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June 26, 2018

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 the intended use of Fructooligosaccharide in Infant Formula**

Dear Sir/Madam:

This is to bring to your kind attention that on January 7, 2016, on behalf of New Francesco Biotechnology Corporation (NFBC), China, we submitted a GRAS notice for use of fructooligosaccharides (FOS) in conventional foods and in infant formula. The agency filed it on February 12, 2016 as GRN 000623. On August 1, 2016, FDA provided a "no questions" letter for use of FOS in conventional foods. However, at the time, NFBC withdrew its intended use of FOS in infant formula due to unresolved questions from FDA for which additional information was required.

Subsequently, as we have obtained the additional information to address FDA's question, on January 25, 2018, we had a pre-GRAS meeting with Dr. Morissette and her team. Based on the discussions with FDA, NFBC decided to submit a new GRAS notice for use of FOS in infant formula. As per FDA recommendations, we hereby submit a new GRAS notice of a claim that the use of FOS 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.

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,

(b) (6)

Madhu G. Soni, Ph.D.

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

**Submitted by:**
New Francisco (Yunfu City) Biotechnology Corporation Limited
Swan-kan-chiau Industrial District
Kaofong Village, Yunfu City,
Guangdong Province,
CHINA 527343

**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

**Contact for Technical and Other Information**
Madhu G. Soni, PhD
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June, 2018

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## 1. Part 1 – SIGNED STATEMENTS AND CERTIFICATION

In accordance with 21 CFR §170 Subpart E consisting of §§170.203 through 170.285, New Francisco (Yunfu City) Biotechnology Corporation Limited (NFBC) hereby informs the FDA that short-chain fructo-oligosaccharides (scFOS), as manufactured by NFBC, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on NFBC'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

Hank Tsai, Ph.D.
New Francisco (Yunfu City) Biotechnology Corporation Limited
Swan-kan-chiau Industrial District
Kaofong Village, Yunfu City,
Guangdong Province,
CHINA 527343

### 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.

### 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). 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

New Francisco (Yunfu City) Biotechnology Corporation Limited (NFBC) 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 Dr. Tsai or Dr. Soni 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).

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### 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

NFBC 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 NFBC, including any favorable or unfavorable information, and pertinent to the evaluation of the safety and GRAS status of the use of scFOS preparation. NFBC 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

Hank Tsai, Ph.D.
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Signature: (b) (6)

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

NFBC 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 2 – 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 *Aspergillus oryzae.*

### 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 King-Prebiotics® FOS.

#### 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 scFOS is 700 daltons (Da), representing the average of the molecular weights of its 3 components (505 Da, 666 Da, and 828 Da, respectively), respectively.

#### 2.1.5. Chemical Structure

Fructo-oligosaccharides (FOS) 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.

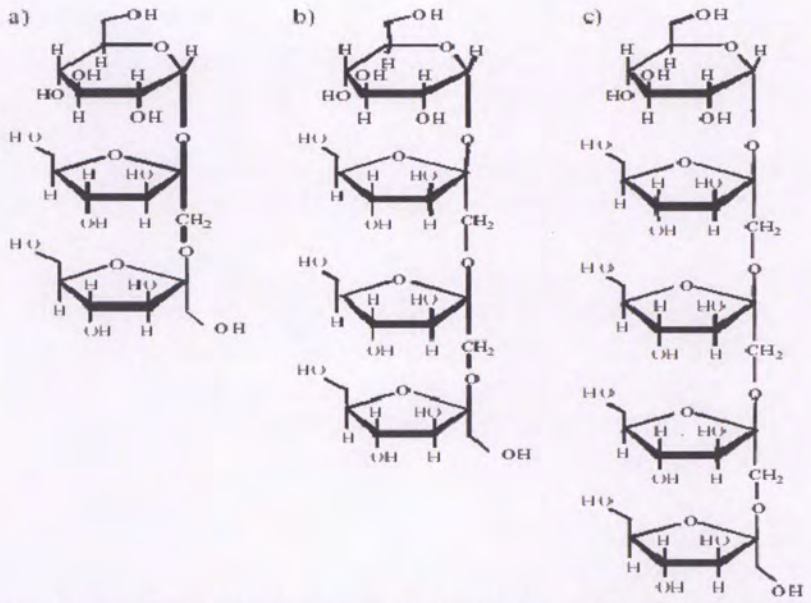

**Figure 1. Structural Formulas of scFOS components (a) 1-Kestose (GF1), (b) Nystose (GF2), and (c) Fructofuranosylnystose (GF3). Fructosyl units are linked at position β-2, 1 of sucrose.**

### 2.1.6. Other Chemically Related Constituents

As described above, the subject of this 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 New Francisco (Yunfu City) Biotechnology Corporation Limited (NFBC). scFOS will be marketed in the U.S. in the form of powder and syrup. The specifications of scFOS-950-P and scFOS-950-S are presented in Table 1. A copy of the specifications sheet provided by NFBC for FOS-950-P and FOS-950-S is included as Appendix I. To demonstrate conformance with the food-grade specifications, NFBC analyzed several lots (Lot means a batch, or a specific identified portion of a batch- 21 CFR 111.3.) of scFOS. Analytical results from five lots (Tables 2 and 3) suggest that scFOS powder as well as syrup is consistently manufactured to meet the standard specifications. The specification parameters comprise physical appearance, purity, total scFOS levels, moisture, sulphated ash, as well as limits for potential chemical and microbiological impurities, and

contaminants. The distribution ratio of scFOS components [1-kestose (GF2), Nystose (GF3) and Fructofuranosylnystose (GF4)] for FOS-950-P and FOS-950-S is presented in Tables 2 and 3, respectively. The results of batch analysis for scFOS demonstrate that the manufacturing process produces oligomers that are characteristic of typical scFOS preparations synthesized from sucrose by enzymatic synthesis with GF2, and GF3 representing the major fructose oligomers and lower quantities of longer chain GF4. Small quantities (~5%) of residual sucrose, glucose and fructose represent 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 reviewed by the FDA without any questions [including GRN 537 (Ingredion, 2014) and GRN 44 (GTC Nutrition, 2000)].

**Table 1. Food Grade Physical and Chemical Specifications of scFOS Powder (FOS-950-P) and Syrup (FOS-950-S)**

| Parameters | Specifications | | Method |
|---|---|---|---|
| | FOS-950-P | FOS-950-S | |
| Appearance | Off white light yellow powder | Off white light yellow syrup | Sensory test |
| Taste | Slightly sweet | Slightly sweet | Sensory test |
| Total scFOS (%) | ≥95 | ≥95 | HPLC |
| 1-kestose (GF2) (%) | NLT 30.0 | NLT 30.0 | HPLC |
| Nystose (GF3) (%) | NLT 40.0 | NLT 40.0 | HPLC |
| Fructofuranosylnystose (GF4) (%) | NLT 5.0 | NLT 5.0 | HPLC |
| Sugars (%) | ≤5 | ≤5 | HPLC |
| pH (30% solution) | 4.5-7.0 | 4.5-7.0 | pH meter |
| Moisture (%) | ≤3.5 | N/A | Moisture meter |
| Ash (%) | ≤0.1 | ≤0.1 | GB/T 20885 |
| Melamine (mg/kg) | ≤0.01 | ≤0.01 | GB/T 22388 |
| **Heavy metals** | | | |
| Lead (mg/kg) | ≤0.02 | ≤0.02 | GB 5009.12 |
| Total Arsenic (mg/kg) | ≤0.05 | ≤0.05 | GB 5009.11 |
| Cadmium (mg/kg) | ≤0.1 | ≤0.1 | GB 5009.15 |
| Total Mercury (mg/kg) | ≤0.01 | ≤0.01 | GB 5009.17 |
| **Microbiological limits** | | | |
| Total Bacterial Count (CFU/g) | ≤500 | ≤500 | GB 4789.2 |
| Yeasts (CFU/g) | ≤20 | ≤20 | GB 4789.15 |
| Molds (CFU/g) | ≤20 CFU/g | ≤20 | GB 4789.15 |
| Coliforms (MPN/g) | ≤3.0 | ≤3.0 | GB 4789.3 |
| *Escherichia coli* (MPN/g) | <3.0 | <3.0 | GB 4789.38 |
| Salmonella | Negative/25g | Negative/25g | GB 4789.4 |
| Shigella | Negative/25g | Negative/25g | GB 4789.5 |
| *Staphylococcus aureus* | Negative/25g | Negative/25g | GB 4789.10 |
| Enterobacteriacea (MPN/g) | <0.3 | <0.3 | GB 4789.41 |
| Listeria | Negative/25g | Negative/25g | GB 4789.30 |
| *Bacillus cereus* (MPN/g) | <3.0 | <3.0 | GB 4789.14 |
| *Cronobacter sakazakii* | Negative/100g | Negative/100g | GB 4789.40 |

NLT = Not less than; CFU = Colony forming units

**Table 2. Food Grade Physical and Chemical Specifications of scFOS Powder (FOS-950-P)**

| Parameters | Standard Specifications | Lot #17005 | Lot #17006 | Lot #18001 | Lot #18002 | Lot #18003 |
|---|---|---|---|---|---|---|
| Appearance | Off white light yellow powder | Off white light yellow powder | Off white light yellow powder | Off white light yellow powder | Off white light yellow powder | Off white light yellow powder |
| Taste | Slightly sweet | Slightly sweet | Slightly sweet | Slightly sweet | Slightly sweet | Slightly sweet |
| Total scFOS (%) | ≥95 | 95.7 | 96.4 | 96.4 | 96.5 | 96.5 |
| 1-kestose (GF2) (%) | NLT 30.0 | 35.7 | 38.3 | 36.2 | 37.7 | 35.0 |
| Nystose (GF3) (%) | NLT 40.0 | 48.0 | 46.7 | 49.0 | 47.1 | 49.2 |
| Fructofuranosylnystose (GF4) (%) | NLT 5.0 | 12.0 | 11.4 | 11.2 | 11.7 | 12.3 |
| Sugars (%) | ≤5 | 4.3 | 3.6 | 3.6 | 3.5 | 3.5 |
| pH (30% solution) | 4.5-7.0 | 5.5 | 5.4 | 6.2 | 5.9 | 5.2 |
| Moisture (%) | ≤3.5 | 3.1 | 2.2 | 3.1 | 2.4 | 3.2 |
| Ash (%) | ≤0.1 | 0.02 | 0.04 | 0.02 | 0.03 | 0.03 |
| Melamine (mg/kg) | ≤0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| **Heavy metals** | | | | | | |
| Lead (mg/kg) | ≤0.02 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Total Arsenic (mg/kg) | ≤0.05 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Cadmium (mg/kg) | ≤0.1 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Total Mercury(mg/kg) | ≤0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| **Microbiological limits** | | | | | | |
| Total Bacterial Count (CFU/g) | ≤500 | 30 | <10 | 10 | <10 | <10 |
| Yeasts (CFU/g) | ≤20 | <10 | <10 | <10 | <10 | <10 |
| Molds (CFU/g) | ≤20 | <10 | <10 | <10 | <10 | <10 |
| Coliforms (MPN/g) | ≤3.0 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 |
| *Escherichia coli* (MPN/g) | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 |
| Salmonella | Negative/25g | Negative | Negative | Negative | Negative | Negative |
| Shigella | Negative/25g | Negative | Negative | Negative | Negative | Negative |
| *Staphylococcus aureus* | Negative/25g | Negative | Negative | Negative | Negative | Negative |
| Enterobacteriaceae (MPN/g) | <0.3 | <0.3 | <0.3 | <0.3 | <0.3 | <0.3 |
| Listeria | Negative/25g | Negative | Negative | Negative | Negative | Negative |
| *Bacillus cereus* (MPN/g) | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 |
| *Cronobacter sakazakii* | Negative/100g | Negative | Negative | Negative | Negative | Negative |

**Table 3. Food Grade Physical and Chemical Specifications of scFOS Syrup (FOS-950-S)**

| Parameters | Standard Specifications | Lot #16001 | Lot #16002 | Lot #16003 | Lot #16004 | Lot #17001 |
|---|---|---|---|---|---|---|
| Appearance | Off white light yellow Syrup | Off white light yellow Syrup | Off white light yellow Syrup | Off white light yellow Syrup | Off white light yellow Syrup | Off white light yellow Syrup |
| Taste | Slightly sweet | Slightly sweet | Slightly sweet | Slightly sweet | Slightly sweet | Slightly sweet |
| Total scFOS (%) | ≥95 | 95.8 | 96.3 | 96.7 | 96.1 | 96.6 |
| 1-kestose (GF2) (%) | NLT 30.0 | 36.2 | 37.5 | 36.3 | 38.6 | 38.2 |
| Nystose (GF3) (%) | NLT 40.0 | 48.1 | 47.1 | 48.0 | 45.6 | 47.1 |
| Fructofuranosylnystose (GF4) (%) | NLT 5.0 | 11.5 | 11.7 | 12.4 | 11.9 | 11.3 |
| Sugars (%) | ≤5 | 4.2 | 3.7 | 3.3 | 3.9 | 3.4 |
| pH (30% solution) | 4.5-7.0 | 6.2 | 5.9 | 6.0 | 5.8 | 6.0 |
| Dry matter (%) | ≥75 | 75.3 | 75.0 | 75.2 | 75.3 | 75.5 |
| Ash (%) | ≤0.1 | 0.03 | 0.04 | 0.02 | 0.03 | 0.05 |
| Melamine (mg/kg) | ≤0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| **Heavy metals** | | | | | | |
| Lead (mg/kg) | ≤0.02 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Total Arsenic (mg/kg) | ≤0.05 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Cadmium (mg/kg) | ≤0.1 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Total Mercury(mg/kg) | ≤0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| **Microbiological limits** | | | | | | |
| Total Bacterial Count (CFU/g) | ≤500 | <10 | <10 | <10 | <10 | <10 |
| Yeasts (CFU/g) | ≤20 | <10 | <10 | <10 | <10 | <10 |
| Molds (CFU/g) | ≤20 | <10 | <10 | <10 | <10 | <10 |
| Coliforms (MPN/g) | ≤3.0 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 |
| *Escherichia coli* (MPN/g) | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 |
| Salmonella | Negative/25g | Negative | Negative | Negative | Negative | Negative |
| Shigella | Negative/25g | Negative | Negative | Negative | Negative | Negative |
| *Staphylococcus aureus* | Negative/25g | Negative | Negative | Negative | Negative | Negative |
| Enterobacteriaceae (MPN/g) | <0.3 | <0.3 | <0.3 | <0.3 | <0.3 | <0.3 |
| Listeria | Negative/25g | Negative | Negative | Negative | Negative | Negative |
| *Bacillus cereus* (MPN/g) | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 | <3.0 |
| *Cronobacter sakazakii* | Negative/100g | Negative | Negative | Negative | Negative | Negative |

NFBC has established the *Cronobacter sakazakii* limits as Negative/100 g. *C. sakazakii* is a bacterium that causes a rare but often fatal infection of the bloodstream and central nervous system. Recently, concerns have been raised because *C. sakazakii* can be present in powdered infant formula. Most cases of *C. sakazakii* come from powdered infant formula contaminated with the bacterium. However, this type of infection is still very rare. High temperatures reached in preparing the formula usually kill the bacteria, but they are known to survive even after preparation. Powdered infant formula is most likely contaminated after production, since the pasteurization process is normally adequate to kill *C. sakazakii* bacteria. However, if the powder is produced using the dry blending process, and not heated, *Cronobacter* bacteria can survive in the formula. However, infant formula manufacturers typically provide instructions with the infant formula indicating that hot water should be used in the preparation of the liquid formula. This, should minimize the potential for *C. sakazakii* being present in the product as served. The presence of *C. sakazakii* in powdered infant formula is most likely due to manufacture under poor Good Manufacturing Practices.

It is noted that the *C. sakazakii* has been identified in the infant formula and not FOS. In fact, a search of the literature using PubMed and other databases using the search terms "FOS and *Cronobacter sakazakii"* and "Frutooligosaccharides and *Cronobacter sakazakii"* do not yield any articles related to these search terms. Nevertheless, NFBC has applied rigorous test procedures to ensure that no *Cronobacter sakazakii* is present in the finished FOS product.

### 2.3. Manufacturing Process

The manufacturing process used in the production of scFOS for use in infant formula is identical to that of NFBC GRAS notice (GRN 623) on scFOS that received no question letter from FDA for the use of scFOS in conventional foods.

scFOS is manufactured according to current good manufacturing practices (cGMP) and ISO standards, as outlined in Figure 2, at New Francisco (Yunfu City) Biotechnology Corporation Limited (NFBC) facilities located at Swan-kan-chiau Ind. Dist., Kaofong Village, Yunfu City, Guangdong, Zip: 527343, China. The manufacturing details of the powder and syrup forms of FOS are shown in Figure 2. scFOS constituents, such as 1-kestose, nystose, and fructosyl-nystose, are produced by the treatment of sucrose with a food-grade preparation of β-fructofuranosidase. In general, β-fructofuranosidase hydrolyzes sucrose to glucose and fructose. At high concentrations of sucrose, some β-fructofuranosidases can transfer the fructosyl residue to the sucrose molecule, in which fructosyl residues are transferred to sucrose by β-2,1 glycosidic bonds.

For the manufacturing of scFOS, the sucrose solution is prepared by dissolving food-grade sucrose in deionized water at an elevated temperature. The enzyme, β-fructofuranosidase, derived from *A. oryzae* is added to the sucrose solution in a fermenter. The pH is adjusted in the enzymatic reaction between sucrose and β-fructofuranosidase by sodium carbonate; the reaction is subsequently terminated by the addition of citric acid to the fermenter solution. This process results in the formation of a solution containing at least 50% of scFOS. For the preparation of high purity scFOS products FOS-950-S, the 50% scFOS solution is followed by decolorization, filtration, purification and evaporation. The scFOS purity is further increased through chromatographic separation for removal of glucose, fructose and sucrose. After purification,

FOS-950-S is packaged. The powder form, FOS-950-P, is obtained by evaporation, spray drying, and packing.

The enzyme, β-fructofuranosidase, used in the manufacturing of FOS is derived from *Aspergillus oryzae*. It is a well-known commercial enzyme commonly used for the production of FOS. The β-D-fructofuranosidases enzyme preparation meets the general and additional requirements for enzyme preparations as outlined in the monograph on Enzyme Preparations in the Food Chemicals Codex. The β-D-fructofuranosidases preparation is produced in accordance with current good manufacturing practices, using ingredients that are acceptable for general use in foods, and under conditions that ensure a controlled fermentation. These methods are based on generally available and accepted methods used for production of microbial enzymes (Aunstrup, 1979; Aunstrup et al., 1979, Enzyme Applications, 1994).

The β-D-fructofuranosidases enzyme preparation is derived from a pure culture of a nonpathogenic, nontoxigenic strain of *Aspergillus oryzae*, which is registered with the American Type Culture Collection (ATCC). The specific *A. oryzae* strain used in the production of FOS is same as described in GRN 623. *A. oryzae* is the source organism for many enzyme preparations that are considered to be GRAS. These include glucose oxidase (GRN 106), lipase (GRN 43, GRN 75, and GRN 103), aspartic proteinase (GRN 34), exopeptidase (GRN 10), pectin esterase (GRN 8), and protease and carbohydrase (GRN 90). All these GRAS notices and FDA responses are available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices. Enzymes from *A. oryzae* are accepted as a constituent of foods. A. *oryzae* has been used to produce soy sauce in the United States prior to 1958 and carbohydrase and protease (GRN 90). Therefore, ingredients from *A. oryzae* meet the criterion of "common use in foods in the US before 1958" and can be considered "generally recognized as safe". The available information on *A. oryzae* and the steps involved in the manufacturing supports the safe use of enzyme β-D-fructofuranosidase derived from *A. oryzae* strain in the production of scFOS.

All raw materials and processing aids used in the manufacture of scFOS are suitable food-grade materials and/or are used in accordance with applicable U.S. Code of Federal Regulations for such uses. The manufacturing facility is registered with FDA under the number 19919474440. Additionally, the facility is ISO certified: ISO9001 2008(2003/08) and ISO 22000 HACCP (2005/08). Furthermore, NFBC has over 20-years experience in saccharides production and as per various international quality management systems, including QS Production, HALAL, OU Kosher, GMO-FREE IP, and SA8000 certification that guarantee premium quality of a series of international-grade oligosaccharides (King-Prebiotics®) products that are manufactured from food grade sucrose (21 CFR 184.1854) and lactose (21 CFR 168.122).

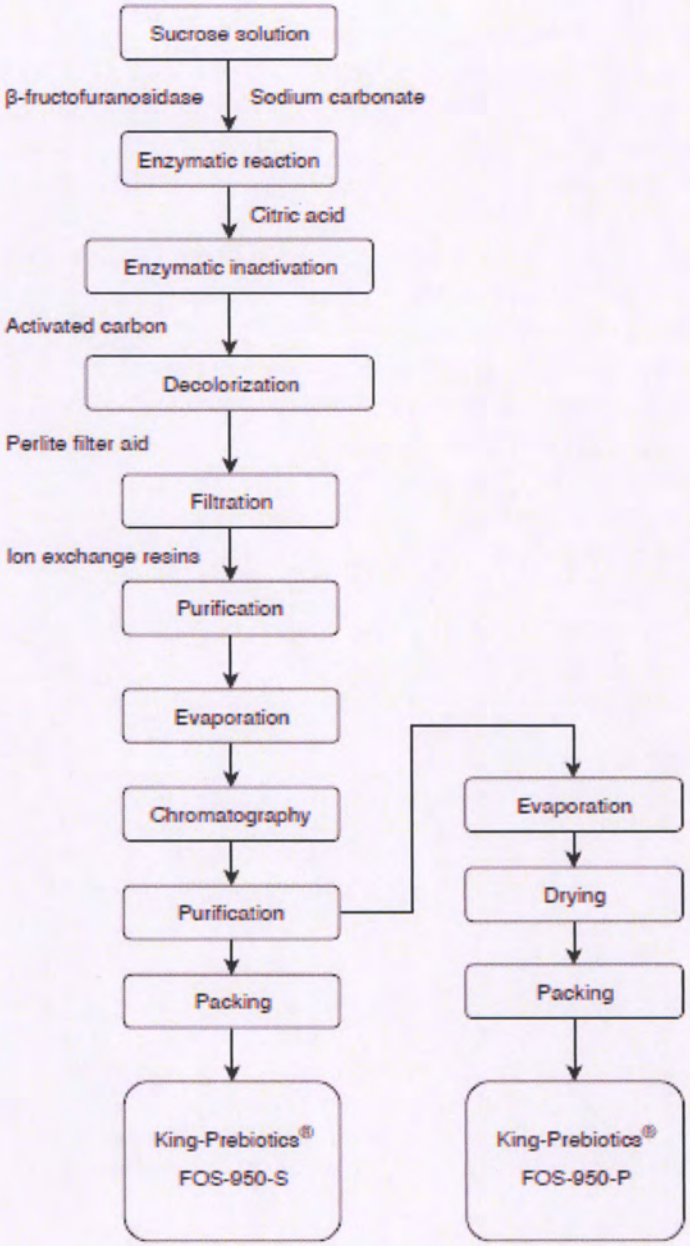


**Figure 2. Manufacturing Process of scFOS**

## 2.4. Technical Effects

NFBC 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

NFBC 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

As mentioned earlier, NFBC intends to market scFOS as an ingredient for addition to term infant formula and follow-on formula at use levels 400 and 500 mg/100 ml of formula as consumed, respectively. The proposed uses of scFOS and the resulting exposures from it have been estimated in the previous GRAS notice (GRN 537) to FDA. The scFOS product described by Ingredion (2014) in GRAS notice (GRN 537) was reported to contain 95% of scFOS. The subject of this present GRAS notice 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).

In the estimates reported by Ingredion (2014) in GRN 537, daily energy intake by formula fed children was considered in determination of FOS intake. For these assessments, daily energy intake of infants fed infant formula provided by Femon (1993) were used. Boys, ages 14-27 days, were found to be the subpopulation of infants with the highest intake 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. The FDA typically uses the 90$^{th}$ percentile of the intake distribution to represent extreme intakes. The energy intake estimates as reported by Femon (1993) are corroborated by a 2008 Feeding Infant and Toddler Study by Butte et al. (2010). In the study by Butte et al. (2010), the 90$^{th}$ percentile energy intake of 779 kcal or approximately 144 kcal/kg bw is similar to the estimates in Fomon (1993).

As the majority of 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. The 90$^{th}$ percentile of formula intake for the two sexes combined is 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, while the maximum (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.

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, intake of scFOS per kg body weight decreases as the infant grows. The estimated 90$^{th}$ percentile intake of scFOS peaks at about 1035 mg/kg bw/day during the first 6 weeks of life,

then begins to decline, reaching about 840 mg/kg bw/day by weeks 8-12. This suggest that the maximum estimated daily intake (EDI) of FOS is unlikely to exceed 1035 mg/kg bw/day. This is a very conservative estimate for long-term exposure because as the infant grows the formula intake increases but at a slower rate than weight gain. Although, historically non-exempt infant formulas provided 20 kcal/fl oz as fed, recent information indicates that several infant formula notifications for infant formulas 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 of 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.

There are no other sources of FOS or fructans in the diets of formula fed infants. Introduction of any foods that might possibly contain FOS or other nondigestible carbohydrates would be at the expense of formula (i.e., to maintain the same caloric intake, formula consumption would necessarily decrease as solid foods are added) and it is quite likely that the result of introduction of other foods would be a net decrease in prebiotic intake – certainly in fructan – including FOS, intake.

In summary, the intended 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 bodyweight 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, the maximum intake of 828 mg scFOS/kg bw/day is considered.

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

No known self-limiting levels of use are associated with the notified ingredient scFOS. As such, users will control the amounts used due to economic reasons.

## 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 not based on common use in food before 1958.

## 6. Part VI – NARRATIVE

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

In the published literature, over 1000 preclinical and clinical studies with FOS have appeared. In the following section, relevant toxicological and efficacy studies on FOS are summarized in the order of their importance and 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. For the present GRAS, attempts have also been made to provide the supporting evidence in the following sequence: published pivotal studies, secondary published studies, corroborative unpublished studies and regulatory agencies assessments. It should be noted that the safety in use of the proposed use of scFOS in infant formula is based on the totality of available evidence.

### 6.1. Pivotal Studies of FOS

#### 6.1.1. Primary Published Studies in Infant

The available studies of scFOS are summarized in Table 4. In a randomized, double-blind, placebo-controlled trial, Paineau et al. (2014) studied 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 FOS or maltodextrins until the age of 4 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; 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. Intake of formula did not differ between the groups, nor did growth.

The most frequent adverse event was abdominal pain, followed by liquid stools (Paineau et al., 2014) 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, 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 assessment.

**Table 4. Studies of scFOS in Infants**

| Reference | Dose, Duration | Study Design, Objective | Subjects | Results |
|---|---|---|---|---|
| Paineau et al. (2014) | 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." |
| Ripoll et al. (2014); unpublished | 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." |

| | | | | |
|---|---|---|---|---|
| Xia et al. (2012) | 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* and *C. difficile*." |
| Lasekan et al. (2010) | 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." |
| Guesry et al. (2000) | 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. |

In another study, 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 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 (Actilight® 950P) 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, height, weight and digestive tolerance (i.e., constipation, crying, soft stool, vomiting and regurgitation, adverse events-AEs and serious adverse events-SAEs) were monitored (Ripoll et al., 2015).

Tolerance and growth parameters were similar in both the groups (Ripoll et al., 2015). Overall, 81% of infants experienced at least one AE, with no significant difference in the number of AEs between groups. The most prevalent AEs in all infants were nasopharyngitis (28%), bronchitis (12%), and gastroenteritis (9%). No difference was observed between groups for diarrhea and gastroenteritis. During the study, 6 different infants suffered from serious adverse events. None of the serious adverse events was related to the study product. Digestive tolerance was evaluated during the 6 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 2 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 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 authors also noted that infants consuming the scFOS supplemented formula have experienced an improvement in vomiting prevalence and in stool consistency. The results of 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 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 4 months of age. The findings from this study support the NFBC proposed use of scFOS in follow on formula.

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 a randomized, double-blind, placebo controlled study, Xia et al. (2012) analyzed intestinal bacterial populations from term infants fed formula supplemented with FOS. In this study, healthy term infants aged ≤6 days were enrolled in a 4-week trial assessing the effects of 4 types of feeding on the intestinal microbiota. The types of feeding included cow's milk (control), human milk (reference), and two FOS groups (240 or 340 mg scFOS/100 ml). Although the publication mentioned use of FOS and not scFOS, based on the additional information obtained

from FDA, under FOIA (2016), it was confirmed that the test article used was 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 split-ups or vomit. The groups did not differ in total bacterial loads, although they tended to be lower in the infants fed human milk than 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 supports the safety of scFOS at the maximum use levels of up to 340 mg/100 ml. Although the use levels in this study are lower as compared to the present GRAS assessment, the findings did not reveal any adverse effects related to scFOS and should be applicable to NFBC GRAS.

In a 28 day parallel feeding randomized, double-blind, trial, Lasekan et al. (2015) compared the effects of soy-based infant formulas containing supplemental scFOS on gastrointestinal (GI) tolerance and hydration in healthy term newborn infants 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 one of two 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 is not mentioned, the investigators clearly mention use of scFOS. No significant study group differences in study completion rates (CF = 81, EF1 = 86, and EF2 = 87%), growth, mean rank stool consistency, stool frequency, formula intake, spit-up/vomit, and safety measures (urine specific gravity, USG; hydration status and adverse events) were noted. A total of six serious adverse events were reported in the study, 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 demonstrated good tolerance and hydration 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. As compared to the present GRAS, the use levels of scFOS in this study is lower (250 mg/ 100 ml) and did not reveal any adverse effects of scFOS.

In a recent prospective, intervention open trial, Vandenplas et al. (2017) tested the safety of a new symbiotic infant formula, supplemented with *Bifidobacterium lactis* and FOS, with lactose and a whey/casein 60/40 protein ratio, in 280 infants for 3 months. Specific for the study formula is the addition of FOS (0.35 g/100 ml) and *B. lactis* ($10^7$ cfu/g powder). The inclusion was based on parents who intended to feed their infants (partially) formula and agreed to feed the new symbiotic formula. The degree of polymerization for FOS was not mentioned in the article. Age at entry was 3.8±3.6 weeks. Of the 280 infants, 75 received study formula from birth and 227 infants fed during trial period received study formula exclusively. The median age of the infants at inclusion was 0.89 months. Weight evolution was in accordance with the World Health Organization growth charts for exclusive breastfed infants. The evolution 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 functional constipation (3.2%), daily regurgitation (10.9%),

infantile crying and colic (10.5%) were all significantly lower than the reported median prevalence for a similar age according to 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). The findings from this study are applicable to the present GRAS, although the use levels are lower.

In a prospective, randomized, double-blind study, Guesry et al. (2000; published as abstract; also described in GRN 537), compared the effects of three concentrations of scFOS in infant formula. In this study, 7 to 20-day-old 53 infants 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 FOS providing daily intakes of 1 g lactose or 1, 2, or 3 g scFOS. In this study, the volume of the formula in each bottle was not stated; so the dietary concentration of FOS in mg/ml cannot be determined. However the actual intake of FOS was reported. The infants were examined and weighed weekly and mothers recorded 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 2 weeks later for measurement of pH and enumeration of bifidobacteria. Drop-out rates did not differ by group. A dose-related increase in stooling frequency with scFOS intake was noted. There were no differences in fecal pH, bifidobacteria counts, or adverse effects. Assuming that the infants were of normal weight for this age range, they would have averaged about 3.7 kg and 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 safety of NFBC proposed uses of scFOS in infant formula.

In summary, the available studies in infant suggest that levels up to 680 mg scFOS/100 ml of infant formula is well tolerated by infant 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 above described studies is applicable to the current scFOS. The findings from these studies support the proposed uses of scFOS by NFBC 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

Some of the below described studies with oligofructose (FOS), derived from other sources such as chicory, are considered secondary pivotal studies as these molecules share the same composition, 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 hydrolysis or bacterial fermentation the distinctions between them become less noteworthy. Additionally, their activity and fate in the gastrointestinal system, while not identical (particularly for fructans of widely different DP), are somewhat similar. 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 GRN 392 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 75% of their total oligosaccharide intake.

In a randomized, double-blind trial, Brunser et al. (2006b) studied the effect of probiotic or prebiotic supplemented milk formulas on fecal microbiota composition of infants. In this study, 116 healthy term infants were given a standard milk-based infant formula, the same formula with 200 mg/100 ml of oligofructose (from chickory), 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 (66% of those enrolled) 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 3 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 3 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 3 formula groups or between the formula-fed and breastfed infants, nor were there any differences in growth measured by gain in weight 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 a randomized, double-blind study in infants, Bettler and Euler (2006) assessed growth and tolerance in healthy full-term infants fed formula supplemented with oligofructose for 12 weeks. In this study, infants aged 14 days or less who were being fed formula were randomized to receive standard milk-based formula or the same formula supplemented with 150 or 300 mg oligofructose/100 ml (n=98 and 101, respectively). Weight, length, and head circumference were recorded at baseline and every 4 weeks. Adverse events and reports of formula acceptance and tolerance were recorded at these same visits as well as during telephone calls between each visit. Blood was drawn at baseline and termination for analysis of albumin, blood urea nitrogen, calcium, magnesium, phosphorus, creatinine, triglycerides, low-density lipoprotein, and total cholesterol. The mean weight, length, and head circumference were not significantly different among the groups; and there were no differences in the mean values for routine blood chemistries at week 12 and the values were within normal reference ranges. 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. There were no significant differences in incidence of diarrhea, loose stools, dehydration or allergic reaction among the three groups. The investigators concluded that the control, probiotic, and ymbiotic formulas were equally tolerable, equally safe, and equally supportive of normal growth over the 28-day feeding period.

In a randomized, double-blind, placebo-controlled study, Kapiki et al. (2007) investigated the effect of a FOS supplemented formula on gut flora of preterm infants. In this study, 56

healthy bottle-fed preterm infants were enrolled and were supplemented with chicory-derived FOS. 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 were randomly assigned to receive preterm formula with 400 mg maltodextrin (placebo)/100 ml formula, while 41 infants were fed similar formula with 400 mg FOS/100 ml formula. The duration of feeding was 14 days. At baseline, the infants were measured anthropometrically and a stool sample was collected for bacterial analysis. During the study a diary was maintained of formula intake, stool frequency and characteristics, and any side effects. Additional stool samples were collected after 7 and 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. 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. The investigators stated that "All infants tolerated well the two formulae," although the evidence supporting this claim was not described. The investigators 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 non-randomized, non-blinded, non-placebo-controlled study, Lugonja et al. (2010) compared the bifidogenic effects of breast milk and prebiotic- supplemented infant formula. In this study, 21 healthy infants aged 5 to 16 weeks (mean = 8.6 weeks) were enrolled. Ten infants (7 boys and 3 girls) were breastfed, while 11 infants (6 boys and 5 girls) received formula containing 400 mg/100 ml of a blend of inulin and oligofructose derived from chicory. The fructans were not further described. The relative proportions of FOS and inulin in the blend was not reported, nor was the rationale for creating the blend. The trial had a duration of 28 days, during which daily measures were taken of weight, length, number of feeds, any indications of intolerance (GI symptoms, flatus, regurgitation, loss of appetite), frequency of stooling, and stool consistency (soft, normal, or hard). At baseline and on days 14 and 28, stool samples were collected and analyzed for pH, organic acids, and numbers of bifidobacteria, lactobacilli, total aerobes, total anaerobes, and fungi/yeasts. The number of daily feeds was significantly higher in the breastfed group. Counts of bifidobacteria increased significantly over the 28 days in both groups. *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. 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 formula-fed and breastfed infants. There were no significant differences between groups in measures of intolerance, stool frequency, or stool consistency.

In a prospective, randomized, double-blind, parallel-group study 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 study, 300 healthy formula-fed term

infants aged 7-14 days were assigned to one of 4 α-lactalbumin-enriched formulas for 8 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. Additional 75 infants served as a human milk-fed reference group. Tolerance was assessed via a parental questionnaire and physician-reported study events. The primary outcome measure was stool soap and mineral content at week 8; secondary outcome measures included stool characteristics and GI tolerance. Among the participants 2 each from the human-milk reference group and the high sn-2 group, 1 each from the control group and 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 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 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 not different among the 4 formula groups and the human-milk reference group; parental reports indicated no increase in the incidence of watery stools, gassiness, or other symptoms of intolerance with the addition of oligofructose. Addition of up to 5.0 g oligofructose/L to formula had no effect on growth (weight, length, head circumference).

In summary, the available studies with oligofructose or FOS derived from other sources, such as chicory, is well tolerated in infants. The findings from these studies suggest that scFOS at the intended use of scFOS in infant formula by NFBC is unlikely to cause adverse effects.

#### 6.1.2.2. Studies in Children and Adults

In multiple clinical studies in children and adult human subjects, safety (tolerance) of scFOS has been studied. These studies are available publicly and has been the subject of several comprehensive evaluations by several GRAS notifiers, independent expert panels and the FDA. These studies are extensively described in GRN 44, 537, 605, 623, 717 (FDA, 2000; 2015; 2016a; 2016b; 2017). Among these GRAS notices on scFOS, GRN 623 was submitted by NFBC. As the available information is described in these previous GRAS notices, NFBC is incorporating by reference all these GRAS notices. In the first GRAS notification by GTC Nutrition (2000), the Acceptable Intake Level for infants less than one year old was determined as 4.2 g/day, while for the general population (excluding infants less than one year of age) it was determined to be 20 g/day. scFOS consumption by children and adults did not result in serious adverse events (SAEs). The available information revealed only mild gastrointestinal side-effects of scFOS consumption that included flatulence, bloating, abdominal discomfort and transient diarrhea. These findings are well-established effects 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 found.

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

The available information suggest that the neonatal piglet is similar in nutritional requirements, intestinal physiology, and metabolism to the human infant, and its body composition is similar to that of the premature human infant. Currently, 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. Given this, the available studies of FOS in neonatal piglets are described first followed by studies in other animal species.

In a study in the neonatal pig model, Howard et al. (1995b) investigated the effect of scFOS on bifidogenic and colonic epithelial cell proliferation (full description of the test article used were not provided). The other information in the article indicate that the product used has to be scFOS. After being allowed to nurse for 36 hours, 20 newborn male pigs were housed individually, fed every 3 hours and given water *ad libitum*. Pigs were randomized into 2 groups of 10 to receive elemental formula supplemented with 0 or 3 g scFOS/L for 15 days. Formula intake and body weight were recorded daily. Following necropsy, cecal and proximal colonic contents were collected; the pH and SCFA concentration of cecal contents were measured. Samples from both sources were analyzed for enumeration of total anaerobic microbiota and *Bifidobacterium* spp. The cecum and proximal and distal colon were subjected to morphological examination of crypt height, leading edge, cell density, labeled cells, proliferation zone, and labeling index. Carcasses received gross macroscopic necropsy to detect any lesions, and samples of liver, lung, small intestine, large intestine, kidney, skeletal muscle, heart, and spleen were examined. In an additional (side) experiment, 12 additional neonatal male pigs were divided into 2 groups (6 pigs/group) and fed one of the 2 previously described diets for 6 days. Fecal samples were collected on days 1, 3, and 6 and analyzed for bifidobacteria.

In the study by Howard et al. (1995b) the mean daily intake of scFOS in the test group was 1.4 g; as the bodyweight of the pigs was not reported this cannot be expressed in mg/kg bw/day. Neither daily intake of formula nor gain in body weight differed significantly between the two groups of pigs in the main experiment, and no significant effect was observed in counts of total anaerobic microbiota or bifidobacteria in the cecal or proximal colonic contest. Similarly, neither cecal SCFA concentrations nor pH were significantly affected by ingestion of scFOS. However, scFOS significantly increased cecal mucosal cell density and labeled cells as well as proximal colonic mucosal crypt height, leading edge, labeled cells, proliferation zone, and labeling index and distal colonic mucosal crypt height, leading edge, cell density, labeling index, and labeled cells.

In the additional experiment by Howard et al. (1995b), pigs receiving scFOS showed a significant linear upward trend in numbers of fecal bifidobacteria while no trend was evident among control pigs. The investigators concluded that, "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 main experiment, one pig receiving scFOS exhibited intestinal lesions "suggestive of bacterial infection," and 6 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 congestion, hemorrhage, 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 in weaning piglets, 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 pigs. In this study six weaning piglets were used. 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 were used as controls. At the end of the experiment, their 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 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 authors concluded that "the beneficial roles of scFOS in the physiology of the large intestine rely on the activity of intestinal microbiota."

In 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 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) studied the effects of fermentable nondigestible carbohydrates in piglets infected with *Salmonella typhimurium*. Forty-eight 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. Bodyweight, physical activity level, and stool consistency were assessed daily and body temperature every other day. The piglets were killed on day 14 and the small intestine and colon were removed, weighed, and measured. The jejunum and ileum and a mid-colon section were isolated for analysis of the SCFA content of the contents, histomorphological analysis, and measurement of disaccharidase activity. *S. typhimurium* infection produced diarrhea in controls and 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 authors 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 a study in neonatal mice, 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* a 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.

Howard et al. (1995a) studied the effects of scFOS, XOS, and gum Arabic on cecal and colonic microbiota in weaning rats and mice. In the weaning mice study, 52 male BALB/c mice (13 mice/group) with average weight 22.3 g were housed individually and given free access to mouse chow and water containing 0 or 30 g/L of scFOS, XOS, or gum Arabic for 14 days. At necropsy, the cecum and colon were excised and bacteria were enumerated. There were no differences in feed and water intake or in weight gain. The mice ingesting scFOS had significantly higher concentrations of bifidobacteria as compared to other 3 groups, both in absolute numbers and as a percent of total bacteria. There were no differences in feed and water consumption or in weight gain. A variable morphological effect was noted as evidenced by significantly greater cecal crypt depth following ingestion of XOS as compared to the other treatments and significantly less with scFOS ingestion compared with the other treatments while in the colon the effects were reversed with scFOS producing the greatest crypt depth and XOS the least. The proliferation zone was significantly less in rats receiving scFOS or XOS than controls or rats receiving gum Arabic. The cell density was lower in rats fed scFOS or gum Arabic than the other diets. However, none of these differences was regarded by the authors as functionally significant.

In the rat study by Howard et al. (1995a), 44 male Sprague-Dawley weanling rats average weight of 51.7 g were individually housed and given *ad libitum* access to rat chow and water containing 0 or 30 g/L of scFOS, XOS, or gum Arabic (n = 11 rats/group) for 14 days. At necropsy, colons and ceca were examined for morphological change (crypt depth, cell density, and proliferation zone). There were no differences in feed and water intake or in weight gain. Variable morphological effects noted were as follows: cecal crypt depth was significantly greater with ingestion of XOS compared to the other treatments and significantly less with scFOS ingestion compared with the other treatments while in the colon the effects were reversed with scFOS producing the greatest crypt depth and XOS the least; the proliferation zone was significantly less in rats receiving scFOS or XOS than controls or rats receiving gum Arabic; and

cell density was lower in rats fed scFOS or gum Arabic than the other diets. However, none of these differences was regarded by the authors as functionally significant.

In two separate experiments, Fukata et al. (1999) investigated the effects of competitive exclusion and ingestion of scFOS on colonization of chicks with *Salmonella enteriditis*. Both experiments used one-day-old White Leghorn Hy-Line cockerel chicks caged in battery brooders. 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. As well as *Bifidobacterium, Bacteroides,* and *Lactobacillus* spp., and *Escherichia coli* 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 while 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 regarded as a surrogate model for human infants, studies conducted in these animals are applicable to present GRAS assessment. In the studies in the 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 the NFBC proposed use of scFOS in infants is unlikely to cause adverse effects.

#### 6.1.2.4. Other Published Studies

##### 6.1.2.4.1. Metabolism

Several non-digestible oligosaccharides and polysaccharides have been shown to act as prebiotic compounds, of which inulin, FOS and GOS are presently the most widely used in food. As described in the published literature and regulatory assessments, pharmacokinetic studies of FOS demonstrate that FOS is not hydrolyzed by human salivary or pancreatic enzymes and passes undigested and unabsorbed to the colon where it 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). Available studies in Wistar rats, as well as *in vitro* studies, using pancreatic and small intestinal homogenates and purified sucrase-isomaltase complex, demonstrate 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. Available human studies in healthy 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) studied 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 2 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 was observed during the treatment period. 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.

#### 6.1.2.4.2. Toxicity Studies

In the published literature, several studies of scFOS derived from sucrose has been described. The scFOS used in these studies appears to be substantially equivalent to the subject of 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* 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 no-observed adverse-effect levels (NOAELs) were the highest doses tested. In these studies, scFOS related effects apparent at high doses included intestinal weight increases, transient diarrhea, and soft/watery stools. 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. No evidence of carcinogenicity was reported in a 2-year study conducted with Fischer 344 rats and a 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 are briefly summarized below.

### 6.1.2.4.3. Acute Toxicity Studies

In the acute oral toxicity studies, effects of scFOS were tested in male and female mice and Sprague Dawley rats. The available details and findings from these studies are summarized in Table 5. 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 5. Acute Toxicity Studies of scFOS in mice and rats**

| Reference | Animal Model | Source & Description of Test Article | Dose & Duration of Feeding | Findings |
|---|---|---|---|---|
| Takeda and Niizato (1982) Mouse study | 48 4-week-old 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) Rat study | 48 6-week-old male and 10-week-old 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. |

### 6.1.2.4.4. Short-term, Subchronic and Chronic Toxicity Studies

Summary of short-term, subchronic and chronic toxicity studies are provided in Table 6. The findings from the available published toxicological studies suggest that various scFOS preparations are of low oral toxicity in repeat dose toxicity studies in rodents. These published and generally available studies pertinent to the safety 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.

Carabin and Flamm (1999) described 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. A dose-related increase in diarrhea, soft stools, cecal distension,

intestine weights for rats fed up to 20400 mg/kg bw/day for 90 days was reported by Meijl Seika Kaisha (1982). 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 observations, hematology, clinical chemistry, or histopathology even at the highest dose tested. The NOAEL was the highest dose tested (4680 mg oligofructose/kg bw/day).

In a 104-week feeding study, Clevenger et al. (1988) studied the chronic toxicity and carcinogenicity of FOS. In this study, male and female Fischer 344 rats (12-13s/sex/dose) were fed diets containing scFOS at levels of 0, 0.8, 2.0, and 5.0% (equivalent to 0, 341, 854, and 2170 mg/kg bw/day for male rats and 0, 419, 1045, and 2664 mg/kg bw/day for female rats) for 2 years. All standard parameters for such studies were measured. 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. There were slight elevations of sodium and chloride in male rats. 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 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 50,000 ppm, the highest concentration tested, equivalent to 2170 mg/kg bw/day for males and 2664 mg/kg bw/day for females.

As regards 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, 8000, 20000, and 50000 ppm 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 (20000 and 50000 ppm) was significantly greater than the incidence in 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 neosugar-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 one to three 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 results this study indicate that FOS is not carcinogenic.

**Table 6. Summary of Short-term, Subchronic and Chronic Toxicity Studies of scFOS Conducted in Rats**

| Reference | Species strain (No./sex/group; age/weight) | Route and Dose (mg/kg bw/day) | Duration | NOAEL (mg/kg bw/day) | Other Observations |
|---|---|---|---|---|---|
| **Short-term Toxicity Studies** | | | | | |
| Takeda and Niizato (1982); summarized in Carabin and Flamm (1999) | 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 10$^{th}$ 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 |
| Tokunaga et al. (1986) | 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 |
| **Subchronic Toxicity Study** | | | | | |
| Meijl Seika Kaisha (1982), cited in GRN 44 (GTC Nutrition, | 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 |

| | | | | | |
|---|---|---|---|---|---|
| 2000) | | | | | stools, cecal distension, intestine weights |
| **Chronic Toxicity and Carcinogenicity Study** | | | | | |
| Clevenger et al. (1988) | 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 |

↑ = 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

### 6.1.2.4.5. Developmental and Reproductive Toxicity Studies

A summary of the available developmental and reproductive toxicity studies of scFOS are described in Table 7. The findings of a developmental toxicity study were also summarized Carabin and Flamm (1999) from an unpublished study conducted by Henquin (1988). The available study related details are provided in Table 7. In this study, dietary administration 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 investigators 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 10000 mg/kg bw/day) were investigated. In this study rats during postcoitum days 0 to 15 were fed diet containing FOS. No treatment related adverse effects (diarrhea), or differences in pregnancy outcome or *in utero* development were noted.

**Table 7. Summary of Developmental and Reproductive Toxicity Studies of scFOS Conducted in Rats**

| Reference | Species strain (No./sex/group; age/weight) | Route and Dose | Duration | Other Observations |
|---|---|---|---|---|
| Henquin (1988); described in Carabin and Flamm (1999) | Wistar rats (29F; n=12 treatment and n=17 control) | Dietary: 0 or 10000 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<br>↓ Body weight during nursing period was reported in the treated pregnant rats and pups<br>Diarrhea observed in treated pregnant rats (number not reported) during the first week and soft stools in weeks 2 and 3 for this group<br>Growth delay in male pups in test group 3 |
| Sleet and Brightwell (1990); 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<br>No deaths or diarrhea reported<br>↓ Body weight on postcoitum Day 2 in all FOS treated rats compared to control<br>Dose related decrease in body weight for FOS treated rats<br>Body weight and body weight changes in 2500 and 5000 mg/kg bw/day groups were similar among groups from Day 12-15<br>No remarkable findings at necropsy<br>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<br>↑ Fetal weights of 10,000 mg/kg bw groups compared to control, no other reduction in litter or fetal weights |

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

### 6.1.2.4.6. Genotoxicity Studies

In a series of genotoxicity assays, Clevenger et al. (1988) tested 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 summarized in Table 8. The results of these studies consistently demonstrate that scFOS is not genotoxic in bacteria and mammalian cells in the presence or absence of metabolic activation.

**Table 8. *In vitro* Genotoxicity Studies on scFOS**

| Reference | Test system | Concentration | Metabolic Activation | Result |
|---|---|---|---|---|
| Clevenger et al., 1988 | 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 |

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

### 6.1.3. Corroborative Evidence

#### 6.1.3.1. Regulatory Agency Review

The available information suggests that several scFOS preparations have received GRAS status for use as a food ingredient in a variety of conventional foods, including infant formula (GRN 44, 537, 605, 623 and 717) (FDA, 2000; 2015; 2016a; 2016b; 2017). The currently marketed FOS products are manufactured using sucrose as a starting material that is converted to FOS using β-fructofuranosidase enzymes obtained from different non-toxigenic or 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 available safety related studies of scFOS have been extensively reviewed and described in the 2014 FDA GRAS notification (GRN, 537) on scFOS (Ingredion, 2014) for its uses in infant formula and also in a more recent GRAS notification (GRN, 717) for uses of scFOS in conventional food (Galam, 2017). In addition to these two GRAS notices, in three additional GRAS notices (GRN 623; GRN, 605; and GRN, 44) also the safety data on scFOS has been extensively described. Furthermore, there is one more GRAS notice on oligofructose (GRN, 392) that also describes the safety related information on FOS. The comparative data and information from all these GRAS notices is provided in Table 9. This comparison of these GRAS notices suggest that all these products are similar. FDA did not question the acceptability and suitability of the available evidence to support the proposed uses described in these eight GRAS notices, including its uses in infant formula, and replied to all these notifications that the agency received with recognition of the notifier's request and a statement that the agency had 'no questions' regarding the conclusions that the scFOS or oligofructose is GRAS for the intended applications. NFBC is hereby incorporating all the toxicology and human tolerance studies discussed in these previous GRAS notices by reference (Galam, 2017; NFBC, 2016; Tata, 2015; Ingredion, 2014; Pfizer, 2011; GTC, 2000).

**Table 9. Comparison of the Subject of Present GRAS, scFOS, with other FDA Accepted GRAS Notices**

| Constituents | Current GRAS | GRN 717 | GRN 623 | GRN 605 | GRN 537 | GRN 392# | GRN 44 |
|---|---|---|---|---|---|---|---|
| Manufacturing | Sucrose + fungal enzyme | Sucrose + fungal enzyme | Sucrose + fungal enzyme | Sucrose + fungal enzyme | Sucrose + fungal enzyme | Chicory inulin + carbohydrase | Sucrose + fungal enzyme |
| Total FOS (%) | ≥95 | 95±2 | ≥95 | 95±2 | NLT 95 | 93.8-96.5* | >95 |
| 1-kestose (GF2) (%) | NLT 30 | NLT 30 | NLT 30 | 35-43* | NLT 30 | 26.0-28.6* | 35±6 |
| Nystose (GF3) (%) | NLT 40 | NLT 40 | NLT 40 | 42-48* | NLT 45 | 25.5-28.7* | 50±6 |
| Fructofuranosylnystose (GF4) (%) | NLT 5 | NLT 5 | NLT 5 | 6-11* | NLT 5 | 16.7-20.0* | 10±4 |
| Sugars (%) | ≤5 | 5±2 | ≤5 | 5±2% | NMT 5 | 3.7-6.1* | <5 |
| Intended uses | Term Infant Formula | Multiple foods | Multiple foods | Multiple foods | Term Infant Formula | Term Infant Formula | Multiple foods |
| Use levels | 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% | 4 g/L starter formula; 5 g/L follow on formula | 3 g/L formula | 0.4 to 6.7% |
| EDI | **828 mg/kg/day (90th %)** | 9.09 g/day (90th %) | 12.8 g/day (90th %) | 12.8 g/day (90th %) | **828 mg/kg/day (90th %)** | 620 mg/kg/day (90th %) | 12.8 g/day (90th %) |
| ADI | At proposed use levels (4 or 5 g/L) | 20 g/day | 20 g/day | 20 g/day | At proposed use levels (4 or 5 g/L) | 3 g/L | 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 |

*Range from batches given in the GRAS notice; #GRN 392 is on Oligofructose that refers to fructans with a DP of < 10 and more specifically with > 25% of the molecules having a degree of polymerization (DP) ≥ 5 and < 75% with a DP ≤ 4. For GRN 393- DP generally in the range of 2 to 8 and predominantly (90%) in the range of 3 to 6.

The subject of present GRAS, 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 a previous GRAS (GRN, 537). These comparisons are provided in Table 10. Additionally, in both these GRAS notices, scFOS is proposed for uses in infant formula at same use levels. In both these GRAS notices, the enzyme, β-fructofuranosidase, derived from microorganism, is used in the manufacturing of FOS. The levels of scFOS (95%) in both these GRAS 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.

As scFOS manufactured by NFBC is chemically similar to scFOS preparations that have been concluded to be GRAS (e.g., GRN 537) by Ingredion (2014), a discussion of publicly available data and information relevant to the safety of scFOS is incorporated by reference to studies discussed in GRN 537. Additionally, during the review of GRN 537, FDA raised some safety related and other question that has been obtained by NFBC under FOIA request. NFBC also incorporate the information obtained under FOIA (2016). Based on all this 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 notice (GRN 537) submitted to FDA by Ingredion (2014) and the subject of this present GRAS assessment, as well as other information available, NFBC has concluded that the scFOS it intends to market for uses in infant formula is safe and GRAS.

In addition to FDA, in 2013, the Foods Standards Australia New Zealand (FSANZ) 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 scFOS 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.

Several other structurally related β2-1 fructan preparations also have GRAS status for use as food ingredients (*e.g.*, GRN 118, 392, 477 and 576- these GRAS notices and FDA responses 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.

**Table 10. Comparison of scFOS specifications with subject of GRN 537**

| Parameters | Specifications | |
|---|---|---|
| | Current GRAS | GRN 537 |
| Appearance | Off white powder | White powder |
| Taste | Slightly sweet taste | Slightly sweet |
| Total scFOS (%) | ≥95 | NLT 95 |
| 1-kestose (GF2) (%) | NLT 30.0 | NLT 30 |
| Nystose (GF3) (%) | NLT 40.0 | NLT 45 |
| Fructofuranosylnystose (GF4) (%) | NLT 5.0 | NLT 5 |
| Sugars (%) | ≤5 | NMT 5 |
| pH | 4.5-7.0 | 5.0-7.0 |
| Moisture (%) | ≤3.5 | NMT 5 |
| Ash (%) | ≤0.1 | NMT 0.05 |
| Melamine (mg/kg) | ≤0.01 | NA |
| **Heavy metals** | | |
| Lead | ≤0.02 mg/kg | NMT 1 mg/kg |
| Total Arsenic | ≤0.05 mg/kg | NMT 1 mg/kg |
| Cadmium | ≤0.1 mg/kg | NA |
| Total mercury | ≤0.01 mg/kg | NA |
| **Microbiological limits** | | |
| Total plate count | ≤1000 CFU/g | NMT 300 CFU/g |
| Yeasts | ≤20 CFU/g | NMT 20 CFU/g |

**Table 10. Comparison of scFOS specifications with subject of GRN 537**

| Parameters | Specifications | |
|---|---|---|
| | Current GRAS | GRN 537 |
| Molds | ≤20 CFU/g | NMT 20 CFU/g |
| Coliforms | <30 MPN/100g | NMT 10 CFU/g |
| *E. coli* | <3.0 MPN/100g | Negative CFU/g |
| Salmonella | Negative/25g | Negative CFU/100 g |
| Shigela | Negative/25g | NA |
| *Staphylococcus aureus* | Negative/25g | Negative CFU/g |
| Enterobacteriaceae | <0.3 MPN/g | Negative CFU/30 g |
| Listeria | Negative/25g | NA |
| *Bacillus cereus* | <3.0 MPN/g | NA |
| *Anaerobic sulfite-reducing clostridia* | <3.0 MPN/g | NA |
| *Cronobacter sakazakii* | Negative/100 g | Negative CFU/10 g |

NLT= not less than; NMT= not more than; CFU= colony-forming unit; NA = Not available

### 6.1.3.2. Unpublished Corroborative Studies

The available unpublished studies with scFOS are summarized in Table 11. An unpublished study from Abbott (1992), described in FSANZ (2013) and in GRN 537 (Ingredion, 2014), on the effects of scFOS in infants did not reveal any adverse effects. In this randomized, double-blind, placebo-controlled study, 63 healthy term infants aged 4-10 weeks were given control formula for 2 weeks, followed by a whey-enriched formula containing 0, 150, or 310 mg scFOS/100 ml formula for 2 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 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 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. 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 scFOS trimers and tetramers were recovered from the urine of most of the infants who had 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 authors concluded that "Infant formulas containing added FOS at the levels provided … are well tolerated and support normal growth in term infants." The investigators did offer any explanation for the appearance of scFOS residues in urine but not in blood. 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 another unpublished randomized, double-blind, placebo-controlled, multicenter trial (Abbott 1993) also described in FSANZ (2013) and GRN 537 (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. Another group of 25 healthy breast-fed infants aged 0-9 days constituted a human-milk reference group. Of the 70 infants receiving formula, 34 (65%) receiving formula without scFOS and 36 (72%) consuming scFOS-containing formula, as well as 23 (92%) 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. 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 11. 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

FOS are oligosaccharides that occur naturally in plants such as onion, chicory, garlic, asparagus, banana, artichoke, Jerusalem artichokes, lettuce, rye, among many others (GTC (Mitsuoka et al. 1987, Spiegel et al. 1994, Tashiro et al. 1992; Bornet et al., 2002; Sabater-Molina et al., 2009). Given their natural presence in commonly consumed vegetables, FOS is regularly consumed by humans in foods. 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

As indicated earlier, 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. The health benefits derived from the colonic fermentation of FOS in humans are well documented (Gibson and Roberfroid 1995). FOS have a number of interesting properties, including a low sweetness intensity. They are also low calorie, non-cariogenic and are considered as soluble dietary fiber. Additionally, FOS has been claimed for physiological effects such as improved mineral absorption and decreased levels of serum cholesterol, triacylglycerols and phospholipids (Sabater-Molina et al., 2009). Because of their prebiotic effects, currently FOS are increasingly included in food products and infant formulas. Their consumption increases fecal bolus and the frequency of depositions, and may help reduce constipation.

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 09, 2018, the agency has received six notices on FOS and provided "no questions" letters to all of the notifiers. These GRAS notices are described earlier. The available information suggest that scFOS is safe as currently used in conventional foods as well as in infant formula.

### 6.1.3.5. 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

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

a deviant finding that contradicts the far larger body of research that has found 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 found that little or no digestion or absorption of fructans occurs in either the stomach or the small intestine.

In both of the studies by Bjorck and Nilsson (1991) and Nilsson and Bjorck (1988), it was found 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.

In addition to the studies in rats, Nilsson and Bjorck (1988) 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. 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.

### 6.1.3.6. Degree of Polymerization (DP) and Safety

#### 6.1.3.6.1. DP and Fermentability

Given the large variation in degree of polymerization (DP) of FOS, the question can arise whether the small chain molecules of scFOS can have easier fermentability (and more and rapid gas formation) or lower fermentability (and lower and slower gas formation).

In a single-blind crossover study, 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 this study, 5 healthy men and 5 women 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. Breath samples were taken every 30 minutes for 12 hours after each test and analyzed for $H_2$. In this study, hydrogen production profiles were used to estimate orocecal transit times. Based on the findings from this study (Table 12), the investigators concluded that orocecal transit time was slower for the long-chain inulin than for the oligofructose, but it is evident 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 12. Effects of Chicory-Derived Long-chain Inulin and Oligofructose on Hydrogen Production and Gastrointestinal Transit.**

| Test article and dose | Hydrogen production ppm . minutes $10^2$ | | 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 |

In another study, Stewart and Slavin (2006) compared the *in vitro* batch fermentability of 6 chain lengths of inulin and FOS with DP ranging from 2 to >20 using human fecal inoculum. 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 yet another *in vitro* study, Bohacenko et al. (2013) compared 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*. While the enterococci fermented the shorter chain substrate more efficiently than 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."

The above described studies illustrate that the available information failed to find a clear association between DP of the oligosaccharide and fermentation rate. There is also a substantial literature that indicates that shorter-chain fructans are fermented more quickly than longer-chain ones, 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. The point is that these differences are subtle, and inconsistent, and highly variable. The variability across individuals, 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. Because of this large variability, differences in fermentation of fructans with different DP has only been shown when the DP difference is extreme, e.g., Rumessen and Gudmand-Hoyer's (1998) comparison of fructans with median DP of 3 and 12 or Stewart and Slavin's (2006) range of DP from 2 to >20—and even in this extreme case, the overall trend was not statistically significant.

In comparison, 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.

### 6.1.3.6.2. DP and Osmolality

As scFOS molecules are expected to be more osmotically active than other oligofructoses with higher DP, including the ones used studies that are used to support or corroborate the safety

of the subject of present GRAS, scFOS, it is important to address potentially higher osmotic activity is not expected to pose a concern, such as increased abdominal distension, pain and laxation, including 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.

The available information suggest that human breast milk contains more than 200 different oligosaccharides (Kunz et al. 2000; Vandenplas 2002; German et al., 2008; Ballard and Morrow 2013), with a total concentration in excess of 1200 mg/100 ml. This level is approximately 3 times higher than the level of intended use of scFOS in starter formula. Vandenplas (2002) reported concentrations of 2000 mg HMO/100 ml milk on the fourth day of life of the infant. In another study, Zivkovic et al. (2011) reported that for some mothers, the most dominant component, lacto-N-neotetraose (LNnT; mass 709.3) can be 10x more intense than the next most abundant lacto-N-fucopentaose I/V (LNFP I/V; mass 855.3). 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. All of this suggest that dominant human milk oligosaccharides (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.

In another study, 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 1 year 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). This also indicates that the predominant HMOs present in human milk at higher levels as compared to the proposed uses of scFOS, are of the same DP.

Based on the available information, galactooligosaccharides (GOS) are commonly used and have been accepted for addition to infant formula at concentrations as high as 800 mg/100 ml by FDA (GRN 236) and international regulatory agencies. In Europe, formula containing a blend of 90% GOS and 10% long-chain FOS at 800 mg/100 ml has been in use for many years. 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 of the subject of present GRAS, scFOS by NFBC is as follows: DP3= 30%; DP4= 40%; DP5= 5%. Similarly, GOS described in other GRAS notices (GRN 286, 489, 495; and 569 submitted by NFBC) for infant formula use have similar DP profiles to Vivinal® GOS. This indicates that GOS added to infant formula is more osmotically active than 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 osmolarity than formula containing the intended 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 osmolarity of infant formula is determined by its total formulation, not merely by its oligosaccharide content (if any). Infant formula manufacturers routinely test formula for osmolarity and adjust its composition as needed to assure that it is within the desired range.

### 6.2. Summary and Discussion

New Francisco (Yunfu City) Biotechnology Corporation Limited (NFBC), China, intends to market 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 *Aspergillus oryzae*. 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 NFBC 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.

The identity and composition of the final product has been well characterized. 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 mean molecular weight of scFOS is 700 Da (average of the three components). NFBC has established food grade specifications for scFOS. NFBC intends to use scFOS in 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. The conservative total daily scFOS exposure of infants 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.

As described earlier, the safety of consumption of scFOS has been supported by many studies conducted on the metabolism of scFOS and other fructans, as well as toxicological studies of scFOS and other fructans. These studies include animals as well as in humans' investigations. Additionally, the safety is also 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 determined to be GRAS for use in infant formula, while in GRN 44 (additional uses) and GRN 537 use of scFOS in infant formula was determined 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 and 717) (FDA, 2000; 2016a;

2016b; 2017). All of these GRAS notifications have consistently concluded that the addition of scFOS to food is GRAS under their respective conditions of intended use.

In several published studies, the digestibility of fructans has been investigated. These studies in rats, as well as *in vitro* studies, demonstrate 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). In one study (Bjork and Nilsson, 1991) inulin was reported to be hydrolyzed by gastric acid in rats 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 also suggest that majority of ingested fructans, including scFOS reach the colon where it is fermented by bacteria. The kinetics of orocecal transit time and bacterial fermentation are inversely proportional to the degree of polymerization of the fructan.

The results of acute toxicity studies (studies discussed by Carabin and Flamm, 1999) suggest that scFOS has a low potential for acute oral toxicity. In a subacute study, feeding Wistar rats 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 (studies 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, 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 (Barnes et al., 2012). In another study in piglets, 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 (Correa-Matos et al., 2003).

In a subchronic toxicity study, 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 (Boyle et al., 2008). No reports of treatment-related adverse effects in terms of food intake, body weight, body weight gain, clinical observations, hematology, clinical chemistry, or histopathology even at the highest dose tested. The NOAEL was the highest dose tested 4680 mg oligofructose/kg bw/day (Table 6). In a published 104-week combined chronic toxicity and carcinogenicity study in male and female Fischer 344 rats were fed scFOS (Clevenger et al., 1988) at levels up to 50,000 ppm. Some statistically significant differences were noticed but there were no toxicologically relevant, test-article-related macro or microscopic changes in either males or females. 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 F-344 rats with highly variable background incidence, the observation was a chance artifact. The findings of this study suggest that scFOS is

not carcinogenic and does not produce chronic toxicity in rats. The NOAEL was determined as 50,000 ppm, the highest concentration tested, equivalent to 2170 mg/kg bw/day for males and 2664 mg/kg bw/day for females (Table 6).

In a reproductive and developmental study of scFOS in rats fed a diet containing 20% scFOS from day 1 to day 21 of gestation, 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) (Table 7). The reduction in body weight of the pregnant rats may be due to a 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 in 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. 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 (Table 8).

In summary, in several acute; subacute; subchronic; chronic; developmental and reproductive; and mutagenicity and genotoxicity studies, the safety of consumption of scFOS and fructans has been investigated. Based on the complete body of toxicological information on scFOS and oligfructose 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 mentioned here.

In a published randomized, double-blind, placebo-controlled trial, 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 current 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.

In another published randomized, controlled, double blind study, 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 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 current 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 NFBC proposed use of scFOS in follow on formula.

In additional studies that used FOS, but not necessarily scFOS (either high dose or for a long period of time) mentioned briefly here also further supports the safety of scFOS in infants. In a published randomized, double-blind, placebo controlled study (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 on the intestinal microbiota- cow's milk (control), human milk (reference), and two FOS groups (240 or 340 mg scFOS/100 ml). The FOS used 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. In another published randomized, double-blind trial in 116 healthy term infants, Brunser et al. (2006b) 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. Seventy-six 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.

In yet another, double-blind study in infants, Bettler and Euler (2006) studied growth and tolerance in healthy full-term infants (aged 14 days or less) fed formula supplemented with oligofructose (150 or 300 mg/100 ml) for 12 weeks. 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 earlier. In these studies no adverse effects were reported.

Based on a consideration of the totality of the 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 that is 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) 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).

### 6.3. Expert Panel Review and Conclusion

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel)[4], qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested New Francisco (Yunfu City) Biotechnology Corporation Limited (NFBC) to evaluate the Generally Recognized As Safe (GRAS) status 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. A comprehensive search of the scientific databases for safety and toxicity information on FOS was conducted through March 2018. Additionally, safety and regulatory evaluations by national and international agencies were also searched and considered for the present assessment.

The Expert Panel independently and critically evaluated materials submitted by NFBC, and other information deemed appropriate or necessary. Following an independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the conclusion described herein. The Expert Panel was selected and convened in accordance with the Food and Drug Administration (FDA)'s guidance for industry on "Best Practices for Convening a GRAS Panel"[5]. NFBC ensured that all reasonable efforts were made to identify and select a balanced Expert Panel with expertise in food safety, toxicology, infant nutrition and nutrition. Efforts were made to identify conflicts of interest or relevant "appearance issues" that could potentially bias the outcome of the deliberations of the Expert Panel. No such conflicts of interest or "appearance issues" were identified. The Expert Panel received a reasonable honorarium as compensation for their time; the honoraria provided to the Expert Panel were not contingent upon the outcome of their deliberations.

Based on a critical evaluation of the publicly available data described and summarized herein, the Expert Panel members whose signatures appear below, have individually and collectively 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).

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[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.

[4]Modeled after that described in section 201(s) of the Federal Food, Drug, and Cosmetic Act, As Amended. See also attachments (curriculum vitae) documenting the expertise of the Panel members.

[5] Available at: https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm583856.htm

It is also our opinion 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.

**Signatures**

(b) (6)

Douglas L. Archer, Ph.D. — June 14, 2018 — Date

(b) (6)

Robert L. Martin, Ph.D. — June 9, 2018 — Date

(b) (6)

Roger A. Clemens, DrPH, FIFT, CFS, FASN, FACN — 15 June 2018 — Date

(b) (6)

Madhusudan G. Soni, Ph.D., F.A.C.N., FA.T.S.
Advisor to Panelists — June 19, 2018 — Date

## 7. Part VII – SUPPORTING DATA AND INFORMATION


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## APPENDIX I

**Product specification sheets for both NFBC FOS products: FOS-950-P; FOS-950-S**



云浮市新金山生物科技股份有限公司
New Francisco (Yunfu City) Biotechnology Corporation Limited

# Specification

Effective Date: 09/01/2018

Approved Date: 09/01/2018

## King-Prebiotics®

### Fructo-oligosaccharides (FOS-950-P)

King-Prebiotics® FOS is manufactured from food grade sucrose via a transfructosylation catalyzed by an official defined GRAS β-fructofuranosidase. FOS is the natural prebiotics that promotes the growth of Bifidobacteria in the colonic microbiome.

### Compositional Specification

| | | | |
|---|---|---|---|
| Moisture | ≤3.5% | Cadmium | ≤0.1mg/kg |
| T.FOS (d.m.) | ≥95% | Total Arsenic | ≤0.05mg/kg |
| Sucrose + monosaccharides (d.m.) | ≤5% | Total Mercury | ≤0.01mg/kg |
| pH (30%) | 4.5–7.0 | Lead | ≤0.02mg/kg |
| Ash | ≤0.1% | Melamine | ≤0.01mg/kg |

### Microbiological Specification

| | | | |
|---|---|---|---|
| T.B.C | ≤500 CFU/g | Coliforms | ≤3.0 MPN/g |
| Enterobacteriaceae | <0.30 MPN/g | E.coli | <3.0 MPN/g |
| Staphylococcus aureus | Neg./25g | Salmonellae | Neg./25g |
| Yeasts and moulds | ≤20 CFU/g | Shigella | Neg./25g |
| Bacillus cereus | <3.0 MPN/g | Listeria | Neg./25g |
| Cronobacter sakazakii | Neg./100g | | |

### Allergens

| | | | |
|---|---|---|---|
| Gluten | Absent | Enzyme | Absent |
| Colza | Absent | Meat/egg derivatives | Absent |
| Folate | Absent | Seed/soy derivatives | Absent |
| Vitamins and minerals | Negligible | Insecticides, pesticides | Absent |
| Nuts, nut components | Absent | Other allergens | Absent |
| Protein | Absent | Fat | Absent |
| Milk | Absent | | |

### Sensory

| | |
|---|---|
| Appearance | Off white light yellow powder |
| Taste | Slightly sweet |

New Francisco (Yunfu City) Biotechnology Corporation Limited
Address:
Swan-Kan-Chiau Ind. Dist., Kaofong Village,
Yunfu City, Guangdong, China (527343)
Tel: +86-766-8750999
Fax : +86-766-8750800
Please visit www.nfbc.com.cn or email service@nfbc.com.cn

云浮市新金山生物科技股份有限公司
New Francisco (Yunfu City) Biotechnology Corporation Limited

## Other Information

# King-Prebiotics®

## Fructo-oligosaccharides (FOS-950-P)

### Nutritional data per 100g

* Note: The nutrition values are for reference only. Please refer to local regulations for actual product labelling.

| | | | |
|---|---|---|---|
| Energy* | 200 Kcal or 835 KJ | Total carbohydrate (g) | 95.0 |
| Protein (g) | 0 | Moisture (g) | 5.0 |
| Fat, Saturated fat, Trans fat (g) | 0 | Fructo-oligosaccharides (g) | 90.3 |
| Sodium (mg) | 0 | Sugar (g) | 4.8 |

***In compliance with EU legislation (EU/1169/2011)**

### Packaging & Storage

| | |
|---|---|
| Package | 25 KG multiple-layered paperbag with inner polyethylene liner |
| Storage condition | Keep in clean, dry and dark conditions.<br>Keep away from strongly odorous materials. |
| Shelf life | 24 months after date of production |

### Safety & Hazard

| | |
|---|---|
| Safety | Safe. Not toxic. Not dangerous.<br>Excessive consumption may cause laxative effects.<br>Like other fine powders, it may explode upon ignition in air. |
| Risk statement | None |
| Hazard category | Not harmful |
| Irradiation | Not irradiated |
| Pesticide residue | Negligible |
| Aflatoxin B1 | ≤5 μg/kg |

### Certification

| | |
|---|---|
| FSSC | 22000 |
| ISO | 9001:2015<br>22000:2005 |
| Kosher | Orthodox Union |
| Halal | Majelis Ulama Indonesia (MUI)<br>Shandong Halal Certification (SHC) |
| Social accountability | SA8000:2015 |
| FDA GRAS | GRN No.623 |

**New Francisco (Yunfu City) Biotechnology Corporation Limited**
Address:
Swan-Kan-Chiau Ind. Dist., Kaofong Village, Yunfu City, Guangdong, China (527343)
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Please visit www.nfbc.com.cn or email service@nfbc.com.cn

云浮市新金山生物科技股份有限公司
New Francisco (Yunfu City) Biotechnology Corporation Limited

# Specification

Effective Date: 09/01/2018

Approved Date: 09/01/2018

## King-Prebiotics®

### Fructo-oligosaccharides (FOS-950-S)

King-Prebiotics® FOS is manufactured from food grade sucrose via a transfructosylation catalyzed by an official defined GRAS β-fructofuranosidase. FOS is the natural prebiotics that promotes the growth of Bifidobacteria in the colonic microbiome.

## Compositional Specification

| | | | |
|---|---|---|---|
| Dry matter | ≥75% | Cadmium | ≤0.1mg/kg |
| T.FOS (d.m.) | ≥95% | Total Arsenic | ≤0.05mg/kg |
| Sucrose + monosaccharides (d.m.) | ≤5% | Total Mercury | ≤0.01mg/kg |
| pH (30%) | 4.5 – 7.0 | Lead | ≤0.02mg/kg |
| Ash | ≤0.1% | Melamine | ≤0.01mg/kg |

## Microbiological Specification

| | | | |
|---|---|---|---|
| T.B.C | ≤500 CFU/g | Coliforms | ≤3.0 MPN/g |
| Enterobacteriaceae | <0.30 MPN/g | E.coli | <3.0 MPN/g |
| Staphylococcus aureus | Neg./25g | Salmonellae | Neg./25g |
| Yeasts and moulds | ≤20 CFU/g | Shigella | Neg./25g |
| Bacillus cereus | <3.0 MPN/g | Listeria | Neg./25g |
| Cronobacter sakazakii | Neg./100g | | |

## Allergens

| | | | |
|---|---|---|---|
| Gluten | Absent | Enzyme | Absent |
| Colza | Absent | Meat/egg derivatives | Absent |
| Folate | Absent | Seed/soy derivatives | Absent |
| Vitamins and minerals | Negligible | Insecticides, pesticides | Absent |
| Nuts, nut components | Absent | Other allergens | Absent |
| Protein | Absent | Fat | Absent |
| Milk | Absent | | |

## Sensory

| | |
|---|---|
| Appearance | Off white light yellow syrup |
| Taste | Slightly sweet |

New Francisco (Yunfu City) Biotechnology Corporation Limited
Address:
Swan-Kan-Chiau Ind. Dist., Kaofong Village, Yunfu City, Guangdong, China (527343)
Tel: +86-766-8750999
Fax : +86-766-8750800
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## Other Information

# King-Prebiotics®

Fructo-oligosaccharides (FOS-950-S)

### Nutritional data per 100g

* Note: The nutrition values are for reference only. Please refer to local regulations for actual product labelling.

| | | | |
|---|---|---|---|
| Energy* | 158 Kcal or 659 KJ | Total carbohydrate (g) | 75.0 |
| Protein (g) | 0 | Moisture (g) | 25.0 |
| Fat, Saturated fat, Trans fat (g) | 0 | Fructo-oligosaccharides (g) | 71.3 |
| Sodium (mg) | 0 | Sugar (g) | 3.8 |

***In compliance with EU legislation (EU/1169/2011)**

### Packaging & Storage

| | |
|---|---|
| Package | 35KG or 1200KG food grade high-density polyethylene |
| Storage condition | Keep in clean, dry and dark conditions.<br>Keep away from strongly odorous materials. |
| Shelf life | 12 months after date of production |

### Safety & Hazard

| | |
|---|---|
| Safety | Safe. Not toxic. Not dangerous.<br>Excessive consumption may cause laxative effects.<br>Like other fine powders, it may explode upon ignition in air. |
| Risk statement | None |
| Hazard category | Not harmful |
| Irradiation | Not irradiated |
| Pesticide residue | Negligible |
| Aflatoxin B1 | ≤5 μg/kg |

### Certification

| | |
|---|---|
| FSSC | 22000 |
| ISO | 9001:2015<br>22000:2005 |
| Kosher | Orthodox Union |
| Halal | Majelis Ulama Indonesia (MUI)<br>Shandong Halal Certification (SHC) |
| Social accountability | SA8000:2015 |
| FDA GRAS | GRN No.623 |

**New Francisco (Yunfu City) Biotechnology Corporation Limited**
Address:
Swan-Kan-Chiau Ind. Dist., Kaofong Village, Yunfu City, Guangdong, China (527343)
Tel: +86-766-8750999
Fax : +86-766-8750800
Please visit www.nfbc.com.cn or email service@nfbc.com.cn

| | |
|---|---|
| **From:** | Madhu Soni |
| **To:** | Morissette, Rachel |
| **Subject:** | RE: missing information for newly received GRAS notice for FOS in infant formula |
| **Date:** | Thursday, July 26, 2018 1:25:21 PM |
| **Attachments:** | image002.png<br>Expert Panelists CVs- FOS IF GRAS.pdf |

Dear Dr. Morissette,

Please accept my apology for the oversight. Please find attached, CVs of all the expert panelists combined in one file. If you have any questions, please let me know.

Best regards

Madhu

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Madhu G. Soni, *PhD, FACN, FATS*
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Vero Beach, FL 32968, USA
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www.soniassociates.net

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**From:** Morissette, Rachel [mailto:Rachel.Morissette@fda.hhs.gov]
**Sent:** Thursday, July 26, 2018 10:24 AM
**To:** Madhu Soni <sonim@bellsouth.net>
**Subject:** missing information for newly received GRAS notice for FOS in infant formula

Dear Dr. Soni,

I am the Consumer Safety Officer assigned to your recent GRAS notice for the intended use of FOS in infant formula. Before I can file this notice, I need to clarify some missing information that I identified. On page 52 of 64, Footnote 4 refers to attachments containing the CVs of the GRAS Panel; however, FDA did not receive a copy of these attachments with the submitted notice. Can you please provide these missing attachments to me via email at your earliest convenience? Once I receive those documents, I can proceed with processing your filing letter.

Best regards,

*Rachel*

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**Rachel Morissette, Ph.D.**
*Consumer Safety Officer*

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

FDA U.S. FOOD & DRUG ADMINISTRATION

Seventy three pages of Curriculum Vitae removed in accordance with the Privacy Act of 1974.

| | |
|---|---|
| **From:** | Kulas, Megan |
| **To:** | Morissette, Rachel; West-Barnette, Shayla |
| **Cc:** | Assar, Carrie; Tonucci, Linda H; Wolcoff, Suzanne |
| **Subject:** | GRN 797 45-day meeting |
| **Date:** | Thursday, September 27, 2018 10:04:46 AM |

Good Morning Rachel,

I'm the CSO assigned to this GRN (with mentoring support from Linda, this is my first one).

1. After reviewing the GRN, we have the one comment: On Page 12 the notification states: "However, infant formulas manufacturers typically provide instructions with the infant formula indicating that hot water should be used in the preparation of the liquid formula. This should minimize the potential for C. sakazakii being present in the product served."

   This is not the practice in the US to label the product with mixing instructions using hot water.

2. Additionally, we would like to discuss the following during the meeting, Page 16 "There are no other sources of FOS or fructans in the diets of formula fed infants. Introduction of any foods that might possibly contain FOS or other nondigestible carbohydrates would be at the expense of formula…"

Please let me know if DBGNR has any questions/comments about ours above. Also, since I am learning the ropes, any advice/feedback is greatly appreciated.

Looking forward to Monday's discussion,
Megan

**Megan Kulas, M.S.**
Consumer Safety Officer
Infant Formula and Medical Foods Staff
Office of Nutrition and Food Labeling
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: (301) 796-8131
megan.kulas@fda.hhs.gov

| | |
|---|---|
| **From:** | Madhu Soni |
| **To:** | Morissette, Rachel |
| **Subject:** | RE: questions to be addressed for GRN 000797 |
| **Date:** | Monday, October 15, 2018 2:49:42 PM |
| **Attachments:** | image001.png<br>FOS GRAS IF FDA Query Response Final-1.pdf |

Dear Dr. Morissette,
Please find attached a pdf file providing a point-by-point response to the agency queries related to GRN 797. I hope the information and clarifications, along with some discussion in the response addresses the queries. If you have any questions or need additional explanation, please let me know. Thank you for the opportunity to provide this explanation.

Best regards

Madhu

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**From:** Morissette, Rachel [mailto:Rachel.Morissette@fda.hhs.gov]
**Sent:** Wednesday, October 3, 2018 12:09 PM
**To:** Madhu Soni <sonim@bellsouth.net>
**Subject:** questions to be addressed for GRN 000797

Dear Dr. Soni,

Please see attached questions to be addressed for GRN 000797.

In addition to the attached questions, we wanted to draw your attention to an issue that was raised by our Infant Formula and Medical Foods Staff (IFMFS), from the office that administers the Infant Formula Notification (IFN) program, who also reviewed the notice. On page 12 of the notice, NFBC states the following:

"However, infant formula manufacturers typically provide instructions with the infant formula indicating that hot water should be used in the preparation of the liquid formula. This, should minimize the potential for C. *sakazakii* being present in the product as served."

IFMFS asked that I convey the message to you that labeling the product with mixing instructions using hot water is NOT the practice in the United States. Please see 21 CFR 107.20, "Directions for use," for labeling requirements for preparation and use. Additionally, please see 21 CFR 106.55(e) for controls to prevent adulteration from microorganisms. While this is not an issue for the current GRAS notice, if a statement like this were included in the Infant Formula Notification itself, it would raise an issue with IFMFS. I wanted to convey that message so you're aware of the fact that the quoted sentence would be problematic in an IFN.

If you have any questions, please let me know.

Best regards,

**Rachel Morissette, Ph.D.**
*Consumer Safety Officer*

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


| | |
|---|---|
| **From:** | Madhu Soni |
| **To:** | Morissette, Rachel |
| **Subject:** | RE: questions to be addressed for GRN 000797 |
| **Date:** | Wednesday, October 17, 2018 12:04:45 PM |
| **Attachments:** | image002.png<br>FOS GRAS IF FDA Query Response Final-R1.pdf |

Dear Dr. Morissette,
As per our discussion, please find attached a revised copy of the response to FDA queries. Hope this is acceptable. If you have any questions, please let me know.

Best regards

Madhu

**From:** Madhu Soni [mailto:sonim@bellsouth.net]
**Sent:** Monday, October 15, 2018 2:49 PM
**To:** 'Morissette, Rachel' <Rachel.Morissette@fda.hhs.gov>
**Subject:** RE: questions to be addressed for GRN 000797

Dear Dr. Morissette,
Please find attached a pdf file providing a point-by-point response to the agency queries related to GRN 797. I hope the information and clarifications, along with some discussion in the response addresses the queries. If you have any questions or need additional explanation, please let me know. Thank you for the opportunity to provide this explanation.

Best regards

Madhu

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**From:** Morissette, Rachel [mailto:Rachel.Morissette@fda.hhs.gov]
**Sent:** Wednesday, October 3, 2018 12:09 PM
**To:** Madhu Soni <sonim@bellsouth.net>
**Subject:** questions to be addressed for GRN 000797

Dear Dr. Soni,

Please see attached questions to be addressed for GRN 000797.

In addition to the attached questions, we wanted to draw your attention to an issue that was raised by our Infant Formula and Medical Foods Staff (IFMFS), from the office that administers the Infant Formula Notification (IFN) program, who also reviewed the notice. On page 12 of the notice, NFBC states the following:

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preparation of the liquid formula. This, should minimize the potential for C. *sakazakii* being present in the product as served."

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If you have any questions, please let me know.

Best regards,

**Rachel Morissette, Ph.D.**
*Consumer Safety Officer*

**Center for Food Safety and Applied Nutrition**
**Office of Food Additive Safety**
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rachel.morissette@fdahhs.gov


Dear Dr. Morissette,

**RE: GRN 797 (Fructo-oligosaccharide GRAS notice for Infant Formula)**

This responds to your email of October 3, 2018, regarding your queries that need to be addressed for fructo-oligosaccharide (FOS) notice (GRN 797) submitted on behalf of New Francisco Biotechnology Corporation (NFBC), China. We are providing a point-by-point response to all your queries along with some relevant clarifications/discussion.

**FDA Query:** (1) On page 7 of the notice under 2.1.4. Chemical Formula and Molecular Weight, NFBC states the following:

> "The molecular weight of scFOS is 700 daltons (Da), representing the average of the molecular weights of its 3 components (505 Da, 666 Da, 828 Da, respectively), respectively."
>
> Please clarify the average molecular weight, since the average of the three numbers given is not 700 Da.

**Response:** Thank you for bringing this to out attention. We apologize for the confusion in our calculations. scFOS is a mixture of sucrose (342 Da), 1-kestose (505 Da), nystose (666 Da), and fructofuranosylnystose (828 Da). Based on scFOS composition from 10 batches listed in Tables 2 and 3 of the GRAS notice (GRN 797), the average ratio of the above mentioned four components will be 3.7%, 37.0%, 47.6% and 11.7%, respectively (please see below Appendix I). Therefore, the weighted average molecular weight of these components of scFOS is 614 Da. The details, along with calculations, are provided in Appendix I.

**FDA Query:** (2) Please clarify the type of ion exchange resin used in the manufacture of FOS and whether it complies with a specific regulation or effective FCN.

**Response:**

Ion exchange resins are widely used in a variety of applications including commercial, industrial water purification, drinking water applications, food processing, and pharmaceutical industries. The ion exchange resins used by NFBC in the manufacture of scFOS are compliant with the FDA Food Additive Regulation 21 CFR § 173.25.

**FDA Query:** (3) On page 16 of the notice, NFBC states the following:

> "There are no other sources of FOS or fructans in the diets of formula fed infants. Introduction of any foods that might possibly contain FOS or other nondigestible carbohydrates would be at the expense of formula (i.e., to maintain the same caloric

intake, formula consumption would necessarily decrease as solid foods are added) and it is quite likely that the result of introduction of other foods would be a net decrease in prebiotic intake - certainly in fructan - including FOS, intake."

The meaning of this sentence is unclear. Please clarify if the meaning is as follows; if not, please clarify what the relevant statement in the notice means:

As an infant consumes complimentary foods, and therefore consumes less infant formula, the level of FOS consumed will decrease due to the complimentary foods not containing the same level of FOS as infant formula.

**Response:** Sorry for the confusion. The meaning provided by FDA is correct.

**FDA Query:** (4) The GRAS Panel members state that a literature search was conducted through March 2018. Please verify if NFBC conducted the same literature search through March 2018. If not, please provide the date range through which NFBC conducted a literature search.

**Response:** The NFBC conducted the same literature search through March 2018. Sorry for not stating this.

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 your questions.

Best regards

Madhu Soni, PhD

Agent for: New Francisco Biotechnology Corporation (NFBC), China

## Appendix I

Determination of the average weighted molecular weight

| | | Table 2 FOS-950-P (GRN 797) | | | | | Table 3 FOS-950-S (GRN 797) | | | | | Calculation | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | **Standard Specifications** | **Lot #17005** | **Lot #17006** | **Lot #18001** | **Lot #18002** | **Lot #18003** | **Lot #16001** | **Lot #16002** | **Lot #16003** | **Lot #16004** | **Lot #17001** | **Average ratio (%)** | **Molecular Weight (Dalton)** | **Average weighted (Dalton)** |
| Total scFOS (%) | ≥95 | 95.7 | 96.4 | 96.4 | 96.5 | 96.5 | 95.8 | 96.3 | 96.7 | 96.1 | 96.6 | 96.3 | | **614** |
| 1-kestose (GF2) (%) | NLT 30.0 | 35.7 | 38.3 | 36.2 | 37.7 | 35 | 36.2 | 37.5 | 36.3 | 38.6 | 38.2 | 37.0 | 505 | 186.7 |
| Nystose (GF3) (%) | NLT 40.0 | 48 | 46.7 | 49 | 47.1 | 49.2 | 48.1 | 47.1 | 48 | 45.6 | 47.1 | 47.6 | 666 | 316.9 |
| Fructofuranosylnystose (GF4) (%) | NLT 5.0 | 12 | 11.4 | 11.2 | 11.7 | 12.3 | 11.5 | 11.7 | 12.4 | 11.9 | 11.3 | 11.7 | 828 | 97.2 |
| Sugars (%) | ≤5 | 4.3 | 3.6 | 3.6 | 3.5 | 3.5 | 4.2 | 3.7 | 3.3 | 3.9 | 3.4 | 3.7 | 342 | 12.7 |
| GF2+GF3+GF4+sugar | Total Carbohydrate | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | | |