GRAS Notice for Resistant Glucan

Prepared for:

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
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Part 1. §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E (consisting of §170.203 through 170.285), Nihon Shokuhin Kako Co., Ltd. (NSK) hereby submits a Generally Recognized as Safe (GRAS) notice for Resistant Glucan to the United States (U.S.) Food and Drug Administration (FDA). It is NSK's view that Resistant Glucan is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act, on the basis of their conclusion that Resistant Glucan is GRAS under the conditions of its intended use, as described in Part 1.3 below. In addition, as a responsible official of NSK, Masayasu Takada hereby certifies that all data and information presented in this notice constitute a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to NSK and pertinent to the evaluation of the safety and GRAS status of Resistant Glucan as an ingredient for addition to food, as described herein.

Signed,

(b) (6)

6 / 1 / 2017

Masayasu Takada, Ph.D.
General Manager, Research Institute
Nihon Shokuhin Kako Co., Ltd.
E-mail: masayasu.takada@nisshoku.co.jp

Date

1.1 Name and Address of Notifier

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417-8530

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1.2 Common Name of Notified Substance

The common name of the notified substance is Resistant Glucan.

1.3 Conditions of Use

The intended food categories and use levels of NSK's Resistant Glucan are summarized in Table 1.3-1. Resistant Glucan is intended for use as a low-calorie bulking agent, formulation aid, humectant, and texturizer in various food and beverage products. For example, the ingredient can be used to replace sugars and/or fats in food products, while still providing the same creaminess and mouth feel. In addition to their technological functions, these ingredients may provide a source of dietary fiber. The intended use levels range from 1.9 to 22.3% on a dry weight basis of the final food or beverage product. Resistant Glucan is also intended for use in dietary supplements as a formulation aid (binder, filler, or excipient), at use levels ranging from 72 to 93% on a dry weight basis.

The Resistant Glucan will not be added to meat and poultry products (including soups and soup mixes containing meat or poultry), or to foods that are specifically marketed towards infants and young children (including infant formula). Additionally, some of the foods listed in Table 1.3-1 have a standard of identity within Title 21 of the *Code of Federal Regulations* (CFR). Resistant Glucan is not intended for addition to standardized foods, unless it is permitted by the applicable standard of identity. The ingredient may be used in products that are similar to foods for which a standard of identity exists. In such cases, the products will not be referred to by their common names (*e.g.*, mayonnaise) to avoid confusion.

Table 1.3-1 Individual Food-Uses and Maximum Use-Levels Intended for Resistant Glucan in the United States[a]

Food Category	Intended Food-Uses	RACC	Maximum Use-Level of Resistant Glucan (g/serving on dwb)	Maximum Use-Level of Resistant Glucan (% dwb)[b]
Baked Goods and Baking Mixes	Breading and Batter Coatings	30 g	2.0	6.5
	Cakes	55 to 125 g	1.6 to 3.6	2.9
	Cookies	30 g	3.4	11.2
	Non-Sweet Baked Goods (breads, rolls, crackers, flour tortillas, pita bread, pizza crust, and English muffins)	30 to 55 g	2.0 to 3.6	6.5
	Wafers	30 g	2.0	6.5
Beverages and Beverage Bases	Carbonated Beverages, Non-Carbonated Beverages and Dry Beverage Mixes	360 mL	6.8	1.9

Table 1.3-1 Individual Food-Uses and Maximum Use-Levels Intended for Resistant Glucan in the United States[a]

Food Category	Intended Food-Uses	RACC	Maximum Use-Level of Resistant Glucan (g/serving on dwb)	Maximum Use-Level of Resistant Glucan (% dwb)[b]
Breakfast Cereals	Breakfast Cereals (Ready-to-Eat)	15 to 60 g	1.0 to 3.9	6.5
	Instant/Cooked Cereals	40 to 55 g (on a dry basis)	2.6 to 3.4	6.5
Chewing Gum	Chewing Gum	3 g	0.1	4.3
Fats and Oils	Fat Spreads	15 g	3.3	22.3
	Mayonnaise	15 g	0.6	4.3
	Salad dressings	30 g	3.4	11.2
Frozen Dairy Desserts and Mixes	Ice Cream	2/3 cup	4.6	2.9
Gelatins, Puddings, and Fillings	Pudding	½ cup prepared	3.5	2.9
	Fillings in baked goods	85 g (pie fillings)	3.7	4.3
Hard Candy	Hard Candy	2 to 15 g	0.2 to 1.3	8.6
Jams and Jellies, commercial	Jam	15 mL	3.3	22.3
	Jelly	15 mL	1.3	8.6
Milk Products	Yogurt	170 g	3.7	2.2
	Yogurt Drinks[c]	240 mL	5.3	2.2
Nuts and Nut Products	Peanut Butter	30 mL	1.3	4.3
Snack Foods	Snack Chips (Corn, Potato, Rice and Pretzels)	30 g	3.4	11.2
Soft Candy	Chocolate Confectionery	30 g	1.3	4.3
	Soft Candy	30 g	2.6	8.6
Soups and Soup Mixes	Soups[d]	245 g	4.7	1.9
Sugar Substitutes	Sugar Substitutes	8 g (reference amount for sugar)	0.5	5.8
Dietary Supplements	Dietary Supplements[e]	As stated on product label	n/a	72 to 93

dwb = dry weight basis; n/a = not applicable; NHANES = National Health and Nutrition Examination Survey; RACC = Reference Amount Customarily Consumed as per 21 CFR § 101.12.

[a] In this table, the maximum intended use level of Resistant Glucan is presented as both grams (g) per serving (RACC), and as the inclusion rate in percentage. The dietary intake assessment (see Part 3) was conducted using the maximum use level of Resistant Glucan on a percentage basis.

[b] NSK intends to market liquid and powder formulations of their Resistant Glucan ingredient, which differ only in their moisture content. The liquid concentrate of Resistant Glucan (Fit Fiber® #80) is standardized to contain at least 72% solids, while the powder formulation (Fit Fiber® #80P) contains at least 93% solids. As such, the maximum proposed use levels for Resistant Glucan are provided on a dried weight basis in this table.

[c] No food codes for yogurt drinks are available in NHANES. Therefore, food codes for smoothie-type dairy-based drinks were selected as surrogates in the dietary exposure assessment for Resistant Glucan (see Part 3).

[d] Exclude soups and soup mixes containing meat or poultry.

[e] Resistant Glucan is proposed for use as a binder, filler, or excipient in dietary supplements. As the amount of Resistant Glucan when used as a formulation aid could theoretically be close to 100%, it is assumed that the dietary supplements contain only Resistant Glucan, which corresponds to maximum levels of 72% on a dried weight basis for the liquid concentrate, and 93% on a dried weight basis for the powder.

1.4 Statutory Basis for GRAS

Pursuant to 21 CFR § 170.30 (a) and (b), Resistant Glucan manufactured by NSK has been concluded to have GRAS status for use as an ingredient in specified foods and beverages, as described in Part 1.3, on the basis of scientific procedures.

1.5 Availability of Information

NSK agrees to make the data and information that are the basis for the conclusion of Resistant Glucan's GRAS status available to the FDA for review and copy upon request, either during or after the evaluation of the GRAS notice, at the address specified below during business hours:

Nihon Shokuhin Kako Co., Ltd.
30 Tajima Fuji
Shizuoka, Japan
417-8530

Upon request, NSK will provide the FDA with a complete copy of the data and information either in an electric format that is accessible for the FDA's evaluation or on paper.

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

It is NSK's view that all data and information presented in Parts 2 through 7 of this GRAS notice do not contain any trade secret, commercial, or financial information that is privileged or confidential. Therefore, none of the data and information presented herein are exempt from the Freedom of Information Act, 5 U.S.C. Section 552.

1.7 Food Safety and Inspection Service Statement

NSK does not intend to use Resistant Glucan in a product or products that are subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA).

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

2.1 Identity

2.1.1 Names

Common Name: Resistant Glucan

Trade Name: Fit Fiber® #80 (liquid concentrate) and Fit Fiber® #80P (spray-dried powder)

Chemical Name: Not applicable

Chemical Abstract Service (CAS) Number: Not applicable

2.1.2 Description

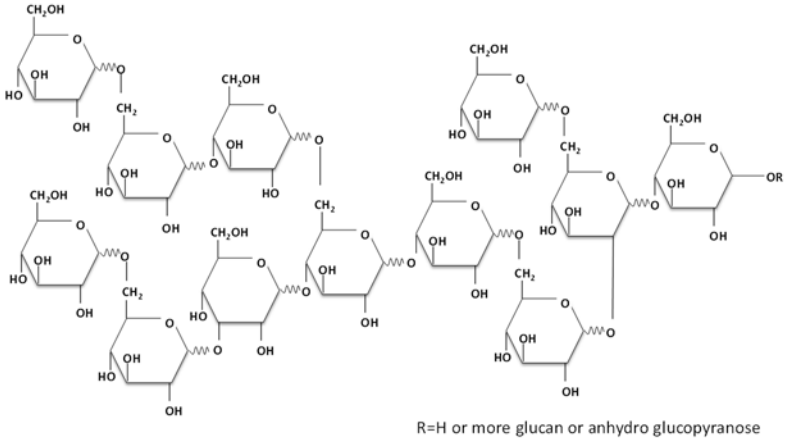
In general, sugars can be polymerized to form polysaccharides when heated. The Resistant Glucan ingredient produced by NSK (Fit Fiber® #80, Fit Fiber® #80P) is a synthetic, water-soluble carbohydrate polymer that is obtained by the bulk melt polycondensation of glucose syrup in the presence of activated carbon, which serves as a catalyst. The resulting material is largely composed of glucose polymers (>90% glucan content on a dried weight basis) containing various forms of glycosidic linkages (*e.g.*, α- and β- 1,2-, 1,3-, 1,4-, and 1,6- linkages) (Figure 2.1.2-1). Resistant Glucan therefore contains a dietary fiber fraction that is indigestible by digestive enzymes (*i.e.*, "resistant glucan")[1]; the ingredient contains at least 75% dietary fiber.

Resistant Glucan is structurally similar to other digestion-resistant carbohydrates that are currently marketed as food ingredients in the U.S. These include for example polydextrose, which is a synthetic, water-soluble carbohydrate polymer that is permitted as a direct food additive in the U.S., specifically as a bulking agent, formulation aid, humectant, and texturizer in all foods (except meat and poultry, baby food, and infant formula), when used consistent with current Good Manufacturing Practice (cGMP) (21 CFR§172.841). Although both Resistant Glucan and polydextrose are produced by bulk melt polycondensation, polydextrose is obtained from a mixture of glucose and sorbitol (approximately in a 9:1 ratio), and thus typically contains 2% free sorbitol (GRN 107; U.S. FDA, 2002), whereas Resistant Glucan is obtained from glucose syrup and contains only glucose monomers.

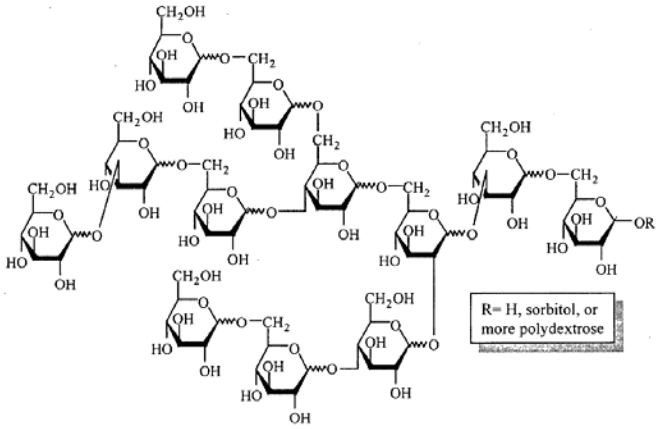
[1] The term "resistant glucan" may be used to solely refer to the indigestible dietary fiber component of this material.

Figure 2.1.2-1 Structural Formula of Resistant Glucan and Polydextrose

A) Representative structure of resistant glucan (dietary fiber fraction)



B) Representative structure of polydextrose (GRN 107; U.S. FDA, 2002)



2.1.3 Structure Analysis

NSK has conducted analyses to demonstrate that Resistant Glucan is structurally similar to polydextrose, as well as other digestion-resistant carbohydrate polymers that are also currently permitted for use in the U.S. as low-caloric bulking agents. These include resistant maltodextrin (marketed as Fibersol®-2) and resistant dextrin (marketed as Nutriose® 6 and Nutriose® 10) that are obtained from the pyrodextrinization of starch, which converts a portion of the naturally occurring α-1,4- and α-1,6-glycosidic linkages to a random mixture of α- and β- 1,2-,1,3-, 1,4- and 1,6-glycosidic linkages.

The structural properties of NSK's Resistant Glucan and other similar digestion-resistant carbohydrates are summarized in Table 2.1.3-1. For comparison, the structural properties of

fully digestible maltodextrin are also presented. The analyses confirm that Resistant Glucan (similar to polydextrose, resistant maltodextrin, and resistant dextrin) is a branched glucan containing a mixture of α- and β-1,2-, 1,3-, 1,4-, and 1,6-glycosidic linkages. In contrast, maltodextrin consists mainly of α-1,4-glycosidic linkages.

Table 2.1.3-1 Structural Properties of Resistant Glucan in Comparison to Other Digestion-Resistant Carbohydrates Marketed in the United States

	Resistant Glucan [Fit Fiber® #80(P)][a]	Polydextrose (Litesse®)[a]	Resistant Maltodextrin (Fibersol®-2)[a]	Resistant Dextrin[b]		Maltodextrin (Pinedex® #1)[a]
				Nutriose® 6	Nutriose® 10	
Total dietary fiber content (AOAC 2001.03) (%)	81.7	80.8	88.0	85	70	0
Average molecular weight (M_w)	2,100	1,900	1,900	4,000 to 6,000	3,500 to 4,500	2,300
Degree of polymerization	12	12[c]	DP >7 (70%)[d] DP 4 to 6 (24%)[d]	12 to 25	4 to 10	3 to 9[e]
Dextrose equivalent	8 to 12	12	8 to 12.5[d]	2.5 to 5.0	8 to 12	Less than 20[f]
Anomeric carbon of glucose	α and β	α and β	α and β	α and β	α and β	α only
Glycosidic linkages (%)						
Non-reducing end Glc*p*	54.1	56.4	71.4	NR	NR	16.3
1,2-linked Glc*p*	3.5	3.2	2.0	NR	NR	0
1,3-linked Glc*p*	0.7	1.1	0.4	NR	NR	0
1,4-linked Glc*p*	9.6	7.0	11.5	NR	NR	78.5
1,6-linked Glc*p*	15.6	16.5	5.9	NR	NR	3.5
Others[g]	16.6	15.9	8.7	NR	NR	1.7
Glycosidic linkages (excluding terminal residues) (%)						
1,2-linked Glc*p*	7.6	7.2	6.9	10	10	0
1,3-linked Glc*p*	1.4	2.5	1.5	10	10	0
1,4-linked Glc*p*	21.0	16.0	40.4	50	50	93.8
1,6-linked Glc*p*	33.9	37.8	20.8	30	30	4.2
Others[g]	36.1	36.4	30.5	NR	NR	2.0

AOAC = Association of Official Analytical Chemists; DP = degree of polymerization; NR = not reported.
[a] Data were obtained from analyses conducted by NSK, unless otherwise stated.
[b] Based on information in a GRAS notice submitted for resistant dextrin (GRN 436; U.S. FDA, 2013). An additional step involving partitioning chromatography is employed in the production of Nutriose® 6, which removes the lower molecular weight fraction, specifically the DP1 and DP2 saccharides (GRN 436; U.S. FDA, 2013). Therefore, Nutriose® 6 has a higher average DP and molecular weight, as well as higher dietary fiber content, in comparison to Nutriose® 10.
[c] Based on information in a GRAS notice submitted for polydextrose (GRN 107; U.S. FDA, 2002).
[d] Based on information presented in product brochure for Fibersol®-2, which is available at: http://www.adm.com/en-US/products/Documents/Fibersol%20Technical%20Brochure.pdf.
[e] Data taken from Cummings and Stephen, 2007.
[f] Based on the specification parameter in the Food Chemicals Codex monograph for maltodextrin (FCC, 2016).
[g] 1,3,4-, 1,2,3-, 1,4,6-, 1,3,6-, 1,3,4,6-, 1,2,3,6-, and 1,2,4,6-linked Glc*p*.

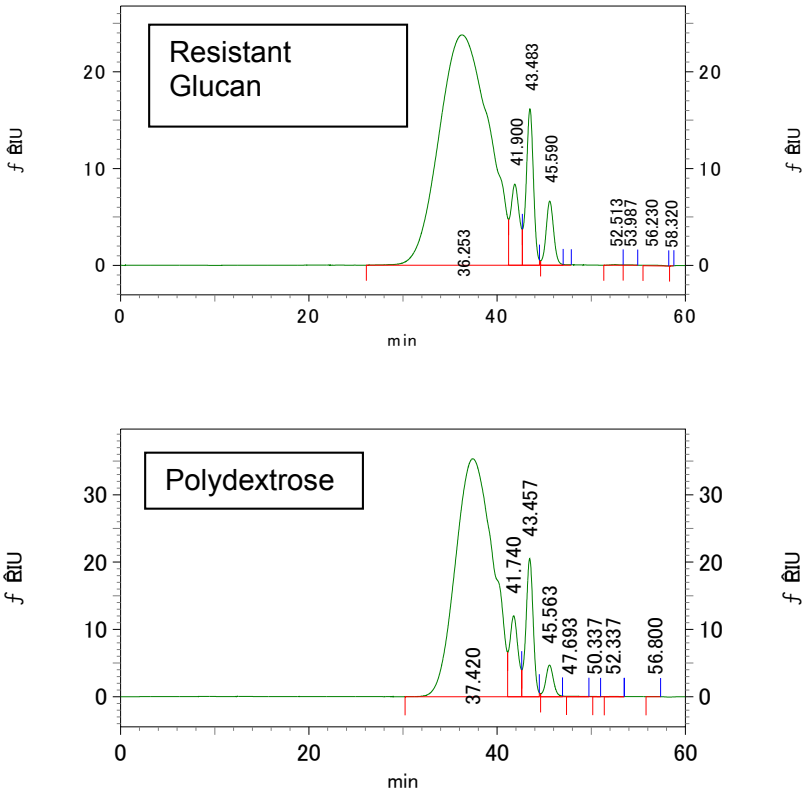
As summarized in Table 2.1.3-2, the majority of the saccharides in NSK's Resistant Glucan have a degree of polymerization (DP) of 3 or greater. The chromatogram from high-performance liquid chromatography (HPLC) analysis of Resistant Glucan and polydextrose is presented in Figure 2.1.3-1. Overall, the molecular weight of the constituents present in Resistant Glucan ranged from 38 to 21,301 (DP 1 to DP 131), while the molecular weight of polydextrose ranged from 36 to 9,573 (DP 1 to DP 59). Although the upper limit of the molecular weight for the polymers in Resistant Glucan is higher than that of polydextrose, the average molecular weight is considered to be similar at approximately 2,000, and both ingredients have an average DP of 12 (see Table 2.1.3-1).

Table 2.1.3-2 Saccharide Composition in Resistant Glucan and Polydextrose[a]

Degree of Polymerization (DP)	Proportion of Saccharides (%)	
	Resistant Glucan [Fit Fiber® #80 (P)]	**Polydextrose**
DP 3 or higher	86.25	85.39
DP 2	7.63	8.27
DP 1	2.87	2.29
Others[b]	3.25	4.05

[a] Data were obtained from analyses conducted by NSK.
[b] Composed predominantly of anhydro-sugar.

Figure 2.1.3-1 HPLC Chromatogram of the Resistant Glucan and Polydextrose

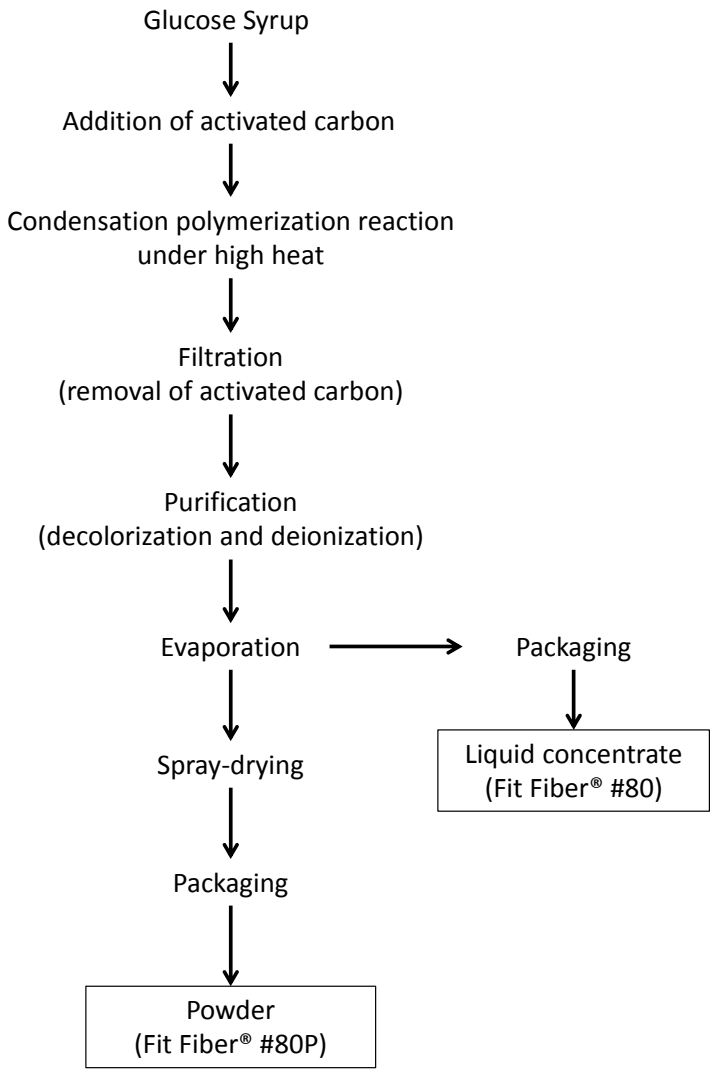


2.2 Method of Manufacturing

A flowchart of the manufacturing process for Resistant Glucan is presented in Figure 2.2-1. Resistant Glucan is produced from glucose syrup that undergoes condensation polymerization reaction under high heat, in the presence of an activated carbon catalyst. The solution is then filtered to remove the activated carbon, and a number of purification steps are employed, including decolorization using fresh activated carbon and deionization with the use of a mixed bed ion-exchange resin. The solution is concentrated by evaporation to produce the liquid formulation of Resistant Glucan (Fit Fiber® #80), which can be subsequently spray-dried to produce the powder formulation (Fit Fiber® #80P).

The manufacturing process for Resistant Glucan is conducted consistent with cGMP and appropriate quality control procedures (*i.e.*, FSSC 22000 certification) are in place. All of the raw materials and processing aids used in the manufacture of NSK's Resistant Glucan are certified to be food-grade and are suitable for use in the U.S. for such purposes.

Figure 2.2-1 Schematic Overview of the Manufacturing Process for the Resistant Glucan



2.3 Product Specifications and Batch Analyses

2.3.1 Product Specifications

The product specifications established for NSK's Resistant Glucan are presented in Table 2.3.1-1. The specifications include parameters related to the identity and composition of the Resistant Glucan, and sets maximum acceptance limits for levels of lead and microbial contaminants. The specifications for the Resistant Glucan are similar to those established for polydextrose in the Food Chemicals Codex (FCC, 2016).

Table 2.3.1-1 Product Specifications for NSK's Resistant Glucan			
Parameter	**Liquid Concentrate**	**Powder**	**Method of Analysis**
Composition			
Appearance	Transparent sticky syrup with pale yellow color	White to cream powder	Internal
Taste/odor	Slightly sweet, odorless		Internal
Moisture	Max. 28%	Max. 7%	Total Solids Method (Appendix X, FCC 6th ed.); Loss on Drying (Appendix II, FCC 6th ed.)
Solid content	Min. 72%	Min. 93%	
pH (10% solution)	2.5 to 6.0		Method for pH determination of Polydextrose (FCC 6th ed.)
Ash	Max. 0.1%		Method for residue on ignition of Polydextrose (FCC 6th ed.)
Total glucan content	Min. 90.0%[a]		Method for identification of polymer of Polydextrose (FCC 6th ed.)
Anhydro-D-Glucose (levoglucosan)	Max. 4.0%[a]		
Residual free glucose monomer	Max 6.0%[a]		HPLC (Appendix II, FCC 6th ed.)
5-hydroxymethylfurfural	Max. 0.1%[a,b]		Method for 5-hydroxymethylfurfural and related compounds of Polydextrose (FCC 6th ed.)
Total dietary fiber content	Min. 75.0%[a]		AOAC Official Method 2001.03 (AOAC, 2005)
Dextrose equivalent	6 to 15		Reducing sugars assay (Appendix X, FCC 6th ed.)
Heavy Metals			
Lead	Max. 0.5 ppm		ICP-MS
Microbiological Contaminants			
Standard plate count	Max. 300 CFU/g		Plate count agar method (Standard Methods of Analysis in Food Safety Regulation - Microorganisms)
Yeast	Max. 100 CFU/g		Potato dextrose agar method (Standard Methods of Analysis in Food Safety Regulation - Microorganisms)
Mold	Max. 100 CFU/g		Potato dextrose agar method (Standard Methods of Analysis in Food Safety Regulation - Microorganisms)
Coliforms	Negative		BGLB method (Standard Methods of Analysis in Food Safety Regulation - Microorganisms)

BGLB = brilliant green lactose bile; CFU = colony forming units; FCC = Food Chemicals Codex; ICP-MS = inductively coupled plasma mass spectrometry; max. = maximum; min. = minimum; ppm = parts per million.

[a] Calculated on an anhydrous, ash-free basis.

[b] Trace amounts of 5-hydroxymethylfurfural is produced as a byproduct of the polymerization process.

2.3.2 Batch Analyses

The results of analysis conducted on 3 non-consecutive batches of the liquid and powder Resistant Glucan formulations are summarized in Tables 2.3.2-1 and 2.3.2-2, respectively. The results indicate that the manufacturing process for NSK's Resistant Glucan produces a consistent product that conforms to the specifications defined in Table 2.3.1-1. The certificates of analysis are provided in Appendix A.

Table 2.3.2-1 Analyses of 3 Non-Consecutive Batches of NSK's Resistant Glucan (Liquid Concentrate – Fit Fiber® #80)

Parameter	Specification	Lot No.		
		15.7.27	**15.9.10**	**15.10.8**
Composition				
Appearance	Transparent sticky syrup with pale yellow color	Passes	Passes	Passes
Taste/odor	Slightly sweet, odorless	Passes	Passes	Passes
Moisture (%)	Max. 28.0	26.4	27.7	27.9
Solid content (%)	Min. 72.0	73.6	72.3	72.1
pH (10% solution)	2.5 to 6.0	4.3	4.7	4.4
Ash (%)	Max. 0.1	0.00	0.00	0.00
Total glucan content (%)[a]	Min. 90.0	97.4	100.0	100.0
Anhydro-D-Glucose (levoglucosan) (%)[a]	Max. 4.0	1.3	1.5	1.3
Residual free glucose monomer (%)[a]	Max 6.0	4.0	3.4	3.9
5-hydroxymethylfurfural (%)[a]	Max. 0.1	0.06	0.06	0.06
Dietary fiber content (%)[a]	Min. 75.0	79.3	78.3	81.0
Dextrose equivalent	6 to 15	10	10	10
Heavy Metals				
Lead (ppm)	Max. 0.5	<0.01	<0.01	<0.01
Microbiological Contaminants				
Standard plate count (CFU/g)	Max. 300	2	0	0
Yeast (CFU/g)	Max. 100	0	0	0
Mold (CFU/g)	Max. 100	0	0	0
Coliforms	Negative	Negative	Negative	Negative

CFU = colony forming units; FCC = Food Chemicals Codex; max. = maximum; min. = minimum; ppm = parts per million.

[a] Calculated on an anhydrous, ash-free basis.

Table 2.3.2-2 Analyses of 3 Non-Consecutive Batches of NSK's Resistant Glucan (Powder – Fit Fiber® #80P)

Parameter	Specification	Lot No.		
		15.7.28	**15.8.27**	**15.9.11**
Composition				
Appearance	White to cream powder	Passes	Passes	Passes
Taste/odor	Slightly sweet, odorless	Passes	Passes	Passes
Moisture (%)	Max. 7.0	3.9	3.3	3.2
Solid content (%)	Min. 93.0	96.1	96.7	96.8
pH (10% solution)	2.5 to 6.0	3.9	4.1	4.0
Ash (%)	Max. 0.1	0.00	0.00	0.00
Total glucan content (%)[a]	Min. 90.0[a]	100.0	100.0	100.0
Anhydro-D-Glucose (levoglucosan) (%)[a]	Max. 4.0	1.4	1.3	1.4
Residual free glucose monomer (%)[a]	Max 6.0	4.0	4.1	3.9
5-hydroxymethylfurfural (%)[a]	Max. 0.1	0.06	0.06	0.06
Dietary fiber content (%)[a]	Min. 75.0	79.1	80.3	79.3
Dextrose equivalent	6 to 15	10	10	10
Heavy Metals				
Lead (ppm)	Max. 0.5	<0.01	<0.01	<0.01
Microbiological Contaminants				
Standard plate count (CFU/g)	Max. 300	0	0	0
Yeast (CFU/g)	Max. 100	0	0	0
Mold (CFU/g)	Max. 100	0	0	0
Coliforms	Negative	Negative	Negative	Negative

CFU = colony forming units; FCC = Food Chemicals Codex; max. = maximum; min. = minimum; ppm = parts per million.
[a] Calculated on an anhydrous, ash-free basis.

2.3.3 Additional Analyses

NSK also conducted analyses on 3 non-consecutive batches of the Resistant Glucan liquid and powder formulations to demonstrate that the levels of other heavy metals aside from lead (*i.e.*, cadmium, arsenic, and mercury) are below the limits of detection. The results of these analyses are summarized in Table 2.3.3-1. The certificates of analysis are provided in Appendix A.

Table 2.3.3-1 Analyses for Heavy Metals in 3 Non-Consecutive Batches of NSK's Resistant Glucan

Resistant Glucan (Liquid Concentrate – Fit Fiber® #80)				
Parameter	**Method of Analysis**	**Lot No.**		
		15.7.27	**15.9.10**	**15.10.8**
Arsenic (ppm)	ICP-MS	<0.01	<0.01	<0.01
Cadmium (ppm)	ICP-MS	<0.01	<0.01	<0.01
Mercury (ppm)	ICP-MS	<0.01	<0.01	<0.01
Resistant Glucan (Powder – Fit Fiber® #80P)				
Parameter	**Method of Analysis**	**Lot No.**		
		15.7.28	**15.8.27**	**15.9.11**
Arsenic (ppm)	ICP-MS	<0.01	<0.01	<0.01
Cadmium (ppm)	ICP-MS	<0.01	<0.01	<0.01
Mercury (ppm)	ICP-MS	<0.01	<0.01	<0.01

ICP-MS = inductively coupled plasma mass spectrometry; ppm = parts per million.

2.4 Stability

NSK conducted studies to investigate the stability of Resistant Glucan when stored at room temperature and away from light in its original package for up to 6 months for the liquid concentrate (Fit Fiber® #80), and for up to 24 months for the powder formulation (Fit Fiber® #80P). An unopened sample was assessed at 0, 3, and 6 months for the liquid concentrate, and at 0, 12, and 24 months for the powder. The results of these studies are presented in Table 2.4-1 and Table 2.4-2. The Resistant Glucan in both the liquid concentrate and powder forms are stable with respect to their carbohydrate structure (*e.g.*, degree of polymerization, and dietary fiber content) and microbial content when kept under the recommended storage conditions.

Table 2.4-1 Stability of the Resistant Glucan in the Liquid Concentrate Form (Fit Fiber® #80) During Bulk Storage

Parameter	Duration of Storage (months)		
	0	**3**	**6**
pH (Brix 10)	4.6	4.4	4.3
Solid (%)	74.1	74.0	73.7
Ash (%)	0.0	0.0	0.0
Dietary fiber content (dwb %)	83.7	82.3	82.6
Carbohydrate Profile (%)			
DP≥3	87.6	87.6	87.8
DP 2	6.3	6.3	6.3
DP 1	2.9	2.7	2.6
Others[a]	3.2	3.4	3.3
Microbiological Contaminants			
Standard plate count (CFU/g)	0	0	0
Yeast (CFU/g)	0	0	0
Mold (CFU/g)	0	0	0
Coliforms	Negative	Negative	Negative

CFU = colony forming units; DP = degree of polymerization; dwb = dried weight basis
[a] Composed predominantly of anhydro-sugar.

Table 2.4-2 Stability of the Resistant Glucan in the Powder Form (Fit Fiber® #80P) During Bulk Storage

Parameter	Duration of Storage (months)		
	0	**12**	**24**
pH (Brix 10)	4.9	5.0	4.7
Solid (%)	97.3	96.1	95.8
Ash (%)	0.0	0.0	0.0
Dietary fiber content (dwb %)	81.8	82.6	80.5
Carbohydrate Profile (%)			
DP≥3	86.7	86.9	86.8
DP 2	6.6	7.1	6.5
DP 1	3.2	2.5	3.1
Others[a]	3.5	3.5	3.6
Microbiological Contaminants			
Standard plate count (CFU/g)	0	0	0
Yeast (CFU/g)	0	0	0
Mold (CFU/g)	0	0	0
Coliforms	Negative	Negative	Negative

CFU = colony forming units; DP = degree of polymerization; dwb = dried weight basis
[a] Composed predominantly of anhydro-sugar.

2.5 Technical Effect

As mentioned, Resistant Glucan is intended for use as a low-calorie bulking agent, formulation aid, humectant, and texturizer in various foods and beverages. For example, the ingredient can be used to replace sugars and/or fats in food products, while still providing the same creaminess and mouth feel. In addition to their technological functions, these ingredients may provide a source of dietary fiber.

Resistant Glucan is intended to replace other digestion-resistant carbohydrates (*e.g.*, polydextrose, resistant dextrin, and resistant maltodextrin) that are currently accepted for use in the U.S. for similar purposes. The current regulatory status of these ingredients in the U.S. is summarized in brief below. The estimated daily intake of the Resistant Glucan ingredient from its intended conditions of use (see Part 3) is comparable to those that have been derived for other similar digestion-resistant carbohydrates. No adverse effects are anticipated from the intended uses of Resistant Glucan, as discussed further in Part 6.

Polydextrose

Polydextrose is approved as a direct food additive in the U.S., as *per* 21 CFR §172.841 (U.S. FDA, 2016a), for use as a bulking agent, formulation aid, humectant, and texturizer in all foods (except meat and poultry, baby food and infant formula) in accordance with cGMP. For all age groups, the cumulative mean and 90th percentile intake of polydextrose from all permitted uses of the additive has been estimated at 16 g/person/day and 31 g/person/day, respectively (72 FR 46562 – U.S. FDA, 2007). In recognition that excessive consumption of polydextrose could have a laxative effect in sensitive individuals, the regulation establishing the approved food additive uses of polydextrose requires that foods containing polydextrose at levels exceeding 15 g per single serving should be labeled with the following: "*Sensitive individuals may experience a laxative effect from excessive consumption of this product*" [21 CFR§172.841(e)] (U.S. FDA, 2016a).

In 2009, the FDA issued a "*no questions*" response to a GRAS notice submitted by Mead Johnson & Co. for the use of polydextrose (*i.e.*, Litesse® Two) in milk-based term infant formula, in a combination of galacto-oligosaccharides (GOS), at levels not to exceed 2 g/L of polydextrose and 2 g/L of GOS (GRN 233; U.S. FDA, 2009).

Resistant Maltodextrin (Fibersol®-2)

Fibersol®-2 is produced from corn starch by pyrolysis and subsequent enzymatic treatment, similar to the process used to manufacture conventional maltodextrin, resulting in a product with a random mixture of α- and β-1,2-, 1,3-, and 1,4- glycosidic linkages, in addition to the naturally

occurring α-1,4- and α-1,6- linkages[2]. According to the product's website[3], Fibersol®-2 meets the requirements for the GRAS status of maltodextrin as prescribed under 21 CFR §184.1444, which can be used in foods with no limitations other than cGMP (U.S. FDA, 2016b). Fibersol®-2 is added to foods as a low-calorie bulking agent and soluble dietary fiber.

Resistant Dextrin (Nutriose®)

The FDA has issued a "*no questions*" response to a GRAS notice submitted for the use of resistant dextrin (Nutriose® 6 and Nutriose® 10) as a bulking agent and dietary fiber ingredient in various foods (excluding meat products, poultry products, and infant formula), at levels ranging from 3 to 9 g/serving (GRN 436; U.S. FDA, 2013). Nutriose® 6 and Nutriose® 10 are "specialty dextrins" that are produced using a highly controlled process of starch dextrinization followed by enzymatic treatment and purification by column chromatography, resulting in a highly indigestible soluble dextrin with a higher fiber content and narrower molecular weight distribution in comparison to traditional dextrins (GRN 436; U.S. FDA, 2013). Based on the proposed uses and use levels, the estimated mean and 90th percentile user intake for the Nutriose® ingredients are estimated at 17.4 g/person/day and 32.6 g/person/day, respectively (GRN 436; U.S. FDA, 2013).

Part 3. §170.235 Dietary Exposure

3.1 Methodology

An assessment of the anticipated dietary exposure to Resistant Glucan as an ingredient in foods and beverages under the intended conditions of use was conducted using data available in 2011-2012 cycle of the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Survey (NHANES) (CDC, 2015).

The NHANES data are collected and released in 2-year cycles with the most recent cycle containing data collected in 2011-2012. Information on food consumption was collected from individuals *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2). In addition to collecting information on the types and quantities of foods being consumed, NHANES contain socio-economic, physiological and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. Sample weights were incorporated with NHANES data to compensate for the potential under-representation of intakes from specific populations and allow the data to be considered nationally representative (USDA, 2014; CDC, 2015). The NHANES

[2] http://www.bdnutritional.com/products/fibersol.
[3] http://www.fibersol.com/products/fibersol-2/faqs/

data were employed to assess the mean and 90th percentile intake of Resistant Glucan for each of the following population groups:

- Infants and young children, ages 0 to 3 years;
- Children, ages 4 to 11;
- Female teenagers, ages 12 to 19;
- Male teenagers, ages 12 to 19;
- Female adults, ages 20 and up;
- Male adults, ages 20 and up; and
- Total population (all age and gender groups combined).

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of Resistant Glucan by the U.S. population. Estimates for the daily intake of Resistant Glucan represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2011-2012 data, and these individual average amounts comprised the distribution from which mean and percentile intake estimates were generated. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. All-person intake refers to the estimated intake of Resistant Glucan averaged over all individuals surveyed, regardless of whether they potentially consumed food products containing Resistant Glucan, and therefore includes individuals with "zero" intakes (*i.e.,* those who reported no intake of food products containing Resistant Glucan during the 2 survey days). All-user intake refers to the estimated intake of Resistant Glucan by those individuals who reported consuming food products for which Resistant Glucan is intended to be used, hence the "all-user" designation. Individuals were considered "users" if they consumed 1 or more food products containing Resistant Glucan on either Day 1 or Day 2 of the survey.

Considering that NSK produces 2 different formulations of Resistant Glucan that differ in their moisture content (*i.e.*, Fit Fiber® #80 liquid concentrate containing at least 72% solids, and Fit Fiber®#80P powder containing at least 93% solids), the intake of Resistant Glucan from its intended uses was estimated on a dried weight basis. Although Resistant Glucan is not intended for use in infant foods or food products marketed to young children, an assessment of dietary exposures in this age group are included as worst-case estimates. It is also assumed that all food and beverage products falling into the proposed food use categories contain the Resistant Glucan ingredients at the maximum intended use level.

3.2 Probable Consumption

A summary of the estimated daily intake of Resistant Glucan from all intended food-uses is provided in Table 3.2-1 on an absolute basis (g/person/day), and in Table 3.2-2 on a body weight basis (g/kg body weight/day). The percentage of surveyed individuals reporting consumption of food products for which the Resistant Glucan is intended to be added (*i.e.*,

"users") was high among the total population (99.1%). The percentage of users was also high among the individual demographic groups evaluated in the current intake assessment, with at least 84.8% of the surveyed individuals within each population groups reporting as users of food products in which Resistant Glucan is intended for use (see Tables 3.2-1 and 3.2-2). Large user percentages within a population group typically lead to similar results for the all-person and all-user consumption estimates. Consequently, only the all-user intake results will be discussed.

Table 3.2-1 Summary of the Estimated Daily Intake of Resistant Glucan (Dried Weight Basis)[a] from Intended Food-Uses in the United States by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (g/day)		All-Users Consumption (g/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Infants and Young Children	0 to 3	8.8	18.3	84.8	711	10.3	19.1
Children	4 to 11	18.1	29.6	100.0	1,348	18.1	29.6
Female Teenagers	12 to 19	17.2	26.0	100.0	533	17.2	26.0
Male Teenagers	12 to 19	22.5	36.5	100.0	518	22.5	36.5
Female Adults	20 and up	20.1	34.7	99.9	2,212	20.1	34.7
Male Adults	20 and up	24.9	43.6	99.9	2,089	24.9	43.6
Total Population	All Ages	20.9	36.6	99.1	7,411	21.1	36.7

NHANES = National Health and Nutrition Examination Survey
[a] The intake estimates were obtained based on the maximum proposed use levels for Resistant Glucan on a dried weight basis, as presented in Table 1.3-1.

Table 3.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Resistant Glucan (Dried Weight Basis)[a] from Proposed Food-Uses in the United States by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (g/kg bw/day)		All-Users Consumption (g/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 3	0.65	1.32	84.7	708	0.76	1.41
Children	4 to 11	0.64	1.09	100.0	1,348	0.64	1.09
Female Teenagers	12 to 19	0.29	0.47	100.0	522	0.29	0.47
Male Teenagers	12 to 19	0.34	0.59	100.0	515	0.34	0.59
Female Adults	20 and up	0.28	0.50	99.9	2,189	0.28	0.50
Male Adults	20 and up	0.29	0.51	99.9	2,070	0.29	0.51
Total Population	All Ages	0.35	0.66	99.1	7,352	0.35	0.66

bw = body weight; NHANES = National Health and Nutrition Examination Survey
[a] The intake estimates were obtained based on the maximum proposed use levels for Resistant Glucan on a dried weight basis, as presented in Table 1.3-1.

On an all-user basis, the resulting mean and 90th percentile intakes of Resistant Glucan by the total U.S. population from all proposed food uses were estimated at 21.1 g/person/day (0.35 g/kg body weight/day) and 36.7 g/person/day (0.66 g/kg body weight/day), respectively. On an absolute basis, male adults were identified as the population group with the highest mean and 90th percentile all-user intakes of 24.9 g/person/day and 43.6 g/person/day, respectively. On a body weight basis, the highest intakes of Resistant Glucan were estimated for children age 11 and younger. Among infants and young children (0 to 3 years of age), the mean and 90th percentile all-user intakes of Resistant Glucan were estimated at 0.76 and 1.41 g/kg body weight/day, respectively. For children age 4 to 11 years old, the mean and 90th percentile all-user intakes were estimated at 0.64 and 1.09 g/kg body weight/day, respectively.

Several conservative assumptions have been included in the present assessment, which means that resulting values should be considered “worst case” estimates of exposure for the target population. For example, it was assumed that all food products within a food category contain the ingredients at the maximum specified level of use. In reality, the levels of Resistant Glucan added to specific foods will vary and are unlikely to have 100% market penetration. In addition, it is well-established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, may overestimate the consumption of food products that are consumed relatively infrequently (Anderson, 1988). It should also be noted that Resistant Glucan will not be added to food and beverage products that are specifically marketed for infants and young children, which minimizes the exposure that could potentially occur in this population group.

Part 4. §170.240 Self-Limiting Levels of Use

The addition of the Resistant Glucan ingredient to foods will be limited in that it will only be added to food products at levels needed to achieve its technological function, and at levels that do not negatively impact organoleptic properties (and thereby consumer acceptability) of foods.

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

Although digestion-resistant carbohydrates that are structurally similar to Resistant Glucan are present in the food supply, the statutory basis for the conclusion that the use of Resistant Glucan is GRAS is through scientific procedure, and not through experience based on common use in food. Therefore, this part is not applicable for the present GRAS notice.

Part 6. §170.250 Narrative

6.1 Introduction

As mentioned, NSK's Resistant Glucan ingredient is a carbohydrate polymer containing various forms of glycosidic linkages (*i.e.*, α- and β- 1,2-, 1,3-, 1,4-, and 1,6- linkages). Carbohydrate polymers that are linked by α-glycosidic linkages, such as the α-1,4- and α-1,6- bonds in starches and glycogen, are readily hydrolyzed in the human gastrointestinal tract into their monosaccharide constituents which are absorbed and processed by the body (Wisker *et al.*, 1985; Hall, 2011). In contrast, limited capacity exists to hydrolyze β-glycosidic bonds (Wisker *et al.*, 1985; Mussatto and Mancilha, 2007). As such, there are some carbohydrates that will escape digestion and absorption in the upper gastrointestinal tract, and consequently pass along to the large intestines where they could potentially serve as a substrate for anaerobic fermentation by the resident microflora, resulting in the production of hydrogen, carbon dioxide, and methane gas, lactic acid, and short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate (Cummings and Macfarlane, 1991; Cummings *et al.*, 2001; Hammer and Hammer, 2012). Carbohydrates that are poorly digested with limited absorption are considered to have a low potential for systemic toxicity; nevertheless, excessive consumption of these compounds can lead to gastrointestinal symptoms among sensitive individuals, such as bloating, abdominal cramps, flatus/gas, borborygmi, and in extreme cases, watery stools and diarrhea (Livesey, 2001; Flood *et al.*, 2004).

A number of product-specific studies have been conducted by NSK to evaluate the digestibility and fermentability, as well as safety and tolerability of their Resistant Glucan ingredient [Fit Fiber® #80(P)]. These studies are described in Parts 6.2 to 6.4 below. Additionally, comprehensive and detailed searches of the published scientific literature pertaining to the safety of other similar digestion-resistant carbohydrates were conducted through February 2016 using the following databases: 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®. The tolerability of polydextrose and other similar digestion-resistant carbohydrates are discussed in Part 6.5 to provide further support for the use of Resistant Glucan.

All of the pivotal data and information used to establish the safety of Resistant Glucan under its intended conditions of use are "generally available" (*i.e.*, in the public domain), and none are exempt from disclosure under the Freedom of Information Act. A listing of the data and information discussed herein is provided in Part 7.

6.2 Digestibility and Fermentability of Resistant Glucan

6.2.1 *In vitro* Digestibility

An *in vitro* digestibility study was conducted to assess the ability of Resistant Glucan (Fit Fiber® #80P) to resist successive digestion by salivary amylase, artificial gastric juices, pancreatic amylase, and intestinal mucosal enzymes (Hamaguchi *et al.*, 2015). The method used to investigate successive digestion was a modification of the method described by Okada *et al.* (1990). Briefly, a sample of Fit Fiber® #80P (final concentrations of 4.55% w/v) was successively incubated with salivary amylase for 30 minutes, artificial gastric juice (consisting of a hydrochloric acid-potassium chloric acid buffer, pH 2) for 100 minutes, pancreatic amylase for 360 minutes, and then intestinal mucosal enzymes for 180 minutes. All incubations were carried out at a temperature of 37°C, and after each incubation, the reactions were stopped with heat treatment at 100°C for 10 minutes (in the case of artificial gastric juice, reversal was achieved with the addition of sodium hydroxide). For comparison, the successive digestion of polydextrose (Litesse®), indigestible dextrin[4] (Fibersol®-2), and maltodextrin (Pinedex No. 2) were also investigated. The rate of hydrolysis (*i.e.,* increment of reducing sugar) as a measure of digestibility was determined *via* the Somogyi-Nelson method.

Fit Fiber® #80P was not digested by salivary amylase and artificial gastric juice, and was only slightly digested by pancreatic amylase and intestinal mucosal enzymes, as indicated by the hydrolysis rate of 0.6 and 7.8%, respectively. Similarly, polydextrose and indigestible dextrin were digested only slightly by salivary amylase (0.1 and 0.8%, respectively), not at all in artificial gastric juice, and slightly by pancreatic amylase (0.1 and 2.7%, respectively) and intestinal mucosa enzymes (6.8 and 13.8%, respectively). In contrast, maltodextrin was almost fully digested by the combined action of salivary amylase (22.4%), pancreatic amylase (5%) and intestinal mucosa enzymes (56.9%). The total rate of hydrolysis for Fit Fiber® #80P, polydextrose, indigestible dextrin, and maltodextrin was determined to be 8.4, 7.0, 17.3, and 84.3%, respectively. The results of this study demonstrate that Fit Fiber® #80P, polydextrose and indigestible dextrin (Fibersol®-2) are only minimally digested by gastrointestinal enzymes *in vitro*.

6.2.2 Studies Conducted in Rats

6.2.2.1 Digestibility in Ileorectostomized Rats

The digestibility of Resistant Glucan, resistant maltodextrin, and polydextrose was investigated in 5-week old ileorectostomized male Sprague-Dawley rats (Kondo *et al.*, 2017). After an adaptation period, ileorectostomy was performed on 24 rats following an overnight fast to allow

[4] Another widely used term for “resistant maltodextrin”.

for the direct collection of undigested food from the terminal ileum digesta as feces. Surgery was performed over 3 consecutive days (8 rats per day). To shorten the recovery period, the ileocecal valve was ligated and the colonic terminal was anastomosed to a stoma in the abdominal wall to allow the cecal and colonic contents to be excreted naturally. Post-operatively, the rats were not permitted food and water for the first 24 hours, and were then fed a control diet for 8 to 11 days. Intramuscular injection of antibiotics was performed at surgery and for 5 days post-surgery.

Following postoperative recovery, the rats (average body weight of 207±2 g) were allocated on the basis of body weight to 4 test diets (6 rats/group): (i) control diet, (ii) 50 g/kg diet of Resistant Glucan (Fit Fiber® #80P), (iii) 50 g/kg diet of resistant maltodextrin, or (iv) 50 g/kg diet of polydextrose for 9 days. Resistant Glucan, resistant maltodextrin, or polydextrose was replaced by an equivalent amount of cornstarch in the control diet. After the 9-day test period, the rats were administered the control diet during a 3-day washout period, following which the rats (average body weight of 283±3 g) were again allocated by body weight to the same 4 test diets administered *ad libitum* during Phase I for an additional 10 days. This phase of the study (Phase II) is identical to Phase I with the exception that the drinking water contained 0.1% neomycin to sterilize the remaining segments of the gastrointestinal tract (*i.e.*, small intestines and rectum) in the ileorectostomized animals. Diets and drinking water were provided *ad libitum* in the study. In both Phase I and II, 50 g/kg diet of cellulose powder was also added to each test or control diet to promote fecal consistency. Fecal cellulose recovery (measured as insoluble dietary fiber by the Prosky method) was also used as an indicator of the completeness of fecal collection. Body weight and food intakes were recorded daily. Feces were collected over the last 3 days of each test period for the analysis of undigested Resistant Glucan, resistant maltodextrin, and polydextrose. Additionally, to assess for the presence of bacterial degradation of the digestion-resistant carbohydrates in the small intestine, fresh fecal samples were collected from all rats on the morning of Day 7 during Phase II and used for the determination of levels of organic acids as a marker of bacterial activity.

In the 2 days following surgery, the rats lost 10 to 13 g (approximately 7%) of body weight; however, all rats gained weight at a constant rate (4 to 6 g/day) from Day 3 onwards. On Day 7 of the experimental period, no organic acids including formate, acetate, propionate, iso-butyrate, n-butyrate, iso-valerate, n-valerate, succinate and lactate were detected in the fresh feces, except in one control rat. No significant differences in food intake and body weight gain among the dietary groups were reported over the duration of the test period in either Phase I or II; however, body weight gain in the resistant maltodextrin group was significantly lower than in the control group during Phase I. During the last 3 days of each experimental period from which fecal matter was collected, no significant differences were reported in the amount of feed, and accordingly the amount of digestion-resistant carbohydrates, consumed. Fecal weight (dry matter) did not significantly differ among animals receiving Resistant Glucan, resistant maltodextrin, or polydextrose in either Phase I or Phase II. Fecal recoveries of undigested

Resistant Glucan, resistant maltodextrin, and polydextrose were also not statistically significant among these groups, being reported at 62.0, 67.7, and 57.5%, respectively, during Phase I and 66.5, 66.4, and 61.3%, respectively, during Phase II. The results of this study suggest that Resistant Glucan, resistant maltodextrin, and polydextrose all similarly undergo limited digestion and absorption in the upper gastrointestinal tract, with the majority (>60%) of an ingested dose reaching the colon intact.

6.2.2.2 Glycemic and Insulinemic Response

A study was conducted to investigate the glycemic and insulinemic responses following ingestion of digestion-resistant carbohydrates in rats (Kondo *et al.*, 2016). Following an adaptation period, 25 male Sprague-Dawley rats (8 weeks old, 290 to 300 g) were divided into 5 groups on the basis of body weight. After a 20-hour fast, the rats were administered by gavage a 20% (w/v) aqueous solution of glucose, Resistant Glucan (Fit Fiber® #80P), resistant maltodextrin, or polydextrose (corresponding to a dose of 1,000 mg/kg body weight of each test article), or saline (the vehicle). Blood samples (50 µL) were collected from the tail vein at 0, 30, 60, and 120 minutes post-administration for the determination of plasma glucose and insulin concentrations.

Plasma glucose at 30 and 60 minutes following the oral administration of Resistant Glucan, resistant maltodextrin, polydextrose, and saline was significantly lower than in animals administered glucose, while plasma glucose in animals receiving Resistant Glucan, resistant maltodextrin, and polydextrose was significantly higher than in animals administered saline at 30 minutes. The area-under-the-curve (AUC) of plasma glucose for the Resistant Glucan, resistant maltodextrin, and polydextrose groups were significantly lower when compared to the glucose group (by approximately 2-folds), and significantly higher when compared to the saline group. However, no significant differences in the plasma glucose at any time point or plasma glucose AUC were reported between the Resistant Glucan, resistant maltodextrin, and polydextrose groups. The AUC of plasma insulin was significantly lower in the Resistant Glucan, resistant maltodextrin, and polydextrose groups as compared to the glucose group (by approximately 2.5-folds), and significantly higher as compared to the saline group. However, no statistically significant differences in plasma insulin at any time point or plasma insulin AUC were reported between the Resistant Glucan, resistant maltodextrin, and polydextrose groups. Overall, the results of this study demonstrate that similar to other digestion-resistant carbohydrates, Resistant Glucan produces an attenuated glycemic and insulinemic response in comparison to the administration of an equivalent dose of glucose.

6.2.2.3 Fermentation

A study was conducted to investigate the fermentability of Resistant Glucan in rats (Kondo *et al.*, 2017). Following an adaptation period, 24 male Sprague-Dawley rats (8 weeks old, 275 to 284 g) were divided into 4 groups of 6 rats on the basis of body weight to receive either:

(i) control diet, (ii) 50 g/kg diet of Resistant Glucan (Fit Fiber® #80P), (iii) 50 g/kg diet of resistant maltodextrin, or (iv) 50 g/kg diet of polydextrose for 4 weeks. Resistant Glucan, resistant maltodextrin, or polydextrose was replaced by an equivalent amount of cornstarch in the control diet. Diets and drinking water were provided *ad libitum*. Body weight and food intake were recorded daily. From Day 8 to 10, feces were collected for the analysis of undigested resistant maltodextrin, polydextrose, and Resistant Glucan. At the end of the experiment, the cecum was removed and weighed, and the cecal contents were homogenized and used for the measurement of pH and organic acids (acetate, n-butyrate, propionate, succinate and lactate).

Food intake and body weight gain did not differ among the dietary groups. The weight of cecal tissue and cecal contents were significantly greater in the Resistant Glucan, polydextrose, and resistant maltodextrin groups when compared to the control group, while cecal pH was significantly lower in these groups than in the control group. No significant differences were reported between the Resistant Glucan, polydextrose, and resistant maltodextrin groups in these parameters, with the exception of significantly greater cecal content weight in the polydextrose group *versus* the Resistant Glucan or resistant maltodextrin groups. The levels of total SCFAs, which include acetate, propionate, and n-butyrate, in the Resistant Glucan group did not differ significantly from the polydextrose, resistant maltodextrin, or control groups. Lactate was detected only at very low levels in all groups. The levels of succinate in the Resistant Glucan group was comparable to controls, but were significantly higher than those in the polydextrose group and significantly lower than those in the resistant maltodextrin group. The fecal recovery of Resistant Glucan (28.6% of the ingested dose) on Day 8 to 10 was comparable to the recovery of polydextrose (33.1%), which were both significantly higher compared to the recovery of resistant maltodextrin (13.3%). These results demonstrate that digestion-resistant carbohydrates undergo fermentation by the colonic microflora, and that the extent of fermentation is similar between Resistant Glucan and polydextrose, with comparable amounts of the ingested dose being recovered in the feces unchanged.

Considering that approximately 67% of an ingested dose of Resistant Glucan escapes digestion and absorption within the small intestines, based on the results of the ileorectostomized study in rats (see Part 6.2.2.1), and that approximately 29% of an ingested dose is excreted in the feces unchanged in intact rats, it is estimated that approximately 38% of an ingested dose of Resistant Glucan undergoes fermentation within the colon.

6.3 Toxicological Studies Conducted with Resistant Glucan

6.3.1 Acute Toxicity

The acute oral toxicity of Resistant Glucan (Fit Fiber® #80P) was investigated in 5-week-old Sprague-Dawley rats (Hamaguchi *et al.*, 2015). The study was performed in accordance with Ministry of Health and Welfare, Japan, Guideline Notification No. 24 (MHLW, 1989). Prior to the test article administration, the test animals were acclimatized for 1 week and fed a stock feed.

After a 16-hour fasting period, male and female rats were divided into 3 groups (5/sex/group) and administered a single dose of 0 (control), 5,000, or 10,000 mg/kg body weight of Fit Fiber® #80P *via* oral gavage. The total volume of administration of each dose was 20 mL/kg body weight. All rats were observed for unusual symptoms for 4 hours post-administration, and once daily thereafter for 14 days. After the 14-day observation period, all animals were necropsied and the major thoracoabdominal organs were examined. No mortalities were reported, and the acute oral medial lethal dose (LD_{50}) for Fit Fiber® #80P in male and female rats was concluded to be greater than 10,000 mg/kg body weight, the highest dose tested.

6.3.2 Sub-Chronic Oral Toxicity

The subchronic oral toxicity of Resistant Glucan [Fit Fiber® #80P, containing 81.8% total dietary fiber as determined by AOAC 2001.03 (AOAC, 2005)] was assessed in a 90-day toxicity study conducted in rats (Bito *et al.*, 2016), which was performed in accordance with Ministry of Health and Welfare, Japan, Guideline Notification No. 29 and No. 655 (MHLW, 1996, 1999). According to NSK, the batch of Fit Fiber® #80P tested in this study contains 97.3% solids. The doses administered in the study were selected based on the results of a 14-day preliminary dose-range finding study in which no adverse effects were reported.

Following an acclimatization period of 2 weeks, 30 male and 30 female 4-week-old Sprague-Dawley rats [Crl:CD(SD) SPF] were allocated to 3 groups (10/sex/group) on the basis of body weight and fed diets containing Fit Fiber® #80P at concentrations of 0 (control), 3, or 5% in the diet. These dietary concentrations correspond respectively to mean intakes of 0, 1,954 and 3,318 mg/kg body weight/day in males, and 0, 2,254, and 3,874 mg/kg body weight/day in females. Food and water were provided *ad libitum* throughout the study. Rats were individually observed for clinical signs every 3 or 4 days (twice per week). Body weights and food consumption were also measured every 3 or 4 days (twice per week). From Day 86 to 87, all animals were put in individual cages, and fasting 4-hour urine samples were collected for urinalysis. Ophthalmological examinations were carried out on 6 animals per group on Day 88. Subsequently, fasting blood samples were obtained from all animals for routine hematology and blood chemistry analyses, after which the rats were euthanized. Complete necropsies were conducted on all animals, and their organs were removed and weighed. Histopathological analyses were performed on rats in the control and high-dose groups; however, if any changes were observed microscopically and suspected to be test article-related, histopathological analyses were also performed on rats in the low-dose group.

One male rat in the high-dose group died on Day 81. During the histopathological examinations for this animal, a mild enlargement of the cecum along with luminal dilatation was observed, and the consistency of the cecal contents was noted to be an almost solid paste, in amounts greater than normal for a rat. However, no other abnormalities were observed in this animal, including changes in its general condition, or changes in body weight and food consumption prior to its death. Also, no abnormal histopathological changes were reported. The study authors noted

that cecal enlargement is characteristic of ingestion of large amounts of poorly digested carbohydrates. In the absence of any other abnormalities though, the death was not considered to be related to the cecal enlargement, and it was concluded to be incidental and unrelated to the administration of Fit Fiber® #80P. No significant differences were reported in body weights between animals in the test and control groups. A statistically significant decrease in food consumption was reported in low-dose males on Day 77, and a statistically significant increase was reported in high-dose females on Day 63. No significant differences were reported in food consumption at any other time points between the test and control group animals, and these changes were considered to be incidental. Thus, the effects reported on food consumption were considered to be transient and not toxicologically relevant. No ophthalmological abnormalities were reported in any of the animals examined.

At the end of the study, a statistically significant decrease was reported in daily urinary excretion of electrolytes (*i.e.*, Na, K, and Cl) in the low-dose males (1.6±0.3, 3.9±0.6, and 2.4±0.4 mmol/day, respectively) in comparison to their control (2.1±0.6, 5.2±1.3, 3.2±0.7 mmol/day, respectively). However, the effect was not observed in the high-dose males or in the females administered either dose of Fit Fiber® #80P, and no other changes indicative of impairments in renal function were observed, such as changes in blood urea nitrogen (BUN), creatinine, or abnormal histopathological changes in the kidneys. Moreover, no significant changes in any other urinalysis parameters were observed. Therefore, these findings were not considered to be attributed to the administration of the test article.

With respect to the hematology parameters assessed at the end of the study, in comparison to their respective control, a statistically significant decrease in mean corpuscular hemoglobin (MCH) was reported in high-dose males (*i.e.*, 17.2±0.5 pg *versus* 17.8±0.5 pg) and a statistically significant decrease in fibrinogen volume (FIB) was reported in high-dose females (*i.e.*, 185±15 mg/dL *versus* 211±28 mg/dL). However, these changes did not occur in both sexes, and the values reported in the high-dose animals remained within the normal range for the rats at the facility. Additionally, the study authors reported no significant changes in other related hematological parameters (such as mean corpuscular volume and mean corpuscular hemoglobin concentration, prothrombin time, and activated partial thromboplastin time). Therefore, these changes were not considered to be toxicologically relevant.

Among the blood chemistry parameters assessed, a statistically significant decrease in aspartate aminotransferase (AST) activity was noted in low-dose males in comparison to their respective control at the end of the study (*i.e.*, 58±6 IU/L *versus* 68±11 IU/L). However, the effect was not observed in the high-dose males or any of the females receiving the test article, and it was not considered to be toxicologically significant. A statistically significantly decrease in alanine phosphatase (ALP) activity was noted in low-dose (290±53 IU/L) and high-dose (294±31 IU/L) males compared to controls (341±43 IU/L). No statistically significant effects in ALP were observed in the females, although a trend towards a decrease was noted. In the

absence of any abnormal histopathological changes, and that the ALP values observed were within the physiological normal range for the rats at the facility, these effects were not considered to be toxicologically relevant.

The only statistically significant change in organ/tissue weights was an increase in relative prostate weights observed in low-dose males compared to the controls. Since this effect was not dose-dependent (*i.e.*, similar changes were not observed in high-dose males), and no histopathological correlates were observed, it was not considered to be toxicologically relevant. No macroscopic changes were apparent in tissue/organs harvested at necropsy, and microscopic analyses of tissues/organs revealed no histopathological changes that could be attributed to the administration of the Fit Fiber®#80P test article.

The authors concluded the no-observed-adverse-effect level (NOAEL) to be 5% of Fit Fiber® #80P in the diet (the highest concentration tested), which corresponded to intakes of 3,318 mg/kg body weight/day in male rats and 3,874 mg/kg body weight/day in female rats.

6.3.3 Mutagenicity/Genotoxicity

The potential mutagenicity of Resistant Glucan [Fit Fiber® #80P, containing 82.6% total dietary fiber as determined by AOAC 2001.03 (AOAC, 2005)] was evaluated in a bacterial reverse mutation test (Ames test) performed according to OECD Test No. 471 (OECD, 1997) (Hamaguchi *et al.*, 2015; Bito *et al.*, 2016). This assay was conducted using the plate incorporation method in *Salmonella typhimurium* TA100, TA1535, TA98, TA1537 and *Escherichia coli* WP2 *uvrA* in the presence and absence of S9 metabolic activation. The negative control consisted of the vehicle (sterile distilled water), and the positive controls consisted of 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, sodium azide, 2-methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acridine dihydrochloride, 2-aminoanthracene, and benzo[*a*]pyrene. Each test was conducted in triplicate at test article concentrations of 313, 625, 1,250, 2,500, and 5,000 µg/plate. Results were deemed to be positive if the number of revertant colonies was at least double the number of natural revertant colonies (*i.e.*, of the negative control) and if reproducibility and concentration-dependence were reported. In both tests, no positive mutagenic responses were reported in any strain at any concentration tested, in the presence or absence of metabolic activation. In contrast, the positive control substances displayed marked mutagenic activity. It was concluded that Fit Fiber® #80P was non-mutagenic under the conditions of this study.

6.4 Clinical Studies Conducted with Resistant Glucan

6.4.1 Ascending-Dose Tolerability Study in Healthy Adults

The safety and tolerability of Resistant Glucan (Fit Fiber® #80P) was investigated in an ascending dose study conducted in healthy adults (Bito *et al.*, 2016). A total of 20 healthy

volunteers (10 males and 10 females) between 20 and 59 years of age (mean±SD = 37.0±8.1 years) and with mean (±SD) body weight of 58.8±9.0 kg were enrolled in this study[5]. Four test doses of Fit Fiber® #80P (82.6% total dietary fiber as determined by AOAC 2001.03) (AOAC, 2005), 0.3, 0.5, 0.7, and 0.9 g/kg body weight on a dried weight basis, were administered on a single occasion in ascending order to each of the subjects with a 1-week washout period between each dose. The test articles were prepared by dissolving Fit Fiber® #80P in 200 mL of water, and it was consumed 2 hours after ingesting lunch prepared at the test facility. Subjects were prohibited from ingesting oligosaccharides, sugar alcohols, yogurt, milk, and alcoholic beverages from the day prior to and the day after ingesting the test article. Within 24 hours of ingestion of the test article, all subjects were asked to record the incidence of abdominal symptoms (abdominal pain, tenesmus, gurgling sounds, abdominal bloating, flatus, vomiting and discomfort, and nausea), defecation frequency, the shape and the consistency of stools, and any other adverse side effects. In addition, subjects were also asked to record any variation in their physical condition, lifestyle, intake of supplements and drugs, and intake of alcohol. The evaluation of stool shape and consistency was performed using a self-reported subjective 6-level scale with the following ratings: (1) very hard (pellet-shaped); (2) hard-solid; (3) normal (banana-shaped); (4) soft, pasty; (5) muddy; and (6) watery. Muddy stools and watery stools were categorized as diarrhea.

Two subjects (1 male and 1 female) dropped out of the study for reasons unrelated to the test article (*i.e.*, personal circumstances). Four subjects reported gurgling sounds at the 0.7 g/kg body weight dose. Among these 4 subjects, 2 of them also reported flatus, and 1 of these 2 subjects reported experiencing tenesmus and abdominal discomfort. These 4 subjects reported the same symptoms at the 0.9 g/kg body weight dose, and gurgling sounds and flatus were also reported in 2 additional subjects at this higher dose. However, all symptoms were reported to be mild and transient, and improved spontaneously. Diarrhea (*i.e.,* presence of muddy or watery stools) was not reported in any subject at any dose level within 24 hours of ingesting the test article. As such, the maximum no-effect dose for diarrhea (defined as the dose at which diarrhea was not observed in any subjects) was estimated to be greater than 0.9 g/kg body weight for Fit Fiber® #80P on a dried weight basis. This is comparable to the maximum no-effect doses for diarrhea that have been established for other poorly digested carbohydrates (see Part 6.5). The results of this study demonstrate Fit Fiber® #80P to be well tolerated (*i.e.*, no diarrhea observed) when consumed by healthy adults at doses of <u>at least</u> 0.9 g/kg body weight (dried weight basis), which corresponds to intakes of approximately 63 g for a 70 kg adult.

[5] The mean and standard deviations of the age and body weights of the participants were not reported in the Bito *et al.* (2016) publication, but were provided upon request from the study investigators.

6.4.2 Efficacy Study on Improvements in Laxation

The safety and tolerability of Resistant Glucan [Fit Fiber® #80P, containing 80.4% total dietary fiber as determined by AOAC 2001.03 (AOAC, 2005)] was investigated in a randomized, single-blinded, placebo-controlled, parallel-group trial in women (Hamaguchi *et al.*, 2016). Sixty (60) female generally healthy volunteers with a tendency for constipation (*i.e.*, 2 to 4 instances of defecation per week) were enrolled in this study. The subjects were between 20 and 60 years of age (mean±SD = 38.9±8.5 years), and the mean body weight (±SD) was reported at 52.8±5.1 kg. The following exclusion criteria were applied: subjects using drugs, functional foods, cosmetics, or instruments that may influence the outcomes of the study; subjects consuming large amounts of foods such as lactic acid bacteria beverages, foods containing lactic acid bacteria or natto bacteria, lactic acid bacteria preparations, dietary fiber-enriched food, sugar alcohols, and oligosaccharides; subjects currently undergoing treatment for digestive diseases that may affect the study outcome; subjects with a history of surgery involving the digestive system (except appendectomy); subjects who were pregnant or suspected to be pregnant; subjects who were considered unsuitable for the study because of an illness or the possibility of developing serious side effects; and, subjects who were deemed to be inappropriate participants by a doctor.

The 60 enrolled subjects were randomly allocated into 4 groups of 15 subjects per group. The test articles administered in the study (*i.e.*, Fit Fiber® #80P or the maltodextrin placebo) were dissolved in water and administered at a total volume of 100 mL. After a 2-week observation period, all subjects were administered the placebo (13.2 g maltodextrin/day) for 2 weeks. Following this, the subjects consumed for 2 weeks test beverages containing 0 (control), 3.3 (low-dose), 6.6 (mid-dose), or 13.2 (high-dose) g per day of Fit Fiber® #80P, which was combined with 13.2, 9.9, 6.6, and 0 g per day of maltodextrin, respectively. As such, a total of 13.2 g of test powder (containing different ratios of Fit Fiber® #80P to maltodextrin) was administered daily, of which 0.7 g is accounted for by moisture. To ensure that the test assignments were not revealed to the study subjects, adequate volumes of caramel were added to the test powders to create equal appearance and flavor. During the study, subjects were requested to maintain their habitual diet, and food products that may have an effect on the gastrointestinal flora were prohibited (*e.g.*, food items containing intentionally added pre- and pro-biotics and fiber). In addition, subjects completed a daily questionnaire on their diet, prescriptions, and health status. A questionnaire related to stool and defecation properties (*i.e.*, defecation days, frequency, fecal volume, color, odor, and excretory feeling) was also completed.

No subjects withdrew from the study. No adverse effects on the physical condition of the subjects were reported. A statistically significant increase in the mean defecation days per week, defecation frequency per week, and fecal volume was reported in the group receiving the high-dose Fit Fiber® #80P compared to the placebo group. No significant changes were

reported in the subjects receiving 3.3 and 6.6 g/day doses of Fit Fiber® #80P. No significant changes in fecal shape, color, odor, or excretory feeling were reported among groups. The significant increases in defecation frequency, defecation days, and fecal volume among constipated individuals are considered to be a beneficial and not adverse response to the ingestion of dietary fiber. The investigator confirmed that diarrhea was not reported in any of the participants. The results of this study demonstrate that the ingestion of Fit Fiber® #80P at a dose of 13.2 g per day for 2 weeks is safe and tolerable in this study population. This dose of Fit Fiber® #80P corresponds to intake of 12.5 g/day on a dried weight basis.

6.5 Tolerability of Digestion-Resistant Carbohydrates

6.5.1 Nature of Gastrointestinal Symptoms

Carbohydrates that are not fully digested by enzymes into absorbable saccharides within the upper gastrointestinal tract enter the large intestines where they can potentially serve as substrates for fermentation by the resident microflora (Grabitske and Slavin, 2009). The various beneficial effects associated with dietary fibers, which are broadly defined as poorly digested carbohydrates, are related to these very properties (IOM, 2005). However, the ingestion of poorly digested carbohydrates, particularly at high levels of intake, has also been associated with a number of undesirable gastrointestinal effects such as acid reflux and heartburn, bloating, belching/burping, flatulence, abdominal distention, nausea, borborygmi/rumbling in the gut, stomachaches/spasmodic abdominal pain, and in extreme cases, watery feces and diarrhea (Livesey, 2001; Grabitske and Slavin, 2009).

As reviewed by Grabitske and Slavin (2009), there are a number of different factors affecting the gastrointestinal acceptability of poorly digested carbohydrates. These include factors related to the individual (*e.g.*, biological predisposition, lifestyle, health status), as well as those related to the chemical structure of the carbohydrate. Colonic fermentation of carbohydrates that are incompletely digested and absorbed in the upper gastrointestinal tract yields organic acids (*i.e.*, lactic acid, SCFAs) and gases such as hydrogen, methane, and carbon dioxide (Grabitske and Slavin, 2009; Hammer and Hammer, 2012). Flatus and bloating can result if the amount of gases produced exceeds the colon's capacity to absorb them (Hammer and Hammer, 2012). Additionally, carbohydrates that are not fermented in the colon, or for which intake exceeds the fermentation capacity of the colonic microflora, will remain in the gastrointestinal tract and increase the osmotic load (Grabitske and Slavin, 2009). The unabsorbed carbohydrates, together with any unabsorbed organic acids and electrolytes, reduce the uptake of water and can result in watery stools or osmotic diarrhea (Grabitske and Slavin, 2009; Hammer and Hammer, 2012). Since osmotic pressure is related to the number of molecules, rather than weight, substances with lower molecular weights (*e.g.*, sugar alcohols) exert a greater osmotic force and have greater potential to induce laxative effects (*i.e.*, induce osmotic diarrhea at a

lower dose) in comparison to higher molecular weight polysaccharides such as polydextrose (Flood *et al.*, 2004; Grabitske and Slavin, 2009).

In general, the gastrointestinal effects associated with excessive intakes of poorly digestible carbohydrates are transient and cease promptly upon cessation or reduction of intake, and subjects may also develop improved tolerance to the material over time (Livesey, 2001; Flood *et al.*, 2004). Nevertheless, even though they are transient, these symptoms can affect the perception of the well-being by the consumers and their acceptability of foods containing the ingredient.

6.5.2 Tolerability of Poorly-Digested Carbohydrates that are Structurally Similar to Resistant Glucan

Polydextrose is only partly fermented within the colon, with approximately 50% of an ingested dose being excreted unchanged (Auerbach *et al.*, 2007). The fermentation pattern of polydextrose is known to be more sustained and slow, resulting in a reduced rate of SCFA production and lower gas production rate, in comparison to short-chain digestion-resistant carbohydrates with lower molecular weight; this likely accounts for the greater tolerability of polydextrose in comparison to these other compounds (Röytiö and Ouwehand, 2014). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has reviewed the use of polydextrose in foods, and derived Acceptable Daily Intake (ADI) of "not specified"[6] (JECFA, 1987). Nevertheless, JECFA indicated that: "*Studies in man have demonstrated that polydextroses, when administered at very high doses, exert a laxative effect, with a mean laxative threshold of 90 g per day or 50 g as a single dose. This factor should be taken into account when considering appropriate levels for the use of polydextroses alone or in combination with other substances causing laxative effects by osmotic action*" (JECFA, 1987). These same conclusions were also subsequently drawn by the Scientific Committee on Food (SCF) of the European Commission following a review of the available data (SCF, 1992), and in a detailed review published by Flood *et al.* (2004). Of note, the diarrhea induced by polydextrose was considered to be isolated and transient, and no changes have been observed with respect to clinical chemistry or nutrient utilization as demonstrated in metabolic balance studies (Flood *et al.*, 2004).

In a tolerability study conducted with resistant maltodextrin (Fibersol®-2), the maximum no-effect dose level (*i.e.*, dose where no diarrhea observed in any subject following ingestion of the test article) was concluded by the study authors to be 1.0 g/kg body weight for men and >1.1 g/kg body weight for women (Kishimoto *et al.*, 2013). Additionally, a number of randomized,

[6] When applied to food an ADI of "not specified" means "*food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological, and other), the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food does not, in the opinion of JECFA, represent a hazard to health*" (WHO, 1987).

double-blind, placebo-controlled studies have been conducted to examine the short- and long-term tolerance of resistant dextrin (Nutriose®) in healthy human subjects (*e.g.*, van den Heuvel *et al.*, 2004; Vermorel *et al.*, 2004; Pasman *et al.,* 2006). In the GRAS notice submitted for the use of Nutriose® 6 and Nutriose® 10 as food ingredients, the "maximum non-laxative dose" was reported as 45 g/day (GRN 436, U.S. FDA, 2013). Although increased incidence and severity of flatulence and bloating were reported in some subjects at doses higher than 45 g/day (*i.e*., 60 and 80 g/day), no diarrhea was reported at these doses (van den Heuvel *et al*., 2004).

6.5.3 Considerations for Resistant Glucan

It is worth noting that the possibility for gastrointestinal disturbances and laxation/diarrhea exists when large amounts of any poorly digested, fermentable carbohydrates are consumed. These include the naturally occurring fiber components that are consumed from various plant sources (*e.g.*, grains, fruits, and vegetables), as well as commercially produced polymers that are purposely added to foods as ingredients. For comparison, NSK's Resistant Glucan ingredient is at least as well tolerated as other structurally and metabolically similar digestion-resistant carbohydrate polymers currently marketed in the U.S., as described in Part 6.5.2.

The level of intake anticipated from the intended uses and use levels of Resistant Glucan are generally well below the maximum no-effect level for diarrhea, which was established as >0.9 g/kg body weight for Resistant Glucan on a dried weight basis (Bito *et al.*, 2016). The 90th percentile intake of Resistant Glucan (on a dried weight basis) is estimated at 0.66 g/kg body weight/day for the total population, and ranges from 0.47 to 0.59 g/kg body weight/day among individual population groups 12 years and older. Although the 90th percentile intake of Resistant Glucan (dried weight basis) is estimated at 1.41 g/kg body weight/day for infants and young children (age 0 to 3 years), and at 1.09 g/kg body weight/day for children age 4 to 11 years, the intended uses of Resistant Glucan are not anticipated to pose any concerns. Resistant Glucan will also not be added to foods that are specifically marketed towards infants and young children (up to 3 years of age), which minimizes the exposure that could potentially occur in this population group. No incidences of diarrhea were reported when Resistant Glucan was administered at doses up to 0.9 g/kg body weight (dried weight basis), the highest dose tested, suggesting that the true laxative threshold for the ingredient is likely to be greater. The maximum no-effect level derived (on a body weight basis) in the adult tolerability is applicable to the general population since there is no evidence to suggest that children are more sensitive to the effects of poorly-digested carbohydrates than adults (Flood *et al.*, 2004). Additionally, a number of conservative assumptions were made during the intakes assessment, which means the calculated consumption levels are "worst-case" estimates that likely exceed actual consumption levels.

It is possible that there may be certain sub-groups who may be particularly sensitive to developing gastrointestinal symptoms following the ingestion of poorly digestible carbohydrates; these can include, for example, individuals with Irritable Bowel Syndrome (IBS). However, such

individuals will likely receive dietary guidance on the types of foods/food products that they should limit or avoid consuming (WGO, 2015). While food products containing polydextrose exceeding 15 g per single serving are required to be specially labeled ("*Sensitive individuals may experience a laxative effect from excessive consumption of this product*") [21 CFR§172.841(e)], this is not required for food products containing resistant dextrins or resistant maltodextrin. Considering that Resistant Glucan was demonstrated to be well tolerated in humans (laxative threshold >0.9 g/kg body weight), and that the addition of Resistant Glucan to foods will not exceed 7 g/serving (see Table 1.3-1), a label statement for food products containing Resistant Glucan is not considered to be warranted.

Overall, the intended uses of NSK's Resistant Glucan (Fit Fiber® #80 and #80P) as ingredients in foods are not anticipated to pose any safety or tolerability concerns.

6.6 Summary

Overall, the GRAS status of the intended uses of Resistant Glucan can be supported by the following:

- Resistant Glucan is structurally similar to other digestion-resistant carbohydrates that are manufactured and widely used as food ingredients (*e.g.*, polydextrose, resistant maltodextrin, resistant dextrins).

- NSK manufactures their Resistant Glucan ingredients (Fit Fiber® #80, #80P) in accordance with cGMP using food-grade materials. Batch analyses demonstrate that the manufacturing process consistently produces a material that meets the defined specifications, and is free from microbial and heavy metal contaminants.

- Stability studies support shelf-life of 6 months for the liquid concentrate of Resistant Glucan (Fit Fiber® #80) and 24 months for the powder formulation (Fit Fiber® #80P).

- The estimated daily intake of Resistant Glucan from its intended uses is comparable to those derived for other similar digestion-resistant carbohydrates it is intended to replace in the diet:

    - The mean intake of Resistant Glucan, polydextrose, and resistant dextrin among the total U.S. population (all ages) has been estimated at 21, 16, and 17 g/day, respectively; and

    - The 90th percentile intake of Resistant Glucan, polydextrose, and resistant dextrin among the total U.S. population (all ages) has been estimated at 37, 31, 33 g/day, respectively.

- Studies conducted *in vitro* and in animals have demonstrated that similarly to polydextrose and resistant maltodextrin, Resistant Glucan is largely resistant to digestion in the upper gastrointestinal tract, and the undigested fraction is subject to fermentation by the colonic microflora (Kondo *et al.*, 2017).

- Resistant Glucan has low potential for systemic toxicity:

    - Oral LD_{50} was determined as >10,000 mg/kg body weight in rats (Hamaguchi *et al.*, 2015);

    - The NOAEL from a 90-day feeding study was determined as 5% inclusion rate in the diet (the highest concentration tested), which corresponded to intakes of approximately 3,318 mg/kg body weight/day in male rats, and 3,874 mg/kg body weight/day in female rats (Bito *et al*., 2016); and

    - No evidence of mutagenicity was observed when Resistant Glucan was tested using the Ames assay (Hamaguchi *et al.*, 2015; Bito *et al*., 2016).

- Excessive consumption of poorly digested, fermentable carbohydrates can produce gastrointestinal symptoms, such as bloating, abdominal cramps, flatus/gas, borborygmi in sensitive individuals, and, in extreme cases, watery stools and diarrhea (Livesey, 2001; Flood *et al*., 2004). Nevertheless, Resistant Glucan is expected to be well tolerated under the intended conditions of use.

    - No adverse effects or undesirable gastrointestinal symptoms were reported when Resistant Glucan (Fit Fiber® #80P) was administered for 2 weeks to a group of women with a tendency for constipation at doses of 13.2 g/day (12.5 g/day on a dried weight basis) (Hamaguchi *et al.*, 2016).

    - In an ascending-dose tolerability study conducted with Resistant Glucan (Fit Fiber® #80P), diarrhea was not reported in any of the subjects at all of the doses tested, and the maximum no-effect dose for diarrhea was established to be at least 0.9 g/kg body weight on a dried weight basis (Bito *et al.*, 2016).

    - Comparable laxative thresholds have been established for other similar digestion-resistant carbohydrates that Resistant Glucan is intended to replace in the diet (*e.g*., polydextrose, resistant dextrin, and resistant maltodextrin).

6.7 Expert Panel Evaluation

A Panel of Experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients has unanimously concluded on the GRAS status of Resistant Glucan under the conditions of its intended use. The Expert Panel consisted of the following qualified scientific experts: Professors Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine); George Fahey, Ph.D. (University of Illinois); and I. Glenn Sipes, Ph.D. (University of Arizona)[7].

The Expert Panel, convened by NSK, independently and critically evaluated all data and information presented herein and concluded that Resistant Glucan, meeting appropriate food-grade specifications and manufactured consistent with cGMP, is safe and suitable for use as an ingredient as specified in Part 1.3, and is GRAS based on scientific procedures. It is believed that other qualified experts would concur with these conclusions. A summary of data and information reviewed by the Expert Panel, and their evaluation of such data as it pertains to the GRAS uses of Resistant Glucan, is presented in Appendix B.

6.8 Conclusion

Based on the data and information presented herein, NSK has concluded that the intended uses of their Resistant Glucan ingredient, as described in Part 1.3, are GRAS based on scientific procedures. NSK is not aware of any data and information that are, or may appear to be, inconsistent with their conclusion that the intended uses of Resistant Glucan is GRAS. The GRAS status of Resistant Glucan is further supported by the unanimous consensus rendered by an independent panel of experts, qualified by experience and scientific training to evaluate the safety of food ingredients, who concluded that the intended uses of Resistant Glucan described herein is GRAS.

Resistant Glucan may therefore be marketed and sold for its intended purpose in the U.S., without the promulgation of a food additive regulation under Title 21 of the CFR.

[7] The panelists participated in their individual capacities. Institutional affiliations are provided for identification purposes only.

Part 7. §170.255 List of Supporting Data and Information

Anderson SA, editor (1988). *Estimation of Exposure to Substances in the Food Supply*. (Contract No. FDA 223-84-2059). Bethesda (MD): Federation of American Societies for Experimental Biology (FASEB), Life Science Research Office (LSRO).

AOAC (2005). AOAC official method 2001.03. Total dietary fiber in foods containing Maltodextrin [45.4.13]. In: *Official Methods of Analysis of the Association of Official Analytical Chemists: Vols. 1&2, 18th edition* (Current through Revision 1, 2006). Arlington (VA): Association of Official Analytical Chemists (AOAC).

Auerbach MH, Craig SA, Howlett JF, Hayes KC (2007). Caloric availability of polydextrose. Nutr Rev 65(12):544-549.

Bito H, Hamaguchi N, Hirai H, Ogawa K (2016). Safety evaluation of a newly-developed dietary fiber: resistant glucan mixture. J Toxicol Sci 41(1):33-44.

CDC (2015). *National Health and Nutrition Examination Survey (NHANES): 2011-2012*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://wwwn.cdc.gov/nchs/nhanes/search/nhanes11_12.aspx [Page last reviewed: November 6, 2015].

Cummings JH, Macfarlane GT (1991). The control and consequences of bacterial fermentation in the human colon. J Appl Bacteriol 70(6):443-459.

Cummings JH, Stephen AM (2007). Carbohydrate terminology and classification. Eur J Clin Nutr 61(Suppl. 1):S5-S18.

Cummings JH, Macfarlane GT, Englyst HN (2001). Prebiotic digestion and fermentation. Am J Clin Nutr 73(2, Suppl.):415S-420S.

FCC (2016). Maltodextrin. In: *Food Chemicals Codex, 10th edition*. Rockville (MD): United States Pharmacopeial Convention (USP), pp. 802-803.

Flood MT, Auerbach MH, Craig SAS (2004). A review of the clinical toleration studies of polydextrose in food. Food Chem Toxicol 42(9):1531-1542.

Grabitske HA, Slavin JL (2009). Gastrointestinal effects of low-digestible carbohydrates. Crit Rev Food Sci Nutr 49(4):327-360.

Hall JE (2011). Digestion and absorption in the gastrointestinal tract. In: *Guyton and Hall Textbook of Medical Physiology, 12th edition*. Philadelphia (PA): W.B. Saunders Company, Elsevier, pp. 789-798.

Hamaguchi N, Hirai H, Aizawa K, Takada M (2015). Production of water-soluble indigestible polysaccharides using activated carbon. J Appl Glycosci 62:7-13.

Hamaguchi N, Hirai H, Bito H, Ogawa K (2016). Effects of resistant glucan mixture on bowel movement in female volunteers. J Nutr Sci Vitaminol (Tokyo) 62(1):62-66 [Errata, 62(2):139].

Hammer HF, Hammer J (2012). Diarrhea caused by carbohydrate malabsorption. Gastroenterol Clin North Am 41(3):611-627.

IOM (2005). Dietary, functional, and total fiber. In: *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids (Macronutrients*). (National Academy of Sciences (NAS), Institute of Medicine (IOM), Food and Nutrition Board (FNB), Panel on Micronutrients, Panel on the Definition of Dietary Fiber, Subcommittee on Upper Reference Levels of Nutrients, Subcommittee on Interpretation and Uses of Dietary Reference Intakes, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes). Washington (DC): National Academy Press (NAP), pp. 339-421. Available at: http://www.nap.edu/openbook.php?record_id=10490&page=339.

JECFA (1987). Polydextroses. In: *Evaluation of Certain Food Additives and Contaminants.* 31st Meeting of the Joint FAO/WHO Expert Committee on Food Additives, Feb. 16-25, 1987, Geneva, Switz. (WHO Technical Report Series, no 759). Geneva, Switz.: World Health Organization (WHO), pp. 31-32, 49. Available at: http://whqlibdoc.who.int/trs/WHO_TRS_759.pdf.

Kishimoto Y, Kanahori S, Sakano K, Ebihara S (2013). The maximum single dose of resistant maltodextrin that does not cause diarrhea in humans. J Nutr Sci Vitaminol (Tokyo) 59(4):352-357.

Kondo T, Handa K, Genda T, Hino S, Hamaguchi N, Morita T (2016) [unpublished]. *Resistant Dextrin Derivatives are Moderately Digested in the Small Intestine and Their Energy Contribution Would be Greater Than in the Large Bowel Fermentation.* Shizuoka City, Japan: Shizuoka University, College of Agriculture, Academic Institute.

Kondo T, Handa K, Genda T, Hino S, Hamaguchi N, Morita T (2017). Digestion-resistant dextrin derivatives are moderately digested in the small intestine and contribute more to energy production than predicted from large-bowel fermentation in rats. J Nutr 147(3):330-336.

Livesey G (2001). Tolerance of low-digestible carbohydrates: a general view. Br J Nutr 85(Suppl. 1):S7-S16.

MHLW (1989). *Guidelines for Toxicity Studies on Drugs*. (Notification no 24, September 11, 1989). Tokyo, Japan: Ministry of Health, Labor and Welfare, Japan (MHLW), Pharmaceuticals and Cosmetics Division, Pharmaceuticals Affairs Bureau.

MHLW (1996). *Guidelines for Designation of Food Additives and for Revision of Standards for Use of Food Additives*. (Notification no 29, March 22, 1996). Tokyo, Japan: Ministry of Health, Labor and Welfare, Japan (MHLW), Environmental Health Bureau.

MHLW (1999). *Partial Revision of Guidelines for Repeated-Dose Toxicity Studies* (Notification no 655, April 5, 1999). Tokyo, Japan: Ministry of Health, Labor and Welfare, Japan (MHLW), Pharmaceutical and Medical Safety Bureau. Cited In: Bito et al., 2016 [As Ministry of Health, Labor and Welfare, 1999, p. 34].

Mussatto S, Mancilha IM (2007). Non-digestible oligosaccharides: a review. Carbohydr Polym 68(3):587-597.

OECD (1997). Bacterial reverse mutation test. In: *OECD Guidelines for the Testing of Chemicals*. (OECD Guideline no 471) [Updated & Adopted 21 July 1997]. Paris, France: Organisation for Economic Co-operation and Development (OECD). Available at: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en;jsessionid=1ijgh8o34k0qd.delta.

Okada K, Yoneyama M, Mandai T, Aga H, Sakai S, Ichikawa T (1990). Digestion and fermentation of pullulan. Nippon Eiyo Shokuryo Gakkaishi [J Jap Soc Nutr Food Sci] 43(1):23-29 [Includes Translation].

Pasman W, Wils D, Saniez MH, Kardinaal A (2006). Long-term gastrointestinal tolerance of NUTRIOSE® FB in healthy men. Eur J Clin Nutr 60(8):1024-1034.

Röytiö H, Ouwehand AC (2014). The fermentation of polydextrose in the large intestine and its beneficial effects. Benef Microbes 5(3):305-313.

SCF (1992). Report of the Scientific Committee for Food on a second series of food additives of various technological functions (Opinion expressed 19 October 1990) [Polydextrose]. In: Report of the Scientific Committee for Food on a second series of food additives of various technological functions (Opinion expressed 19 October 1990). In: *Food Science and Techniques*. (Reports of the Scientific Committee for Food, Fourth Series). Brussels, Belgium: Commission of the European Communities (EEC), Scientific Committee for Food (SCF), pp. 1-19. Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_reports_26.pdf.

U.S. FDA (2002). *Agency Response Letter GRAS Notice No. GRN No. 000107 [Polydextrose, Ardsley (NY): Danisco Cultor America].* 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=107 [Oct. 17, 2002].

U.S. FDA (2007). Food additives permitted for direct addition to food for human consumption; polydextrose; Final rule (21 CFR Part 172) [Docket No. 2006F–0059]. Fed Regist (US) 72(161):46562-46564. Available at: https://www.gpo.gov/fdsys/granule/FR-2007-08-21/E7-16322.

U.S. FDA (2009). *Agency Response Letter GRAS Notice No. GRN 000233 [Combination of galacto-oligosaccharides and polydextrose, Evansville (IN): Mead Johnson & Company]*. 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/?set=GRASNotices&id=233 [Sep. 4, 2009].

U.S. FDA (2013). *Agency Response Letter GRAS Notice No. GRN 000436 [Enzyme-modified dextrins, Lestrem, France: Roquette Frères]*. Silver Spring (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=436 [Jan 18, 2013].

U.S. FDA (2016a). Part 172—Food additives permitted for direct addition to food for human consumption. §172.841—Polydextrose. In: *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs* (U.S. Food and Drug Administration). Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

U.S. FDA (2016b). Part 184—Direct food substances affirmed as generally recognized as safe. §184.1444—Maltodextrin. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration).* Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

USDA (2014). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2011-2012.* Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release [Last Modified: 10/2/2014].

van den Heuvel EGHM, Wils D, Pasman WJ, Bakker M, Saniez MH, Kardinaal AF (2004). Short-term digestive tolerance of different doses of NUTRIOSE®FB, a food dextrin, in adult men. Eur J Clin Nutr 58(7):1046-1055.

Vermorel M, Coudray C, Wils D, Sinaud S, Tressol JC, Montaurier C et al. (2004). Energy value of a low-digestible carbohydrate, NUTRIOSE® FB, and its impact on magnesium, calcium and zinc apparent absorption and retention in healthy young men. Eur J Nutr 43(6):344-352.

WGO (2015). *Irritable Bowel Syndrome: A Global Perspective*. (World Gastroenterology Organisation Global Guideline). Milwaukee (WI): World Gastroenterology Organisation (WGO). Available at: http://www.worldgastroenterology.org/irritable-bowel-syndrome.html.

WHO (1987). Section 5.5: Setting the ADI. In: *Principles for the Safety Assessment of Food Additives and Contaminants in Food*. (Environmental Health Criteria, no 70). Geneva, Switz.: World Health Organization (WHO) / International Programme on Chemical Safety (IPCS) / United Nations Environment Programme (UNEP), pp. 75-85. Available at: http://www.inchem.org/documents/ehc/ehc/ehc70.htm#SectionNumber:5.5.

Wisker E, Feldheim W, Pomeranz Y, Meuser F (1985). Dietary fiber in cereals. In: Pomeranz Y, editor. *Advances in Cereal Science and Technology. Vol. VII*. St. Paul (MN): American Association of Cereal Chemists, pp. 169-238.

Appendix A

Certificates of Analysis for Fit Fiber® #80 and #80P

NIHON SHOKUHIN KAKO CO., LTD.

Tel 03(3212)9111
Fax 03(3212)9131

Marunouchi kitaguchi Bldg. 20F, 6-5, Marunouchi 1-chome, Chiyoda-ku Tokyo 100-0005, Japan

CERTIFICATE OF ANALYSIS

February 17, 2016

Product: Fit Fiber® #80 (Resistant glucan) - 25 kg square can
Lot Number: (b) (6)

Characteristic		Lower Limit	Upper Limit	Value	Unit
Appearance - Transparent sticky syrup with pale yellow color		-	-	Passes Test	
Taste - Slightly sweet / odor - odorless		-	-	Passes Test	
Moisture		-	28.0	26.4	%
Solid content		72.0	-	73.6	%
Total glucan content		90.0	-	97.4	%
Anhydro-D-Glucose (Levoglc)		-	4.0	1.3	%
Residual free glucose monomer		-	6.0	4.0	%
5-Hydroxymethylfurfural		-	0.10	0.06	%
Dietary fiber content		75.0	-	79.3	%
Dextrose equivalent		6	15	10	
pH (10%solution)		2.5	6.0	4.3	
Ash		-	0.10	0.00	%
Lead		-	1.0	<0.05	ppm
Microbiological	Standard plate count	-	300	2	CFU/g
	Yeast	-	100	0	CFU/g
	Mold	-	100	0	CFU/g
	Coliforms - Negative to test	-	-	Passes Test	

[redacted]

NIHON SHOKUHIN KAKO CO., LTD.

Tel 03(3212)9111
Fax 03(3212)9131

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CERTIFICATE OF ANALYSIS

February 17, 2016

Product: Fit Fiber® #80 (Resistant glucan) - 25 kg square can
Lot Number: (b) (6)

Characteristic		Lower Limit	Upper Limit	Value	Unit
Appearance - Transparent sticky syrup with pale yellow color		-	-	Passes Test	
Taste - Slightly sweet / odor - odorless		-	-	Passes Test	
Moisture		-	28.0	27.7	%
Solid content		72.0	-	72.3	%
Total glucan content		90.0	-	100.0	%
Anhydro-D-Glucose (Levoglc)		-	4.0	1.5	%
Residual free glucose monomer		-	6.0	3.4	%
5-Hydroxymethylfurfural		-	0.10	0.06	%
Dietary fiber content		75.0	-	78.3	%
Dextrose equivalent		6	15	10	
pH (10%solution)		2.5	6.0	4.7	
Ash		-	0.10	0.00	%
Lead		-	1.0	<0.05	ppm
Microbiological	Standard plate count	-	300	0	CFU/g
	Yeast	-	100	0	CFU/g
	Mold	-	100	0	CFU/g
	Coliforms - Negative to test	-	-	Passes Test	

[redacted]

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CERTIFICATE OF ANALYSIS

February 17, 2016

Product: Fit Fiber® #80 (Resistant glucan) - 25 kg square can
Lot Number: (b) (6)

Characteristic		Lower Limit	Upper Limit	Value	Unit
Appearance - Transparent sticky syrup with pale yellow color		-	-	Passes Test	
Taste - Slightly sweet / odor - odorless		-	-	Passes Test	
Moisture		-	28.0	27.9	%
Solid content		72.0	-	72.1	%
Total glucan content		90.0	-	100.0	%
Anhydro-D-Glucose (Levoglc)		-	4.0	1.3	%
Residual free glucose monomer		-	6.0	3.9	%
5-Hydroxymethylfurfural		-	0.10	0.06	%
Dietary fiber content		75.0	-	81.0	%
Dextrose equivalent		6	15	10	
pH (10%solution)		2.5	6.0	4.4	
Ash		-	0.10	0.00	%
Lead		-	1.0	<0.05	ppm
Microbiological	Standard plate count	-	300	0	CFU/g
	Yeast	-	100	0	CFU/g
	Mold	-	100	0	CFU/g
	Coliforms - Negative to test	-	-	Passes Test	

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NIHON SHOKUHIN KAKO CO., LTD.

Tel 03(3212)9111
Fax 03(3212)9131

Marunouchi kitaguchi Bldg. 20F, 6-5, Marunouchi 1-chome, Chiyoda-ku Tokyo 100-0005, Japan

CERTIFICATE OF ANALYSIS

February 17, 2016

Product: Fit Fiber® #80P (Resistant glucan) - 15 kg bag
Lot Number: (b) (6)

Characteristic		Lower Limit	Upper Limit	Value	Unit
Appearance - White to cream powder		-	-	Passes Test	
Taste - Slightly sweet / odor - odorless		-	-	Passes Test	
Moisture		-	7.0	3.9	%
Solid content		93.0	-	96.1	%
Total glucan content		90.0	-	100	%
Anhydro-D-Glucose (Levoglc)		-	4.0	1.4	%
Residual free glucose monomer		-	6.0	4.0	%
5-Hydroxymethylfurfural		-	0.10	0.06	%
Dietary fiber content		75.0	-	79.1	%
Dextrose equivalent		6	15	10	
pH (10%solution)		2.5	6.0	3.9	
Ash		-	0.10	0.00	%
Lead		-	1.0	<0.05	ppm
Microbiological	Standard plate count	-	300	0	CFU/g
	Yeast	-	100	0	CFU/g
	Mold	-	100	0	CFU/g
	Coliforms - Negative to test	-	-	Passes Test	

[redacted]

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CERTIFICATE OF ANALYSIS

February 17, 2016

Product: Fit Fiber® #80P (Resistant glucan) - 15 kg bag
Lot Number: (b) (6)

Characteristic		Lower Limit	Upper Limit	Value	Unit
Appearance - White to cream powder		-	-	Passes Test	
Taste - Slightly sweet / odor - odorless		-	-	Passes Test	
Moisture		-	7.0	3.3	%
Solid content		93.0	-	96.7	%
Total glucan content		90.0	-	100.0	%
Anhydro-D-Glucose (Levoglc)		-	4.0	1.3	%
Residual free glucose monomer		-	6.0	4.1	%
5-Hydroxymethylfurfural		-	0.10	0.06	%
Dietary fiber content		75.0	-	80.3	%
Dextrose equivalent		6	15	10	
pH (10%solution)		2.5	6.0	4.1	
Ash		-	0.10	0.00	%
Lead		-	1.0	<0.05	ppm
Microbiological	Standard plate count	-	300	0	CFU/g
	Yeast	-	100	0	CFU/g
	Mold	-	100	0	CFU/g
	Coliforms - Negative to test	-	-	Passes Test	

[redacted]

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CERTIFICATE OF ANALYSIS

February 17, 2016

Product: Fit Fiber® #80P (Resistant glucan) - 15 kg bag
Lot Number: (b) (6)

Characteristic		Lower Limit	Upper Limit	Value	Unit
Appearance - White to cream powder		-	-	Passes Test	
Taste - Slightly sweet / odor - odorless		-	-	Passes Test	
Moisture		-	7.0	3.2	%
Solid content		93.0	-	96.8	%
Total glucan content		90.0	-	100.0	%
Anhydro-D-Glucose (Levoglc)		-	4.0	1.4	%
Residual free glucose monomer		-	6.0	3.9	%
5-Hydroxymethylfurfural		-	0.10	0.06	%
Dietary fiber content		75.0	-	79.3	%
Dextrose equivalent		6	15	10	
pH (10%solution)		2.5	6.0	4.0	
Ash		-	0.10	0.00	%
Lead		-	1.0	<0.05	ppm
Microbiological	Standard plate count	-	300	0	CFU/g
	Yeast	-	100	0	CFU/g
	Mold	-	100	0	CFU/g
	Coliforms - Negative to test	-	-	Passes Test	

[redacted]

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Japan Food Research Laboratories
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No. 16136614001-0101 1/1
Date issued: December 23, 2016

CERTIFICATE OF ANALYSIS

Client: NIHON SHOKUHIN KAKO CO., LTD.
30 Tajima, Fuji-shi, Shizuoka 417-8530, Japan

Sample name: Fit Fiber #80 Lot No. (b) (6)

Received date: December 09, 2016

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Arsenic (as As)	Not detected	0.01 ppm		1
Lead	Not detected	0.01 ppm		1
Cadmium	Not detected	0.01 ppm		1
Mercury	Not detected	0.01 ppm		1

QL: Quantitation limit N: Notes M: Method

Method

1: Inductively coupled plasma mass spectrometry

JAPAN FOOD RESEARCH LABORATORIES

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Received date: December 09, 2016

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Mercury	Not detected	0.01 ppm		1

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1: Inductively coupled plasma mass spectrometry

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Appendix B

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Resistant Glucan (Fit Fiber® #80, #80P) for Use as an Ingredient in Food

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Resistant Glucan (Fit Fiber® #80, #80P) for Use as an Ingredient in Food

July 15, 2016

Nihon Shokuhin Kako Co. Ltd. (referred to hereafter as "NSK") intends to market resistant glucan (Fit Fiber® #80, #80P) as a low-calorie bulking agent, formulation aid, humectant, and texturizer in various food and beverage products in the United States (U.S.), at levels ranging between 1.9 to 22.3% on a dry weight basis. The Fit Fiber® ingredients are also intended for use as a formulation aid (binder, filler, or excipient) in dietary supplements. An Expert Panel of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened by NSK to conduct a critical and comprehensive evaluation of the available pertinent data and information concerning resistant glucan (Fit Fiber® #80, #80P). The Expert Panel was asked to determine whether the intended uses of resistant glucan would be Generally Recognized as Safe (GRAS), based on scientific procedures. For purposes of the Expert Panel's evaluation, "safe" or "safety" indicates 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 Expert Panel consisted of the below-signed qualified scientific experts: Professors Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine); George Fahey, Ph.D. (University of Illinois); and I. Glenn Sipes, Ph.D. (University of Arizona).

The Expert Panel, independently and collectively, critically evaluated a supporting dossier submitted by NSK [Documentation Supporting the Determination that Resistant Glucan (Fit Fiber® #80, #80P) is Generally Recognized as Safe (GRAS) for Use as an Ingredient in Food, July 4, 2016]. This dossier is a comprehensive package of data and information including the method of manufacture, product specifications and analytical data, stability, intended conditions of use, and estimated intake of resistant glucan based on all intended uses. Additionally, the dossier contains a compilation of the scientific information and data pertinent to the safety of resistant glucan, including data provided by NSK, as well as data identified from the literature and other published sources through February 2016.

Following its independent and collaborative critical evaluation of the data and information, the Expert Panel convened *via* teleconference on July 15, 2016. The Expert Panel reviewed its findings and following discussion unanimously concluded that the intended uses described herein of resistant glucan (Fit Fiber® #80, #80P), meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practices (cGMP), are GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion is provided in the following section.

Summary and Basis for GRAS Determination

NSK manufactures resistant glucan, a synthetic, water-soluble carbohydrate polymer produced by the bulk melt polycondensation of glucose syrup in the presence of activated carbon, which serves as a catalyst. Resistant glucan is marketed as a liquid concentrate (Fit Fiber® #80) and as a spray-dried powder (Fit Fiber® #80P). The only difference between these two formulations is the moisture content; the spray-dried powder is specified to contain 93% solids and not more than 7% water, while the liquid concentrate is specified to contain at least 72% solids and not more than 28% water. The Fit Fiber® ingredients are largely composed of carbohydrates (>90% glucan content on a dried weight basis) containing various forms of glycosidic linkages (*i.e.*, α- and β- 1,2-, 1,3-, 1,4-, and 1,6- linkages). Since glycosidic linkages other than α-1,4- and α-1,6- bonds are not readily hydrolyzed by human digestive enzymes (Cummings and Stephen, 2007), resistant glucan largely escapes digestion within the upper gastrointestinal tract. The Fit Fiber® ingredients are specified to contain at least 75% total dietary fiber, as measured by the Association of Official Analytical Chemists (AOAC) method 2001.03.

Resistant glucan is considered structurally similar to other digestion-resistant carbohydrates currently marketed in the U.S. For example, polydextrose is another synthetic, water-soluble carbohydrate polymer containing various forms of glycosidic linkages (*i.e.*, α- and β- 1,2-, 1,3-, 1,4-, and 1,6- linkages) that is produced by the bulk melt polycondensation of a glucose and sorbitol mixture (approximately 9:1 ratio) in the presence of catalytic amounts of citric acid or phosphoric acid. It is permitted as a direct food additive in the U.S., specifically as a bulking agent, formulation aid, humectant, and texturizer in all foods (except meat and poultry, baby food, and infant formula), when used consistent with cGMP (21 CFR§172.841) (U.S. FDA, 2016b). Other digestion-resistant carbohydrate polymers (*e.g.*, resistant maltodextrin marketed as Fibersol®-2, and resistant dextrin marketed as Nutriose® 6 and Nutriose® 10) that are obtained from the pyrodextrinization of starch, which converts a portion of the naturally occurring α-1,4- and α-1,6-glycosidic linkages to a random mixture of α- and β- 1,2-,1,3-, 1,4- and 1,6- glycosidic linkages, are also marketed for use in the U.S. as food ingredients. Analyses conducted by NSK demonstrate that the distribution of glycosidic linkages and the molecular weight profiles are generally similar between Fit Fiber® and these other digestion-resistant carbohydrates.

Resistant glucan is produced from glucose syrup that undergoes condensation polymerization reaction under high heat (180 to 230°C) in the presence of an activated carbon catalyst. The solution is then filtered to remove the activated carbon; this is followed by a number of purification steps including decolorization using fresh activated carbon and deionization with the use of a mixed bed ion-exchange resin. Subsequently, the solution is concentrated by evaporation to produce the liquid formulation (Fit Fiber® #80), which can then be spray-dried to produce the powder formulation (Fit Fiber® #80P). The manufacturing process for the Fit Fiber® ingredients is conducted consistent with cGMP and appropriate quality control procedures (*i.e.*,

FSSC 22000 certification) are in place. All raw materials and processing aids used in the manufacture of Fit Fiber® are food-grade and suitable for use in the U.S. for such purposes.

NSK has established physical and chemical specifications for Fit Fiber® #80 and #80P, which include parameters related to its identity and composition, and lead and microbial contaminants. The Expert Panel reviewed analytical data conducted on 3 non-consecutive batches of Fit Fiber® #80 and #80P, which demonstrate that the manufacturing process consistently produces a product that complies with the established product specifications. The Expert Panel also reviewed data on the stability of the Fit Fiber® ingredients when kept in bulk storage for up to 6 months for the liquid concentrate (Fit Fiber® #80), and up to 24 months for the powder formulation (Fit Fiber® #80P). The results of these studies demonstrate that the ingredients are stable with respect to their carbohydrate structure (*e.g.*, degree of polymerization, dietary fiber content), and are free from microbial contaminants when kept for the durations tested under the recommended storage conditions (*i.e.*, in its unopened original packaging at room temperature and away from light).

Similarly to other digestion-resistant carbohydrates which they are intended to replace, NSK's Fit Fiber® #80 and #80P will be used as a low-calorie bulking agent, formulation aid, humectant, and texturizer in various foods and beverages. The Fit Fiber® ingredients are also intended for use as a formulation aid (binder, filler, or excipient) in dietary supplements. The food categories and use levels at which NSK's Fit Fiber® #80 and #80P are intended to be added are summarized in Attachment 1. The Fit Fiber® ingredients will not be added to meat and poultry products, or to foods that are specifically marketed towards infants and young children (including infant formula). An assessment of the anticipated dietary exposure to Fit Fiber® as an ingredient in foods and beverages under the intended conditions of use was conducted using data available in 2011-2012 cycle of the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Survey (NHANES) (CDC, 2015). For the total population, the all-user mean and 90th percentile intakes of Fit Fiber® on a dried weight basis were estimated at 21.1 g/person/day (0.35 g/kg body weight/day) and 36.7 g/person/day (0.66 g/kg body weight/day), respectively. These intake levels are comparable to those estimated for other similar digestion-resistant carbohydrates that Fit Fiber® is intended to replace in the diet; for example, the mean and 90th percentile intakes for polydextrose have been estimated at 16 g/person/day and 31 g/person/day, respectively (72 FR 46562), while the mean and 90th percentile intakes for Nutriose® have been estimated at 17 g/person/day and 33 g/person/day, respectively (GRN 436; U.S. FDA, 2013).

On an absolute basis, the highest mean and 90th percentile all-user intake of Fit Fiber® (dried weight basis) was estimated for male adults at 24.9 and 43.6 g/person/day, respectively. On a body weight basis, the highest intakes of Fit Fiber® were estimated for children age 11 and younger. Among infants and young children (0 to 3 years of age), the mean and 90th percentile all-user intakes of Fit Fiber® were estimated at 0.76 and 1.41 g/kg body weight/day,

respectively. For children age 4 to 11 years old, the mean and 90th percentile all-user intakes were estimated at 0.64 and 1.09 g/kg body weight/day, respectively. However, the Expert Panel considers these intakes to be a gross over-estimation given that a number of conservative "worst-case" scenario assumptions are made during the assessment. For example, it is assumed that all food products within a food category contain the ingredients at the maximum specified level of use. It should also be noted that Fit Fiber® will not be added to foods that are specifically marketed towards infants and young children, which minimizes the exposure that could potentially occur in this population group.

Carbohydrate polymers that are linked by α-glycosidic linkages, such as the α-1,4- and α-1,6-bonds in starches and glycogen, are readily hydrolyzed in the human gastrointestinal tract into their monosaccharide constituents which are absorbed and processed by the body (Wisker *et al.*, 1985; Hall, 2011). The carbohydrates that escape digestion and absorption in the upper gastrointestinal tract will enter the large intestine where they could potentially undergo fermentation by the resident microflora, producing hydrogen, carbon dioxide, and methane gas, lactic acid, and short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate (Cummings and Macfarlane, 1991; Cummings *et al.*, 2001; Hammer and Hammer, 2012). NSK's resistant glucan ingredient (Fit Fiber® #80 and #80P) is expected to undergo similar metabolic processes as other partially digestible carbohydrates that are widely consumed in the diet. Experiments conducted *in vitro* demonstrate that Fit Fiber® #80P is not digested by salivary amylase or artificial gastric juices, and is only minimally digested by pancreatic amylase (0.6% hydrolysis rate) and intestinal mucosal enzymes (7.8% hydrolysis rate) (Hamaguchi *et al.,* 2015). Similar results were obtained for polydextrose and resistant maltodextrin, which were minimally hydrolyzed by salivary amylase (0.1 and 0.8%, respectively), not at all hydrolyzed in artificial gastric juice, and only slightly hydrolyzed by pancreatic amylase (0.1 and 2.7%, respectively) and intestinal mucosa enzymes (6.8 and 13.8%, respectively) (Hamaguchi *et al.,* 2015).

NSK examined the digestibility and fermentability of Fit Fiber® using rat models (Kondo *et al.*, 2016). A study was conducted in which ileorectostomized male Sprague-Dawley rats (6/group) were allocated by body weight to diets containing: i) 50 g/kg feed of Fit Fiber®, (ii) 50 g/kg feed of resistant maltodextrin, or (iii) 50 g/kg feed of polydextrose for 9 days. A group of control animals received diets substituted by an equivalent amount of cornstarch. The experiment was repeated after a 3-day washout period (control diet only for all animals), in which the animals received the same test diets for 10 days but with drinking water containing 0.1% neomycin. Neomycin was added to the drinking water to sterilize the segments of the gastrointestinal tract (*i.e.*, small intestines and rectum) remaining in the ileorectostomized animals. The fecal recovery of undigested Fit Fiber®, resistant maltodextrin, and polydextrose were reported at 62.0, 67.7, and 57.5%, respectively, during the first phase of the study (*i.e.*, no neomycin in drinking water), and at 66.5, 66.4, and 61.3%, respectively, when neomycin was added to the drinking water. Therefore, similar to other digestion-resistant carbohydrates, a large proportion

of ingested Fit Fiber® (approximately 67%) escapes digestion in the upper gastrointestinal tract. This is further supported by a study conducted in fasted male Sprague-Dawley rats (5/group) where the administration of Fit Fiber® at 1,000 mg/kg body weight by gavage produced an attenuated glycemic and insulinemic response in comparison to rats receiving an equivalent glucose dose, in a manner that was comparable to those produced by an equivalent dose of other poorly digested carbohydrates (*i.e*., polydextrose and resistant maltodextrin).

The fermentability of Fit Fiber® has also been examined in male Sprague-Dawley rats (6/group) fed diets containing: i) 50 g/kg feed of Fit Fiber®, (ii) 50 g/kg feed of resistant maltodextrin, or (iii) 50 g/kg feed of polydextrose for 4 weeks. A group of control animals received diets substituted by an equivalent amount of cornstarch. Although cecal pH was significantly lower in animals fed diets containing the digestion-resistant carbohydrates in comparison to controls, the levels of total short-chain fatty acids (SCFAs) in the Fit Fiber® group did not significantly differ from the polydextrose, resistant maltodextrin, or control groups. The fecal recovery of Fit Fiber® on Day 8 to 10 (28.6% of the ingested dose) was comparable to the fecal recovery of polydextrose (33.1%), which were both significantly higher compared to the recovery of resistant maltodextrin (13.5%). These results demonstrate that digestion-resistant carbohydrates undergo fermentation by the colonic microflora, and that the extent of fermentation is similar between resistant glucan and polydextrose. Together with the results from the digestibility study conducted in ileorectostomized rats described above (Kondo *et al.*, 2016), it is estimated that approximately 33% of an ingested dose of Fit Fiber® is digested and absorbed within the small intestines, while approximately 38% of an ingested dose undergoes fermentation in the colon. The remainder (approximately 29%) will be excreted in the feces unchanged.

NSK has conducted a number of preclinical toxicity studies with their Fit Fiber® ingredient. Fit Fiber® #80P was not mutagenic when tested using an Ames assay conducted in accordance with OECD Guideline No. 471 (Hamaguchi *et al.*, 2015; Bito *et al*., 2016). An acute oral toxicity study and 90-day oral toxicity study were conducted with Fit Fiber® #80P; these studies were performed consistent with guidelines established by Japan's Ministry of Health and Welfare. Fit Fiber® #80P is of low acute oral toxicity, with the median lethal dose (LD_{50}) reported at >10,000 mg/kg body weight in rats (Hamaguchi *et al.*, 2015). In an 90-day oral toxicity study, 4-week-old Sprague-Dawley rats (10/sex/group) were administered diets containing Fit Fiber® #80P at concentrations of 0 (control), 3%, or 5% (Bito *et al.*, 2016). No significant differences in body weights were reported in animals administered Fit Fiber® #80P in comparison to controls. Some changes in food consumption were reported, including a significant decrease in males receiving Fit Fiber® #80P at 3% in the diet on Day 77, and significant increase in females receiving Fit Fiber® #80P at 5% in the diet on Day 63, in comparison to controls. However, given that these changes were transient and did not occur consistently throughout the study, they were considered to be incidental and not toxicologically relevant. Some statistically significant changes were reported in animals administered Fit Fiber® #80P when compared to control animals with respect to organ weights, and serum chemistry, hematology, and urinalysis

parameters. However, these were not related to the dietary concentration of Fit Fiber® #80P administered, remained within the ranges reported for the historical control rats at the facility, and/or did not occur together with related histopathological correlates, and therefore were not considered to be toxicologically relevant. Additionally, no abnormalities attributable to the Fit Fiber® #80P test article were reported during histopathological analysis. One male rat administered 5% Fit Fiber® #80P in the diet died on Day 81; histopathological examination revealed mild enlargement of the cecum along with luminal dilatation, and the cecal content was reported to be a solid paste. No other abnormalities were reported in this animal, including changes in its general condition, or changes in body weight and food consumption prior to its death, and there were no other abnormal histopathological findings. The authors noted that cecal enlargement is characteristic of ingestion of large amounts of poorly digested carbohydrates. However, in the absence of any other abnormalities, the death was not considered to be related to the cecal enlargement, and it was concluded to be incidental and unrelated to the administration of Fit Fiber®. The no-observed-adverse-effect level (NOAEL) in this study was determined to be 5% dietary inclusion rate for Fit Fiber® #80P, which corresponds to intake of 3,318 mg/kg body weight per day in male rats and 3,874 mg/kg body weight/day in female rats, the highest dose tested in the study (Bito *et al*., 2016).

Similar to other carbohydrates that are poorly digested and absorbed, NSK's Fit Fiber® ingredient was demonstrated to have a low potential for systemic toxicity. Nevertheless, excessive consumption of these compounds can lead to undesirable gastrointestinal symptoms among sensitive individuals, such as bloating, abdominal cramps, flatus/gas, borborygmi, and in extreme cases, watery stools and diarrhea (Livesey, 2001; Flood *et al.*, 2004). NSK has conducted 2 clinical studies investigating the effects of Fit Fiber® #80P following oral administration. The ability of Fit Fiber® #80P to improve laxation was evaluated in a randomized, single-blind, placebo-controlled, parallel-group study involving 60 generally healthy women (15/group) between 20 to 60 years of age with a tendency for constipation (*i.e.*, 2 to 4 instances of defecation per week) (Hamaguchi *et al.*, 2016). For 2 weeks, the participants received test beverages containing 0 (control), 3.3, 6.6, or 13.2 g/day of Fit Fiber® #80P, which was combined with 13.2, 9.9, 6.6, and 0 g/day of maltodextrin, respectively. As such, a total of 13.2 g of test powder (containing different ratios of Fit Fiber® #80P to maltodextrin) was administered daily, of which 0.7 g is moisture. There was a statistically significant increase in the mean defecation days per week, defecation frequency per week, and fecal volume in the group receiving the highest dose of Fit Fiber® #80P tested compared to the placebo group. These findings, in this particular group of subjects, were considered to be beneficial (*i.e.*, improvement in laxation). No adverse effects or undesirable gastrointestinal symptoms, including diarrhea, were reported in any of the subjects and Fit Fiber® #80P is considered to be well-tolerated when administered at doses up to 13.2 g/day (*i.e.*, up to 12.5 g/day of Fit Fiber® on a dried weight basis).

An ascending-dose study has been conducted to examine the tolerability of Fit Fiber® #80P in 20 healthy adults (10 males and 10 females between 20 to 59 years of age) (Bito *et al.*, 2016). Four test doses of Fit Fiber® #80P (82.6% total dietary fiber as determined by AOAC 2001.03), of 0.3, 0.5, 0.7, and 0.9 g/kg body weight on a dried weight basis, were administered on a single occasion in ascending order to each of the subjects with a 1-week washout period in between each dose. The test articles were prepared by dissolving the Fit Fiber® #80P powder in 200 mL of water, and it was consumed 2 hours after ingesting lunch prepared at the test facility. Two subjects (1 male and 1 female) dropped out of the study, which according to the authors, were due to circumstances that were unrelated to the test article. Gastrointestinal symptoms such as gurgling sounds, flatus, tenesmus and abdominal discomfort were reported in several of the subjects within 24 hours of ingesting doses of 0.7 or 0.9 g/kg body weight. Four subjects reported gurgling sounds at the 0.7 g/kg body weight dose. Among these 4 subjects, 2 of them also reported flatus, and 1 of these 2 subjects reported experiencing tenesmus and abdominal discomfort. These 4 subjects reported the same symptoms at the 0.9 g/kg body weight dose, and gurgling sounds and flatus were also reported in 2 additional subjects at this higher dose level. However, all symptoms were noted to be mild and transient, and they improved spontaneously. No diarrhea (defined as the presence of muddy or watery stools) was reported in any subject at any dose level within 24 hours of ingesting the test article. As such, the maximum no-effect dose for diarrhea (*i.e.*, the dose at which diarrhea was not observed in any subjects) was established to be 0.9 g/kg body weight for Fit Fiber® #80P on a dried weight basis, which corresponds to intakes of approximately 63 g for a 70 kg adult (Bito *et al*., 2016). Comparable laxative thresholds have been established for other similar digestion-resistant carbohydrates. The mean laxative threshold for polydextrose, which is the mean dose at which the participants in a given study first experienced diarrhea, has been established as 90 g/day (1.3 g/kg body weight/day) or 50 g (0.7 g/kg body weight) as a single dose (JECFA, 1987; SCF, 1992; Flood *et al.*, 2004). For resistant maltodextrin (Fibersol®-2), the maximum no-effect dose level for diarrhea has been established at 1.0 g/kg body weight for men and >1.1 g/kg body weight for women (Kishimoto *et al.*, 2013). Diarrhea was not reported when doses up to 80 g/day of resistant dextrin (Nutriose®) were consumed (van den Heuvel *et al.*, 2004; Vermorel *et al.*, 2004; Pasman *et al.,* 2006).

The intakes anticipated from the intended uses and use levels of Fit Fiber® are generally below the maximum no-effect level for diarrhea of >0.9 g/kg body weight for Fit Fiber® on a dried weight basis (Bito *et al.*, 2016). Even though the tolerability study for Fit Fiber® was conducted in adults, the maximum no-effect level derived is considered applicable to the general population given that there is no evidence to suggest that children are more sensitive to the effects of poorly-digested carbohydrates than adults (Flood *et al.*, 2004). The 90th percentile intake of Fit Fiber® (dried weight basis) is estimated at 0.66 g/kg body weight/day for the total population, and from 0.47 to 0.59 g/kg body weight/day among individual population groups 12 years and older. On a body weight basis, the intake of Fit Fiber® is estimated to be highest among infants and children, with the 90th percentile intake of Fit Fiber® (dried weight basis)

estimated at 1.41 g/kg body weight/day for infants and young children (age 0 to 3 years), and at 1.09 g/kg body weight/day for children age 4 to 11 years. The Expert Panel recognized that these intake estimates are considered to be "worst-case" and likely to exceed actual consumption levels, given that a number of conservative assumptions are made during the assessment (*e.g.*, all food products within a food category contain the Fit Fiber® ingredients at the maximum specified level of use). Moreover, no incidences of diarrhea were reported when Fit Fiber® was administered at doses up to 0.9 g/kg body weight (dried weight basis), the highest dose tested, suggesting that the true laxative threshold for the ingredient may be greater. Although gastrointestinal symptoms such as gurgling sounds, flatus, tenesmus, and abdominal discomfort were reported in some subjects within 24 hours of ingesting the 0.7 and 0.9 g/kg body weight doses, these were mild and transient, and they are known to occur following the ingestion of large quantities of any poorly digested, fermentable carbohydrates.

In summary, the Expert Panel noted that the safety of NSK's Fit Fiber® ingredient is supported by a number of product-specific studies that have been conducted with the ingredient, including a clinical study investigating its gastrointestinal tolerability. Furthermore, the estimated daily intakes of the Fit Fiber® ingredients are comparable to those determined for other similar digestion-resistant carbohydrates with comparable laxative thresholds. These digestion-resistant carbohydrates are already marketed as food ingredients in the U.S., and Fit Fiber® #80 and #80P are intended to replace these existing ingredients in the diet. Based on the information and data reviewed, the intended uses of NSK's resistant glucan (Fit Fiber® #80 and #80P) as an ingredient in conventional foods and beverages are not anticipated to pose any safety or tolerability concerns.

CONCLUSION

We, as members of the Expert Panel, have independently and collectively critically evaluated the data and information deemed pertinent to the safety of the intended conditions of use for NSK's resistant glucan (Fit Fiber® #80, #80P) ingredient. We unanimously conclude that resistant glucan (Fit Fiber® #80, #80P), when produced consistent with current Good Manufacturing Practices and meeting appropriate food grade specifications, is Generally Recognized as Safe (GRAS) under its intended conditions of use as an ingredient in foods and beverages at levels up to 22.3% on a dry weight basis and as a formulation aid in dietary supplements, based on scientific procedures.

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

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REFERENCES

Bito H, Hamaguchi N, Hirai H, Ogawa K (2016). Safety evaluation of a newly-developed dietary fiber: resistant glucan mixture. J Toxicol Sci 41(1):33-44.

CDC (2015). *National Health and Nutrition Examination Survey (NHANES): 2011-2012*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://wwwn.cdc.gov/nchs/nhanes/search/nhanes11_12.aspx [Page last reviewed: November 6, 2015].

Cummings JH, Macfarlane GT (1991). The control and consequences of bacterial fermentation in the human colon. J Appl Bacteriol 70(6):443-459.

Cummings JH, Stephen AM (2007). Carbohydrate terminology and classification. Eur J Clin Nutr 61(Suppl. 1):S5-S18.

Cummings JH, Macfarlane GT, Englyst HN (2001). Prebiotic digestion and fermentation. Am J Clin Nutr 73(2, Suppl.):415S-420S.

Flood MT, Auerbach MH, Craig SAS (2004). A review of the clinical toleration studies of polydextrose in food. Food Chem Toxicol 42(9):1531-1542.

Hall JE (2011). Digestion and absorption in the gastrointestinal tract. In: *Guyton and Hall Textbook of Medical Physiology, 12th edition*. Philadelphia (PA): W.B. Saunders Company, Elsevier, pp. 789-798.

Hamaguchi N, Hirai H, Aizawa K, Takada M (2015). Production of water-soluble indigestible polysaccharides using activated carbon. J Appl Glycosci 62:7-13.

Hamaguchi N, Hirai H, Bito H, Ogawa K (2016). Effects of resistant glucan mixture on bowel movement in female volunteers. J Nutr Sci Vitaminol (Tokyo) 62(1):62-66 & [Errata, 62(2):139].

Hammer HF, Hammer J (2012). Diarrhea caused by carbohydrate malabsorption. Gastroenterol Clin North Am 41(3):611-627.

JECFA (1987). Polydextroses. In: *Evaluation of Certain Food Additives and Contaminants.* 31st Meeting of the Joint FAO/WHO Expert Committee on Food Additives, Feb. 16-25, 1987, Geneva, Switz. (WHO Technical Report Series, no 759). Geneva, Switz.: World Health Organization (WHO), pp. 31-32, 49. Available at: http://whqlibdoc.who.int/trs/WHO_TRS_759.pdf.

Kishimoto Y, Kanahori S, Sakano K, Ebihara S (2013). The maximum single dose of resistant maltodextrin that does not cause diarrhea in humans. J Nutr Sci Vitaminol (Tokyo) 59(4):352-357.

Kondo T, Handa K, Genda T, Hino S, Hamaguchi N, Morita T (2016). *Resistant Dextrin Derivatives are Moderately Digested in the Small Intestine and Their Energy Contribution Would be Greater Than in the Large Bowel Fermentation [Confidential].* Shizuoka City, Japan: Shizuoka University, College of Agriculture, Academic Institute.

Livesey G (2001). Tolerance of low-digestible carbohydrates: a general view. Br J Nutr 85(Suppl. 1):S7-S16.

Pasman W, Wils D, Saniez MH, Kardinaal A (2006). Long-term gastrointestinal tolerance of NUTRIOSE(®) FB in healthy men. Eur J Clin Nutr 60(8):1024-1034.

SCF (1992). Report of the Scientific Committee for Food on a second series of food additives of various technological functions (Opinion expressed 19 October 1990) [Calcium disodium EDTA]. In: Report of the Scientific Committee for Food on a second series of food additives of various technological functions (Opinion expressed 19 October 1990). In: *Food Science and Techniques*. (Reports of the Scientific Committee for Food, Fourth Series). Brussels, Belgium: Commission of the European Communities (EEC), Scientific Committee for Food (SCF), pp. 1-19. Available at: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_26.pdf.

U.S. FDA (2013). *Agency Response Letter GRAS Notice No. GRN 000436 [Enzyme-modified dextrins, Lestrem, France: Roquette Frères]*. Silver Spring (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=436 [Jan 18, 2013].

U.S. FDA (2016a). Part 170—Food additives. §170.3—Definitions. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration).* Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

U.S. FDA (2016b). Part 172—Food additives permitted for direct addition to food for human consumption. §172.841—Polydextrose. In: *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs* (U.S. Food and Drug Administration). Washington (DC): U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

van den Heuvel EGHM, Wils D, Pasman WJ, Bakker M, Saniez MH, Kardinaal AF (2004). Short-term digestive tolerance of different doses of NUTRIOSE© FB, a food dextrin, in adult men. Eur J Clin Nutr 58(7):1046-1055.

Vermorel M, Coudray C, Wils D, Sinaud S, Tressol JC, Montaurier C et al. (2004). Energy value of a low-digestible carbohydrate, NUTRIOSE® FB, and its impact on magnesium, calcium and zinc apparent absorption and retention in healthy young men. Eur J Nutr 43(6):344-352.

Wisker E, Feldheim W, Pomeranz Y, Meuser F (1985). Dietary fiber in cereals. In: Pomeranz Y, editor. *Advances in Cereal Science and Technology. Vol. VII*. St. Paul (MN): American Association of Cereal Chemists, pp. 169-238.

Attachment 1

Intended Uses and Use Levels for Fit Fiber®

Table 1 Summary of the Individual Food-Uses and Maximum Use-Levels Intended for Fit Fiber® in the U.S.

Food Category	Intended Food-Uses	Maximum Use-Level of Fit Fiber® (% dwb)[a]
Baked Goods and Baking Mixes	Breading and Batter Coatings	6.5
	Cakes	2.9
	Cookies	11.2
	Non-Sweet Baked Goods (breads, rolls, crackers, flour tortillas, pita bread, pizza crust, and English muffins)	6.5
	Wafers	6.5
Beverages and Beverage Bases	Carbonated Beverages, Non-Carbonated Beverages and Dry Beverage Mixes	1.9
Breakfast Cereals	Breakfast Cereals (Ready-to-Eat)	6.5
	Instant/Cooked Cereals	6.5
Chewing Gum	Chewing Gum	4.3
Fats and Oils	Fat Spreads	22.3
	Mayonnaise	4.3
	Salad dressings	11.2
Frozen Dairy Desserts and Mixes	Ice Cream	2.9
Gelatins, Puddings, and Fillings	Pudding	2.9
	Fillings in baked goods	4.3
Hard Candy	Hard Candy	8.6
Jams and Jellies, commercial	Jam	22.3
	Jelly	8.6
Milk Products	Yogurt	2.2
	Yogurt Drinks[b]	2.2
Nuts and Nut Products	Peanut Butter	4.3
Snack Foods	Snack Chips (Corn, Potato, Rice and Pretzels)	11.2
Soft Candy	Chocolate Confectionery	4.3
	Soft Candy	8.6
Soups and Soup Mixes	Soups	1.9
Sugar Substitutes	Sugar Substitutes	5.8
Dietary Supplements	Dietary Supplements[c]	72 to 93

Abbreviations: dwb = dry weight basis

[a] NSK intends to market liquid and powder formulations of Fit Fiber®, which differ only in their moisture content. Fit Fiber® #80 (liquid concentrate) is standardized to contain at least 72% solids, while the Fit Fiber® #80P (powder) contains at least 93% solids (see Table 3.3-1). As such, the maximum proposed use levels for Fit Fiber® are provided on a dried weight basis in this table.

[b] No food codes for yogurt drinks are available in NHANES. Therefore, food codes for smoothie-type dairy-based drinks were selected as surrogates.

[c] Resistant glucan is proposed for use as a binder, filler, or excipient in dietary supplements. As the amount of Fit Fiber® when used as a formulation aid could theoretically be close to 100%, it is assumed that the dietary supplements contain only Fit Fiber®, which corresponds to maximum levels of 72% on a dried weight basis for the liquid concentrate, and 93% on a dried weight basis for the powder.