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April 1, 2021

Via FedEx & CD-ROM

Dr. Susan Carlson
Director, Division of Biotechnology and
GRAS Notice Review
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notification for Ingredion Incorporated's Short-Chained Fructooligosaccharides

Dear Dr. Carlson:

We respectfully submit the attached GRAS Notification on behalf of our client, Ingredion Incorporated, for Short-Chained Fructooligosaccharides ("scFOS"). scFOS is intended for use as a bulking agent and a general-purpose food ingredient in food categories that have previously been considered in past GRAS Notifications for scFOS, with one higher use level for the bars category, one expanded use category and use level for dairy beverages, and two new use categories in meal replacement shakes and dairy analog beverages. More detailed information regarding product identification, intended use levels, the manufacturing process, and safety of the ingredient is set forth in the attached GRAS Notification.

Ingredion has determined that its scFOS is GRAS for its intended uses based on scientific procedures in accordance with 21 C.F.R. § 170.30(b) and in conformance with the guidance issued by the Food and Drug Administration (FDA) under 21 C.F.R. § 170.36, 81 Fed. Reg. 54960 (Aug. 17, 2016). Therefore, the use of the scFOS as described in this GRAS Notification is exempt from the requirement of premarket approval as set forth in the Federal Food, Drug, and Cosmetic Act.

The analytical data, published studies, and information that are the basis for this GRAS Notification are available for FDA review and copying at reasonable times at Keller and Heckman LLP, 1001 G Street, NW, Suite 500W, Washington, DC 20001, or will be sent to FDA upon request.

We look forward to the Agency's review of this submission and would be happy to provide Agency officials with any information they may need to complete their assessment. Thank you for your attention to this matter.

Cordially yours,

Evangelia C. Pelonis

Enclosure

GRAS Notice for Short-Chained Fructooligosaccharides

Prepared for: Office of Food Additive Safety (FHS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Dr.
College Park, Maryland 20740

Submitted by: Keller and Heckman LLP
1001 G St., NW
Suite 500W
Washington, DC 20001

On behalf of our client:

Ingredion Incorporated
10 Finderne Ave.
Bridgewater, New Jersey 08807

Date: April 1, 2021

Table of Contents

List of Tables

List of Figures

Appendix

Part 1. Signed statements and certification

1. Applicability of 21 C.F.R. part 170, subpart E

We submit this generally recognized as safe (GRAS) notice in accordance with 21 C.F.R. part 170, subpart E.

2. Name and address of the notifier

Company: Ingredion Incorporated
Name: Debra Levine
Address: 10 Finderne Avenue, Bridgewater, New Jersey 08807
Phone: (908) 575-6203
Email: Debra.Levine@ingredion.com

All communications on this matter are to be sent to Counsel for Ingredion.

Evangelia C. Pelonis
Keller and Heckman LLP
1001 G Street, NW
Suite 500W
Washington, DC 20005
Tel: 202-434-4106
Fax: 202-434-4646
Email: pelonis@khlaw.com

3. Name of the notified substance

The common or usual name of the notified substance will be short-chain fructooligosaccharides (scFOS). It is also commonly called short-chain fructan. This substance is chemically identical to other fructan products on the market, consisting of a linear chain of f3(21)-linked fructose residues with a glucose termination. It is sold by Ingredion under the trade name NutraFlora®. The systematic name of all fructans is {α-D-glucopyranoside-(1-2)-}f3-D-fructofuranosyl-[(1-2)-f3-D-fructofuranosyl]$_n$. The brace around the terminal glucopyranoside is placed there to recognize that the presence of this residue is not invariable in fructans, although it is always present in scFOS. While fructans can have degrees of polymerization (DP; the number of fructose or glucose residues) ranging from 2 to more than 60, scFOS consists entirely of molecules with DP between 3 and 5, consisting of 2 to 4 fructose residues and a single terminal glucose residue. These molecules may also be regarded as consisting of a single sucrose molecule with 1-3 fructose units linked by f3(2-1) linkages.

4. Applicable conditions of use of the notified substance

Ingredion intends to market scFOS as a bulking agent and a general-purpose food ingredient in food categories that have previously been considered in past GRAS Notices with one new use level for the bars category, one expanded use category and use level for dairy beverages, and two new food categories: meal replacement shakes and dairy analog beverages.

5. Basis for the GRAS determination

Keller and Heckman LLP, on behalf of Ingredion, hereby notifies the Agency of its determination that Ingredion's scFOS is GRAS for its intended use, consistent with Section 201(s) of the Federal Food, Drug, and Cosmetic Act (FD&C Act). This GRAS conclusion is based on scientific procedures in accordance with 21 C.F.R. §170.30(a) and (b) and conforms to the guidance issued by the Food and Drug Administration (FDA) under 21 C.F.R. §170.36, 81 Fed. Reg. 54,960 (Aug. 17, 2016). The statutory basis for our conclusion of GRAS status is through scientific procedures in accordance with proposed 21 C.F.R. § 170.36. The GRAS status of scFOS is based on data generally available in the public domain.

6. Exclusion from premarket approval

The notified substance is not subject to the premarket approval requirements of the FD&C Act based on our conclusion that the notified substance is GRAS under the conditions of its intended use.

7. Availability of data and information

The information for this GRAS conclusion including analytical data, published studies, and information that are the basis for this GRAS determination are available to FDA upon request as required by 21 C.F.R. § 170.225(c)(7)(ii)(A) or (B) by contacting Keller and Heckman LLP at the below address.

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8. Applicability of FOIA Exemptions

Ingredion is not claiming any information in Parts 2 through 7 of this document as trade secret, confidential or financial information that is privileged or confidential. Thus, all information and data in this submission are not exempt from the Freedom of Information Act (FOIA), 5 U.S.C. Section 552.

9. Certification

We certify on behalf of our client, Ingredion, that this GRAS conclusion is based on representative data from Ingredion required to support the safety and GRAS status of scFOS. To the best of our knowledge, it is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

10. Signature and name and title of the person signing this GRAS notice:

Date: April 1, 2021

Evangelia C. Pelonis
Partner
Keller and Heckman LLP

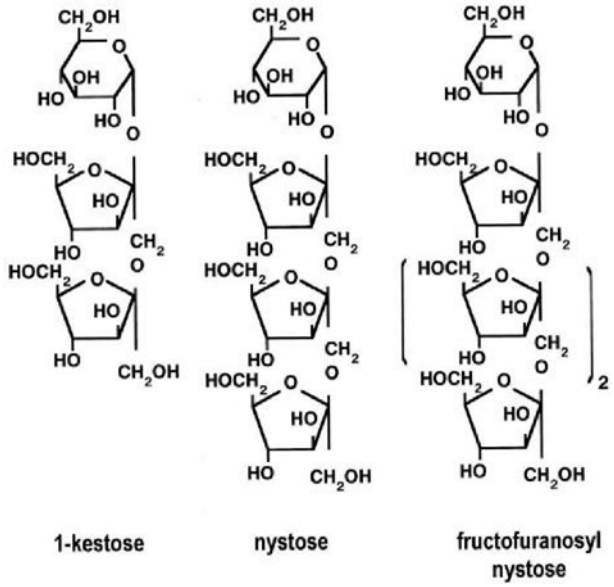
Part 2. Identity, method of manufacture, specifications, and physical or technical effect

1. Identity

The subject of the current GRAS Notice is short-chain fructooligosaccharides (scFOS) (Chemical Abstracts Registry Number (CASRN) 308066-66-2), marketed under the tradename NutraFlora®. Short-chain FOS is one member of a group of f3(2-1) oligo- and polysaccharides designated fructans. The common feature of the fructan oligosaccharides is that they comprise essentially linear chains of fructose units linked by f3(2-1) fructosyl-fructose linkages, sometimes with a single terminal glucose molecule; this terminal glucose residue is invariably present in scFOS. The systematic name of all fructans, including scFOS, is {α-D-glucopyranoside-(1-2)-}f3-D-fructofuranosyl-[(1-2)-f3-D-fructofuranosyl]$_n$. The brace around the terminal glucopyranoside is placed there to recognize that the presence of this residue is not invariable in fructans other than scFOS. Since the f3(2-1) linkage joining the glucose residue with the neighboring fructose residue is the same as is found in the disaccharide sucrose, the configuration may also be viewed as a sucrose molecule linked to a chain of fructose units by f3(2-1) fructosyl-fructose linkages.

NutraFlora® scFOS consists of 3 different molecules, each containing a terminal glucose residue and 2, 3, or 4 fructose residues, designated GF_2, GF_3, and GF_4, with the respective names 1-kestose, nystose, and fructofuranosylnystose. The molecular formula of NutraFlora® scFOS is the same as that of all fructans: $C_6H_{11}O_5(C_6H_{10}O_5)_nOH$. The formulas of its three components are: 1-kestose—$C_{18}H_{32}O_{16}$, nystose—$C_{24}H_{42}O_{21}$, and fructofuranosylnystose—$C_{30}H_{52}O_{26}$. The molecular weight (MW) of scFOS is 700, representing the average of the molecular weights of its 3 components (505, 666, and 828, respectively), which are present at approximately 37%, 53%, and 10%, respectively. The structural formulas of the three components are shown in Figure 1.

Figure 1. Structural Formulas of 1-Kestose, Nystose, and Fructofuranosylnystose



NutraFlora® scFOS is available in both liquid (syrup) and powder forms. A brief summary of the physical and chemical properties of both forms of the product is provided in Table 1.

Table 1. Physical and Chemical Properties of NutraFlora® scFOS

Physical/Chemical Property	Value	
	Syrup	Powder
Appearance	Slightly viscous fluid	Colorless powder
Appearance in solution	Colorless	Colorless
Taste	Slightly sweet	Slightly sweet
Odor	None	None
pH (10% solution)	5.0 – 7.5	5.0 – 7.0
Solubility in water	High	High
Solubility in ethanol	Insoluble	Insoluble
Viscosity (cP, 70% solution at 30ºC)	1007 - 1759	1000

In May 2000, GTC Nutrition Company (subsequently acquired by Ingredion) submitted GRAS Notice No. 44 and received a "no objection letter" (NOL) from FDA in November 22, 2000 for scFOS's use in various food categories at various use levels.[1] Additional correspondence regarding intended uses was submitted for GRAS Notice No. 44 and FDA responded with a NOL on June 1, 2007. Ingredion also submitted GRAS Notice No. 537 and received an NOL from FDA on February 6, 2015 for scFOS's use in infant formula.[2]

2. Method of Manufacture

To produce scFOS, purchased dry or liquid sucrose is diluted with water, and the pH is adjusted. The β-fructofuranosidase enzyme (produced by *Aspergillus fijiensis*) is added at a controlled temperature. *A. fijiensis* is a synonym for *A. japonicus*, which is a member of the *niger* group. The enzyme has an optimum temperature of 60ºC and pH of 5.5; it is stable within

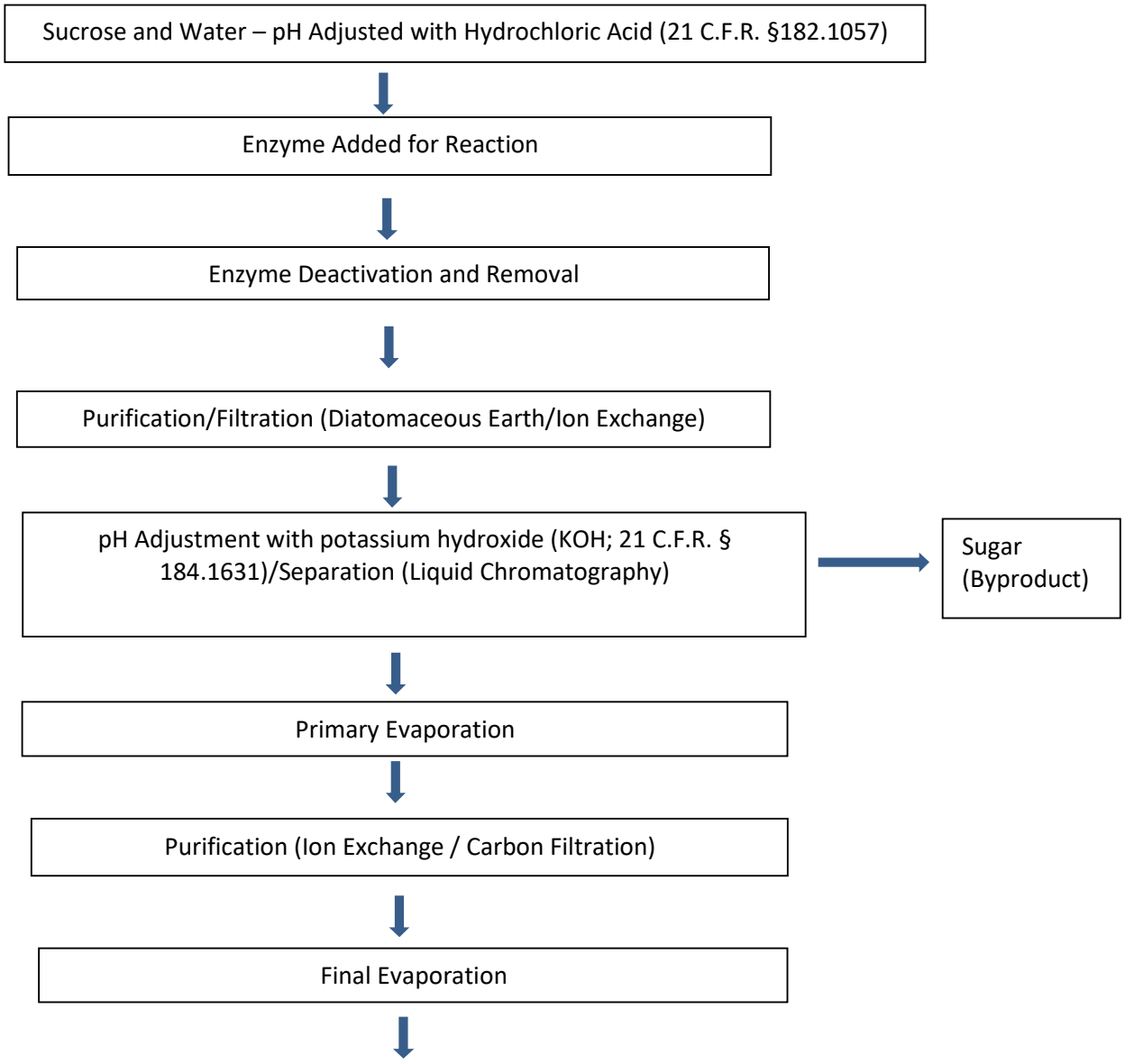
[1] *See* FDA, GRAS Notice (GRN) 44, available at https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=44&sort=GRN_No&order=DESC&startrow=101&type=basic&search=44.

[2] *See* FDA, GRAS Notice (GRN) 537, available at https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=537&sort=GRN_No&order=DESC&startrow=1&type=basic&search=537.

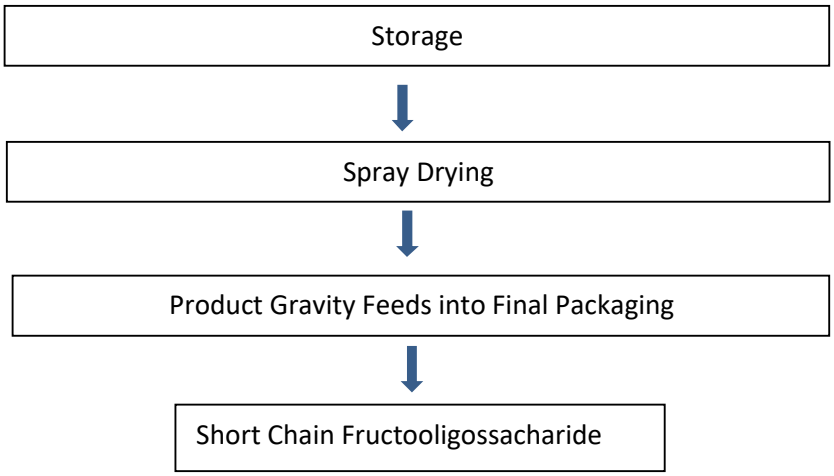
a pH range of 4 to 8.[3] The enzyme acts as an invertase on sucrose molecules and a fructosyltransferase between both sucrose molecules and fructofuranosylsucrose molecules. The invertase activity cleaves sucrose into glucose and fructose units, while the fructofuranosidase activity transfers the fructose unit from sucrose to a growing fructose chain to yield 1-kestose (GF2), nystose (GF3), and fructofuranosylnystose (GF4) molecules. One glucose residue is released as each fructose unit is added to the chain.

The manufacturing process is shown in Figure 2 below.

Figure 2: Current Manufacturing Process



[3] Quang, D.N., *et al.*, 2005. Purification and some properties of β-fructofuranosidase from *A. niger* IM1303386. *Process Biochem* 40:2461-2466.



As noted above, Ingredion obtained a no questions letter for GRAS Notice 44 and 537 for scFOS produced in a slightly different manner for use in related products.

1. scFOS was produced by dissolving granular sucrose in water, held at a constant temperature, followed by addition of the β-fructofuranosidase enzyme (produced by *A. fijiensis)*. In the current process, scFOS may be produced with dry or liquid sucrose diluted with water, followed by pH adjustment with hydrochloric acid.[4]

2. There were steps described as "decolorization," which were achieved with active carbon. In the current process, there are two purification steps. The first purification step is achieved with diatomaceous earth and ion exchange, and the second purification step is achieved with carbon filtration.

3. After the first active carbon and decolorization steps, the material was filtered, desalted, concentrated, treated with active carbon, and underwent chromatography before the second decolorization step. In the current process, only pH adjustment with potassium hydroxide (KOH)[5] and separation of scFOS from the sugar byproduct occurs between the first and second purification steps.

4. In the original process, potassium hydroxide (KOH) was added during the reaction phase to maintain the pH within the desired range. In the current process, KOH is added (a) after the reaction phase, (b) after the second ion

[4] HCL is GRAS listed for use in food as buffer and neutralizing agent under 21 C.F.R. § 182.1057.

[5] KOH is affirmed as GRAS for processing aid use under 21 C.F.R. § 184.1631.

exchange before carbon filtration, and (c) in the sugar separation step for pH control.

5. After the second decolorization step in the original process, there was filtration (after which scFOS syrup was derived), followed by concentration and drying to produce scFOS powder. In the current process, the second purification step is followed by evaporation, storage (after which scFOS syrup may be derived), and spray drying to produce scFOS powder.

While the current manufacturing process differs slightly from the process described in past GRAS Notices, the finished product identity remains essentially identical.

3. Product Specifications and Batch Analyses

(a) Product Specifications

Ingredion has established various physical, compositional, and microbiological specifications for scFOS. The specifications, which have been selected to ensure a consistent food-grade product, are summarized in Table 2 together with the methods. All analytical methods have been validated for their intended use.

Table 2. Specifications for Food Grade scFOS

Parameter	Unit	Specification	Method[1]
Appearance		White Powder	Internal Method (Visual)
Flavor		Slightly Sweet	Internal Method (Sensory)
Odor		None	
Total scFOS	%	NLT[1] 95.0	Internal Method (High-Performance Liquid Chromatography)
1-Kestose (GF2)	%	NLT 30.0	
Nystose (GF3)	%	NLT 45.0	
Fructofuranosylnystose (GF4)	%	NLT 5.0	
Sugars	%	NMT[2] 5.0	
Protein	%	NMT 0.01	Kjedahl
Ash	%	NMT 0.05	USP
Moisture	%	NMT 5.00	Internal Method (Refractive Index)
pH		5.0-7.0	Internal Method
Lead	mg/kg	NMT 1.0	ICP MS Heavy Metals AOAC 2015.01 Mod<2232
Arsenic	mg/kg	NMT 1.0	
Standard plate count	cfu[3]/g	NMT 300	Internal Methods (based on AOAC methods)
Yeast	cfu/g	NMT 20	
Mold	cfu/g	NMT 20	
Coliforms	cfu/g	NMT 10	
Escherichia coli	cfu/10 g	Negative	Current USP/NF,62
Anaerobic Mesospores	cfu/g	NMT 10	Compendium of Methods for the Microbiological Examination of Foods (CMMEF), 5th ed.
Aerobic Thermophilic Spores	cfu/10 g	NMT 10	

Anaerobic thermophilic spores	Tubes Positive	NMT 10	CMMEF, 4th ed.
Aerobic mesophilic spores	cfu/g	NMT 10	CMMEF, 5th ed.
Salmonella spp. PCR	cfu/100 g	Negative	AOAC-RI100201
Coagulase-positive *Staphylococci*	cfu/10 g	Negative	Current USP/NF,62
Enterobacteriaceae MPN[4]	cfu/30 g	Negative	ISO21528-1:2017

[1] – Internal methods have been validated: NLT: Not less than; NMT: Not more than; cfu: Colony forming unit; MPN: Most probable number.

Table 3 below provides the results of three non-consecutive lots of product demonstrating that the specifications are consistently met.

Table 3. Batch Analyses for Food Grade scFOS

Parameter	**Unit**	**Specification[1]**	**7280203300**	**7280338308**	**7280288307**
Appearance		White Powder	Passes	Passes	Passes
Flavor		Slightly Sweet	Passes	Passes	Passes
Odor		None	Passes	Passes	Passes
Total scFOS	%	NLT 95.0	96.1	95.8	95.8
1-Kestose (GF2)	%	NLT 30.0	37.2	36.5	37.0
Nystose (GF3)	%	NLT 45.0	47.9	48.5	47.8
Fructofuranosylnystose (GF4)	%	NLT 5.0	11.0	10.8	11.0
Sugars	%	NMT 5.0	3.9	4.2	4.22
Protein	%	NMT 0.01	0.00	0.00	0.00
Ash	%	NMT 0.05	0.00	0.00	0.00
Moisture	%	NMT 5.00	3.9	3.4	3.21
pH		5.0-7.0	6.1	6.2	6.0
Lead	mg/kg	NMT 1.0	<0.01	<0.01	<1.0
Arsenic	mg/kg	NMT 1.0	<0.01	<0.01	<0.01
Standard plate count	cfu/g	NMT 300	<10	<10	<10
Yeast	cfu/g	NMT 20	<10	<10	<10
Mold	cfu/g	NMT 20	<10	<10	<10
Coliforms	cfu/g	NMT 10	<10	<10	<10
Escherichia coli	cfu/10 g	Negative	Negative/10	Negative/10g	Negative/10g
Anaerobic Mesospores	cfu/g	NMT 10	<3	<3	<3
Aerobic thermophilic spores	cfu/10 g	NMT 10	<5	<5	<5

Anaerobic thermophilic spores	Tubes positive	NMT 10	0/6 tubes positive	0/6 tubes positive	0/6 tubes positive
Aerobic mesophilic spores	cfu/g	NMT 10	<1	<1	<1
Salmonella spp. PCR	cfu/100 g	Negative	Negative	Negative	Negative
Coagulase-positive *Staphylococci*	cfu/10 g	Negative	Negative	Negative	Negative
Enterobacteriaceae MPN	cfu/30 g	Negative	<0.3	<0.3	<0.3

[1] NLT: Not less than; NMT: Not more than; cfu: Colony forming unit; MPN: Most probable number.

Part 3. Dietary exposure

1. Estimate of Dietary Exposure

Ingredion intends to market scFOS as a bulking agent and a general-purpose food ingredient, as detailed below. We note that scFOS is described as a source of dietary fiber in FDA's Review of the Scientific Evidence on the Physiological Effects of Certain Non-Digestible Carbohydrates (June 2018).[6,7] The uses addressed in this submission do not include infant formula or use in USDA-regulated meat and poultry products. Maximum daily intakes of scFOS have been calculated to be no more than approximately 20 g/day for a 90th percentile consumer.

2. Cumulative Estimated Dietary Exposure

Keller and Heckman had a consumption estimate done on behalf of Ingredion, which is attached as Appendix 1. [8] The consumption estimate was generated based on 2015-2016 NHANES data, to address the applications in Table 4 below. While many of these applications mirror those in previous GRAS notices, there are the following differences:

1) a higher use level for the bars category,
2) one expanded category and higher use level for dairy beverages (including cultured dairy, rather than only unflavored and flavored milk), and
3) two new food categories: meal replacement shakes and dairy analog beverages.

The bars category was included in GRN 44 with a use level of up to 1.4-2.5% per 40 gram or 70 gram RACC for a total use level of 0.56-1.75 grams scFOS per serving. Here, we have included a use level of 15% scFOS per 40 gram bar for a total use level of 6 grams scFOS per serving. GRN 44 previously included "milk, flavored and unflavored" with a use level of 0.4% per 240 mL RACC for a total use level of approximately 1 gram per serving. Here, we have expanded the category to include 1.3% in all types of dairy beverages including cultured dairy beverages with a 240 mL serving size and 1.5-3.2% in drinkable yogurts with a standard serving of 93-207 grams for a total level of use of 3 grams per serving. The two new categories added are: (1) meal replacement shakes (milk-based and non-milk based) with a 2.5% use level, which based on a 240 mL RACC yields 6 grams per serving; (2) dairy product analogs with a 1.3% use level, which based on a 240 mL RACC yields 3 grams per serving. The rest of the

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6 In FDA's Review of the Scientific Evidence on the Physiological Effects of Certain Non-Digestible Carbohydrates (June 2018), FDA included short chain FOS as an "inulin-type fructan" found to meet the definition of "dietary fiber." *See* page 18 at https://www.fda.gov/media/113659/download.

7 Mobley, A.R. *et al.* (2014). Identifying Practical Solutions to Meet America's Fiber Needs: Proceedings from the Food & Fiber Summit. Nutrients 6: 2540-2551.

8 Estimated Daily Intake of Short Chain Fructooligosaccharide by the U.S. Population from Permitted and Proposed Food Uses (2015-2016 NHANES), August 14, 2020, Appendix 1.

product categories and use levels are the same as those covered in GRN 44 and the additional correspondence to GRN 44.

The estimates should be seen as conservative from the standpoint that—where ranges were provided for a given serving size and/or level of use, the largest of the potential combinations was used. In fact, it is not likely for various foods that the upper level use level would be used in the larger serving-size product. For example, there is a category in Table 4 below for "Sauces, Gravies, and Condiments" for which the serving size is provided as "30 to 125 g." The relevant serving size for condiments is 30 grams, whereas 125 grams is the largest serving size for any sauces under Section 101.12 of the food additive regulations for "Major main entree sauces, *e.g.*, spaghetti sauce." The use levels of interest in the table are reported as "0.8 to 3.3" percent. Ingredion's research indicates that it is the products with the lower serving size (*i.e.*, the condiments) that would be most likely to employ the highest scFOS use level of 3.3% (*e.g.*, 30 g × 3.3% = 0.99 g scFOS), and the larger serving size items would be employ the lowest scFOS use level (*e.g.*, 125 g × 0.8% = 1 g). However, the consumption estimate takes the approach of multiplying the lower end of the serving size range with the lower end of the use level range (*e.g.*, 30 g × 0.8% = 0.24 g scFOS) and the higher end of the use level range by the high end of the serving size range (*e.g.*, 125 g × 3.3% = 4.13 g scFOS) to calculate the scFOS levels in the right-hand column (*i.e.*, in this case, 0.24 to 4.13 g). Accordingly, the estimates in the table are expected to generally overestimate actual uses, as it is not expected that the products with the larger serving sizes will employ scFOS at the highest use level described for that food category.

Table 4. Typical Use Levels of scFOS

Food Category[a,b]	Standard Serving Size	Level of Use/Serving (percent)	Level of Use/Serving (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[d]	40 g	15.0	6 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

Food Category[a,b]	Standard Serving Size	Level of Use/Serving (percent)	Level of Use/Serving (mL or g /serving)[c]
Dairy Beverages[e]	240 mL (all types) 93 to 207[f] (drinkable yogurts only)	1.3 1.5 to 3.2	3 g
Dairy Product Analogs	240 mL	1.3	3 g
Dessert Toppings and Fillings	30 g	3.3	0.99 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)[g]	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
Meal Replacement Shakes (milk-based and non-milk based)[h]	240 mL	2.5	6 g
Milk, Flavored and Unflavored[i]	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 fructooligosaccharide

[a] The food use categories, standard serving sizes and proposed use levels (%) are adapted from GRAS Notice No. 44 (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] Bars were included at up to 2.5% as a typical use level in GRN 44; however, bars are included at a higher proposed use level of 15.0% in the current assessment.

[e] The category of dairy beverages includes milk (flavored and unflavored), cultured dairy beverages like Kefir, and drinkable yogurts).

[f] RACC has not been established for yogurt drinks; however, an approximate serving size was established based on products currently on the U.S. market.
[g] This category excludes infant formula.
[h] The category of meal replacement shakes (milk-based and non-milk based) were included under beverages and juices in GRN 44; however, they are currently included as a separate category at a proposed use level of up to 2.5%.
[i] Milk (flavored and unflavored) were included at up to 0.4% as a typical use level in GRN 44; however, they are currently included under the category of dairy beverages at a proposed use level of up to 1.3%.

Table 5: Summary of the Estimated Daily Intake of scFOS from Typical and Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	**Age Group (Years)**	***Per Capita* Intake (g/day)**		**Consumer-Only Intake (g/day)**			
		Mean	**90th Percentile**	**%**	**n**	**Mean**	**90th Percentile**
Infants	0 to <1	3	7	64.2	204	4	9
Toddlers	1 to 2	10	17	99.9	369	10	17
Children	3 to 11	10	15	100	1,176	10	15
Female Teenagers	12 to 19	8	12	100	473	8	12
Male Teenagers	12 to 19	10	17	99.4	491	10	17
Female Adults	20 and up	9	15	100	2,205	9	15
Male Adults	**20 and up**	**11**	**20**	**99.9**	**1,994**	**11**	**20**
Total Population	**All ages (ages 2 and up)**	**10**	**17**	**99.5**	**6,912**	**10**	**17**
n = sample size							

Table 6: Summary of the Estimated Daily Per Kilogram Body Weight Intake of scFOS from Typical and Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (g/kg bw/day)		Consumer-Only Intake (g/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to <1	0.30	0.86	64.2	204	0.47	1.03
Toddlers	1 to 2	0.80	1.32	99.9	364	0.80	1.32
Children	3 to 11	0.38	0.65	100	1,171	0.38	0.65
Female Teenagers	12 to 19	0.14	0.26	100	465	0.14	0.26
Male Teenagers	12 to 19	0.15	0.26	99.4	490	0.15	0.26
Female Adults	20 and up	0.12	0.21	100	2,191	0.12	0.21
Male Adults	20 and up	0.13	0.24	99.9	1,968	0.13	0.24
Total Population	All ages (ages 2 and up)	0.17	0.35	99.5	6,853	0.18	0.35
n = sample size							

Thus, based on the consumption estimate report in Appendix 1, the estimated daily intake (EDI) for scFOS for the total populations (ages 2 and up) is 10 grams per person per day (10 g/p/day) at the mean consumption level and 18 grams per person per day (18 g/p/day) at the 90th percentile consumption level. The highest rate of exposure was seen in the male adult population, which was 11 g/p/day for the mean and 20 g/p/day for the 90th percentile male adult population. In comparison, the additional correspondence to GRN 44 dated June 1, 2007 considered 20 grams per person per day at the 90th percentile in the general population. Thus, there is no increased exposure to scFOS as a result of these higher use levels and new product categories.

As discussed in GRN 44 and subsequently cited by other notices submitted for scFOS (GRNs 537, 605, 623, 717, and 797), multiple clinical studies in children and adult human subjects of scFOS have confirmed the non-toxic nature of this ingredient.[9] The published

[9] *See* FDA, GRN 44, available at, https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=44&sort=GRN_No&order=DESC&startrow=101&type=basic&search=44; see GRN 605, available at, https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=605&sort=GRN_No&order=DESC&startrow=1&type=basic&search=605; see GRN 623 available at, https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=623&sort=GRN_No&order=DESC&startrow=1&type=basic&search=623; see GRN 717 available at,

studies describing the safety of scFOS described the acceptable intake level of intake described in the cited GRNs for infants less than one year old was 4.2 g/day, while for the general population older than one year of age the intake with no adverse effect was determined to be 20 g/day scFOS. FDA had no questions regarding these safe reference levels regarding the GRAS status of the intended uses of scFOS. The available information in the published literature reveals only mild gastrointestinal side-effects of scFOS consumption that included flatulence, bloating, abdominal discomfort, and transient diarrhea. These findings are well-described in the scientific literature, and updated searches of the recent scientific literature as of March 23, 2021 did not identify any new studies relevant to the safety of scFOS in children or adults or studies that would question or modify conclusions reached in previous GRAS notices.

https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=717&sort=GRN_No&order=DESC&startrow=1&type=basic&search=717; see GRN 797 available at, https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=797&sort=GRN_No&order=DESC&startrow=1&type=basic&search=797.

Part 4. Self-limiting Levels of Use

The use of scFOS is not self-limiting.

Part 5. Experience based on common use in food before 1958

The basis of this GRAS assessment is not common use in food before 1958.

Part 6. Safety Narrative: Comprehensive Discussion of the Basis of GRAS Status of ScFOS

Since GRAS Notice 537, additional GRAS Notices (*i.e.*, GRAS Notice 605 (filed by Tata Chemicals Limited for FOS), GRAS Notice 623 (filed by New Francisco Biotechnology Corporation (NFBC) for FOS), and GRAS Notice 717 (filed by Galam, Ltd. for scFOS)) have been filed regarding FOS and scFOS when used in a wide array of foods other than infant formula (*e.g.*, acidophilus milk, nutritional bars, baby food, nutritional beverages, biscuits, cakes, confectionery, cookies, crackers, flavored and unflavored milks, hard candy, ice cream and frozen yogurt, jams and jellies, muffins, ready-to-eat cereals, sorbet, soup and yogurt), and GRAS Notice 797 addressed the use of NFBC's FOS in infant formula at the same use levels as GRAS Notice 537.[10] In its most recent a "no questions letter" regarding FOS, issued to NFBC on November 15, 2018 for GRAS Notice 797, FDA stated with respect to the safety of FOS:[11]

> NFBC discusses the safety of FOS and incorporates into the notice published toxicity studies cited in GRNs 000044, 000537, 000605, 000623, and 000717.[1] These published toxicity studies on FOS include acute, short-term (6-8 weeks), subchronic, chronic, carcinogenicity, developmental, and reproductive, as well as *in vitro* genotoxicity assays. NFBC states that these studies did not reveal any toxicologically relevant treatment-related adverse effects, including a 104-week study where male and female rats were fed FOS through the diet at up to 2170 mg/kg bw/d and 2664 mg/kg bw/d, respectively. NFBC states that an updated literature search was conducted through March 2018 and did not reveal any new data or information on the effects of FOS in adults or children.
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> _____
>
> FN1 FOS was the subject of GRNs 000044, 000537, 000605, 000623, and 0007 17 . We evaluated these notices and responded in letters dated November 22, 2000, February 6, 2015, March 17, 2016, August 1, 2016, and February 13, 2018, respectively, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

Galam Ltd. provided a comprehensive summary of available safety data concerning scFOS when used in foods for the general population in its GRAS Notice (No. 717), submitted

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10 *See* FDA, Inventory of GRAS Notices, at https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices (searching for "oligosaccharides," and reviewing "substance name" for both "fructooligosaccharides" and "fructo-oligosaccharides").

11 FDA, Agency Response Letter to GRAS Notice 797, available at https://www.fda.gov/media/119454/download.

to FDA on June 18, 2017.[12] We have summarized that safety discussion below, followed by our updated literature search results.

The available body of toxicology information indicates that scFOS has low to no toxicological effects. scFOS has been evaluated in a battery of *in vitro* genotoxicity studies, animal feeding studies, and studies in infants and adult humans. These studies are briefly summarized here and indicate no concern for oral toxicity for scFOS at any dose tested.

1. Genotoxicity

Clevenger *et al.* (1988) describes a series of *in vitro* genotoxicity studies on scFOS. In these studies, the scFOS was negative for genotoxicity in bacterial (Ames), mouse lymphoma, and unscheduled DNA synthesis assays, both with and without metabolic activation.[13]

2. Animal Studies

Carabin and Flamm (1999) summarizes results of acute and repeat dose toxicity studies showing that scFOS. In the acute studies, scFOS has a low potential for acute oral toxicity when given in single doses up to 9,000 mg/kg bw in mice and rats. In two subacute studies reported by Carabin and Flamm, feeding two different strains of Wistar rats up to 4,500 or 5,000 mg kg bw/day for 6 weeks did not produce any adverse treatment related effects. In a reproductive and developmental study, rats were fed a diet containing 20% scFOS from day 1 to day 21 of gestation. The authors report reduction in body weight of the pregnant rats and growth delays for the male pups in the test group during nursing. No other effects on the pregnancy and development of fetuses was reported.[14]

Boyle *et al.* fed Sprague-Dawley rats 0, 0.55, 1.65, 4.96, or 9.91 % oligofructose in feed for 13 weeks.[15] The authors reported no treatment-related adverse at any dose tested, and determined the NOAEL to be the highest dose tested which equates to 4,680 mg/kg bw/day.

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[12] GRAS Notice 797, submitted by New Francisco (Yunfu City) Biotechnology Corporation was closed on November 15, 2018 but addressed safety in infant populations because the intended use was limited to infant formula.

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

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

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

Studies in neonatal mice at 5% in the diet and 2-day old piglets at up to 10 g/L in the colostrum produced no adverse effects.[16]

In a published 104-week combined chronic toxicity and carcinogenicity study Fischer 344 rats were fed scFOS at up to 50,000 ppm in their feed. The study authors reported some statistically significant differences but that none were toxicologically relevant or test-article-related. The NOAEL was determined as 50,000 ppm, the highest concentration tested, equivalent to 2,170 mg/kg bw/day for males and 2,664 mg/kg bw/day for females.[17]

3. Human Studies

In two randomized, double-blind, placebo-controlled studies healthy infants aged 0-7 days received formula supplemented with 400 mg/100 ml scFOS to the age of 4 months, or 500 mg/100 mL scFOS from 4 to 10 months of age. Neither formula consumption nor growth differed between groups fed scFOS or placebo. No treatment related adverse events were reported in either study.[18] Additional studies in infants using FOS other than scFOS further supports the safety of scFOS in infants. Three such studies fed infants a range of FOS up to 340 mg in 100 mL formula for up to 13 weeks. No differences in test groups in comparison to

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[16] Nakamura, Y., Nosaka, S., Suzuki, M., Nagafuchi, S., Takahashi, T., Yajima, T., Takenouchiohkubo, N., lwase, T., Moro, I. 2004. Dietary fructooligosaccharides up-regulate immunoglobulin A response and polymeric immunoglobulin receptor expression in intestines of infant mice. *Clin Exp Immunol* 137:52-58; Barnes, J.L., Hartmann, B., Holst, J.J., Tappenden, K.A. 2012. Intestinal adaptation is stimulated by partial enteral nutrition supplemented with the prebiotic short-chain fructooligosaccharide in a neonatal intestinal failure piglet model. *J Parenter Enteral Nutr* 36:524-537; Correa-Matos, N.J., Donovan, S.M., Isaacson, R.E., Gaskins, H.R., White, B.A., Tappenden, K.A. 2003. Fermentable fiber reduces recovery time and improves intestinal function in piglets following Salmonella typhimurium infection. *J. Nutr*. 133:1845-1852

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

[18] Paineau, D., Respondek, F., Menet, V., Sauvage, R., Bornet, F., Wagner, A. 2014. Effects of short-chain fructooligosaccharides on faecal bifidobacteria and specific immune response in formula-fed infants: a randomized, double-blind, placebo-controlled trial. *J Nutr Sci Vitaminol* 60(3): 167-75; Ripoll, C., Chappuis, E., Respondek, F., Wanger, A., Gottarand, F. 2015. scFOS supplemented follow-on formula in healthy infants: Impact on vaccine specific faecal secretory IGA response, faecal bifidobacteria, growth and digestive tolerance. *Bioactive Carb Dietary Fiber* 5(2): 169-178

control with regard to formula consumption or weight gain and no serious treatment related adverse events were reported.[19]

Studies in adults, both healthy and those who have undergone ileostomy, report that scFOS ingestions did not result in any reported adverse events, is well tolerated and that ingested scFOS reach the colon and there are fermented by resident bacteria.[20]

4. Updated Literature Search

We conducted an updated literature search for new information on FOS in the public literature that captured information published through March 23, 2021 and found no additional studies that evaluated the safety of FOS in humans or animals. We did identify a publication from November 2018 that evaluated the use of FOS to reduce constipation in infants ages six to twenty-four months. Infants were given dietary supplements containing 6, 9, or 12 grams of FOS daily for four weeks with no significant adverse effects.[21] Two reports of abdominal discomfort and flatulence were reported, as well as two episodes of vomiting after treatment. We further identified several studies which evaluated the effects of scFOS on gut microbiota

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[19] Xia, Q., Williams, T., Hustead, D., Price, P., Morrison, M., Yu, Z. 2012. Quantitative analysis of intestinal bacterial populations from term infants fed formula supplemented with fructooligosaccharides. *J Pediatr Gastroenterol Nutr* 55:314-320; Brunser, 0., Figueroa, G., Gotteland, M., Haschke-Becher, E., Magliola, C., Rochat, F., Cruchet, S., Palframan, R., Gibson, G., Chauffard, F., Haschke, F. 2006. Effects of probiotic or prebiotic supplemented milk formulas on fecal microbiota composition of infants. *Asia Pac J Clin Nutr* 15:368-376; Bettler, J., Euler, A.R. 2006. An evaluation of the growth of term infants fed formula supplemented with fructooligosaccharide. *Int J Probiot Prebiot* 1: 19-26

[20] Bach Knudsen, K.E., and Hessov, I. 1995. Recovery of inulin from Jerusalem artichoke (Helianthustuberosus L.) in the small intestine of man. *Br J Nutr* 74: 101-113; Ellegard, L., Andersson, H., Bosaeus, I. 1997. Inulin and oligofructose do not influence the absorption of cholesterol or the excretion of cholesterol, Ca, Mg, Zn, Fe, or bile acids but increases energy excretion in ileostomy subjects. *Eur J Clin Nutr* 51: 1-5; Rumessen, J.J., Gudmand-Hoyer, E. 1998. Fructans of chicory: Intestinal transport and fermentation of different chain lengths and relation to fructose and sorbitol malabsorption. *Am J Clin Nutr* 68:357-364; van Dokkum, W., Wezendonk, B., Srikumar, T.S., van den Heuvel, E.G.H.M. 1999. Effect of nondigestible oligosaccharides on large-bowel functions, blood lipid concentrations and glucose absorption in young healthy male subjects. *Eur J Clin Nutr* 53:1-7

[21] Souza, D.D.S. *et al*. (2018). Randomized, Double-Blind, Placebo-Controlled Parallel Clinical Trial Assessing the Effect of Fructooligosaccharides in Infants with Constipation. Nutrients 10(11), at https://www.ncbi.nlm.nih.gov/pubmed/30388751.

in pigs[22], dogs[23], and cats[24]. We reviewed these studies and concluded that, while they do not provide further support for scFOS's safety, neither do they detract from or raise questions regarding the GRAS status of scFOS for its intended use.

5. Allergenicity

As noted above, Ingredion's scFOS contains not more than 0.01% protein. There is no indication that scFOS poses a risk of allergenicity. There are no reports of allergic reaction in the public literature. Because scFOS products are oligosaccharides and contain essentially no protein, they present an insignificant risk of potential allergenic effects.

6. Summary

The safety of scFOS has been well-established. Consistent with GRAS Notices 44, 537, 605, 623, 717, and 797, the existing acute, short-term, subchronic, chronic, carcinogenicity, developmental, reproductive, and *in vitro* genotoxicity test data showing no adverse effects from the use of scFOS in food support the safety of scFOS for the intended uses that are considered in this GRAS Notice.

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[22] Yan H. *et al.* (2020) Short-chain fructo-oligosaccharides alleviates oxidized oil-induced intestinal dysfunction in piglets associated with the modulation of gut microbiota. *J Funct Foods*. 64: 103661; Le Bourgot C. *et al.* (2019) Perinatal short-chain fructooligosaccharides program intestinal microbiota and improve enteroinsular axis function and inflammatory status in high-fat diet-fed adult pigs. *FASEB J.* 33: 301-13.

[23] Apper E., Privet L., Taminiau B., Le Bourgot C., and Svilar L. (2020) Relationships between gut microbiota, metabolome, body weight, and glucose homeostasis of obese dogs fed with diets differing in prebiotic and protein content. *Microorganisms*. 8:513.

[24] Hall J.A., Jewell D. E., and Ephraim E. (2020) Changes in the fecal metabolome are associated with feeding fiber not health status in cats with chronic kidney disease. *Metabolites*. 10(7): 281; Hall J.A., Jewell D.E., and Ephraim E. (2020) Chronic kidney disease in cats alters response of the plasma metabolome and fecal microbiome to dietary fiber. *PLOS One*. 15(7): e0235480.

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FDA, GRAS Notice (GRN 605), available at, https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=605&sort=GRN_No&order=DESC&startrow=1&type=basic&search=605.

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October 8, 2021

Via Electronic Mail

Ellen T. Anderson
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration

Re: Responses to FDA Questions regarding GRN 1006 (Short-Chain Fructooligosaccharides (scFOS))

Dear Ms. Anderson,

The purpose of this letter is to provide responses to FDA's September 24, 2021 questions regarding GRN 1006 for Short-Chain Fructooligosaccharides (scFOS). For clarity and convenience, we have repeated FDA's questions in bold below, followed by our responses.

1. **Please provide a statement confirming that all raw materials and processing aids (e.g., ion exchange resins, etc.) used in the manufacture of scFOS are food grade and used in accordance with applicable U.S. regulations.**

All raw materials and processing aids used in the manufacture of scFOS are food grade and used in accordance with applicable U.S. regulations.

2. **The specifications for scFOS provided in the notice appear to only apply to the powder form of scFOS. Please provide specifications for the syrup form of scFOS.**

Specifications for Food Grade scFOS - Liquid

Parameter	Unit	Specification	Method
Appearance	-	Pale yellow syrup	Visual
Flavor	-	Standard, clean slightly sweet	Internal Method (Sensory)
Odor	-	Standard, odorless	
Total scFOS	% db	NLT 95.0	Internal Method (High-Performance Liquid Chromatography)
1-Kestose (GF2)	% db	30-42	
Nystose (GF3)	% db	45-57	
Fructofuranosylnystose (GF4)	% db	5-15	
Sugars (sucrose, glucose, fructose)	% db	NMT 5.0	
Ash	%	NMT 0.05	Internal Method (conductivity)
Dry Substance	%	70-73	Internal Method (Refractive Index)
pH (as is)	-	5.0-7.5	Internal Method
Lead	mg/kg	NMT 1.0	ICP MS Heavy Metals AOAC 2015.01 Mod<2232
Arsenic	mg/kg	NMT 1.0	
Standard plate count	cfu^3/g	NMT 300	Internal Methods (based on AOAC methods)
Yeast	cfu/g	NMT 20	
Mold	cfu/g	NMT 20	
Coliforms	cfu/g	NMT 10	
Escherichia coli	cfu/10 g	Negative	Current USP/NF,62
Anaerobic Mesospores	cfu/g	NMT 10	Compendium of Methods for the Microbiological Examination of Foods (CMMEF), 5th ed.
Aerobic Thermophilic Spores	cfu/10 g	NMT 10	
Anaerobic thermophilic spores	Tubes Positive	NMT 10	CMMEF, 4th ed.
Aerobic mesophilic spores	cfu/g	NMT 10	CMMEF, 5th ed.
Salmonella spp. PCR	cfu/100 g	Negative	AOAC-RI100201
Coagulase-positive *Staphylococci*	cfu/10 g	Negative	Current USP/NF,62
Enterobacteriaceae MPN[4]	cfu/30 g	Negative	ISO21528-1:2017

Internal methods have been validated
[1]NLT = Not less than
[2]NMT= Not more than
[3]Cfu: Colony forming unit
[4]MPN: Most probable number.

3. For the specifications provided, please indicate when the determination is on a dry matter basis.

The Carbohydrate Profile including sugars (fructose, glucose, sucrose), GF2 (1-Kestose), GF3 (Nystose), GF4 (Fructofuranosylnystose), and total Fructooligosaccharides are analyzed on a dry matter basis for both the dry and liquid forms of scFOS.

4. The specifications provided in the notice include a limit for "sugars." Please clarify the identity of the sugars that are included for this specified limit (e.g., glucose, fructose, and sucrose).

The sugar content in both the liquid and powder scFOS products include sucrose, glucose, and fructose.

5. We note that the stability of scFOS is not discussed in the notice. Please provide a discussion of the stability of scFOS, including any studies of stability conducted with the powder and syrup forms of scFOS produced by the method described in the notice.

Stability data for dry scFOS

A 24-months shelf-life study was conducted at 25°C and 33% relative humidity to ensure the integrity of the scFOS. Testing for microbial identification and growth was also conducted to verify that parameters are maintained within specification. The integrity of the ingredient was measured using an HPLC method. Results for all parameters tested were within specification throughout the 24 months shelf-life study.

Lot #	Test	Specification	Storage Time (Months)					
			0	1	3	12	18	24
MSA-434-359	SPC/g*	SPC 300	$3.0x10^1$	$4.0x10^1$	$1.0x10^1$	$2.0x10^1$	$1.0x10^1$	$2.0x10^1$
	Coliforms	NMT 10	Negative	Negative	Negative	Negative	Negative	Negative
	scFOS (% db)**	NLT 95	97.6	97.4	97.9	97.8	97.8	97.4
	SPC/g*	SPC 300	$2.0x10^1$	$2.0x10^1$	$4.0x10^1$	$0.5x10^1$	$1.0x10^1$	$3.0x10^1$

MS-510-069	Coliforms	NMT 10	Negative	Negative	Negative	Negative	Negative	Negative
	scFOS (% db)**	NLT 95	96.0	96.0	96.3	95.8	96.0	95.7

NLT = Not less than
NMT= Not more than
*Standard plate count
**Reported as % on a dry basis

Stability data for liquid scFOS

A shelf-life study was conducted for a period of 9 months for liquid scFOS with added buffers (citric acid monohydrate and sodium phosphate dibasic dihydrate).[1] The purpose of the study was to ensure that the product stability is maintained when stored under the recommended storage temperature of 68 - 86°F (20°C - 30°C) during its shelf life. The chemical stability of the liquid scFOS (with 70-72% dry substance) was evaluated by measuring its total scFOS content and pH using HPLC and pH meter, respectively. Microbial testing of the product was also conducted to verify that parameters are maintained within specification. As shown in the table below, the results for all parameters tested were within specification throughout the 9 months of shelf-life stability study.

Lot #	Test	Specification	Storage Time (Months)		
			0	6	9
3036251200	Total scFOS (% db)*	NLT 95.0	95.9	95.8	96.1
	pH (as is)	5.0-7.5	7.1	6.6	6.6
	TPC (cfu/g)**	NMT 300	<10	<10	<10
	Coliforms (cfu/g)	NMT 10	<1	<1	<1
3030016300	Total scFOS (% db)*	NLT 95.0	96.0	96.2	96.1
	pH (as is)	5.0-7.5	7.0	6.5	6.4
	TPC (cfu/g)**	NMT 300	40	40	15
	Coliforms (cfu/g)	NMT 10	<10	<10	<10

NLT = Not less than
NMT= Not more than
*Reported as % on a dry basis
**Total plate count

6. In the notice, Ingredion provides an updated assessment of the dietary exposure to scFOS from previously notified and proposed intended uses in foods other than use in

[1] Liquid FOS is blended with buffers to extend the shelf life to 9 months. The buffers, citric acid monohydrate and sodium phosphate dibasic dihydrate are used in accordance with 184.1033 and 182.1778, respectively.

infant formula. Given that scFOS has existing uses in infant formula, we request that, in the narrative, Ingredion address whether they considered the potential cumulative dietary exposure to scFOS in infants resulting from both the intended use in foods combined with intended uses of scFOS in infant formula that have been the subject of other GRAS notices. Furthermore, please provide a short narrative explaining why exposure stemming from infants that may consume both infant formula and conventional foods is not expected to be a safety concern.

Ingredion is aware of other GRAS notices which cover the use of scFOS in infant formula. The most recent of these to receive a "no questions" letter from the FDA is GRN 797 which calculated a 90th percentile exposure to scFOS from the intended use in infant formula of 1,035 to 1,090 mg/kg bw/day during the period of highest formula consumption (first 6 weeks of life) and decreasing as the infant grows to ~800 mg/kg bw/day from 6-12 months of life. Ingredion has conducted an exposure assessment for scFOS for the intended uses described in GRN 1006 of 1,030 mg/kg bw/day for infants (0-1 years of age) as shown in Table 6 of the GRAS notice. Caloric needs of infants ≤ 1 year of age are well defined. As such any exposure to scFOS from the intended uses described in GRN 1006 in this target population will necessarily be substitutional (i.e., intake of formula with scFOS will result in a corresponding decrease to scFOS from other sources). As the maximum exposure calculated in GRN 797 is virtually the same (though slightly lower) than that calculated for GRN 1006, the overall exposure to scFOS in the target population which consumes both formula and other foods containing scFOS as described in these two GRNs would be very similar to, or slightly less than, the 90th percentile exposure of 1,035-1,090 mg/kg bw/day described in GRN 797.

7. **On page 15 of the notice, the adult male subpopulation is described to have the highest estimated dietary exposure on a per person basis. We note that in Table 6 of the notice, toddlers (aged 1 to 2) are the subpopulation with the highest dietary exposure on a per body weight basis. Please provide a short narrative explaining why there is no safety concern in this potentially vulnerable subpopulation from your intended use.**

In GRN 1006, the notifier calculates a mean exposure to scFOS for toddlers (1-2 years old) of 7 g/day (0.8 g/kg bw/day) and a 90th percentile exposure of 17 g/day (1.32 g/kg bw/day). As a non-digestible fiber, scFOS primarily acts as a "prebiotic" and is fermented in the digestive tract by intestinal microflora. Reported adverse events from the published literature are typically very mild and are consistent across all age groups and are typically described as mild gastrointestinal discomfort (distention, bloating, gas, etc.). Further, literature reports of human trials which fed children in this age range scFOS or other similar long and short-chain oligosaccharides indicate these exposures would be expected to be safe for the target population.

- da Silva Souza et al. 2018[2] randomized 36 children between 6 months and 24 months in age to either placebo or scFOS groups. Children in the treatment group received an amount of scFOS based on their bodyweight for 4 weeks. Bodyweight groups were 6.0-8.9 kg, 9.0-11.9 kg, and 12.0+ kg. These groups were given 6, 9 or 12 g/day scFOS respectively, which equates to 0.67-1 g/kg bw/day. Reported adverse events consisted of two children with mild gastrointestinal effects (abdominal distension and flatulence) and one child with vomiting, however these children continued treatment.

- Chatchatee et al. 2014[3] randomized 767 children 11 to 29 months in age to control (cow's milk) or "growing up milk (GUM)" formulations with or without long-chain fructo-oligosacchariades (lcFOS), short-chain galacto-oligosaccharides (scGOS), and long-chain polyunsaturated fatty acids. The treatment group received GUM containing 1.2 g/100 mL scGOS and lcFOS at a 9:1 ratio. Children received up to 750 mL (containing 9 g/day combined scGOS and lcFOS) of these preparations for 52 weeks. The authors report a total of 2,217 adverse events including 78 serious adverse events. Only 29 adverse events were judged to be related to the treatment, none of them serious, and consisted of mild GI symptoms.

It is clear, from the totality of the evidence, that scFOS exposure to children 1-2 years of age is safe for its intended uses as described in GRN 1006.

Please let us know if you have any questions or need anything else.

Best regards,

Evangelia Pelonis

[2] da Silva Souza *et al.* (2018) Randomized, double-blind, placebo-controlled parallel clinical trial assessing the effect of fructooligosaccharides in infants with constipation. *Nutrients*. 10:1602; doi:10.3390/nu1011602

[3] Chatchaee P. *et al.* (2014) Effects of growing-up milk supplemented with prebiotics and LCPUFAs on infections in young children. *JPGN* 58:428-37.

From: Pelonis, Evangelia C.
To: Anderson, Ellen
Subject: RE: [EXTERNAL] FW: GRN 001006
Date: Thursday, December 16, 2021 9:01:01 AM
Attachments: image002.png
image003.png
image007.png

Hello Ellen,

Thanks for your email.

We have confirmed that the production strain for the β-fructofuranosidase enzyme used to manufacture scFOS as described in GRN 1006 is the same strain used in GRN 44 and GRN 537. The production strain, *Aspergillus fijiensis,* is classified under the American Type Culture Collection (ATCC) number 20611, it is not genetically engineered, and it is considered non-pathogenic and non-toxic.

Please let us know if you need anything else and we look forward to receiving FDA's response.
Happy Holidays!

Best regards,
Eve

Evangelia C. Pelonis
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From: Anderson, Ellen <Ellen.Anderson@fda.hhs.gov>
Sent: Wednesday, December 15, 2021 2:07 PM
To: Pelonis, Evangelia C. <pelonis@khlaw.com>
Subject: RE: [EXTERNAL] FW: GRN 001006

Hello Eve,

We are finishing up our review of GRN 001006 and have this follow-up question for the notifier:

The notifier states that the β-fructofuranosidase enzyme used to manufacture scFOS is produced by *Aspergillus fijiensis*, and that this "…enzyme acts as an invertase on sucrose molecules and a fructosyltransferase between both sucrose molecules and fructofuranosylsucrose molecules." The notifier also states that, "*A. fijiensis* is a synonym for *A. japonicus*, which is a member of the *niger* group." We note that there is no additional information about the enzyme or the production organism in the notice. The notifier further states that their scFOS is produced in a slightly different manner than the scFOS described in GRN 000044 and GRN 000537. For the administrative record, please clarify if the production strain *A. fijiensis* is the same strain described in GRNs 000044 and 000537, noting the strain's identity (i.e., the ATCC number), whether it is genetically engineered,

and whether it is non-pathogenic and non-toxigenic.

We would appreciate a response at your earliest convenience; however, with the holidays approaching, we understand if a response is delayed.

Thank you!

Sincerely,
Ellen

Ellen Anderson
Regulatory Review Scientist

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1309
ellen.anderson@fda.hhs.gov

Pronouns: she/her/hers

From: Anderson, Ellen
Sent: Friday, October 08, 2021 1:43 PM
To: Pelonis, Evangelia C. <pelonis@khlaw.com>
Subject: RE: [EXTERNAL] FW: GRN 001006

Hello Eve,

Thank you for providing the responses to our questions. I will let you know if there are any follow-up questions after our review is complete.

Sincerely,
Ellen

Ellen Anderson
Regulatory Review Scientist

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1309
ellen.anderson@fda.hhs.gov

Pronouns: she/her/hers

From: Pelonis, Evangelia C. <pelonis@khlaw.com>
Sent: Friday, October 08, 2021 1:09 PM
To: Anderson, Ellen <Ellen.Anderson@fda.hhs.gov>
Subject: [EXTERNAL] FW: GRN 001006

Dear Ms. Anderson,

Please find attached our responses to the additional information requested for GRN 1006.

Best,
Eve

Evangelia C. Pelonis
Partner

direct 202.434.4106 pelonis@khlaw.com

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From: Anderson, Ellen <Ellen.Anderson@fda.hhs.gov>
Sent: Friday, September 24, 2021 12:18 PM
To: Pelonis, Evangelia C. <pelonis@khlaw.com>
Subject: GRN 001006

Dear Ms. Pelonis,

Please see the attached letter regarding GRAS notice GRN 001006.

Sincerely,
Ellen

Ellen Anderson
Regulatory Review Scientist

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1309
ellen.anderson@fda.hhs.gov

Pronouns: she/her/hers