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 3’-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 3’-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 3’-Sialyllactose Sodium Salt in Selected Conventional Foods and Enteral Tube Feeding Formulas
References
GRAS Determination for the Use of 3’-Sialyllactose Sodium Salt in Selected Conventional Foods and Enteral Tube Feeding Formulas

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

Prepared by:
Spherix Consulting Group, Inc.
751 Rockville Pike, Unit 30-B
Rockville, MD 20852
USA

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
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
IFN\(\gamma\): 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-\(N\)-neotetraose
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-neohexaose
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

3’-Sialyllactose sodium salt (3’-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 3’-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>
<thead>
<tr>
<th>Intended Uses</th>
<th>Intended Use Level (g/kg or g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toddler milks (Go and Grow by Similac®)</td>
<td>0.28</td>
</tr>
<tr>
<td>Milk-based meal replacement beverages for children (Pediasure®)</td>
<td>0.9</td>
</tr>
<tr>
<td>Cereals, dry instant, for infants and young children</td>
<td>1</td>
</tr>
<tr>
<td>Cereals, prepared, ready-to-serve, for infants and young children</td>
<td>1</td>
</tr>
<tr>
<td>Bars, including snack bars, meal-replacement bars, and breakfast bars</td>
<td>1</td>
</tr>
<tr>
<td>Meal replacement drinks for adults (including dairy and non-dairy drinks for weight reduction); including formulas for pregnant women</td>
<td>0.9</td>
</tr>
<tr>
<td>Non-carbonated drinks (e.g. fitness water, thirst quenchers, sports and isotonic drinks)</td>
<td>0.45</td>
</tr>
<tr>
<td>Enteral tube feeds used as sole source nutrition (Ensure®, Glucerna®, and Boost®)</td>
<td>1.5</td>
</tr>
<tr>
<td>Oral Electrolyte Solutions</td>
<td>0.1</td>
</tr>
</tbody>
</table>
F. BASIS FOR GRAS DETERMINATION

This GRAS determination for the use of 3’-SL 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 3’-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 3’-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 88% 3’-SL dry weight. The remaining components include carbohydrate by-products, ash, and moisture.
   a. 3’-Sialyllactose is a naturally occurring acidic oligosaccharide in human milk.
   b. Published studies showing that the amount of 3’-SL in breast milk ranges from 0.04 to 2.89 g/L.
   c. Human milk oligosaccharides, including 3’-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 GRAS Notice 921, which received a “no questions” letter on October 30, 2020 for the use 3’-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. 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.

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

f. Use of the subject of this GRAS Determination in the intended selected conventional foods and enteral tube feeding formulas results in mean and 90\(^{th}\) percentile estimated daily intakes (EDIs) of 0.153 and 0.356 g/day (0.002 and 0.005 g/kg bw/day) for consumers not less than 2 years old.

g. Use of the subject of this GRAS Determination in selected conventional foods and enteral tube feeding formulas results in mean and 90\(^{th}\) percentile cumulative estimated daily intakes (EDIs) of 1.05 and 3.78 g/day (0.016 and 0.056 g/kg bw/day) for consumers not less than 2 years old.

h. Use of the subject of this GRAS Determination in oral electrolyte solutions results in an estimated daily intake of 0.1 – 0.2 g of 3’-SL (equivalent to 0.7 – 1.4 mg of 3’-SL /kg bw/day assuming a 13.5 kg toddler and 0.1 – 0.3 mg of 3’-SL/kg bw/day assuming a 70 kg adult). Because OESs are intended for short term use, intake of 3’-SL from OESs will not impact the cumulative 3’-SL intake resulting from the use of 3’-SL in select conventional foods and enteral tube feeding formulas.

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

4. The safety of exposure to Chr. Hansen A/S’s 3’-SL at its intended use level is supported by:
a. Data demonstrating the qualitative and quantitative similarities between the subject of this GRAS Notice and the 3’-SL ingredient tested in the pivotal genotoxicology and subchronic toxicology studies conducted by Phipps et al. (2019), which is also the subject of GRN 880;

b. The lack of genotoxicity and no observed adverse effect level (NOAEL) for 3’-SL established in the 90-day subchronic dietary toxicology conducted by Phipps et al. (2019);

c. Published genotoxicology, 90-day subchronic dietary toxicology, and neonatal piglet studies conducted with 3’-SL or a mixture of HMOs containing the subject of this GRAS Determination (Parschat et al., 2020; Monaco et al., 2019; Hanlon, 2020);

d. Clinical data showing the ingestion of HMOs are well tolerated in infants up to 1.0 g/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 921);

g. The GRAS status of other 3’-SL products for use in selected conventional foods (GRN 766; GRN 880).

Therefore, 3’-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. 3’-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.
II. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT OF THE NOTIFIED SUBSTANCE

A. COMMON OR USUAL NAME

3’-Sialyllactose sodium salt (3’-SL; CAS No. 128496-08-5)

B. CHEMICAL NAME

3’-Sialyl-D-lactose; N-acetylneuraminoyllactose sodium salt

C. MOLECULAR FORMULA AND MASS

C\textsubscript{23}H\textsubscript{39}NNaO\textsubscript{19}; 633.55 g/mol

D. STRUCTURAL FORMULA

![Structural Formula]

E. DESCRIPTION OF 3’-SIALYLLACTOSE

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 nonreducing end via an α2,3 or α2,6 bonding, respectively.

The subject of this GRAS Determination is the 3’-sialyllactose sodium that is the subject of GRAS Notice (GRN) 921, which is GRAS for use in infant formula and received a “no questions” letter from FDA on October 30, 2020. It is produced by Chr. Hansen A/S using fermentation and a genetically engineered strain of \textit{Escherichia coli} BL21 (DE3). The 3’-SL sodium salt is purified from the culture medium and spray-dried, producing a powdered finished product. Residual impurities include lactose and carbohydrate by-products. Importantly, the structure of the 3’-SL product produced by this process is identical to the structure of 3’-SL as confirmed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), \textsuperscript{1}H,
13C, double-quantum filtered \(^1H\(^1H\)-COSY, phase-sensitive \(^1H\(^13C\)-heteronuclear single quantum correlation (HSQC), and phase-sensitive \(^1H\(^13C\)-heteronuclear multiple bond correlation (HMBC) NMR spectroscopy.

Chr. Hansen A/S intends to expand the intended uses of its 3’-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.

F. PRODUCTION PROCESS

Because the production process was extensively reviewed in GRN 921, the description of the production strain and manufacturing process are incorporated by reference (see pages 6 – 10 of GRN 921). The only difference is that cobalt is no longer added to the fermentation medium. Briefly, 3’-SL is produced by fermentation using the genetically engineered strain of \(E. coli\) BL21(DE3) \(JBT-3SL\) 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, 3’-SL is purified from the fermentation medium and the resulting concentrate is spray-dried into a powder. All other manufacturers involved in the 3’-SL manufacturing are Chr. Hansen A/S-qualified and either GMP-, ISO-, and International Featured Standards Food 6.1-compliant.

G. FINISHED PRODUCT SPECIFICATIONS

To ensure a consistent food-grade product that is free of genetically modified ingredients, each batch of 3’-SL is evaluated against the same product specifications as those specified in GRN 921 using compendial or validated methods that are fit-for-use. Data from five non-consecutive batches of 3’-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 921. Therefore, the summaries of the genomic and finished product stability are incorporated by reference (see pages 12 -15 of GRN 921). 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 3’-SL

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Analytical method</th>
<th>Specification</th>
<th>Batch number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>11027019</td>
</tr>
<tr>
<td><strong>Physical Parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appearance (Color)⁴</td>
<td>Visual</td>
<td>White to ivory-colored</td>
<td>Complies</td>
</tr>
<tr>
<td>Appearance (Form)⁴</td>
<td>Spray-dried powder</td>
<td>Complies</td>
<td>Complies</td>
</tr>
<tr>
<td><strong>Chemical Parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3’-Sialyllactose⁴</td>
<td>HPAEC-PAD</td>
<td>≥ 88% (%DW)</td>
<td>93.9</td>
</tr>
<tr>
<td>Other carbohydrates⁴</td>
<td>HPAEC-PAD</td>
<td>≤ 12% (% Area)</td>
<td>1.4</td>
</tr>
<tr>
<td>Lactose⁴</td>
<td>KF titration</td>
<td>≤ 5% (% Area)</td>
<td>0.6</td>
</tr>
<tr>
<td>Sialic Acid⁴</td>
<td>HPAEC-PAD</td>
<td>≤ 10% (% Area) &lt; LOQ</td>
<td>0.5</td>
</tr>
<tr>
<td>N-Acetylglucosamine⁴</td>
<td>Nanoquant</td>
<td>≤ 5% (% Area) &lt; LOQ</td>
<td>&lt; LOQ</td>
</tr>
<tr>
<td>Protein content⁴</td>
<td>Nanoquant (modified Bradford)</td>
<td>≤ 100 µg/g</td>
<td>14.2</td>
</tr>
<tr>
<td>Ash¹</td>
<td>ASU L 06.00-4</td>
<td>≤ 8.5 %</td>
<td>3.7</td>
</tr>
<tr>
<td>Moisture¹</td>
<td>PV-347 ICP-MS</td>
<td>≤ 9.0 %</td>
<td>8.3</td>
</tr>
<tr>
<td>Sodium¹</td>
<td>ASU L 00.00-135</td>
<td>≤ 4.2 %</td>
<td>3.0</td>
</tr>
<tr>
<td>Endotoxins³</td>
<td>Ph. Eur. 2.6.14</td>
<td>≤ 10 EU/mg</td>
<td>0.018</td>
</tr>
<tr>
<td>Aflatoxin M1¹</td>
<td>DIN EN ISO 14501</td>
<td>≤ 0.025 µg/kg</td>
<td>&lt; 0.025</td>
</tr>
<tr>
<td>GMO residues²</td>
<td>PCR</td>
<td>≤ 0.01%</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Heavy Metals</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arsenic¹</td>
<td>ASU L 00.00-135</td>
<td>≤ 0.2 mg/kg</td>
<td>ND</td>
</tr>
<tr>
<td>Cadmium¹</td>
<td>ASU L 00.00-135</td>
<td>≤ 0.1 mg/kg</td>
<td>ND</td>
</tr>
<tr>
<td>Lead¹</td>
<td>ASU L 00.00-135</td>
<td>≤ 0.02 mg/kg</td>
<td>ND</td>
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<tr>
<td>Mercury¹</td>
<td>ASU L 00.00-135</td>
<td>≤ 0.5 mg/kg</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Microbiology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard Plate Count¹</td>
<td>ISO 4833-2</td>
<td>≤ 10000 cfu/g</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Yeast and Mold¹</td>
<td>ISO 21527-2</td>
<td>≤ 100 cfu/g</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Enterobacteriaceae¹</td>
<td>ISO 21528-1</td>
<td>≤ 10 cfu/g</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Salmonella¹</td>
<td>ISO 6579</td>
<td>Absent/25 g</td>
<td>Absent</td>
</tr>
<tr>
<td>Cronobacter sakazakii¹</td>
<td>ISO/TS 22964</td>
<td>Absent/10g</td>
<td>Absent</td>
</tr>
</tbody>
</table>
Table 2. Product Specifications and Batch Data 3’-SL

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Analytical method</th>
<th>Specification</th>
<th>Batch number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

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 3’-SL powder 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 is to increase 3’-SL intake.

B. HISTORY OF EXPOSURE

3’-Sialyllactose is a naturally occurring acidic oligosaccharide found in human and bovine milk. Synthetic forms of 3’-SL have been approved for use in infant formula and conventional foods (GRN 766, 2018; GRN 880, 2020; GRN 921, 2020).

Acidic oligosaccharides make up 15-20% of all HMOs found in human milk (Bode, 2012). The concentration of 3’-SL in human milk has been determined in numerous studies and as summarized in GRN 921, ranges from 0.04 – 2.89 g/L (Aakko et al., 2017; Asakuma et al., 2007; Austin et al., 2016; 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., 2017; Leo et al., 2010; Ma et al., 2018; Martin-Sosa et al., 2003; McGuire et al., 2017; Nakhla et al., 1999; Nijman et al., 2018; Olivares et al., 2015; Smilowitz et al., 2013; Sprenger et al., 2017; Spevacek et al., 2015; Sumiyoshi et al., 2003; Thurl et al., 2010). Unlike other HMOs, such as 2’-fucosyllactose (2’-FL), 3-fucosyllactose (3-FL), and lacto-N-tetraose (LNT), 3’-SL levels remain relatively constant over the course of lactation and do not differ between Secretor status of the mother. Additionally, a systematic review conducted by Thurl et al. (2017) reported means and 95% percentile confidence limits for the amount of 3’-SL in the milk of mothers of term and preterm infants of 0.16 g/L and 0.12 – 0.19 g/L and 0.24 g/L and 0.2 – 0.28 g/L, respectively.

3’-Sialyllactose is also found in bovine milk at concentrations ranging from 0.092-0.867 g/L (Lee et al., 2015; McJarrow et al., 2019; Nakamura et al., 2003). However, unlike human milk, the levels of 3’-SL levels decrease in bovine milk over lactation time.

In the United States, synthetic forms of 3’-SL are used in infant formula at levels up to 0.28 g/L, baby food products up to 1.6 g/kg for, foods for special dietary use up to 25 g/kg, and in conventional foods and beverages up to 12.5 g/kg (GRN 766, 2018; GRN 880, 2020; GRN 921, 2020).

Thus, humans are exposed to 3’-SL either through the ingestion of human milk, bovine milk, and/or products containing synthetic forms of 3’-SL.
C. INTENDED USES

3’-Sialyllactose is GRAS for use in non-exempt infant formula at 0.28 g/L and selected conventional foods at 0.1 to 12.5 g/kg (GRN 766, 2018; GRN 880, 2020; GRN 921, 2020). The 3’-SL ingredient manufactured by Chr. Hansen A/S is GRAS for use in only non-exempt term infant formula at 0.28 g/L (GRN 921). Chr. Hansen A/S intends to expand the use of the subject of GRN 921 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 0.1 to 1 g/L (Table 3). Importantly, these expanded uses include new uses, substitutional uses for other forms of 3’-SL that are GRAS for use in infant formula and conventional foods, and increases to the 3’-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 expanded intended uses increase 3’-SL overall exposure.

<table>
<thead>
<tr>
<th>Uses That Are GRAS</th>
<th>Use Levels That Are GRAS (g/kg or g/L)</th>
<th>Intended Uses</th>
<th>Intended Use Level (g/kg or g/L)</th>
<th>Maximum Use Level Used for Cumulative EDI Calculations (g/kg or g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-exempt infant formula</td>
<td>0.28</td>
<td>Toddler formula (Go and Grow by Similac®)</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>Toddler formula</td>
<td>0.24</td>
<td>Milk-based meal replacement beverages for children (Pediasure®)</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Baby food, instant cereal</td>
<td>1.6</td>
<td>Cereals, dry instant, for infants and young children</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Baby food, jugged cereal</td>
<td>1.6</td>
<td>Cereals, prepared, ready-to-serve, for infants and young children</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Baby food, cereal bar</td>
<td>1.6</td>
<td>-</td>
<td>-</td>
<td>1.6</td>
</tr>
<tr>
<td>Baby food, snacks</td>
<td>1.6</td>
<td>-</td>
<td>-</td>
<td>1.6</td>
</tr>
<tr>
<td>Baby food, vegetables</td>
<td>1.6</td>
<td>-</td>
<td>-</td>
<td>1.6</td>
</tr>
<tr>
<td>Baby food, fruit</td>
<td>1.6</td>
<td>-</td>
<td>-</td>
<td>1.6</td>
</tr>
<tr>
<td>Drinks for children</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
<td>0.15</td>
</tr>
<tr>
<td>Imitation milk</td>
<td>0.117</td>
<td>-</td>
<td>-</td>
<td>0.117</td>
</tr>
<tr>
<td>Non-dairy yogurt</td>
<td>0.533</td>
<td>-</td>
<td>-</td>
<td>0.533</td>
</tr>
<tr>
<td>Unflavored, pasteurized milk</td>
<td>0.25</td>
<td>-</td>
<td>-</td>
<td>0.25</td>
</tr>
<tr>
<td>Flavored milk</td>
<td>0.25</td>
<td>-</td>
<td>-</td>
<td>0.25</td>
</tr>
<tr>
<td>Buttermilk</td>
<td>0.25</td>
<td>-</td>
<td>-</td>
<td>0.25</td>
</tr>
<tr>
<td>Yogurt</td>
<td>2.5</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Table 3. Comparison of Uses and Use levels That Are GRAS with the Intended Uses and Use Levels

<table>
<thead>
<tr>
<th>Uses That Are GRAS</th>
<th>Use Levels That are GRAS (g/kg or g/L)</th>
<th>Intended Uses</th>
<th>Intended Use Level (g/kg or g/L)</th>
<th>Maximum Use Level Used for Cumulative EDI Calculations (g/kg or g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen yogurt</td>
<td>1.6</td>
<td>-</td>
<td>-</td>
<td>1.6</td>
</tr>
<tr>
<td>Meal replacement bars for weight loss</td>
<td>5</td>
<td>Bars, including snack bars, meal-replacement bars, and breakfast bars</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Bars, snack</td>
<td>2.5</td>
<td>-</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Meal replacement drinks (including dairy and non-dairy drinks for weight reduction)</td>
<td>0.5</td>
<td>Meal replacement drinks for adults (including dairy and non-dairy drinks for weight reduction); including formulas for pregnant women</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Sports, isotonic, and energy drinks</td>
<td>0.25</td>
<td>Non-carbonated drinks (e.g. fitness water, thirst quenchers, sports and isotonic drinks)</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>Herbal tea</td>
<td>12.5</td>
<td>-</td>
<td>-</td>
<td>12.5</td>
</tr>
<tr>
<td>Cappuccino</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>Sugar substitute</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>Enteral tube feeding</td>
<td>1.6</td>
<td>Enteral tube feeds used as sole source nutrition (Ensure®, Glucerna®, and Boost®)</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Oral Electrolyte Solutions</td>
<td>1.6</td>
<td>Oral Electrolyte Solutions</td>
<td>0.1</td>
<td>-</td>
</tr>
</tbody>
</table>

1 Obtained from GRN 766, GRN 880, and GRN 921.
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 3’-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 that the ingestion of 1-2 L of OES, such as Pedialyte, per day may be needed 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 0.1 g/L of 3'-SL would result in a daily intake of 0.1–0.2 g of 3'-SL (equivalent to 7.4 – 14.8 mg of 3'-SL/kg bw/day, assuming a 13.5 kg toddler and 1.4 – 2.8 mg of 3'-SL/kg bw/day, assuming a 70 kg adult). Because OESs are intended for short term use, intake of 3'-SL from OESs will not impact the cumulative 3'-SL intake resulting from the use of 3’-SL in select conventional foods and enteral tube feeding formulas.

2. Estimated Daily Intake of 3’-SL from Selected Conventional Foods and Enteral Tube Feeding Formula

Estimates for the intake of Chr. Hansen A/S’s intended uses of 3’-SL were based on the food uses and Chr. Hansen A/S’s use level in Table 3, 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 3’-SL.

To determine the impact of Chr. Hansen A/S’s intended uses on the cumulative estimated daily intake of 3’-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 685 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 3’-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 3’-SL by the U.S. population. Estimates for the daily intake of 3’-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 3’-SL by those individuals consuming food products containing 3’-SL. Individuals were considered users if they consumed one or more food products containing 3’-SL on either Day 1 or Day 2 of the survey.

4. **Food Usage**

The estimated “all-user” total intakes of 3’-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 3’-SL in the 2015-2016 survey. The mean intakes by 3’-SL consumers age 2+ from Chr. Hansen A/S’s intended food uses were estimated to be 0.153 g/person/day or 0.002 g/kg body weight/day. The heavy consumer (90th percentile) intakes were estimated to be 0.356 g/person/day or 0.005 g/kg body weight/day. The highest consumers on a mean EDI by body weight basis were ages 13 months to 2 years and 2 to 5 years at 0.004 g/kg body weight/day (0.055 and 0.068 g/day, respectively).

The cumulative estimated “all-user” total intakes of 3’-SL from 685 proposed food uses listed in NHANES in the U.S. by population group is described in Table 5. In summary, 54.9% of the total U.S. population 2+ years was identified as consumers of 3’-SL from the selected food uses in the 2015-2016 survey. The mean intakes by all 3’-SL consumers age 2+ from all 3’-SL food uses were estimated to be 1.05 g/person/day or 0.016 g/kg body weight/day. The heavy consumer (90th percentile) intakes were estimated to be 3.78 g/person/day or 0.056 g/kg body weight/day. The highest consumers on a mean EDI by body weight basis were ages 20 years and up at 0.018 g/kg body weight/day (1.42 g/day).

Importantly, a comparison of the mean and 90th percentile EDIs of 3’-SL ages 2+ from Chr. Hansen A/S’s food uses and all food uses shows that the EDI increases from 0.153 and 0.356 to 1.05 to 3.78 g/day (compare Tables 4 and 5, respectively), which is likely due to a limited number of uses for 3’-SL and users consuming a variety of foods all of which contain 3’-SL.
### Table 4. Estimated “All-user” Daily Intake (EDI) of 3’-SL from Chr. Hansen A/S’s Food Uses by Population Group (2015-2016 NHANES Data)

<table>
<thead>
<tr>
<th>Population Group</th>
<th>N users</th>
<th>N population</th>
<th>% Users</th>
<th>Mean mass (kg)</th>
<th>Mean EDI (g)</th>
<th>90th % EDI (g)</th>
<th>Mean EDI (g/kg)</th>
<th>90th % EDI (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ages 0-6 months</td>
<td>49</td>
<td>197</td>
<td>24.87</td>
<td>7.00</td>
<td>0.009</td>
<td>0.015</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>ages 7-12 months</td>
<td>72</td>
<td>207</td>
<td>34.78</td>
<td>9.44</td>
<td>0.027</td>
<td>0.060</td>
<td>0.003</td>
<td>0.006</td>
</tr>
<tr>
<td>ages 13 months-2 years</td>
<td>44</td>
<td>535</td>
<td>8.22</td>
<td>12.56</td>
<td>0.055</td>
<td>0.089</td>
<td>0.004</td>
<td>0.007</td>
</tr>
<tr>
<td>ages 2-5 years</td>
<td>69</td>
<td>915</td>
<td>7.54</td>
<td>16.92</td>
<td>0.068</td>
<td>0.129</td>
<td>0.004</td>
<td>0.008</td>
</tr>
<tr>
<td>ages 6-12 years</td>
<td>146</td>
<td>1505</td>
<td>9.70</td>
<td>36.58</td>
<td>0.116</td>
<td>0.275</td>
<td>0.003</td>
<td>0.008</td>
</tr>
<tr>
<td>ages 13-19 years</td>
<td>145</td>
<td>1143</td>
<td>12.69</td>
<td>67.35</td>
<td>0.157</td>
<td>0.363</td>
<td>0.002</td>
<td>0.005</td>
</tr>
<tr>
<td>ages 20 years and up</td>
<td>513</td>
<td>5748</td>
<td>8.92</td>
<td>80.76</td>
<td>0.168</td>
<td>0.370</td>
<td>0.002</td>
<td>0.005</td>
</tr>
<tr>
<td>ages 2 years and up</td>
<td>873</td>
<td>9311</td>
<td>9.38</td>
<td>67.35</td>
<td>0.153</td>
<td>0.356</td>
<td>0.002</td>
<td>0.005</td>
</tr>
</tbody>
</table>

### Table 5. Cumulative Estimated “All-user” Daily Intake (EDI) of 3’-SL in All Food Uses by Population Group (2015-2016 NHANES Data)

<table>
<thead>
<tr>
<th>Population Group</th>
<th>N users</th>
<th>N population</th>
<th>% Users</th>
<th>Mean mass (kg)</th>
<th>Mean EDI (g)</th>
<th>90th % EDI (g)</th>
<th>Mean EDI (g/kg)</th>
<th>90th % EDI (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ages 0-6 months</td>
<td>142</td>
<td>197</td>
<td>72.08</td>
<td>7.00</td>
<td>0.041</td>
<td>0.068</td>
<td>0.006</td>
<td>0.010</td>
</tr>
<tr>
<td>ages 7-12 months</td>
<td>171</td>
<td>207</td>
<td>82.61</td>
<td>9.44</td>
<td>0.055</td>
<td>0.131</td>
<td>0.006</td>
<td>0.014</td>
</tr>
<tr>
<td>ages 13 months-2 years</td>
<td>380</td>
<td>535</td>
<td>71.03</td>
<td>12.56</td>
<td>0.104</td>
<td>0.106</td>
<td>0.008</td>
<td>0.008</td>
</tr>
<tr>
<td>ages 2-5 years</td>
<td>567</td>
<td>915</td>
<td>61.97</td>
<td>16.92</td>
<td>0.146</td>
<td>0.118</td>
<td>0.009</td>
<td>0.007</td>
</tr>
<tr>
<td>ages 6-12 years</td>
<td>881</td>
<td>1505</td>
<td>58.54</td>
<td>36.58</td>
<td>0.338</td>
<td>0.52</td>
<td>0.009</td>
<td>0.014</td>
</tr>
<tr>
<td>ages 13-19 years</td>
<td>664</td>
<td>1143</td>
<td>58.09</td>
<td>67.35</td>
<td>0.981</td>
<td>3.29</td>
<td>0.015</td>
<td>0.049</td>
</tr>
<tr>
<td>ages 20 years and up</td>
<td>2995</td>
<td>5748</td>
<td>52.11</td>
<td>80.76</td>
<td>1.42</td>
<td>5.03</td>
<td>0.018</td>
<td>0.062</td>
</tr>
<tr>
<td>ages 2 years and up</td>
<td>5107</td>
<td>9311</td>
<td>54.85</td>
<td>67.35</td>
<td>1.05</td>
<td>3.78</td>
<td>0.016</td>
<td>0.056</td>
</tr>
</tbody>
</table>
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 3’-SL, which is a non-digestible oligosaccharide found in human milk, also known as a human milk oligosaccharide (HMO). Acidic oligosaccharides, including 3’-SL make up 15-20% of all HMOs found in human milk (Bode, 2012). As summarized in GRN 921, published studies indicate that 3’-SL levels in human milk range from 0.04 – 2.89 g/L.

To obtain a thorough and comprehensive understanding of the safety of 3’-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 3’-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 3’-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, three synthetic 3’-SL products are GRAS (GRN 766, 2018; GRN 880, 2020; GRN 921, 2020). The subject of GRN 766 is enzymatically synthesized by GeneChem, Inc. and is GRAS for use in non-exempt term infant formulas and selected conventional foods. The subject of GRN 880 is manufactured 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 921 is manufactured by Chr. Hansen A/S, GRAS for use in non-exempt term infant formula and is also the subject of this GRAS determination. Importantly, as summarized in GRN 921, the subjects of GRN 880 and this GRAS determination are structurally identical to the 3’-SL found in human milk, qualitatively comparable and quantitatively similar to each other, and supported by a battery of published genotoxicology, subchronic toxicology, and neonatal piglet tolerance studies conducted with 3’-SL and mixtures containing 3’-SL. 3’-Sialyllactose is not genotoxic and has a no observed adverse effect level (NOAEL) of at least 5.0 g/kg bw/day, which was the highest dose tested in the pivotal 90-day subchronic toxicity study (Phipps et al., 2019). Additionally, publicly available clinical data show that the ingestion 3’-SL, mixtures of 3’- and 6’-SL, 2’-fucosyllactose (2’-FL), and other non-digestible carbohydrates is also 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 3’-SL tested by Phipps et al. (2019) and the subject of GRN 880 (Scientific Committee on Food, 1998; GRN 880, 2020; GRN 921, 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(D3) and the production organism was
thoroughly summarized in GRN 921. 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
921). Importantly, because *JBT-3SL* was engineered with genes with known function, which do
not confer toxicogenicity, virulence, or DNA transfer, using site-specific homologous
recombination or transposition, *JBT-3SL* 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-3SL* and its comprehensive characterization, use of *JBT-3SL* 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 Notices and opinions published by worldwide authoritative bodies, including GRN 921
which summarizes the GRAS status of the use of Chr. Hansen A/S’s 3’-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 880, 2020; GRN 815, 2019;
GRN 833, 2019; GRN 919, 2020; GRN 921, 2020; GRN 923, 2020; EFSA Panel on Dietetic
Products, 2015; EFSA Panel on Nutrition et al., 2019). As summarized on page 25 of GRN 921,
HMOs, including 3’-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 3’-SL 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 include
a battery of genotoxicology and subchronic toxicology studies conducted with a 3’-SL
containing ingredient manufactured by Glycom A/S and published by Phipps et al. (2019).
Additional genotoxicology and subchronic toxicology studies with a 3’-SL ingredient
manufactured by GeneChem Inc. and published by Kim et al. (2018), and a mixture containing
2’-FL, 3’-FL, 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 921 and therefore their summaries are incorporated by reference (see pages 25 – 38 of GRN 921). Briefly, Phipps et al. (2019) conducted an OECD-compliant bacterial reverse mutation assay, an OECD-compliant in vitro micronucleus assay, and an OECD-compliant 90-day subchronic oral toxicity study in neonatal rats with a product containing 90.3% 3’-SL manufactured by fermentation by Glycom A/S to support the GRAS status of the subject of GRN 880. Kim et al. (2018) conducted an OECD-compliant bacterial reverse mutation assay, an OECD-compliant in vitro chromosome aberration assay, an in vivo micronucleus assay, an acute toxicity study in rats, a 28-day subchronic toxicity study in rats, an OECD-compliant 90-day subchronic oral toxicity studies in rats, a single dose escalation study in Beagle dogs with a product containing 98.8% 3’-SL manufactured by enzymatic synthesis by GeneChem, Inc. to support the GRAS status of the subject of GRN 766. Parschat et al. (2020) evaluated the genotoxicity and subchronic toxicity of Chr. Hansen A/S’s 3’-SL in combination with 2’-FL, 3’-FL, LNT, and 6’-SL, all of which are manufactured using fermentation, 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. 3’-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 in the subchronic toxicology study conducted by Phipps et al. (2019). Similar results were reported by Kim et al. (2018) and Parschat et al. (2020).

As summarized in GRN 921 (pages 26 and 27), both the 3’-SL sodium salts tested by Phipps et al. (2019) and the subject of this GRAS Determination are manufactured by fermentation using genetically engineered strains of *E. coli* and contain similar amounts of 3’-SL (90.3 vs 93.68 % (average 3’-SL content; see Table 2), respectively). 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 3’-SL product is qualitatively comparable and quantitatively similar to the 3’-SL product tested by Phipps et al. (2019), the toxicology studies conducted by Phipps et al. (2019) are pivotal to supporting the safety of Chr. Hansen A/S’s 3’-SL product. Thus, based on the results reported by Phipps et al. (2019), adverse effects resulting from the ingestion of 3’-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 3’-SL in the neonatal piglet, which is an appropriate model for understanding the tolerance of food ingredients in infants (Litten-Brown et al., 2010). Donavan (2017) evaluated the safety and tolerance of a 3’-SL sodium salt (> 98% purity) manufactured by enzymatic synthesis by GeneChem whereas Hanlon (2020) evaluated the safety and tolerance of a mixture of HMOs containing 2’-FL, 3’-FL, lacto-N-
tetraose (LNT), 3’-SL, and 6’-SL manufactured by Chr. Hansen A/S. Both studies are extensively summarized in previous GRAS notices and therefore their summaries are incorporated by reference.

As summarized on pages 53-56 of GRN 766, Donavan (2017) fed two-day-old male and female piglets one of four diets (n=12/group) containing varying amounts of a 3’-SL sodium salt (> 98% purity) manufactured by enzymatic synthesis by GeneChem for 21 days. Since the filing of GRN 766, this study was published by Monaco et al. (2019). The control diet was a commercially-available non-medicated sow-milk replacer formula (Advance Liqui-Wean, Milk Specialties Co., Dundee, IL, USA). The 3’-SL-containing diets were the control diet supplemented with 140, 200, or 500 mg/L 3’-SL, equivalent to 135.3, 193.3, and 483.2 mg/L 3’-SL, respectively, after correcting for the sodium content. 3′-Sialyllactose was well-tolerated at all doses over the 21-day treatment period. Three piglets were removed from the 140 mg/L-treated group due to watery diarrhea and the authors concluded that this was incidental and not of toxicological concern due to the lack of a dose response. The 3’-SL containing diets also supported growth and development at levels comparable to those observed in the piglets fed the control diet, and had no significant effects on serum chemistry, hematology and organ microscopic structure. Donovan et al. concluded that supplementing 3’-SL to swine milk replacer up to 500 mg/L is safe and supports normal growth and development.

As summarized on pages 38 to 70 of GRN 921, a mixture of HMOs containing 2’-FL, 3’-FL, LNT, 3’-SL, and 6’-SL was administered to two-day-old Yorkshire crossbred piglets for 21 days. Thirty-six 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 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 between 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, these changes were considered not adverse as there were no microscopic correlates. Importantly, the underlying infection did not affect the validity of the results. 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 HMO Mix 1 (0.13 and 0.13 g 3’-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 921, this study was published by Hanlon (2020).

E. CORROBORATIVE ANIMAL STUDIES

The additional neonatal piglet and rat studies that corroborate the safe use of 3’-SL in foods were conducted by Obelitz-Ryom et al. (2018), Monaco et al. (2018), Wang et al. (2019), Obelitz-Ryom et al. (2019) and Chleilat et al. (2020). Except for Chleilat et al. (2020), all of these studies were summarized in GRN 921 and their summaries are incorporated by reference. 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 3’-SL supplementation. Only the endpoints relevant to the safety and tolerability of sialyllactose and 3’-SL supplementation are briefly summarized below.

As summarized in GRN 921, 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.

As summarized in GRN 921, 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 for glutamate dehydrogenase. This difference was not dose dependent and was not considered treatment related or adverse.

As summarized in GRN 921, 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.

As summarized in GRN 921, Obelitz-Ryom et al. (2019) fed preterm piglets either intact unpasteurized Jersey cow’s milk supplemented with lactose, intact unpasteurized Jersey cow’s milk supplemented with 4.5% sialyllactose (a 6:1 ratio of 3’-SL and 6’-SL), or their mother’s milk only for 19 days. In addition to assessing cognitive performance, clinical outcomes and growth were evaluated. Although the growth of the pigs receiving the cow’s milk supplemented with either lactose or sialyllactoses was less than the pig receiving their mother’s milk, as judged by weight gain, no adverse events related to the experimental diet were reported in the study.

Chleilat et al. (2020) fed 3 week-old Sprague Dawley rats either a control diet or diets containing either 0.625% 2’-FL, 0.625% 3’-SL, or 0.625% 2’-FL and 0.625% 3’-SL for eight weeks. Body composition, intestinal permeability, serum cytokines, fecal microbiota composition, and messenger RNA (mRNA) expression of selected genes involved in gut barrier function in the gastrointestinal tract (5 males and 5 females/group) were assessed after the 8 week treatment period. There were no differences in body composition among the groups. Males in the HMO-fed groups had a small, but significant decrease in body weight at week 8 of the study (p=0.03), as well as significantly lower levels of the proinflammatory cytokine interleukin 18 (IL-18) in their serum (p=0.01). Female rats fed the diet containing both 2’-FL and 3’-SL had significantly increased cecum weight compared to the control (p=0.002), and significantly decreased colon weight compared to the control (p=0.03) and the 3’-SL fed groups (p=0.02). All females fed HMOs had significant reductions in intestinal permeability compared to controls whereas no significant differences were observed among the different male groups. All HMO-fortified diets also altered gut microbiota composition and mRNA expression in the gastrointestinal tract, albeit differently according to sex. Importantly, the authors concluded that supplementation with a fraction of the HMOs found in breast milk has a complex sex-dependent risk/benefit profile. The weight of the evidence reported in this study suggests that HMO...
supplementation, in general, has functional benefits, such as lowering proinflammatory cytokine gene expression and reducing intestinal permeability. Additionally, the increase in cecum weight reported in this study is consistent with the results of other studies that have administered non-digestible carbohydrates to rats for extended periods of time (Zhou et al., 2017; Adam et al., 2015; Konishi et al., 1984; Oku, T, 1997; Nzeusseu et al., 2006; Jacobs and Schneeman, 1981).

F. CLINICAL STUDIES

Additional support for the safe use of 3’-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

3’-Sialyllactose is a non-digestible HMO that is GRAS for use in infant formula and conventional foods (GRN 766, 2018; GRN 880, 2020; GRN 921, 2020). Numerous studies have evaluated the tolerability of ingesting HMOs including 3’-SL, 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), were conducted in infants with 3’-SL alone or in combination with 6’-SL, galactooligosaccharides, and live microorganisms. Three of these clinical studies, Parente et al. (2003), Rasko et al. (2000), and Gurung et al. (2018), were conducted in adults with H. pylori infections where only 3’-SL was ingested. The remaining thirteen studies were conducted in infants or adults with 2’-FL, LNnT 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 925, 2021). Therefore, their summaries are incorporated by reference and the studies are briefly summarized in tabular format below along with new studies conducted 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 their publication, 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 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 nondigestible 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 or 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 3’-SL, are not expected.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design and Population</th>
<th>Groups (Numbers of Subjects)</th>
<th>Duration</th>
<th>Safety Parameters</th>
<th>GRN Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="#">Radke et al., 2017</a></td>
<td>Multicenter, randomized placebo-controlled, double-blind study</td>
<td>Healthy term infants 0-14 days old</td>
<td>Group 1: Control formula; (n=207)</td>
<td>6 months Follow-up at 12 months, no test formula 6-12 months</td>
<td>GRN 766, pages 62-64</td>
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<td>Group 2: Test formula containing 5.8 ± 1.0 g BMOs*/100 g powdered formula (8 g/L in the reconstituted formula) and 1x10⁷ cfu/g <em>B. lactis</em> CNCM I-3446; (n=206)</td>
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<td>Group 3: Breastfed reference group; (n=63)</td>
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<td>*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</td>
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<tr>
<td><a href="#">Cooper et al., 2017</a></td>
<td>Multicenter, randomized, placebo-controlled, double-blind study</td>
<td>Healthy term infants born to HIV+ mothers</td>
<td>Group 1: Cesarean-delivered infants consuming standard formula; (n=101)</td>
<td>4 months</td>
<td>Four hundred and thirty infants were randomized into the study.</td>
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<td>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 1x10⁷ cfu/g <em>B. lactis</em> CNCM I-3446; (n=92)</td>
<td>4 months</td>
<td>Four hundred and thirty infants were randomized into the study.</td>
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<td>o Nine (2.1%) infants were lost to follow-up after randomization but before starting the study formulas.</td>
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<td>o Eight infants were found to be HIV infected, seven at the 4-week visit (v2) and one became positive at 6 months (v5).</td>
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<td>o Of the eight that were HIV infected, three infants died and one discontinued the study.</td>
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<td>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 <em>B. lactis</em> CNCM I-3446, vaginal group starter formula, cesarean starter formula containing BMOs and <em>B. lactis</em> CNCM I-3446, and cesarean starter formula groups, respectively.</td>
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<tr>
<td>Reference</td>
<td>Study Design and Population</td>
<td>Groups (Numbers of Subjects)</td>
<td>Duration</td>
<td>Safety Parameters</td>
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<td>Simeoni et al., 2016</td>
<td>Randomized, placebo-controlled, double-blind study</td>
<td>Group 1: Standard formula; (n=37)</td>
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<td>• No difference in compliance or tolerability was observed among the three groups.</td>
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<td></td>
<td>Healthy 5-day old, term infants</td>
<td>Group 2: Standard formula plus 5.7±1.0 g/100 g bovine milk oligosaccharides (BMOs*; 8.0 g/L</td>
<td>12 weeks</td>
<td>o 10 infants discontinued in the human milk/breastfed group (5 withdrew voluntarily and 5 for other reasons)</td>
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<td>reconstituted formula) and 1x10⁷ cfu/g of <em>B. lactis</em> CNCM I-3446; (n=39)</td>
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<td>o 7 infants discontinued in the standard formula group (2 withdrew due to GI symptoms, 4 withdrew voluntarily, and 2 were lost to follow-up)</td>
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<td>Group 3: Human milk; (n=37)</td>
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<td>o 7 infants discontinued in the standard formula with the BMOS and <em>B. lactis</em> CNCM I-3446 group (3 withdrew due to GI symptoms, 2 withdrew voluntarily, and 3 were lost to follow-up)</td>
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<td>• There were no differences in anthropometric measures among the three groups.</td>
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<td>• There were no differences in the standard formula and standard formula with BMOS and <em>B. lactis</em> CNCM I-3446 groups in ‘spitting up’, vomiting, crying, colic, flatulence and irritability.</td>
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</table>
### Table 6. Clinical Studies with Human Milk Oligosaccharides and Infants

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design and Population</th>
<th>Groups (Numbers of Subjects)</th>
<th>Duration</th>
<th>Safety Parameters</th>
<th>GRN Reference</th>
</tr>
</thead>
</table>
| Meli et al., 2014 | Randomized, double-blind, single-center study | Healthy term infants (<14 days old)                                                           | 4 months | • 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. | GRN 766, pages 62-64 |
Table 6. Clinical Studies with Human Milk Oligosaccharides and Infants

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design and Population</th>
<th>Groups (Numbers of Subjects)</th>
<th>Duration</th>
<th>Safety Parameters</th>
<th>GRN Reference</th>
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</thead>
<tbody>
<tr>
<td>Riechmann et al., 2020</td>
<td>Non-randomized, open-label, prospective study</td>
<td>Group 1: Formula-fed infants (n=82)</td>
<td>8 weeks</td>
<td>• 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).</td>
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<td>Healthy term infants 7 days to 2 months old</td>
<td>Group 2: Infants consuming formula and human milk; the formula contained 1.0g/L of 2'-FL, 0.5 g LNN, and Lactobacillus reuteri (n=62)</td>
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<td>• Fourteen subjects dropped out of Group 2 (eight were excluded due to protocol deviations, three dropped out due to adverse events, and three were lost to follow-up).</td>
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<td>Group 3: Breast-fed infants (n=63)</td>
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<td>• 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).</td>
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<td></td>
<td>• There were no significant differences between any of the groups for any of the anthropometric measures.</td>
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<td></td>
<td>• 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.</td>
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<td>• There were no significant differences among the groups in the gassiness, fussiness, crying or spitting-up/vomiting domains of the IGSQ.</td>
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<td>• For the stooling domain, Group 2 were significantly different than Group 3 at baseline and 8 weeks.</td>
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<td>• 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</td>
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<td>• 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.</td>
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<td>• 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.</td>
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</tbody>
</table>
| Nowak- Wegrzyn et al., | Double-blind, placebo-controlled food challenges | Treatment #1: Whey-based extensively hydrolyzed formula                                      | Not applicable |                                                             | GRN 919, page 33

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### Table 6. Clinical Studies with Human Milk Oligosaccharides and Infants

<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>Storm et al., 2019</td>
<td>Randomized, placebo-controlled double-blind study Healthy term infants 14 days old ±5 days.</td>
<td>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)</td>
<td>6 weeks</td>
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</tbody>
</table>
  - 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.  
    - 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). |
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</thead>
<tbody>
<tr>
<td>Puccio et al., 2017</td>
<td>Prospective, randomized, placebo-controlled study Healthy, term infants 0 to 14 days old</td>
<td>Group 1: Formula (n=87) Group 2: Formula with 1.0 g/L 2'-FL and 0.5 g/L LNnT (n=88)</td>
<td>6 months (after 6 months, all infants were switched to a non-HMO containing formula)</td>
<td>• 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).&lt;br&gt;• 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.&lt;br&gt;• 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.&lt;br&gt;• 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.</td>
<td>GRN 650, page 38</td>
</tr>
<tr>
<td>Goehring et al., 2016</td>
<td>Prospective, randomized, placebo-controlled study Healthy, term infants 5 days old</td>
<td>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)</td>
<td>16 weeks</td>
<td>• 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.&lt;br&gt;• 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 ≤ 0.05).&lt;br&gt;• 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.</td>
<td>GRN 735, page 62</td>
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</table>
### Table 6. Clinical Studies with Human Milk Oligosaccharides and Infants

<table>
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<tr>
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<tbody>
<tr>
<td>Marriage et al., 2015</td>
<td>Prospective, randomized, placebo-controlled study</td>
<td>Group 1: Formula with GOS (n=101)</td>
<td>17 weeks</td>
<td>• 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.</td>
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<tr>
<td></td>
<td>Healthy, term infants 5 days old</td>
<td>Group 2: Formula with GOS + 0.2 g/L 2’-FL (n=104)</td>
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<td>• 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.</td>
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<td>Group 3: Formula with GOS + 1.0 g/L 2’-FL (n=109)</td>
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<td>• 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.</td>
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<td>Group 4: human milk (HM) (n=106)</td>
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<td>• 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.</td>
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<td>• 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.</td>
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<td>• There were no significant differences in the overall percentage of subjects experiencing adverse events or serious adverse events in the formula-treated groups.</td>
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<td>• 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&lt;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&lt;0.05)</td>
</tr>
</tbody>
</table>

GRAS Notification for the Use of 3’-Sialyllactose Sodium Salt
Prepared for Chr. Hansen A/S
May 18, 2021

-33-   SPHERIX CONSULTING GROUP, INC.
<table>
<thead>
<tr>
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</table>
| Kajzer et al., 2016       | Prospective, randomized, double-blind, placebo-controlled study | 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. |
| Alliet et al., 2016       | Randomized, placebo controlled, study                            | 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.                                                                                           |
| Steenhout et al., 2016    | Randomized, placebo controlled, study                            | 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.                                                                                           |
### Table 7. Clinical Studies with Human Milk Oligosaccharides and Adults

<table>
<thead>
<tr>
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</thead>
</table>
| 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. |
| 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. |
| 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 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. |
<table>
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<tbody>
<tr>
<td>Ryan et al., 2021</td>
<td>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</td>
<td>Group 1: 4 g of 2’-FL in combination with micronutrients, macronutrients, amino acids, and isomaltooigosaccharide (n=20)</td>
<td>6 weeks</td>
<td>Twelve subjects completed the study.</td>
<td>Not previously reviewed</td>
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<td>Eight subjects withdrew from the study</td>
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<td>Two dropped out/declined to participate</td>
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<td>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</td>
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<td>Three were lost to follow-up.</td>
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<tr>
<td>Palsson et al., 2020</td>
<td>Open-label, single arm study Adult male and female patients (18 and older) with IBS</td>
<td>Group 1: 5 g of 2’-FL/LNnT (4:1 ratio) (n=317)</td>
<td>12 weeks</td>
<td>Thirteen subjects were discontinued after completing the baseline survey because they did not start the intervention. Therefore, 273 patients completed the study.</td>
<td>Not previously reviewed</td>
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<td>Eight subjects withdrew due to an adverse event.</td>
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<td>Four subjects withdrew consent.</td>
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<td>Nineteen subjects were lost to follow-up.</td>
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<td>The authors reported that there were no incidents causing safety concerns and the patients generally reported that the intervention was well-tolerated</td>
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<td>Forty-seven patients reported a total of 87 adverse events (AEs) in the study</td>
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<td>Sixty-one of the AEs were related to the gastrointestinal tract.</td>
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<td>The most common side effect was passing gas, followed by abdominal distension and pain.</td>
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<td>One serious AE occurred (hospitalization due to colitis) but was determined to be unrelated to the intervention by the study’s medical safety officer.</td>
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</table>
| 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 |
| 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) | 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. | GRN 735, page 61 |
## Table 7. Clinical Studies with Human Milk Oligosaccharides and Adults

<table>
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<tr>
<td></td>
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<td>Group 7: 20 g LNnT (n=10)</td>
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<td>Group 8: 3.3 g 2′-FL; 1.7 g LNnT (n=10)</td>
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<td>Group 9: 6.7 g 2′-FL; 3.4 g LNnT (n=10)</td>
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<td>Group 10: 13.3 g 2′-FL; 6.7 g LNnT (n=10)</td>
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</table>

- 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.
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 3’-SL, published clinical studies administering other non-digestible, poorly absorbed carbohydrates in enteral tube feeding formulas are relevant to understanding the tolerance of 3’-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 3’-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**

<table>
<thead>
<tr>
<th>Citation</th>
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<th>Duration</th>
<th>Safety-Related Findings</th>
</tr>
</thead>
</table>
| Lampe et al., 1992 | Prospective, randomized, placebo-controlled, double-blind, crossover study | 1. Self-selected diet<br>2. Enteral formula containing no added fiber (maltodextrin)<br>3. Enteral formula containing 15 g PHGG/day<br>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 | 1. Standardized normal diet<br>2. Liquid formula diet<br>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>
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</tr>
</thead>
</table>
| Homann et al., 1994 | Prospective, randomized, double-blind, placebo-controlled trial | 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 | 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>
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<th>Duration</th>
<th>Safety-Related Findings</th>
</tr>
</thead>
</table>
| Peters and Davidson, 1996| Prospective, randomized, double-blind cross-over study | 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 | 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 | 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

<table>
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<tr>
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<tbody>
<tr>
<td>Rushdi et al., 2004</td>
<td>Prospective, randomized, double-blind, controlled study</td>
<td>1. Standard fiber-free feed</td>
<td>4 days</td>
<td>• 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.</td>
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<td>2. Enteral feed enriched with 222 g PHGG/L (22 to 37 g PHGG/day)</td>
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<td>• Supplementation with PHGG significantly reduced the number of liquid stools.</td>
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<td></td>
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<td></td>
<td>• There were no differences in the incidence or severity of gastrointestinal symptoms between the two groups.</td>
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<td></td>
<td>• 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.”</td>
</tr>
<tr>
<td>Galactomannan</td>
<td>Open-label study</td>
<td>A semi-digested formula containing galactomannan</td>
<td>4 weeks</td>
<td>• No adverse effects were reported.</td>
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<td>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</td>
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<td>• Serum diamine oxidase activity significantly increased following the treatment with the semidigested formula containing galactomannan.</td>
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<td></td>
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<td>• The water content of the feces decreased, and the frequency of normal stools increased with the semidigested formula containing galactomannan.</td>
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<td></td>
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<td>• 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.</td>
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<td></td>
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<td>• All effects reversed after termination of the galactomannan supplementation.</td>
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<td>• 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.</td>
</tr>
<tr>
<td>Citation</td>
<td>Study Design</td>
<td>Treatments</td>
<td>Duration</td>
<td>Safety-Related Findings</td>
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<tr>
<td>Karakan et al., 2007</td>
<td>Randomized, double-blind, placebo-</td>
<td>1. Diet</td>
<td>2 days</td>
<td>• Both enteral feeding solutions were well tolerated with no reported adverse effects.</td>
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<td></td>
<td>controlled study</td>
<td>2. Diet</td>
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<td>• The median duration of enteral feeding and the hospital stay was significantly shorter</td>
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<td></td>
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<td>containing 0.7</td>
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<td>in the group receiving the fiber-containing diet.</td>
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<td>g/soluble fiber and</td>
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<td>• The fiber-containing diet also significantly improved the pancreatitis severity scores.</td>
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<td>0.8g/100 g insoluble fiber (24 g/day)</td>
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<td>• The authors reported that fiber supplementation in severe AP improves hospital stay,</td>
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<td>duration of nutrition therapy, acute phase response and overall complications compared to</td>
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<td>standard EN therapy.</td>
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<td>Khoshoo et al., 2010</td>
<td>Randomized, double-blind crossover</td>
<td>1. Formula</td>
<td>2 weeks</td>
<td>• There were nine patients with neurological disorders; 3 patients with inflammatory bowel</td>
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<td></td>
<td>study</td>
<td>2. Formula</td>
<td>with a</td>
<td>disease; and 2 patients with short bowel syndrome</td>
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<td>with 3.5 g</td>
<td>5-day</td>
<td>• There were no withdrawals.</td>
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<td>FOS/L (approximately</td>
<td>washout</td>
<td>• There was no significant difference in the daily number of bowel movements between</td>
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<td>3.5 g FOS/ day)</td>
<td>period</td>
<td>children receiving either the fiber or control formulas when evaluating the three</td>
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<td>between</td>
<td>diagnoses groups combined or the short bowel syndrome group alone.</td>
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<td></td>
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<td>treatment</td>
<td>• The children with neurological impairments had more frequent bowel movements when fed</td>
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<td>periods</td>
<td>the control formula than when fed fiber formula whereas the inflammatory bowel disease</td>
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<td>group had more daily bowel movements when fed the fiber-containing formula.</td>
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<td>• Stools were in the “mushy” category when the participants consumed the fiber containing</td>
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<td>formula.</td>
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<td>• Children with neurological impairment had a significantly lower proportion of stools</td>
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<td>(P&lt;0.05) characterized as hard nuts and a significantly lower proportion of stools.</td>
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<td>• In the inflammatory bowel disease group, stool frequency was higher with the fiber</td>
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<td>formula, but there was no change in consistency.</td>
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<td>• There was no difference in the occurrence of vomiting between the two treatments in</td>
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<td>any of the groups.</td>
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<td>• For nine children with a neurological disorder, the mean grade of flatulence/gas was</td>
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<td>significantly less (P&lt;0.05) when participants consumed the fiber formula whereas there</td>
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<td>was no difference in flatulence in the other groups.</td>
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</table>
Table 8. Clinical Studies of Non-digestible Carbohydrates Administered Via Enteral Feeding¹

<table>
<thead>
<tr>
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<th>Duration</th>
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</tr>
</thead>
</table>
| Garleb et al., 1996 | Randomized, double-blind, controlled study | 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        | • 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.”  
• 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 | 1. Control formula  
2. Test formula with 2.6 g/L of oligo-fructose/mulin 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

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</table>
| Majid et al., 2014 | Randomized, double-blind, placebo-controlled study | 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 | A minimum of 3 days | • 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. |
|          |              | 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 | | |
## Table 8. Clinical Studies of Non-digestible Carbohydrates Administered Via Enteral Feeding

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</table>
| Modi et al., 2010 | Prospective, randomized, double-blind, placebo-controlled, multi-center study | 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 | 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

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</thead>
<tbody>
<tr>
<td>Armanian et al., 2016</td>
<td>Prospective, randomized, double-blind, placebo-controlled study</td>
<td>1. Distilled water</td>
<td>1 week</td>
<td>• No adverse effects were reported.</td>
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<td>2. A supplement containing scGOS/lc FOS in a 9:1 ratio</td>
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<td>• Stool frequency was significantly increased in the scGOS/lc FOS-treated group.</td>
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<td>*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</td>
<td></td>
<td>• The authors concluded that scGOS/lc FOS increases stool frequency, improve feeding tolerance and reduce bilirubin level in preterm neonates and therefore can be efficacious for the management of neonatal hyperbilirubinemia.”</td>
</tr>
<tr>
<td>Van den Berg et al., 2015</td>
<td>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 &lt;32 weeks or birth-weight &lt;1500 g</td>
<td>1. Placebo/maltodextrin (n=58)</td>
<td>4 weeks</td>
<td>• Nine infants died in the placebo-treated group whereas six infants died in the scGOS/lc FOS/pAOS-treated group.</td>
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<td>2. scGOS/lc FOS/ pectin-derived acidic oligosaccharides (pAOS) (n=55)</td>
<td></td>
<td>• Adverse events were not reported.</td>
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<td>• 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.”</td>
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</table>

1Incorporated 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 3’-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 3’-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 3’-SL to OESs are not expected.

c. **Lack of Impact of 3’-SL on Osmolarity**

The WHO current standard OES osmolarity is 245 mOsm/L; Pedialyte® from Abbott is 250 mOsm/L (Oféi 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 0.1 g/L of 3’-SL to OES, such as Pedialyte®, is calculated on the basis of molar weight to add 4.6 mOsm/L (0.2 mOsm/L 3’-SL and 4.4 mOsm/L sodium). Thus, the addition of 1.2 g/L 3’-SL will not impact the osmolarity of the typical OES.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Trial Design</th>
<th>Test Article</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alam et al., 2015</td>
<td>Randomized, double-blind placebo controlled clinical trial of 126 children</td>
<td>Group 1: Standard hypotonic oral rehydration solution (ORS)</td>
<td>The mean duration of diarrhea was significantly shorter in children in Group 2 compared to Group 1.</td>
</tr>
<tr>
<td></td>
<td>(male and female) (weight for length/weight for age &lt;3 Z-score with or without pedal edema), aged 6-36 months with acute diarrhea</td>
<td>Group 2: Standard hypotonic ORS with 15 g/L partially hydrolyzed guar gum</td>
<td>Adverse events/tolerance related to test article not reported by authors.</td>
</tr>
<tr>
<td>Passariello et al., 2011</td>
<td>Single-blind, prospective, controlled trial including children (age range, 3-36 months with acute diarrhea</td>
<td>Group 1: Standard hypotonic oral rehydration solution (ORS)</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 2: hypotonic ORS with zinc, 0.35 g/L fructooligosaccharides and 0.35 g/L xylooligosaccharides</td>
<td>Total ORS intake in the first 24 hours of rehydration therapy was statistically significantly lower in Group 1 than Group 2.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No adverse events related to the use of the ORS were observed in the study groups.</td>
</tr>
<tr>
<td>Vandenplas et al., 2011</td>
<td>Randomized, prospective, double-blind placebo-controlled trial in children between 3 and 186 months (males and females) with acute diarrhea</td>
<td>Group 1: Standard hypotonic oral rehydration solution (ORS)</td>
<td>Children in Group 2 had significantly reduced duration of diarrhea compared with Group 1.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group 2: Standard hypotonic ORS with a symbiotic blend (Streptococcus thermophilus, Lactobacillus rhamnosus, Lactobacillus acidophilus, Bifidobacterium lactis, Bifidobacterium infantis, fructo-oligosaccharides).</td>
<td>Adverse events/tolerance related to test article not reported by authors.</td>
</tr>
<tr>
<td>Raghupathy et al., 2006</td>
<td>Randomized, double-blind, placebo-controlled study including boys aged 6 months to 3 years with acute diarrhea with clinically detectable dehydration</td>
<td>Group 1: Standard hypotonic oral rehydration solution (ORS) (311 mOsm/kg)</td>
<td>Statistically significant shortened duration of diarrhea in Group 2 compared to Group 1.</td>
</tr>
<tr>
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<td></td>
<td>Group 2: Standard hypotonic ORS with 50 g/L high-amylose maize starch</td>
<td>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.</td>
</tr>
</tbody>
</table>
## Table 9. Studies of Oral Electrolyte Solutions (OES) with Added Non-digestible Carbohydrate

<table>
<thead>
<tr>
<th>Reference</th>
<th>Trial Design</th>
<th>Test Article</th>
<th>Results</th>
</tr>
</thead>
</table>
| 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 3’-SL ingredient was extensively reviewed in GRN 921. Therefore, the allergenicity summary in GRN 921 is incorporated by reference (see page 76 of GRN 921). Allergic reactions resulting from the exposure to Chr. Hansen A/S’s 3’-SL product are not expected based on the following:

- 3’-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-3SL* 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 3SL is controlled with a specification of ≤ 0.01 % protein.

H.  REGULATORY APPROVALS AROUND THE WORLD

In the United States, 3’-SL is GRAS and the subject of GRAS Notifications 766, 880, 921. 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_2019-0957.pdf; accessed on January 4, 2021). Although an opinion by the European Commission has not been published, following their review of the 3’-SL Novel Food application submitted by Glycom A/S, the European Food Safety Authority agreed that the NOAEL for Glycom’s 3’-SL is 5,000 mg/kg bw/day, which was the highest dose tested in the 90-day oral toxicity study conducted by Phipps et al. (2018), intake of 3’-SL at the proposed use levels is unlikely to exceed the intake level of naturally occurring 3’-SL in breastfed infants on a body weight basis, the intake of other carbohydrates structurally related to 3’-SL is not a safety concern, and that 3’-SL is safe for use in infant and follow-on formulas at 0.2 and 0.15 g/L, respectively, and in selected conventional foods and food supplements up to 2.5 g/kg (EFSA Panel on Nutrition et al., 2020; EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) et al., 2019).
VII. SUPPORTING DATA AND INFORMATION

A. REFERENCES

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


GRN 852 (2019). 2’-Fucosyllactose. BASF SE.


GRN 895 (Pending). Lacto-N-neotetraose. Glycom A/S.


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 3’-sialyllactose sodium salt (3’-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 3’-SL 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 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 3’-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 88% 3’-SL dry weight. The remaining components include carbohydrate by-products, ash, and moisture.
   a. 3’-Sialyllactose is a naturally occurring acidic oligosaccharide in human milk.
   b. Published studies showing that the amount of 3’-SL in breast milk ranges from 0.04 to 2.89 g/L.
   c. Human milk oligosaccharides, including 3’-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 GRAS Notice 921, which received a “no questions” letter on October 30, 2020 for the use 3’-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. 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.

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

f. 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 0.153 and 0.356 g/day (0.002 and 0.005 g/kg bw/day) for consumers not less than 2 years old.

g. 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 1.05 and 3.78 g/day (0.016 and 0.056 g/kg bw/day) for consumers not less than 2 years old.

h. Use of the subject of this GRAS determination in oral electrolyte solutions results in an estimated daily intake of 0.1–0.2 g of 3’-SL (equivalent to 0.7–1.4 mg of 3’-SL/kg bw/day assuming a 13.5 kg toddler and 0.1–0.3 mg of 3’-SL/kg bw/day assuming a 70 kg adult). Because OESs are intended for short term use, intake of 3’-SL from OESs will not impact the cumulative 3’-SL intake resulting from the use of 3’-SL in select conventional foods and enteral tube feeding formulas.
3. Genotoxicology and subchronic toxicology studies published by Phipps et al. (2019) show that 3’-SL is not genotoxic and has a NOAEL (no observed adverse effect level) of 5 g/kg bw/day, which was the highest dose tested.

4. The safety of exposure to Chr. Hansen A/S’s 3’-SL at its intended use level is supported by:

   a. Data demonstrating the qualitative and quantitative similarities between the subject of this GRAS Notice and the 3’-SL ingredient tested in the pivotal genotoxicology and subchronic toxicology studies conducted by Phipps et al. (2019), which is also the subject of GRN 880;

   b. The lack of genotoxicity and no observed adverse effect level (NOAEL) for 3’-SL established in the 90-day subchronic dietary toxicology conducted by Phipps et al. (2019);

   c. Published genotoxicology, 90-day subchronic dietary toxicology, and neonatal piglet studies conducted with 3’-SL or a mixture of HMOs containing the subject of this GRAS Determination (Parschat et al., 2020; Donovan et al., 2017; Hanlon, 2020);

   d. Clinical data showing the ingestion of HMOs are well tolerated in infants up to 1.0 g/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 921);

   g. The GRAS status of other 3’-SL products for use in selected conventional foods (GRN 766; GRN 880).
Therefore, 3’-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. 3’-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, GRAS Expert Panel Member
Medicine Public Health & Nutrition
The Daedalus Foundation

Signature: __________________________
Date: May 18, 2021

A. Wallace Hayes, PhD, DABT, FATS, ERT
GRAS Expert Panel Member
Harvard School of Public Health

Signature: __________________________
Date: May 18, 2021

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

Signature: __________________________
Date: May 18, 2021

Claire Kruger, PhD, DABT
Scientific Advisor to the Panel

Signature: __________________________
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)  

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**

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<td>☐ Amendment to GRN No.</td>
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<tr>
<td>☐ Supplement to GRN No.</td>
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| ☑ All electronic files included in this submission have been checked and found to be virus free. (Check box to verify) |

| ☑ Yes | If yes, enter the date of response to a communication from FDA (yyyy/mm/dd): |
| No    | |

**SECTION B – Information About the Notifier**

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<tr>
<td>Name of Contact Person</td>
<td>Kate Urbain</td>
</tr>
<tr>
<td>Position or Title</td>
<td>Head of Regulatory Affairs North America</td>
</tr>
<tr>
<td>Organization (if applicable)</td>
<td>Chr. Hansen A/S</td>
</tr>
<tr>
<td>Mailing Address (number and street)</td>
<td>9015 W Maple St.</td>
</tr>
<tr>
<td>City</td>
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<tr>
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<td>E-Mail Address</td>
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<th>1b. Agent or Attorney (if applicable)</th>
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<tr>
<td>Name of Contact Person</td>
<td>Dietrich B. Conze</td>
</tr>
<tr>
<td>Position or Title</td>
<td>Managing Partner</td>
</tr>
<tr>
<td>Organization (if applicable)</td>
<td>Spherix Consulting Group, Inc.</td>
</tr>
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<td>Mailing Address (number and street)</td>
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<tr>
<td>E-Mail Address</td>
<td><a href="mailto:dconze@spherixgroup.com">dconze@spherixgroup.com</a></td>
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SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term
3'-Sialyllactose sodium salt (3'-SL)

2. Submission Format: (Check appropriate box(es))
   - [ ] Electronic Submission Gateway
   - [x] 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)
   - [x] 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)
   - [x] a) GRAS Notice No. GRN 921
   - [ ] b) GRAS Affirmation Petition No. GRP __________
   - [ ] c) Food Additive Petition No. FAP __________
   - [ ] d) Food Master File No. FMF __________
   - [x] e) Other or Additional (describe or enter information as above) GRN 546, 571, 650, 659, 735, 749, 766, 815, 852, 880, 897, 919, 925

6. Statutory basis for conclusions of GRAS status (Check one)
   - [x] 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)
   - [x] 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 3'-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  [x] 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

3'-Sialyllactose sodium salt (3'-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

(name of notifier)

conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

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

Dietrich B. Conze, PhD

Dietrich B. Conze, PhD, Managing Partner

Date (mm/dd/yyyy)

06/04/2021
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.

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