with neurological impairments had more frequent bowel movements when fed the control formula than when fed fiber formula whereas the inflammatory bowel disease group had more daily bowel movements when fed the fiber-containing formula • Stools were in the "mushy" category when the participants consumed the fiber containing formula • Children with neurological impairment had a significantly lower proportion of stools ($P<0.05$) characterized as hard nuts and a significantly lower proportion of stools. • In the inflammatory bowel disease group, stool frequency was higher with the fiber formula, but there was no change in consistency. • There was no difference in the occurrence of vomiting between the two treatments in any of the groups • The nine children with a neurological disorder, the mean grade of flatulence/gas was significantly less ($P<0.05$) when participants consumed the fiber formula whereas there was no difference in flatulence in the other groups.

Table 8. Clinical Studies of Non-digestible Carbohydrates Administered Via Enteral Feeding[1]

Citation	Study Design	Treatments	Duration	Safety-Related Findings
				• There were no differences in abdominal pain or weight gain among the different groups. • The authors concluded, "This study showed that a peptide-based formula containing fiber was as well-tolerated as a fiber-free formula in a small population of children with gastrointestinal impairments."
Garleb et al., 1996	Randomized, double-blind, controlled study 27 healthy male college students (n=9/treatment group)	1. Formula 2. Formula with 5 g scFOS/L (approx. 15 g scFOS/day) 3. Formula with 10 g scFOS/L (approx. 30 g scFOS/day)	14 days	• One subject dropped out of the study after one day due to intolerance to the liquid product. The subject was replaced with an alternate. • There were no differences in body weight or deviations from the normal range of blood chemistry values among the three treatment groups. • Although there were no differences in propionate or butyrate, fecal pH, or fecal percent dry matter, fecal acetate, isobutyrate, and isovalerate concentrations were higher among students ingesting scFOS. • Consumption of scFOS also increased fecal bifidobacteria. • Complaints of nausea, cramping, distension, vomiting, diarrhea, and regurgitation were similar across all groups and were present on fewer than 5% of participant-days. • Flatus was reported more frequently by those consuming 30 g scFOS/day, but most complaints occurred during the first 4 days. • The authors concluded that "these results indicate that [scFOS] does not compromise serum chemistry profiles, is well tolerated particularly at an intake of 15 g/d and would serve as a bifidogenic factor when incorporated into a liquid enteral product."
Simakachorn et al., 2011	Randomized, double-blind, placebo-controlled study 94 critically ill children age 1-3 years under mechanical ventilation and enteral feeding (n=47/groups)	1. Control formula 2. Test formula with 2.6 g/L of oligo-fructose/inulin and 2.8 g/L of acacia gum in combination with 2 strains of live microorganisms	7 days of enteral feeding followed by 14 days of oral feeding	• 6 children withdrew from the test formula group; 8 children withdrew from the control formula group. One child withdrew consent in the test formula group, 5 children withdrew consent in the control formula group. • One child was lost to follow-up in the test formula group (moved to another hospital) and one child was lost to follow-up in the control formula group (no reason given). Four children discontinued the intervention in the test formula group due to death whereas two children discontinued the intervention in the control formula group due to death. • There were no significant differences in adverse events between the two groups and no reported secondary infections during the ICU stay.

Table 8. Clinical Studies of Non-digestible Carbohydrates Administered Via Enteral Feeding[1]

Citation	Study Design	Treatments	Duration	Safety-Related Findings
				• Abdominal distension, vomiting, and stool frequency were also unaffected by the fiber. • The authors concluded that the experimental enteral formula is safe and well tolerated by children in intensive care receiving enteral nutrition.
Majid et al., 2014	Randomized, double-blind, placebo-controlled study 47 adults in the intensive care unit	1. Control formula containing soy polysaccharides, resistant starch, Arabic gum, cellulose, inulin, and oligofructose (0.7 g/100 ml soluble fiber and 0.8 g/100 ml insoluble fiber, equivalent to 6.75 g/day)); n=23 2. Formula containing soy polysaccharides, resistant starch, Arabic gum, cellulose, inulin, and oligofructose (0.7 g/100 ml soluble fiber and 0.8 g/100 ml insoluble fiber; equivalent to 6.75 g/day) with and additional 7 g oligofructose/inulin; n=24	A minimum of 3 days	• 12 patients discontinued the study before the intervention (7 in the placebo group and 5 in the oligofructose/inulin group) • 6 patients discontinued the intervention in the control formula group (1 patient transferred to an oral diet and five transferred to palliative care) vs 7 patients discontinued in the oligofructose/inulin group (5 transferred to palliative care and 2 were discharged to another hospital) • There was no significant difference in short-chain fatty acid concentrations at baseline or follow-up between the two groups. • Fecal pH was similar in the two groups at baseline and at follow-up. • There were no significant differences in fecal frequency or the daily fecal score between the two groups. • There was no difference between the two groups in the mean number of days of diarrhea or in the number of patients experiencing diarrhea on either one or two or more consecutive days.

Table 8. Clinical Studies of Non-digestible Carbohydrates Administered Via Enteral Feeding[1]

Citation	Study Design	Treatments	Duration	Safety-Related Findings
Galactooligosaccharides or GOS/FOS				
Modi et al., 2010	Prospective, randomized, double-blind, placebo-controlled, multi-center study 160 preterm infants (gestational age <33 weeks) receiving enteral feeding	1. Standard formula 2. Test formula with 8 g/L of scGOS/lc FOS in a 9:1 ratio	~8 weeks or until discharge	• 83 infants received the standard formula; 77 infants received the test formula containing GOS/FOS. The parents of two and four infants withdrew consent in the standard and test formula groups, respectively. One infant in the standard formula group died before reaching the primary outcome and two infants in the test formula group died before reaching the primary outcome. One infant in the standard formula treated group was discharged before reaching the primary outcome. • Six adverse events were reported by one infant, five of which were not considered related to the trial. • There were three cases of necrotizing enterocolitis (one in the standard formula group vs 2 in the test formula group). • Nineteen infants develop at least one episode of a blood stream infection (10 in the standard formula group vs 9 in the test formula group. • There was no overall difference in tolerance between control and test formula, but the addition of scGOS/lc FOS to formula improved tolerance for the most immature infants. There were no differences in gains in weight, length, or head circumference; in stooling frequency, stool characteristics, or fecal microbiota; or in GI signs or water balance (based on concentrations of serum sodium and creatinine). • The authors concluded that scGOS/lc FOS supplementation is safe.
Akatsu et al., 2016	Prospective, randomized, double-blind, placebo-controlled study 36 elderly individuals	1. Oral feeding (n=13) 2. Enteral formula (n=11) 3. Enteral formula w/ GOS and bifidogenic growth stimulator (BGS; 2-amino-3-carboxy-1,4-naphtho-quinone) (n=12) Products were delivered via percutaneous endoscopic gastrostomy	10 weeks	• No adverse effects were reported.

Table 8. Clinical Studies of Non-digestible Carbohydrates Administered Via Enteral Feeding[1]

Citation	Study Design	Treatments	Duration	Safety-Related Findings
Armanian et al., 2016	Prospective, randomized, double-blind, placebo-controlled study 25 hyper-bilirubinemic preterm neonates who had reached 30 ml/kg bw/day enteral feeding volume	1. Distilled water 2. A supplement containing scGOS/lc FOS in a 9:1 ratio *The supplement was initially administered by 0.5 g/kg/day and then increased to 1 g/kg/day and 1.5 g/kg/day	1 week	• No adverse effects were reported. • Stool frequency was significantly increased in the scGOS/lc FOS-treated group. • The authors concluded that oligosaccharides increase stool frequency, improve feeding tolerance and reduce bilirubin level in preterm neonates and therefore can be efficacious for the management of neonatal hyperbilirubinemia.
Van den Berg et al., 2015	Prospective, randomized, double-blind, placebo-controlled study to determine the effect of combined short-chain galacto-oligosaccharides (scGOS), long-chain fructo-oligosaccharides (lcFOS) and pectin-derived acidic oligosaccharides (pAOS) on antibody concentrations after pneumococcal conjugate vaccination in very preterm infants. 113 infants with a gestational age of <32 weeks or birth-weight <1500 g	1. Placebo/maltodextrin (n=58) 2. scGOS/lc FOS/ pectin-derived acidic oligo-saccharides(pAOS) (n=55)	4 weeks	• Nine infants died in the placebo-treated group whereas six infants died in the scGOS/lc FOS/pAOS-treated group. • Adverse events were not reported. • The authors concluded "Short-term supplementation of scGOS/lcFOS/pAOS during day 3–30 of life decreased the pneumococcal vaccine antibody response after the primary series of PCV7 at 5 months in preterm infants to levels which are similar in term infants from a Dutch population study. However, after the booster vaccination at 12 months, this effect of the scGOS/lcFOS/pAOS on the PCV response had disappeared."

[1]Incorporated by reference from the amendment to GRN 897.

3. Clinical Studies with Other Non-digestible Carbohydrates and Oral Electrolyte Solutions

a. Background

Oral electrolyte solutions (OESs) are liquid products that facilitate rapid and effective rehydration. OESs contain, at a minimum, a digestible carbohydrate such as dextrose and sodium in water to facilitate water absorption from the lumen of the gastrointestinal tract. Specifically, dextrose absorption facilitates sodium ion absorption, which thereby raises the concentration of sodium ions in the blood stream, pulling water from the lumen of the gastrointestinal tract into the blood stream. Importantly, this is all accomplished through a balance between the amount of carbohydrate and the electrolytes in the OES. Additionally, although sodium absorption improves as the dextrose concentration of the oral fluid is increased up to about 2.5% w/w, higher concentrations of dextrose can increase the osmotic load in the gut, pulling water out of the blood stream, further exacerbating dehydration. Simple sugars such as dextrose and fructose have also been shown to be more effective than larger, more complex carbohydrates in facilitating electrolyte absorption and many oligosaccharides are not stable in acidic mediums such as OESs. As a result, conventional OESs generally do not include oligosaccharides or polysaccharides (Patent 10,695,358, date issued June 30, 2020 Abbott Laboratories).

Importantly, non-digestible carbohydrates, such as 6'-SL, 2'-FL, GOS, FOS and LNnT stimulate the growth or activity, or both, of Bifidobacterium in the gastrointestinal tract (reviewed in Gibson and Roberfroid, 1995). Non-digestible carbohydrates are also fermented by the colonic bacteria to short-chain fatty acids (SCFA), which are rapidly absorbed in the colon and further promote fluid and sodium absorption (reviewed in Binder et al., 2014). Thus, OESs supplemented with non-digestible carbohydrates, such as 6'-SL, may facilitate rehydration, as well as maintenance of the microbiota.

b. Use of Non-Digestible Carbohydrates in Acute Diarrhea and As an Ingredient in Oral Electrolyte Solutions

The safety and tolerance of numerous non-absorbable carbohydrates (GOS, FOS, xylooligosaccharides (XOS)) have been extensively reviewed and been the subject of numerous GRAS Notices (GRNs 44, 172, 233, 236, 246, 285, 286, 334, 343, 370, 458, 484, 495, 518, 537, 569, 605, 620, 623, 671, 674, 717, 721, 729, 779, 797, 816, 818, 896); human milk oligosaccharides have also been extensively reviewed and the subjects of numerous GRAS Notices (2'-FL: GRNs 546, 571, 650,735, 749, 815, 852, 859, 897; 3-FL: GRN 925; 3'-SL and 6'-SL: GRNs 766, 880, 881, 921, 922; LNT: GRN 923; LNnT: GRNs 919, 895).

During diarrhea, pathogenic bacteria may either grow and colonize the gastrointestinal (GI) tract and then invade the host tissues or, alternatively, they may secrete toxins which may disrupt the function of the intestinal mucosa, causing nausea, vomiting, and diarrhea. Oli et al., (1998) showed that in a pig model, adding fructo-oligosaccharides (FOS) to an OES accelerated the recovery of lactobacilli and reduced bacterial counts of Enterobacteriaceae. Brunser et al. (2006) studied the effect of FOS on the intestinal microbiota during treatment with amoxicillin and reported an increase in bifidobacteria in patients receiving FOS after seven days of antibiotic treatment compared to a control group. These authors reported that the effect of FOS on the occurrence of antibiotic-related diarrhea episodes was not significant. Vaisman et al. (2010) investigated the effect of a mixture of long-chain FOS, GOS, and acidic oligosaccharides on the number and consistency of stools and on immune system biomarkers in 104 supplemented and non-supplemented subjects (aged 9–24 months) with acute diarrhea. No treatment-related adverse effects were reported. Additionally, studies of OESs supplemented with non-digestible carbohydrates and/or sources of non-digestible carbohydrates, such as guar gum, FOS, XOS, and high amylose maize starch, indicate that non-digestible carbohydrates do not exacerbate acute diarrhea (Table 8; Alam et al., 2015; Passariello et al., 2011; Vandenplas et al., 2011; Raghupathy et al., 2006; Hoekstra et al., 2004; Alam et al., 2000). Therefore, based on the weight of the evidence, adverse effects resulting from the addition of 6'-SL to OESs are not expected.

c. Lack of Impact of 6'-SL on Osmolarity

The WHO current standard OES osmolarity is 245 mOsm/L; Pedialyte® from Abbott is 250 mOsm/L (Ofei et al., 2019). Despite common perceptions that sport drinks can be used for dehydration, liquid products such as sports beverages and juices are hyperosmolar (330–730 mOsm/L) and inappropriate as rehydration solutions for diarrhea and dehydration because they increase fluid losses and worsen the diarrheal disease. It is critical that the addition of any ingredient to an OES not impact the osmolarity. The addition of 0.15 g/L of 6'-SL to OES, such as Pedialyte®, is calculated on the basis of molar weight to add 6.5 mOsm/L (0.24 mOsm/L 6'-sialyllactose and 6.25 mOsmL/l sodium). Thus, the addition of 1.2 g/L of 6'-SL will not impact the osmolarity of the OES.

Table 9. Studies of Oral Electrolyte Solutions (OES) with Added Nondigestible Carbohydrate

Reference	Trial Design	Test Article	Results
Alam et al., 2015	Randomized, double-blind placebo controlled clinical trial of 126 malnourished children (male and female) (weight for length/weight for age <3 Z-score with or without pedal edema), aged 6-36 months with acute diarrhea	• Group 1: Standard hypotonic oral rehydration solution (ORS) • Group 2: Standard hypotonic ORS with 15 g/L partially hydrolyzed guar gum	• The mean duration of diarrhea was significantly shorter in children in Group 2 compared to Group 1. • Adverse events/tolerance related to test article not reported by authors.
Passariello et al., 2011	Single-blind, prospective, controlled trial including children (age range, 3-36 months) with acute diarrhea	• Group 1: Standard hypotonic oral rehydration solution (ORS) • Group 2: hypotonic ORS with zinc, 0.35 g/L fructooligosaccharides and 0.35 g/L xylooligosaccharides	• Resolution of diarrhea at 72 hours, number of daily outputs at 24, 48, and 72 hours was statistically significantly improved in Group 2 compared to Group 1. • Total ORS intake in the first 24 hours of rehydration therapy was statistically significantly lower in Group 1 than Group 2. • No adverse events related to the use of the ORS were observed in the study groups.
Vandenplas et al., 2011	Randomized, prospective, double-blind placebo-controlled trial in children between 3 and 186 months (males and females) with acute diarrhea	• Group 1: Standard hypotonic oral rehydration solution (ORS) • Group 2: Standard hypotonic ORS with a symbiotic blend (*Streptoccoccus thermophilus*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Bifidobacterium lactis*, *Bifidobacterium infantis*, fructo-oligosaccharides).	• Children in Group 2 had significantly reduced duration of diarrhea compared with Group 1. • Adverse events/tolerance related to test article not reported by authors.
Raghupathy et al., 2006	Randomized, double-blind, placebo-controlled study including boys aged 6 months to 3 years with acute diarrhea with clinically detectable dehydration	• Group 1: Standard hypotonic oral rehydration solution (ORS) (311 mOsm/kg) • Group 2: Standard hypotonic ORS with 50 g/L high-amylose maize starch	• Statistically significant shortened duration of diarrhea in Group 2 compared to Group 1. • Before the start of this study high-amylose maize starch ORS was administered orally to 6 children with acute diarrhea and found to be well tolerated. It did not induce vomiting or significant increase in diarrhea.

Table 9. Studies of Oral Electrolyte Solutions (OES) with Added Nondigestible Carbohydrate

Reference	Trial Design	Test Article	Results
Hoekstra et al., 2004	Randomized, double-blind, placebo-controlled multicenter study including boys aged 1 to 36 months with acute diarrhea	• Group 1: Standard hypotonic oral rehydration solution (ORS) • Group 2: Standard hypotonic ORS with a mixture of non-digestible carbohydrates (soy polysaccharide 25%, alpha-cellulose 9%, gum arabic 19%, fructooligosaccharides 18.5%, inulin 21.5%, resistant starch 7%)	• No significant differences in mean 48 hours stool volume or duration of diarrhea in Group 2 compared to Group 1. • No significant adverse effects, as compared to ORS with placebo, were noted.
Alam et al., 2000	Double-blind, randomized, placebo controlled clinical trial of 150 male children aged 4 to 18 months who had acute diarrhea	• Group 1: Standard hypotonic oral rehydration solution (ORS) • Group 2: Standard hypotonic ORS with 15 g/L partially hydrolyzed guar gum	• Children in Group 2 had significantly reduced duration of diarrhea compared with Group 1. • Adverse events/tolerance related to test article not reported by authors.

G. ALLERGENICITY

The allergenicity of Chr. Hansen A/S's 6'-SL ingredient was extensively reviewed in GRN 922. Therefore, the allergenicity summary in GRN 922 is incorporated by reference (see page 72 of GRN 922). Allergic reactions resulting from the exposure to Chr. Hansen A/S's 6'-SL product are not expected based on the following:

- 6'-SL is a component of human milk;
- Allergic reactions to HMOs have not been reported;
- Genetically engineered strains of *E. coli* BL21(DE3) are safely used in the production of food and pharmaceutical ingredients;
- Cross-reactivity of the genes used to engineer *JBT-6SL* with known allergens is not expected based on the results of FASTA amino acid alignments with the AllergenOnline Database maintained by the University of Nebraska – Lincoln;
- The protein content of Chr. Hansen A/S's 6'-SL is controlled with a specification of ≤ 0.01 % protein.

H. REGULATORY APPROVALS ACROSS THE WORLD

In the United States, 6'-SL is GRAS for use in infant formula and conventional foods and the subject of two GRAS Notifications 881 and 922. It is also the subject of a Novel Food application in the European Union for use in infant and follow-on formulas, conventional foods, foods for special medical purposes, and food supplements (https://ec.europa.eu/food/sites/food/files/safety/docs/novel-food_sum_ongoing-app_2019-0881.pdf; accessed on January 1, 2021), although an opinion by the European Commission has not been published. Following their review of the 6'-SL Novel Food application submitted by Glycom A/S, the European Food Safety Authority stated that the NOAEL for Glycom's 3'-SL is 3,000 mg/kg bw/day because there was no clear explanation for the small and soft testes characterized by severe unilateral tubular atrophy and absence of sperm in the epididymis in one testis of each of four rats in the 5000 mg/kg treated group (EFSA Panel on Nutrition et al., 2020). Importantly, EFSA opined that the intake of 6'-SL at the proposed use levels is unlikely to exceed the intake level of naturally occurring 6'-SL in breastfed infants on a body weight basis, the intake of other carbohydrates structurally related to 6'-SL is not a safety concern, and that 6'-SL is safe for use in infant and follow-on formulas at 0.4 and 0.3 g/L, respectively, and in selected conventional foods and food supplements up to 2.5 g/kg.

VII. SUPPORTING DATA AND INFORMATION

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 6'-SL in toddler formulas, foods for infants and young children, meal replacements drinks for adults, non-carbonated drinks, bars, oral electrolyte solutions, and enteral tube feeding formulas 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 6'-SL in non-exempt term infant formula 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 6'-SL as an ingredient for the intended use in toddler formulas, foods for infants and young children, meal replacements drinks for adults, non-carbonated drinks, bars, oral electrolyte solutions, and enteral tube feeding formulas has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. The subject of this GRAS Determination is a spray-dried, powdered food ingredient that contains not less than 90% 6'-SL dry weight. The remaining components include carbohydrate by-products, ash, and moisture.

    a. 6'-Sialyllactose is a naturally occurring acidic oligosaccharide in human milk.

    b. Published studies showing that the amount of 6'-SL in breast milk ranges from 0.01 to 1.7 g/L.

    c. Human milk oligosaccharides, including 6'-SL, are resistant to the digestive enzymes in the gastrointestinal tract, poorly absorbed, and pass through the gastrointestinal tract where they are either fermented by the microbiota or excreted unchanged.

2. The subject of this GRAS determination is the subject of GRN 922, which received a "no question" letter on April 23, 2021 for the use of 6'-SL in non-exempt term infant formula.

    a. The subject of this GRAS Determination is manufactured using a genetically engineered strain of *Escherichia coli* BL21(DE3) by Chr. Hansen A/S in a Food Safety System Certification (FSSC) 22000-, ISO 9001:2015-, GMP-,

and International Featured Standards Food 6.1-compliant facility. Chr. Hansen A/S is an FDA-registered food facility.

b. The genetically engineered strain of *E. coli* BL21(DE3) used by Chr. Hansen A/S is not toxigenic and not capable of DNA transfer to other organisms and has the same virulence profile as *E. coli* BL21(DE3).

c. All raw materials, processing aids, and food contact substances are GRAS and/or conform to the specifications stated in 21 CFR and/or the Food Chemicals Codex (FCC).

d. Fermentation by-products include lactose, sialic acid, and *N*-acetylglucosamine which are known human milk oligosaccharides; their presence in the finished ingredient is not of toxicological concern.

e. Process procedures and product specifications are in place to control the levels of residual impurities and carbohydrate by-products, as well as heavy metals, microbes, and production organism-derived DNA and possible endotoxin, ensuring a consistent, safe, food-grade finished ingredient.

f. The available stability studies indicate a shelf-life of one year when stored from the date of production under ambient conditions.

g. Use of the subject of this GRAS determination in the intended selected conventional foods and enteral tube feeding formulas results in mean and 90th percentile estimated daily intakes (EDIs) of 0.258 and 0.706 g/day (0.004 and 0.010 g/kg bw/day) for consumers not less than 2 years-old.

h. Use of the subject of this GRAS determination in selected conventional foods and enteral tube feeding formulas results in mean and 90th percentile cumulative estimated daily intakes (EDIs) of 0.208 and 0.42 g/day (0.003 and 0.006 g/kg bw/day) for consumers not less than 2 years-old.

i. Use of the subject of this GRAS determination in oral electrolyte solutions results in an estimated daily intake of 0.15 – 0.3 g of 6'-SL (equivalent to 1.1 – 2.2 mg of 6'-SL /kg bw/day assuming a 13.5 kg toddler and 0.2 – 0.4 mg of 6'-SL /kg bw/day assuming a 70 kg adult). Because OESs are intended for short term use, intake of 6'-SL from OESs will not impact the cumulative 6'-SL intake resulting from the use of 6'-SL in select conventional foods and enteral tube feeding formulas.

3. Genotoxicology and subchronic toxicology studies published by Phipps et al. (2019) show that 6'-SL is not genotoxic and has a no observed adverse effect level (NOAEL) of 5 g/kg bw/day, which was the highest dose tested.

4. The safety of exposure to Chr. Hansen A/S's 6'-SL at its intended use level is supported by:

    a. Data demonstrating the qualitative and quantitative similarities between the subject of this GRAS Determination and the 6'-SL ingredient tested in the pivotal genotoxicology and subchronic toxicology studies conducted by Phipps et al. (2019), which is also the subject of GRN 881;

    b. The lack of genotoxicity and no observed adverse effect level (NOAEL) for 6'-SL established in the 90-day subchronic dietary toxicology conducted by Phipps et al. (2019);

    c. Published genotoxicology and 90-day subchronic toxicology and neonatal piglet studies conducted with 6'-SL or a mixture of HMOs containing the subject of the GRAS determination (Parschat et al., 2020; Monaco et al., 2020; Hanlon, 2020);

    d. Clinical data showing the ingestion of HMOs are well tolerated in infants up to 1.0g/day and adults up to 20 g/day;

    e. Clinical data showing that the use of other non-digestible carbohydrates in infants, adults, enteral tube feeding products, and oral electrolyte solutions is well tolerated up to 63 g/day;

    f. The GRAS status of the subject of this GRAS determination for use in infant formula (GRN 922);

    g. The GRAS status of other 6'-SL products for use in selected conventional foods (GRN 881).

Therefore, 6'-SL is safe and GRAS at the proposed level of addition to the intended toddler formulas, foods for infants and young children, meal replacements drinks for adults, non-carbonated drinks, bars, oral electrolyte solutions, and enteral tube feeding formulas. 6'-Sialyllactose 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.

Peter Pressman, MD, MS, FACN, Signature: [redacted]
GRAS Expert Panel Member
Medicine Public Health & Nutrition Date: May 18, 2021
The Daedalus Foundation

A. Wallace Hayes, PhD, DABT, FATS, ERT Signature: [redacted]
GRAS Expert Panel Member
Harvard School of Public Health Date: May 18, 2021

Thomas E. Sox, PhD, JD Signature: [redacted]
GRAS Expert Panel Member
Principal, Pondview Consulting LLC Date: May 18, 2021

Claire Kruger, PhD, DABT Signature: [redacted]
Scientific Advisor to the Panel
Date: May 18, 2021

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
001016	Jun 8, 2021
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	Position or Title
Kate Urbain	Head of Regulatory Affairs North America

Organization *(if applicable)*: Chr. Hansen A/S

Mailing Address *(number and street)*: 9015 W Maple St.

City	State or Province	Zip Code/Postal Code	Country
West Allis	Wisconsin	53214	United States of America

Telephone Number	Fax Number	E-Mail Address
414-607-5819		USKAUR@chr-hansen.com

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position or Title
Dietrich B. Conze	Managing Partner

Organization *(if applicable)*: Spherix Consulting Group, Inc.

Mailing Address *(number and street)*: 751 Rockville Pike, Unit 30-B

City	State or Province	Zip Code/Postal Code	Country
Rockville	Maryland	20852	United States of America

Telephone Number	Fax Number	E-Mail Address
240-367-6089		dconze@spherixgroup.com

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

6'-Sialyllactose Sodium Salt (6'-SL)

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 922

☐ 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)* GRN 546, 571, 650, 659, 735, 749, 766, 815, 852, 880, 897, 919, 921

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.

Chr. Hansen A/S intends to use 6'-SL as an ingredient in toddler formulas, foods for infants and young children, meal replacements drinks for adults, non-carbonated drinks, bars, oral electrolyte solutions, and enteral tube feeding formulas.

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 Chr. Hansen A/S

(name of notifier)

has concluded that the intended use*(s)* of 6'-Sialyllactose Sodium Salt (6'-SL)

(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. Chr. Hansen A/S 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)

9015 W Maple St, West Allis, WI 53214

(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, PhD Digitally signed by Dietrich B. Conze, PhD Date: 2021.06.04 11:07:13 -04'00'	Dietrich B. Conze, PhD, Managing Partner	06/04/2021

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)
	Chr Hansen 6'-SL GRAS to FDA.pdf	Submission
	References	Submission

Spherix Consulting Group

January 28, 2022

Jason Downey, Ph.D.
Regulatory Review Scientist
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive, HFS-225
College Park, MD 20740

RE: Questions Regarding GRN 001016

Dear Dr. Downey:

Below are our responses to your requests for additional information regarding GRN 001016 as stated in your email on January 3, 2022. Your requests are in italicized text and our responses are in plain text below.

1) *In Table 1 (page 1), you state that 6'-SL is intended for use in milk-based meal replacement beverages for children such as Pediasure. Please specify if by the term "milk," you are referring to cow and/or soy milk or other milk analogs.*

The intended use in milk-based meal replacement beverages encompasses meal replacement beverages for children, such as PediaSure or Boost Kid Essentials. These products often contain a combination of milk and soy protein ingredients, in addition to other macro- and micro-nutrients. These products are covered in previous GRAS notifications for other HMOs such as 2'-fucosyllactose (GRN 000897; GRN 000852; GRN 000735), referenced as meal-replacement beverages.

2) *In Table 1 (page 1), please specify the age ranges that correspond to certain populations indicated under the intended uses (i.e., "children" and "young children"). Please also specify the uses attributed to the different age groups listed in detail in the dietary exposure section (Tables 4 and 5; page 15).*

The intended uses listed in Table 1 and Table 3 that specify "for infants and young children" and "for children" refer to products listed as "baby food" or "baby juice" in the NHANES database. The food codes for these products, which were used to calculate the dietary exposures presented in in Table 4, are 56210000, 5780XXXX, and 5782XXXX, where X denotes any digit from 0-9. The food codes for these products, which were used to calculate the dietary exposures presented in Table 5, are 114800XX, 13310000, 13311000, 13312000, 200000XX, 21701XXX, 22810010, 22820000, 23410010, 23420010, 24701XXX, 247030XX, 24705010, 24706010, 27601000, 27610XXX,

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

27640XXX, 27641000, 276421XX, 27644110, 5380XXXX, 54350XXX, 54360000, 54408100, 56210000, 5780XXXX, 5782XXXX, 57830100, 58503XXX, 58508XX, 58509XXX, 67100XXX, 67101XXX, 67102XXX, 67104XXX, 67105030, 67106XXX, 67108XXX, 67109XXX, 671130XX, 671140XX, 672020XX, 67203XXX, 67204XXX, 67205000, 67211000, 67212000, 67230000, 67230500, 67250100, 67250150, 67260000, 67304XXX, 67307XXX, 67308XXX, 673090XX, 67404XXX, 67405XXX, 67408XXX, 6741XXXX, 67430000, 67430500, 67501XXX, 67600100, 76102010, 76102030, 7620XXXX, 7640XXXX, 76420000, 76501000, 76502000, 7660XXXX, and 7661XXXX, where X denotes any digit from 0-9.

Importantly, the NHANES database does not specify an age range for these products. Additionally, the estimated daily intakes provided in Tables 4 and 5 were determined by querying the NHANES database with food codes that approximate the intended uses. The unique users of the food codes identified in the query were then binned according to age group (noted in Table 4 and 5), and the 6'-SL intakes for each unique user and each food code was then calculated by multiplying amount of the food consumed for each food code by the 6'-SL inclusion rate. The 6'-SL intakes for each unique user and each food code within each age group were then compiled to obtain the distribution of total 6'-SL intakes within each age group. Because the data in the NHANES database relies on real-world use of the foods associated with each food code, the method provides an accurate representation of the estimated daily intake of the ingredient. The method also prevents bias that may occur if specific uses and/or food codes were assigned to specific age group. Therefore, it is not possible to specify age ranges that correspond to certain populations indicated under the intended uses and the uses attributed to the different age groups listed in detail in the dietary exposure section.

3) *On page 3 of the notice, under 2(i), you provide the dietary exposure for the intended use of 6'-SL in oral electrolyte solutions (OES) as 0.15-0.3 g/d and state that this is equivalent to 1.1-2.2 mg/kg bw/d assuming a 13.5 kg toddler. We note that this conversion is incorrect. Please provide the correct conversion of the dietary exposure based on your weight assumption.*

Yes, the dietary exposures for the intended use of 6'-SL in OESs stated on page 3 of the Notice are incorrect. The correct dietary exposures for the intended use of 6'-SL in OESs are stated on page 11 of the Notice. Item "i." on page 3 should read, "Use of the subject of this GRAS Determination in oral electrolyte solutions results in an estimated daily intake of 0.15 – 0.3 g of 6'-SL (equivalent to 11.1 – 22.2 mg of 6'-SL /kg bw/day assuming a 13.5 kg toddler and 2.1 – 4.2 mg of 6'-SL /kg bw/day assuming a 70 kg adult). Because OESs are intended for short term use, intake of 6'-SL from OESs will not impact the cumulative 6'-SL intake resulting from the use of 6'-SL in select conventional foods and enteral tube feeding formulas."

4) In Table 2 (page 8),

a. you list the specifications of "other carbohydrates" as ≤ 10%. Please provide the levels of the "other carbohydrates" present in your ingredient.

Chr. Hansen's 6'-SL is a high purity ingredient specified to contain ≥90% 6'-SL, with acceptable limits also established for the small amounts of residual carbohydrates that may be present, namely lactose (≤5%), sialic acid (≤10%), and N-acetylglucosamine (≤5%). The "other carbohydrates" parameter in the specifications captures residual carbohydrates that are structurally related to 6'-SL. The level of "other carbohydrates" across five representative batches of the 6'-SL ingredient is below the limit of quantitation (0.5%), as presented in Table 2 (pg. 8) of the current GRAS notice.

b. you list the monosaccharides in 6'-SL that are analyzed by HPAEC-PAD. Please provide a representative chromatogram for each monosaccharide.

A representative chromatogram for 6'-SL (Batch No. 11020039) analyzed by HPAEC-PAD, along with an overlay of the chromatograms for commercially purchased standards for residual carbohydrates, is provided in Figure 1 below.

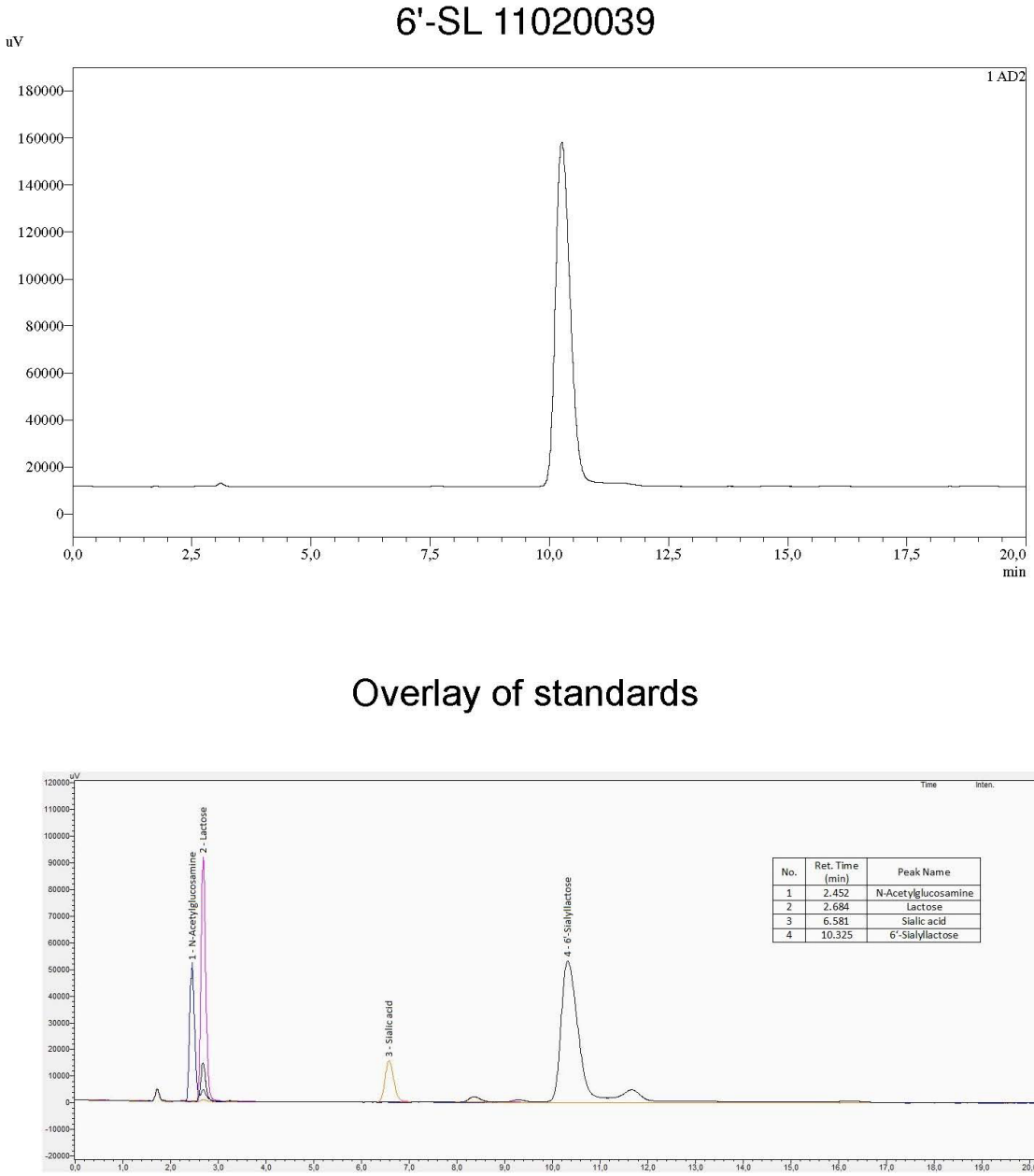

Figure 1. Representative HPAEC-PAD Chromatogram for 6'-SL (Batch No. 11020039), Along with an Overlay of the Chromatograms for the Carbohydrate Standards

c. Please provide the LOQ for the analytical method used to determine the mono- and polysaccharides via HPAEC-PAD.

The LOQ for carbohydrate by-products measured *via* the HPAEC-PAD method is 0.5% (see Footnote 4 of Table 2 in the GRAS notice).

5) Please clarify if 6'-SL will be added to OES as stated on page 11 of the notice and is not a stand-alone use. Also, please provide the formulation of the OES containing 6'-SL that is intended as the article of commerce.

There is a typographical error in first sentence of the first paragraph of Chapter III, Section D.1, entitled "Estimated Daily Intake of 6'-SL from Oral Electrolyte Solutions". 6'-SL is intended to be added to OES intended to prevent dehydration caused by vomiting, diarrhea, or heat exhaustion. This is a stand-alone use. The intended uses of 6'-SL in drinks intended for rehydration (e.g., after exercise or travel) are separately covered by the category "sports, isotonic, and energy drinks" specified in Table 3 of the Notice, which was included in the estimated daily intake assessments provided in Tables 4 and 5 of the Notice.

Regarding the formulation of the OES containing 6'-SL, the formulation of the OES that is intended to be used to prevent dehydration caused by vomiting, diarrhea, or heat exhaustion has an osmolarity of 250 mOsm/L, which approximates the osmolarity recommended by the World Health Organization for oral rehydration solutions (245 mOsm/L). OESs are composed of a combination of water, dextrose, and minerals (electrolytes), and in some formulations, additional ingredients such as galactooligosaccharides, flavorings, sweeteners, and color additives.

6) You provide a dietary exposure estimate to 6'-SL based on food consumption data from the 2015–2016 National Health and Nutrition Examination Survey (NHANES). We note that you have provided the dietary exposure for OES independently of the exposures from other intended uses, based on the statement that the OES (such as Pedialyte) that you intend to add 6'-SL to will not be part of the regular diet. We note that Pedialyte can be consumed in place of other fluid replacement electrolyte solutions. Please provide a cumulative dietary exposure estimate that includes the OES use of 6'-SL.

As discussed in our response to Question 5, the use of 6'-SL in OESs intended to prevent dehydration caused by vomiting, diarrhea, or heat exhaustion is a stand-alone use. The intake assessment was provided separately in Chapter 3, Section 3.D.1 because of its infrequent use and low number of users (i.e., 1 user) in the NHANES database (see pg. 11 of the GRAS Notice). Therefore, a cumulative dietary exposure estimate that includes all products that could be considered under the food category of OES for this use of 6'-SL cannot be justified. With respect to the consideration that OES can be consumed in place of other fluid replacement electrolyte solutions, the intended use of 6'-SL in drinks intended for rehydration after exercise or travel is covered by the "sports, isotonic, and energy drinks" category specified in Table 3 of the Notice and was included in the estimated daily intake and cumulative estimated daily intake assessments provided in Tables 4 and 5 of the Notice, respectively.

7) On page 11 of the notice, you state that the estimated daily intake of 6'-SL is 0.15-0.3 g/p/d in OES (based on 1-2 L consumption/p/d) and 3.8 g/p/d in enteral feeding (Table 3). Further on page 48, you describe the lack of impact of the addition of 6'-SL to the osmolality of OES based on the assumption that a person can consume 1 L OES/d.

a. On page 48, you state, "… the addition of 1.2 g/L of 6'-SL will not impact the osmolarity of the OES". Please clarify the intended use level for 6'-SL in OES. If the intended use level is 1.2 g/L of 6'-SL, please explain why this would not result in a significant change in the osmolarity of the typical OES.

b. Please provide the range of iso-osmolar concentration that is expected in the final food based on the maximum consumption of 6'-SL (0.3 g/p/d) from OES.

c. Please provide the range of iso-osmolar concentration that is expected in the final food based on the maximum consumption of 6'-SL (3.8 g/p/d) from enteral feeding.

Osmolality is a measure of the number of particles of a substance per kilogram of solvent (mOsm/kg), whereas the osmolarity is the measure of the number of particles of a substance per liter of solution (mOsm/L). Osmolarity is not commonly used in osmometry as it is temperature dependent: the volume of a solution can change with temperature. However, assuming that the targeted OESs and enteral feeding formulas are at room temperature, the osmolality should approximate the osmolarity of a solution. Importantly, the use of 6'-SL in OESs and enteral feeding formulas at 0.15 and 3.8 g/L, respectively, will result in fixed osmolarities and osmolalities of the resulting products. Therefore, the exposure to 6'-SL will depend on the amount of the OES or enteral feeding formula consumed.

There is a typographical error in the last sentence on pg. 48 of the GRAS notice, which should read, "Thus, the addition of 0.15 g/L 6'-SL sodium salt will not impact the osmolarity of the typical OES", not "Thus, the addition of 1.2 g/L 6'-SL sodium salt will not impact the osmolarity of the typical OES".

While addressing this question, we found an error in our calculations for the resulting osmolarity of OESs containing 6'-SL-sodium salt that was described in Chapter 6, Section F.3.c (page 48) of the GRAS Notice. The addition of 0.15 g/L of 6'-SL-sodium salt to OESs, such as Pedialyte®, will add approximately 0.44 mOsm/L to the OESs, not 6.5 mOsm/L, based on the molecular weight of 6'-SL-sodium salt of 655.5 g/mol and the dissociation of 6'-SL-sodium salt to 6'-SL and sodium in solution. The values presented in the GRAS notice were calculated using the addition rates of 0.15 g/L for 6'-SL and sodium and the molecular weights of 6'-SL and sodium, which is not correct. Therefore, the resulting osmolarity of OESs containing 6'-SL sodium salt will be approximately 250.44 mOsm/L, which does not significantly change the osmolarity of the typical OES and is comparable to the World Health Organization standard for OESs of 245 mOSm/L. Importantly, because the correct osmolarity of the resulting OES is lower that what was originally stated in the GRAS Notice, the lower osmolarity does affect Chr. Hansen's conclusion regarding the GRAS status of 6'-SL for its intended uses.

Regarding the osmolality of enteral feeding formulas, this typically ranges from approximately 200 to 750 mOsm/kg depending on the formulation (Forchielli et al., 2003). The addition of 3.8 g 6'-SL/kg enteral feeding formula will add approximately 11.6 mOsm/kg to the final formulation. Considering the wide range of osmolality of commercial formulas, and the potential for 6'-SL to replace other carbohydrates in the formulation, the addition of the 6'-SL ingredient is expected to have minimal impact on the osmolality of the formula. Moreover, enteral formulas are expected to be used under medical supervision, with the health care provider selecting a formula with an osmolality that is appropriate for the individual's medical condition.

8) *On page 19, you state, "The ADME … have been extensively summarized in previous GRAS Determination and opinions published by worldwide authoritative bodies …" Each GRAS notice stands on its own. Therefore, while you may refer to previous information using "incorporation by reference", you still need to provide a gist of the main information in the GRAS notice. Please briefly discuss the ADME of 6'-SL, including:*

a. what fraction of 6'-SL is absorbed in the gut,

b. the physiological fate of 6'-SL absorbed in the gut,

c. what fraction of 6'-SL is bacterially metabolized, and

d. what short chain fatty acids are formed, and their effects on enterocytes/colonocytes.

6'-SL is one of the most predominant acidic oligosaccharides in human milk (reviewed in Bode et al., 2012). HMOs, including 6'-SL, are highly resistant to the digestive enzymes of the gastrointestinal (GI) tract and only small amounts are absorbed. Specifically, in vitro studies have shown that less than 5% of ingested HMOs are digested and in vivo studies conducted in rats and infants have reported that 1 to 2% of the total amount of the ingested HMOs is absorbed and excreted unchanged in urine (Brand-Miller et al., 1998; Engfer et al., 2000; Gnoth et al., 2000; Goehring et al., 2014; Ruhaak et al., 2014; Santos-Fandila et al., 2014; Dotz et al., 2014; Obermeier et al., 1999; Rudloff et al., 2012). Although the exact mechanisms by which HMO absorption occurs have not been fully elucidated, data from in vitro studies suggest that neutral HMOs are transported across the intestinal epithelium by receptor-mediated transcytosis and paracellular transport, whereas acidic HMO, such as 6'-SL, are absorbed via non-specific paracellular transport only (Gnoth et al., 2001). Importantly, the unabsorbed HMOs pass through the GI tract to the large intestine where approximately 45% are fermented by the microbiota into short chain fatty acids and 40-50% are excreted in the feces (reviewed in Zhang et al., 2021; Moubareck, 2021). The primary short chain fatty acids produced by fermentation include acetate, butyrate and propionate, which are all readily absorbed (reviewed in Wong et al., 2006). Following absorption, acetate, and propionate are then transported to and metabolized by the liver whereas butyrate is the preferred fuel for colonocytes. Butyrate also plays a major role in colonocyte proliferation and differentiation. Studies also suggest that sialyllactose can be metabolized by microbial and intestinal neuraminidases

in the GI tract, producing sialic acid and lactose, both of which are components of human milk and the diet (reviewed in Röhrig et al., 2017). Because the 6'-SL that is the subject of this GRAS Notice is structurally identical to the 6'-SL found in human milk, there is reasonable certainty that the absorption, distribution, metabolism, and excretion of 6'-SL ingested from the intended uses at the intended use levels will mimic that of infants consuming human milk.

9) *The vast majority of the cited studies in support of the use of 6'-SL in enteral tube feeding and OESs were conducted using long-chain nondigestible carbohydrates with a degree of polymerization much higher than 3, which is in contrast to 6'-SL (a trisaccharide). For example, PHGG has been used in many such studies; it is a galactomannan with an average MW of about 25,000-30,000 Da. This implies that the long chain nondigestible carbohydrates may be better suited in such conditions. Please explain how the studies conducted with nondigestible carbohydrates that are chemically and structurally different from 6'-SL scientifically support the use of 6'-SL in enteral tube feeding and OESs at the intended use levels. Please also address how these studies support the general recognition of safety of the intended use of 6'-SL.*

Per GRN 000897, which received a "no questions" letter from FDA in 2020, the HMO 2'-fucosyllactose is GRAS for use in enteral feeding formulas at 20 g/L. Importantly, the notifier of GRN 000897 cited and relies on the same studies summarized in this GRAS Notice to support the safety and GRAS status of their HMO product in enteral feeding formulas. Additionally, the studies conducted with the non-digestible carbohydrates that are chemically and structurally different from 6'-SL support the safe use of the subject of this GRAS Notice in enteral tube feeding formulas and OESs at the proposed levels because all the non-digestible carbohydrates used in the cited studies have a similar metabolic fate as 6'-SL and they were administered at levels that exceed the proposed use levels of the subject of this Notice (up to 63 g/day). Specifically, the non-digestible carbohydrates PHGG, galactomannan, and fructooligosaccharides used in the studies cited in GRN 000897 and summarized in Table 8 of this Notice are chains of carbohydrates that are resistant to the digestive enzymes in the mammalian gastrointestinal tract. As a result, they pass through the gastrointestinal tract into the large intestine relatively intact where they are fermented by anaerobic bacteria to carbon dioxide, methane, hydrogen, and short-chain fatty acids, the latter of which are readily absorbed and/or used by the colonic mucosa and other tissues for energy. The remaining, unfermented carbohydrates are then excreted in the feces. Therefore, consumers of enteral feeding formulas and OESs containing the subject of this Notice will be exposed to the same profile of metabolites as consumers of enteral feeding formulas and OES products containing other long-chain non-digestible carbohydrates or other forms of 6'-SL and Chr. Hansen has reached the same conclusion as the notifier of GRN 000897; studies conducted with long-chain nondigestible carbohydrates support the general recognition of safety of the use of 6'-SL in enteral tube feeding formulas and OESs and there is reasonable certainty that the use of 6'-SL enteral tube feeding formulas and OESs is safe.

10) Infants aged 7-12 months will be exposed to 6'-SL from a combination of infant formula and conventional foods (baby/toddler foods) raising the exposure level, which is reflected in Tables 4 and 5. Please explain how the 90th percentile dietary exposure to 6'-SL in this age group (7-12 months), which is comparable to or higher than those of some older age groups, is safe. Because the progression from 6 months of age to 7 months of age and beyond is not expected to cause an immediate remodeling of the gut architecture and gut microbiota composition, please incorporate in your discussion the perspective of gut and gut microbiota development in infants of this age group.

As discussed in Chapter 3, Section C, 6'-SL is GRAS for use in infant and toddler formulas; other infant and toddler foods and drinks, and conventional foods (GRN 000881; GRN 000922). The 6'-SL ingredient manufactured by Chr. Hansen is GRAS for use in only non-exempt term infant formula at 0.4 g/L (GRN 000922), and Chr. Hansen now intends to expand the conditions of use to include toddler formulas, foods for infants and young children, meal replacements drinks, non-carbonated drinks, bars, oral electrolyte solutions, and enteral tube feeding formulas. Importantly, the 6'-SL exposures in infants 0-6 and 7-12 months old that result from the intended uses and use levels of the subject of this Notice are less than those that have already been determined to be safe and GRAS for other 6'-SL products that intended for use in infant formula, toddler formulas, and conventional foods (GRN 000881). Therefore, the safe use of the subject of this Notice for the proposed uses and use levels is similar to the safe use and use levels of other 6'-SL products that are GRAS for use in infant formula, toddler formulas, and conventional foods.

Regarding the safety of the increase 6'-SL exposure in infants aged 0 - 6 months to 7 – 12 months and older consumers, it is generally accepted that the colonization of the gastrointestinal tract with the microbiota begins at birth and expands rapidly thereafter, driven primarily by the term of pregnancy, mode of delivery, type of feeding (breast feeding vs. formula feeding), and use of antibiotics. Moreover, microbiota colonization plays an integral role in promoting the development of the gastrointestinal tract architecture and function, as well as the development and education of the host immune system, including vascular and smooth muscle development, epithelial cell development, gastrointestinal motility, intestinal barrier function, protection from enteric infections, and the gut-associated lymphoid tissue (GALT) development (reviewed in Goulet, 2015; Gensollen et al., 2016). Consistent with this, the American Academy of Pediatrics recommends "exclusive breast feeding for approximately 6 months, followed by continued breast feeding as complementary foods are introduced, with continuation of breastfeeding for 1 year or longer as mutually desired by mother and infant" (Section on Breastfeeding, American Academy of Pediatrics, 2012). Because the gastrointestinal architecture and microbiota of infants 7 – 12 months-old and older consumers are more mature than those of infants 0 - 6 months and can tolerate other foods that may contain other non-digestible carbohydrates, which have a similar metabolic fate as 6'-SL, it is reasonable to conclude that the increase in 6'-SL exposures from 0 - 6 months to 7 – 12 months and beyond is safe.

11) Both electrolytes and non-electrolytes contribute to cellular osmotic concentration and osmotic balance. As 6'-SL is a small trisaccharide, please explain why feeding very large amounts of 6'-SL, even for limited time, will not result into osmotic imbalance and adverse effects, particularly in individuals receiving 6'-SL through OES and enteral tube feeding.

As discussed in our response to Question 7, OESs containing 6'-SL at the intended use level will have an osmolarity/osmolality that approximates the World Health Organization standard for OESs (250.22 mOsm/L vs 245 mOSm/L). Additionally, because enteral feeding formulas have a wide range of osmolalities (~200 to 750 mOsm/kg) (Forchielli et al., 2003), the addition to 6'-SL to enteral feeding formulas at the intended use level will not result in enteral feeding formulas that have osmolalities outside the general range of osmolalities for enteral feeding formulas. Therefore, osmotic imbalance or adverse effects resulting from the use of 6'-SL in OESs and enteral tube feeding formulas are not expected. Importantly, enteral formulas containing 6'-SL will be used under medical supervision, with the health care provider selecting a formula with an osmolality that is appropriate for the individual's medical condition.

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

Sincerely,

Dietrich B. Conze, Ph.D.
Managing Partner

References

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From: Dietrich Conze
To: Downey, Jason
Cc: Claire Kruger; Kathy Brailer
Subject: [EXTERNAL] Re: GRN 001016 (Chr. Hansen, 6"-SL)
Date: Tuesday, April 26, 2022 3:55:28 PM

Hi Jason,

The notifier is in agreement to remove the use of 6'-SL in OES.

Regards.
Dietz

Dietrich Conze, PhD
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> On Apr 25, 2022, at 10:40 AM, Downey, Jason <Jason.Downey@fda.hhs.gov> wrote:
>
> Hi Dietz,
>
> This is regarding GRN 001016, Chr. Hansen's 6'-siallyllactose (6'-SL) notice, and its amendment. The information provided in the notice does not support general recognition of safety for the use of 6'-SL in oral electrolyte solutions (OES) in populations with acute gastroenteritis at risk for dehydration. Further, we still have questions regarding the tolerability of this ingredient for all individuals with diarrhea/dehydration. Additionally, we evaluate ingredients intended for use in medical foods in collaboration with the Office of Nutrition and Labeling, Infant Formula and Medical Foods Staff within our Center (CFSAN/ONFL/IFMFS). Due to the issues associated with OES that can be deemed as a medical food, and the intended use of 6'-SL in this food category, we still have a few outstanding questions for the experts in ONFL/IFMFS. We cannot readily resolve these issues within the provided evaluation timeframe. To continue with the evaluation of the other intended uses included in GRN 001016, we request that the notifier remove the intended use of 6'-SL in OES. After the evaluation of GRN 001016 is complete, we suggest that the notifier request a GRAS

pre-submission meeting with FDA. Such a meeting would allow the notifier to discuss the safety of the intended use of 6'-SL in OES, subsequent to our internal discussions with ONFL/IFMFS and possible outcomes thereon.

Please contact me by close of business May 2, 2022, to let us know if the notifier is in agreement with removing the use of 6'-SL in OES. You may send this to me via email.

Jason

Jason Downey, Ph.D. (he/him/his)
Regulatory Review Scientist

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

From: Dietrich Conze
To: Downey, Jason
Cc: Claire Kruger; Kathy Brailer
Subject: [EXTERNAL] Re: Question about specifications in Chr. Hansen HMO GRNs
Date: Tuesday, May 31, 2022 2:00:04 PM

Hi Jason.

Chr. Hansen's response to your question is below.

To clarify, Chr. Hansen does indeed test for the presence of *Cronobacter* spp. using ISO 22964. So, the specifications in GRNs 1014-1017 should refer to "*Cronobacter* spp." and not "*Cronobacter sakazakii* spp." or "*Cronobacter sakazakii*". If any *Cronobacter* spp. are detected, the batch will not be released and further testing will be done to determine the exact species.

Regards.
Dietz

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> On May 26, 2022, at 12:05 PM, Downey, Jason <Jason.Downey@fda.hhs.gov> wrote:
>
> Hi Dietz,
>
> I hope you've been doing well.
>
> We have a couple of quick questions about the Cronobacter specifications in this round of Chr. Hansen HMO notices (GRNs 001014-001017). In GRN 001016, the notifier lists a specification for "Cronobacter sakazakii spp." (Table 2, page 8). The notifier states that the method used is ISO 22964. The current version of this method is ISO 22964:2017, which corresponds to "Microbiology of the Food Chain - Horizontal Method for the Detection of Cronobacter spp." For the administrative record, please clarify whether the notifier tests for the presence of Cronobacter spp. or C. sakazakii, specifically. If it is the former, please state whether presumptive positives are further analyzed to determine if the isolate is

C. sakazakii. Please clarify this for GRNs 001014-1017.

Thank you!

Jason

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