Soni & Associates Inc.

749 46th Square
Vero Beach, FL 32968, USA
Telephone: 772-299-0746
Facsimile: 772-299-5381
E-mail: msoni@soniassociates.net

January 15, 2021

Paulette M. Gaynor, Ph.D.
Senior Policy Advisor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD 20740



**Subject: GRAS Notification for use of short-chain Fructooligosaccharides in Infant Formula**

Dear Dr. Gaynor:

We respectfully submit the attached GRAS notice, on behalf of Tata Chemicals Limited (India) for use of short-chain fructooligosaccharides (scFOS) in infant formula. As regards submission of this GRAS notice, please note that on March 8, 2018, we had a pre-GRAS meeting with Dr. Morissette and her team.

Based on the discussions with FDA and FDA recommendations (memorandum of meeting- March 15, 2018), we have prepared the attached GRAS notice of a claim that the use of scFOS in infant formula, described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be GRAS, based on scientific procedures.

Please also note that the attached GRAS notice is a follow-up to Tata Chemical's GRN 000605, which was for the intended use of FOS in conventional foods and received a No Questions letter from FDA on March 17, 2016.

As required, please find enclosed three copies of the GRAS notification. If you have any questions or require additional information, please feel free to contact me by phone at 772-299-0746 or by email at sonim@bellsouth.net .

Sincerely,

Madhu G. Soni, Ph.D.

Enclosure: Three copies of the GRAS notification

# GENERALLY RECOGNIZED AS SAFE (GRAS) EVALUATION OF SHORT CHAIN FRUCTO-OLIGOSACCHARIDES FOR USES IN TERM INFANT FORMULA

**Submitted by:**
Tata Chemicals Limited
Innovation Centre
Ambedveth (V), Paud road, Mulshi,
Pune, Maharashtra - 412108
INDIA

**Submitted to:**
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
HFS-200
5100 Campus Drive
College Park, MD 20740
USA

December, 2020

# GENERALLY RECOGNIZED AS SAFE (GRAS) EVALUATION OF SHORT CHAIN FRUCTO-OLIGOSACCHARIDES FOR USES IN TERM INFANT FORMULA

## TABLE OF CONTENTS

## 1. Part I – SIGNED STATEMENTS AND CERTIFICATION

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through §170.285, Tata Chemicals Limited (Tata), India hereby informs the FDA that short-chain fructo-oligosaccharides (scFOS), as manufactured by Tata, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Tata's view that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Section 1.3 below.

### 1.1. Name and Address of Notifier

Tata Chemicals Limited
Bombay House, 24 Homi Modi Street,
Fort, Mumbai Maharashtra – 400001
INDIA

### 1.2. Name of Notified Substance

The common name of the substance of this Generally Recognized As Safe (GRAS) assessment is short-chain fructo-oligosaccharides (scFOS) or oligofructose. scFOS for food uses will be marketed as standardized (to the content of FOS) powder. scFOS will be marketed under the tradename- FOSSENCE™.

### 1.3. Intended Conditions of Use

Short-chain fructo-oligosaccharides (scFOS) is intended for use as an ingredient in non-exempt term infant formula at the maximum intended addition levels of 400 mg scFOS/100 ml in starter formula (from birth to approximately 6 months) as consumed and 500 mg scFOS/100 ml in follow-on formula (infants older than approximately 6 months) as consumed. FOS is not intended for addition to pre-term formula. The intended uses and levels of scFOS in term infant formula are identical to those described in GRN 537 (Ingredion, 2014) and GRN 797 (NFBC, 2018). Based on energy intakes and the energy content of infant formula, the 90$^{th}$ percentile formula intake for males and females combined is estimated as 207 ml/kg body weight (bw)/day. The 90$^{th}$ percentile intake of scFOS is estimated as 828 mg/kg bw/day from starter formula within the first month of life and about 800 mg/kg bw/day from the follow-on formula thereafter.

### 1.4. Statutory Basis for GRAS Determination

This GRAS conclusion is based on scientific procedures in accordance with 21 CFR 170.30(a) and 170.30(b).

### 1.5. Exclusion from Premarket Approval

Tata has determined that the use of scFOS derived from enzymatic conversion of sucrose is Generally Recognized As Safe, under the conditions of its intended use in non-exempt infant formula, consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This GRAS conclusion has been reached in accordance with requirements in 21 CFR 170.220. Therefore, the use of FOS derived from enzymatic conversion of sucrose is exempt from the premarket approval requirements of the FD&C Act.

### 1.6. Availability of Data & Information

The data and information that are the basis for this GRAS conclusion will be made available to FDA upon request by contacting Mr. Dipak Bagad at the below addresses. The data and information will be made available to FDA in a form in accordance with that requested under 21 CFR 170.225I(7)(ii)(A) or 21 CFR 170.225(c)(7)(ii)(B).

Mr. Dipak Bagad
Manager - Regulatory Affairs
Tata Chemicals Limited
Innovation Centre, Tata Chemicals Limited
Ambedveth (V), Paud road, Mulshi,
Pune, Maharashtra – 412108
INDIA

Tel: +91- 020 66549772
Fax: +91-020 66549735
Email: dbagad@tatachemicals.com

### 1.7. Data Exemption from Disclosure

Parts II through VII of this GRAS notification do not contain data or information that is exempt from disclosure under the Freedom of Information Act. There is no privileged or confidential information such as trade secrets and/or commercial or financial information in this document and the information contained in this dossier can be made publicly available.

### 1.8. Certification

Tata certifies that, to the best of its knowledge, this GRAS conclusion is based on a complete, representative, and balanced dossier that includes all relevant information, available and obtainable by TATA, including any favorable or unfavorable information, and pertinent to the evaluation of the safety and GRAS status of the use of scFOS preparation. Tata accepts responsibility for the GRAS determination that has been made for FOS derived from enzymatic conversion of sucrose as described in this dossier.

### 1.9. Name, Position/Title of Responsible Person who Signs the Dossier and Signature

Mr. Rahul Gupta
Business Head – Nutritional Solutions
Tata Chemicals Limited
Mumbai, Maharashtra
INDIA

Tel: +91- 8976056249
Fax: +91- NA
Email: rgupta@tatachemicals.com

Signature: 

### 1.10. FSIS/USDA – Use in Meat and/or Poultry

Tata does not intend to add scFOS to any meat and/or poultry products that come under USDA jurisdiction. Therefore, 21 CFR 170.270 does not apply.

## 2. Part II – IDENTITY, SPECIFICATION, MANUFACTURING AND TECHNICAL EFFECTS

Short-chain fructo-oligosaccharides (scFOS) are derived from food grade sucrose via a transfructosylation catalyzed by β-fructofuranosidase enzyme derived from a non-pathogenic and non-toxigenic strain of *Aureobasidium pullulans*.

### 2.1. Identity

#### 2.1.1. Description

The scFOS product is white to light yellow syrup or off white to light yellow powder with slight sweet taste and no odor.

#### 2.1.2. Synonyms and Trade Names

FOS; Oligofructose; short-chain fructo-oligosaccharides (scFOS or FOS); Neosugar. The systematic name of all fructans, including scFOS, is [α-D-glucopyranoside-(1-2)-]-β-D-fructofuranosyl-[(1-2)-β-D-fructofuranosyl]$_n$.

The subject of this GRAS assessment will be marketed under the trade name FOSSENCE™.

#### 2.1.3. Chemical Abstract Registry Number

The CAS Registry Number for fructo-oligosaccharides (FOS) is 308066-66-2.

#### 2.1.4. Chemical Formula and Molecular Weight

The molecular formula for all fructans is $C_6H_{11}O_5(C_6H_{10}O_5)nOH$. The formulas of its three components are: 1-kestose – $C_{18}H_{32}O_{16}$, nystose – $C_{24}H_{42}O_{21}$, and fructofuranosylnystose – $C_{30}H_{52}O_{26}$. The molecular weight of individual three components of scFOS is as follows: 1-kestose- 505 Da; nystose- 666 Da; and fructofuranosylnystose- 828 Da.

#### 2.1.5. Chemical Structure

scFOS are a mixture of oligosaccharides consisting of a sucrose molecule (glucose – fructose disaccharide, GF1) linked to one (GF2; degree of polymerization or DP3), or two (GF3; DP4) or three (GF4; DP5) additional fructose units added by β2-1 glycosidic linkages to the fructose unit of the sucrose. Fructans can have degrees of polymerization (the number of fructose or glucose residues) ranging from 2 to over 60. scFOS consists entirely of molecules with degrees of polymerization between 3 and 5, consisting of 2 to 4 fructose residues and a single terminal glucose residue. scFOS, the subject of this present GRAS dossier, primarily consists of 3 different molecules, each containing a terminal glucose residue and 2, 3, or 4 fructose residues, designated as GF2, GF3, and GF4, also called as 1-kestose, nystose, and fructofuranosylnystose, respectively. The structural formulas of 1-kestose, nystose, and fructofuranosylnystose are shown in Figure 1.

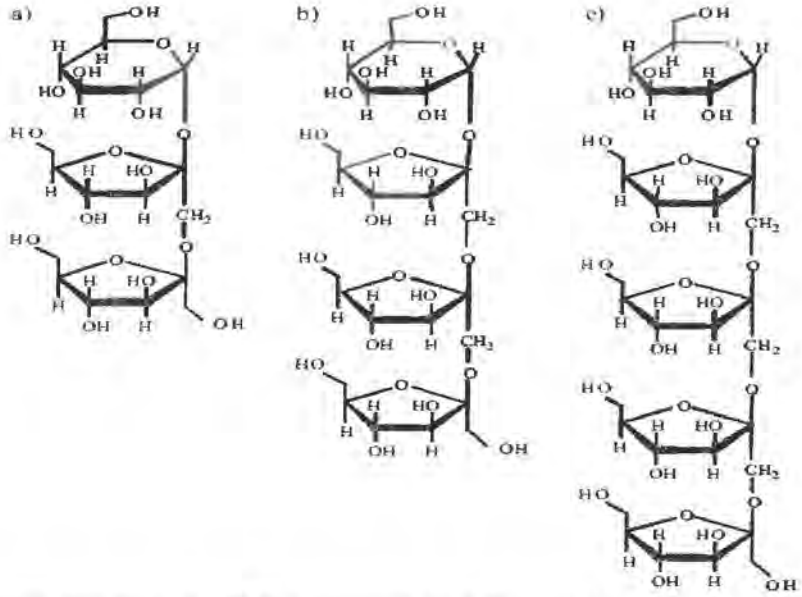


**Figure 1. Chemical Structure of scFOS components (a) 1-Kestose (GF1), (b) Nystose (GF2), and (c) Fructofuranosylnystose (GF3). Fructosyl units are linked at position β-2, 1 of sucrose.**

### 2.1.6. Other Chemically Related Constituents

As described above, the subject of present GRAS assessment primarily contains small-chain fructo-oligosaccharides. Similar to scFOS, the longer chain chemically related fructans, such as oligofructose and inulin, of β2-1 linked fructose molecules that may or may not have a terminal glucose molecule are primarily derived by isolation and/or partial enzymatic hydrolysis of inulin from chicory root. The term oligofructose has been typically used to characterize linear oligosaccharides, ranging 3 to 6 saccharides in length. The term inulin is typically used to define long-chain polymers of β2-1 linked fructose molecules with degrees of polymerization ranging from 10 to 60 or more saccharides in length. These related polymers have similar chemical composition to scFOS and are likely to have similar toxicological and physiological characteristics following ingestion. These oligomers display a higher molecular weight distribution. Given these differences between scFOS and other inulin type fructans, the subject of this GRAS dossier has been primarily limited to discussion of scFOS produced from sucrose by enzymatic synthesis. As some fructans product also contain relatively high levels of scFOS, these products are also considered in this GRAS assessment.

## 2.2. Specifications

Food grade specifications of scFOS have been established by Tata Chemicals Limited (Tata). scFOS for uses in infant formula will be marketed in the form of powder and liquid. The specifications of scFOS-P95 and scFOS-L95 are presented in Table 1. To demonstrate conformance with the food-grade specifications, Tata analyzed three batches of scFOS. Analytical results from three lots (Appendix I) suggest that scFOS powder ((Appendix I A) as well as liquid (Appendix I B) is consistently manufactured to meet the standard specifications. The distribution ratio of scFOS components [1-kestose (GF2), Nystose (GF3) and Fructofuranosylnystose (GF4)] for FOS-P95 and FOS-L95 is provided in Appendix I A and B. The batch analysis data for scFOS demonstrate that the manufacturing process produces oligomers that are characteristic of typical scFOS preparations derived from sucrose by enzymatic action with GF2, and GF3 representing the major fructose oligomers and lower

quantities of longer chain GF4. The final product also contains small amounts (~5%) of residual sucrose, glucose and fructose representing the major by products or residues in the ingredient. The subject of this GRAS assessment, scFOS, is substantially equivalent to the scFOS that was the subject of the GRAS notified substances for uses in infant formula, reviewed by the FDA with no questions [including GRN 797 (NFBC, 2018) GRN 537 (Ingredion, 2014) and GRN 44 (GTC Nutrition, 2000)].

**Table 1. Food Grade Specifications of scFOS Powder (FOS-P95) and Syrup (FOS-L95)**

| Parameters | Specifications | | Method |
|---|---|---|---|
| | FOS-P95 | FOS-L95 | |
| Description | Fine white free flowing hygroscopic powder (clear in solution) | Colorless to sunshine yellow color syrupy liquid | Sensory test |
| Taste and Aroma | Sweet, without foreign tastes / odors | Sweet, without foreign tastes / odors | Sensory test |
| Total solids (%) | NLT 97.0 | - | FCC (Fructooligosaccharides short chain) |
| Moisture (Karl Fisher) (%) | NMT 5.0 (w/w) | NMT 25 | In-house |
| Brix (Refractometer) °Bx | - | NLT 75 | In-house |
| Residue on ignition (sulphated ash) (%) | NMT 0.1 | NMT 0.1 | FCC (Fructooligosaccharides short chain) |
| pH (pH meter with 10% solution @ 25°C) | 5.0 - 7.5 | 5.0 - 7.5 | In-house |
| **Carbohydrate composition** (a) Identification | | | |
| Fructose (% dry basis) | NLT 67.0 | NLT 67.0 | FCC (Fructooligosaccharides short chain) |
| Glucose (% dry basis) | NMT 33.0 | NMT 33.0 | |
| (b) Assay | | | |
| Total Fructooligosaccharides (%) | NLT 95.0 | NLT 95.0 | |
| -- Trimer (GF2) | Informative | Informative | |
| -- Tetramer (GF3) | Informative | Informative | |
| -- Pentamer and larger (GF4 and higher) | Informative | Informative | |
| (c) Sucrose + Glucose + Fructose | NMT 5.0 | NMT 5.0 | AACC 80-04.01 |
| **Heavy metals** | | | |
| Lead (as Pb) (ppm) | NMT 0.02 | NMT 0.02 | SO-IN-MUL-TE-063A By ICPMS |
| Arsenic (as $As_2O_3$) (ppm) | NMT 0.1 | NMT 0.1 | SO-IN-MUL-TE-063A By ICPMS |
| Cadmium (Cd) (ppm) | NMT 0.01 | NMT 0.01 | SO-IN-MUL-TE-063A By ICPMS |
| Mercury (as Hg) (ppm) | NMT 0.01 | NMT 0.01 | SO-IN-MUL-TE-063A By ICPMS |
| Chromium (as Cr) (ppm) | NMT 0.05 | NMT 0.05 | SO-IN-AFL-MNR-C-TE-006 |
| Tin (as Sn) (ppm) | NMT 50.0 | NMT 50 | SO-IN-AFL-MNR-C-TE-006 |
| Copper (as Cu) (ppm) | NMT 30.0 | NMT 30 | SO-IN-AFL-MNR-C-TE-006 |

**Table 1. Food Grade Specifications of scFOS Powder (FOS-P95) and Syrup (FOS-L95)**

| Parameters | Specifications | | Method |
|---|---|---|---|
| | FOS-P95 | FOS-L95 | |
| Methyl Mercury (Calculated as the element) (ppm) | NMT 0.25 | NMT 0.25 | SO-IN-AFL-MNR-C-TE-006 |
| **Microbiological limits** | | | |
| Total Plate Count (cfu/g) | NMT 300 | NMT 300 | IS 5402 : 2012 |
| Enterobacteriacea (MPN/g) | NMT 3 | NMT 3 | IS 7402 |
| Yeasts & Mould (cfu/g) | NMT 20 | NMT 20 | IS 5403 :1999 (Reaff.2013) |
| *Escherichia coli* (MPN/g) | Absent 10 g | Absent 10 g | IS 5887 (Part I) 1976 (Reaff. 2013) |
| *Staphylococcus aureus* | Absent 10 g | Absent 10 g | IS 5887 (Part II) 1976 (Reaff. 2013) |
| Salmonella spp | Absent 100 g | Absent 100 g | IS 5887 (Part III) 1999 (Reaff. 2013) |
| Shigella spp | Absent 25 g | Absent 25 g | IS 5887 (Part VII) 1999 (Reaff. 2013) |
| Listeria monocytogenes | Absent 25 g | Absent 25 g | ISO 11290 (Part I) 2017 |
| Sulphite reducing Clostridia (cfu/g) | NMT 10 | NMT 10 | ISO 15213: 2003 |
| *Cronobacter sakazakii* | Absent 300 g | Absent 300 g | ISO 22964: 2017 |
| *Bacillus cereus* (cfu/g) | NMT 100 | NMT 100 | IS 5887 (Part VI) 1999 (Reaff. 2005) |
| **Mycotoxins** | | | |
| *Aflatoxin B1 (ppb)* | NMT 0.5 | NMT 0.5 | AOAC 999.07 by HPLC using immunoaffinity column and Kobra cell |
| *Aflatoxin B2 (ppb)* | NMT 0.5 | NMT 0.5 | |
| *Aflatoxin G1(ppb)* | NMT 0.5 | NMT 0.5 | |
| *Aflatoxin G2 (ppb)* | NMT 0.5 | NMT 0.5 | |
| *Aflatoxin M1(ppb)* | NMT 0.025 | NMT 0.025 | SO-IN-AFL-MNR-C-TE-065 |
| *Melamine (ppm)* | NMT 0.5 | NMT 0.5 | SO-IN-AFL-MNR-C-TE-023 (Ref: USFDA) |

NLT = Not less than; NMT = Not more than; CFU = Colony forming units

## 2.3. Manufacturing Process

scFOS (FOSSENCE™) is produced by the action of microbial enzyme; β-fructofuranosidase/fructosyltransferase on sucrose syrup. β-Fructofuranosidase is an intracellular enzyme produced by a wild type (natural) strain of the fungus, *Aureobasidium pullulans*, referred here as Culture. The microorganism (*A. pullulans*) used in the production of scFOS, which is intended to be used in infant formula is the same that has been used in the manufacturing of scFOS, subject of GRN 605 that received no question letter from FDA for the use in conventional foods. In the food industry, *A. pullulan* is used in the production of food ingredients. *A. pullulan* is used in the production of pullulan. *A. pullulans* used in the production of scFOS is non-toxigenic and non-pathogenic and is registered under the Microbial Type Culture Collection (MTCC), Chandigarh in India with accessions no MTCC 5490. The production process of scFOS is developed by using whole cell microbial biotransformation technique utilizing the membrane bound enzyme; β-Fructofuranosidase. The production process of the FOS contains three major steps:

1. Microbial fermentation for the production of cell biomass, which is used as source of enzyme this process also referred as Upstream Process (USP).
2. Sugar Solution Preparation & Biotransformation of sucrose to FOS (BT)
3. Purification and Concentration of FOS or Downstream Process (DSP).

**1. Microbial fermentation for the production of enzyme and cell biomass or Upstream Process (USP):**

In the first step of FOS production, microbial Culture (biomass) is generated by fermentation technique by inoculating of seed-culture in the main fermenter. The Lyophilized vial (master culture) from culture bank is used to prepare a mother culture from which the stock culture is prepared subsequently. These stock cultures are stored at -80°C and used to prepare the working cultures for the production. The culture is prepared using growth media consisting of anhydrous glucose, yeast extract powder, peptone and polypropylene glycol (antifoaming agent). Flasks with the media are inoculated and incubated at 27-28°C for the period 24-48 hours. In addition, the culture is also inoculated into sterile nutrient broth (NB) tubes for sterility checking, that are incubated at 37°C. Following confirmation of desired growth pattern and purity (i.e., no contamination), the culture is used as the seed inoculum for the next stage. The quantity of the microbial biomass is built up to the production level through successive stages of culturing of the seed culture, verifying at each step for desired characteristics and purity. On the last step, the growth medium is slightly modified to include sucrose in order to stimulate the production of the intracellular enzyme by the microbial culture. The biomass required for production is separated using a plate and frame filter press under sterile conditions. The separated biomass is kept frozen (-80 $^0$C) till needed for production.

**2. Sugar Solution Preparation & Biotransformation (BT):**

In the second step, initially prepared the sugar solution from the purified cane sugar (50 brix) and pasteurized at 72$^0$C for 30 seconds. The generated biomass of Culture-A is then reacted with the sugar solution (sucrose 50%) in a bioreactor and the reaction is carried out at optimum temperature, pH & agitation speed. The progress of the reaction is monitored with the help of HPLC by analyzing the reaction mixture at various time intervals. The reaction is terminated after complete conversion of sucrose to FOS which would be around 55 to 60%. The termination of reaction is carried by heating whole fermentation broth at 80$^0$C for 1 hours, this treatment inactive the enzyme and culture biomass. Subsequently, biomass is harvested by filtration with filter press and the clear filtrate is subjected to downstream operations.

**3. Purification and Concentration of FOS or Downstream Process (DSP) of the FOS.**

Recovered dilute, partly pure FOS solution from the biotransformation step is subjected to various downstream processing operations for the purification and concentration purpose as mentioned below:

A. Activated Carbon Treatment

B. Resin Treatment

C. Chromatography treatment

D. Polishing treatment

E. Concentration and Pasteurization

F. Spray drying and packaging

**A. Activated carbon treatment**:

Activated carbon treatment is carried out to remove color and organic impurities generated during the biotransformation process. In this process activated carbon is added in the enzyme inactivated FOS solution and the mixture is stirred for the 4 hours at $60^0$C and then carbon is separated from the FOS solution with help of plate filtration system. Subsequently FOS solution is filtered through 0.2 micron filtration to removes carbon traces.

**B. Resin treatment:**

Resin treatment is carried out by using ion exchange resins for the removal of color, organic, metal and mineral (ash) impurities. There are two types of the food grade polymeric resins; cations and anions are used for the treatment. Both the resins are filled in a mixed bed resin column and the column is regenerated with acid and alkali solutions. The FOS solution collected after carbon treatment is passed from the column at $40^0$C temperature and subsequently collected in a clean tank.

**C. Chromatography treatment:**

The resin purifies FOS solution is further passes through the chromatography column, which is filled with the gel filtration type food grade polymeric resins to improve the FOS content and remove other saccharides. The outcome of the chromatography will be more than 95% purity FOS solution.

**D. Polishing treatment:**

The polishing treatment is carried out by using mixed bed resin column, which is filled with anionic and cationic food grade polymer resins, which further removes the traces of organic, metal and mineral (ash) impurities. The resin treatment is carried out at 40 $^0$C temperature. The clean FOS solution is further passed through 0.2 micron polish filter to remove any of the fine particles and contaminants.

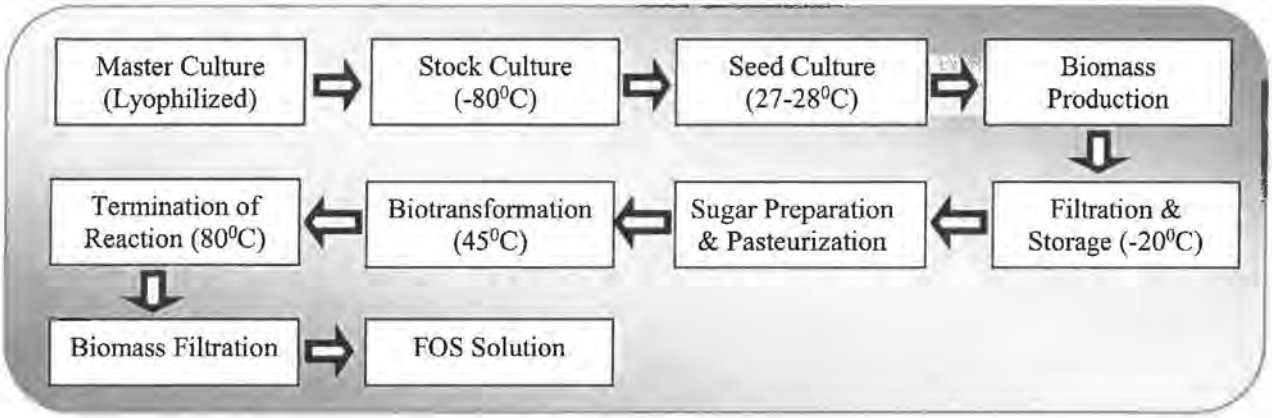
**E. Concentration and pasteurization:**

Concentration is mainly carried out to remove the water content of the liquid dilute FOS solution collected after polishing step. FOS solution is subjected to vacuum evaporation system and concentration is performed at $65^0$C to achieve the brix of the solution to 50% & 75%, for the manufacturing of powder product and liquid products, respectively. The concentrated solution is then pasteurized at $76^0$C temperature for 15 seconds with the help of heat exchanger, which ultimately reduces the microbial contaminations to the accepted levels.

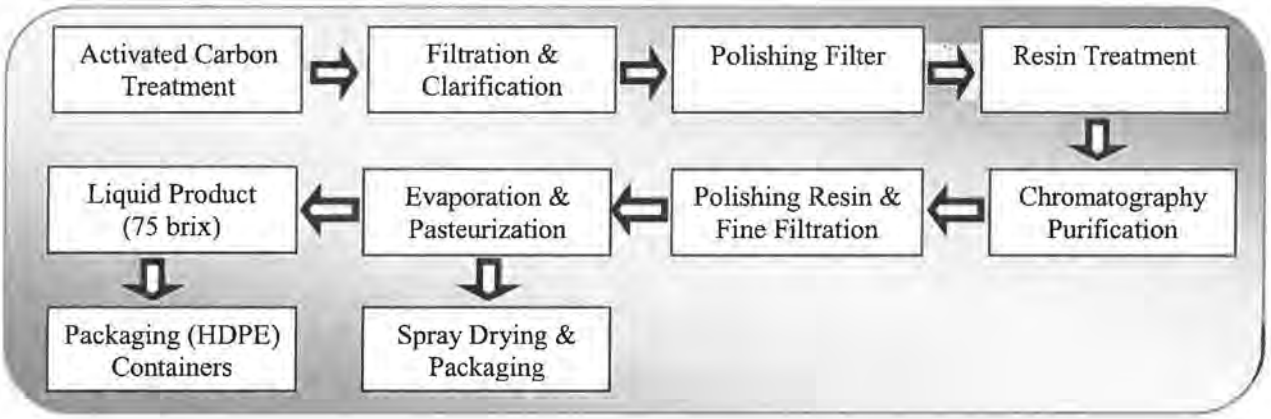
**F. Spray drying and packaging:**

The concentrated and pasteurized 50 brix solutions is finally spray dried in a spray drier and maintained the moisture content below 3% to reduce the possibility of lump formation and microbial contaminations. The powder product is then packed in a clean 20 Kg Nylon bag. Whereas the liquid product is packed in 25 Kg Jerry Cans, made up of food grade containers (HDPE). The final product is then stored in dry place at ambient temperature.

**Process Flow Diagram – Upstream Process –**




**Process Flow Diagram – Downstream Process –**




All raw materials and processing aids used in the manufacturing process for scFOS such as hydrochloric acid, sodium hydroxide, and activated carbon, are suitable, food grade, and are used in accordance with current good manufacturing practices. Hydrochloric acid and sodium hydroxide are GRAS for use in food production, limited only by current Good Manufacturing Practice (21 CFR §182.1057 and §184.1631, respectively). Food-grade activated carbon is an unlisted GRAS substance with a long history of safe use in food processing. The resins and microfiltration used are in compliance with FDA guidelines. The manufacturing facility is certified with FSSC 22000 (Version 5) (2020/23).

### 2.4. Technical Effects

Tata intends to add scFOS to infant formula in order to enhance the organoleptic properties and palatability of formula, and to provide a non-digestible oligosaccharide that may improve stool consistency, reduce the risk of constipation, serve as a source of colonic fermentation, and modulate colonic bacterial colonization in the infant receiving the formula containing scFOS.

## 3. Part III – DIETARY EXPOSURE

### 3.1. Intended Use Levels and Food Categories

Tata intends to use scFOS in non-exempt infant formula at maximum addition levels of 400 mg/100 ml in starter formula (from birth to approximately 6 months) as consumed and 500 mg/100 ml in follow-on formula (infants older than approximately 6 months) as consumed.

### 3.1.1. Estimated Daily Intake from the Proposed Uses

The proposed uses of scFOS, by Tata, as a food ingredient in term infant formula and follow-on formula at use levels 400 and 500 mg/100 ml of formula as consumed, respectively, are identical to those described in the previous GRAS notices that received no question letter from FDA. The resulting exposures of scFOS from its proposed uses have been estimated in the previous GRAS notices (GRN 797; GRN 537) submitted to FDA. The scFOS product described by Ingredion (2014) in GRAS notice (GRN 537) and NFBC in GRAS notice (GRN 797) was reported to contain 95% of scFOS. The subject of this present GRAS notice also contains the same levels of FOS. Furthermore, composition of the three primary constituents of scFOS (1-Kestose, Nystose and Fructofuranosylnystose) in the subject of present GRAS notification is substantially equivalent to the subject of GRN 537 (Ingredion, 2014) and GRN 797 (NFBC, 2018).

In determining the FOS intake, Ingredion (2014) in GRN 537 considered daily energy intake of formula fed children. In these estimates of intake, daily energy consumption of infants fed infant formula provided by Femon (1993) were considered. The subpopulation of infants, boys in the age range 14-27 days, were found to have the highest intake of energy per kg body weight. The 90$^{th}$ percentile energy intake in this age group was reported as 141.3 kcal/kg bw/day. The highest energy intake in girls in the same age group, 14–27 days, was reported as 138.9 kcal/kg bw/day that was similar to boys. In order to represent extreme intake, the FDA typically uses the 90$^{th}$ percentile of the intake distribution. In a 2008 Feeding Infant and Toddler Study by Butte et al. (2010) further corroborated the energy intake estimates reported by Femon (1993). In the study by Butte et al. (2010), the reported 90$^{th}$ percentile energy intake of 779 kcal or approximately 144 kcal/kg bw is similar to the estimates reported by Fomon (1993).

The available information suggest that majority of the standard ready to consume formulas contain 67 kcal/100 ml. In order to obtain 141.3 kcal energy/kg bw, an infant boy must consume 209 ml formula/kg bw. Similarly, for an infant girl, to reach her 90$^{th}$ percentile of energy consumption of 138.9 kcal/kg bw/day, she will need to consume 205.5 ml formula/kg bw. Based on these values, the 90$^{th}$ percentile of formula intake for the two sexes combined will be about 207 ml formula/kg bw/day. Based on these assumptions, the 90$^{th}$ percentile daily intake of FOS, added at a maximum concentration of 400 mg/100 ml to the starter formula is estimated to be 828 mg/kg bw/day. Similarly, the 90$^{th}$ percentile daily intake of FOS from follow on formula (containing 500 mg FOS/100 ml) is estimated as 1035 mg/kg bw/day. It should be noted that by the time follow on formula is introduced, consumption of infant formula (on a body weight basis) has decreased by about 20% and, even though the maximum intended addition level of scFOS is increased to 500 mg/100 ml, the 90$^{th}$ percentile intake of scFOS is only about 800 mg/kg bw/day.

It is recognized that as the infant grows, formula intake increases, but more slowly than weight gain, so that consumption assessed as ml formula per kg body weight is lower for infants

older than 27 days. As a result of this and as the infant grows, intake of scFOS per kg body weight decreases. The estimated intake of scFOS at 90$^{th}$ percentile peaks at about 1035 mg/kg bw/day during the first 6 weeks of life, then begins to decline and by weeks 8-12, it reaches to approximately 840 mg/kg bw/day. This suggest that the maximum estimated daily intake (EDI) of FOS is unlikely to exceed 1035 mg/kg bw/day.

For long-term exposure, the assumptions made in these estimates are quite conservative, because as the infant grows the formula intake increases but at a slower rate than weight gain. Historically non-exempt infant formulas provided 20 kcal/fl Oz as fed. However, recent information indicates that several infant formula notifications provide only 19 kcal/fl Oz. Given this, at a maximum use level of 500 mg scFOS/100 ml of infant formula, and maintaining the same energy intake, the 90$^{th}$ percentile daily intake of scFOS would increase approximately 54.5 mg scFOS (increase from 1035 mg/kg bw/day to 1090 mg/kg bw/day) with consumption of the lower 19 kcal/100 ml infant formula. As an infant grows and starts consuming complimentary foods, and thus reduces the intake of infant formula, the level of scFOS consumed will decrease due to the complimentary foods that are unlikely to contain the same level of scFOS as infant formula.

In summary, the proposed use level of scFOS in starter formula is 400 mg/100 ml formula, resulting in a 90$^{th}$ percentile intake of 828 mg scFOS/kg bw/day during the period from 14 to 27 days of age, the period of highest formula intake. By the time follow-on formula is introduced, consumption of infant formula (on a body weight basis) has decreased by about 20% and, even though the maximum intended addition level of scFOS is increased to 500 mg/100 ml, the 90$^{th}$ percentile intake of scFOS is only about 800 mg/kg bw/day. For safety assessment purposes of scFOS in infant formula, the maximum intake of 828 mg scFOS/kg bw/day is considered.

## 4. Part IV – SELF LIMITING LEVELS OF USE

There are no known self-limiting levels of use that are associated with the use of notified ingredient scFOS.

## 5. Part V – EXPERIENCE BASED ON COMMON USE IN FOODS BEFORE 1958

Not applicable. The statutory basis for the conclusion of GRAS status of scFOS in this document is based on scientific procedures and not based on common use in food before 1958.

## 6. Part VI – NARRATIVE

Non-digestible oligosaccharides, including FOS, have received considerable attention in recent year for their potential health effects such as reducing constipation, decreasing levels of serum lipids (cholesterol, triacylglycerols, phospholipids), stimulating growth of bifidobacterial in the human colon, improving mineral absorption, as a non-cariogenic, and as a low-calorie sweetener, etc. As a result of these properties of oligosaccharides, FOS is increasingly included in food products and infant formulas. Given their potential health benefits and increased uses in foods, scFOS have been extensively investigated for its safety and efficacy. The toxicity potential of FOS have been summarized in several published experimental studies and review articles. These studies include short and long-term toxicity studies in experimental animals, metabolic (*in vitro* and *in vivo*) experiments, and human clinical studies, including studies in infants. Additionally, the safety in use of FOS have been extensively and critically evaluated by national and international regulatory agencies such as the FDA, FSANZ, and EFSA (SCF). These agency reviews demonstrate that FOS is safe for its intended use as an ingredient in food, including infant formula.

In the published literature, several preclinical and clinical studies with FOS have appeared. In the following section, relevant efficacy and toxicological studies on FOS are summarized in support of the conclusions drawn in this GRAS assessment. Efforts have been made to present both the data supporting the safety as well as any data on the adverse effects of FOS. In this GRAS assessment, attempts have been made to summarize the available information, related to safety of FOS, in the order of their importance. First, the published pivotal studies are described, followed by secondary published studies, then corroborative unpublished studies and finally regulatory agencies assessments are summarized. The safety in use of the proposed use of scFOS in infant formula is based on the totality of available evidence.

### 6.1. DATA PERTAINING TO SAFETY

#### 6.1.1. Pivotal or Primary Published Clinical Studies of scFOS in Infant

The available pivotal studies of scFOS in infants related to growth and safety are summarized in Table 2 as well as further described below.

In a prospective, randomized, double-blind study, Guesry et al. (2000; published as abstract; also described in GRN 537) investigated the effects of 3 doses of scFOS in infants. In this study, 53 infants (age- 7 to 20 day old) were randomized to receive five bottles of formula per day for two weeks. Each bottle provided either 200 mg lactose or 200, 400, or 600 mg scFOS providing daily intakes of 1 g lactose or 1, 2, or 3 g scFOS. As, the volume of the formula in each bottle was not stated, the dietary concentration of FOS in mg/ml could not be determined. However, the actual intake of FOS was reported. The infants were examined and weighed weekly and mothers were asked to record daily formula consumption, stooling patterns, diaper rash, spitting up, vomiting, or other events. Stool samples were collected at baseline, at the end of feeding period, and two weeks later for pH measurement and enumeration of bifidobacteria. Drop-out rates did not differ by group. A dose-related increase in stooling frequency with scFOS intake was observed. There were no differences in bifidobacterial counts, fecal pH, or adverse effects. Assuming that the infants were of normal weight for this age range, they would have averaged about 3.7 kg; this level of intake would provide 811 mg scFOS/kg bw/day. This amount is the mean daily intake of FOS that would result from addition of 680 mg scFOS/100 ml

formula. The findings from this study support the safety in use of scFOS in infant formula proposed by Tata.

**Table 2. Pivotal or Primary Studies of scFOS in Infants**

| Dose, Duration | Study Design, Objective | Subjects | Results | Reference |
|---|---|---|---|---|
| scFOS - 200, 400, or 600 mg/day for 2 weeks | Prospective, randomized double-blind study comparing the effects of 3 concentration levels of scFOS in infant formula | 53 infants aged 7-20 days | Drop-out rates did not differ by group. Stooling frequency increased dose-dependently with scFOS intake. There were no differences in fecal pH, bifidobacteria counts, or adverse effects | Guesry et al. (2000) |
| scFOS – 0 or 2.5 g/L formula until 35 days of age | Randomized, double-blind, placebo-controlled, multi- center study of tolerance to soy-based infant formulas with scFOS and mixed carotenoids | 186 healthy term infants aged 0- 8 days | There were no significant differences between formula groups in completion rates, formula intake, growth, stool frequency or consistency, feeding-associated spit-up or vomit, urine specific gravity, hydration status, adverse events, or serious adverse events. Two serious adverse events were reported in each formula group, but all were considered not study related. The authors concluded that, "This study demonstrated that the addition of FOS at 2.5 g/L and mixed carotenoids to soy protein-based formulas, with or without sucrose, was safe and well tolerated in healthy term newborn infants." | Lasekan et al. (2015) |
| scFOS – 0, 2.4, or 3.4 g/L formula for 4 weeks | Randomized, double-blind, placebo-controlled, multi- center study of the effects of feeding on the intestinal microbiota | 97 healthy term infants aged ≤ 6 days (mean = 2.3±0.3 days) | Dropouts from each group were: Control group—10 drop-outs, 1 due to parental report of intolerance; 2.4-g scFOS group—11 drop-outs, 3 due to parental report of intolerance, 2 withdrawn by investigators due to non-test-article related adverse events; 3.4 g scFOS group - 6 drop-outs, 1 due to parental report of intolerance.<br>No differences were reported among groups in stool frequency or consistency, frequency of feedings with spit-ups or vomit, or total bacterial loads. The highest abundance of bifidobacteria was in the high-scFOS group, but differences among groups were not significant. Lactobacilli, bacteroides, *E. coli*, and *C. difficile* levels were not significantly different across groups. The authors concluded that infant formula is similar to human milk in its ability to support bifidobacteria and lactobacilli, but suggested that "future improvement of infant formula should be directed to reduce the abundance of potentially harmful bacteria including *E. coli* | Xia et al. (2012) |

| | | | | |
|---|---|---|---|---|
| | | | and *C. difficile*." | |
| scFOS 5 g/L for 6 months | Prospective, randomized, double-blind, placebo-controlled, multicenter study of the effect of scFOS on growth, digestive tolerance, fecal bifidobacteria count, and specific poliovirus secretory IgA | 75 healthy 4-month-old infants | 81% of the infants suffered adverse events, but there were no significant differences between groups receiving scFOS or maltodextrin placebo; few were regarded as feeding-related and these did not differ between groups. No differences were observed between groups in the incidence or severity of intolerance symptoms, growth (weight and height), or secretory IgA levels. A significantly greater number of fecal bifidobacteria was noted in the scFOS group as compared to controls after one month of feeding, but the difference was no longer significant after 2 months. The authors concluded that, "The overall digestive tolerance of the scFOS supplemented follow-on milk formula is very good and confirms that scFOS can be used safely at 5 g/L in infants older than 4 months." | Ripoll et al. (2015) |
| scFOS 4 g/L to age 4 months | Prospective, randomized, double-blind, placebo controlled, multicenter trial of effect of scFOS on bifidogenesis and antipoliovirus IgA | 61 healthy term infants aged 0-7 days (mean age = 4.1±0.8 days) | Formula consumption and growth did not differ between the group receiving scFOS and a control group that received maltodextrin. There was no difference in incidence or severity of adverse effects between groups. Fecal bifidobacteria counts were significantly higher among infants receiving scFOS than those receiving maltodextrins, but no significant difference was seen in poliovirus-specific IgA. The authors concluded that, "This study demonstrates that a milk- based infant formula supplemented with scFOS at 4 g/L will increase the fecal content of Bifidobacteria in healthy term infants in comparison to a placebo formula without inducing any problem of digestive tolerance." | Paineau et al. (2014) |

In a parallel feeding randomized, double-blind, 28-day trial in healthy term newborn infants, Lasekan et al. (2015) compared the effects of soy-based infant formulas containing supplemental scFOS on gastrointestinal (GI) tolerance and hydration. In this study, the infants were fed either a commercialized soy formula (with history of safe use) containing sucrose as 20% of total carbohydrate, no supplemental scFOS and no mixed carotenoids (lutein, lycopene,

beta-carotene) as a control (CF, n=62 infants) or 1 of 2 experimental soy-based formulas, EF1 (n=64) and EF2 (n=62) containing scFOS (2.5 g/L) and mixed carotenoids (lutein = 53 μg/L, lycopene = 81 μg/L and beta-carotene = 30 μg/L). EF1 differed from EF2 by containing sucrose. Although the degree of polymerization of supplemental FOS was not described, the investigators clearly stated the use of scFOS. No significant study group differences in study completion rates (CF=81, EF1=86, and EF2=87%), growth, stool frequency, formula intake, spit-up/vomit, mean rank stool consistency, and safety measures (urine specific gravity, USG; hydration status and adverse events) were noted.

In the study by Lasekan et al. (2015), a total of six serious adverse events were reported, two in each study group and were rated by investigators as "not related" or "probably not related" to the study formulas. The number of parental reports of loose/watery stools in the CF, EF1 and EF2 were 4, 7 and 2, respectively. However, these were not significantly different and the hydration status and urine specific gravity for these subjects were normal. The findings from this study suggest that term infants fed soy-based formulas supplemented with scFOS and mixed carotenoids, with or without sucrose in the first 35 days of infancy showed good tolerance and hydration that was comparable to the control soy-based formula with history of safe use. The investigators also noted that a higher level of scFOS may be needed to produce a softer stool consistency. The findings from this study did not reveal any adverse effects of scFOS. The use levels of scFOS used in this study is lower (250 mg/ 100 ml) as compared to proposed uses by Tata.

Xia et al. (2012) analyzed intestinal bacterial populations from term infants fed formula supplemented with FOS. In this randomized, double-blind, placebo-controlled 4-week trial, healthy term infants aged $\leq$ 6 days were enrolled to investigate the effects of four types of feeding on the intestinal microbiota. The types of feeding included cow's milk (control), two FOS groups (240 or 340 mg FOS/100 ml), and human milk (reference). Although the publication mentioned use of FOS and not scFOS, the available information from other sources suggest that the test article used was scFOS. A total of 65 infants completed the study. No differences were reported among groups in stool consistency or frequency, or in the frequency of feedings with spit-ups or vomit. The groups did not differ in total bacterial loads, although they tended to be lower in the infants fed human milk as compared to formula-fed infants. The investigators concluded that infant formula is similar to human milk in its ability to support bifidobacteria and lactobacilli, but suggested that future improvement of infant formula should be directed to reduce the abundance of potentially harmful bacteria including *E. coli* and *C. difficile*. The results of this study support the safety of scFOS at the maximum use levels of up to 340 mg/100 ml. Although the use levels of scFOS in this study are lower as compared to the present GRAS, the findings did not reveal any adverse effects related to scFOS.

In a randomized, controlled, double blind trial, Ripoll et al. (2015) studied the effect of scFOS on digestive tolerance and growth parameters in infants up to 10 months of age. In this study, 75 formula-fed healthy infants were enrolled at the age of four months received either a placebo or scFOS supplemented formula for six months. Infants meeting all eligibility criteria were randomized (1:1 ratio) either in the scFOS group (follow-on milk formula supplemented with scFOS at 500 mg/100 ml – 3.5% in replacement of maltodextrins in the powder) or in the control group (follow-on milk formula without scFOS supplementation). Fecal poliovirus sIgA after vaccination and bifidobacteria concentration, weight, height, and digestive tolerance (i.e.,

constipation, crying, soft stool, vomiting and regurgitation, adverse events and serious adverse events) were monitored.

In the study by Ripoll et al. (2015), tolerance and growth parameters were similar in both the groups. Overall, 81% of infants experienced at least one adverse event, with no significant difference in the number of adverse events between groups. The most prevalent adverse event in all infants were bronchitis (12%), gastroenteritis (9%), and nasopharyngitis (28%). No difference was observed between groups for diarrhea and gastroenteritis. During the study, six different infants suffered from serious adverse events. None of the serious adverse event was related to the study product. Digestive tolerance was evaluated during the six month-study for infants who received at least one feeding of follow-on milk per day, (equivalent to at least 2.5 g/day). There was no difference between the two groups in terms of prevalence of digestive symptoms except for the number of days with vomiting that was lower and the number of days with soft stools that was higher in the scFOS group. The investigators reported that after six months of supplementation, the strict follow-up of adverse events and digestive tolerance criteria have demonstrated the good tolerance of scFOS follow-on milk, as no difference was observed between groups for gastroenteritis, constipation, diarrhea, prevalence of infections, regurgitation, and crying while these conditions are common at this life-stage. The authors also noted that infants consuming the scFOS supplemented formula have experienced an improvement in vomiting prevalence and in stool consistency.

The results of Ripoll et al. (2015) study show that a follow-on milk formula supplemented with 500 mg/100 ml scFOS is safe and well tolerated leading to normal growth in infants after the age of four months and promotes fecal bifidobacteria levels after one month in infants who had never been breast-fed. scFOS addition elicited normal digestive tolerance and normal growth suggesting it can be used safely at 500 mg/100 ml in infants after four months of age. The findings from this study support the proposed use of scFOS in follow on formula by Tata. Ripoll et al. (2015) also suggested that findings from their study (described above) compliments the data from previous studies by Euler et al. (2005) and Veereman-Wauters et al. (2011) that revealed no negative impact on growth following supplementation with FOS (oligofructose from chicory- by partial enzymatic hydrolysis) at dose from 3 to 8 g/L in younger infants after 4 and 5 weeks of supplementation.

In yet another randomized, double-blind, placebo-controlled trial, Paineau et al. (2014) investigated the effects of scFOS on fecal bifidobacteria and specific immune response in formula-fed infants. In this study, 61 healthy term infants aged 0-7 days (mean age=4.1±0.8 days) were allocated to receive formula supplemented with 400 mg/100 ml of either scFOS or maltodextrins until the age of four months. The scFOS used had a degree of polymerization between 3 and 5 that is substantially equivalent to the scFOS that is the subject of this GRAS notice. Stool samples were collected prior to clinic visits at baseline and at the ages of 2, 3, and 4 months for analysis of bifidobacteria and antipoliovirus IgA. Additionally, weight and length of the infant were also measured at each clinic visit. Parents were asked to maintain diaries on formula consumption, digestive tolerance (assessed by incidence of abdominal pain, diarrhea, and vomiting), and adverse effects.

In the study by Paineau et al. (2014), the amount of formula consumed did not differ between the groups, nor did growth. The most frequent adverse event was abdominal pain, followed by liquid stools without any difference in incidence or severity between the feeding groups. Only one serious adverse event of an episode of bronchitis unrelated to feeding was

reported. In infants receiving scFOS, fecal bifidobacteria counts were significantly higher as compared to receiving maltodextrins, but no significant difference was seen in poliovirus-specific IgA. The investigators concluded that the findings from this study demonstrates that a milk-based infant formula supplemented with scFOS at 400 mg/100 ml will increase the fecal content of bifidobacteria in healthy term infants in comparison to a placebo formula without inducing any problem of digestive tolerance. The findings from this study support the safety and tolerance of formula containing scFOS at levels of 400 mg/100 ml in infants and are applicable to the present GRAS.

In a prospective, interventional open label trial, Vandenplas et al. (2017) investigated the effects of a new symbiotic infant formula, supplemented with *Bifidobacterium lactis* and FOS, with lactose and a whey/casein 60/40 protein ratio, administered to 280 infants for three months. The study formula was added with FOS (350 mg/100 ml) and *B. lactis* ($10^7$ cfu/g powder). The inclusion infant in the study was based on parents who intended to feed their infants (partially) formula and agreed to feed them the new symbiotic formula. The degree of polymerization for FOS was not mentioned in the study. The age of infant at entry was 3.8 ± 3.6 weeks. Of the 280 infants, 75 received the study formula from birth and 227 infants fed during the trial period received the study formula exclusively. The median age of the infants at inclusion was 0.89 months. Weight 'evolution' (as mentioned in publication) was in accordance with the World Health Organization growth charts for exclusive breastfed infants.

In the study by Vandenplas et al. (2017), the measurement of all anthropometric parameters (weight-for-length z score and body mass index-for-age z score) was within the normal range. The incidence of daily regurgitation (10.9%), infantile crying and colic (10.5%), and functional constipation (3.2%) were all significantly lower as compared to the reported median prevalence for a similar age according to the literature (median value of 7.8% for functional constipation, 26.7% for regurgitation, 17.7% for infantile colic). No serious adverse event related to the study product was reported. The investigators concluded that new symbiotic infant starter formula (containing 0.35 g FOS/100 ml) was safe, resulted in normal growth and was well tolerated. The results of this study support the safety of scFOS at use levels of up to 350 mg/100 ml.

In summary, the available studies in infants suggest that levels up to 680 mg scFOS/100 ml of infant formula is well tolerated by infants without any adverse effects. The test articles used in the above described studies is substantially equivalent to the subject of present GRAS. The minor differences in the scFOS product is unlikely to cause any difference in toxicological or clinical effects. Thus, the clinical evidence from the above described studies is applicable to the current scFOS. The findings from these studies support the proposed uses of scFOS by Tata in term infants as stated in this GRAS assessment.

### 6.1.2. Secondary Published Studies

#### 6.1.2.1. Studies in Infant with Similar Substances

In the published literature there are several studies with oligofructose (FOS) derived from other sources such as chicory. These studies are considered as secondary pivotal studies as the molecules are similar to scFOS, i.e., linear chains of fructose units linked by β(2,1) fructosyl-fructose linkages, sometimes with a glucose endcap also linked by a β(2,1) bond. Following fermentation with microorganisms or hydrolysis the distinctions between them become less noteworthy. Additionally, their activity and fate in the gastrointestinal tract is somewhat similar,

although not identical particularly for fructans of widely different DP. From a safety perspective, both oligofructose from chicory or inulin and scFOS are compositionally and metabolically similar. Indeed, all fructans contain molecules with DP of 3, 4, and 5, the components of scFOS, usually in substantial quantities. Although detailed information on the DP distribution of fructans is not always publicly available, in a GRAS notice (GRN 392) on oligofructose derived from chicory that received a no question letter for addition of oligofructose to infant formula reported percentages of the total oligosaccharide content provided by fractions of DP 3, 4, and 5 ranges narrowly from 74.2 to 77.2%. This indicates that the infants in studies with oligofructose and similar FOS were ingesting scFOS as much as 75% of their total oligosaccharide intake. Given this the studies with oligofructose are applicable to the present GRAS assessment.

Yao et al. (2010) investigated the effects of infant formula containing oligofructose from chicory at levels 0, 3, or 5 g/L on stool characteristics and composition. In this prospective, randomized, double-blind, parallel-group study, 300 healthy formula-fed term infants aged 7-14 days were assigned to one of four, α-lactalbumin-enriched formulas for eight weeks: standard term infant formula; formula with 40% of the palmitate in the sn-2 position; formula with high sn-2 and 3.0 g oligofructose/L; or formula with high sn-2 and 5.0 g oligofructose/L. Additionally, 75 infants fed human milk served as a reference group. Tolerance was assessed via a parental questionnaire and physician-reported study events. The primary outcome measure was mineral content and stool soap at week 8; secondary outcome measures included stool characteristics and GI tolerance.

In the study by Yao et al. (2010), 2 participants from the human-milk reference group, 2 from the high sn-2 group, 1 from the control group, 1 from the 3.0-g oligofructose group, and 0 from the 5.0 g oligofructose group withdrew. The infants receiving the high sn-2 formula, whether with or without oligofructose had significantly less stool palmitate soaps and higher bifidobacteria counts as compared to the control infants, resembling the human-milk reference group. There was no difference in stool frequency. The high sn-2 group also had significantly softer stools compared to the control infants, and the addition of oligofructose resulted in a further dose-dependent increase in stool softness. The 5.0 g oligofructose group was not significantly different from the human-milk reference infants. Similarly, the addition of oligofructose significantly decreased stool calcium in a dose-dependent manner. Physician reported GI events were few and were not different among the four formula groups and the human-milk reference group; parental reports indicated no increase in the incidence of gassiness, watery stools, or other symptoms of intolerance with the addition of oligofructose. The addition of up to 5.0 g oligofructose/L to formula had no effect on growth (weight, length, head circumference).

Lugonja et al. (2010) compared the bifidogenic effects of breast milk and prebiotic-supplemented infant formula. In this non-randomized, non-blinded, non-placebo-controlled trail, 21 healthy infants aged 5 to 16 weeks (mean = 8.6 weeks) were divided in to two groups. Group one with 10 infants (7 boys and 3 girls) were breastfed, while other group with 11 infants (6 boys and 5 girls) received formula containing 400 mg/100 ml of a blend of inulin and oligofructose derived from chicory. Additional details of the fructans were not described. The relative proportions of FOS and inulin in the blend was not reported, nor was the rationale for creating the blend. During the trial duration of 28 days, daily measures of infants were taken for weight, length, number of feeds, frequency of stooling, stool consistency (soft, normal, or hard), and any indications of intolerance (loss of appetite, regurgitation, GI symptoms, and flatus). At baseline,

and on Days 14 and 28, stool samples were collected and analyzed for pH, organic acids, and numbers of lactobacilli, total aerobes, total anaerobes, bifidobacteria, and fungi/yeasts. The number of daily feeds was significantly higher in the breastfed group. *Lactobacilli* increased in both groups while aerobes, anaerobes, and fungi and yeasts decreased, but there were no significant differences between the formula and breastfed groups. The counts of bifidobacteria increased significantly over the 28 days in both groups. Total organic acids increased and pH decreased over time in both groups. Most stools from infants in both groups were of normal consistency. The mean water content of the stools of infants receiving formula containing inulin + oligofructose was 77.9%, non-significantly lower than the mean water content of breastfed infants' stools (81.2%). All infants grew at normal rates and there was no difference between the groups. There were no significant differences between groups in measures of intolerance, stool frequency, or stool consistency.

In another study, Kapiki et al. (2007) investigated the effect of a FOS supplemented formula on gut flora of preterm infants. In this randomized, double-blind, placebo-controlled study, 56 healthy bottle-fed preterm infants were enrolled. For this study, FOS was described as having been produced by partial enzymatic hydrolysis of chicory inulin. All enrolled infants were less than 14 days old (mean age = 7.0 ± 4/5 days), had gestational ages less than 36 weeks (mean = 33.7 ± 1.6 weeks), and had been admitted to a neonatal unit, but were otherwise healthy. Of the 56 infants, 24 received preterm formula with 400 mg maltodextrin (placebo)/100 ml formula, while 41 infants received similar formula with 400 mg FOS/100 ml for 14 days. In this study, 9 infants failed to complete the study, 5 from the FOS group and 4 from the placebo group, for reasons not related to the study. Over the full 14 days, infants in the placebo group gained significantly more weight and had significantly greater arm circumference, while those in the FOS group gained non-significantly greater length. Both formulas were well tolerated. The intake of the FOS-supplemented formula produced a significantly higher frequency of defecation and softer stools as well as significantly greater concentrations of fecal bifidobacteria and bacteroides and significantly lower numbers of *E. coli* and enterococci. In the publication, it is stated that "All infants tolerated well the two formulae," although the evidence supporting this claim was not described. The investigators also stated, "We have documented that the addition of a small quantity of FOS in the normal diet of preterm infants was well tolerated and resulted in a rapid increase in the numbers of bifidobacteria and the proportion of infants colonized by bifidobacteria."

In a randomized, double-blind study in infants, Bettler and Euler (2006) evaluated growth and tolerance in infants fed formula supplemented with oligofructose (FOS) from chicory. Healthy term infants were randomly assigned to 1 of 3 formulas (a bovine milk–based control formula or identical experimental formulas supplemented with either 1.5 g/L or 3.0 g/L FOS) *ad lib* for 12 weeks. Anthropometric measurements were recorded at baseline and at 4, 8, and 12 weeks. Adverse events and tolerance were recorded throughout the study, and blood samples were drawn at baseline and at 12 weeks for a clinical chemistry panel. The study enrolled 297 infants, of whom 212 completed the trial. The infants were found to have grown appropriately. All 3 formulas were judged to be safe and well tolerated based on growth, laboratory data, and adverse event profiles. The high dose (3.0 g/L FOS) group had less constipation than the other study groups. The investigators concluded that Bovine milk–based term formula supplemented with either 1.5 g/L or 3.0 g/L FOS is safe and supports normal growth.

Brunser et al. (2006) investigated the effect of probiotic or prebiotic supplemented milk formulas on fecal microbiota composition of infants. In this randomized, double-blind trial, 116 healthy term infants were given a standard milk-based infant formula, the same formula with 200 mg/100 ml of oligofructose (from chicory), the same formula with $10^8$ cfu *L. johnsonii* NCC533 (La1)/g powder, or breast feeding, for a period of 13 weeks, followed by a 2-week washout with standard formula. Parents maintained a record of formula intake and any adverse effects and returned to the clinic every 15 days for health status evaluation and anthropometric measurements. Seventy-six formula-fed infants completed the entire study; primary reasons for withdrawal were failure to follow the protocol, antibiotic use, or illness. The investigators stated that withdrawal rates did not differ across the three formula groups and none of the withdrawals were associated with adverse reaction to the formula. All formulas were well tolerated and average formula intake was similar for all three groups, resulting in an average intake of oligofructose of 252 mg/kg bw/day. The number of adverse events per infant did not differ between the three formula groups or between the formula-fed and breastfed infants, nor were there any differences in growth measured by weight gain and length. The investigators concluded that the study confirms a predominance of bifidobacteria in breastfed infants, and that the concentration of oligofructose used in this study (200 mg/100 ml formula) was too small to have a significant effect on the host microbiota.

In summary, the findings from the studies conducted with oligofructose or FOS derived from other sources, such as chicory, shows that these ingredients are well tolerated in infants. The findings from these studies suggest that scFOS, derived from sucrose and the subject of present GRAS, at the intended use levels in infant formula is unlikely to cause adverse effects.

#### 6.1.2.2. Studies in Children and Adults

Safety of scFOS has been described in several published clinical trials in children and adult human subjects. These studies have been the subject of several comprehensive evaluations, including several GRAS notices [GRN 44 (FDA, 2000), 537 (FDA, 2015), 605 (FDA, 2016a), 623 (FDA, 2016b), 717 (FDA, 2017), 797 (2018)] that have been reviewed by independent expert panels and the FDA. Among these GRAS notices on scFOS, GRN 605 was submitted by Tata. As the available information is extensively described in these previous GRAS notices, including GRN 605 Tata, all these GRAS notices are incorporated in the present GRAS by reference. The first GRAS notice on scFOS, GTC Nutrition (2000) established the ADI of 4.2 g/day scFOS for infant (<1 year old). For the general population, scFOS ADI was established as 20 g/day. In these studies no serious adverse events of scFOS were reported. The available information revealed only mild GI side-effects of scFOS consumption that included bloating, abdominal discomfort, flatulence, and transient diarrhea. These GI effects are consistent with the effects associated with intake of high levels of non-digestible fibers. Updated searches of the recent scientific literature were conducted to identify any new studies relevant to the safety of scFOS in children and adults. No recent studies on the effects of scFOS in adults or children were located since the submission of last GRAS notice in 2018.

#### 6.1.2.3. scFOS Studies in Piglets and Other Weaning Animals

The neonatal piglet is considered the best surrogate model to human infants with regards to assessing the ability of test infant formula to support infant growth and development. The available evidence indicate that neonatal piglet is similar in nutritional requirements, intestinal physiology, and metabolism to the human infant. Additionally, the body composition of piglet is

similar to that of the premature human infant. Given this, the available studies of FOS in neonatal piglets are described first followed by studies in other animal species.

In two experiments with neonatal pigs, Howard et al. (1995b) investigated the effects of feeding scFOS on cecal and colonic microbiota, proliferation of cecal and colonic epithelial mucosa, and short-chain fatty acid concentrations in the cecum. Although full description of test article was not provided, the information described indicate that the product used in the study appears to be scFOS. In the first experiment, male neonatal pigs (10/group) were fed diets containing either 0 or 3 g scFOS/L of formula for 15 days and then the large intestine were examined for changes in cecal and proximal colonic microbiota; cecal pH; short-chain fatty acid concentrations; morphology of cecal, proximal, and distal colonic epithelial mucosa; gross necropsy; and histopathology. Supplementation with scFOS did not alter cell counts of viable bifidobacterial organisms or total anaerobic microbiota, cecal pH, or concentrations of short-chain fatty acids. Cecal mucosal cell density and labeled cells increased with FOS consumption. Proximal colonic mucosal crypt height, leading edge, labeled cells, proliferation zone, and labeling index increased with scFOS consumption. Distal colonic mucosal crypt height, leading edge, cell density, labeling index, and labeled cells increased with FOS consumption. Gross necropsy and histopathology found no significant lesions. In the second experiment, neonatal pigs were fed diets containing either 0 or 3 g scFOS/L of formula for 6 days. Fecal samples were collected on the first full day of feeding and on days 3 and 6 after initiation of feeding. On days 1 and 3, concentrations of bifidobacteria were similar between diets. However, on day 6, pigs consuming FOS tended to have greater numbers of bifidobacteria. These data suggest dietary consumption of FOS will enhance bifidobacteria populations and prevent colonic epithelial mucosa atrophy in neonates fed an elemental diet.

In the first experiment by Howard et al. (1995b), one pig receiving scFOS exhibited intestinal lesions "suggestive of bacterial infection," and six pigs (5 receiving scFOS) showed mild hepatocellular vacuolation. These hepatic changes were nonspecific and were attributed by the pathologist to a variety of factors including hypoxia, stress, metabolic imbalance, and anorexia. The investigators concluded that these effects were not significant. Of the 20 pigs, 16 showed pulmonary lesions of hemorrhage, congestion, or atelectasis, which were regarded as acute lesions most likely due to handling during sample collection prior to sacrifice. The groups assignments of the 16 pigs were not reported, but the authors reported that they "were not associated with dietary factors" (Howard et al., 1995b). In this study, 36-hour-old piglets were put on formula containing 0 or 3 g scFOS/L and no adverse effects were reported that were attributed to the test article.

In another study, Tsukahara et al. (2003) investigated the effect of dietary scFOS supplementation on luminal SCFA production and its influence on the morphometrical variables of mucosa of the large intestine in six weaning piglets. After 7 days of adaptation, three pigs were given a test diet containing scFOS (10%) *ad libitum* for 10 days. The other three remained on the basal diet and served as controls. At the end of the experiment, the large intestines were removed, and the cecum, gyri centripetales, gyri centrifugales, and rectum were separated. The contents of each portion were collected and measured for SCFA concentration, pH, and moisture. A micrometer was used to measure the crypt depth. The numbers of epithelial and mitotic cells in the crypt columns were also counted. The concentration of SCFA was significantly higher in piglets fed FOS than in the controls. The concentration of n-butyrate was markedly stimulated by FOS. As compared to the control, the number of epithelial mitotic, and mucin-containing cells

was higher in piglets fed scFOS. Accordingly, the crypt depth was larger in the scFOS-fed piglets. The luminal n-butyrate concentration showed a significantly positive correlation with the crypt depth and the number of epithelial, mitotic, and mucin-containing cells. The investigators concluded that "the beneficial roles of scFOS in the physiology of the large intestine rely on the activity of intestinal microbiota."

In yet another study in piglets, Barnes et al. (2012) investigated the effects of partial enteral nutrition, supplemented with the prebiotic scFOS in a neonatal intestinal failure piglet model. In this study, male and female neonatal piglets (2 day old, n = 87) underwent placement of a jugular catheter and an 80% jejunoileal resection and were randomized to one of the following treatment groups: control (20% standard enteral nutrition/80% standard parenteral nutrition PN), control plus prebiotic (10 g/L- scFOS), control plus probiotic ($1x10^9$ CFU *Lactobacillus rhamnosus* GG [LGG]), or control plus symbiotics (scFOS + LGG). Animals (7-8 piglets/group) received infusions for 24 hours, 3 days, or 7 days, and markers of intestinal adaptation were assessed. Prebiotic treatment increased ileal mucosa weight compared with all other treatments and ileal protein compared with the control, regardless of day. Ileal villus length increased in the prebiotic and symbiotics group, regardless of day, specifically due to an increase in epithelial proliferation. In the 7-day prebiotic group, peptide transport was upregulated in the jejunum, whereas glutamine transport was increased in both the jejunum and colon. The investigators concluded that scFOS prebiotic and/or symbiotics supplementation resulted in enhanced structure and function throughout the residual intestine. No adverse effects were noted from administration of 10 g scFOS/L in the parenteral formula, and the prebiotic was regarded as "highly effective at inducing adaptation in the residual jejunum, ileum, and colon."

Correa-Matos et al. (2003) investigated the effects of fermentable nondigestible carbohydrates in piglets infected with *Salmonella typhimurium*. In this study, 2-day-old colostrum-fed piglets (12 piglets/treatment) were randomly assigned to receive sow's-milk replacer formula alone (control) or control formula supplemented with 7.5 g/L of methylcellulose, soy polysaccharides (soy fiber), or an undefined FOS for 14 days. The source and composition of the supplements were not described. On day 7, half of the piglets in each treatment group received an oral gavage of *S. typhimurium* 798 (originally isolated from a pig) or saline. *S. typhimurium* infection produced diarrhea in the controls and in the methylcellulose groups, but not in the soy polysaccharides or FOS groups. Ileal lactase activity and physical activity were significantly lower in the controls than in other groups after infection. Ileal mucosal barrier function was significantly impaired by *S. typhimurium* infection in the control and soy polysaccharide groups, but was unaltered in the jejunum and colon. Overall, consumption of FOS shortened recovery time and improved infection-associated symptoms in piglets infected with *S. typhimurium*. The investigators concluded that, "because fermentable fiber enhances intestinal function and reduces the severity of *S. typhimurium* infection–associated symptoms, it may be a cost-effective way in which to reduce the severity of pathogenic infection–associated symptoms in infants."

In another publication, Howard et al. (1995a) studied the effects of scFOS, XOS, and gum Arabic on cecal and colonic microbiota in weaning rats and mice. In this study also two experiments were conducted to determine if supplementing soluble fiber [FOS, xylooligosaccharide (XOS) or gum arabic] to a semi-elemental diet would affect cecal and colonic microbiota. Experiments 1 and 2 used identical dietary regimens; mice and rats were given free access to a powdered semi-elemental diet. Animals were assigned to one of the four

following treatment groups: control, no supplemental dietary fiber, FOS, XOS and gum arabic. Dietary fiber was supplied via drinking water at 30 g/L. In the first experiment, populations of *Bifidobacteria* and total anaerobic flora were enumerated from the contents of the cecum and colon of weanling mice. Consumption of FOS increased the concentrations of *Bifidobacteria* and the ratio of *Bifidobacteria* to total anaerobic flora. In the second experiment, tissue from the cecum and distal colon of weanling rats was examined for morphological changes of the mucosa. Consumption of XOS increased cecal crypt depth and labeling index relative to the other three treatments. Consumption of gum arabic and the control diet increased cecal proliferation zone. Consumption of XOS and the control diet increased cecal cell density. Distal colonic crypt depth was greatest in controls and rats fed FOS, intermediate in those fed gum arabic, and smallest in those fed XOS. These results suggest that FOS effectively stimulates growth of *Bifidobacteria* and XOS supports a modest enhancement of cecal epithelial cell proliferation.

Nakamura et al. (2004) investigated the effects of scFOS on the mucosal immune system in infancy using neonatal BALB/c mice. In this study, at 2 days of age, litter sizes were adjusted to 4-6 pups and the pups and their dam were housed together and fed *ad libitum* diet containing 0 or 5% scFOS. Pups were weaned at 21 days of age and fed the same diets *ad libitum* to age 23, 30, 38, or 44 days. On days 28, 36, and 42, twenty-four-hour fecal samples were collected and analyzed for IgA level. Following euthanasia, the small intestine and colon were removed, luminal contents were flushed and analyzed for SCFA, segments were weighed, and the tissue was homogenized and centrifuged for analysis of IgA. Feed intake and body weight did not differ between the groups. Mice receiving scFOS had significantly higher levels of IgA in the jejunum, ileum, and colon, as well as in the feces, and significantly higher levels of cecal acetate, butyrate, and propionate. No adverse effects were observed.

Fukata et al. (1999) investigated the effects of competitive exclusion and ingestion of scFOS on colonization of chicks with *Salmonella enteriditis,* in two separate experiments. Both experiments used 1-day-old White Leghorn Hy-Line cockerel chicks caged. In both the experiments, 60 chicks were divided into 4 groups (n=15): a control group; a competitive-exclusion group that received the control diet but was inoculated with an undefined bacterial preparation; an scFOS group for which the feed was supplemented with 0.1% scFOS; and a combination-treatment group that received both interventions. In experiment 1, all chicks were inoculated with *S. enteriditis* on day 7, while in experiment 2, chicks were inoculated on day 21. Following inoculation, on day 1, week 1, and week 2, five birds from each group were euthanized and their ceca evaluated for *Salmonella* spp., *Escherichia coli, Lactobacillus* spp., *Bifidobacterium,* and *Bacteroides* using plating techniques. In experiment 1, the enumeration of *S. enteriditis* in the chicks inoculated with the competitive-exclusion preparation was significantly decreased compared with the other three groups. In experiment 2, *S. enteriditis* was significantly decreased in the scFOS group and the combination-treatment group. No significant differences between groups were noted on cecal numbers of total bacteria, *Bifidobacterium, Bacteroides, Lactobacillus*, or *E. coli*. The investigators concluded that low-dose feeding of scFOS in the diet of chicks with a competitive-exclusion treatment is unlikely to a shift the intestinal gut microbiota but may result in reduced susceptibility to *Salmonella* colonization. The results of this study show that feeding of scFOS at 0.1% dietary concentration to 1-day-old chicks for up to 35 days did not reveal adverse effects.

In summary, the available studies in weaning pigs, rats, mice and chicks indicate that scFOS is unlikely to cause adverse effects. As the piglet is considered as a surrogate model for

human infants, studies conducted in these animals are applicable to the present GRAS assessment. In the studies using piglet model, the exposure to scFOS was as follows: diet containing scFOS (10%) *ad libitum* for 10 days; 3 g scFOS/L for 15 days, intestinal failure model-10 g/L for 7 day; and 7.5 g/L in formula for 14 days. In these studies, no adverse effects of scFOS were reported. Additional studies in chicks (0.1% scFOS in diet), mice (water containing 30 g scFOS/L for 14 days) and rats (water containing 30 g scFOS/L for 14 days) also did not reveal adverse effects of scFOS. These findings from neonatal animal studies suggest that proposed use of scFOS in infants by Tata is unlikely to cause adverse effects.

#### 6.1.2.4. Specific Animal Toxicity Studies of scFOS

In an attempt to investigate safety and establish the no observed adverse effect level (NOAEL) of scFSO, subject of present GRAS (FOSSENCE™), Jain et al. (2019) conducted the acute toxicity, 14-day dose range finding study, and subchronic (90-day) toxicity in Wistar rats.

##### 6.1.2.4.1. Specific Acute Toxicity

In order to determine maximum tolerable dose (MTD), young adult healthy Wistar rats (HsdHanTM) were administered a single oral dose of scFOS (dissolved in water) at dose levels of 0, 2000, 5000, and 9000 mg/kg (n=5 rats/sex/group) (Jain et al., 2019). The rats were observed for clinical signs or mortality, and body weights and feed consumption were measured. All the rats were euthanized under isoflurane anesthesia on day 15, and gross pathological examinations were performed. Oral gavage administration of scFOS to Wistar rats did not reveal any clinical signs, mortality, on body weight changes, and feed consumption changes at 2000, 5000, and 9000 mg/kg bw. Necropsy at the end of study (day 15 post-dose) did not reveal any gross pathological abnormalities. Based on these findings the MTD was considered to be more than 9000 mg/kg bw. The $LD_{50}$ of scFOS following oral administration to rats was more than 9000 mg/kg bw.

##### 6.1.2.4.2. Specific Dose-Range Finding Study

For these investigations, young adult healthy Wistar rats (HsdHanTM) were orally (gavage) administered scFOS at dose levels of 0, 2000, 5000, and 9000 mg/kg bw/day (n=5 rats/sex/group) for 14 consecutive days (Jain et al., 2019). The rats were observed for clinical signs or mortality, and body weights and feed consumption were measured. On Day 15, all the rats were euthanized under isoflurane anesthesia and blood samples were collected by retro-orbital puncture for clinical pathology (hematology, coagulation, and clinical chemistry), and organ weights and gross pathological examination. Based on the increased cecum weight observed in both sexes at all the doses tested, microscopic examination was performed on cecum, colon, duodenum, jejunum, and ileum from all dose group animals.

No clinical signs or mortality were observed at the tested dose levels of 2000, 5000, and 9000 mg/kg bw/day. The body weights were unaffected at all the doses tested. A slight decrease in feed consumption observed during treatment days 4-8 and 8-11 at the highest dose. However, feed consumption during days 11–14 was comparable to the control group. Hence slight decrease in feed consumption that was observed during initial days of the treatment was considered as transient non-adverse finding. There were no scFOS related changes observed in hematology, coagulation, and clinical chemistry parameters as well as in organs weight and gross pathology. There were no scFOS related microscopic changes observed in cecum, colon, duodenum, jejunum, and ileum of both the sexes. The findings from this study reveals that 14-day repeat

dose oral gavage administration of scFOS to Wistar rats did not cause any adverse toxicological changes on the evaluated parameters at doses up to 9000 mg/kg bw/day.

**6.1.2.4.3. Specific Subchronic Toxicity Study**

The 90-day study was performed as per OECD guidelines for testing of chemicals (Test Guideline No. 408, "Repeated Dose 90-Day Oral Toxicity Study in Rodents" adopted on September 21, 1998) (Jain et al 2019). For these investigations, young adult healthy Wistar rats (HsdHanTM) (n=10 rats/sex/group) were administered scFOS through oral gavage route at doses of 0, 2000, 5000, and 9000 mg/kg bw/day for 90 consecutive days. In addition, two recovery groups (n=5 rats/sex/group) such as control recovery and high dose recovery were included. All standard parameters as per OECD guidelines were measured. These parameters included clinical signs, body weights, feed consumption, ophthalmological examination, functional observation battery, clinical pathology (hematology, clinical chemistry), urine analysis, necropsy, organ weights, and histopathology.

There were no deaths, relevant clinical signs, or abnormal ophthalmological findings noticed at any of the dose levels in this study (Jain et al., 2019). Few clinical signs and other changes noted were considered incidental and not considered related to scFOS treatment. There were no treatment related changes observed in neurological/functional examination carried out at the end of treatment period for the main toxicity treatment groups and at the end of recovery period for the toxicity recovery groups. Body weights were unaffected at 2000 and 5000 mg/kg bw/day doses in males and at all the doses tested in females as compared to the control group. The statistically significant lower body weights were observed on day 90 in animals treated at 9000 mg/kg bw/day when compared to the control group in males. The body weights were slightly lower (without statistical significance) at 9000 mg/kg/day in both main and recovery group males for the most part of the treatment period from week 7 till the end of the treatment period and considered partially reversible at the end of the recovery period. The feed consumption was unaffected at 2000 mg/kg bw/day (G2) in males and females as compared to the control group. The statistical significant changes (decrease) in feed consumption were observed at the doses of 5000 and 9000 mg/kg/day in males and females during the treatment period and considered reversible during the recovery period (Jain et al., 2019).

No scFOS treatment related biologically significant adverse effects were noted in hematological and coagulation parameters of both the sexes across the groups. There were few statistically significant differences in hematology parameters in scFOS treated animals compared to controls. These changes included decreased hemoglobin at 5000 mg/kg bw/day in males; decreased mean corpuscular hemoglobin concentration at all FOS-treated groups in males and at 5000 and 9000 (main and recovery) mg/kg bw/day in females; increased mean platelet volume at 2000 and 5000 mg/kg/day in males and 9000 mg/kg bw/day recovery in males and females; decreased absolute eosinophils at 9000 mg/kg bw/day in males; and decreased reticulocytes (both absolute and %) at 9000 mg/kg bw/day recovery females. In the coagulation parameters, decreased prothrombin time values at 9000 mg/kg bw/day recovery males were noted (Jain et al., 2019). All the statistically significant changes observed were considered incidental and toxicologically insignificant as the alterations were of minimal in magnitude and/or lacked the dose progression and also microscopic correlation.

As regards changes in clinical chemistry parameters, no scFOS treatment related biologically significant adverse effects were observed in of both the sexes across the groups.

There are occasional sporadic findings of statistically significant differences in the following parameters from FOS-treated rats. These changes included decreased total cholesterol, total proteins, and globulin at 5000 and 9000 mg/kg/day in males and at 9000 mg/kg/day in females; alanine aminotransferase (ALT) at 9000 mg/kg/day recovery in males; decreased potassium at 9000 mg/kg/day recovery in males; and increased alkaline phosphatase at 9000 mg/kg/day in females. There were no test item-related changes in the urinalysis parameters in treated rats as compared to controls (Jain et al., 2019).

As regards organ weights, increase in absolute and relative cecum weight (with and without content) was observed at 9000 mg/kg/day in both the sexes. However, this change was not associated with any microscopic findings and hence considered as test item-related non-adverse effect. The cecum weight change was completely reversed in the recovery males, whereas in females, it was partially recovered. Similar increase in cecum weight was also present at 2000 and 5000 mg/kg/day in both the sexes and was attributed to test item administration. The large doses of scFOS may result in cecal enlargement indicative of higher cecum weight, which was considered to be a trophic effect and not a toxic effect. All other statistically significant differences observed in organ weight and their ratios were considered incidental as the changes were minimal in magnitude and/or lacked the microscopic correlation. There were no test item-related gross changes observed in male and female rats (Jain et al., 2019).

Histopathology findings from control and high dose (9000 mg/kg/day) groups did not reveal any scFOS related microscopic changes observed in male and female rats at all the doses tested. All the microscopic findings observed in males and females at 9000 mg/kg/day dose were considered incidental/spontaneous and not related to test item administration, as they were distributed randomly across the groups and/ or normally present in rats of this age. In addition, observed microscopic findings were comparable to vehicle control group. Based on the findings from this study, the oral gavage administration of scFOS (FOSSENCE™) at levels up to 9000 mg scFOS/kg bw/day is safe in Wistar rats without any adverse toxicological findings when administered for 90 consecutive days. The no-observed adverse-effect level (NOAEL) can be established as 9000 mg scFOS/kg bw/day (Jain et al., 2019). The findings from these specific studies with the subject of present GRAS indicate that the proposed use of scFOS in infant formula is unlikely to cause adverse effects.

### 6.1.2.5. Other Published Studies

#### 6.1.2.5.1. Metabolism

The available published studies suggest that several non-digestible oligosaccharides and polysaccharides act as prebiotic compounds. Among these, inulin, FOS and GOS are the most commonly used in food. Pharmacokinetic studies of FOS demonstrate that it is not hydrolyzed by human salivary or pancreatic enzymes and passes undigested and unabsorbed to the colon. In colon, FOS is fermented by colonic microflora to short-chain fatty acids, carbon dioxide, methane and hydrogen gases (Hidaka et al., 1986, Tomomatsu, 1994; Gibson and Roberfroid, 1995; Rumessen et al., 1990, 1998; Hess et al., 2011). The available studies in Wistar rats, as well as *in vitro* studies, using pancreatic and small intestinal homogenates and purified sucrase-isomaltase complex, suggest that scFOS, like other fructans, is not hydrolyzed by the intestinal enzymes but is fermented by gut microbiota (Oku et al., 1984; Tsuji et al., 1986; Tokunaga et al., 1989; Bjork and Nilsson, 1991). The unfermented dietary FOS is excreted in the feces. The kinetics of bacterial fermentation is inversely proportional to the degree of polymerization of the

fructan. The available evidence from studies in healthy human subjects (Stone-Dorshow and Levitt, 1987; Rumessen et al., 1990; Molis et al., 1996; Alles et al., 1996; Rumessen and Gudmand-Hoyerr, 1998; Castiglia-Delavaud et al., 1998; van Dokkum et al., 1999), as well as in compromised adults with ileostomy (Bach Knudsen and Hessov, 1995; Ellegard et al., 1997) suggest that nearly all ingested fructans, such as inulin, oligofructose, and scFOS reach the colon where they are fermented by colonic bacteria.

Sivieri et al. (2014) investigated the prebiotic effect of FOS in the simulator of the human intestinal microbial ecosystem (SHIME® model). The model was used to study the effect of FOS on the fermentation pattern of the colon microbiota. Initially, an inoculum prepared from human feces was introduced into the reactor vessel and stabilized over two weeks using a culture medium. This stabilization period was followed by a 2-week control period during which the microbiota was monitored. The microbiota was then subjected to a 4-week treatment period by adding 5 g/day FOS to vessel one (the "stomach" compartment). A significant increase in the *Lactobacillus* spp. and *Bifidobacterium* spp. populations during the treatment period was noted. Overall microbial community was changed in the ascending colon compartment of the SHIME reactor. FOS induced an increase of the SCFA concentration during the treatment period, mainly due to significant increased levels of acetic and butyric acids. However, ammonium concentrations increased during the same period. This study indicates the usefulness of *in vitro* methods that simulate the colon region as part of research towards the improvement of human health.

#### 6.1.2.5.2. Toxicity Studies of FOS

In addition to above described specific toxicity studies with the subject of present GRAS assessment, several studies of scFOS derived from sucrose have been described in the published literature. The scFOS used in these studies appear to be substantially equivalent to the subject of the present GRAS. These studies included acute oral toxicity studies in mice and rats, three subacute studies, one subchronic study, one chronic study and two studies evaluating developmental and maternal toxicity in rats. Additionally, *in vitro* mutagenicity and genotoxicity studies in bacterial or mammalian cell models in the presence and absence of metabolic activation have also been conducted with scFOS. In the repeat-dose toxicity studies, no consistent treatment-related adverse effects of scFOS were noted and the NOAELs were the highest doses tested. In these studies, scFOS related effects apparent at relatively high doses included transient diarrhea, soft/watery stools, and intestinal weight increases. These effects are well-established and consistent with the effects associated with intake of high-levels of non-digestible fibers and are considered to not be toxicologically relevant to humans. Decreases in body weight in rats receiving high doses of scFOS are expected as a result of the decreased caloric value of the diets rather than a direct toxic effect. In a 2-year study conducted with Fischer 344 rats no evidence of carcinogenicity was reported and the NOAEL was determined to be the highest dietary concentration tested of 5% (equivalent to 2170 and 2664 mg/kg bw/day for males and females, respectively). No developmental or reproductive adverse effects were associated with FOS consumption. Results of genotoxicity studies conducted with scFOS consistently demonstrate the lack of a genotoxic effect in bacteria and mammalian cells in the presence or absence of metabolic activation. These studies of scFOS are briefly described below.

#### 6.1.2.5.3. Acute Toxicity Studies

In the acute oral toxicity studies, the effects of scFOS were tested in male and female mice and Sprague Dawley rats. The available study details and findings from these studies are summarized in Table 3. The results of these studies demonstrate that scFOS is of low acute oral toxicity with median lethal dose ($LD_{50}$) values exceeding 9000 mg/kg bw (highest dose tested) in both mice and rats.

**Table 3. Acute Toxicity Studies of scFOS in mice and rats**

| Test Species | Description of Test Article | Dose & Duration | Findings | Reference |
|---|---|---|---|---|
| Male and female JcL-IcR mice (6 mice/sex/dose) | scFOS | Single gavage doses of 0, 3, 6, or 9 g scFOS/ kg bw | No deaths occurred and there were no differences in body weight gain between the test and the control animals. No abnormalities were seen in either sex. The LD50 for oral administration of scFOS to rats in this study was > 9000 mg/kg bw. | Takeda and Niizato (1982); mouse study |
| Male and female Sprague Dawley rats (6 rats/sex/ dose) | scFOS | Single gavage doses of 0, 3, 6, or 9 g scFOS/ kg bw | There were no deaths and no abnormalities or changes in body weight of animals of either sex. The LD50 for oral administration of scFOS to rats in this study was > 9000 mg/kg bw. | Takeda and Niizato (1982); rat study |

### 6.1.2.5.4. Repeat-Dose Toxicity Studies

A summary of short-term, subchronic and chronic toxicity studies of scFOS is provided in Table 4. The findings from the available published toxicological studies suggest that various scFOS preparations are of low oral toxicity in repeat dose studies in rodents. These published and commonly available toxicity studies of scFOS have been the subject of several critical and independent evaluations by regulatory and other agencies. These repeat-dose published toxicity studies did not reveal any toxicologically significant effects of relevance to humans following oral administration of scFOS. In these studies, NOAEL determinations have been consistently reported as the highest doses tested.

In a review article, Carabin and Flamm (1999) described the findings from subacute studies that were conducted by Takeda and Niizato (1982). The findings from these 6-week gavage and feeding studies of scFOS in Wistar rats support NOAELs of 4500 to 5000 mg/kg bw/day (highest doses tested). Tokunaga et al. (1986) reported that male Wistar rats consuming FOS at dietary concentrations of 10 and 20% (equivalent to approximately 4185 and 7795 mg/kg bw/day, respectively) experienced transient watery stools during the first few days of administration and increased small and large intestine weights, and increased fecal and decreased gastrointestinal transit time when in the diet for 6 to 8 weeks. Meijl Seika Kaisha (1982) reported dose-related increase in diarrhea, soft stools, cecal distension, and intestine weights for rats fed up to 20400 mg/kg bw/day for 90 days. Additional details of the study were not reported.

In a subchronic toxicity study (Boyle et al., 2008), Sprague-Dawley rats were fed standard rodent chow for 13 weeks with 0, 0.55, 1.65, 4.96, or 9.91% oligofructose, replacing cornstarch. In this study, there were no reports of treatment-related adverse effects in terms of food intake, body weight, body weight gain, clinical chemistry, hematology, clinical

observations, or histopathology even at the highest dose tested. The NOAEL was the highest dose tested (4680 mg oligofructose/kg bw/day).

In a chronic feeding study, Clevenger et al. (1988) investigated the toxicity and carcinogenicity of FOS in rats following exposure for 104-weeks (Table 4). In this study, male and female Fischer 344 rats (12-13/sex/dose) were fed diets containing scFOS at levels of 0, 0.8, 2.0, and 5.0% (equivalent to 0, 341, 854, and 2,170 mg/kg bw/day for male rats and 0, 419, 1,045, and 2,664 mg/kg bw/day for female rats) for two years. All standard parameters for such studies were studied. In all groups, some mortality was observed; however, it was not considered treatment-related. Exposure to scFOS did not affect feed intake, body weight gain, feed conversion efficiency, absolute organ weights, or any hematology outcomes. A slight elevations in sodium and chloride in male rats was noted. In male rats in the mid-dose group, exposure to scFOS showed slightly elevated levels of blood glucose and creatinine, but the creatinine levels in males in the high-dose group decreased. Other outcomes did not significantly differ between test groups and the controls. In female rats, except for a slight elevation of uric acid in the low- and mid-dose groups, all blood chemistry parameters were similar to those of the controls. No test-article-related macro- or microscopic changes were found in either males or females. The NOAEL was established as 5%, the highest concentration tested, equivalent to 2170 mg/kg bw/day for males and 2664 mg/kg bw/day for females.

As regards the carcinogenicity-related observations, Clevenger et al. (1988) reported similar numbers of neoplastic lesions (e.g., pheochromocytomas, thyroid C-cell adenomas, leukemias, and pituitary adenomas) in the scFOS-treated animals and controls, with the exception of pituitary adenomas. In male rats, the incidence of pituitary adenomas for the 0, 0.8, 2.0 and 5.0% dose groups was 20, 26, 38, and 44%, respectively. The historic incidence of pituitary adenomas in F-344 male rats from the test laboratory ranges from 1 to 49%. While the incidence of this tumor was well within historical range for all male rats, the incidence in the two highest dose groups (2.0 and 5.0%) was significantly greater than the incidence in the controls. In the female rats, a negative trend in the incidence of pituitary adenomas was recorded. The significance of a dose-related trend was equivocal in that one trend test showed a significant trend, whereas another test did not. If males are compared to females, a similar but opposite dose-response trend is noted. This dichotomy has no apparent biological basis. If male and female pituitary adenoma incidences are combined, no significant across-dose group difference are found. All of these observations point toward the conclusion that the higher incidence of pituitary adenomas in FOS-treated male rats is a chance artifact. Such chance artifacts can arise when large numbers of statistical comparisons are made. In this study, 54 comparisons were made, and 1 - 3 significant results would be expected by chance alone at the significance levels of 0.01 and 0.05, respectively. These observations suggest that higher incidence of pituitary adenomas in males was not treatment related. The findings from this study indicate that FOS is not carcinogenic.

**Table 4. Summary of Short-term, Subchronic and Chronic Toxicity Studies of scFOS Conducted in Rats**

| Species strain (No./sex/group; age/weight) | Route and Dose (mg/kg bw/day) | Duration | NOAEL (mg/kg bw/day) | Other Observations | Reference |
|---|---|---|---|---|---|
| **Short-term Toxicity Studies** | | | | | |
| Wistar rats (6M/group; 40-50 g) | Dietary: 0 (control), ~4185, ~7795[c] scFOS Neosugar® | 6-8 weeks | NR | ↓ Body weight in 10,000 group<br>↑ Cecum and colon weights in both treatment groups<br>↑ Small intestine weights in 10,000 group<br>↑ Fecal weight and ↓ GI transit time in both treatment groups<br>↓ Serum triacylglycerol and ↑ fecal excreted neutral sterols and volatile fatty acids<br>During the first few days FOS administration transient watery stools | Tokunaga et al. (1986) |
| Wistar SPF rats (18M/group; 6-7 weeks old) | Gavage: 0 (control), 1500, 3000 or 4500 scFOS ($DP_{av}$ = 3.5) | 6 weeks | 4500 | No mortalities or abnormalities<br>Minor ↑ body weight in 3000 and 4500 groups (stat. sig. not reported)<br>No consistent, treatment-related findings in serum chemistry parameters (occasional fluctuations reaching statistical significance were considered spurious – further details not reported)<br>Swollen appendix in rats receiving treatment (number/group not reported) | Takeda and Niizato (1982); summarized in Carabin and Flamm (1999) |
| Wistar SDP rats (18M/group; 6-7 weeks old) | Gavage: 0 (control), ~2500, ~5000[a] scFOS ($DP_{av}$ = 3.5) | 6 weeks | 5000[b] | No mortalities or treatment-related abnormalities<br>Diarrhea reported on the 10th day of FOS administration (no additional details reported)<br>↓ body weight in FOS treated animals [(week 1-5) – stat. sig. not reported)], normalized near completion of study<br>FOS related ↓ in cholesterol (stat. sig. not reported)<br>Swollen appendices were reported at Week 2 and Week 6 necropsies (number/group not reported)<br>No treatment related toxicity compared to controls | Takeda and Niizato (1982); summarized in Carabin and Flamm (1999) |
| **Subchronic Toxicity Study** | | | | | |
| Rats (strain, number, sex, age not identified) | Dietary: Up to 20,400 scFOS (no further details reported) | 90 days | NR | No significant changes in clinical chemistry, hematological or urine parameters and no abnormalities upon gross or histopathological examination<br>Dose related ↑ in diarrhea, soft | Meijl Seika Kaisha (1982), cited in GRN 44 (GTC |

| | | | | | |
|---|---|---|---|---|---|
| | | | | stools, cecal distension, intestine weights | Nutrition, 2000) |
| **Chronic Toxicity and Carcinogenicity Study** | | | | | |
| Fischer 344 rats (50/sex/group; 4 weeks old) | Dietary: Male: 0, 341, 854, and 2170 Female: 0, 419, 1045, and 2664 scFOS Neosugar® (DP = 2-4) | 104 weeks | 2170 (males)[b] 2664 (females)[b] | No dose-related effects on survival, growth, hematological or clinical chemistry parameters, organ weights or neoplastic lesions | Clevenger et al. (1988) |

↑ = Increase; ↓ = decrease; DP = degree of polymerization; $DP^{av}$ = average degree of polymerization; GI = gastrointestinal; GRN = GRAS registration notification; F = female; FOS = fructo-oligosaccharide; M = male; NOAEL = no observed adverse effect level; NR = not reported; [a] Calculated using U.S. FDA, 1993; [b]Study authors did not provide a NOAEL, values were derived based on reported study findings; [c]Calculated using the food intake values presented in the study report and weight of rats from U.S. FDA, 1993

### 6.1.2.5.5. Developmental and Reproductive Toxicity Studies

In addition to above described toxicity studies, the findings of a developmental toxicity study in rats were also summarized Carabin and Flamm (1999) from an unpublished study by Henquin (1988). The available study related details are provided in Table 5. In this study, dietary exposure of scFOS at concentrations up to 20% (equivalent to approximately 10000 mg/kg bw/day) did not result in developmental toxicity. In the study summary, fetal markers other than body weight were not further described. During the nursing period, 'a growth delay was observed for the pups (specifically males) in the test group,' which was attributed to the restricted nutritional status of the lactating mothers (who were consuming a diet with an essentially non-caloric content of 20%, far above recommended levels to avoid nutritional disturbances). The reviewers concluded that 'a diet containing 20% FOS has no significant effects on the course of pregnancy in rats and on the development of their fetuses and newborns.'

In another study by Sleet and Brightwell (1990) also summarized in Carabin and Flamm (1999), maternal and developmental toxicity of FOS at dietary concentrations up to 20% (equivalent to approximately 10,000 mg/kg bw/day) were investigated. In this study, rats during postcoitum days 0 to 15 were fed a diet containing FOS. No treatment related adverse effects (diarrhea), or differences in pregnancy outcome or *in utero* development were noted.

**Table 5. Summary of Developmental and Reproductive Toxicity Studies of scFOS Conducted in Rats**

| Species strain (No./sex/group) | Route and Dose | Duration | Other Observations | Reference |
|---|---|---|---|---|
| Wistar rats (29F; n=12 treatment and n=17 control) | Dietary: 0 or 10,000 mg scFOS/kg bw/day (no further details reported) | Gestation days 1-21 | No treatment effect on number of pregnancies or fetus or newborn weights; ↓ Body weight during nursing period was reported in the treated pregnant rats and pups; Diarrhea was observed in treated pregnant rats (number not reported) during the first week and soft stools in weeks 2 and 3 for this group; Growth delay in male pups in test group 3 | Henquin (1988); described in Carabin and Flamm (1999) |

| | | | | |
|---|---|---|---|---|
| Sprague Dawley (CrL CD (SD) BR) rats Pregnant female rats (24-27/group) | Dietary: 0 or 2375[a] mg scFOS/kg bw/day (Day 0 – 6 postcoitum)<br><br>Dietary: 0, 2500, 5000, or 10,000[a] mg scFOS/kg bw/day (Day 6 – 15 postcoitum)<br><br>scFOS (no further details reported) | Days 0-15 postcoitum | No treatment related adverse events; No deaths or diarrhea reported; ↓ Body weight on postcoitum Day 2 in all FOS treated rats compared to control; Dose related decrease in body weight for FOS treated rats; Body weight and body weight changes in 2,500 and 5,000 mg/kg bw/day groups were similar among groups from Day 12-15<br>No remarkable findings at necropsy; No treatment related effects on number of pups/litter, the sex ratio, and viability of both the embryo and the fetus or structural development of fetuses; ↑ Fetal weights of 10,000 mg/kg bw groups compared to control, no other reduction in litter or fetal weights | Sleet and Brightwell (1990); described in Carabin and Flamm (1999) |

↑ = Increase; ↓ = decrease; [a] Calculated using U.S. FDA, 1993

### 6.1.2.5.6. Mutagenicity and Genotoxicity Studies

Clevenger et al. (1988) investigated the genotoxic potential of commercially available scFOS (Neosugar®). These assays included, microbial reverse mutation assays (Ames assay) in *Salmonella typhimurium* and *Escherichia coli* WP2 uvrA, mammalian cell mutation assay with mouse lymphoma L5178Y cells; and induction of unscheduled DNA synthesis (UDS) in human epithelioid cells (HeLa S3). The reverse mutation and unscheduled DNA repair assays were conducted in accordance with the OECD guidelines and the mammalian cell mutation assay conducted according to recognized methods. The findings from these assays are provided in Table 6. The results of these studies suggest that scFOS is not genotoxic in bacteria and mammalian cells in the presence or absence of metabolic activation.

**Table 6. *In vitro* Genotoxicity Studies on scFOS**

| Test system | Concentration | Metabolic Activation | Result | Reference |
|---|---|---|---|---|
| Bacterial reverse mutation assay (*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and TA 1538 and *Escherichia coli* WP2 *uvrA* | 0, 50, 150, 500, 1500, or 5000 µg/plate | ± S9* | Negative | Clevenger et al., 1988 |
| Mammalian cell mutation assay (mouse lymphoma L5178Y cells) | 2000, 3000, 4000 or 5000 µg/ml | ± S9* | Negative | Clevenger et al., 1988 |
| Unscheduled DNA synthesis [Human epithelioid cells (HeLa S3)] | 25, 50, 100, 200, 400, 800, 1600, 3200, 6400, 12,800, 25,600, 51, 200 µg/ml | - | Negative | Clevenger et al., 1988 |

*S9= Metabolic activation with Aroclor 1254-induced rat liver S9; scFOS – short chain fructo-oligosaccharides

### 6.1.3. Available Corroborative Safety Evidence

#### 6.1.3.1. Regulatory Agency Review

Based on FDA GRAS Notices inventory website, the FDA has received six GRAS notices on FOS preparations, all of which have received GRAS status for use of FOS as a food ingredient in a variety of conventional foods, including infant formula (GRN 44, 537, 605, 623, 717 and 797) (FDA, 2000; 2015; 2016a; 2016b; 2017; 2018). The currently marketed FOS products are derived from sucrose is enzymatically converted to FOS following action of β-fructofuranosidase obtained from different non-toxigenic and non-pathogenic strains of microorganisms. Given the use of similar manufacturing processes, the differences between various FOS products would be limited to minor variations in the compositional distribution of the glucose-fructose disaccharides (FOS), and to differences in the residual levels of other sugars. This also suggests that the safety information on FOS products can be interchangeably used.

The safety related information of scFOS have been extensively described in the 2014 FDA GRAS notification (GRN, 537) on scFOS for its uses in infant formula by Ingredion (2014). In a subsequent GRAS notification (GRN, 717), Galam (2017) has also described available safety information on uses of scFOS in conventional foods (Galam, 2017). In addition to these two GRAS notices, in four additional GRAS notices (GRN, 979; GRN 623; GRN, 605; and GRN, 44) the safety data on scFOS has been summarized. Among these GRAS notices on the use of scFOS, GRN 605 was submitted by Tata. The comparative data and information from all these GRAS notices is provided in Table 7. This comparison of these GRAS notices suggest that all these products are similar. Furthermore, there is one more GRAS notice on oligofructose (GRN, 392) that also describes the safety related information on FOS derived from chicory. FDA did not question the acceptability and suitability of the available evidence to support the proposed uses described in these seven GRAS notices, including its uses in infant formula, and replied to all these notifications, including the GRAS notice by Tata, that the agency had 'no questions' regarding the conclusions that the scFOS or oligofructose is GRAS for the intended uses. Tata is hereby incorporating all the toxicology and human tolerance studies discussed in these previous GRAS notices by reference (NFBC, 2018; Galam, 2017; NFBC, 2016; Tata, 2015; Ingredion, 2014; Pfizer, 2011; GTC, 2000).

As can be noted from the comparison given in Table 7, the subject of present GRAS assessment, scFOS for use in infant formula is substantially equivalent in its specification and composition (including disaccharide polymers as well as degree of polymerization) to that of previous GRAS notices, GRN 797 and GRN 537, for uses of scFOS in infant formula. Additionally, in these three GRAS notices, scFOS is proposed for uses in infant formula at same use levels. In these three GRAS notices, the enzyme, β-fructofuranosidase, derived from microorganism, is used in the manufacturing of FOS. The levels of scFOS (95%) in these three GRAS notices is same. The individual scFOS molecules such as 1-kestose (GF2), Nystose (GF3), and Fructofuranosylnystose (GF4), as well as residual levels of sugars is very similar.

**Table 7. Comparison of the Subject of Present GRAS, scFOS, with other FDA Accepted GRAS Notices**

| Constituents | Current GRAS* | GRN 797* | GRN 537* | GRN 717 | GRN 623 | GRN 605 | GRN 44 |
|---|---|---|---|---|---|---|---|
| Manufacturing | Sucrose + fungal enzyme | Sucrose + fungal enzyme | Sucrose + fungal enzyme | Sucrose + fungal enzyme | Sucrose + fungal enzyme | Sucrose + fungal enzyme | Sucrose + fungal enzyme |
| Total FOS (%) | ≥95 | ≥95 | NLT 95 | 95±2 | ≥95 | 95±2 | >95 |
| 1-kestose (GF2) (%) | NLT 30 | NLT 30 | NLT 30 | NLT 30 | NLT 30 | 35-43** | 35±6 |
| Nystose (GF3) (%) | NLT 45 | NLT 40 | NLT 45 | NLT 40 | NLT 40 | 42-48** | 50±6 |
| Fructofuranosylny stose (GF4) (%) | NLT 5 | NLT 5 | NLT 5 | NLT 5 | NLT 5 | 6-11** | 10±4 |
| Sugars (%) | ≤5 | ≤5 | NMT 5 | 5±2 | ≤5 | 5±2% | <5 |
| Intended uses | Term Infant Formula | Term Infant Formula | Term Infant Formula | Multiple foods | Multiple foods | Multiple foods | Multiple foods |
| Use levels | 4 g/L starter formula; 5 g/L follow on formula | 4 g/L starter formula; 5 g/L follow on formula | 4 g/L starter formula; 5 g/L follow on formula | 0.4 to 6.7% | 0.4 to 6.7% | 0.4 to 6.7% | 0.4 to 6.7% |
| EDI | 828 mg/kg/day (90$^{th}$ %) | 828 mg/kg/day (90$^{th}$ %) | 828 mg/kg/day (90$^{th}$ %) | 9.09 g/day (90$^{th}$ %) | 12.8 g/day (90$^{th}$ %) | 12.8 g/day (90$^{th}$ %) | 12.8 g/day (90$^{th}$ %) |
| ADI | At proposed use levels (4 or 5 g/L) | At proposed use levels (4 or 5 g/L) | At proposed use levels (4 or 5 g/L) | 20 g/day | 20 g/day | 20 g/day | 20 g/day |
| Safety determination | Totality of evidence | Totality of evidence | Totality of evidence | Totality of evidence | Totality of evidence | Totality of evidence | Totality of evidence |

*The three shaded columns are for GRAS notices for use of scFOS in Infant Formula, while the other GRAS notices are for use of scFOS in conventional foods. **Based on ranges from batches given in the GRAS notice.

Given the structural and chemical similarity of scFOS preparations that have been concluded GRAS (e.g., GRN 797 and 537) by NFBC (2018) and Ingredion (2014) with the current GRAS (Table 7), a discussion of publicly available data and information relevant to the safety of scFOS is incorporated by reference to studies described in GRN 797 and 537. Additionally, in GRN 797 some safety related and other question raised by FDA for GRN 537 are discussed. Based on all available information, there exists no evidence in the available information on scFOS that demonstrates, or suggests reasonable grounds to suspect, a hazard to infants when scFOS is added as a prebiotic ingredient to non-exempt infant formula at levels up to 400 mg/100 ml in starter formula as consumed and 500 mg/100 ml in follow-on formula as consumed. Additionally, given the similarity between the GRAS notices (GRN 797 and GRN 537) and the subject of this present GRAS assessment, as well as other information available, Tata has concluded that the scFOS it intends to market for uses in infant formula is safe and GRAS.

The Foods Standards Australia New Zealand (FSANZ) has evaluated the safety of FOS for its uses as an alternative to inulin (FSANZ, 2013). Based on published information

characterizing the metabolism of FOS, published studies characterizing the toxicity of FOS in animal models and published studies evaluating the safety and tolerance of FOS in humans (children and infants), FSANZ concluded that FOS is technologically suited to its proposed use and complies with international specifications. FSANZ noted that no adverse effects on growth, hydration status, nutrient intake, frequency and nature of adverse events, gastrointestinal intolerance, stool consistency and frequency, or fecal flora were observed in studies conducted in healthy infants or young children at amounts of FOS up to 3.0 g/L for periods ranging from 1 week to approximately 3 months.

In addition to above described information on FOS, several other structurally related β2-1 fructan preparations also have received GRAS status for use as food ingredients (*e.g.*, GRN 118, 392, 477 and 576- all these GRAS notices and FDA responses to these GRAS assessments are available at FDA GRAS Inventory webpage). Although the related inulin type fructans have similar chemical composition to scFOS and are expected to have a similar toxicological and physiological profile following ingestion, these oligomers typically display a higher molecular weight distribution.

**6.1.3.2. Unpublished Corroborative Studies**

In GRN 537 (Ingredion, 2014) on scFOS and also in FSANZ (2013) on FOS, two unpublished on the effects of scFOS in infants are summarized. These publicly available unpublished studies with scFOS are summarized in Table 8.

In the first unpublished randomized, double-blind, placebo-controlled trial, 63 healthy term infants (age 4-10 weeks) were fed control formula for 2 weeks, followed by a whey-enriched formula containing 0, 150, or 310 mg scFOS/100 ml for additional two weeks. The groups as enrolled included 21, 22, and 20 infants, respectively, with a mean age of 43±4 days. Formula intake, growth, stool characteristics, and tolerance were assessed on days 1, 15, and 29. Urine was collected on days 15 and 29 and blood was collected on day 29 for analysis. One infant from the control group, 5 from the low-scFOS group, and 4 from the high-scFOS group failed to complete the study. Withdrawal of one infant from control-group, two from the low-scFOS infants, and three from the high-FOS infants was due to intolerance, while the remainder was attributed to protocol failures. Intolerance withdrawals were based on vomiting or spit-up, diarrhea or watery stools, fussiness, increased stool frequency, or weight loss; there were no differences in reported adverse events among feeding groups.

In this first study (Ingredion, 2014; FSANZ, 2013), no significant differences among groups were reported in formula intake, growth, stooling patterns, tolerance, or in any of the outcomes measured in blood or urine. The blood analysis did not reveal the presence of kestose or nystose, but these molecules were found in the urine of most of the infants who received scFOS-containing formula for 2 weeks (GF2 in 55% and GF3 in 64%). The only statistically significant difference in the microbiota was a reduction in *Clostridium* spp., in infants receiving scFOS as compared to the control group. The investigators concluded that "Infant formulas containing added FOS at the levels provided ... are well tolerated and support normal growth in term infants." No explanation for the appearance of scFOS residues in urine but not in blood was provided. However, the levels found in urine exceeded the detection limits by only small amounts and, although the analytical methods and limits of detection in the blood analyses were not described, it may be that these limits were higher for the blood analyses than for those in urine and scFOS residue levels simply failed to reach detection limits.

In the second unpublished randomized, double-blind, placebo-controlled, multicenter trial (Abbott 1993; FSANZ; 2013; Ingredion, 2014), 102 healthy term infants aged 1-8 days were randomized to receive formula containing 0 (n = 52) or 300 (n = 50) mg scFOS/100 ml formula for approximately 16 weeks (to 112 days of age). Additional group of 25 healthy breast-fed infants aged 0-9 days served as human-milk reference group. Of the 70 infants receiving formula, 34 infants that were fed formula without scFOS and 36 consuming scFOS-containing formula, as well as 23 of the 25 fed human milk-fed infants completed the study. Protocol errors were responsible for the loss of 12, 6, and 2 infants from the non-scFOS formula group, the scFOS formula group, and the human milk group, respectively. Six infants were withdrawn from the non-scFOS formula group and 8 from the scFOS group due to adverse events: symptoms of milk intolerance (2 and 4 infants, respectively), diarrhea or watery stools (2 and 1 infants), constipation (2 and 1 infants), and colic or gassiness (1 scFOS-group infant each). Differences among groups were not statistically significant.

In the second study, there were no differences among groups in measures of weight, length, or head circumference at any time during the study, nor did the formula groups differ in feeding frequency or intake, feedings with spit-up or vomit, stool frequency, or stool consistency, although the human milk-fed infants had significantly softer and more frequent stools than the 2 formula groups. Levels of total cholesterol in blood were significantly higher in the human milk group than in either formula group, but levels of AST and ALT were similar in all groups. No blood samples from any infant had detectible scFOS trimers or tetramers, but they were consistently found in urine from infants receiving formula containing scFOS. No urine sample contained detectible ketones. The investigators concluded that "infant formulas containing added FOS at ... up to 3 g/L are well tolerated and support normal growth in term infants. The addition of the fermentable fiber at these levels, however, has only small effects on fecal microflora."

**Table 8. Unpublished studies with scFOS in Infants***

| Reference | Dose, Duration | Study Design, Objective | Subjects | Results |
|---|---|---|---|---|
| Abbott (1993) | scFOS 0 or 3 g/L formula for about 16 weeks (to 112 days of age) | Randomized, double-blind, placebo-controlled, multicenter study of the safety and bifidogenic effect of scFOS in infant formula | 102 healthy term infants aged 1-8 days (and 25 healthy breast-fed infants aged 0-9 days as a human-milk reference group) | Six infants were withdrawn from the non-scFOS formula group and 8 from the scFOS group due to adverse events: symptoms of milk intolerance (2 and 4 infants, respectively), diarrhea or watery stools (2 and 1 infants), constipation (2 and 1 infants), and colic or gassiness (1 scFOS-group infant each). Differences between groups were not statistically significant. There were no differences between groups in measures of weight, length, head circumference, feeding frequency or intake, feedings with spit-up or vomit, stool frequency, or stool consistency, although the human- milk-fed infants had significantly softer and more frequent stools than the 2 formula groups. Levels of AST and ALT were similar in all groups. No blood samples from any infant had detectible scFOS trimers or tetramers. No urine sample contained detectible ketones.<br>No differences were seen between the groups in populations of *Bifidobacteria, Bacteroides,* or *Clostridia* spp., or *C. difficile*, but counts of |

| | | | | |
|---|---|---|---|---|
| | | | | *Lactobacillus* spp. were significantly higher among infants receiving the scFOS-supplemented formula. The authors concluded that "infant formulas containing added FOS at ... up to 3 g/L are well tolerated and support normal growth in term infants." |
| Abbott (1992) | scFOS 0, 1.5, or 3.1 g/L formula for 2 weeks | Randomized, double-blind, placebo-controlled study of the safety and bifidogenic effect of scFOS in infant formula | 63 healthy term infants aged 4-10 weeks with a mean age of 43±4 days | One infant from the control group, 5 from the low-scFOS group, and 4 from the high-scFOS group failed to complete the study; withdrawal of the single control- group infant, 2 of the low-scFOS infants, and 3 of the high-FOS infants was due to intolerance, while the remainder were attributed to protocol failures.<br>Intolerance withdrawals were based on vomiting or spit- up, diarrhea or watery stools, fussiness, increased stool frequency, or weight loss; there were no differences in reported adverse events among feeding groups.<br>No significant differences among groups were reported in formula intake, growth, stooling patterns, tolerance, or in any of the outcomes measured in blood or urine. No kestose or nystose was detected in the blood of any infant. Infants receiving scFOS had significantly reduced *Clostridia* spp. as compared to the control group. The authors concluded that "Infant formulas containing added FOS at the levels provided ... are well tolerated and support normal growth in term infants." |

*Adapted from GRN537*

### 6.1.3.3. Natural Occurrence

Oligosaccharides, including FOS, occur naturally in plants and are commonly consumed by humans in foods. FOS occurs in a number of plants such as onions, Jerusalem artichokes, bananas, lettuce, asparagus, rye, garlic and wheat (rough and bran forms) (GTC Nutrition, 2000; Bornet et al., 2002). Some grains and cereals, such as wheat and barley, also contain FOS (Campbell et al., 1997). The Jerusalem artichoke and its relative yacon[1] together with the Blue Agave plant have been reported to contain the highest concentrations of FOS of cultured plants. Campbell et al. (1997a) extensively analyzed and characterized the naturally occurring FOS levels in a variety of plants. Of the 25 samples analyzed for FOS content, 20 showed detectable levels of FOS. In these samples, the FOS content ranged from 0.1-0.2 mg/g for most (12/20) of the fruits. The highest FOS content was found in ripe bananas, which contained 2.0 mg/g FOS. Of the 40 vegetable samples analyzed, 16 did not contain FOS. An additional 6 vegetables contained 0.1 or 0.2 mg/g FOS, while the remaining 16 vegetables contained from 0.3 to 58.4 mg/g FOS.

The available information suggests that humans consume FOS on a daily basis following ingestion of plants that naturally contain FOS. An estimate of FOS intake from commonly

[1] The yacón is a species of perennial daisy traditionally grown in the northern and central Andes from Colombia to northern Argentina for its crisp, sweet-tasting, tuberous roots.

consumed plants was provided in GRN 44 (GTC Nutrition, 2000). For this analysis, data provided by Campbell et al. (1997) for the content of FOS was used along with food intake data available for the U.S. population from the 1994-96 United States Department of Agriculture's (USDA) Continuing Survey of Food Intakes by Individuals (CSFII). Based on the foods included in the analysis reported by Campbell et al. (1997), the mean FOS intake for adults in the U.S. was estimated as 114 mg/day. For adults, an upper bound estimate of daily FOS intake, based on the 90$^{th}$ percentile food intake was determined as 248 mg/day. The food types that contributed the most to FOS consumption were onions, bananas, lettuce, and wheat (in rough and bran forms).

#### 6.1.3.4. Current Uses

FOS and other prebiotic ingredients are increasingly being recognized as useful dietary tools for the modulation of the colonic microflora toward a healthy balance. FOS represents only a fraction of the inulin class of carbohydrates known as fructans. This class includes different chain length polymers such as inulin, oligofructose and FOS. Thus, inulin is a composite oligosaccharide that contains several FOS molecules. These polymers are chemically similar entities and share the same basic structure of β (2-1) linked fructosyl units, sometimes ending with a glucosyl unit. As all these fractions are mixtures of molecules that differ only in chain length, they can be described by their range and average degree of polymerization. Various terms describing fructans have been used interchangeably in the published literature. Currently, there are several commercial sources of FOS, inulin, and oligofructose. These products are sold and consumed as fat replacements and sugar substitutes for use in a variety of foods such dairy products, candies and chocolates, spreads, baked goods and breakfast cereals, meat products, ice cream and frozen yogurt (GTC Nutrition, 2000). In the U.S., FOS is sold as a nutritional supplement at recommended doses of up to 4 to 8 g/day to promote the growth of bifidobacteria, and as an ingredient in nutritional supplement liquids as a source of dietary fiber.

Based on information from FDA's GRAS Notice Inventory[2] website as of April 28, 2015, the agency has received three notices on FOS and provided "no questions" letters to all of the notifiers. In May 01, 2000, GTC Nutrition Company submitted GRAS notification (GRN 44) to FDA for use of FOS in different food categories (GTC Nutrition 2000). On November 22, 2000, FDA issued "no questions" letter for this GRAS notice (FDA, 2000). Subsequently, two GRAS notifications were submitted to FDA for use of FOS in infant formulas by: Pfizer Nutrition (2011; GRN 392) and by Ingredion Incorporated (2014; GRN 537). Both these firms received a "no questions" letter from FDA (FDA, 2011, 2015). A closely related oligosaccharide, galacto-oligosaccharide, has also been determined to be GRAS for use in a variety of foods in nine GRAS notifications to the FDA. All these GRAS notices are available at FDA's GRAS Notice Inventory.

#### 6.1.3.5. scFOS Safety and Degree of Polymerization

##### 6.1.3.5.1. Degree of Polymerization and Fermentability

As described earlier, generally FOS have a degree of polymerization (DP) of 2 to 10 and can be produced from sucrose by transfructosylation and from inulin by controlled hydrolysis. Inulin, extracted from chicory roots, has a more heterogeneous DP, ranging from 3 to 60. Given the large variation in degree of polymerization (DP) of FOS, it is important to address, whether

[2]Accessible at: http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true.

the small chain molecules of scFOS with lower DP can have easier fermentability (and more and rapid gas formation) or lower fermentability (and lower and slower gas formation).

Rumessen and Gudmand-Hoyer (1998) studied the intestinal transport and fermentation of chicory-derived long-chain inulin (median DP=12) and oligofructose (median DP=3) in a single-blind crossover study. In this study, 10 healthy subjects (5/sex; aged 18-25 years) received single dose tests in random order, separated by 48 hours or more, of 10, 20, and 30 g oligofructose and 20 g long-chain inulin. Following dose administration, breath samples were collected every 30 minutes for 12 hours after each test and analyzed for hydrogen ($H_2$). In this study, hydrogen production profiles were used to estimate orocecal transit times (Table 9). The investigators concluded that orocecal transit time was slower for the long-chain inulin as compared to the oligofructose. The findings also suggest that the difference is not great as compared to the extremely large amount of variability. Hydrogen production was not significantly different between the two fructans, even with substantial differences in DP profiles.

**Table 9. Effects of Chicory-Derived Long-chain Inulin and Oligofructose on Hydrogen Production and Gastrointestinal Transit***

| Test article and dose | AUC – $H_2$ production ppm . min/$10^2$ | | Orocecal Transit Time (minutes) | |
|---|---|---|---|---|
| | Mean | Range | Mean | Range |
| Short chain, 10 g | 139 | 110-186 | 105 | 60-240 |
| Short chain, 20 g | 306 | 241-570 | 30 | 15-105 |
| Short chain, 30 g | 368 | 256-615 | 53 | 0-165 |
| Long chain, 10 g | 247 | 118-491 | 75 | 15-180 |

*Adapted from information based on GRN 537

In an *in vitro* study using human fecal inoculum, Stewart and Slavin (2006) compared the batch fermentability of 6 chain lengths of inulin and FOS with DP ranging from 2 to >20. In this study, samples were removed at 0, 4, 8, 12, and 24 hours and total SCFA, acetate, propionate, and butyrate were measured. The investigators reported, "...individual sample chain length did not follow a clear trend with fermentability," although a statistically significant difference was detected in the speed of fermentation when the samples were grouped into FOS (DP <10) and inulin (DP >10). In another *in vitro* study, the fermentability of Orafti® GR chicory inulin (DP = 2-60) and Orafti® P95 oligofructose (DP = 2-8) by *Lactobacillus rhamnosus, Lactobacillus acidophilus, Enterococcus durans,* and *Enterococcus faecium* was compared (Bohacenko et al., 2013). While the enterococci fermented the shorter chain substrate more efficiently as compared to the higher-DP oligosaccharide, both species of *Lactobacillus* fermented both fructans equally well, leading the authors to conclude that "no significant difference at both lactobacilli species was observed in respect of utilization of prebiotics with different chain length."

Based on above described studies, it appears that there is no clear association between fermentation rate and DP of the oligosaccharide. The available information indicates that as compared to longer chain oligosaccharides, shorter-chain fructans are fermented rather quickly, and that there may be differences in the specific small chain fatty acids produced. Based on the available evidence, this is most likely the case. These differences are so delicate or precise, it is difficult to analyze or describe, and inconsistent, and highly variable. Additionally, intra-individual variation, and even within an individual over time, is likely a function of many factors including the luminal pH, the presence of calcium or other buffers, the microbiota profile, etc. Given this large variability, differences in fermentation of fructans with different DP has only

been shown when the DP difference is extreme, as elucidated by Rumessen and Gudmand-Hoyer's (1998) in comparison of fructans with median DP of 3 and 12 or by Stewart and Slavin's (2006) in range of DP from 2 to >20—and even in this extreme case, the overall trend was not statistically significant.

The available information suggest that DP differences among FOS, including scFOS, are minor. The weighted mean DP of oligofructose (Orafti® P95) and that of scFOS are 4.29 and 3.73, respectively. The median DP of these 2 substances are both = 4. Given all this, it is unlikely that any difference could be demonstrated in the gastrointestinal handling, fermentation rate, SCFA production, or fate of these substantially similar substances (polymers). This is not surprising given that the gut microbiota varies amongst individuals in significant ways.

**6.1.3.5.2. Osmolality and Degree of Polymerization**

scFOS molecules with low DP are likely to be more osmotically active as compared to other oligofructoses with higher DP, including the ones used in studies that are used to support or corroborate the safety of the subject of present GRAS, scFOS. Hence, it is important to address whether potentially higher osmotic activity from scFOS is not expected to pose a concern, including increased abdominal distension, pain and laxation, as well as the possibility of severe laxation/diarrhea. Both breast-fed and bottle-fed infants are currently exposed to human milk and to infant formula that impose greater osmotic loads than does infant formula with the intended level of scFOS.

It is well known that human breast milk contains over 200 different oligosaccharides (Kunz et al. 2000; Vandenplas 2002; German et al., 2008; Ballard and Morrow 2013), with a total concentration in excess of 1.2 g/100 ml. This level is approximately 3 times higher as compared to the proposed uses of scFOS in starter formula. The concentration of human milk oligosaccharides (HMO) has been reported to be 2 g/100 ml of milk on the fourth day of life of the infant (Vandenplas, 2002). For some mothers, the most dominant component, lacto-N-neotetraose (LNnT; mass 709.3) can be 10 times more intense than the next most abundant lacto-N-fucopentaose I/V (LNFP I/V; mass 855.3) (Zivkovic et al., 2011). For others, the three most abundant components, lacto-N-neotetraose, lacto-N-tetraose (LNT; mass 709.3) and lacto-N-fucopentaose I/V make up over 50% of the total. Among all samples analyzed to date, a neutral oligosaccharide with neutral mass 709.3 Da (3Hex, 1HexNAc;LNnT) is the most prominent. Thus the available information suggest that dominant HMOs are short-chain oligosaccharides with DP 3-5 (the same as scFOS) and with a molecular weight of 709.3 Da, nearly identical with the 700 Da molecular weight of scFOS.

Chaturvedi et al. (2001) analyzed HMOs from 12 donors and reported that the mean total oligosaccharide concentration for the 11 typical donors (a total of 77 samples) was approximately 9 g/L for the first 14 weeks of lactation followed by a gradual decline to approximately 4 g/L at year one postpartum. These investigators further noted that the predominant oligosaccharides for the first few months of lactation were 2'-FucLac and LNF-1. For the first 3 months of lactation, 2'-FucLac, at approximately 3 g/L, was the oligosaccharide present in the largest concentration. The description of these 2 oligosaccharides was as follows: 2'-FucLac = 2'-fucosyllactose = Fuc-α(1,2)-Gal-β(1,4)-Glc (DP=3); and LNF-1 = lacto-N-fucopentaose-I = Fuc-α(1,2)-Gal-β(1,3)-Glc-N-Ac-β(1,3)-Gal-β(1,4)Glc (DP=5). All this information indicates that the predominant HMOs present in human milk at higher levels is similar to the intended uses of scFOS.

The available information from FDA GRAS notice (GRN 236) and international regulatory agencies reviews show that galactooligosaccharides (GOS) are commonly used and have been accepted for addition to infant formula at concentrations as high as 800 mg/100 ml. Infant formula containing a blend of 90% GOS and 10% long-chain FOS at 800 mg/100 ml has been in use for many years in the European countries. The GOS added to infant formula actually has a significantly lower DP as compared to scFOS. As described in GRN 233, a GRAS notice on combination of GOS and polydextrose (Vivinal®), the DP profile of GOS (mean DP = 3.10) was as follows: DP2= 33%; DP3= 39%; DP4= 18%; DP5= 7%; DP6, 7, and 8= 3%. The DP profile (Table 7) of the subject of present GRAS, scFOS by Tata is as follows: DP3= 30%; DP4= 45%; DP5= 5%. Similarly, GOS described in other GRAS notices (GRN 286, 489, 495; and 569) for infant formula use have similar DP profiles to Vivinal® GOS. This indicates that GOS added to infant formula is more osmotically active as compared scFOS. Although the extensive literature regarding the safety of the addition of GOS to infant formula (at a level twice that proposed uses for scFOS) was not reviewed in the current notice, it is widely available and has been provided to FDA in numerous submissions. Thus, the available information indicates that scFOS supplemented formula is unlikely to cause adverse effects as result of any osmotic activity.

In summary, human milk-fed infants, as well as infants consuming formula with added GOS have long been exposed to fluids with higher osmolality as compared to formula containing the proposed addition level of scFOS with no reported adverse effects. Given this, there is no reason to expect an adverse osmotic effect with the intended use of scFOS. Additionally, the osmolality of infant formula is determined by its total formulation, not merely by its oligosaccharide content (if any). Infant formula manufacturers routinely test formula for osmolality and adjust its composition as needed to assure that it is within the desired range.

**6.1.3.6. scFOS and Changes in Colonic pH**

Nilsson and Bjorck (1988) reported significant acid hydrolysis of inulin (fructan) that increases with time. In addition to this, some animal and human studies also show approximately 10-20% acid hydrolysis of all FOS. It has been also reported that scFOS lowers the colonic pH. The lowering of colonic pH may lead to more hydrolysis of scFOS in infants. Thus, it is important to address whether the generation of fructose through increased acid hydrolysis of scFOS (that could be absorbed) would be of any consequence to infant health, including increased gastrointestinal discomfort. In the rat study by Nilsson and Bjorck (1988), significant gastric-acid hydrolysis of inulin has been reported. In a subsequent study, Bjorck and Nilsson (1991) repeated the study to produce a similar effect (Bjorck and Nilsson 1991). However, this is a deviant finding that contradicts the far larger body of findings suggesting little or no acid hydrolysis of fructans during gastric passage. This body of research includes at least two studies (Bach Knudsen and Hessov 1995, and Ellegard et al. 1997), in patients with ileostomies. These studies revealed that little or no digestion or absorption of fructans occurs in either the stomach or the small intestine. In both of the studies, Bjorck and Nilsson (1991; 1988) it was reported that a small amount of inulin was apparently hydrolyzed by gastric acid in rats. However, in both these studies the rats were restricted to only 10 g feed/day, producing abnormally low gastric pH. It is not clear whether similar phenomenon would be observed, if fructans are consumed *ad libitum* or with food - especially with dairy-based food - which is likely to provide significant buffering. The investigators did not explain in either report why they adopted this approach.

Besides the above reported *in vivo* experiments in rats, these investigators also performed an *in vitro* study in which inulin was incubated for 2 hours in a solution with HCl molarity of 0.10 or 0.05 and observed some degree of acid hydrolysis (Nilsson and Bjorck, 1988). Both the *in vivo* and *in vitro* studies involve a pH of around 1-2, far more acidic than could ever be produced in the colon by SCFA production, which would be unlikely to reduce the luminal pH to less than about 5.5. It is also important to note that complete hydrolysis of scFOS produces substantially less fructose than does hydrolysis of inulin or inulin-derived FOS. This is because all scFOS includes a glucose endcap, so that, for example, the breakdown of the scFOS DP=3 molecule is only 2/3 fructose and 1/3 glucose. In all, complete hydrolysis of scFOS, with DP=3-5, produces 72.4% fructose and 27.6% glucose. In contrast, breakdown of inulin with, say, DP=20, or of FOS derived from such inulin, would be 95% fructose and 5% glucose. Given all this, free fructose is unlikely to be an issue with scFOS.

### 6.2. Summary, Discussion and Conclusion

Tata Chemicals Limited (Tata) intends to use small chain fructo-oligosaccharides (scFOS) as a food ingredient in non-exempt term infant formula. The manufacturing process of scFOS involves the biotransformation of sucrose by the action of a microbial derived enzyme β-D-fructofuranosidase from *Aureobasidium pullulans*. The scFOS are prepared using raw materials and processing aids that are food-grade and comply with applicable U.S. federal regulations. The scFOS is manufactured according to cGMP in both a liquid (syrup) and powder form. The scFOS manufactured by Tata is composed of sucrose molecules (glucose-fructose disaccharides, GF) to which one, two, or three additional fructose units have been added by β 2-1 glycosidic linkages to the fructose unit of sucrose.

Tata has fully developed and characterized the identity and composition of the final product. scFOS primarily consists of 3 different molecules, each containing a terminal glucose residue and 2, 3, or 4 fructose residues, designated as GF2, GF3, and GF4. The food grade specifications for scFOS have been established by Tata. The scFOS in infant formula will be used at the maximum use levels of 400 mg scFOS/100 ml in starter formula (from birth to approximately 6 months) as consumed and 500 mg scFOS/100 ml in follow-on formula (infants older than approximately 6 months) as consumed. The conservative total daily intake of scFOS by infants is estimated at the 90$^{th}$ percentile from maximum concentration of 500 mg/100 mL, to be 1035 mg/kg bw/day. This estimated intake is very conservative for long-term exposure because as the infant grows the formula intake increases but at a slower rate than weight gain. Hence, the 90$^{th}$ percentile intake of scFOS is highest during the first 6 weeks of life and begins to decline and reach about 840 mg/kg bw/day by 8-12 weeks.

The safety of consumption of scFOS has been supported by several studies conducted on the metabolism of scFOS and other fructans, as well as safety/toxicity studies in animals and humans with scFOS and other fructans. Additionally, the safety in use of scFOS is supported from other GRAS notices on FOS that have been reviewed by FDA and had no questions. Several fructans, including scFOS, are already GRAS for use in food, including use in infant formula. In GRN 392, the use of oligofructose was concluded to be GRAS for use in infant formula, while in GRN 44 (additional uses) and GRN 537 use of scFOS in infant formula was concluded as GRAS. These uses of scFOS and fructans did not report any adverse effects. In addition to these GRAS uses in infant formula, several scFOS preparations have GRAS status for use as a food ingredient in a variety of conventional food and beverage categories (GRN 44, 605, 623, 717 and 797) (FDA, 2000; 2016a; 2016b; 2017; 2018). All of these GRAS notifications

have consistently concluded that the addition of scFOS to food is GRAS under their respective conditions of intended use.

The digestibility of fructans has been investigated in multiple *in vitro* and animal (rats) studies. These studies demonstrate that scFOS, unlike like other fructans that are hydrolyzed by the intestinal enzymes, scFOS is fermented by gut microbiota (Oku et al., 1984; Tsuji et al., 1986; Tokunaga et al., 1989; Bjork and Nilsson, 1991). Bjork and Nilsson, 1991 reported that inulin was hydrolyzed in rats by gastric acid due to low gastric pH because of food restriction in rats to only 10 g feed/day. Therefore, this phenomenon is not expected under conditions of normal food intake. This is a deviant finding that contradicts the far larger body of research that has found little or no acid hydrolysis of fructans during gastric passage. Animal studies demonstrated that scFOS is not absorbed.

In a number of published human studies in healthy adults (Stone-Dorshow and Levitt, 1987; Rumessen et al., 1990; Molis et al., 1996; Alles et al., 1996; Rumessen and Gudmand-Hoyerr, 1998; Castiglia-Delavaud et al., 1998; van Dokkum et al., 1999), as well as in compromised adults with ileostomy (Bach Knudsen and Hessov, 1995; Ellegard et al., 1997) effects of scFOS were investigated. The available information from human studies in both healthy subjects and subjects with ileostomy suggest that majority of ingested fructans, including scFOS reach the colon where it is fermented by gut microbiota. The kinetics of orocecal transit time and bacterial fermentation are inversely proportional to the degree of polymerization of the fructan.

In a summary report on several studies discussed by Carabin and Flamm (1999), findings from the acute toxicity studies suggest that scFOS has a low potential for acute oral toxicity. In a subacute study, feeding Wistar rats at levels up to 4500 mg scFOS/kg bw/day for 6 weeks did not produce any adverse effects, as evaluated by hematology, clinical chemistry and histopathology. Studies in neonatal animals demonstrate the lack of adverse effects of scFOS as discussed by Carabin and Flamm (1999). Inclusion of scFOS in the diets of neonatal BALB/c mice at 5% dietary concentration for up to 44 days did not reveal adverse effect on feed intake or body weight gain (Nakamura et al., 2004). In a study in piglets, Barnes et al. (2012) reported that addition of 10 g scFOS/L to enteral and parenteral feed of 2-day-old male and female piglets did not reveal adverse effects on weight gain, weights of stomach, pancreas, liver, and kidney; and gut morphology. In another study in piglets, Correa-Matos et al. (2003) reported that addition of 7.5 g FOS/L to the colostrum formula fed to 2-day-old piglets revealed enhanced intestinal function without any adverse effects.

In a repeat-dose 90-day toxicity study by Boyle et al. (2008), Sprague-Dawley rats were fed standard rodent chow for 13 weeks with 0, 0.55, 1.65, 4.96, or 9.91% oligofructose, replacing cornstarch. In this study no treatment related adverse effects as evaluated by food intake, body weight, body weight gain, clinical observations, hematology, clinical chemistry, or histopathology even at the highest dose tested, were noted. The NOAEL was the highest dose tested 4680 mg oligofructose/kg bw/day. In a chronic toxicity and carcinogenicity study (104 week), Clevenger et al. (1988) investigated the effects of feeding of scFOS (at levels up to 50,000 ppm) to male and female Fischer 344 rats. In this study, some statistically significant differences were noted but there were no toxicologically relevant, test-article-related macro or microscopic changes in either sex. The incidence of spontaneous tumors in the scFOS-treated animals was comparable to that of controls, with the exception of pituitary adenomas in male rats. However, as the pituitary adenoma is one of the most frequently occurring spontaneous

tumors in F-344 rats with highly variable background incidence, the observation was a chance artifact. The findings from this study suggest that scFOS is not carcinogenic and does not produce chronic toxicity in rats. Based on the findings from this study, the NOAEL was determined as 50,000 ppm (equivalent to 2170 mg/kg bw/day for males and 2664 mg/kg bw/day for females, the highest dose tested.

In a reproductive and developmental toxicity study, rats were fed a diet containing 20% scFOS from day 1 to 21 of gestation. In this study, except for the reduction in body weight of the pregnant rats and a growth delay for the male pups in the test group during nursing, there were no other effects on the pregnancy and development of fetuses (Carabin and Flamm 1999). The reduction in body weight of the pregnant rats appears to be related to lower caloric value for scFOS, decreased intake of food for this group, or diarrhea observed in the first week and softer stools in the second and third weeks. The results of this study suggest that feeding of rats at a 20% dietary concentration of scFOS has no significant effects on the course of pregnancy in rats and on the development of their fetuses and newborns. In another study, also described by Carabin and Flamm (1999), scFOS at dietary concentrations up to 20% did not adversely affect the pregnancy outcome or *in utero* development of the rat. In additional studies, scFOS was found to be non-mutagenic in bacterial reverse mutation assays, and non-genotoxic in a number of genotoxicity assay, such as mouse lymphoma assay, unscheduled DNA synthesis (UDS) assay, and chromosome aberration assay.

In summary, in multiple toxicity studies the safety of scFOS and fructans has been investigated. These studies included acute, subacute, subchronic, chronic, developmental and reproductive, and mutagenicity and genotoxicity. Based on the totality of toxicological information on scFOS and oligofructose it is concluded that the oral toxicity of these substances is extremely low. In addition to the studies described in animals, the effects of scFOS or oligofructose (FOS) have been investigated in a number of studies in infants. These studies are extensively described earlier. Some of the relevant studies in infants are briefly described here.

In a published randomized, double-blind, placebo-controlled trial by Paineau et al. (2014), 61 healthy term infants aged 0-7 days received formula supplemented with 400 mg/100 ml of either scFOS (DP 3-5) or maltodextrins to the age of 4 months. The scFOS in this study is substantially equivalent in terms of DP to the scFOS that is the subject of the present GRAS notice. Formula consumption did not differ between the groups, nor did growth, and the most frequent adverse event was abdominal pain, followed by liquid stools, but there was no statistically significant difference in the incidence or severity between the feeding groups. The findings from this study demonstrates that a milk-based infant formula supplemented with scFOS at 400 mg/100 ml will increase the fecal content of Bifidobacteria in healthy term infants in comparison to a placebo formula without inducing any problem of digestive tolerance.

Ripoll et al. (2015) investigated the effect of scFOS on digestive tolerance and growth parameters in infants up to 10 months of age. In this randomized, controlled, double blind study, 75 formula-fed healthy infants were included at the age of 4 months and received either a placebo or scFOS (500 mg/100 ml) supplemented formula for six months. The scFOS in this study is substantially equivalent in terms of DP to the scFOS that is the subject of the present GRAS notice. Tolerance and growth parameters were similar in both the groups. No difference was observed between groups for diarrhea and gastroenteritis. The results after 6 months of supplementation, the strict follow-up of adverse events and digestive tolerance criteria have demonstrated the good tolerance of scFOS follow-on milk, as no difference was observed

between groups for diarrhea, gastroenteritis, prevalence of infections, regurgitation, constipation and crying while these conditions are common at this life-stage. The findings from this study, show that a follow-on milk formula supplemented with 500 mg/100 ml scFOS is safe and well tolerated leading to normal growth in infants after the age of 4 months and promotes fecal bifidobacteria levels after one month in never breast fed infants. scFOS addition elicited normal digestive tolerance and normal growth suggesting it can be used safely at 500 mg/100 ml in infants after 4 months of age. The findings from this study support the proposed use of scFOS in follow on formula.

In additional studies in which FOS, but not necessarily scFOS (either high dose or for a long period of time) further supports the safety of scFOS in infants. In a published randomized, double-blind, placebo controlled study by Xia et al. (2012), healthy term infants aged ≤6 days were enrolled in a 4-week trial assessing the effects of 4 types of feeding that included cow's milk (control), human milk (reference), and two FOS groups (240 or 340 mg scFOS/100 ml) on the intestinal microbiota. The FOS used in this study was confirmed as scFOS. A total of 65 infants completed the study. No differences were reported among groups in stool frequency or consistency, or in the frequency of feedings with spitups or vomit.

Brunser et al. (2006) compared the effects on infants' fecal microbiota of a standard milk-based infant formula, the same formula with 200 mg/100 ml of oligofructose, the same formula with $10^8$ cfu *L. johnsonii* NCC533 (La1)/g powder, or breast feeding, for a period of 13 weeks, followed by a 2-week washout with standard formula. In this randomized, double-blind trial of the 116 healthy term infants, 76 formula-fed infants (66% of those enrolled) completed the entire study. Primary reasons for withdrawal were failure to follow the protocol, antibiotic use, or illness. The withdrawal rates did not differ across the 3 formula groups and none of the withdrawals was associated with adverse reaction to the formula. All formulas were well tolerated.

Bettler and Euler (2006) investigated growth and tolerance of in healthy full-term infants (aged 14 days or less) fed formula supplemented with oligofructose (150 or 300 mg/100 ml) for 12 weeks in a double-blind study in infants. In this study, overall, at least one adverse event was reported for 55% of the infants, but the lowest incidence of formula related adverse events was in the group receiving the higher dose of oligofructose (300 mg/100 ml), and none of the formula-related adverse events was considered to be serious. Additionally, there were no differences among groups in formula acceptance and tolerance. The investigators concluded that the experimental cow's milk-based formula supplemented with either 1.5 or 3.0 g oligofructose/L is safe, well-tolerated and supports normal infant growth. In addition to these studies, several other published studies in which infants, including preterm infants, were given scFOS or oligofructose are available and summarized previously. In these studies no adverse effects were reported.

For the present GRAS assessment of scFOS, a comprehensive search of the scientific literature for safety and toxicity information on scFOS and other similar molecules was conducted through December 2020 and utilized. Based on the totality of the available published evidence, it is concluded that there is sufficient qualitative and quantitative scientific evidence to determine the safety-in-use of scFOS in term infant formula. FOS products have been used in food for over 18 years with no evidence of adverse effects related to the safety of its use. The use of a similar manufacturing process in the preparation of the scFOS with similar compositional analysis, the subject of this GRAS assessment and those that has been the subject of FDA notifications suggests that the differences between various scFOS products would be limited to

minor variations in the compositional distribution of the FOS oligomers, and to differences in the residual levels of other sugars. These observations also suggest that the safety information on FOS products can be interchangeably used. The FDA responses to GRAS notification (GRN 537; GRN 797) on scFOS indicate that the agency is satisfied with the safety-in-use of scFOS at use levels of 400 mg scFOS/100 ml in starter formula as consumed and 500 mg scFOS/100 ml in follow-on formula as consumed. The safety determination of scFOS is based on the totality of available evidence, including current approved uses, *in vitro* and *in vivo* metabolism studies, and a variety of animal studies and, human and infant studies that supports the safety-in-use of scFOS.

In summary, on the basis of scientific procedures[3], the use of scFOS derived from sucrose as a food ingredient in infant formula at levels of 400 mg scFOS/100 ml in starter formula as consumed and 500 mg scFOS/100 ml in follow-on formula as consumed is considered as safe. The proposed uses are compatible with current regulations, *i.e.*, scFOS is used in infant formula at use levels of 400 mg scFOS/100 ml in starter formula as consumed and 500 mg scFOS/100 ml in follow-on formula and is produced according to current good manufacturing practices (cGMP).

Based on a critical evaluation of the publicly available data described and summarized herein, Tata Chemicals Limited has concluded that short-chain fructo-oligosaccharides (scFOS), meeting the specifications cited above, and when used as a food ingredient in infant formula at use levels of 400 mg scFOS/100 ml in starter formula (from birth to approximately 6 months) as consumed and 500 mg scFOS/100 ml in follow-on formula (infants older than approximately 6 months) as consumed is Generally Recognized As Safe (GRAS).

It is also the opinion of Tata Chemicals Limited that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that short-chain fructo-oligosaccharides (scFOS), when used as described, is GRAS, based on scientific procedures.

[3] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

## 7. Part VII – SUPPORTING DATA AND INFORMATION


Abbott Laboratories. 1993. Growth, tolerance, and stool characteristics of term infants consuming short-chain fructooligosaccharides in infant formula. [Unpublished report submitted in support of Application A1055 from GTC Nutrition to Food Standards Australia New Zealand to permit the optional addition of scFOS to Infant Formula Products, Foods for Infants, and Formulated Supplementary Foods for Young Children; available online at: http://www.foodstandards.gov.au/Pages/default.aspx website]

Abbott Laboratories. 1992. Growth, tolerance, and stool characteristics of term infants consuming short-chain fructooligosaccharides in infant formula. [Unpublished report submitted in support of Application A1055 from GTC Nutrition to Food Standards Australia New Zealand to permit the optional addition of scFOS to Infant Formula Products, Foods for Infants, and Formulated Supplementary Foods for Young Children; available online at: http://www.foodstandards.gov.au/Pages/default.aspx website]

Alles, M.S., Hautvast, G.J.A., Nagengast, F.M., Hartemink, R., Van Laere, K.M.J., Jansen, J.B.M.J. 1996. Fate of fructo-oligosaccharides in the human intestine. Br J Nutr 76:211-221.

Aunstrup, K. 1979. Production, Isolation, and Economics of Extracellular Enzymes in Applied Biochemistry and Bioengineering, Vol. 2, Enzyme Technology, eds. L. B. Wingard, E. Katchalski-Katzir, and L. Goldstein, pp. 28-68.

Aunstrup, K., Anderson, O., Falch, E.A., Nielsen, T.K. 1979. Production of Microbial Enzymes in Microbial Technology, 2nd ed., Vol. 1, eds H.J. Peppler and D. Perlman. Ch. 9, pp. 282-309.

Bach Knudsen, K.E., and Hessov, I. 1995. Recovery of inulin from Jerusalem artichoke (*Helianthustuberosus* L.) in the small intestine of man. Br J Nutr 74:101-113.

Ballard, O., Morrow, A.L. 2013. Human milk composition: Nutrients and bioactive factors. Pediatr Clin North Am 60:49-74.

Barnes, J.L., Hartmann, B., Holst, J.J., Tappenden, K.A. 2012. Intestinal adaptation is stimulated by partial enteral nutrition supplemented with the prebiotic short-chain fructooligosaccharide in a neonatal intestinal failure piglet model. J Parenter Enteral Nutr 36:524-537.

Bettler, J., Euler, A.R. 2006. An evaluation of the growth of term infants fed formula supplemented with fructooligosaccharide. Int J Probiot Prebiot 1:19-26.

Bjorck, I., Nilsson, U. 1991. On the possibility of acid hydrolysis of inulin in the rat stomach. Food Chem 41:243-250.

Bohacenko, I., Pinkrova, J., Kopicova, Z., Kunova, G., Peroutkova, J., Pechacova, M. 2013. Fermentability of commercial inulin-type fructans by lactobacilli and enterococci. Poster presented at VUPP.

Bornet, F.R., Brouns, F., Tashiro, Y., Duvillier, V. 2002. Nutritional aspects of short-chain fructooligosaccharides: natural occurrence, chemistry, physiology and health implications. Dig. Liver Dis. 34(Suppl 2):S111-120.

Boyle, F.G., Wrenn, J.M., Marsh, B.B., Anderson, W.I., Angelosanto, F.A., McCartney, A.L., Lien, E.L. 2008. Safety evaluation of oligofructose: 13-week rat study and *in vitro* mutagenicity. Food Chem Toxicol 46:3132-3139.

Brunser, O., Figueroa, G., Gotteland, M., Haschke-Becher, E., Magliola, C., Rochat, F., Cruchet, S., Palframan, R., Gibson, G., Chauffard, F., Haschke, F. 2006. Effects of probiotic or prebiotic supplemented milk formulas on fecal microbiota composition of infants. Asia Pac J Clin Nutr 15:368-376.

Butte, N.F., Fox, M.K., Briefel, R.R., Siega-Riz, A.M., Dwyer, J.T., Deming, D.M., Reidy, K.C. 2010. Nutrient intakes of US infants, toddlers, and preschoolers meet or exceed dietary reference intakes. J Am Diet Assoc 110(Suppl):S27-37.

Campbell, J.M., Bauer, L.L., Fahey, G.C., Hogarth, A.J.C., Wolf, B.W., Hunter, D.E. 1997a. Selected fructooligosaccharide (1-kestose, nystose, and 1F-ß-fructofuranosylnystose) composition of foods and feeds. J. Agric. Food Sci. 45(8):3076-3082.

Campbell, J.M., Fahey, G.C. Jr., Wolf, B.W. 1997. Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. *J. Nutr.* 127(1):130-136.

Carabin, I.G., Flamm, W.G. 1999. Evaluation of safety of inulin and oligofructose as dietary fiber. Reg Toxicol Pharmacol 30:268-282.

Castiglia-Delavaud, C., Verdier, E., Besle, J.M., Vernet, J., Boirie, Y., Beaufrere, B., Baynast, R.D., Vermorel, M. 1998. Net energy value of non-starch polysaccharide isolates (sugarbeet fibre and commercial inulin) and their impact on nutrient digestive utilization in healthy human subjects. Br J Nutr 80:343-352.

Chaturvedi, P., Warren, C.D., Altaye, M., Morrow, A.L., Ruiz-Palacios, G., Pickering, L.K., Newburg, D.S. 2001. Fucosylated human milk oligosaccharides vary between individuals and over the course of lactation. Glycobiology 11:365-372.

Clevenger, M.A., Turnbull, D., Inoue, H., Enomoto, M., Allen, J.A., Henderson, L.M., Jones, E. 1988. Toxicological evaluation of neosugar: Genotoxicity, carcinogenicity, and chronic toxicity. J Am Coll Toxicol 7:643-662.

Correa-Matos, N.J., Donovan, S.M., Isaacson, R.E., Gaskins, H.R., White, B.A., Tappenden, K.A. 2003. Fermentable fiber reduces recovery time and improves intestinal function in piglets following *Salmonella typhimurium* infection. J. Nutr. 133:1845-1852.

Ellegard, L., Andersson, H., Bosaeus, I. 1997. Inulin and oligofructose do not influence the absorption of cholesterol or the excretion of cholesterol, Ca, Mg, Zn, Fe, or bile acids but increases energy excretion in ileostomy subjects. Eur J Clin Nutr 51:1-5.

Enzyme Applications. 1994. In Encyclopedia of Chemical Technology, 4th ed., edt. J. I. Kroschwitz, Vol. 9, pp. 597-620.

Euler, A.R., Mitchell, D.K., Kline, R., Pickering, L.K. 2005. Prebiotic effect of fructo-oligosaccharide supplemented term infant formula at two concentrations compared with unsupplemented formula and human milk. J Pediatr Gastroenterol Nutr 40:157-164.

FDA. 2018. Agency Response Letter GRAS Notice No. GRN 000797 (Fructooligosaccharide). November 15, 2018. Available at: https://www.fda.gov/media/119454/download

FDA. 2017. Agency Response Letter GRAS Notice No. GRN 000717 (Fructooligosaccharide). February 13, 2018. Available at: https://www.fda.gov/media/111649/download

FDA. 2016a. Agency Response Letter GRAS Notice No. GRN 000605 [Fructo-oligosaccharides, Pune, India: Tata Chemicals Limited]. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=605.

FDA. 2016b. Agency Response Letter GRAS Notice No. GRN 000623 [Fructooligosaccharides, Guangdong Province, China: New Francisco Biotechnology Corporation]. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=623.

FDA. 2015. Agency Response Letter GRAS Notice No. GRN 000537 (Fructooligosaccharide). February 5, 2015. Available at: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm440653.

FDA. 2012. Agency Response Letter GRAS Notice No. GRN 000392 (Oligofructose). May 7, 2012. Available at: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm307720.htm.

FDA. 2003. Agency response letter: GRAS notice no. GRN 000118. May 5. Available at http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true

FDA. 2000. Agency Response Letter GRAS Notice No. GRN 000044 (Fructooligosaccharide). November 22, 2000. Available at: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm154122.htm.

FDA. 1993. Appendix I. Table 14. Conversion table for test chemical treatment doses used in PAFA. In: Priority Based Assessment of Food Additives (PAFA) Database. Washington (DC): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), p. 58. Available at: http://www.fda.gov/ohrms/DOCKETS/DOCKETS/95s0316/95s-0316-Rpt0272-36-Appendix-DReference-F-FDA-vol205.pdf.

Fomon, S.J. 1993. Energy intake by normal infants. In Nutrition of Normal Infants, p. 104–111. Baltimore, MD: Mosby.

FOIA. 2016. Freedom of Information Act. GRN 537- FOIA-Responsive Documents for FOIA 2016-3635 obtained by Soni & Associates Inc.

Food Standards Australia New Zealand (FSANZ). 2013. Approval report- application A1055, short chain fructo-oligosaccharides. May 23, 2013.

Fukata, T., Sasai, K., Miyamoto, T., Baba, E. 1999. Inhibitory effects of competitive exclusion and fructooligosaccharide, singly and in combination, on Salmonella colonization of chicks. J Food Protect 62:229-233.

Galam Ltd., 2017. Short-chain fructo-oligosaccharides. GRN 717. Available at: https://www.fda.gov/media/110038/download

German, J.B., Freeman, S.L., Lebrilla, C.B., Mills, D.A., 2008. Human milk oligosaccharides: evolution, structures and bioselectivity as substrates for intestinal bacteria. Nestle Nutr. Workshop Ser. Pediatr. Program 62:205-218; discussion 218-222.

Gibson, G. R., Roberfroid, M. B. 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J. Nutr 125:1401-1412.

GTC Nutrition. 2000. Fructo-oligosaccharides. GRN No. 44. Available online: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm261587

Guesry, P.R., Bodanski, H., Tomsit, E., Aeschimann, J.M. 2000. Effect of 3 doses of fructooligosaccharides in infants. J Pediatr Gastroenterol Nutr 31(Suppl 2):S252. [Abstract]

Henquin, J.C. 1988. Reproduction toxicity: study on the influence of fructooligosaccharides on the development of foetal and postnatal rat. Raffinerie Tirlemontoise internal report. [Unpublished report presented in Carabin and Flamm 1999]

Hess, J.R., Birkett, A.M., Thomas, W., Slavin, J.L. 2011. Effects of short-chain fructooligosaccharides on satiety responses in healthy men and women. Appetite 56:128-134.

Hidaka, H., Eida, T., Takizawa, T., Tokunaga, T., Tashiro, Y. 1986. Effects of fructooligosaccharides on intestinal flora and human health. Bifidobacteria Microflora 5:37-50.

Howard, M.D., Gordon, D.T., Garleb, K.A., Kerley, M.S. 1995a. Dietary fructooligosaccharide, xylooligosaccharide and gum arabic have variable effects on cecal and colonic microbiota and epithelial cell proliferation in mice and rats. J Nutr 125:2604-2609.

Howard, M.D., Gordon, D.T., Pace, L.W., Garleb, K.A., Kerley, M.S. 1995b. Effects of dietary supplementation with fructooligosaccharides on colonic microbiota populations and epithelial cell proliferation in neonatal pigs. J Pediatr Gastroenterol Nutr 21:297-303.

Ingredion Corporation. 2014. Fructo-oligosaccharides. GRN No. 537. Available online: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM422895.

Jain, M., Gote, M., Dubay, A.K., Narayanan, S., Krishnappa, H., Kumar, DP. S., Ravi, G.S., Vijaysarthi, S.K., Shankar, S., 2019. Safety evaluation of fructooligosaccharide (FOSSENCE™): Acute, 14-day, and subchronic oral toxicity study in Wistar rats. Toxioclogy Research Application 2:1-20.

Kapiki, A., Costalos, C., Oikonomidou, C., Triantafyllidou, A., Loukatou, E., Pertrohilou, V. 2007. The effect of a fructo-oligosaccharide supplemented formula on gut flora of preterm infants. Early Hum Devel 83:335-339.

Kunz, C., Rudloff, S., Baier, W., Klein, N., Strobel, S. 2000. Oligosaccharides in human milk: Structure, functional, and metabolic aspects. Ann Rev Nutr 20:699–722.

Lasekan, J., Baggs, G., Acosta, S., Mackey, A. 2015. Soy protein-based infant formulas with supplemental fructooligosaccharides: Gastrointestinal tolerance and hydration status in newborn infants. Nutrients 7(4):3022-3037.

Lugonja, N.M., Martinov, O.B., Rasovic, M.R., Spasic, S.D., Gojgic, G.D., Vrvic, M.M. 2010. A comparative investigation of an *in vitro* and clinical test of the bifidogenic effect of an infant formula. J Clin Biochem Nutr 47:208-216.

Meiji Seika Kaisha (1982) [unpublished]. Subacute toxicity of Neosugar G. Meiji Seika Kaisha. Cited In: GTC Nutrition, 2000 [As: Meiji Seika Kaisha, 1982a].

Mitsuoka, T., Hidaka, H., Eida T. 1987. Effects of fructo-oligosaccharides on intestinal microflora. Die Nahrung 31:427-436.

Molis, C., Flourie, B., Ouarne, F., Gailing, M-F., Lartigue, S., Guibert, A., Bornet, F., Galmiche, J-P. 1996. Digestion, excretion, and energy value of fructooligosaccharides in healthy humans. Am J Clin Nutr 64:324-328.

Nakamura, Y., Nosaka, S., Suzuki, M., Nagafuchi, S., Takahashi, T., Yajima, T., Takenouchi-ohkubo, N., Iwase, T., Moro, I. 2004. Dietary fructooligosaccharides up-regulate immunoglobulin A response and polymeric immunoglobulin receptor expression in intestines of infant mice. Clin Exp Immunol 137:52-58.

NFBC. 2015. New Francesco Biotechnology Corporation. Fructo-oligosacchardie GRAS notice. Complete GRAS notice submitted to FDA is available at: https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm504609.pdf

NFBC. 2018. New Francesco Biotechnology Corporation. Fructo-oligosacchardie GRAS notice. Complete GRAS notice submitted to FDA is available at: http://www.fda.gov/media/132054/download

Nilsson, U., Bjorck, I. 1988. Availability of cereal fructans and inulin in the rat intestinal tract. J Nutr 118:1482-1486.

Oku, T., Tokunaga, T., Hosoya, N. 1984. Nondigestibility of a new sweetener, "Neosugar," in the rat. J Nutr 114:1574-1581.

Paineau, D., Respondek, F., Menet, V., Sauvage, R., Bornet, F., Wagner, A. 2014. Effects of short- chain fructooligosaccharides on faecal bifidobacteria and specific immune response in formula-fed infants: a randomized, double-blind, placebo-controlled trial. J Nutr Sci Vitaminol 60(3):167-75.

Pfizer Nutrition. 2011. Oligofructise. GRN No. 392. Available online: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm277112.

Ripoll, C., Chappuis, E., Respondek, F., Wanger, A., Gottarand, F. 2015. scFOS supplemented follow-on formula in healthy infants: Impact on vaccine specific faecal secretory IGA response, faecal bifidobacteria, growth and digestive tolerance. Bioactive Carb Dietary Fiber 5(2):169-178.

Rumessen, J.J., Gudmand-Hoyer, E. 1998. Fructans of chicory: Intestinal transport and fermentation of different chain lengths and relation to fructose and sorbitol malabsorption. Am J Clin Nutr 68:357-364.

Rumessen, J.J., Bode, S., Hamberg, O., Gudmand-Hoyer, E. 1990. Fructans of Jerusalem artichokes: Intestinal transport, absorption, fermentation, and influence on blood-glucose, insulin, and C-peptide responses in healthy-subjects. Am J Clin Nutr 52:675-681.

Sabater-Molina, M., Larqué, E., Torrella, F., Zamora, S. 2009. Dietary fructooligosaccharides and potential benefits on health. J. Physiol. Biochem. 65(3):315-28.3

Sivieri, K., Morales, M.L., Saad, S.M., Adorno, M.A., Sakamoto, I.K., Rossi, E.A. 2014. Prebiotic effect of fructooligosaccharide in the simulator of the human intestinal microbial ecosystem (SHIME® model). J. Med. Food 17(8):894-901.

Sleet, R., Brightwell, J. 1990. FS-teratology study in rats. Raffinerie Tirlemontoise Internal Report. [Unpublished report presented in Carabin and Flamm 1999]

Spiegel, J. E., Rose, R., Karabell, P., Frankos, V. H., Schmitt D. F. 1994. Safety and benefits of fructooligosaccharides as food ingredients. Food Technol. 48:85-89.

Stewart, M.L., Slavin, J.L. 2006. *In vitro* fermentability of inulin and fructooligosaccharides (FOS) is dependent on chain length. FASEB J 20:A600 (Abstract

Stone-Dorshow, T., Levitt, M.D. 1987. Gaseous response to ingestion of a poorly absorbed fructooligosaccharide sweetener. Am J Clin Nutr 46:61-65.

Takeda, U., Niizato, T. 1982. Acute and subacute safety tests. Proceedings of the 1st Neosugar Research Conference. Tokyo: Meija-Seika Publications. [Presented in Carabin and Flamm 1999]

Tashiro, Y., Eida, T., Hidaka, H. 1992. Distribution and quantification of fructooligosaccharides in food materials. Sci. Rep. Meiji Seika Kaisha 31:35-40.

Tata. 2015. Tata Chemicals Ltd. Fruct-oligosacchardie GRAS notice. Complete GRAS notice submitted to FDA is available at: https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm495918.pdf

Tokunaga, T., Oku, T., Hosoya, N. 1989. Utilization and excretion of a new sweetener, fructooligosaccharide (Neosugar), in rats. J Nutr 119:553-559.

Tokunaga, T., Oku, T., Hosoya, N. 1986. Influence of chronic intake of new sweetener fructooligosaccharide (Neosugar) on growth and gastrointestinal function of the rat. J Nutr Sci Vitaminol 32:111-121.

Tomomatsu, H. 1994. Health effects of oligosaccharides. Food Technol 48:61-65.

Tsuji, Y., Yamada, K., Hosoya, N., Moriuchi, S. 1986. Digestion and absorption of sugars and sugar substitutes in rat small intestine. J Nutr Sci Vitaminol 32:93-100.

Tsukahara, T., Iwasaki, Y., Nakayama, K., Ushida, K. 2003. Stimulation of butyrate production in the large intestine of weaning piglets by dietary fructooligosaccharides and its influence GRAS Monograph for Short-Chain 232 JHEIMBACH LLC Fructooligosaccharides in Infant Formula on the histological variables of the large intestinal mucosa. J Nutr Sci Vitaminol 49:414- 421.

Vandenplas, Y. 2002. Oligosaccharides in infant formula. Br J Nutr 87:Suppl. 2:S293–S296.

Vandenplas, Y., Analitis, A., Tziouvara, C., Kountzoglou, A., Drakou, A., Tsouvalas, M., Mavroudi, A., Xinias, I. 2017. Safety of a new synbiotic starter formula. Pediatr. Gastroenterol. Hepatol. Nutr 20(3):167-177.

van Dokkum, W., Wezendonk, B., Srikumar, T.S., van den Heuvel, E.G.H.M. 1999. Effect of nondigestible oligosaccharides on large-bowel functions, blood lipid concentrations and glucose absorption in young healthy male subjects. Eur J Clin Nutr 53:1-7.

Veereman-Wauters, G., Staelens, S., Van de Broek, H., Plaskie, K., Wesling, F., Roger, L.C., McCartney, A.L., Assam, P. 2011. Physiological and bifidogenic effects of prebiotic supplements in infant formulae. J Pediatr Gastroenterol Nutr 52:763-771.

Xia, Q., Williams, T., Hustead, D., Price, P., Morrison, M., Yu, Z. 2012. Quantitative analysis of intestinal bacterial populations from term infants fed formula supplemented with fructo-oligosaccharides. J Pediatr Gastroenterol Nutr 55:314-320.

Yao, M., Capeding, R., Fitzgerald, M., Ramanujam, K., Yachetti, S., DeRusso, P. 2010. High 2-palmitate and oligofructose in lower protein *alpha*-lactalbumin-enriched term infant formula: Effects on stool characteristics and stool composition. J Pediatr Gastroenterol Nutr 50 (Suppl 2):E207-208.

Zivkovic, A.M., German, J.B., Lebrilla, C.B., Mills, D.A. 2011. Human milk glycobiome and its impact on the infant gastrointestinal microbiota. Proc Natl Acad Sci 108 Suppl 1:4653-4658.

## APPENDIX I

Analytical results from three lots each for Powder and Liquid batches

**Appendix I A: Food Grade Specifications of scFOS Powder (FOS-P95)**

| Parameters | Specification | Batch numbers | | |
|---|---|---|---|---|
| | FOS-P95 | FP9D120T04 | FP9D120T06 | FP9D120T08 |
| Description | Fine white free flowing hygroscopic powder (clear in solution) | Complies | Complies | Complies |
| Taste and Aroma | Sweet, without foreign tastes / odors | Complies | Complies | Complies |
| Total solids (%) | NLT 97.0 | 98.69 | 98.0 | 97.90 |
| Moisture (Karl Fisher) (%) | NMT 5.0 (w/w) | 1.23 | 2.0 | 2.10 |
| Residue on ignition (sulphated ash) (%) | NMT 0.1 | 0.02 | 0.06 | 0.02 |
| pH (pH meter with 10% solution @ 25°C) | 5.0 - 7.5 | 7.06 | 5.02 | 6.33 |
| **Carbohydrate composition** (a) Identification | | | | |
| Fructose (% dry basis) | NLT 67.0 | 69.57 | 69.41 | 71.27 |
| Glucose (% dry basis) | NMT 33.0 | 23.49 | 22.46 | 22.73 |
| (b) Assay | | | | |
| Total Fructooligosaccharides (%) | NLT 95.0 | 95.25 | 95.17 | 95.26 |
| -- Trimer (GF2) | Informative | 35.47 | 31.05 | 28.97 |
| -- Tetramer (GF3) | Informative | 50.66 | 52.21 | 49.51 |
| -- Pentamer and larger (GF4 and higher) | Informative | 9.14 | 11.96 | 16.99 |
| (c) Sucrose + Glucose + Fructose | NMT 5.0 | 4.75 | 4.83 | 4.74 |
| **Heavy metals** | | | | |
| Lead (as Pb) (ppm) | NMT 0.02 | < 0.02 | < 0.02 | < 0.02 |
| Arsenic (as $As_2O_3$) (ppm) | NMT 0.1 | < 0.05 | < 0.05 | < 0.05 |
| Cadmium (Cd) (ppm) | NMT 0.01 | < 0.01 | < 0.01 | < 0.01 |
| Mercury (as Hg) (ppm) | NMT 0.01 | < 0.01 | < 0.01 | < 0.01 |
| Chromium (as Cr) (ppm) | NMT 0.05 | < 0.05 | < 0.05 | < 0.05 |
| Tin (as Sn) (ppm) | NMT 50 | < 0.50 | < 0.50 | < 0.50 |
| Copper (as Cu) (ppm) | NMT 30 | < 0.50 | < 0.50 | < 0.50 |
| Methyl Mercury (Calculated as the element) (ppm) | NMT 0.25 | < 0.02 | < 0.02 | < 0.02 |
| **Microbiological limits** | | | | |
| Total Plate Count (cfu/g) | NMT 300 | < 10 | < 10 | < 10 |
| Enterobacteriacea (MPN/g) | NMT 3.0 | < 3 | < 3 | < 3 |
| Yeasts & Mould (cfu/g) | NMT 20 | < 10 | < 10 | < 10 |
| *Escherichia coli* (MPN/g) | Absent 10 g | Absent | Absent | Absent |

| Parameters | Specification | Batch numbers | | |
|---|---|---|---|---|
| | **FOS-P95** | **FP9D120T04** | **FP9D120T06** | **FP9D120T08** |
| *Staphylococcus aureus* | Absent 10 g | Absent | Absent | Absent |
| Salmonella spp | Absent 100 g | Absent | Absent | Absent |
| Shigella spp | Absent 25 g | Absent | Absent | Absent |
| Listeria monocytogenes | Absent 25 g | Absent | Absent | Absent |
| Sulphite reducing Clostridia (cfu/g) | NMT 10 | < 10 | < 10 | < 10 |
| *Cronobacter sakazakii* | Absent 300 g | Absent | Absent | Absent |
| *Bacillus cereus* (cfu/g) | NMT 100 | < 10 | < 10 | < 10 |
| **Mycotoxins** | | | | |
| *Aflatoxin B1 (ppb)* | NMT 0.5 | < 0.5 | < 0.5 | < 0.5 |
| *Aflatoxin B2 (ppb)* | NMT 0.5 | < 0.5 | < 0.5 | < 0.5 |
| *Aflatoxin G1 (ppb)* | NMT 0.5 | < 0.5 | < 0.5 | < 0.5 |
| *Aflatoxin G2 (ppb)* | NMT 0.5 | < 0.5 | < 0.5 | < 0.5 |
| *Aflatoxin M1 (ppb)* | NMT 0.025 | < 0.022 | < 0.024 | < 0.023 |
| *Melamine (ppm)* | NMT 0.5 | < 0.5 | < 0.5 | < 0.5 |

**Appendix I B: Food Grade Specifications of scFOS Liquid (FOS-L95)**

| Parameters | Specification | Batch numbers | | |
|---|---|---|---|---|
| | **FOS-L95** | **FL9T220003** | **FL9T220005** | **FL9T220007** |
| Description | Colorless to sunshine yellow color syrupy liquid | Colourless syrupy liquid | Light Yellow color syrupy liquid | Colourless syrupy liquid |
| Taste and Aroma | Sweet, without foreign tastes / odors | Complies | Complies | Complies |
| Moisture (Karl Fisher) (%) | NMT 25 | 22.90 | 24.22 | 22.38 |
| Brix (Refractometer) °Bx | NLT 75 | 75.85 | 76.91 | 75.67 |
| Residue on ignition (sulphated ash) (%) | NMT 0.1 | 0.05 | 0.06 | 0.04 |
| pH (pH meter with 10% solution @ 25°C) | 5.0 - 7.5 | 6.26 | 5.93 | 5.88 |
| **Carbohydrate composition** (a) Identification | | | | |
| Fructose (% dry basis) | NLT 67.0 | 68.30 | 68.67 | 67.38 |
| Glucose (% dry basis) | NMT 33.0 | 26.73 | 26.42 | 26.91 |
| (b) Assay | | | | |
| Total Fructooligosaccharides (%) | NLT 95.0 | 95.30 | 95.47 | 95.11 |
| -- Trimer (GF2) | Informative | 50.34 | 46.73 | 51.3 |
| -- Tetramer (GF3) | Informative | 40.60 | 43.38 | 39.87 |
| -- Pentamer and larger (GF4 and higher) | Informative | 4.36 | 5.37 | 3.94 |
| (c) Sucrose + Glucose + Fructose | NMT 5.0 | 4.70 | 4.53 | 4.89 |
| **Heavy metals** | | | | |
| Lead (as Pb) (ppm) | NMT 0.02 | < 0.02 | < 0.02 | < 0.02 |
| Arsenic (as As) (ppm) | NMT 0.1 | < 0.03 | < 0.03 | < 0.03 |
| Cadmium (Cd) (ppm) | NMT 0.01 | < 0.01 | < 0.01 | < 0.01 |
| Mercury (as Hg) (ppm) | NMT 0.01 | < 0.01 | < 0.01 | < 0.01 |
| Chromium (as Cr) (ppm) | NMT 0.05 | < 0.05 | < 0.05 | < 0.05 |
| Tin (as Sn) (ppm) | NMT 50.0 | < 0.05 | < 0.05 | < 0.05 |
| Copper (as Cu) (ppm) | NMT 30.0 | < 0.13 | < 0.09 | < 0.05 |
| Methyl Mercury (Calculated as the element) (ppm) | NMT 0.25 | < 0.01 | < 0.01 | < 0.01 |
| **Microbiological limits** | | | | |
| Total Plate Count (cfu/g) | NMT 300 | < 10 | < 10 | < 10 |
| Enterobacteriacea (MPN/g) | NMT 3 | < 3 | < 3 | < 3 |
| Yeasts & Mould (cfu/g) | NMT 20 | < 10 | < 10 | < 10 |
| *Escherichia coli* (MPN/g) | Absent 10 g | Absent | Absent | Absent |
| *Staphylococcus aureus* | Absent 10 g | Absent | Absent | Absent |
| Salmonella spp | Absent 100 g | Absent | Absent | Absent |
| Shigella spp | Absent 25 g | Absent | Absent | Absent |

| Parameters | Specification | Batch numbers | | |
|---|---|---|---|---|
| | **FOS-L95** | **FL9T220003** | **FL9T220005** | **FL9T220007** |
| Listeria monocytogenes | Absent 25 g | Absent | Absent | Absent |
| Sulphite reducing Clostridia (cfu/g) | NMT 10 | < 10 | < 10 | < 10 |
| *Cronobacter sakazakii* | Absent 300 g | Absent | Absent | Absent |
| *Bacillus cereus* (cfu/g) | NMT 100 | < 10 | < 10 | < 10 |
| **Mycotoxins** | | | | |
| *Aflatoxin B1 (ppb)* | NMT 0.5 | < 0.5 | < 0.5 | < 0.5 |
| *Aflatoxin B2 (ppb)* | NMT 0.5 | < 0.5 | < 0.5 | < 0.5 |
| *Aflatoxin G1(ppb)* | NMT 0.5 | < 0.5 | < 0.5 | < 0.5 |
| *Aflatoxin G2 (ppb)* | NMT 0.5 | < 0.5 | < 0.5 | < 0.5 |
| *Aflatoxin M1(ppb)* | NMT 0.025 | < 0.023 | < 0.021 | < 0.023 |
| *Melamine (ppm)* | NMT 0.5 | < 0.5 | < 0.5 | < 0.5 |

| | |
|---|---|
| **From:** | Madhu Soni |
| **To:** | Morissette, Rachel |
| **Cc:** | "Dipak Bagad" |
| **Subject:** | RE: [EXTERNAL] RE: questions for GRN 990 |
| **Date:** | Monday, July 19, 2021 10:52:51 AM |
| **Attachments:** | image001.png<br>scFOS GRAS infant formula-GRN 990-FDA Query responses final.pdf |



Dear Dr. Morissette,

As mentioned, please find attached a pdf copy of the responses to FDA questions raised by your team for GRN 990. We apologize for the slight delay. If you need any further clarification please let me know.

Thank you for this opportunity.

Best regards,

Madhu

---
Madhu Soni, *PhD, FACN, FATS*
Soni & Associates Inc
749 46th Square
Vero Beach, FL 32968, USA
Phone: +1-772-299-0746
Cell: +1-772-538-0104
www.soniassociatesnet

**From:** Morissette, Rachel [mailto:Rachel.Morissette@fda.hhs.gov]
**Sent:** Monday, July 19, 2021 8:23 AM
**To:** Madhu Soni <sonim@bellsouth.net>
**Subject:** RE: [EXTERNAL] RE: questions for GRN 990

Dear Dr. Soni,

We have not received responses to our questions for GRN 000990 yet. Can you please let me know when you except to send those?

Thank you for your consideration.

*Rachel*

---
**Rachel Morissette, Ph.D.**
*Regulatory Review Scientist*

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

**From:** Madhu Soni <sonim@bellsouth.net>
**Sent:** Saturday, July 3, 2021 8:06 AM
**To:** Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
**Subject:** [EXTERNAL] RE: questions for GRN 990



Thank you Dr. Morissette for the email and the FDA queries

I will try my best to get you the responses in time.

Best regards. Have a great 4th of July weekend.

Madhu

**From:** Morissette, Rachel [mailto:Rachel.Morissette@fda.hhs.gov]
**Sent:** Friday, July 2, 2021 12:38 PM
**To:** Madhu Soni <sonim@bellsouth.net>
**Subject:** questions for GRN 990

Dear Dr. Soni,

Please see attached our questions for GRN 990. Have a good weekend.

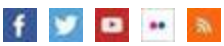
Best regards,

*Rachel*

---

**Rachel Morissette, Ph.D.**
*Regulatory Review Scientist*

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



Dear Dr. Morissette,

**RE: GRN 990 (short-chain Fructo-oligosaccharide GRAS notice)**

This responds to your email of July 2, 2021 regarding clarifications required for our short-chain fructooligosaccharides (scFOS) GRAS notice (GRN 000990) for use as an ingredient in cow milk-based, non-exempt infant formulas for term infants, as consumed, at a level up to 400 mg scFOS/100 mL starter formula and up to 500 mg scFOS/100 mL follow-on formula. We are providing a point-by-point response to your queries along with some relevant clarifications/discussion.

**Regulatory:**

FDA Query 1. The notice refers to many prior GRNs, along with the dates and notifiers for those GRNs. There are a number of instances throughout the notice where the GRNs do not line up with the date of the response letter or notifier cited for that notice in the GRAS notice inventory, which makes it difficult to determine which notice is actually being referenced. Additionally, in at least one example, a notice is cited for a completely unrelated ingredient (i.e., p. 39 cites GRN 979). Please go through the notice and correct any errors when citing prior GRNs.

**Response:** We are sorry for the oversight. The GRN 979 should have been GRN 797. We have checked all the GRN citations and a list with correct links is given in the attached Table 1 (please see Appendix I). The confusion appears to be due to changes to the FDA GRAS Notice inventory website and we did not check the links that were used earlier in our previous Tata Chemicals GRAS notification on FOS (GRN 605) for uses in conventional foods. Again please accept our apology for the oversight.

FDA Query 2. We note that the notice discusses health benefits related to the use of Tata Chemicals' scFOS. In those discussions, the terms "prebiotic" and "probiotic" are used several times throughout the notice. As you are aware, we do not evaluate any purported health benefits in a GRAS notice, and FDA does not have regulatory definitions for "prebiotic" and "probiotic". Please remove any references to those terms in the notice.

**Response:** We agree with FDA suggestion that in the absence of a regulatory definition and given the purported health benefits, we request to remove the terms "prebiotic" and

"probiotic" from the GRAS notification. We note that the prebiotic term appeared 20 times (including 6 times in references) in the GRAS notice and we agree to remove the term "prebiotic" from the description except those from the reference list. The "probiotic" term appeared three times in the GRAS notice, including once in the reference list. We agree to remove the term "probiotic" except the one mentioned in reference list.

**FDA Query 3.** On p. 13 of the notice, the citation for sodium hydroxide is listed as 21 CFR 184.1631; however, this regulation is for the use of potassium hydroxide. Please provide the correct citation for sodium hydroxide.

**Response:** Sorry for the oversight, the correct citation for sodium hydroxide is as follows: 21 CFR 184.1763

**FDA Query 4.** The terms "starter formula" and "follow-on formula" are used in the notice. FDA does not have a definition for these terms. Please specify the intended age ranges in months for these intended uses.

**Response:** The intended age ranges for the proposed use of scFOS are as follows: at a level up to 400 mg scFOS/100 mL formula for infants 0-6 months and up to 500 mg FOS/100 mL formula for infants >6 months of age.

**FDA Query 5.** On p. 14 of the notice, the citation "Femon (1993)" is used. However, there is no author with this name in the reference section. Please clarify if "Fomon (1993)" is intended instead.

**Response:** Thank you for bringing this to our attention and we are sorry for the oversight. We intended to use Fomon (1993).

**FDA Query 6.** On p. 10 of the notice, Tata Chemicals states that the β-fructofuranosidase enzyme used in the manufacture of scFOS is membrane bound. However, this aspect of the process is not elaborated in the manufacturing section of the notice. Instead, Tata Chemicals states that the process involves the use of the culture biomass that contains the β-fructofuranosidase enzyme. Please clarify the use of the enzyme and

whether it is membrane bound and if it is expected to be in the final product.

**Response:** Please note that the enzyme that catalyzes the reaction in the production process of scFOS manufacturing is an intracellular enzyme, which is present in the cell membrane and doesn't get released in the reaction mixture. Hence, Tata Chemicals used culture biomass instead of the free enzyme for the conversion of cane sugar to scFOS. Once the reaction is completed the culture biomass is removed by fine filtration. In this process, the enzymes are not present in the final product.

Fine Filtration: A four stage filtration system is used for the separation of the biomass. In the first stage, culture biomass is filtered and separated by a plate filtration system with a 20 micron pore size cloth as the filtering membrane. In the second, third and fourth phases, respectively, 15 micron, 5 micron and 3 micron filter membranes are used to remove any residual fine parts of the biomass or any other particles, precipitate, etc.

**FDA Query 7.** Please confirm that the enzyme is GRAS for its intended use and meets the Joint FAO/WHO Expert Committee on Food Additives and Food Chemicals Codex (FCC) specifications for enzymes used in food.

**Response:** As mentioned above, please note it is not the enzyme but the biomass is used. Tata chemicals consider that the biomass containing the enzyme, used in the production of scFOS is GRAS. Please note that this is the same biomass containing the enzyme that was used in Tata Chemicals GRAS notification on FOS (GRN 605) for its use in conventional foods in which FDA did not object to the use of the biomass.

The biomass used in the scFOS production as a source of enzyme is from the microorganism *Aureobasidium pullulans*, the same microorganism used in the earlier GRAS notification GRN605. This microorganism is well known for its application in biotechnology, such as production of *Pullulan* and beta-glucan (GRN 99, 605 and 309). It is yeast like fungi and generally recognized as safe. Our toxicology study of the scFOS further conferred its safety in production of food ingredients (Jain et al., 2019).

**FDA Query 8.** In describing the methodologies used for specifications, please provide complete citations for referenced methods and provide confirmation that all methods, including "in house" methods, are validated and appropriate for the respective analytes.

**Response:** We confirm that all methods, including "in house" methods, are validated and appropriate for the respective analytes. FOS analysis is done as per FCC 11th edition (year 2018) page no. 499 to 500.

Please note that, we have got the testing's done through accredited laboratory (SGS India Private Limited) and below are the details of the test parameters and respective validation status.

| Test Parameter | SGS Internal SOP | Base Reference method | Validation status |
|---|---|---|---|
| Lead | SO-IN-MUL-TE-063A By ICPMS | AOAC 2015.01 and AOAC 2015.06 | Yes |
| Arsenic | | | |
| Cadmium | | | |
| Mercury | | | |
| Chromium | SO-IN-AFL-MNR-C-TE-006 | 1. Metals and other elements in plants and pet foods – AOAC 985.01 | Yes |
| | | 2. Metals in plants and pet foods – AOAC 975.03 | |
| | | 3. Metals in solid waste – AOAC 990.08 | |
| | | 4. Method for fortified foods-AOAC 2011.14 | |
| Tin | | 5. Method for heavy metals in food-AOAC 2013.06 | |
| Copper | | 6. Heavy Metals In food AOAC Official method 2015.01 | |
| Methyl Mercury | | 7. Method for Minerals & Trace Elements- AOAC 2015.06 | |
| Aflatoxin M1 | SO-IN-AFL-MNR-C-TE-065 | Instruction Manual of RIDASCREEN Aflatoxin M1 Art No.-R1121 | Yes |
| Melamine | SO-IN-AFL-MNR-C-TE-023 | Simultaneous determination and confirmation of melamine and cyanuric acid in animal feed by zwitterionic hydrophilic interaction chromatography and tandem mass spectrometry. David N. Heller and Cristina B. Nochetto, Rapid Commun. Mass Spectrom., Volume 22, Issue 22,30 November 2008 ,Pages 3624–3632 | Yes |
| | | LIB No. 4421,Volume 24, October 2008, Division of Field Science, Office of Regulatory Affairs, U.S. Food and Drug Administration Determination of Melamine and Cyanuric Acid Residues in Infant Formula using LC-MS/MS | |
| | | ISO/TS 15495:2010 (IDF RM 230:2010) Milk, Milk Products And Infant Formulae -- Guidelines For The Quantitative Determination Of Melamine And Cyanuric Acid By LC-MS/MS | |
| | | Laboratory Information Bulletin LIB No. 4422,October 2008, Division of Field Science, Office of Regulatory Affairs, U.S. Food and Drug Administration, Interim Method for Determination of Melamine and Cyanuric Acid Residues In Foods using LC-MS/MS | |

**FDA Query 9.** Please clarify when specifications are on a dry basis. For example, limits for fructose and glucose levels are listed on a dry basis; however, other specified limits, such as total fructooligosaccharides and other carbohydrates, do not include that designation.

**Response:** Please note that the Specifications provided in the Tata Chemicals notice are all on the dry basis.

**FDA Query 10.** We note that the specifications for 1-kestose, nystose, and fructofuranosylnystose are listed as "informative." Please elaborate on this parameter and provide quantitative limits if applicable. We note that FCC specifications for scFOS include limits for trimer (≥ 30.0%), tetramer (≥ 45.0%), and pentamer and larger (≥ 5.0%).

**Response:** All the batches follow the FCC 11th edition (year 2018) page no. 499 to 500 specifications. While in the shared batch no. FP9D120T08, the trimer (GF2) value is 28.97% (Below 30%), we will ensure that all future batches fall within the FCC specifications. Please find below the data of additional batches that meet the FCC Specifications.

| Purity (%) | Specification | FP9D121035 | FP9D121036 | FP9D121037 | FP9D121038 | FP9D121039 | FP9D121040 | FP9D121041 | FP9D121042 | FP9D121043 | FP9D121044 | FP9D121045 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **GF2** | **NLT 30** | 38.63 | 38.33 | 38.39 | 39.63 | 41.24 | 39.63 | 38.28 | 37.61 | 38.7 | 37.95 | 38.14 |
| **GF3** | **NLT 45** | 48.91 | 49.26 | 49.03 | 48.42 | 47.58 | 48.42 | 48.95 | 48.93 | 48.14 | 48.43 | 48.36 |
| **GF4** | **NLT 5** | 7.93 | 8.18 | 8.25 | 7.82 | 7.38 | 7.82 | 8.34 | 8.62 | 8.49 | 8.89 | 8.72 |
| **Purity** | **NLT 95** | 95.47 | 95.77 | 95.67 | 95.87 | 96.2 | 95.87 | 95.57 | 95.16 | 95.33 | 95.27 | 95.22 |

Please note that for the liquid samples, while maintaining GF2, GF3 and GF4 ratio, it was leading to crystallization; hence, these specifications have been referred as informative.

**FDA Query 11.** The notice includes specified limits for methyl mercury, melamine, and aflatoxins, as well as relatively high limits for tin and copper compared to the results of the batch analyses. Please discuss whether Tata Chemicals has reason to expect the presence of these substances in scFOS.

**Response:** Please note that in the Tata Chemical GRAS notice, specified limits for heavy metals and Aflatoxin are on the basis of Food Safety and Standard Authority of India (FSSAI, 2020) Food Safety Standards (Contaminants, Toxins and Residues) Regulations 2011, where limits for Fructo-oligosaccharides are not mentioned; hence, the limits for "Foods not specified" or "All foods" is taken into consideration for establishing the specification parameters. In addition to this, we have also considered other international markets for establishing these specifications. We do not have any particular reason to expect the presence of these substances in scFOS. Nevertheless, Tara Chemicals intends to test for these substances on a periodic basis to ensure their absence or very low levels in our products.

**FDA Query 12.** Please confirm that the batch analyses of powder and liquid scFOS that are reported in Appendix I of the notice are non-consecutive lots of scFOS.

**Response:** We confirm that the batch analysis of powder and liquid reported in Appendix I and II are from non-consecutive lots of scFOS.

**FDA Query 13.** On p. 13 of the notice, Tata Chemicals states that "The resins and microfiltration used are in compliance with FDA guidelines." Please provide a citation for these guidelines and confirm that all materials and processing aids meet applicable U.S. regulations for use in the production of food ingredients.

**Response:** The resin and microfiltration used as per the guidelines provided in the CFR-21 document -Ref is as below:

**Resins:**
Resin complying with FDA in Code of Federal Regulations 21 CFR 173.25.

**Microfiltration:**
The materials of construction meet the FDA requirements for food contact use as detailed in Code of Federal Regulations, 21 CFR paragraphs 170-199 in that:
- Polypropylene to 21 CFR section 177.1520 (Olefin polymers)
- Ethylene Propylene Rubber and Silicone Elastomeric seal materials to 21 CFR section 177.2600 (Rubber articles intended for repeated use, excluding milk and edible oils)

**FDA Query 14.** In the notice, Tata Chemicals discusses potential dietary exposure to scFOS from intended uses other than infant formula. The

notice states that as infants grow and begin to consume other foods, infant formula intake decreases, along with exposure to scFOS due to other foods being unlikely to contain scFOS at levels comparable to infant formula. However, this point regarding the use level in other foods is incomplete, since it does not address the amount of scFOS-containing food consumed in the infant background diet. Please address the cumulative dietary exposure to scFOS in infants resulting from both the intended use in infant formula and the background uses of scFOS in conventional foods that have been described in other GRAS notices. For example, GRN 000717 includes the use of scFOS in infant and toddler foods in addition to infant formula, as well as other foods that may be consumed.

**Response:** We note that to some extent this query related to intake of scFOS from background and proposed uses has been addressed in an earlier GRAS notice. Following completion of GRN 44 in 2000 and subsequently in 2007, GTC notified FDA (additional correspondence) that it had determined that the addition of scFOS to foods in general, including infant and toddler foods but excluding infant formula, at levels resulting in intakes up to 20 g/day in the general population and up to 4.2 g/day in infants less than one year of age, is also GRAS. Following its review, FDA (2007) had no questions regarding this conclusion. Also, please note that the proposed use levels of scFOS in infant formula (starter or follow-on) by Tata Chemicals is at the same levels as described by Ingredion (2014) in GRAS notice (GRN 537) and NFBC in GRAS notice (GRN 797). Also, the subjects of these three GRAS notices (including GRN 990) contains the same levels of scFOS, i.e., 95%. Given this, the intake of scFOS for formula (only) fed infants will be same and there will not be any increase in the overall consumption of scFOS resulting from this use. As regards infants who start consuming complimentary foods, the intake of scFOS from infant formula will be reduced. Thus there will be a decrease in the level of scFOS consumed. The amount of scFOS-containing food consumed in the infant background diet is likely to differ. However, it is unlikely to be of any safety concern. As indicated earlier scFOS has been determined to be safe at levels up to 4.2 g/day in infants less than one year of age.

As scFOS manufactured by Tata Chemicals will serve as an alternative source of scFOS to existing GRAS sources of scFOS described in GRN 44, GRN 537, GRN 797 and GRN 717 (including infants less than 1 year old), the introduction of scFOS by Tata Chemicals is unlikely to further increase dietary intake of scFOS in an additive manner. The proposed uses of scFOS by Tata Chemicals will serve as an alternative to existing GRAS sources and, therefore, will not change the current dietary exposure to scFOS among U.S. consumers of foods to which FOS may be added. Any additional intake is considered as safe.

## Toxicology

**FDA Query 15.** On p. 18 of the notice, Tata Chemicals states, "In this GRAS assessment, attempts have been made to summarize the available information, related to safety of FOS, **in the order of their importance**" (emphasis added). However, in Section 6.1.1 (Pivotal or Primary Published Clinical Studies of scFOS in Infant), the initial study described is Guesry et al., 2000, which the notice indicates has been published as an abstract. The second and third studies described, Lasekan et al., 2015 and Xia et al., 2012, with tested use levels for scFOS below the levels proposed by Tata Chemicals.

We note that data published as an abstract cannot be considered primary evidence for a GRAS conclusion. Similarly, it is unclear how Lasekan et al., 2015 and Xia et al., 2012 can be considered pivotal studies to support the intended use given the use levels are below Tata Chemicals' proposed use level.

Additionally, on p. 50 of the notice in Section 6.2 (Summary, Discussion and Conclusion), Tata Chemicals states, "Some of the relevant studies in infants are briefly described here." However, the initial studies discussed in this section are Paineau et al., 2104 and Ripoll et al., 2015. The studies by Guesry et al., 2000 and Lasekan et al., 2015 are not summarized in this section of the notice.

Therefore, it is unclear which infant studies Tata Chemicals considers to be pivotal to the GRAS conclusion. Please provide a discussion that addresses which infant studies are considered pivotal to the current GRAS conclusion and why.

**Response:** Sorry for our confusion regarding the use of the term "pivotal" in the GRAS dossier and describing the studies accordingly. Instead of pivotal we should have just described the available relevant studies and its support to the GRAS assessment based on all available evidence. As mentioned in the GRAS notice, the safety determination of scFOS for use in infant formula at the proposed use levels is based on the totality of the available evidence, including current approved uses, *in vitro* and *in vivo* metabolism studies, and a variety of animal studies and human and infant studies that supports the safety-in-use of scFOS.

We agree that Guesry et al. (2000) study is published as an abstract and can only be considered as supportive evidence. Similarly, the studies by Lasekan et al. (2015) and Xia et al. (2012) cannot be considered as pivotal as the levels of scFOS tested in these studies are below the levels proposed by Tata Chemicals. However, given this, these studies also provide the supportive evidence and are not pivotal.

In Section 6.2., we missed mentioning about two relevant studies by Guesry et al. (2000) and Lasekan et al. (2015). Although published as an abstract, the study by Guesry et al. (2000) supports the safety of the proposed uses of scFOS. The study by Lasekan et al. (2015) used lower doses as compared to the proposed doses; however, no adverse effects were noted. The

findings from this study indicate that scFOS is unlikely to cause adverse effects. We would like to incorporate both these studies described in Section 6.1.1. as supportive evidence in the Summary Discussion and Conclusion section 6.2.

We apologize for creating this confusion. We request that the agency consider the totality of the available evidence as the basis to support the present GRAS assessment as we believe that all of the cited studies contribute to the overall safety of scFOS at the intended use levels.

**FDA Query 16.** On p. 18 of the notice, Tata Chemicals indicates that non-digestible oligosaccharides, including FOS, may decrease serum lipids, including cholesterol. On pp. 32 and 36 of the notice, Tata Chemicals indicates that there were decreases in cholesterol reported in rats following consumption of scFOS (i.e., Jain et al., 2019 and Takeda and Niizato, 1982). Similarly, on p. 42 of the notice, when discussing corroborative studies in infants, Tata Chemicals states, "Levels of total cholesterol in blood were significantly higher in the human milk group than in either formula group…." Please provide a brief narrative that specifically discusses the impact, if any, of scFOS consumption on serum cholesterol levels in infants and why this is not expected to be a safety concern.

**Response:** The available evidence from rat studies indicate that exposure to scFOS results in decreased levels of cholesterol (Jain et al., 2019; Takeda and Niizato, 1982). However, the findings from unpublished study in infants fed human milk revealed increase in blood cholesterol levels, while in infants fed formula (with and without scFOS) no such increase was noted. The publicly available information shows that breast milk contains more cholesterol as compared to infant formula (Friedman and Goldberg, 1975) and breastfed infants have higher blood cholesterol (Owen et al., 2002; Wong et al., 1993). It has been also reported that higher neonatal dietary cholesterol is associated with different cholesterol metabolism and less endogenous cholesterol synthesis in infants who are breastfed (Wong et al., 1993; Demmers et al., 2005). There is lack of evidence as to whether a change in synthesis or metabolism of cholesterol in the neonatal period persists beyond weaning and into adulthood (Demmers et al., 2005). The available evidence indicates that scFOS is unlikely to be of safety concern. Thus, we would consider any impact to be negligible.

Please note that the study discussed on page 42 was an unpublished study conducted by Abbott (1993) and reported in the previous GRAS notice GRN 537 (Ingredion, 2014). Additional details of this study were not available for independent review.

**FDA Query 17.** (a) On p. 26 of the notice, Tata Chemicals states "The first GRAS notice on scFOS, GTC Nutrition (2000) established the ADI of 4.2 g/day scFOS for infant [sic] (<1 year old)." However, we note that the dietary exposure to scFOS from the intended use in infant formula at the 90th percentile may exceed the stated ADI depending on body weight (bw). Please provide a safety narrative that compares total dietary exposure to scFOS to Tata Chemicals' stated ADI and discuss why it does not pose a safety concern for infants. Please provide a safety narrative that discusses why consuming scFOS at levels above Tata Chemicals' stated ADI does not pose a safety concern for infants aged 0-6 months.

**Response:** It should be noted that the ADI of 4.2 g/day for scFOS in infant reported in GRN 44 was determined based on a 1987 survey of over 20,000 infants. In this survey, the safety and tolerance of FOS consumption in infants was examined (Yamamoto and Yonekubo, 1993; cited in GRN 44). Based on the FOS concentrations reported in Japanese infant formula and estimates of formula intake in the U.S., the mean and 90$^{th}$ percentile FOS intakes were estimated to be 3.0 and 4.2 g FOS/day, respectively. In this survey, no statistically significant differences between breastfed infants and those fed formula were observed for growth, mothers' perception of health of the baby, or any other adverse effects included in the survey. This shows that the ADI was established based on findings from EDI. The actual ADI has not been determined and may be higher. It is almost 34 years, since the recognition of the ADI of 4.2 g/day and the infant formulas containing FOS are still marketed without any safety concerns. Thus, the accumulating evidence for over three decades indicate that the proposed use of scFOS in infant formula and its addition to baby foods for infants is unlikely to result in adverse effects.

As described in our GRAS notice (GRN 990), the 90$^{th}$ percentile EDI from the proposed uses of scFOS in "starter" and "follow-on" infant formula ranges from 828 to 1035 mg/kg bw/day, respectively. As described in GRN 44, for infants 5 through 11 months the 90$^{th}$ percentile intake is estimated as 3.1 g/day (337 mg/kg bw/day). This additional intake of scFOS is unlikely to be additive, as the infants starts the intake of complimentary foods that may contain scFOS, the intake of scFOS from infant formula decreases. It is also unlikely that the total intake of scFOS from complimentary foods will significantly increase and will be of safety concern in infants receiving infant formula and complimentary foods. The EDIs determined in the GTC Nutrition GRAS notice (GRN 44) assume that scFOS will be used at the proposed use level in all 18 food categories to which scFOS is intended to be added. As such, the EDIs derived are considered highly conservative estimates of potential scFOS intake. Thus, any additional intake of scFOS from complimentary formula is considered as safe. The proposed use levels by Tata Chemicals in infant formula are identical to those described in GRN 797 and GRN 537, and both these GRAS notices received a "no questions" letter.

**Query 17. (b)** In Table 7 (p. 40 of the notice), Tata Chemicals lists the ADI for scFOS as "At proposed use levels (4 or 5 g/L)" for the current notice, as well as for GRNs 000797 and 000537. This statement is unclear, as ADI values are usually expressed in mg/kg bw/d. Please provide an explanation that clarifies this statement.

**Response:** Sorry for the confusion, as such in both of these GRAS notices (GRN 797 and GRN 537), the ADI values were not established. The safety was established for the intended use level of 400 mg/100 ml (4 g/L) for "starter formula" (within the first month of life) that results in a 90$^{th}$ percentile intake of 828 mg/kg bw/day and at 500 mg/100 ml (5 g/L) in "follow-on formula" (infants older than 1 month) that results in the 90$^{th}$ percentile intake of scFOS is about 800 mg/kg bw/day. In both of these GRAS notices, based on the totality of the evidence, the notifiers concluded that the intended use of scFOS in term infant formulas is GRAS.

**FDA Query 18.** In several sections of the notice (listed below), Tata Chemicals incorporates into the notice data and information from previous notices. However, we note that each GRAS notice must independently support the safety of the notified ingredient for its intended use. For each study Tata Chemicals considers critical to the GRAS conclusion for scFOS in infant formula for term infants and that they intend to incorporate into the notice, please provide a summary of the study, along with the complete citation and the specific GRAS notice from where the study came.

Page 26: "These studies have been the subject of several comprehensive evaluations, including several GRAS notices [GRN 44 (FDA, 2000), 537 (FDA, 2015), 605 (FDA, 2016a), 623 (FDA, 2016b), 717 (FDA, 2017), 797 (2018)] that have been reviewed by independent expert panels and the FDA. Among these GRAS notices on scFOS, GRN 605 was submitted by Tata. As the available information is extensively described in these previous GRAS notices, including GRN 605 Tata, all these GRAS notices are incorporated in the present GRAS by reference."

Page 39: "Tata is hereby incorporating all the toxicology and human tolerance studies discussed in these previous GRAS notices by reference (NFBC, 2018; Galam, 2017; NFBC, 2016; Tata, 2015; Ingredion, 2014; Pfizer, 2011; GTC, 2000)."

Page 40: "Given the structural and chemical similarity of scFOS preparations that have been concluded GRAS (e.g., GRN 797 and 537) by NFBC (2018) and Ingredion (2014) with the current GRAS (Table 7), a discussion of

publicly available data and information relevant to the safety of scFOS is incorporated by reference to studies described in GRN 797 and 537."

**Response:** We are sorry for the lack of our understanding as regards citing the previous GRAS notices that were submitted to FDA by other notifiers and received no question letters. We agree that each GRAS notice must independently support the safety of the notified ingredient for its intended use. Please note that all relevant data and safety studies mentioned in all these GRAS notices and critical to the GRAS conclusion for scFOS in infant formula for term infants are appropriately described in our GRAS notice (GRN 990). As per our understanding there are no additional studies or information in these previous GRAS notices that is not described in our GRAS notice.

**FDA Query 19.** On p. 18 of the notice, the intake of scFOS in the Guesry et al., 2000 study is given as 1, 2, or 3 g/day. However, in Table 2 (p. 19 of the notice), the intakes for this study are listed as 200, 400, or 600 mg/day. Please clarify this discrepancy.

**Response:** Thank you for bringing this to our attention and sorry for the oversight. In this study, Guesry et al. (2000) compared the effects of 3 concentrations of scFOS in infant formula. Infants received 5 bottles of formula per day for 2 weeks; each bottle provided either 200 mg lactose or 200, 400, or 600 mg scFOS providing daily intakes of 1.0 g lactose or 1.0, 2.0, or 3.0 g scFOS/day. In Table 2 the intake values should have been 1000, 2000 or 3000 mg/day. Please accept our apology for the discrepancy.

**FDA Query 20.** On p. 26 of the notice, Tata Chemicals states that updated literature searches were conducted to identify new studies relevant to the safety of scFOS in children and adults. However, no end date was provided for the search. Please provide an end date (i.e., month and year) through which Tata Chemicals searched the published literature.

**Response:** The end date for the updated searches was October 2020. Sorry, we forgot to mention this.

**FDA Query 21.** In Section 6.1.2.3. (scFOS Studies in Piglets and Other Weaning Animals), Tata Chemicals discusses studies in piglets and other weaning animals. For studies in which the test article was administered in

the feed (or drinking water) to piglets, rats, or mice (see below), please provide equivalent dose levels on a bw basis (i.e., mg/kg bw/d). If Tata Chemicals is unable to provide this information, please provide an explanation how the following studies can be used to support the GRAS conclusion:

Howard et al., 1995(a) and (b)

Tsukahara et al., 2003

Correa-Matos et al., 2003

Nakamura et al., 2004

**Response:** We attempted to calculate (please see below, the last paragraph of this response) the doses for one of the study. However, as these studies were conducted in weaning animals, it is bit difficult to determine the equivalent dose on body weight basis, given the rapid growth or body weight gain. Hence, we are providing discussion as regards any relevance of these studies from a safety point of view.

It should be noted that all these studies were conducted to investigate the efficacy of FOS in piglets (Howard et al., 1995a; Correa-Matos et al., 2003; Tsukahara et al., 2003) or weaning rats (Howard et al., 1995b) and weaning mice (Howard et al., 1995b, Nakamura et al., 2004). These studies in weaning pigs, rats, and mice indicate that scFOS is unlikely to cause adverse effects. In general, the piglet is considered as a surrogate model for human infants. In the studies using the piglet model, the exposure to scFOS was as follows: diet containing FOS (10%) *ad libitum* for 10 days (Tsukahara et al., 2003); 3 g FOS/L for 15 days (Howard et al., 1995b); and 7.5 g/L in formula for 14 days (Correa-Matos et al., 2003). In these studies, no adverse effects of scFOS were reported. In additional studies, in mice (drinking water containing 30 g scFOS/L for 14 days) and rats (drinking water containing 30 g scFOS/L for 14 days) also, no adverse effects were reported. These findings from neonatal animal studies indicate that proposed use of scFOS in infants is unlikely to cause adverse effects.

In the first study, with two separate experiments, Howard et al. (1995b) investigated the abilities of soluble dietary fiber (including scFOS) to stimulate Bifidobacteria populations and promote large intestinal mucosal cell proliferation in rats and mice. In these experiments, the FOS intake in mice was reported as 0.29 g/day, while in the rats it was reported as 0.51 g/day. The initial weight of mice was provided as 22.3 g while for rat it was 51.7 g. The daily increase in weight of rat was given as 4.4 g/day, so at the end of experiment (14 days) the weight will be 51.7 + 61.6 = 113.3 g. Based on the information provided in this publication, the dose of FOS in mice will be approximately 12.6 g/kg bw/day, while in rats it can range from 4.5 to 9.85 g/kg bw/day.

**FDA Query 22.** On p. 44 of the notice, Tata Chemicals states "Based on information from FDA's GRAS Notice Inventory website as of April 28,

2015, the agency has received three notices on FOS and provided "no questions" letters to all of the notifiers." We note that this statement and the subsequent paragraph are out of date and incorrect. Please provide an updated paragraph that corrects and updates this information.

**Response:** Thank you for bringing this to our attention. We are sorry for the oversight, as this description got inserted from our previous GRAS notice and needed to be corrected. The corrected paragraph should be as follows:

Based on information from FDA's GRAS Notice Inventory[1] website as of July 9, 2021, the agency has received six notices on FOS and provided "no questions" letters to all the notifiers. The details of these notices along with the GRN number, date of closure and FDA's letter are provided in the below table. A closely related oligosaccharide, galacto-oligosaccharide, has also been determined to be GRAS for use in a variety of foods in thirteen GRAS notifications to the FDA. All these GRAS notifications are available at FDA's website on GRAS Notices.

| GRN No. | Substance | Date of closure | FDA's Letter |
|---|---|---|---|
| 797 | Fructooligosaccharides | Nov 15, 2018 | FDA has no questions (in PDF) |
| 717 | Short-chain fructo-oligosaccharides | Feb 13, 2018 | FDA has no questions (in PDF) |
| 623 | Fructooligosaccharides | Aug 1, 2016 | FDA has no questions |
| 605 | Fructo-oligosaccharides | Mar 17, 2016 | FDA has no questions (previous GRAS notice by Tata Chemicals) |
| 537 | Short-chain fructo-oligosaccharides | Feb 6, 2015 | FDA has no questions |
| 44 | Fructooligosaccharide | Nov 22, 2000 | FDA has no questions (additional correspondence available) |

**FDA Query 23.** On p. 58 of the notice, the citation for Tsukahara et al., 2003 contains a typographical error. Please provide the correct citation.

[1]Accessible at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&sort=GRN_No&order=DESC&showAll=true&type=basic&search=

**Response:** Thank you for bringing this to our attentions and we are sorry for the oversight related to the typographical error. The correct reference is as follows:

Tsukahara, T., Iwasaki, Y., Nakayama, K., Ushida, K. 2003. Stimulation of butyrate production in the large intestine of weaning piglets by dietary fructooligosaccharides and its influence on the histological variables of the large intestinal mucosa. J Nutr Sci Vitaminol (Tokyo) 49:414- 421.

## Microbiology

**FDA Query 24.** On p. 10 of the notice, Tata Chemicals states, "A. pullulans used in the production of scFOS is non-toxigenic and non-pathogenic…" Please provide a brief summary discussing the safety of A. pullulans.

**Response:** As this same microorganism (*A. pullulans*) that was used in the manufacturing of scFOS in our previous GRAS notice (GRN 605), we did not further elaborate on this. However, we provide a brief summary of *A. pullulans below*.

*A. pullulans,* used in the production of scFOS is registered with the Microbial Type Culture Collection and Gene Bank (MTCC) under the number MTCC 5490. The characteristics of *A. pullulans*, as well as the development, safety, and identity of the production strain has been established. The production strain, *A. pullulans* MTCC 5490, was subjected to genetic identification by 16S ribosomal RNA gene, partial sequence for confirmation. *A. pullulans* strain MTCC 5490 is maintained in the Microbial Type Culture Collection and Gene Bank. The phylogenetic tree based on 16S rRNA and as compared to other related species and designates was developed for *A. pullulans*.

*A. pullulans* is a common black saprobic mould with a world-wide distribution in both indoor and outdoor environments. It can be found in lake water, on leaves and wood, as well as in used cosmetics and on foods such as fruits, cereals, tomatoes, and cheese. In the food industry, *A. pullulans* is used in the production of food ingredients, including pullulan (GRN 99), beta-glucan (GRN 309). The fungus contains multiple life forms (polymorphic) including blastospores, hyphae, chlamydospores, and swollen cells. The chlamydospores and swollen cells are considered resting forms. The fungus produces a green melanin which turns black over time.

Early clinical studies either failed to establish a pathogenic association or the taxonomic procedures failed to distinguish their isolates from *Exophialia* spp. In the past several decades there have been a few additional reports (Salkin et al., 1986) on the pathogenicity of *A. pullulans* for seriously immunocompromised patients, a phenomenon that is considered possible for most fungi including the baker's yeast *Saccharomyces cerevisiae*. Indeed there are far more reports associating this beneficial and safe industrial yeast with various disease syndromes than the rare associations indicated for *A. pullulans*. In another case report, Hawkes et al. (2005) reported a case of *A. pullulans* fungemia with invasive infection in an

infant. The authors reviewed the previously reported 23 cases of human infection from the literature (1966-2003). This case in an infant is also, the first case of documented invasive pulmonary infection and the first patient with a recently repaired cardiac lesion as the identified risk factor.

Host debilitation is by far the primary factor in the opportunistic or adventitious involvement of saprobic fungi with humans. Nevertheless, the available evidence for the past three decades with yeasts and moulds in environmental, industrial and clinical settings, the involvement of *A. pullulans* with any adverse human health related problems is extremely rare.

Based on above, *A. pullulans* used in the production of scFOS is considered as non-toxigenic and non-pathogenic.

**FDA Query 25.** In Table 1 on p. 9 of the notice, Tata Chemicals provides sampling specifications for Salmonella spp. and Cronobacter sakazakii (C. sakazakii).

**a)** Please state whether Tata Chemicals is analyzing multiple 25 g samples of product or one 100 g sample for Salmonella spp. We recommend that Salmonella testing be performed on sample sizes no larger than 25 g to prevent the possibility of false negatives, unless the method used is validated for larger samples. If analysis is performed on a sample size larger than 25 g, please discuss the method and how it was validated.

**Response:** Please note that Salmonella spp. has been analyzed on multiple 25 g samples (25 g x 4) and not as single 100 g sample. Sorry for our oversight in not mentioning this.

**b)** The notice cites method ISO 22964: 2017 for C. sakazakii as "absent 300g." We note a discrepancy in that this method is validated for test sample sizes of 10 g. Please clarify this discrepancy and state whether Tata Chemicals is analyzing multiple 10 g samples of product or one 300 g sample for C. sakazakii. We recommend that C. sakazakii testing be performed on sample sizes no larger than 10 g to prevent the possibility of false negatives, unless the method used is validated for larger samples. If analysis is performed on a sample size larger than 10 g, please discuss the method and how it was validated.

**Response:** Please note that *Cronobacter sakazakii* has been analyzed as multiples of 50 g (50 g x 6) and method was validated using in cerelac matrix (by SGS India). For the future batches, we will adopt the testing methodology with sample size no larger than 10 g.

**FDA Query 26.** Please state whether any of the raw materials used in the fermentation process are major allergens or are derived from major allergens. If any of the raw materials used are major allergens or derived from major allergens, please discuss why these materials do not pose a safety concern.

**Response:** The raw materials used in the fermentation and scFOS production neither fall under the major allergen category nor are they derived from major allergens.

We hope the above information and clarification addresses your queries. If you have any questions or need additional explanation, please let me know.

Thank you for the opportunity to provide this explanation to the agency queries.

Best regards

Madhu Soni, PhD

Agent for: Tata Chemicals Limited, India

## References


Demmers, T.A., Jones, P.J., Wang, Y., Krug, S., Creutzinger, V., Heubi, J.E., 2005. Effects of early cholesterol intake on cholesterol biosynthesis and plasma lipids among infants until 18 months of age. Pediatrics 115(6):1594-1601.

FDA, 2007. Frucrooligosaccharide, GRAS. Agency response additional correspondence letter: GRAS notice no. GRN 000044. June 1, 2007. Available at: https://wayback.archive-it.org/7993/20171031035213/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm154400.htm

FSSAI, 2020. Food Safety and Standard Authority of India. Food Safety Standards (Contaminants, Toxins and Residues) Regulations 2011. Available at: https://fssai.gov.in/upload/uploadfiles/files/Compendium_Contaminants_Regulations_20_08_2020.pdf

Friedman, G., Goldberg, S.J., 1975. Concurrent and subsequent serum cholesterol of breast- and formula-fed infants. Am J Clin Nutr. 28(1):42-45.

Hawkes, M., Rennie, R., Sand, C., Vaudry, W., 2005. *Aureobasidium pullulans* infection: fungemia in an infant and a review of human case. Diagn. Microbiol. Infect. Dis. 51:209-213.

Jain, M., Gote, M., Dubay, A.K., Narayanan, S., Krishnappa, H., Kumar, DP. S., Ravi, G.S., Vijaysarthi, S.K., Shankar, S., 2019. Safety evaluation of fructooligosaccharide (FOSSENCE™): Acute, 14-day, and subchronic oral toxicity study in Wistar rats. Toxioclogy Research Application 2:1-20.

Owen, C.G., Whincup, P.H., Odoki, K., Gilg, J.A., Cook, D.G., 2002. Infant feeding and blood cholesterol: a study in adolescents and a systematic review. Pediatrics 110(3):597–608.

Salkin, I. F., Martinez, J. A., Kemna, M. E., 1986. Opportunistic infection of the spleen caused by *Aureobasidium pullulans*. J. Clin. Microbiol. 23(5):828-831.

Wong, W.W., Hachey, D.L., Insull, W., Opekun, A.R., Klein, P.D., 1993. Effect of dietary cholesterol on cholesterol synthesis in breast-fed and formula-fed infants. J Lipid Res. 34(8):1403-1411.

Yamamota, Y., A. Yonekubo. 1993. A Survey of Physical Growth, Nutritional intake, Fecal Properties and Morbidity of Infants as Related to Feeding Methods. “Japanese Infant Formula Survey.” Central Research Laboratories, Meiji Milk Products Co., Ltd. (Cited in GRN 44).

## Appendix I

**Table 1. FDA query 1 - GRN citations and list with correct links**

| Page No. in GRAS notice | GRN No.; notifier name and year; FDA response year | Correct FDA response letter link | Correct Notifier reference link |
|---|---|---|---|
| Page 4 | GRN 537 (Ingredion, 2014) | NA (Not applicable) | Web-link not correct- correct link is as follows: http://wayback.archive-it.org/7993/20171031055001/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm422895.pdf |
| Page 4 | GRN 797 (NFBC, 2018) | NA (Not applicable) | Web-link not correct- correct link is as follows: https://www.fda.gov/media/132054/download |
| Page 9 | GRN 797 (NFBC, 2018) | NA (Not applicable) | Correct link provided above for Page 4. |
| Page 9 | GRN 537 (Ingredion, 2014) | NA (Not applicable) | Correct link provided above for Page 4. |
| Page 9 | GRN 44 (GTC Nutrition 2000) | NA (Not applicable) | Web-link not correct- correct link is as follows: http://wayback.archive-it.org/7993/20171031055001/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm261587.pdf |
| Page 10 | GRN 605 | https://www.fda.gov/food/gras-notice-inventory/agency-response-letter-gras-notice-no-grn-000605 | https://wayback.archive-it.org/7993/20190208035755/https:/www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm495918.pdf |
| Page 14 | GRN 797 (NFBC, 2018) | NA (Not applicable) | Correct link provided above for Page 4. |
| Page 14 | GRN 537 (Ingredion, 2014) | NA (Not applicable) | Correct link provided above for Page 4. |
| Page 18 | GRN 537 | NA | Correct link provided above for Page 4. |
| Page 24 | GRN 392 | NA | http://wayback.archive-it.org/7993/20171031055001/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm277112.pdf |
| Page 26 | GRN 44 (FDA, 2000) | Web-link not correct; correct link is as follows https://wayback.archive-it.org/7993/20171031035213/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm154122.htm | NA (Not applicable) |

| | | | |
|---|---|---|---|
| Page 26 | GRN 537 (FDA, 2015) | Correct web-link is as follows https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=GRASNotices&id=537&sort=GRN_No&order=DESC&startrow=1&type=basic&search=537 | NA (Not applicable) |
| Page 26 | GRN 605 (FDA, 2016a) | Weblink is correct | NA (Not applicable) |
| Page 26 | GRN 623 (FDA, 2016b) | Weblink is correct | NA (Not applicable) |
| Page 26 | GRN 717 (FDA, 2017) | Correct web-link is as follows https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=GRASNotices&id=717&sort=GRN_No&order=DESC&startrow=1&type=basic&search=717 | NA (Not applicable) |
| Page 26 | GRN 797 (FDA, 2018) | Correct web-link is as follows https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=GRASNotices&id=797&sort=GRN_No&order=DESC&startrow=1&type=basic&search=797 | NA (Not applicable) |
| Page 26 | GRN 605 (Tata, 2015) | NA (Not applicable) | Correct web-link is as follows https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=GRASNotices&id=605&sort=GRN_No&order=DESC&startrow=1&type=basic&search=605 |
| Page 36 | GRN 44 (GTC Nutrition, 2000) | NA (Not applicable) | Correct link provided above for page 9 |
| Page 39 | GRN 44 (FDA, 2000) | Correct link provided above for page 26 | NA (Not applicable) |
| Page 39 | GRN 537 (FDA, 2015) | Correct link provided above for page 26 | NA (Not applicable) |
| Page 39 | GRN 605 (FDA, 2016a) | Correct link provided above for page 26 | NA (Not applicable) |
| Page 39 | GRN 623 (FDA, 2016b) | Correct link provided above for page 26 | NA (Not applicable) |
| Page 39 | GRN 717 (FDA, 2017) | Correct link provided above for page 26 | NA (Not applicable) |
| Page 39 | GRN 797 (FDA, 2018) | Correct link provided above for page 26 | NA (Not applicable) |
| Page 39 | GRN 537 (Ingredion, 2014) | NA (Not applicable) | Correct link provided above for Page 4. |
| Page 39 | GRN 717 (Galam, 2017) | NA (Not applicable) | Link is correct |
| Page 39 | GRN 979; This should be 797- sorry for the typo | NA (correct link provided above) | NA (correct link provided above) |
| Page 39 | GRN 623 | NA (correct link provided above) | NA (correct link provided above) |

| | | | |
|---|---|---|---|
| Page 39 | GRN 605 (Tata, 2015) | NA (Not applicable) | Correct link provided above for page 26 |
| Page 39 | GRN 44 | NA (correct link provided above) | NA (correct link provided above) |
| Page 39 | GRN 392 | NA (correct link provided above) | NA (correct link provided above) |
| Page 39 | GRN 797 | NA (correct link provided above) | NA (correct link provided above) |
| Page 39 | GRN 537 | NA (correct link provided above) | NA (correct link provided above) |
| Page 40 | GRN 797 (NFBC, 2018) | NA (Not applicable) | Correct link provided above for Page 4. |
| Page 40 | GRN 537 (Ingredion, 2014) | NA (Not applicable) | Correct link provided above for Page 4. |
| Page 41 | GRN 537 (Ingredion, 2014) | NA (Not applicable) | Correct link provided above for Page 4. |
| Page 44 | GRN 44 (GTC Nutrition, 2000) | NA (Not applicable) | Correct link provided above for Page 9 |
| Page 44 | GRN 392 (Pfizer, 2011) | NA (Not applicable) | Web-link not correct- correct link is as follows: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=GRASNotices&id=392&sort=GRN_No&order=DESC&startrow=1&type=basic&search=392 |
| Page 44 | GRN 537 (Ingredion, 2014) | NA (Not applicable) | Correct link provided above for Page 4. |
| Page 47 | GRN 236. This should be GRN 334- sorry for the typo | Available at: https://wayback.archive-it.org/7993/20171031035213/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm233093.htm | Available at: http://wayback.archive-it.org/7993/20171031055001/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm269519.pdf |
| Page 47 | GRN 233 | Available at: https://wayback.archive-it.org/7993/20171031035213/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm185685.htm | Available at: http://wayback.archive-it.org/7993/20171031055001/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm269127.pdf |
| Page 47 | GRN 286 | Available at: https://wayback.archive-it.org/7993/20171031035213/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm186158.htm | Available at: http://wayback.archive-it.org/7993/20171031055001/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm269263.pdf |
| Page 47 | GRN 489 | Available at: https://wayback.archive-it.org/7993/20171031035213/ht | Available at: http://wayback.archive-it.org/7993/20171031055001/https://www.fda.gov/downloads/Food/IngredientsPacka |

| | | | |
|---|---|---|---|
| | | tps://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm401233.htm | gingLabeling/GRAS/NoticeInventory/ucm381400.pdf |
| Page 47 | GRN 495 | Available at: https://wayback.archive-it.org/7993/20171031035213/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm400803.htm | Available at: http://wayback.archive-it.org/7993/20171031055001/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm386769.pdf |
| Page 47 | GRN 569 | Available at: https://wayback.archive-it.org/7993/20171031035213/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm484518.htm | Available at: http://wayback.archive-it.org/7993/20171031055001/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm475293.pdf |
| Page 48 | GRN 392 | Available at: https://wayback.archive-it.org/7993/20171031035213/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm307720.htm | Available at: http://wayback.archive-it.org/7993/20171031055001/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm277112.pdf |
| Page 48 | GRN 44 | For additional uses- available at: https://wayback.archive-it.org/7993/20171031035213/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm154400.htm | NA |
| Page 48 | GRN 537 | NA | Correct link provided above for page 4 |
| Page 48 | GRN 44 (FDA, 2000) | Correct link provided above for page 26 | NA |
| Page 48 | GRN 605 (FDA, 2016a) | Correct link provided above for page 26 | NA |
| Page 48 | GRN 623 (FDA, 2016b) | Correct link provided above for page 26 | NA |
| Page 48 | GRN 717 (FDA, 2017) | Correct link provided above for page 26 | NA |
| Page 48 | GRN 797 (FDA, 2018) | Correct link provided above for page 26 | NA |
| Page 52 | GRN 537 | Correct link provided above for page 26 | NA |
| Page 52 | GRN 797 | Correct link provided above for page 26 | NA |

NA=Not applicable; please note for some GRNs both the “FDA has no questions” letter link as well as link to full GRAS notice submitted by Notifier is provided.

**From:** Madhu Soni
**To:** Morissette, Rachel
**Subject:** [EXTERNAL] RE: questions for GRN 000990 to be addressed
**Date:** Wednesday, September 1, 2021 4:15:14 PM
**Attachments:** image003.png
image007.png
image011.png
image014.png
image016.png
image018.png
scFOS GRAS infant formula-GRN 990-FDA Query-2 responses final.pdf

---



Dear Dr. Morissette,

As per your below email, please find attached responses to FDA queries for GRN 990. If you need any further clarification please let me know.

Thank you for this opportunity.

Best regards,

Madhu

---
Madhu Soni, *PhD, FACN, FATS*
Soni & Associates Inc
749 46th Square
Vero Beach, FL 32968, USA
Phone: +1-772-299-0746
Cell: +1-772-538-0104
www.soniassociatesnet

---

**From:** Morissette, Rachel [mailto:Rachel.Morissette@fda.hhs.gov]
**Sent:** Thursday, August 26, 2021 10:28 AM
**To:** Madhu Soni <sonim@bellsouth.net>
**Subject:** questions for GRN 000990 to be addressed

Dear Dr. Soni,

Below are some additional questions to be addressed for GRN 000990. Please provide your responses within 5 business days or let me know if you have any further questions.

1. Thank you for your response to our question 15 in the July 19, 2021 amendment. We note the totality of the available clinical evidence to support the current GRAS conclusion. However, please state which clinical studies are critical/pivotal to your GRAS conclusion for scFOS.

2. In the response to question 18 in the July 19, 2021 amendment, Tata Chemicals states, “Please note that all relevant data and safety studies mentioned in all

these GRAS notices and critical to the GRAS conclusion for scFOS in infant formula for term infants are appropriately described in our GRAS notice (GRN 000990). As per our understanding, there are no additional studies or information in these previous GRAS notices that is not described in our GRAS notice." However, Table 4 (Studies of Fructans in Infants) in GRN 000537 appears to contain information and studies not discussed in the current notice (e.g., Kim et al., 2007,[1] Moore et al., 2003[2]). Additionally, GRN 000537 contains an extensive discussion of EFSA's conclusion on FOS. In this opinion EFSA stated, in part, "There was an increased prevalence of adverse effects, including loose stools, in infants fed formula with added fructooligosaccharides."[3] A similar discussion of EFSA's opinion on FOS is not included in the current safety narrative for GRN 000990.

a. Please provide an explanation how Tata Chemicals can reach a GRAS conclusion for scFOS in infant formula without information discussed in previous GRAS notices for scFOS.

b. If Tata Chemicals is intending to incorporate information from previous GRAS notices into the current notice, please provide the following information for each study and/or any information being incorporated into the notice: a brief discussion of the specific data/information being incorporated, the GRN number that contains the referenced information, and the page numbers in the GRN where the referenced information can be found. If Tata Chemicals does not intend to incorporate any information into the notice, please provide revised statements as identified in question 18 (i.e., on pages 26, 39, and 40 of the notice) clarifying that no information is being incorporated.

3. In the response to question 21 from the July 19, 2021 amendment, Tata Chemicals states, "However, as these studies were conducted in weaning animals, it is bit [sic] difficult to determine the equivalent dose on body weight basis, given the rapid growth or body weight gain." While we are not asking for any additional scientific information to support your response to question 21, we do ask that you confirm your agreement or disagreement with the following statement: Even though actual body weights may not be provided in the published studies, a dose on a body weight basis is possible to calculate in weaning piglets (eg., Hanlon and Thorsrud, 2014[4]) and piglets in a feeding study are often weighed each day to determine the volume of formula to dispense (e.g., Monaco et al., 2020[5]).

[1]Kim S-H, Lee DH, Meyer D (2007) Supplementation of baby formula with native inulin has a prebiotic effect in formula-fed babies. *Asia Pac J Clin Nutr*. 16(1):172-177

[2]More et al. (2003) Effects of fructo-oligosaccharide-supplemented infant cereal: a double-blind, randomized trial. *Br J Nutr*. 90(3):581-587. doi: 10.1079/bjn2003950

[3]EFSA (2004) Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission relating to the safety and suitability for particular nutritional use by infants of fructooligosaccharides in infant formulae and follow-on formulae. The EFSA Journal, 31:1-11.

[4]Hanlon PR and Thorsrud BA (2014) A 3-week pre-clinical study of 2'-fucosullactose in farm piglets. *Food Chem Toxicol.*, 74:343-348. doi: 10.1016/j.fct.2014.10.025

[5]Monaco et al. (2020) Evaluation of 6'-sialyllactose sodium salt supplementation to formula on growth and clinical parameters in neonatal piglets. *Nutrients* 12(4):1030. doi:

10.3390/nu12041030

Best regards,

Rachel

---

**Rachel Morissette, Ph.D.**
*Regulatory Review Scientist*

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

FDA U.S. FOOD & DRUG ADMINISTRATION

---

[1] Kim S-H, Lee DH, Meyer D (2007) Supplementation of baby formula with native inulin has a prebiotic effect in formula-fed babies. *Asia Pac J Clin Nutr*. 16(1):172-177

[2] More et al. (2003) Effects of fructo-oligosaccharide-supplemented infant cereal: a double-blind, randomized trial. *Br J Nutr*. 90(3):581-587. doi: 10.1079/bjn2003950

[3] EFSA (2004) Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission relating to the safety and suitability for particular nutritional use by infants of fructooligosaccharides in infant formulae and follow-on formulae. The EFSA Journal, 31:1-11.

[4] Hanlon PR and Thorsrud BA (2014) A 3-week pre-clinical study of 2'-fucosullactose in farm piglets. *Food Chem Toxicol*., 74:343-348. doi: 10.1016/j.fct.2014.10.025

[5] Monaco et al. (2020) Evaluation of 6'-sialyllactose sodium salt supplementation to formula on growth and clinical parameters in neonatal piglets. *Nutrients* 12(4):1030. doi: 10.3390/nu12041030

Dear Dr. Morissette,

**RE: Additional Queries for GRN 990 (Short-chain fructooligosaccharide GRAS notice)**

This responds to your email of August 26, 2021 regarding additional clarifications required for Tata Chemicals's short-chain fructooligosaccharides (scFOS) GRAS notice (GRN 000990) for use as an ingredient in infant formulas for term infants. We are providing a point-by-point response to your queries along with some relevant clarifications/discussion.

**FDA Query 1:** Thank you for your response to our question 15 in the July 19, 2021 amendment. We note the totality of the available clinical evidence to support the current GRAS conclusion. However, please state which clinical studies are critical/pivotal to your GRAS conclusion for scFOS.

**Response:** We consider the following two studies as being critical to the GRAS conclusion for scFOS: Ripoll et al. (2015) and Paineau et al. (2014)

**FDA query 2:** In the response to question 18 in the July 19, 2021 amendment, Tata Chemicals states, "Please note that all relevant data and safety studies mentioned in all these GRAS notices and critical to the GRAS conclusion for scFOS in infant formula for term infants are appropriately described in our GRAS notice (GRN 000990). As per our understanding, there are no additional studies or information in these previous GRAS notices that is not described in our GRAS notice." However, Table 4 (Studies of Fructans in Infants) in GRN 000537 appears to contain information and studies not discussed in the current notice (e.g., Kim et al., 2007,[1] Moore et al., 2003[2]). Additionally, GRN 000537 contains an extensive discussion of EFSA's conclusion on FOS. In this opinion EFSA stated, in part, "There was an increased prevalence of adverse effects, including loose stools, in infants fed formula with added fructooligosaccharides."[3] A similar discussion of EFSA's opinion on FOS is not included in the current safety narrative for GRN 000990.

a. Please provide an explanation how Tata Chemicals can reach a GRAS conclusion for scFOS in infant formula without information discussed in previous GRAS notices for scFOS.

[1] Kim S-H, Lee DH, Meyer D (2007) Supplementation of baby formula with native inulin has a prebiotic effect in formula-fed babies. *Asia Pac J Clin Nutr*. 16(1):172-177

[2] More et al. (2003) Effects of fructo-oligosaccharide-supplemented infant cereal: a double-blind, randomized trial. *Br J Nutr*. 90(3):581-587. doi: 10.1079/bjn2003950

[3] EFSA (2004) Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission relating to the safety and suitability for particular nutritional use by infants of fructooligosaccharides in infant formulae and follow-on formulae. The EFSA Journal, 31:1-11.

**Response:** Sorry for missing the studies by Kim et al. (2007) and Moore et al. (2003), as well as the discussion of EFSA's conclusion provided in the previous GRAS notice GRN 000537. We agree to the need for discussing these studies as well as the EFSA's conclusion, for the GRAS determination of scFOS. Thank you for bringing this to our attention. We are incorporating Kim et al. (2007) study described on pages 50, 51 and 67 of the GRN 000537 and the Moore et al. (2003) study described on page 46 and 68 of the GRN 000537. We are also incorporating the discussion of EFSA's opinion on FOS described in GRN 000537 given on pages 205 and 206. Please note that some of this information and discussion from these publications is further described below.

b. If Tata Chemicals is intending to incorporate information from previous GRAS notices into the current notice, please provide the following information for each study and/or any information being incorporated into the notice: a brief discussion of the specific data/information being incorporated, the GRN number that contains the referenced information, and the page numbers in the GRN where the referenced information can be found. If Tata Chemicals does not intend to incorporate any information into the notice, please provide revised statements as identified in question 18 (i.e., on pages 26, 39, and 40 of the notice) clarifying that no information is being incorporated.

**Response:** As indicated above we intend to incorporate information from previous GRAS notices, particularly GRN 000537, in GRN 000990. For the studies by Kim et al. (2007) and Moore et al. (2003), as well as for EFSA's conclusion, a brief discussion is as follows:

*Kim et al. (2007)*: In this prospective, randomized, double-blind, crossover study, the effects of chicory inulin on the gut microbiome, the frequency of defecation, and the pH and consistency of feces was investigated. In this study, 14 healthy term infants averaging 12.4±6.4 weeks of age were randomly assigned to receive control formula for 3 weeks, followed by inulin-supplemented formula, or the 2 treatments in reverse order. Inulin from chicory roots was added to the experimental formula at a concentration of 1.5±0.3 g/100 g powder (~200 mg/100 ml hydrated formula). The defecation frequency and amount and consistency of stools daily were recorded. Anthropometric measures were recorded and fecal samples were collected at the end of each feeding period. The mean intake of inulin was 1.5 g/day. No

infants were withdrawn from the study, no formula-related adverse effects were observed, and there were no differences in growth between control and inulin feeding periods. Stool characteristics during inulin feeding apparently changed in the expected directions (toward increased frequency, softer stools, and lower pH) but none of the changes reached statistical significance. There were no differences between control and inulin feeding in total anaerobic bacteria or bacteroides, but both bifidobacteria and lactobacilli increased significantly during inulin intake periods. The investigators concluded that the addition of native inulin to infant formula elicits a prebiotic response.
The above information is incorporated from GRN 000537 described on pages 50, 51 and 67.

*Moore et al. (2003)*: In this randomized, double-blind, placebo-controlled, 28-day trial, gastrointestinal (GI) effects of FOS-supplemented infant cereal were investigated. In this study, 56 healthy term infants aged 4-11 months with demonstrated tolerance for rice cereal and milk-based formula were assigned to receive infant cereal with 750 mg of either FOS (n=27) or maltodextrin/serving (n=29). A daily diary recording of cereal intake, stooling pattern and characteristics, flatulence, vomiting, spitting up, crying, and abdominal cramping, was maintained by parents. Anthropometric measures were taken at baseline and study completion. One infant receiving the FOS-supplemented cereal and 4 infants receiving the placebo dropped out. The mean daily consumption of FOS was 740 mg/day and was as high as 3000 mg/day. There were no tolerance issues with the FOS-supplemented cereal, and infants receiving FOS had more significantly frequent stools with more regular and softer consistency as compared to those receiving placebo. No serious adverse events were reported, but 17 infants in the experimental group had 24 reported nonserious events as compared to 16 infants with 21 non-serious events in the placebo group. No adverse event was regarded as being related to the intake of FOS. There were no differences between the 2 groups in growth. The investigators stated that the present study is one of few studies documenting tolerance to increased fiber intake in the form of FOS as part of a weaning food.
The above information is incorporated from GRN 000537 described on pages 46 and 68.

*Discussion of EFSA's opinion*: European Food Safety Authority (EFSA, 2004) reviewed an application for the use of only 300 mg oligofructose/100

ml and concluded (described in GRN 000537) that: 1. “There was an increased prevalence of adverse effects, including loose stools, in infants fed formula with added fructooligosaccharides.” 2. “As no measures were made to demonstrate satisfactory water balance, the possibility of increased risk of dehydration cannot be excluded, raising concerns with respect to the safety of such formulae.” 3. “There is no evidence of benefits to infants from the addition of fructooligosaccharides to infant formula at the conditions specified by the manufacturer while there are reasons for safety concerns.”

In GRN 000537 (pages 205, 206 and 207), the concern expressed by EFSA for water balance has been extensively discussed and addressed, while two other aspects of the above conclusion are less central to evaluating the safety of the intended addition of scFOS to infant formula. The increased prevalence of “loose stools” is regarded as a beneficial effect of the formula supplemented with fructans in that the infants receiving these formulas exhibited stooling performance more closely matching that of breastfed infants than did infants receiving control formulas without fructans.

As described in GRN 000537, it appears that EFSA (2004) has interpreted loose, poorly formed, or watery stools as reported by a parent as being equivalent to clinically diagnosed diarrhea. Generally, diarrhea would indeed be properly regarded as an adverse effect. However, it has not been reported in any of the many controlled studies of ingestion of fructans-supplemented formula by infants (or studies of formula supplemented with GOS or other oligosaccharides). In the study by Yao et al. (2010), stool composition and consistency was a primary outcome measure of feeding infant formula supplemented with 300 or 500 mg oligofructose/100 ml for 8 weeks. No infant consuming these formulas was reported as having diarrhea, and even the incidence of parentally reported watery stools was not increased by the addition of oligofructose, indicating that there was no increase in water loss.

Additionally, the absence of statistically significant long-term benefit in short-term studies of scFOS or other fructans added at an average of 300 mg/100 ml (compared with an oligosaccharide content of about 800-1200 mg/100 ml in human milk) does not bear upon the safety of the formula and is not relevant to a determination of whether the intended use is GRAS. Few of these studies were designed or powered to detect long-term beneficial effects from the tested interventions.

Furthermore, in GRN 000537 (pages 206, 207), extensive discussion on the EFSA (2004) considerations of parental classification of stool consistency in the studies reviewed has been provided. This discussion, along with other studies and the Food Standards Australia New Zealand (FSANZ, 2008) critical evaluation of concerns expressed by EFSA relating to water balance, suggest that the intended use of scFOS by Tata Chemicals is unlikely to cause adverse effects. The 2008 conclusions of FSANZ regarding the safety of inulin-supplemented infant formula were repeated in this authoritative body's subsequent conclusion that infant formula supplemented with scFOS is equally safe (FSANZ, 2013).

**FDA query 3:** In the response to question 21 from the July 19, 2021 amendment, Tata Chemicals states, "However, as these studies were conducted in weaning animals, it is bit [sic] difficult to determine the equivalent dose on body weight basis, given the rapid growth or body weight gain." While we are not asking for any additional scientific information to support your response to question 21, we do ask that you confirm your agreement or disagreement with the following statement: Even though actual body weights may not be provided in the published studies, a dose on a body weight basis is possible to calculate in weaning piglets (eg., Hanlon and Thorsrud, 2014[4]) and piglets in a feeding study are often weighed each day to determine the volume of formula to dispense (e.g., Monaco et al., 2020[5]).

**Response:** Sorry for the confusion. We do confirm our agreement with the statement, "Even though actual body weights may not be provided in the published studies, a dose on a body weight basis is possible to calculate in weaning piglets (eg., Hanlon and Thorsrud, 2014) and piglets in a feeding study are often weighed each day to determine the volume of formula to dispense (e.g., Monaco et al., 2020)."

We hope the above information and clarification addresses agency queries. If you have any questions or need additional explanation, please let me know.

Thank you for the opportunity to provide this explanation to the agency queries.

Best regards

Madhu Soni, PhD
Agent for: Tata Chemicals Limited, India

[4] Hanlon PR and Thorsrud BA (2014) A 3-week pre-clinical study of 2'-fucosullactose in farm piglets. *Food Chem Toxicol*., 74:343-348. doi: 10.1016/j.fct.2014.10.025
[5] Monaco et al. (2020) Evaluation of 6'-sialyllactose sodium salt supplementation to formula on growth and clinical parameters in neonatal piglets. *Nutrients* 12(4):1030. doi: 10.3390/nu12041030

**References:**

FSANZ, 2008. Food Standards Australia New Zealand. Final assessment report: proposal P306, addition of inulin/FOS & GOS to food. July 16.

FSANZ, 2013. Food Standards Australia New Zealand. Approval report-application A1055, short chain fructo-oligosaccharides. May 23.

Yao et al. (2010). High 2- palmitate and oligofructose in lower protein alpha-lactalbumin-enriched term infant formula: effects on stool characteristics and stool composition. J Pediatr Gastroenterol Nutr 50(Suppl 2):E207-208.