GRAS NOTICE FOR SHORT-CHAIN FRUCTO-OLIGOSACCHARIDES (scFOS)

PREPARED FOR:
Office of Food Additive Safety (FHS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD
20740

DATE:
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GRAS Notice for Short-Chain Fructo-Oligosaccharides (scFOS)

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GRAS Notice for Short-Chain Fructo-Oligosaccharides (scFOS)

Part 1. §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §§170.203 through 170.285, Galam Ltd. hereby informs the U.S. (United States) Food and Drug Administration (FDA) that short-chain fructo-oligosaccharides (scFOS), as manufactured by Galam, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Galam'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. In addition, as a responsible official of Galam, Fernando Schved hereby certifies that all data and information presented in this notice represents a complete, representative, and balanced submission, and which considered all unfavorable as well as favorable information known to Galam and pertinent to the evaluation of the safety and GRAS status of the use of Galam's scFOS as an ingredient for addition to food.

Signed,

(b) (6)

18-6-17

Fernando Schved, Ph.D.
VP R&D Support and Chief Scientist - CTO
Galam Ltd.
shvedf@galam.co.il

Date

1.1 Name and Address of Notifier

Galam Ltd.
Kibbutz Maanit
M.P. Menashe
3785500, Israel

1.2 Common Name of Notified Substance

short-chain fructo-oligosaccharides (scFOS)

1.3 Conditions of Use

The scFOS manufactured by Galam is intended for use in the same food categories and use levels to those previously determined to be GRAS in GRN 44 (U.S. FDA, 2007). Accordingly, scFOS produced by Galam is intended to be marketed for "*...use in foods in general, excluding meat and poultry products and infant formula, at levels up to 20 grams (g) per day in the general population and at levels up to 4.2 g per day in infants less than one year of age*" (GRN 44). Galam's scFOS preparations will serve as an alternative to existing GRAS sources of FOS available in the U.S. marketplace and the introduction of the ingredient would not change the dietary exposure to scFOS among U.S. consumers of foods to which FOS may be added. As described in

GRN 44 examples of typical food uses and use levels of scFOS in conventional food and beverage products are shown in Table 1.3-1. Galam considers these food types and use levels to represent the same types of foods and use levels that scFOS manufactured by Galam will be used in.

Table 1.3-1 Summary of the Typical Food-Uses and Use Levels for scFOS in the U.S.

Food Category[a,b]	Standard Serving Size (mL or g)	Typical Use Level of scFOS	
		(%)	(mL or g /serving)[c]
Acidophilus Milk	240 mL	0.4	0.96
Analogs and Substitutes for Meat, Poultry or Fish	15 to 85 g	1.2 to 6.7	0.18 to 5.70 g
Bars	40 to 70 g	1.4 to 2.5	0.56 to 1.75 g
Breakfast Cereals	40 to 55 g	1.8 to 2.5	0.72 to 1.38 g
Beverages and Juices	240 mL	0.4	0.96 mL
Cakes	55 g	1.8	0.99 g
Cheese	30 to 110 g	0.9 to 3.3	0.27 to 3.63 g
Cream	15 to 30 g	3.3 to 6.7	0.50 to 2.01 g
Confectionery	40 g	2.5	1.00 g
Cookies	30 g	3.3	0.99 g
Crackers	15 to 30 g	3.3 to 6.7	0.50 to 2.01 g
Dessert Toppings and Fillings	30 g	3.3	0.50 g
Hard Candy	15 g	6.7	1.01 g
Ice Cream	68 g	1.5	1.02 g
Infant Foods (0 to 12 Months)[d]	7 to 60 g	0.4 to 3.6	0.03 to 2.16 g
Jams and Jellies	20 g	5.0	1.00 g
Milk, Flavored and Unflavored	240 mL	0.4	0.96 mL
Milk, Evaporated and Condensed	30 mL	2.6 to 3.1	0.78 to 0.93 mL
Muffins and Quick Bread	50 to 55 g	1.8 to 2.0	0.90 to 1.10 g
Sauces, Gravies, and Condiments	30 to 125 g	0.8 to 3.3	0.24 to 4.13 g
Snacks	30 g	3.3	0.99 g
Sorbet and Sherbet	85 g	1.2	1.02 g
Soup	245 g	0.4	0.98 g
Toddler Foods (12 to 24 Months)	15 to 125 g	0.8 to 6.7	0.12 to 8.38 g
Yogurt	225 mL	0.4	0.90 mL

RTD = ready-to-drink; RTE = ready-to-eat; scFOS = short-chain fructo-oligosaccharides

[a] The food use categories, standard serving sizes and proposed use levels (%) are adapted from GRN 44 additional correspondence (U.S. FDA, 2007).

[b] Use levels may be different from the intended use in the original notice.

[c] Calculated based on standard serving size and proposed % used level.

[d] This category excludes infant formula.

1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a) and (b) of the Code of Federal Regulations (CFR) (U.S. FDA, 2016a), short-chain fructo-oligosaccharides (scFOS) manufactured by Galam, has been concluded to have GRAS status for general food and beverage uses described in Section 1.3 on the basis of scientific procedures.

1.5 Availability of information

The data and information that serve as the basis for this GRAS Notification will be made available to the FDA for review and copying upon request during business hours at the offices of:

Galam Ltd.
Kibbutz Maanit
M.P. Menashe
3785500, Israel

In addition, should the FDA have any questions or additional information requests regarding this notification during or after the Agency's review of the notice, Galam will supply these data and information.

1.6 Freedom of Information Act, 5 U.S.C. 552

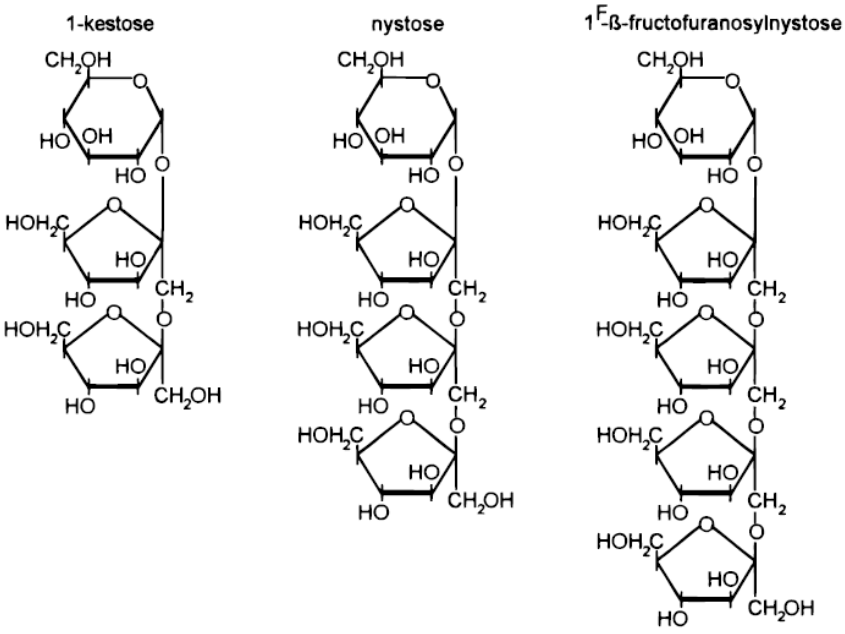
It is Galam's view that all data and information presented in parts 2 through 7 of this notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore all data and information presented herein are not exempt from the Freedom of Information Act, 5 U.S.C. 552.

Part 2. §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Chemical and Physical Characteristics

Short-chain FOS preparations currently used as food ingredients are generally produced by enzymatic synthesis from sucrose (glucose + fructose; GF) and are characterized as short-chain mixtures of fructose oligomers of which one (GF2; 1-kestose), two (GF3; nystose), or three (GF4; β-fructofuranosyInystose) additional fructose units have been added by β2-1 glycosidic linkages to the fructose unit of sucrose (Figure 2.1-1). Accordingly, ingredients produced in this manner are typically defined by the common name short-chain FOS (scFOS). Galam's scFOS preparation will be marketed as a high-purity (95% ±2) powder or syrup (FOS 95).

Figure 2.1-1 Molecular Structure of scFOS Components (from Campbell *et al.,* 1997)



In addition to scFOS, the chemically related fructans, oligofructose and inulin are defined as longer chain oligomers/polymers of β2-1 linked fructose molecules that may or may not have a terminal glucose molecule and 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. Several of these structurally related β2-1 fructan preparations also have GRAS status for use as food ingredients (*e.g.*, GRN 118, 392, 477 and 576) (U.S. FDA, 2003, 2012, 2014, 2015a). 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. Due to qualitative and quantitative differences between scFOS and other inulin type fructans, the subject of this GRAS dossier has been limited to discussion of scFOS produced from sucrose by enzymatic synthesis.

2.1.1 Purity Analysis of FOS 95

Purity analysis of Galam's scFOS powder (FOS 95; Lot No. (b) manufactured under conditions representative of those described herein were conducted using high performance liquid chromatography (HPLC) with Refractive Index (RI) detection. The HPLC-RI chromatogram for FOS 95 is provided in Figure 2.1.1-1 and the results of analysis for FOS 95 is also provided in Table 2.1.1-1. The results of the analysis 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, dextrose and fructose represent the major impurities in the ingredient.

Figure 2.1.1-1 FOS 95 HPLC-RI Chromatogram

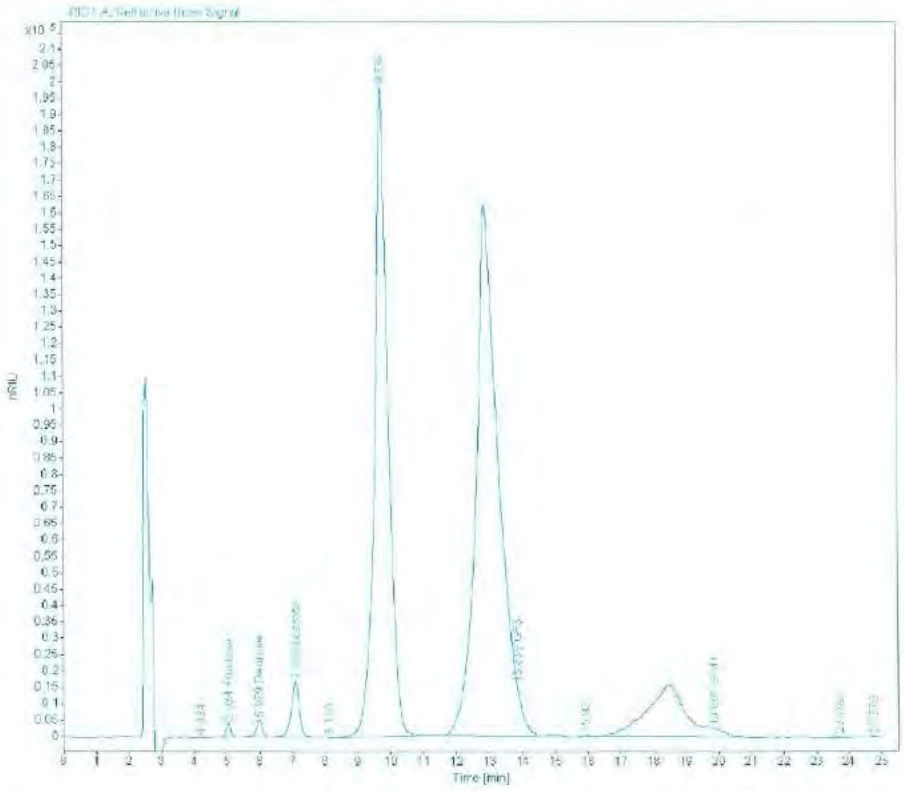


Table 2.1.1-1 FOS 95 HPLC with Refractive Index Detection Results

Peak Number	Name	Area (%)
2	Fructose	0.27
3	Dextrose	0.55
4	Sucrose	1.85
6	GF2	37.25
7	GF3	51.22
9	GF4	8.75

GF2 = 1-kestose; GF3 = nystose; GF4= β-fructofuranosyl-nystose; HPLC = high performance liquid chromatography

2.2 Manufacturing

2.2.1 Raw Materials and Processing Aids

The raw materials and processing aids used in the manufacture of the scFOS ingredients are listed in Table 2.2.1-1. All raw materials and processing aids used in the manufacture of Galam's scFOS ingredients meet food-grade quality specifications[1] and are permitted for use in food by U.S. federal regulation or are GRAS for their respective uses.

Table 2.2.1-1 Raw Materials and Processing Aids Used in the Manufacture of Galam's scFOS Ingredients

Material	Function	Regulatory Status (U.S. FDA, 2016a)
Sucrose	Reactant	21 CFR § 184.1854
Reverse osmosis water		-
Enzyme preparation with fructosyltransferase activity from *Aspergillus aculeatus* (standard and Passover preparations)	Processing aid	GRAS (see Section 3.2)
Ion exchange resin	Resin for enzyme immobilization	21 CFR § 173.25 21 CFR § 177.2710
Chromatography resin	Resin for chromatography	21 CFR § 173.25 21 CFR § 177.1655
Acetic acid	Buffer for immobilization	21 CFR § 184.1005
Citric acid	Buffer for immobilization	21 CFR § 184.1033
Activated carbon	Filtration aid	GRAS based on history of safe use
Calcium hydroxide	pH adjustment	21 CFR § 184.1205
Potassium hydroxide	pH adjustment	21 CFR § 184.1631

GRAS = Generally Recognized as Safe; scFOS = short-chain fructo-oligosaccharide

2.2.2 Production Enzyme

The enzyme used in the production of scFOS manufactured by Galam is an enzyme preparation with fructosyltransferase activity, which is derived from a non-genetically modified strain of *Aspergillus aculeatus*. The enzyme preparation is immobilized on a cross-linked polystyrene divinylbenzene (DVB) ion exchange resin and will not be introduced to the finished ingredient. The enzyme preparation has GRAS status, is food-grade and complies with relevant the Food Chemicals Codex (FCC, 2012) and Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006) specifications for food enzyme preparations.

2.3 Production Details and Schematics

scFOS ingredients that have GRAS status, including the preparations described in GRN 44, 537, 605, and 623, are produced in a similar manner involving the enzymatic transfructosylation of sucrose to produce a characteristic scFOS mixture of GF2, GF3, GF4 oligomers (U.S. FDA, 2000, 2015b, 2016b,c). The FOS solution is then typically filtered to remove the enzyme, de-colored with activated carbon, purified using ion-exchange, chromatography or nano-filtration methods, heat sterilized, and then concentrated to a syrup and/or spray dried to a powder. scFOS manufactured by Galam is produced in a similar manner, with an optimization

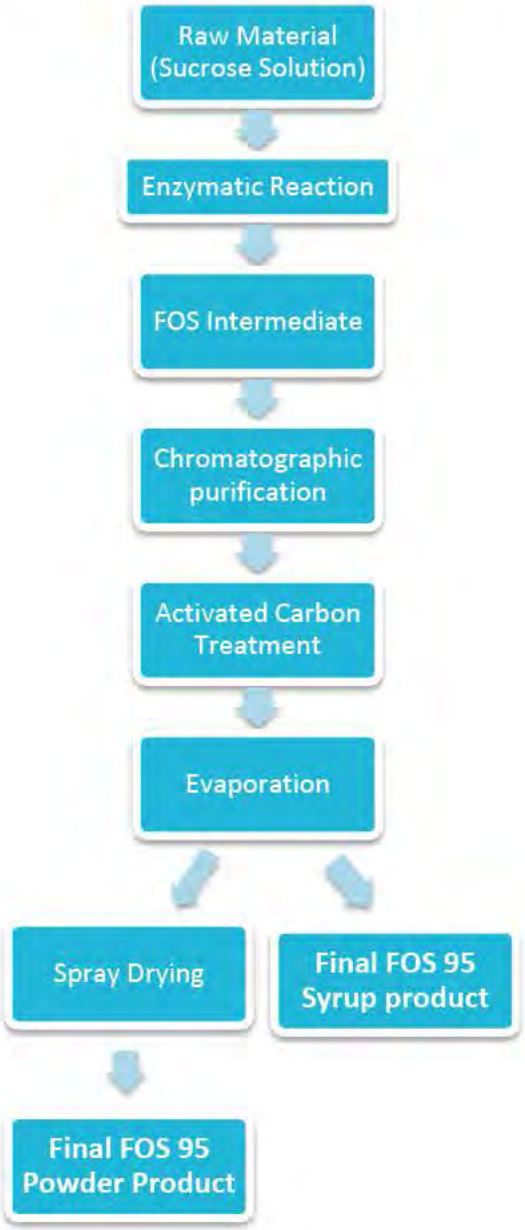
[1] Compliant with the specifications set forth in the Food Chemicals Codex or equivalent international food or pharmacopeia standard (*e.g.*, JECFA).

modification to incorporate the use of enzyme immobilization system during FOS synthesis. The manufacturing process is conducted in accordance with cGMP, the principles of Hazards Analysis and Critical Control Point (HACCP) and ISO 9001:2008 standards. The manufacturing process is described briefly below.

In the first step, a sucrose syrup solution is passed through an ion exchange resin containing immobilized enzyme. The enzyme is immobilized onto the ion exchange resin based on cross-linked polystyrene DVB following a short incubation period with acetate buffer/citrate at slightly acidic to neutral pH. The eluate produced from the reaction column contains >55% scFOS and the reaction flow rate is optimized in-process based on reaction efficiency which is assayed using HPLC-RI analysis. Next, the FOS intermediate solution is purified by column chromatography to reduce the monosaccharide content to the desired concentrations. The FOS 95 solution is passed through an activated carbon to remove potential traces of color bodies and other organic impurities, and then the FOS 95 solution undergoes an evaporation step to produce a concentrated syrup or a spray-drying step to produce a high purity powdered ingredient. A schematic of the manufacturing process for the scFOS ingredients are provided in Figure 2.3-1.

The corresponding batch analyses confirm that the manufacturing process produces a product that is consistent with the established product specifications for Galam's scFOS ingredients (see Section 2.4).

Figure 2.3-1 Schematic Overview of the Manufacturing Process for Galam's scFOS



2.4 Product Specifications and Batch Analysis

2.4.1 Product Specifications

The proposed product specifications for FOS 95 is provided in Table 2.4.1-1.

Table 2.4.1-1 Product Specifications for FOS 95

Parameter	Specification	Method of Analysis
Chemical and physical parameters		
Appearance (FOS 95 Powder)	White powder	Visual Inspection
Appearance (FOS 95 Syrup)	Clear syrup	Visual Inspection
Moisture (%) (FOS 95 Powder)	NMT 5 (powder)	Vacuum Oven
Moisture (%) (FOS 95 Syrup)	NMT 25 (syrup)	
Ash (%)	NMT 0.1	AOAC 942.05
pH	5.0 to 7.0	Internal method
Sugar composition (% dry weight basis)		
Sugar (S, G, F) (%)	5 ± 2	HPLC-RI
Fructooligosaccharides (%)	95 ± 2	
GF2 (%)	NLT 30	
GF3 (%)	NLT 40	
GF4 (%)	NLT 5	
Heavy metals		
Lead (ppm)	NMT 1	ICP
Arsenic (ppm)	NMT 1	ICP
Microbiological parameters		
Standard plate count (CFU/g)	NMT 300	Israeli standard 885/3
Yeast (CFU/g)	NMT 20	Israeli standard 885/8
Mold (CFU/g)	NMT 20	Israeli standard 885/8
Coliforms (CFU/g)	NMT 10	Israeli standard 885/4
Escherichia coli	NMT 10	ISO 16649
Salmonella	Negative	ISO 6479

CFU = colony-forming units; G = glucose; GF2 = 1-kestose; GF3 = nystose; GF4= β-fructofuranosyl-nystose; F = fructose; NLT = not less than; NMT = not more than; S = sucrose

2.4.2 Batch Analyses

Analysis of 4 non-consecutive lots of FOS 95 demonstrate that the manufacturing process produces a consistent product that meets specifications (Table 2.4.2-1).

Table 2.4.2-1 Physical, Chemical and Microbiological Analysis of FOS 95 Powder

Parameter	Specification Value	Manufacturing Lot			
		Syrup		Powder	
		(b) [redacted]		[redacted]	[redacted]
Chemical and physical parameters					
Appearance (FOS 95 Powder)	White powder	-	-	White powder	White powder
Appearance (FOS 95 Syrup)	Clear syrup	Clear Syrup	Clear Syrup	-	-
Moisture (%) (FOS 95 Powder)	NMT 5	-	-	2.30	2.20
Moisture (%) (FOS 95 Syrup)	NMT 25	21.85	23.26	-	-
Ash (%)	NMT 0.1	< 0.1	< 0.1	< 0.1	< 0.1
pH	5.0 to 7.0	6.17	6.30	6.90	6.85
Sugar composition (% dry weight basis)					
Sugar (S, G, F) (%)	5 ± 2	3.35	3.81	2.76	3.17
Fructooligosaccharides (%)	95 ± 2	96.65	96.19	97.24	96.83
GF2 (%)	NLT 30	34.73	31.73	36.67	34.71
GF3 (%)	NLT 40	50.47	49.84	51.36	51.04
GF4 (%)	NLT 5	11.45	14.62	9.21	11.08
Heavy metals					
Lead (ppm)	NMT 1	< 1.0	< 1.0	< 1.0	< 1.0
Arsenic (ppm)	NMT 1	< 1.0	< 1.0	< 1.0	< 1.0
Microbiological parameters					
Standard plate count (CFU/g)	NMT 300	< 10	< 10	70	120
Yeast (CFU/g)	NMT 20	< 10	< 10	< 10	< 10
Mold (CFU/g)	NMT 20	< 10	< 10	< 10	< 10
Coliforms (CFU/g)	NMT 10	< 10	< 10	< 10	< 10
Escherichia coli	NMT 10	< 10	< 10	< 10	< 10
Salmonella	Negative	Negative	Negative	Negative	Negative

CFU = colony-forming units; G = glucose; GF2 = 1-kestose; GF3 = nystose; GF4= β-fructofuranosyl-nystose F = fructose; NLT = not less than; NMT = not more than; S = sucrose.

2.4.3 Additional Analytical Information

2.4.3.1 Residual Protein Analysis

Analysis of 4 non-consecutive lots of FOS 95 ((b) (4) [redacted] demonstrates that the product is free of residual protein that could potentially be carried over from the manufacturing process. Residual protein was analyzed using the Bradford method and the results of analysis are provided in Table 2.4.3.1-1. The results of these analyses demonstrate that the production enzyme is absent from the final product (below the limit of detection) during the manufacturing process.

Table 2.4.3.1-1 Residual Protein Analysis of FOS 95 Powder and Syrup

Specification Parameter	Method	Limit of Detection	Manufacturing Lot			
			Syrup		Powder	
			(b) [redacted]	[redacted]	[redacted]	[redacted]
Protein	Bradford	< 0.01%	< 0.01%	< 0.01%	< 0.01%	< 0.01%

Part 3. §170.235 Dietary Exposure

3.1 Estimated Intake of FOS

Estimates of FOS consumption from the proposed uses were determined by GTC Nutrition in GRN 44 using the 1994-1996 Continuing Survey of Food Intakes by Individuals (CSFII) conducted by the USDA (GTC Nutrition, 2000). FOS intake estimates were conducted for various age groups within the U.S. population including infants (5 to 11 months of age), toddlers (1 year of age), children (2 to 12 years of age), teenagers (13 to 19 years of age) and adults (20 years of age and older) and are provided in full in GRN 44 (U.S. FDA, 2000). A brief summary of the estimated mean and 90th percentile intakes of FOS within the various age categories from GRN 44 is provided in Table 3.1-1 below.

Table 3.1-1 Summary of the Estimated Daily Intake of FOS from Proposed Food-Uses in the U.S. by Population Group (1994-1996 CSFII survey data)[a,b]

Population Group	Age Group	Mean Daily Intake per User		90th Percentile Daily Intake per User	
		(g)	(mg/kg body weight)	(g)	(mg/kg body weight)
Infants	5 to 11 months	1.624	186	3.085	337
Toddlers	1 year	3.896	336	7.054	614
Children	2 to 12 years	5.407	222	10.023	426
Teenagers	13 to 19 years	6.216	102	12.795	211
Adults	20 years and older	4.370	60	9.085	127

CSFII = Continuing Survey of Food Intakes by Individuals; FOS = fructo-oligosaccharides

[a] Adapted from GRN 44 (GTC Nutrition, 2000)

[b] "Food intake data source: USDA 1994-96 Continuing Survey of Food Intakes by Individuals; intake estimates based on proposed use levels in 18 categories of food [outlined in GRN 44]. Analyses include pregnant and/or lactating females and breast-feeding infants; individuals with missing bodyweight data were excluded from all analyses. WesVar Complex Samples 3.0 and CSFII sampling weights were used to calculate estimates." (GTC Nutrition, 2000)

In summary, the resulting mean and 90th percentile intakes of scFOS by the total U.S. population from all proposed food-uses in the U.S., were estimated to range from 1.624 to 6.216 g/person/ day (186 to 336 mg/kg body weight/day) and 3.085 to 12.795 g/person/day (127 to 614 mg/kg body weight/day). Among the individual population groups, the highest mean and 90th percentile intakes of scFOS were determined to be 6.216 g/person/day (102 mg/kg body weight/day) and 12.795 g/person/day (211 mg/kg body weight/day), respectively among teenagers. When intakes were expressed on a body weight basis, toddlers had the highest mean and 90th percentile all-user intakes of 336 and 614 mg/kg body weight/day, respectively.

Additional correspondence was submitted to the FDA by GTC Nutrition regarding revisions to the intended uses provided in the original notice (U.S. FDA, 2007). The letter to the Agency revised the intended uses of FOS to include "*... foods in general, excluding meat and poultry products and infant formula, at levels up to 20 grams (g) per day in the general population and at levels up to 4.2 g per day in infants less than one year of age. GTC*

Nutrition provided a table of the typical use levels of fructooligosaccharide …". Accordingly, general food uses of scFOS manufactured by Galam also could provide up to 20 grams per day in the general population, and with levels up to 4.2 g per day in infants less than one year of age. As scFOS manufactured by Galam will serve as an alternative to existing GRAS sources of galacto-oligosaccharides (GOS) described in GRN 44, the introduction of scFOS manufactured by Galam to the U.S. food supply will not increase dietary intake of scFOS in an additive manner.

Part 4. §170.240 Self-Limiting Levels of Use

No known self-limiting levels of use are associated with the notified ingredient.

Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable

Part 6. §170.250 Narrative and Safety Information

6.1 Introduction

Several scFOS preparations have GRAS status for use as a food ingredient in a variety of conventional food and beverage categories, including infant formula (GRN 44, 537, 605 and 623) (U.S. FDA, 2000, 2015b, 2016b,c). These scFOS preparations are produced in a similar manner using food-grade sucrose as the substrate and the transfrucosylation activity of a food grade fungal enzyme to produce a defined mixture of scFOS. Accordingly, the GRAS status of scFOS preparations produced using a revised or new manufacturing process typically utilize an equivalence approach based on an evaluation of chemical similarities between a GRAS comparator (e.g., GRN 44) and scFOS produced using the revised/new manufacturing process (*e.g.*, GRN 537, 605 and 623) (U.S. FDA, 2000, 2015b, 2016b,c). A similar approach was used by Galam to support the GRAS status of the company's scFOS ingredient. Short-chain FOS manufactured by Galam is produced from sucrose using the transfructosylation activity of a food grade enzyme preparation from derived from *Aspergillus aculeatus*. The enzyme preparation is derived from a safe and suitable organism (*i.e.*, non-pathogenic/non-toxicogenic organism), has GRAS status, and a long history of safe use in fruit juice processing. The enzyme preparation is immobilized on an ion-exchange column and is not present in the ingredient. As described in Section 2.5, scFOS manufactured by Galam contains a mixture of fructose oligomers of which one (GF2; 1-kestose), two (GF3; nystose), or three (GF4; β-fructofuranosyInystose) additional fructose units have been added by β2-1 glycosidic linkages to the fructose unit of sucrose. The ingredient is purified by resin chromatography and is purified using activated carbon to remove organic impurities, evaporative concentration and spray-dried to produce a final powder. Analyses of Galam's scFOS using HPLC-RI demonstrate that the ingredient is of high purity, and is compositionally representative of other GRAS scFOS preparations. There are no novel manufacturing processes employed during the production of Galam's scFOS that would introduce novel reaction products or impurities to the ingredient.

Since scFOS manufactured by Galam is chemically representative of other scFOS preparations that have been determined to be GRAS (e.g., GRN 44), a discussion of publicly available data and information relevant to the safety of scFOS is incorporated by reference to pivotal studies discussed in GRN 44 (U.S. FDA, 2000). Brief summaries of the published literature pertaining to the absorption, distribution, metabolism and excretion of

scFOS are presented in Section 6.2, and an overview of published studies characterizing the toxicity in animal models and safety in humans is discussed in Sections 6.3 through 6.4. To identify new data pertinent to the safety of scFOS published since the GRAS status of scFOS was last evaluated in 2014 (*i.e.*, GRN 537) (U.S. FDA, 2015b), a comprehensive search of the published scientific literature was conducted for the period spanning from April 2014 through October 2016 (see Appendix A for literature search report). The search was conducted using the electronic search tool, Proquest Dialog™, with several databases, including: Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and Toxfile®. Results of the pertinent toxicological studies from prior GRAS notifications and newly identified studies relevant to scFOS safety and tolerance in humans are summarized in their respective sections below. Consistent with the requirements of the GRAS standard, conclusions on the GRAS status of scFOS have considered all publicly available sources of information including favorable and potentially unfavorable information. Based on Galam's updated search of the literature, the company is not aware of newly published studies to suggest the scFOS is unsafe for use as a food ingredient.

The totality of publicly available scientific literature relevant to the safe use of scFOS as an ingredient in food has been comprehensively evaluated, using scientific procedures, by a number of independent scientific experts, including the FDA (GRN 44, 537, 605 and 623) (U.S. FDA, 2000, 2015b, 2016b,c). These GRAS notifications have consistently concluded that the addition of scFOS to food is GRAS under their respective conditions of intended use. The safety of scFOS from sucrose for use as an alternative to inulin derived FOS was evaluated by the Foods Standards Australia New Zealand (FSANZ) in 2013 (FSANZ, 2013). Based on published information characterizing the metabolism of FOS, published studies characterizing the toxicity of scFOS in animal models and published studies evaluating the safety and tolerance of scFOS in humans (children and infants), FSANZ concluded that

"… scFOS produced by invertase catalysed condensation of sucrose is technologically justified and is as safe as IDS [inulin derived substances] already permitted to be added to foods generally, and infant formula products, infant foods and FSFYC [Formulated Supplementary Foods for Young Children] alone or in combination with IDS and GOS [galacto-oligosaccharides] up to the currently permitted maximum amounts. Additionally, scFOS has the potential to soften infant stools and may reduce the incidence of constipation, both of which are considered beneficial effects."

In Japan, scFOS (Neosugar®) has a long-history of safe use as a general food use low-calorie sweetener since 1983 (Benkeblia, 2014). Based on conclusions from previous expert panels on the GRAS status of scFOS, corresponding no objection letters issued by the FDA, the widespread history of use of scFOS as a food ingredient globally, and conclusions from other authoritative bodies on the safety of scFOS as a food ingredient (*e.g.*, FSANZ), Galam has concluded that the current GRAS status of scFOS as described in GRN 44, can be extended to scFOS manufactured by Galam. Galam has therefore concluded that the company's scFOS ingredient, as described herein, is GRAS for the specified uses in conventional food products based on scientific procedures.

6.2 Metabolic Fate

The absorption, distribution, metabolism and excretion of scFOS along with the physiological effects on the gastrointestinal tract related to scFOS ingestion is well characterized and has been previously described in detailed (GRN 44, 537, 605, 623) (U.S. FDA, 2000, 2015b, 2016b,c). Generally, scFOS and related β2-1 fructans are not absorbed and are resistant to digestion by salivary amylase, human pancreatic or intestinal enzymes. FOS reaches the large intestine primarily intact where microbial fermentation occurs. scFOS are fermented in

the colon forming methane, hydrogen and carbon dioxide. Unfermented FOS is excreted in the feces. Since all scFOS preparations are substantially chemically equivalent they are ultimately expected to be substantially physiologically equivalent.

6.3 Toxicological Studies

scFOS derived from sucrose has been subjected to toxicological studies including acute oral toxicity studies in mice and rats as well as 3 subacute studies, 1 subchronic study, 1 chronic study and 2 studies evaluating developmental and maternal toxicity conducted in rats. *In vitro* genotoxicity studies in bacterial or mammalian cell models in the presence and absence of metabolic activation have also been conducted with scFOS. No consistent treatment related adverse effects were reported in the repeat dose studies and the no-observed-adverse-effect levels (NOAELs) were the highest doses tested. scFOS related effects apparent at high doses in these studies (*e.g.* intestinal weight increases, transient diarrhea, and soft/watery stools) are well-established effects consistent with the effects associated with intake of high-levels of non-digestible fibers and are considered to not be toxicologically relevant to humans (WHO, 1987). 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 determined to be the highest dietary concentration tested of 5% [equivalent to 2,170 and 2,664 mg/kg body weight/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 summarized in Sections 6.3.1 through 6.3.4 below.

6.3.1 Acute Toxicity

Acute oral toxicity studies conducted with scFOS in male and female JcL-IcR mice and Sprague Dawley rats have been described in detail in prior GRAS notifications including GRN 44, 537, 605 and 623 (U.S. FDA, 2000, 2015b, 2016b,c). The results of these studies demonstrate that scFOS is of low acute oral toxicity with median lethal dose (LD_{50}) values exceeding 9,000 mg/kg body weight (highest dose tested) in both mice and rats. The results of these studies are summarized in Table 6.3.1-1 below. No additional acute oral toxicity studies were identified in the literature.

Table 6.3.1-1 Summary of Acute Oral Toxicity Studies Conducted with scFOS

Animal Model	Dose (mg/kg bw) and Duration	LD_{50} (mg/kg bw)	Additional Findings	Reference
JcL-IcR mice (4-week-old; 6/sex/dose)	Single dose (gavage) 0, 3,000, 6,000, or 9,000	>9,000 mg/kg	- No mortalities reported. - No abnormalities or differences in body weight were reported in comparison to the controls.	Takeda and Niizato (1982); summarized in Carabin and Flamm (1999)
Sprague-Dawley rats (6-week old male; 10-week-old female; 6/sex/dose)	Single dose (gavage) 0, 3,000, 6,000, or 9,000	>9,000 mg/kg	- No mortalities reported. - No abnormalities or differences in body weight were reported in comparison to the controls.	Takeda and Niizato (1982); summarized in Carabin and Flamm (1999)

bw= body weight; LD_{50}= median lethal lose; scFOS = short-chain fructo-oligosaccharide

6.3.2 Repeat Dose Toxicity

Toxicological evaluations of various scFOS preparations, produced from sucrose *via* enzymatic synthesis, are of low oral toxicity in repeat dose toxicity studies in rodents (see Table 6.3.2-1). All generally available published studies that are pertinent to the safety of scFOS have been the subject of several critical and independent evaluations as discussed in prior GRAS notifications (*i.e*., GRN 44 and GRN 537) (U.S. FDA, 2000, 2015b). No toxicologically significant effects of relevance to humans have been reported in toxicological investigations of scFOS that have been published in the literature; NOAEL determinations have been consistently reported as the highest doses tested. Carabin and Flamm (1999) cited findings from unpublished subacute studies were conducted by Takeda and Niizato (1982) supporting NOAELs of 4,500 to 5,000 mg/kg body weight/day (highest doses tested) following 6-week gavage and dietary administration of scFOS to Wistar rats. Tokunaga *et al.* (1986) reported that male Wistar rats consuming FOS at dietary concentrations of 10 and 20% [equivalent to approximately 4,185 and 7,795 mg/kg body weight/day, respectively (U.S. FDA, 1993)] experienced transient watery stools during the first few days of administration and increased small and large intestine weights, and increased fecal and decreased gastrointestinal transit time when in the diet for 6 to 8 weeks. Meijl Seika Kaisha (1982) cited in GRN 44 (GTC Nutrition, 2000) reported a dose-related increase in diarrhea, soft stools, cecal distension, intestine weights for rats fed up to 20,400 mg/kg body weight/day for 90 days (further study details not reported). The results of a 104-week chronic study conducted with Fischer 344 rats administered dietary scFOS supports NOAELs of 2,170 and 2,664 mg/kg body weight/day for males and females, respectively (Clevenger *et al.*, 1988).

Carabin and Flamm (1999) summarized the findings of an unpublished study conducted by Henquin (1988) and reported that dietary administration of scFOS at concentrations up to 20% [equivalent to approximately 10,000 mg/kg body weight/day (U.S. FDA, 1993)] did not result in developmental toxicity[2]. In another study evaluating the maternal and developmental toxicity of FOS, dietary concentrations up to 20% [equivalent to approximately 10,000 mg/kg body weight/day (U.S. FDA, 1993)] provided to rats during postcoitum days 0 to 15 did not result in treatment related adverse effects (*e.g.* diarrhea), or differences in pregnancy outcome or *in utero* development (Sleet and Brightwell, 1990; summarized in Carabin and Flamm, 1999).

[2] Fetal markers other than body weight were not further described in the study summary.

Table 6.3.2-1 Summary of Repeat Dose Toxicity Studies of scFOS Conducted in Rodents

Species Strain, (No./Sex/Group; age/weight)	Route and Dose (mg/kg bw/day)	Duration	NOAEL (mg/kg bw/day)	Other Observations	Reference
Subacute Studies					
Wistar SPF rats (18 M/group; 6-7 weeks of age)	Gavage: 0 (control), 1,500, 3,000 or 4,500 scFOS (DP_{av} =3.5)	6 weeks	4,500	- No mortalities or abnormalities - Minor ↑ body weight in 3,000 and 4,500 groups (stat. sig. not reported) - No consistent, treatment-related findings in serum chemistry parameters (occasional fluctuations reaching statistical significance were considered spurious– further details not reported) - Swollen appendix in rats receiving treatment (number/group not reported)	Takeda and Niizato (1982), summarized in Carabin and Flamm (1999)
Wistar SDP rats (18 M/group; 6-7 weeks of age)	Dietary: 0 (control), ~2,500, or ~5,000[a] scFOS (DP_{av} =3.5)	6 weeks	5,000[b]	- No mortalities or treatment-related abnormalities - Diarrhea reported on 10th day of FOS administration (no additional details reported) - ↓ body weight in FOS treated animals [(Week 1 to 5) – stat. sig. not reported)], normalized near completion of study - FOS related ↓ in cholesterol (stat. sig. not reported) - Swollen appendices were reported at Week 2 and Week 6 necropsies (number/group not reported) - No treatment related toxicity compared to controls	Takeda and Niizato (1982), summarized in Carabin and Flamm (1999)
Wistar rats (6 M/group; 40-50g)	Dietary: 0 (control), ~4,185, ~7,795[c] scFOS Neosugar®	6 to 8 weeks	NR	- ↓Body weight in 10,000 group - ↑Cecum and colon weights in both treatment groups - ↑ small intestine weights in 10,000 group - ↑ fecal weight and ↓ GI transit time in both treatment groups - ↓ serum triacylglycerol and ↑ fecal excreted neutral sterols and volatile fatty acids - During first few days FOS administration transient watery stools	Tokunaga *et al.* (1986)
Subchronic Studies					
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 - Dose related ↑ in diarrhea, soft stools, cecal distension, intestine weights	Meijl Seika Kaisha (1982) cited in GRN 44 (GTC Nutrition, 2000)

Table 6.3.2-1 Summary of Repeat Dose Toxicity Studies of scFOS Conducted in Rodents

Species Strain, (No./Sex/Group; age/weight)	Route and Dose (mg/kg bw/day)	Duration	NOAEL (mg/kg bw/day)	Other Observations	Reference
Chronic Studies					
Fischer 344 rats (50/sex/group; 4 weeks of age)	Dietary: Male: 0, 341, 854, and 2,170 Female: 0, 419, 1,045, and 2,664 scFOS Neosugar® (DP = 2 to 4)	104 weeks	2,170 (males)[b] 2,664 (females)[b]	-No dose-related effects on survival, growth, hematological or clinical chemistry parameters, organ weights or neoplastic lesions.	Clevenger *et al.* (1988)
Developmental and Reproductive Toxicity Studies					
Wistar rats (29 F; n=12 treatment and n=17 control)	Dietary: 0 or 10,000[a] scFOS (no further details reported)	Gestation days 1 to 21	-	- No treatment effect on number of pregnancies or fetus or newborn weights - ↓Body weight during nursing period was reported in the treated pregnant rats and pups - Diarrhea observed in treated pregnant rats (number not reported) during the first week and soft stools in weeks 2 and 3 for this group - Growth delay in male pups in test group[3]	Henquin (1988); summarized in Carabin and Flamm (1999)

[3] Fetal markers other than body weight were not further described in the study summary.

Table 6.3.2-1 Summary of Repeat Dose Toxicity Studies of scFOS Conducted in Rodents

Species Strain, (No./Sex/Group; age/weight)	Route and Dose (mg/kg bw/day)	Duration	NOAEL (mg/kg bw/day)	Other Observations	Reference
Sprague-Dawley (Crl CD (SD) BR) rats Pregnant female rats (24 to 27/group)	Dietary: 0 or 2,375[a] (Day 0 to 6 postcoitum) Dietary: 0, 2,500, 5,000 or 10,000[a] (Day 6 to 15 postcoitum) scFOS (no further details reported)	Days 0 to 15 Postcoitum	-	- No treatment related adverse effects - No deaths or diarrhea reported - ↓Body weight on postcoitum Day 2 in all FOS treated rats compared to control - Dose-related decrease in body weight for FOS treated rats. Body weight and body weight changes in 2,500 and 5,000 mg/kg body weight/day groups were similar among groups from Day 12 to 15 - No remarkable findings at necropsy - No treatment related effects on number of pups/litter, the sex ratio, and viability of both the embryo and the fetus or structural development of fetuses - ↑ fetal weights of 10,000 mg/kg body weight groups compared to control, no other reduction in litter or fetal weights	Sleet and Brightwell (1990); summarized in Carabin and Flamm (1999)

↑ = increase; ↓ = decrease; bw = body weight; DP = degree of polymerization; DPav = average degree of polymerization; GI = gastrointestinal; GRN = GRAS Registration Notification; F = female; FOS = fructo-oligosaccharides; M = male; NOAEL = no-observed-adverse-effect-level; NR = not reported; scFOS = short-chain fructo-oligosaccharides

[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.

6.3.3 Genotoxicity

The genotoxicity of commercially available scFOS (Neosugar®) has been evaluated in *in vitro* genotoxicity assays including a bacterial reverse mutation assay and an unscheduled DNA repair assay conducted in accordance with guidelines established by the Organization for Economic Cooperation and Development (OECD) and a mammalian cell mutation assay conducted according to recognized methods. The results of these studies consistently demonstrate that scFOS are not genotoxic in bacteria and mammalian cells in the presence or absence of metabolic activation. These studies are described in detail in GRN 44, 537 and 605 and summarized in Table 6.3.3-1 below (U.S. FDA, 2000, 2015b, 2016b). No additional genotoxicity studies were identified in the literature.

Table 6.3.3-1 Summary of *in vitro* Genetic Toxicity Studies on scFOS (Neosugar®)

Compound	Test (and test system)	Concentration	Metabolic Activation	Result	Reference
scFOS (Neosugar®)	Bacterial reverse mutation assay (*Salmonella* Typhimurium TA98, TA100, TA1535, TA1537, and TA1538 and *Escherichia coli* WP2 *uvrA*)	0, 50, 150, 500, 1,500, or 5,000 µg/plate	±S9[a]	Negative	Clevenger *et al.* (1988)
scFOS (Neosugar®)	Mammalian cell mutation assay (Mouse lymphoma L5178Y cells)	2,000, 3,000, 4,000, or 5000 µg/mL	±S9[a]	Negative	Clevenger *et al.* (1988)
scFOS (Neosugar®)	Unscheduled DNA synthesis [Human epithelioid cells (HeLa S3)]	25, 50, 100, 200, 400, 800, 1,600, 3,200, 6,400, 12,800, 25,600, 51,200 µg/mL	-	Negative	Clevenger *et al.* (1988)

S9 = metabolic activation with Aroclor 1254-induced rat liver S9; scFOS = short-chain fructo-oligosaccharides
[a] Aroclor 1254-induced rat liver S9

6.3.4 Other Animal Studies

Updated searches of publicly available literature published since the GRAS status of scFOS was last evaluated in GRN 537[4] identified a limited number of new studies evaluating safety-related endpoints in various animal models. No new evidence was found that would suggest that the use of scFOS as food ingredients would present unsafe or undesirable effects. The full texts and/or abstracts are provided in Appendix A for further supporting information.

6.4 Clinical Studies

The totality of the publicly available literature investigating the consumption of scFOS in human subjects has been the subject of several comprehensive evaluations by several notifiers, independent expert panels and the FDA during previous conclusions on the GRAS status of scFOS as described in GRN 44, 537, 605, 623 (U.S. FDA, 2000, 2015b, 2016b,c). In the first GRAS notification for FOS submitted to the offices of the FDA 16 years ago, GTC Nutrition concluded *"The AIL [Acceptable Intake Level] for FOS ingestion for the general population,*

[4] At the time of this dossier preparation, GRN 537 was the most recent FOS GRAS to receive a "no questions" letter from the U.S. FDA which summarized literature prior to April 2014 (U.S. FDA, 2015b). FOS from Tata Chemicals Limited and New Francisco Biotechnology Corporation has since received a "no questions" letter (GRN 605 and 623) (U.S. FDA, 2016b,c).

excluding infants less than one year of age, is determined to be 20 g/day; the AIL for infants less than one year old is 4.2 g/day" (GTC Nutrition, 2000). Updated searches of the scientific literature published since GRN 537[5] was conducted to identify new studies relevant to the safety of scFOS that have not been previously evaluated with respect to their relevance to the use of scFOS as a GRAS food ingredient. Several studies conducted in children and adults were identified that contain parameters relevant to the safety of scFOS consumption and the findings are summarized in Section 6.4.1 below.

6.4.1 Studies Conducted in Healthy Children and Adults

A comprehensive literature search was conducted to identify studies published from April 2014 to October 2016 evaluating safety and/or tolerability related endpoints of scFOS consumption by children and adults. The search identified 1 study conducted in children and 5 conducted with adults. These studies are tabulated in Table 6.4.1-1 below. The results of these studies are supportive of the findings in the previous GRAS determinations and reiterate the safe use of scFOS as a food ingredient.

scFOS consumption by children and adults did not result in serious adverse events (SAEs). Only mild gastrointestinal side-effects of scFOS consumption were reported which 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. scFOS as part of a 9:1 mixture of 1.2 g/ 100 mL short chain galacto-oligosaccharides (scGOS)/scFOS + 19.2 mg/100 mL n-3 long-chain poly-unsaturated fatty acids provided to children aged 11 to 29 months for 52 weeks was reported to reduce the likelihood of upper respiratory tract or gastrointestinal infections (Chatchatee *et al*., 2014).

[5] At the time of this dossier preparation, GRN 537 was the most recent FOS GRAS to receive a "no questions" letter from the FDA which summarized literature prior to April 2014 (U.S. FDA, 2015b). FOS from Tata Chemicals Limited and New Francisco Biotechnology Corporation has since received a "no questions" letter (GRN 605 and 623) (U.S. FDA, 2016b,c).

Table 6.4.1-1 Summary of Recent Studies of FOS Consumption in Children and Adults – Updated Literature Search April 2104 to October 2016

Population (number of subjects)	Study design	Duration	Test Article	Safety and Tolerance Related Results[a,b]	
Children					
Chatchatee *et al.* (2014) - Effects of growing-up milk supplemented with prebiotics and LCPUFAs on infections in young children					
767 healthy children ages 11 to 29 months Active arm: n=213M/175F Control arm: n=222M/157F	Randomized, double-blind, controlled, parallel, multi-country intervention	52 weeks	**Active arm:** Group 1 (n=388): Grow up milk with added 1.2g/100 mL scGOS/scFOS (9:1) (Immunofortis) and 19.2 mg/100 mL n-3 LCPUFAs **Control arm:** Group 2 (n=342): Grow up milk without added scGOS/scFOS (9:1) (Immunofortis) and n-3 LCPUFAs Group 3 (n= 37): Cow's milk (this group was not randomized	Adverse events	• 2,217 AE including 78 SAE occurred during the study • SAE were not related to study product and none were unexpected • ~49% of AE related to respiratory system (*e.g.* fever, cough, discharge, blocked/runny nose, *etc.*) • 29 AE were possibly or probably related to study product during run-in or intervention period. Primarily mild GI symptoms that occurred in active and control arms evenly.
				Upper respiratory and/or gastrointestinal infections	• ↓ likelihood of experiencing an infectious episode in Group 1
Healthy Adults					
Respondek *et al.* (2014) - Digestive tolerance and postprandial glycaemic and insulinaemic responses after consumption of dairy desserts containing maltitol and fructo-oligosaccharides in adults					
36 Healthy subjects (12M/24F; 18 to 60 y; BMI 18.5 to 30.0 kg/m^2) ITT population for glycemic and insulinaemic responses n=18 32 subjects completed trial without study deviation	Randomized, double-blind reference-controlled, six-period cross-over study	Single dose 2-week washout between treatments	Group 1: Control (Dextrose 35g/210g) Group 2: Dextrose (24g/210g) + scFOS (11.2g/210g) Group 3: Maltitol (35g/210g) Group 4: Maltitol (30g/210g) + scFOS (5g/210g)	Adverse events	• No SAE • No diarrhea was reported and intensity of digestive symptoms were mild • 11/88 adverse events potentially linked to research or study products • NSD adverse events between treatments
				GI Symptoms at 24 and 48h Flatulence, Borborygmi, Bloating,	• NSD in GI symptoms in Group 2 • ↑ Flatulence (0 to 24 and 24 to 48h post treatment) and borborygmi (0 to 24 h post treatment) in group 4 and 5

Table 6.4.1-1 Summary of Recent Studies of FOS Consumption in Children and Adults – Updated Literature Search April 2104 to October 2016

Population (number of subjects)	Study design	Duration	Test Article	Safety and Tolerance Related Results[a,b]	
			Group 5: Maltitol (24g/210g) + scFOS (11g/210g) Group 6: Maltitol (17.5g/210g) + scFOS (17.7g/210g) Doses provided in a chocolate dairy dessert	Discomfort scored on a 1 to 10 scale	• ↑ Flatulence (0 to 24 and 24 to 48h post treatment) and ↑ borborygmi, bloating and discomfort (0 to 24 h post treatment) in group 6
				Stool frequency and consistency	• ↑ Stool frequency and consistency in groups 3, 4, 5, 6 (0 to 24 h post treatment) NSD in consistency or frequency (24 to 48 h post treatment)
				Glycemic and insulinaemic responses	• ↓ Glucose AUC in group 3, 4, 5, 6 • ↓ Glucose C_{max} in groups 2, 3, 4, 5, 6 • ↓ Insulin AUC in groups 2, 3, 4, 5, 6 • ↓ Insulin C_{max} in groups 3, 4, 5, 6
Lecerf *et al.* (2015) - Postprandial glycaemic and insulinaemic responses in adults after consumption of dairy desserts and pound cakes containing short-chain fructo-oligosaccharides used to replace sugars					
Study 1: Dairy Deserts 24 healthy subjects (28% male/72% female; mean age of 32.3 (SD 8.7) years, average BMI = 22.3 (SD 1.9) kg/m^2) [give actual numbers not %s]	Randomized, double-blind, placebo-controlled, crossover,	Single dose At least 1-week washout between treatments	**Study 1: Dairy Deserts** ***Test article:*** Dairy desert containing 11 g scFOS + 24 g dextrose (scFOS replacement of ~30% dextrose in diet) ***Control:*** Dairy desert containing 35 g dextrose	Adverse events	• No SAEs • 6 AEs reported (specific details NR) the author's reported not to be related to study treatments
Study 2: Pound Cakes 31 healthy subjects (29% male/71% female; mean age of 31.9 (SD8.1) years, average BMI = 21.9 (SD 1.9) kg/m^2)			**Study 2: Pound Cakes** ***Test article:*** Pound cake containing 9 g scFOS + 19 g dextrose (scFOS replacement of ~30% dextrose in diet) ***Control:*** Pound cake containing 28 g dextrose	Glycaemic and insulinaemic responses	• ↓ glucose and inulin AUC (Study 1) • NSD in glucose and inulin C_{max} (Study 1) • NSD in glucose and inulin AUC or C_{max} (Study 2)

Table 6.4.1-1 Summary of Recent Studies of FOS Consumption in Children and Adults – Updated Literature Search April 2104 to October 2016

Population (number of subjects)	Study design	Duration	Test Article	Safety and Tolerance Related Results[a,b]	
Azpiroz *et al.* (2016) - Effects of scFOS on the composition of fecal microbiota and anxiety in patients with irritable bowel syndrome: a randomized, double blind, placebo controlled study					
79 IBS patients with rectal hypersensitivity (18 to 60 y Group 1: n= 41 (9M/32F) Group 1: n= 38 (10M/28F)	Parallel, placebo-controlled, randomized, double-blind study	4 weeks	Group 1: Placebo (maltodextrin) Group 2: 5g/day scFOS	Adverse events	• 2 subjects excluded due to non-authorized medical treatment. Treatment was well tolerated with similar number of AE reported between groups
				Clinical outcomes	• NSD in IBS severity composite score at study completion • ↓ abdominal distention in group 2 compared to baseline • ↑ improvement of both the global score and the anxiety score was reported in Group 2 compared to placebo
				Rectal sensitivity (defined as discomfort threshold ≤44 g)	• NSD between treatments at study completion; however, both groups showed improvement compared to baseline
				Fecal microbiota	• ↑ *Bifidobacteria* in group 2 compared to baseline
Slevin *et al.* (2014) - Supplementation with calcium and short-chain fructo-oligosaccharides affects markers of bone turnover but not bone mineral density in postmenopausal women					
300 non-osteoporotic postmenopausal women (45 to 75 years of age; < 136 kg)	Randomized, double-blind, controlled	24 months	***Treatments:*** 3.6g/day scFOS + 800mg/day calcium 800mg/day calcium ***Control:*** 9 g/day maltodextrin	Adverse events	• NSD in treatment compliance between groups • No AE on liver function or electrolytes (no further details reported)
				Bone mineral density and bone turnover markers	• NSD in mean serum 25(OH)D concentrations between treatments throughout the duration of the study • NSD in bone mineral density at study completion. A smaller decline in total body bone mineral density was reported in the scFOS + Calcium group *vs.* Calcium group

Table 6.4.1-1 Summary of Recent Studies of FOS Consumption in Children and Adults – Updated Literature Search April 2104 to October 2016

Population (number of subjects)	Study design	Duration	Test Article	Safety and Tolerance Related Results[a,b]	
					• NSD after 24 months in urinary and serum CTX, serum osteocalcin • NSD in DPD over time between the groups
Cronin *et al.* (2016) - Effects of supplementation with a calcium-rich marine-derived multi-mineral supplement and short-chain fructo-oligosaccharides on serum lipids in postmenopausal women					
300 non-osteoporotic postmenopausal women (45 to 75 years of age; < 136 kg) (Same study population as Slevin *et al.* (2014)	Randomized, double-blind, controlled	24 months with a 4-year follow-up	***Treatments:*** 3.6 g/day scFOS + 800 mg/day calcium 800 mg/day calcium ***Control:*** 9 g/day maltodextrin	Adverse events	• Digestive problems resulted in a higher dropout rate in the CaFOS group (no specific details reported – not reported if this observation was considered treatment-related)
				Anthropological measurements	• NSD in bw, BMI, FMI, FFMI
				Lipid measurements	• ↓ LDL, total cholesterol concentrations at 24-months compared to maltodextrin • NSD in HDL-cholesterol, TAG or LDL:HDL
				Blood pressure	• NSD in SBP or DBP
				Inflammatory measures	• NSD for ICAM-1, VCAM-1 or the inflammatory cytokines CRP, IL-6, IL-10, TNF-α, adiponectin and leptin. • ↑ IL-4 in the CaFOS group compared to maltodextrin after 24 months
				4-Year follow-up	• NSD in each of the diagnosed conditions assessed: change in blood pressure, heart attack, stroke, cancer or bone fracture

25(OH)D = Calcifediol AE = adverse effects; AUC = area under the curve; BMI = body mass index; C_{max} = maximum concentration; CTX = C-terminal telopeptide; DBP = diastolic blood pressure; FOS = fructo-oligosaccharides; F= female; FFMI = fat free mass index FMI = fat mass index; GI = gastrointestinal; HDL = high-density lipoprotein h = hours; IL = interleukin; IBS = irritable bowel syndrome; ITT = intent to treat LCPUFA = long chain polyunsaturated fatty acids; LDL = low-density lipoprotein M= male; n= number of subjects; NSD = no significant difference; SAE = serious adverse effect; SBP = systolic blood pressure; SD = standard deviation; scFOS= short chain fructo-oligosaccharides; scGOS = short chain galacto-oligosaccharides

[a] Results are tabulated if statistically significant compared to controls unless otherwise states.

6.5 Allergenicity

Analytical data on Galam's finished scFOS ingredients have demonstrated the absence of any protein in the final product (see Section 2.4.3); therefore, the potential for allergenicity of FOS 95 is low.

6.6 Expert Panel Evaluation

Galam Ltd., has concluded that scFOS as described herein meeting appropriate food grade specifications and manufactured consistent with current Good Manufacturing Practices, is GRAS for use as an ingredient in specified conventional food and beverage products, as described in Part 1.3, on the basis of scientific procedures.

This GRAS determination is based on data generally available in the public domain pertaining to the safety of scFOS and on a unanimous opinion among a panel of experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients. The Expert Panel consisted of the below-signed qualified scientific experts: Prof. Emer. Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine); Prof. Emer. George C. Fahey Jr, Ph.D. (University of Illinois), and Prof. Emer. Robert Nicolosi, Ph.D. (University of Massachusetts Lowell).

The Expert Panel, convened by Galam, independently and critically evaluated all data and information presented herein, and concluded that scFOS as manufactured by Galam Ltd, was GRAS for use in food as described in Section 1.3 based on scientific procedures. A summary of data and information reviewed by the Expert Panel, and evaluation of such data as it pertains to the proposed GRAS uses of scFOS is presented in Appendix B.

6.7 Conclusions

The data and information summarized in this dossier demonstrate that scFOS, as manufactured by Galam, produced using current Good Manufacturing Practices (cGMP) and meeting appropriate food-grade specifications, is Generally Recognized as Safe (GRAS), based on scientific procedures, under the conditions of intended use in foods, as described herein.

Part 7. §170.255 List of Supporting Data and Information

Azpiroz F, Dubray C, Bernalier-Donadille A, Cardot J.M, Accarino A, Serra J et al. (2016). Effects of scFOS on the composition of fecal microbiota and anxiety in patients with irritable bowel syndrome: a randomized, double blind, placebo controlled study. Neurogastroenterol Motil [epub ahead of print – Jul. 31]. DOI:10.1111/nmo.12911.

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Part	§	Section Title
170—Food additives	170.30	Eligibility for classification as generally recognized as safe (GRAS).
173—Secondary direct food additives permitted in food for human consumption	173.25	Ion-exchange resins [activated carbon]
177—Indirect food additives: Polymers	177.1655	Polysulfone resins
	177.2710	Styrene-divinylbenzene resins, cross-linked
	184.1005	Acetic acid

Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	§	Section Title
184—Direct food substances affirmed as generally recognized as safe	184.1033	Citric acid
	184.1205	Calcium hydroxide
	184.1631	Potassium hydroxide
	184.1854	Sucrose

U.S. FDA (2016b). *Agency Response Letter GRAS Notice No. GRN 000605 [Fructo-oligosaccharides, Pune, India: Tata Chemicals Limited]*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=605 [Mar. 17, 2016].

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Appendix A:

Identification of Pertinent Scientific Literature Regarding the Metabolism and Pre-clinical and Clinical Safety of Short-Chain Fructo-Oligosaccharides (scFOS) Produced from Sucrose

Identification of Pertinent Scientific Literature Regarding the Metabolism and Pre-clinical and Clinical Safety of Short-Chain Fructo-Oligosaccharides (scFOS) Produced from Sucrose

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Identification of Pertinent Scientific Literature Regarding the Metabolism and Pre-clinical and Clinical Safety of Short-Chain Fructo-Oligosaccharides (scFOS) Produced from Sucrose

1.0 INTRODUCTION

To identify pertinent scientific literature regarding the metabolism and pre-clinical and clinical safety of short-chain fructo-oligosaccharides (scFOS) produced from sucrose, a comprehensive search of the scientific literature was conducted using the electronic search tool ProQuest Dialog™.

The methods used to identify the scientific literature and the results of the literature search are provided in Section 2.0 and 3.0, respectively.

2.0 IDENTIFICATION OF PERTINENT STUDIES

2.1 Literature Search Strategy

To retrieve relevant literature on the metabolism and pre-clinical and clinical safety of scFOS (produced from sucrose), 13 literature databases were searched in October and November of 2016 using the electronic search tool ProQuest Dialog™. The databases that were searched, as well as the search terms that were used, are listed in Table 2.1-1 and Tables 2.1-2 to 2.1.4, respectively. To increase the relevance and specificity of the literature search, the search terms were selected to reflect the exposure to the isolated fiber of interest (scFOS produced from sucrose) in combination with metabolism and pre-clinical/clinical parameters. The search was also limited to articles with full texts in English language and publication dates after April 1, 2014[1]. Studies published as abstracts, commentaries, *etc.* were also excluded.

Table 2.1-1 Electronic Databases Used to Retrieve Literature

Electronic Database	Date Range	Update Frequency
Adis Clinical Trials Insight	1990 to present	Weekly
AGRICOLA	1970 to present	Monthly
AGRIS	1975 to present	Monthly
Allied & Complimentary Medicine™	1985 to present	Monthly
BIOSIS® Toxicology	1969 to present	Weekly
BIOSIS Previews®	1926 to present	Weekly
CAB ABSTRACTS	1910 to present	Weekly
EMBASE®	1947 to present	Daily
Foodline®: SCIENCE	1972 to 2016	Stopped updating April 2016; previously twice weekly

[1] At the time of this dossier preparation, GRN 537 was the most recent FOS GRAS to receive a "no questions" letter from the U.S. FDA which summarized literature prior to April 2014. FOS from Tata Chemicals Limited and New Francisco Biotechnology Corporation has since received a "no questions" letter (GRN 605 and 623).

Table 2.1-1 Electronic Databases Used to Retrieve Literature

Electronic Database	Date Range	Update Frequency
FSTA®	1969 to present	Weekly
MEDLINE®	1946 to present	Daily with annual refresh
NTIS: National Technical Information Service	1964 to present	Weekly
ToxFile®	1946 to present	Daily with annual refresh

The literature search strategy to identify pre-clinical safety data related scFOS produced from sucrose is provided below in Table 2.1-2.

Table 2.1-2 Keywords Used to Retrieve Pre-clinical Safety Literature During the Updated Literature Search (April 1, 2014 to October, 2016)

Strategy[a]	
Set 1: Substance terms	Searched for records containing the following chemical names and synonymsb: • fructooligosaccharide* OR fructo-oligosaccharide* OR "scFOS" OR "sc-FOS" OR oligofructose* OR oligfructan* OR inulin OR inulins
Set 2: Animal study terms	Within the results from Set 1, searched for records containing the following terms within the publication: • animal or rat or mouse or mice or dog or rabbit or pig or hamster or monkey or rodent or pig or piglet
Set 3: Route of administration terms	Within the results from Set 2, searched for records containing the following terms within the publication: • oral* or gavage or feeding or diet or dietary or intub* or "drinking water" or intragastric
Set 4: Safety terms	Within the results from Set 3, searched for records containing the following terms within the publication: • toxic* or mortal* or lethal* or adverse* or safe* or risk* or hazard*
Set 5: Acute/repeat dose study terms	Within the results from Set 4, searched for records containing the following terms within the publication: • acute* or subacute or "sub acute" or "single dose" or "short term" or subchronic* or "sub chronic*" or chronic* or "long term" or day or week or month or year
Set 6: Safety factors terms	Within the results from Set 3, searched for records containing the following terms within the publication: • LD50 or NOAEL or LOAEL or "no observed adverse effect*" or "low* observed adverse effect*" or NOEL or LOEL or "no observed effect level" or "low* observed effect level" or "maximum tolerated dose" or safety NEAR/2 assess* or risk NEAR/2 assess*
Set 7: Carcinogenicity terms	Within the results from Set 3, searched for records containing the following terms within the publication: • carcino* or tumor* or tumour* or neoplas* or oncogen* or cancer*
Set 8: Reproductive toxicity terms	Within the results from Set 3, searched for records containing the following terms within the publication: • teratol* or teratogen* or reproduct* NEAR/5 toxic* or development* NEAR/5 toxic* or reproduct* NEAR/5 effect* or development* NEAR/5 effect* or fetus or foetus or fetal or foetal or prenatal* or postnatal* or perinatal* or litter or litters or "2 generation*" or "two generation*" or "multi generation*"
Set 9: Genotoxicity terms	Within the results from Set 1, searched for records containing the following terms within the publication: • genotox* or genetox*or mutagen* or mutat* or Ames or "dna repair" or "dna lesion*" or micronucle* or clastogen* or "DNA adduct*" or "comet assay*"

[a] Syntax for the search strategy is as follows: " " = search terms must appear directly beside each other in the exact order; * = truncation; NEAR/5 = search terms may appear within 5 words of each other with either term appearing first within the record
[b] Due to the vast amount of titles retrieved, search terms were limited to appear in the titles and/or abstracts of the study article for Sets 5 to Set 8

The literature search strategy to identify clinical safety data related scFOS produced from sucrose is provided below in Table 2.1-3.

Table 2.1-3 Keywords Used to Retrieve Clinical Safety Literature During the Updated Literature Search (April 1, 2014 to October, 2016)

Strategy[a]	
Set 1: Substance terms	Searched for records containing the following chemical names and synonyms within the titles and abstractsb: • fructooligosaccharide* OR fructo-oligosaccharide* OR "scFOS" OR "sc-FOS" OR oligofructose* OR oligfructan* OR inulin OR inulins
Set 2: Keywords used to identify human studies	Within the results from Set 1, searched for records containing the following terms within the publication: • human or humans or subject or subjects or patient* or clinical* or volunteer* or men or women or "double blind*" or "single blind*" or "open label*" or "cross over" or crossover or cohort or randomiz* or randomis* or "placebo control*"
Set 3: Keywords used to identify route of administration	Within the results from Set 2, searched for records containing the following terms within the publication: • oral* or diet or dietary or ingest* or capsule or tablet or supplement* or consum*
Set 4: Safety terms	Within the results from Set 3, searched for records containing the following terms within the titles and abstractsb: • safe* or risk or "adverse effect*" or "adverse event*" or "adverse reaction*" or "maximum tolerated dose" or "permissible dose level" or "maximum dose level" or threshold or tolerability or tolera*)

[a] Syntax for the search strategy is as follows: " " = search terms must appear directly beside each other in the exact order; * = truncation; NEAR/5 = search terms may appear within 5 words of each other with either term appearing first within the record
[b] The search terms were limited to titles and abstracts due to the vast amount of titles returned

The literature search strategy to identify nutritive safety and metabolism data related scFOS produced from sucrose is provided below in Table 2.1-4.

Table 2.1-4 Keywords Used to Retrieve Nutritive Safety and Metabolism Literature During the Updated Literature Search (April 1, 2014 to November, 2016)

Strategy[a]	
Set 1: Keywords used for exposure (FOS produced from sucrose)	Searched for records containing the following chemical names and synonyms within the publication: • fructooligosaccharide* OR fructo-oligosaccharide* OR "scFOS" OR "sc-FOS") AND (Sucrose or 57-50-1 or sugar* or actilight or neosugar or nutraflora or Meioligo)
Set 2: Animal study terms	Within the results from Set 1, searched for records containing the following terms within the publication: • animal or rat or mouse or mice or dog or rabbit or pig or hamster or monkey or rodent or pig or piglet
Set 3: Route of administration terms	Within the results from Set 2, searched for records containing the following terms within the publication[b]: • oral* or gavage or feeding or diet or dietary or intub* or "drinking water" or intragastric
Set 4: Metabolism terms	Within the results from Set 1, searched for records containing the following terms within the publication: • metabolis* or metaboliz* or "metabolic* fate*" or "metabolic* path*" or hydroly* or absorb* or absorp* or excret* or eliminat* or pharmacokinetic* or pharmacodynamic* or toxicokinetic* or toxicodynamic* or bioavailab* or biotransform* or ferment* or digest* or fecal or feacal or bowel or "short chain fatty acid*" or SCFA or mineral* or colon or caecum or cecum or fecal or faecal or bile or micro*

[a] Syntax for the search strategy is as follows: " " = search terms must appear directly beside each other in the exact order; * = truncation;
[b] This search is intended to identify any repeat dose study conducted in animals provided scFOS produced from sucrose

2.2 Literature Filtration

Once the search strategy was implemented and the publication titles were retrieved, the relevance of the publications was determined at 3 stages using the titles, abstracts, and the full-text of publications. At each stage, the output was manually reviewed against the inclusion/exclusion criteria listed in Table 2.2-1 were applied to determine literature relevance. The 3 stages are outlined below in greater detail.

- Stage 1: Titles of articles were reviewed, and abstracts of titles determined to be potentially relevant were retrieved.
- Stage 2: Abstracts were reviewed, and full-length articles of abstracts determined to be potentially relevant were retrieved.
- Stage 3: Full-length articles were reviewed, and those determined not to meet all the inclusion criteria specified in Table 2.2-1 were excluded.

Table 2.2-1 Inclusion and Exclusion Criteria Used to Filter the Identified Literature

Inclusion Criteria
• The food/food constituent studied was FOS derived from sucrose • A full-length article published in a peer-reviewed journal • The report analyzed safety-related parameters or metabolism parameters
Exclusion Criteria
• The food/food constituent studied was not FOS derived from sucrose • A full-length article published in a non-peer-reviewed source (*e.g.*, website, magazine, *etc.*) • Published in abstract form only or as a short communication (*e.g.*, conference abstract, letter to the editor, commentary, *etc.*) • A research synthesis study (*e.g.*, narrative review, systematic review, meta-analysis, *etc.*) • The report did not consider safety-related parameters or metabolism parameters • The study was a duplicate record in the literature search • Full publication study report not in English language

3.0 LITERATURE SEARCH RESULTS

The literature searches resulted in the identification of 596 potentially relevant titles, and abstracts were retrieved for 95 records. Following review of the 95 abstracts and recalling the potentially relevant full texts, 6 relevant clinical trials were identified for scFOS produced from sucrose conducted with children and adults. These studies are tabulated in the GRAS dossier. Following review of the potentially relevant 95 abstracts, 11 appeared to be conducted with animals provided scFOS produced from sucrose and including minor safety and/or metabolism related outcomes; although the animal data identified primarily analyzed the efficacious effects of FOS administration. The results from relevant animal studies identified during the literature search did not present findings that are inconsistent with the GRAS status of scFOS for use as a food ingredient. The full texts and/or abstracts for these articles are provided below in Table 3-1.

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Animal Studies Identified in the Updated Literature Search (April 2014 to November 2016)

Reference[2]	Electronic Copy of Publication
Mouse studies	

[2] It should be noted that the reference and abstracts for the potentially relevant full texts have been copied directly from the published abstract.

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Animal Studies Identified in the Updated Literature Search (April 2014 to November 2016)

Reference[2]	Electronic Copy of Publication
Daily Feeding of Fructooligosaccharide or Glucomannan Delays Onset of Senescence in SAMP8 Mice **Authors:** Sadako Nakamura, Naoyuki Kondo, Yoshitake Yamaguchi, Michiru Hashiguchi, Kenichi Tanabe, Chihiro Ushiroda, Miho Kawahashi-Tokuhisa, Katsuyuki Yui, Mana Miyakoda, and Tsuneyuki Oku **Journal:** Gastroenterology Research and Practice Volume 2014, Article ID 303184 We hypothesized that daily intake of nondigestible saccharides delays senescence onset through the improvement of intestinal microflora. Here, we raised senescence accelerated mice prone 8 (SAMP8) on the AIN93 diet (CONT), with sucrose being substituted for 5% of fructooligosaccharide (FOS) or 5% of glucomannan (GM), 15 mice per group. Ten SAMR1 were raised as reference of normal aging with control diet. Grading of senescence was conducted using the method developed by Hosokawa, and body weight, dietary intake, and drinking water intake were measured on alternate days. Following 38 weeks of these diets we evaluated learning and memory abilities using a passive avoidance apparatus and investigated effects on the intestinal microflora, measured oxidative stressmarkers, and inflammatory cytokines. Continuous intake of FOS and GMsignificantly enhanced learning and memory ability and decelerated senescence development when compared with the CONT group. Bifidobacterium levels were significantly increased in FOS and GM-fed mice. Urinary 8OHdG, 15-isoprostane, serum TNF-ı , and IL-6 were also lower in FOS-fed mice, while IL-10 in FOS and GM groups was higher than in CONT group. These findings suggest that daily intake of nondigestible saccharides delays the onset of senescence via improvement of intestinal microflora.	Nakamura et al 2014.pdf
Long term ingestion of a preload containing fructo-oligosaccharide or guar gum decreases fat mass but not food intake in mice **Authors:** Hadri, Z; Chaumontet, C; Fromentin, G; Even, P C; Darcel, N; Bouras, A D; Tome, D; Rasoamanana, R **Journal:** Physiology & Behavior 147 (2015): 198-204. Fermentable dietary fibre such as fructo-oligosaccharide and viscous dietary fibers such as guar gum and alginate affect energy homeostasis. The goal of this study was to compare the impact of long term intake of these three dietary fibers on food intake, meal pattern, body weight and fat accumulation in mice. Over a period of 3 weeks, the mice were fed daily with a preload containing 32 mg of fructo-oligosaccharide or alginate or 13 mg of guar gum. Food intake and body weight were monitored weekly, while meal patterns, adiposity and the expression of hypothalamic neuropeptide genes were evaluated at the end of the study period. The 3 dietary fibers produced a similar decrease in total daily food intake (14 to 22%) at the end of the first week, and this effect disappeared over time. The 3 dietary fibers induced a slight variation in satiation parameters. Body weight and expression of hypothalamic neuropeptide genes were not affected by any of the treatment. Preload of fructo-oligosaccharide and guar gum induced a similar and substantial decrease in the development of adiposity (17% and 14%, respectively), while alginate had no effect. Our results demonstrate mainly that the inhibitory effect of dietary fiber on food intake is lost over time, and that guar gum limits fat storage.	Full text not reviewed
Nondigestible fructans alter gastrointestinal barrier function, gene expression, histomorphology, and the microbiota profiles of diet-induced obese C57BL/6J mice. **Authors:** Liu TzuWen; Cephas, K D; Holscher, H D; Kerr, K R; Mangian, H F; Tappenden, K A; Swanson, K S **Journal:** J Nutr. 2016 May;146(5):949-56. doi: 10.3945/jn.115.227504. Epub 2016 Apr 6. Obesity is associated with compromised intestinal barrier function and shifts in gastrointestinal microbiota that may contribute to inflammation. Fiber provides benefits, but impacts of fiber type are not understood. Objective: We aimed to determine the impact of cellulose compared with fructans on the fecal microbiota and gastrointestinal physiology in obese mice. Methods: Eighteen-wk-old male diet-induced obese C57BL/6J	Full text not reviewed

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Animal Studies Identified in the Updated Literature Search (April 2014 to November 2016)

Reference[2]	Electronic Copy of Publication
mice (n=6/group; 40.5 g) were fed high-fat diets (45% kcal fat) containing 5% cellulose (control), 10% cellulose, 10% short-chain fructooligosaccharides (scFOS), or 10% inulin for 4 wk. Cecal and colon tissues were collected to assess barrier function, histomorphology, and gene expression. Fecal DNA extracts were subjected to 16S ribosomal RNA amplicon-based Illumina MiSeq sequencing to assess microbiota. Results: Body weight gain was greater (P<0.05) in scFOS-fed than in 10% cellulose-fed mice. Both groups of fructan-fed mice had greater (P<0.05) cecal crypt depth (scFOS: 141 μm; inulin: 145 μm) than both groups of cellulose-fed mice (5% and 10%: 109 μm). Inulin-fed mice had greater (P<0.05) cecal transmural resistance (101 Ω × cm2) than 5% cellulose-fed controls (45 Ω × cm2). Inulin-fed mice had lower (P<0.05) colonic mRNA abundance of Ocln (0.41) and Mct1 (0.35) than those fed 10% cellulose (Ocln: 1.28; Mct1: 0.90). Fructan and cellulose groups had different UniFrac distances of fecal microbiota (P<0.05) and α diversity, which demonstrated lower (P<0.01) species richness in fructan-fed mice. Mice fed scFOS had greater (P<0.05) Actinobacteria (15.9%) and Verrucomicrobia (Akkermansia) (17.0%) than 5% controls (Actinobacteria: 0.07%; Akkermansia: 0.08%). Relative abundance of Akkermansia was positively correlated (r=0.56, P<0.01) with cecal crypt depth. Conclusions: Fructans markedly shifted gut microbiota and improved intestinal physiology in obese mice, but the mechanisms by which they affect gut integrity and inflammation in the obese are still unknown.	
Rat studies	
Continuously Ingesting Fructooligosaccharide Can't Maintain Rats' Gut *Bifidobacterium* at a High Level **Authors:** Shaoting Li, Lijuan Gao, Long Chen, Shiyi Ou, Wang Y, and Xichun Peng **Journal:** Journal of Food Science Vol. 80, Nr. 11, 2015 Fructooligosaccharide (FOS) has been reported to increase Lactobacillus and Bifidobacterium populations in animal and human gut. Hence, it has been utilized to regulate the balance of gut microbiota. In this study, we compared the effects of high-FOS (HFOS) diet on normal and obese rats' gut Lactobacillus and Bifidobacterium, with high-soybeanfibers (HSF) diet as control. The results showed that the level of Bifidobacterium population substantially increased at week 4 in groups of rats fed the HFOS diet (P < 0.05), but significantly reduced to a small level at week 8 (P < 0.05); the abundance of Lactobacillus was increased in normal rats (P < 0.05), but decreased in obese rats (P < 0.05). The HSF diet did not promote the growth of Lactobacillus and Bifidobacterium in rats' gut. The findings suggested that Bifidobacterium population could not be maintained at a high level when the rats continuously ingested the HFOS diet for 8 wk; additionally, Lactobacillus population could adapt to a relatively stable level with the consumption of HFOS diet.	Li et al 2015.pdf
Lean rats gained more body weight from a high-fructooligosaccharide diet. **Author:** Li ShaoTing; Gu YingYi; Long, Chen; Gao LiJuan; Ou ShiYi; Peng XiChun **Journals:** Food and Function 6.7 (2015): 2315-2321. Fructooligosaccharides (FOS) are believed to be beneficial to the host growth and its gut health. This article is intended to investigate the different influences of a high-fructooligosaccharide (FOS) diet on the growth and gut microbiota of lean and obese rats. Diet-induced lean and obese rats were fed a high-FOS diet for 8 weeks. Rats' body weight (BW) and feed intake were recorded weekly, and their gut microbiota was analyzed by 16S rDNA sequencing. The results showed that the lean rats gained more BW than the obese ones from the high-FOS diet. In the meanwhile, the gut microbiota in both lean and obese rats was altered by this diet. The abundance of Bacteroidetes was increased significantly (P<0.05) in the lean rats, while no significant alteration in Firmicutes was observed in all rats after the consumption of a high-FOS diet. In conclusion, this study first reported that the lean rats gained more body weight from a high-FOS diet than the obese ones, and the increase of Bacteroidetes might help rats harvest more energy from the high-FOS diet.	Full text not reviewed

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Animal Studies Identified in the Updated Literature Search (April 2014 to November 2016)

Reference[2]	Electronic Copy of Publication
Digestibility of new dietary fibre materials, resistant glucan and hydrogenated resistant glucan in rats and humans, and the physical effects in rats **Authors:** Tsuneyuki Oku1,2*, Kenichi Tanabe2,3, Shigeki Morita2, Norihisa Hamaguchi4, Fumio Shimura1 and Sadako Nakamura1 **Journal:** British Journal of Nutrition (2015), 114, 1550–1559 Resistant glucan (RG) and hydrogenated resistant glucan (HRG) are newly developed non-digestible carbohydrate materials that decrease lifestyle-related diseases. The bioavailability of RG and HRG was investigated by in vitro experiments using human and rat small intestinal enzymes and by in vivo experiments using rats in the present study. Oligosaccharides, which are minor components of RG and HRG, were hydrolysed slightly by small intestinal enzymes of humans and rats, and the hydrolysing activity was slightly higher in rats than in humans. The amount of glucose released from HRG was greater than that from RG. However, the high-molecular-weight carbohydrates of the main components were hardly hydrolysed. Furthermore, neither RG nor HRG inhibited disaccharidase activity. When rats were raised on a diet containing 5 % of RG, HRG, resistant maltodextrin or fructo-oligosaccharide (FOS) for 4 weeks, all rats developed loose stools and did not recover during the experiment, except for the FOS group. Body weight gain was normal in all groups and was not significantly different compared with the control group. Caecal tissue and content weights were significantly increased by feeding RG or HRG, although other organ and tissue weights were not significantly different among the groups. In conclusion, RG and HRG consist of small amounts of glucose and digestible and non-digestible oligosaccharides, and large amounts of glucose polymers, which were hardly hydrolysed by α-amylase and small intestinal enzymes. RG and HRG, which were developed newly as dietary fibre materials, had no harmful effects on the growth and development of rats.	Oku et al 2015.pdf
Short-chain fructooligosaccharides do not alter glucose homeostasis but improve the lipid profile in obese rats. **Authors**: Silva-Morita, F. S. da; Balbo, S L; Mendes, M C; Kadowaki, M K; Yassuda Filho, P; Bonfleur, M L **Journal**: Acta Scientiarum - Health Science 37.2 (2015): 119-125. The present study investigated the effects of short-chain fructooligosaccharides (scFOS) feeding on body weight, fat accumulation, glucose homeostasis and lipid profile in cafeteria (CAF) obese rats. Male Wistar rats were divided randomly into two groups: control group (CTL, n=10), which received a chow diet and water and CAF (n=20), which received the cafeteria diet, standard chow and soda. After 30 weeks of diet, 10 animals of CAF group received scFOS in the diet (50 g kg-1 of diet) over a period of 50 days, forming the CAF FOS group. Were evaluated the body weight, fat pad as well as, quantity of feces, glucose tolerance, insulin resistance (IR) and serum lipids levels. Animals submitted to the CAF diet displayed obesity, hyperglycemia, glucose intolerance, hyperinsulinemia and IR. The scFOS feeding not altered obesity, glucose intolerance, hyperinsulinemia and IR. CAF rats also presented hypertriglyceridemia and lower levels of HDLcholesterol. The CAF FOS animals had reduced serum triglycerides (TG) and increased HDLcholesterol. Thus, the use of scFOS in the diet can be considered as a hypolipidemic agent in the obese state.	Soares da Silva-Morita et al 201
Short-term supplementation with dietary fructooligosaccharide and dietary mannitol elevated the absorption of calcium and magnesium in adult rats. **Author:** Xiao, J; Sakaguchi, E; Bai, G **Journal:** Czech Journal of Animal Science 61.6 (2016): 281-289. The effects of dietary fructooligosaccharide (FOS) and dietary mannitol on the absorption of Ca and Mg in a short term feeding trial were studied. Adult Wistar rats were divided into three groups and fed diets containing 0, 8% FOS or 8% mannitol for seven days. Daily intake and feces were monitored for three days to	Full text not reviewed

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Animal Studies Identified in the Updated Literature Search (April 2014 to November 2016)

Reference[2]	Electronic Copy of Publication
determine the apparent absorption of Ca and Mg. At the last day of the feeding trial, blood sample was collected from cecal vein to assess Ca and Mg levels. The cecum and colon were removed to analyze the parameters. The results showed that both dietary FOS and dietary mannitol significantly increased the apparent absorptions of Ca and Mg. Both dietary FOS and dietary mannitol significantly increased Ca concentration in cecal vein plasma, but did not affect Mg concentration. They significantly decreased Ca concentration and significantly increased soluble Ca concentration in cecal content dry matter (DM). The Mg concentration in colonic content DM was significantly decreased by feeding dietary FOS and dietary mannitol. FOS fermentation in cecum led to low cecal pH and increases in cecal organic acids concentration. Mannitol was fermented in cecum to induce low cecal pH and cecal wall extension. In conclusion, short-term supplementation with dietary FOS and dietary mannitol improved the apparent absorption of Ca and Mg. FOS and mannitol were fermented in cecum to elevate Ca absorption from cecum and to elevate Mg absorption in colon in rats.	
The salivary IgA flow rate is increased by high concentrations of short-chain fatty acids in the cecum of rats ingesting fructooligosaccharides. **Authors:** Yamamoto, Y; Takahahi, T; To, M.; Nakagawa, Y; Hayashi, T; Shimizu, T; Kamata, Y; Saruta, J; Tsukinoki, K **Journal:** Nutrients 8.8 (2016): 500. Salivary immunoglobulin A (IgA) serves as a major effector in mucosal immunity by preventing submucosal invasion of pathogens. However, the mechanism by which consumption of fermentable fibers increases IgA in saliva was not fully elucidated. This study investigated the effects of fructooligosaccharides (FOS) intake and time after feeding on IgA levels in the saliva and cecal digesta and on the concentration of short-chain fatty acids (SCFA) in the cecum in rats. Five-week-old rats were fed a fiber-free diet or a diet with 50 g/kg FOS for zero, one, four, and eight weeks. Ingestion of FOS at one and eight weeks led to a higher IgA flow rate of saliva per weight of submandibular gland tissue ($p<0.05$), which positively correlated with the concentration of SCFA in the cecal digesta ($rs=0.86$, $p=0.0006$, $n=12$), but showed no correlation with the concentration of IgA in the cecal digesta ($rs=0.15$, $p=0.3$, $n=48$). These results suggested that ingestion of FOS increased salivary IgA secretion through high levels of SCFA in the large intestine, which was produced by fermentation of FOS. Thus, continuously ingesting FOS for more than one week could increase secretion of salivary IgA.	Full text not reviewed
Pig studies	
Maternal Short-Chain Fructooligosaccharide Supplementation Influences Intestinal Immune System Maturation in Piglets **Authors:** Cindy Le Bourgot1, Ste´phanie Ferret-Bernard1, Laurence Le Normand1, Ge´rard Savary1, Enrique Menendez-Aparicio1, Sophie Blat1, Emmanuelle Appert Bossard2, Fre´de´ rique Respondek2, Isabelle Le Hue¨rou-Luron1* **Journal:** PLOS ONE | Peripartum nutrition is crucial for developing the immune system of neonates. We hypothesized that maternal short-chain fructooligosaccharide (scFOS) supplementation could accelerate the development of intestinal immunity in offspring. Thirty-four sows received a standard or a scFOS supplemented diet (10 g scFOS/d) for the last 4 weeks of gestation and the 4 weeks of lactation. Colostrum and milk immunoglobulins (Ig) and TGFb1 concentrations were evaluated on the day of delivery and at d 6 and d 21 postpartum. Piglet intestinal structure, the immunologic features of jejunal and ileal Peyer's patches, and mesenteric lymph node cells were analysed at postnatal d 21. Short-chain fatty acid concentrations were measured over time in the intestinal contents of suckling and weaned piglets. Colostral IgA (P,0.05) significantly increased because of scFOS and TGFb1 concentrations tended to improve (P,0.1). IFNc secretion by stimulated Peyer's patch and mesenteric lymph node cells, and secretory IgA production by unstimulated Peyer's patch cells were increased (P,0.05) in	Le Bourgot et al 2014.pdf

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Animal Studies Identified in the Updated Literature Search (April 2014 to November 2016)

Reference[2]	Electronic Copy of Publication
postnatal d 21 scFOS piglets. These differences were associated with a higher proportion of activated CD25+CD4a+ T cells among the CD4+ helper T lymphocytes (P,0.05) as assessed by flow cytometry. IFNc secretion was positively correlated with the population of activated T lymphocytes (P,0.05). Total short-chain fatty acids were unchanged between groups during lactation but were higher in caecal contents of d 90 scFOS piglets (P,0.05); specifically propionate, butyrate and valerate. In conclusion, we demonstrated that maternal scFOS supplementation modified the intestinal immune functions in piglets in association with increased colostral immunity. Such results underline the key role of maternal nutrition in supporting the postnatal development of mucosal immunity.	
Short-chain fructooligosaccharide supplementation during gestation and lactation or after weaning differentially impacts pig growth and IgA response to influenza vaccination. **Authors:** Bourgot, C le; Ferret-Bernard, S; Blat, S; Apper, E; Huërou-Luron, I le **Journal:** Journal of Functional Foods 24 (2016): 307-315. Short-chain fructooligosaccharides (scFOS) in the maternal diet during gestation and lactation positively modulate gut microbiota and maturation of the intestinal immune system in the offspring. The effect of maternal and post-weaning scFOS supplementation on the efficiency of the humoral response to influenza vaccination was evaluated. Seventeen sows received a standard or a scFOS supplemented diet for the last 4 weeks of gestation and lactation. From weaning, 128 pigs were fed a standard or a scFOS supplemented diet for 7 weeks. Post-weaning scFOS diet increased anti-influenza IgA levels in pig serum and faeces whereas maternal scFOS supplementation moderated the decrease of piglet growth induced by sow seropositivity to influenza during lactation and further resulted in a higher body weight at 10 weeks of age. This study confirms a potential interest of early scFOS supplementation to enhance vaccine response and to promote growth.	Full text not reviewed

Appendix B:

Expert Panel Statement

EXPERT PANEL CONSENSUS STATEMENT

Scientific Opinion on the Generally Recognized as Safe (GRAS) Status of short-chain Fructo-Oligosaccharides (scFOS) for Use as a Food Ingredient

April 27, 2017

Introduction

At the request of Galam Ltd. (Galam), an independent panel of scientists (the "Expert Panel"), qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients, was convened to conduct a critical and comprehensive evaluation of the available pertinent data concerning the use of short-chain fructo-oligosaccharides (scFOS) manufactured by Galam as a food ingredient. The Expert Panel consisted of the below-signed qualified scientific experts: Prof. Emer. Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine); Prof. Emer. George C. Fahey Jr, Ph.D. (University of Illinois), and Prof. Emer. Robert Nicolosi, Ph.D. (University of Massachusetts Lowell). For purposes of the Expert Panel's evaluation, "safe" or "safety" means that there is a reasonable certainty of no harm under the intended conditions of use of the ingredient in foods, as stated in 21 CFR §170.3(i) (U.S. FDA, 2016a).

The Panel, independently and collectively, critically evaluated a dossier titled "Documentation Supporting the Generally Recognized as Safe (GRAS) Status of short-chain Fructo-Oligosaccharides (scFOS) for Use as a Food Ingredient", which included a summary of the scientific information on scFOS prepared from a comprehensive search of the scientific literature, including both favorable and unfavorable data and information, as well as details pertaining to the method of manufacture and product specifications, supporting analytical data, intended conditions of use of scFOS in food, estimated exposure under the proposed food-uses, and a comprehensive assessment of the available scientific literature pertaining to the safety of the ingredient. In addition, the Panel evaluated other information deemed appropriate or necessary.

Following its independent, critical evaluation of such data and information, the Panel convened on Tuesday, April 18th, 2017, and unanimously concluded that scFOS as manufactured by Galam, meeting appropriate food-grade specifications, and manufactured according to current Good Manufacturing Practice (cGMP), is GRAS under all conditions of intended use as described GRN 44. This GRAS determination was based on scientific procedures, and a summary of the basis for the Expert Panel's conclusion is provided below.

Summary and Basis for GRAS

The ingredient evaluated by the Expert Panel is scFOS produced by Galam (Galam scFOS). Galam's scFOS preparations will be marketed as a high-purity (95 ± 2%) powder and syrup (Trade named FOS 95). FOS preparations are characterized as short-chain mixtures of fructose oligomers of which 1 (GF2; 1-kestose), 2 (GF3; nystose), or 3 (GF4; β fructofuranosylnystose) fructose units have been added by β2-1 glycosidic linkages to the fructose unit of sucrose. Several scFOS preparations have GRAS status for use as food ingredients for addition to specific conventional food and beverage products across multiple categories, including infant formula (GRN 44, 537, 605, 623) (U.S. FDA, 2000, 2015, 2016b,c). These FOS ingredients are manufactured in a similar manner utilizing the activity of a food grade enzyme to convert sucrose to fructose oligomers and produce a characteristic scFOS mixture of GF2, GF3, GF4 oligomers extended by β2-1 glycosidic linkages to the fructose unit of sucrose. Based on the chemical and compositional similarities of all scFOS preparations, published studies supporting the safety of other FOS preparations, including animal toxicity

studies and human clinical trials, were considered applicable to the general category of FOS and are therefore relevant to Galam's scFOS preparations.

Chemistry and Manufacturing

Short chain FOS ingredients that have GRAS status (*i.e.*, GRN 44, 537, 605, and 623), are produced in a similar manner involving the enzymatic transfructosylation of sucrose to produce a characteristic scFOS mixture of GF2, GF3, GF4 oligomers (U.S. FDA, 2000, 2015, 2016b,c). The scFOS solution is then typically filtered to remove the enzyme, de-colored with activated carbon, purified using ion-exchange, chromatography or nano-filtration methods, heat sterilized, and then concentrated to a syrup and/or spray dried to a powder.

Galam scFOS is produced in a similar manner, with an optimization modification to incorporate the use of enzyme immobilization system during FOS synthesis. The manufacturing process is conducted in accordance with current Good Manufacturing Practices (cGMP), the principles of Hazards Analysis and Critical Control Point (HACCP) and ISO 9001:2008 standards. In brief, Galam's manufacturing process involves the processing of food-grade sucrose syrup through an ion-exchange resin containing an immobilized mixed carbohydrase preparation from *Aspergillus aculeatus* with transfructosylation activity. The enzyme preparation has a long-history of safe use in fruit, and fruit juice processing and has GRAS status (GRASP 5G0297; U.S. FDA, 1985). The scFOS eluate is purified by column chromatography and activated carbon treatment to remove residual monomers and to reduce color bodies or other organic impurities. The scFOS solution is then concentrated by evaporation to produce a syrup or can be spray-dried to high purity powdered ingredient. Galam's scFOS is manufactured using raw materials and processing aids that meet food-grade quality specifications[1] and are permitted for use in food by U.S. federal regulation or are GRAS for their respective uses.

Based on the raw materials, production methods, and available compositional analyses, the Expert Panel agrees that Galam's scFOS manufacturing process produces a product that is consistent with the composition of other food-grade scFOS preparations as discussed above and is chemically representative of other GRAS sources of scFOS. The Panel also concluded that there are no novel manufacturing processes employed during the production of Galam scFOS that would introduce new reaction products or impurities to the ingredient.

Product Analysis

Galam has established food-grade chemical and microbiological specifications for both the FOS 95 syrup and spray dried powder. Batch analysis conducted with 4 non consecutive manufacturing lots of FOS 95 (2 lots of FOS 95 syrup and 2 lots of FOS 95 powder) demonstrate that scFOS produced by Galam's manufacturing process is a consistent product complying with the defined food-grade specifications. The results of the analytical testing confirm the oligosaccharide profile of Galam scFOS is consistent with other FOS oligomers (*e.g.*, GRN 44, 537 and 623). Furthermore, residual protein analysis conducted with 4 non-consecutive batches of FOS 95 (2 lots of FOS 95 syrup and 2 lots of FOS 95 powder) demonstrate the production enzyme is consistently excluded from the final product (below the limit of detection) during the manufacturing process. The Expert Panel agrees that the results of the batch analysis demonstrate a consistent product free of chemical and microbial impurities, and residual protein.

Intended Food Uses and Estimated Exposures

The scFOS manufactured by Galam is intended for use in the same food categories and use levels to those that have previously been determined to be GRAS by GTC Nutrition (GRN 44) and others (GRN 605, 623). Galam

[1] Compliant with the specifications set forth in the Food Chemicals Codex or equivalent international food or pharmacopeia standard (e.g., JECFA)

scFOS preparations will serve as an alternative to existing GRAS sources of FOS available in the U.S. marketplace and the introduction of the ingredient would not change the dietary exposure to FOS among U.S. consumers of foods to which FOS may be added.

Estimates of FOS consumption from the proposed uses were previously determined by GTC Nutrition in GRN 44 using the 1994-1996 CSFII conducted by the USDA (GTC Nutrition, 2000). The resulting mean and 90th percentile intakes of FOS by the total U.S. population from all proposed food-uses in the U.S., were estimated to range from 1.6 to 6.2 g/person/day (186 to 336 mg/kg body weight/day) and 3.1 to 12.8 g/person/day (127 to 614 mg/kg body weight/day). Among the individual population groups, the highest mean and 90th percentile intakes of FOS was determined to be 6.2 g/person/day (102 mg/kg body weight/day) and 12.8 g/person/day (211 mg/kg body weight/day), respectively, as identified among teenagers. When intakes were expressed on a body weight basis, toddlers had the highest mean and 90th percentile all-user intakes of 336 and 614 mg/kg body weight/day, respectively.

Additional correspondence was submitted to the U.S. Food and Drug Administration (FDA) by GTC Nutrition informing the Agency of GTC Nutrition's conclusion that "*…the addition of fructooligosaccharide is GRAS for use in foods in general, excluding meat and poultry products and infant formula, at levels up to 20 grams (g) per day in the general population and at levels up to 4.2 g per day in infants less than one year of age. GTC Nutrition provided a table of the typical use levels of fructooligosaccharide (Table 1).*"

The Expert Panel concluded that Galam's scFOS preparation will serve as an alternative to existing GRAS sources and therefore will not change the current dietary exposure to FOS among U.S. consumers of foods to which FOS may be added.

Information to Establish Safety

Several scFOS preparations have GRAS status for use as a food ingredient in a variety of conventional food and beverage categories, including infant formula (GRN 44, 537, 605 and 623) (U.S. FDA, 2000, 2015, 2016b,c). All of these FOS preparations are produced in a similar manner using food-grade sucrose as the substrate and the transfrucosylation activity of a food grade fungal enzyme to produce a defined mixture of scFOS. Accordingly, the GRAS status of scFOS preparations produced using a revised or new manufacturing process typically utilize an equivalence approach based on an evaluation of chemical similarities between a GRAS comparator (*e.g.*, GRN 44) and scFOS produced using the revised/new manufacturing process (*e.g.*, GRN 537, 605 and 623) (U.S. FDA, 2000, 2015, 2016b,c). Analysis of Galam's scFOS using HPLC-RI demonstrates that the ingredient is of high purity, and is compositionally representative of other scFOS preparations with GRAS status.

Since scFOS manufactured by Galam is chemically representative of other scFOS preparations that have been concluded to be GRAS (*e.g.*, GRN 44), a discussion of publically available data and information relevant to the safety of scFOS is incorporated by reference to pivotal studies discussed in GRN 44 (U.S. FDA, 2000). To identify new data pertinent to the safety of scFOS published since the GRAS status was last evaluated in 2014 (*i.e.*, GRN 537[2]), a comprehensive search of the published scientific literature was conducted for the period spanning from April 2014 through October 2016. Results of the pertinent toxicological studies from prior GRAS notifications and newly identified studies relevant to FOS safety and tolerance in humans are summarized below. Consistent with the requirements of the GRAS standard, conclusions on the GRAS status of scFOS have considered all publically available sources of information including favorable and potentially unfavorable

[2] At the time of this dossier preparation, GRN 537 was the most recent FOS GRAS to receive a "no questions" letter from the FDA which summarized literature prior to April 2014. FOS from Tata Chemicals Limited and New Francisco Biotechnology Corporation has since received a "no questions" letter (GRN 605 and 623).

information. Based on Galam's updated search of the literature, the company is not aware of newly published studies to suggest the scFOS is unsafe for use as a food ingredient.

The totality of publically available scientific literature relevant to the safe use of scFOS as an ingredient in food has been comprehensively evaluated, using scientific procedures, by a number of independent scientific experts, including the FDA (GRN 44, 537, 605 and 623) (U.S. FDA, 2000, 2015, 2016b,c). These GRAS notifications have consistently concluded that the addition of FOS to food is GRAS under their respective conditions of intended use. The safety of scFOS from sucrose for use as an alternative to inulin derived FOS was evaluated by the Foods Standards Australia New Zealand (FSANZ) in 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

"… scFOS produced by invertase catalysed condensation of sucrose is technologically justified and is as safe as IDS [inulin derived substances] already permitted to be added to foods generally, and infant formula products, infant foods and FSFYC [Formulated Supplementary Foods for Young Children] alone or in combination with IDS and GOS [galacto-oligosaccharides] up to the currently permitted maximum amounts. Additionally, scFOS has the potential to soften infant stools and may reduce the incidence of constipation, both of which are considered beneficial effects."

In Japan, scFOS (Neosugar) has a long-history of safe use as a general food use low-calorie sweetener since 1983 (Benkeblia, 2014). Based on conclusions from previous expert panels on the GRAS status of scFOS, corresponding no objection letters issued by the FDA, the widespread history of safe use of scFOS as a food ingredient globally, and conclusions from other authoritative bodies on the safety of scFOS as a food ingredient (*e.g.*, FSANZ), Galam has concluded that the current GRAS status of scFOS as described in GRN 44, can be extended to scFOS manufactured by Galam. Galam has therefore concluded that the company's scFOS ingredient, as described herein, is GRAS for the specified uses in conventional food products based on scientific procedures.

Metabolic Fate and Toxicity

The absorption, distribution, metabolism and excretion of scFOS along with the physiological effects on the gastrointestinal tract related to scFOS ingestion is well characterized and has been previously described in detailed (GRN 44, 537, 605, 623) (U.S. FDA, 2000, 2015, 2016b,c). Generally, FOS and related β2-1 fructans are not absorbed and are resistant to digestion by salivary amylase, human pancreatic or intestinal enzymes. FOS reaches the large intestine primarily intact where microbial fermentation occurs. Colonic fermentation products including short-chain fatty acids, methane, hydrogen and carbon dioxide are produced. Unfermented scFOS is excreted in the feces. Since all scFOS preparations are qualitatively equivalent, they are handled in a physiologically equivalent manner.

Toxicological Studies

Traditional toxicological studies conducted with FOS derived from sucrose include acute oral toxicity studies in mice and rats as well as 3 subacute studies, 1 subchronic study, 1 chronic study and 2 studies evaluating developmental and maternal toxicity conducted in rats.

The results of acute oral toxicity studies conducted with scFOS in male and female JcL-IcR mice and Sprague-Dawley rats demonstrate that scFOS is of low acute oral toxicity with median lethal dose values exceeding 9,000 mg/kg body weight (highest dose tested) in both mice and rats (Takeda and Niizato, 1982; summarized in Carabin and Flamm, 1999).

No toxicologically significant effects of relevance to humans have been reported in toxicological investigations of scFOS that have been published; no-observed-adverse-effect level (NOAEL) determinations are consistently reported to be the highest doses tested. Carabin and Flamm (1999) cited findings from unpublished subacute studies conducted by Takeda and Niizato (1982) supporting NOAELs of 4,500 to 5,000 mg/kg body weight/day (highest doses tested) following 6-week gavage or dietary administration of scFOS to Wistar rats. Tokunaga *et al.* (1986) reported that male Wistar rats consuming FOS at dietary concentrations of 10 and 20% [equivalent to approximately 4,185 and 7,795 mg/kg body weight/day, respectively (U.S. FDA, 1993)] for 6 to 8 weeks experienced transient watery stools during the first few days of administration and increased small and large intestine weights, and increased fecal and decrease gastrointestinal transit time. Meijl Seika Kaisha (1982), cited in GRN 44 (GTC Nutrition, 2000), reported a dose-related increase in diarrhea, soft stools, cecal distension, and increased large intestine weights for rats fed up to 20,400 mg/kg body weight/day for 90 days. The results of a 104-week chronic study conducted with Fischer 344 rats administered dietary scFOS supports NOAELs of 2,170 and 2,664 mg/kg body weight/day for males and females, respectively (Clevenger *et al.*, 1988). Short-chain FOS related effects apparent at high dose levels in these studies (*e.g.* intestinal weight increases, transient diarrhea, and soft/watery stools) are well-established effects consistent with the effects associated with intake of high-levels of non-digestible fibers and are considered to not be toxicologically relevant to humans (WHO, 1987).

Carabin and Flamm (1999) summarized the findings of an unpublished study conducted by Henquin (1988) and reported that dietary administration of scFOS to Wistar rats at concentrations up to 20% [equivalent to approximately 10,000 mg/kg body weight/day (U.S. FDA, 1993)] did not result in developmental toxicity[3]. In a another study evaluating the maternal and developmental toxicity of scFOS, dietary concentrations up to 20% [equivalent to approximately 10,000 mg/kg body weight/day (U.S. FDA, 1993)] provided to Sprague-Dawley (Crl CD (SD) BR) rats during postcoitum days 0 to 15 did not result in treatment related adverse effects (*e.g.* diarrhea), or differences in pregnancy outcome or *in utero* development (Sleet and Brightwell, 1990; summarized in Carabin and Flamm, 1999).

Genotoxicity

The genotoxicity of commercially available scFOS (Neosugar®) has been evaluated in *in vitro* genotoxicity assays including a bacterial reverse mutation assay and an unscheduled DNA repair assay conducted in accordance to guidelines established by the Organization for Economic Cooperation and Development (OECD) and a mammalian cell mutation assay conducted according to recognized methods (Clevenger *et al.*,1988). No evidence of genotoxicity was reported in a bacterial reverse mutation assay conducted with *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and TA1538 and *Escherichia coli* WP2 uvrA at doses up to 5,000 µg/plate in the presence and absence of Aroclor 1254-induced rat liver S9 metabolic activation. Similarly, scFOS at levels up to 5,000 mg/mL did not result in genotoxicity in a mammalian cell mutation assay conducted with Mouse lymphoma L5178Y cells in the presence and absence of Aroclor 1254-induced rat liver S9 metabolic activation. Negative results were also reported in an unscheduled DNA synthesis assay conducted with human epithelioid cells (HeLa S3) at concentrations up to 51,200 µg/mL. The results of these studies consistently demonstrate that scFOS are not genotoxic in bacteria and mammalian cells in the presence or absence of metabolic activation.

[3] Fetal markers other than body weight were not further described in the study summary.

Clinical trials

The totality of the publically available literature investigating the consumption of scFOS in human subjects has been the subject of several comprehensive evaluations by several notifiers, independent expert panels and the FDA during previous deliberations on the GRAS status of scFOS as described in GRN 44, 537, 605, 623 (U.S. FDA, 2000, 2015, 2016b). In the first GRAS notification for scFOS submitted to the offices of the FDA 16 years ago, GTC Nutrition concluded "The AIL [Acceptable Intake Level] for FOS ingestion for the general population, excluding infants less than one year of age, is determined to be 20 g/day; the AIL for infants less than one year old is 4.2 g/day" (GTC Nutrition, 2000).

Studies Identified in the Updated Literature Search (2014-2016)

To identify new data pertinent to the safety of FOS published since the GRAS status of FOS was last evaluated in 2014 (*i.e.*, GRN 537[4]), a comprehensive search of the published scientific literature was conducted for the period spanning from April 2014 through October 2016. From the literature search, 596 potentially relevant titles were identified, and abstracts were retrieved for 95. The search identified 1 study conducted in children and 5 conducted with adults, and the results of these studies did not result in adverse effects that would suggest scFOS are unsuitable or unsafe for food use. Only mild gastrointestinal side-effects were reported and these included flatulence, bloating, abdominal discomfort and transient diarrhea; findings that are well-established effects consistent with the effects associated with intake of high levels of non-digestible fibers.

Eleven relevant scFOS efficacy studies conducted with animals (3 mouse, 6 rat, and 2 piglet) provided outcomes relevant to safety. The results from safety parameters assessed did not reveal any toxicologically relevant adverse effects of scFOS.

The Expert Panel concluded that Galam's scFOS preparations are compositionally representative of other GRAS scFOS preparations with GRAS status and published studies supporting the safety of other FOS preparations, including animal toxicity studies and human clinical trials, are applicable to the general category of FOS and are therefore relevant to Galam scFOS preparations. No new evidence was found that would suggest that the use of scFOS as food ingredients would result in unsafe or undesirable effects.

Allergy

No reports of allergenicity associated with food uses of scFOS or cross reactivity to major food allergens have been identified. Analytical data on Galam's finished scFOS ingredients have demonstrated the absence of any protein in the final product (detection limit 0.01% or 100 ppm); therefore, the potential for allergenicity of scFOS manufactured by Galam is low. The production enzyme has GRAS status for general food use (U.S. FDA, 1985, and has a long-history of safe food use (*i.e.*, fruit and vegetable juice processing, beer and wine). The Expert Panel concludes that Galam's finished scFOS ingredients are unlikely to pose allergenic concerns.

[4] At the time of this dossier preparation, GRN 537 was the most recent FOS GRAS to receive a "no questions" letter from the U.S. FDA which summarized literature prior to April 2014. FOS from Tata Chemicals Limited and New Francisco Biotechnology Corporation has since received a "no questions" letter (GRN 605 and 623).

Conclusion

We, the members of the Expert Panel, have independently and collectively, critically evaluated the information summarized above and conclude that short-chain fructo-oligosaccharides (scFOS) as manufactured by Galam, using current Good Manufacturing Practices, and meeting appropriate food-grade specifications, is safe and suitable and GRAS based on scientific procedures, under the conditions of intended use in foods described in GRN 44.

It is our opinion that other qualified experts would concur with these conclusions.

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Professor Emeritus Joseph F. Borzelleca, Ph.D.
Virginia Commonwealth University School of Medicine

01 May 2017
Date

(b) (6)

Professor Emeritus Robert J. Nicolosi, Ph.D.
University of Massachusetts Lowell

02 May 2017
Date

(b) (6)

Professor Emeritus George C. Fahey Jr, Ph.D.
University of Illinois

5/3/17
Date

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