NutraSource, Inc.
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December 19, 2017

GRN 000755
RECEIVED
DEC 28 2017
OFFICE OF
FOOD ADDITIVE SAFETY

Dr. P. Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

Subject: GRAS Notice for D-allulose As a Food Ingredient

Dear Dr. Gaynor,

On behalf of SamYang Corp., we are submitting a GRAS notification for D-allulose as a food ingredient. The attached document contains the specific information that addresses the safe human food uses for the notified substance. We believe that this determination and notification are in compliance with Pursuant to 21 C.F.R. Part 170, subpart E.

We enclose an original copy of this notification for your review. Please feel free to contact me if additional information or clarification is needed as you proceed with the review. We would appreciate your kind attention to this matter.

Sincerely,

(b) (6)

12/19/2017

Susan Cho, Ph.D.
Susanscho1@yahoo.com
Agent for SamYang Corp.

Enclosure

GENERALLY RECOGNIZED AS SAFE (GRAS) NOTICE FOR D-ALLULOSE (D-PSICOSE) AS A FOOD INGREDIENT

On behalf of SamYang Corp.
295 Pangyo-ro, Bundang-gu, Seongnam-si
Gyeonggi-do, Republic of Korea

Prepared by: NutraSource, Inc.
6309 Morning Dew Court
Clarksville, MD 21029
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GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS FOR D-ALLULOSE (D-PSICOSE) AS A FOOD INGREDIENT

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PART 1. SIGNED STATEMENTS AND A CERTIFICATION

Pursuant to 21 C.F.R. Part 170, subpart E, SamYang Corp. submits a Generally Recognized as Safe (GRAS) notice and claims that the use of D-allulose in foods, as described in Parts 2 through 7 of this GRAS notice, is not subject to the premarket approval requirements of the FD&C Act based on its conclusion that the substance is GRAS under the conditions of its intended use.

1.A. Name and Address of the Notifier

Contact person: Dr. Chong-Jin Park
Company name: SamYang Corp.

1.B. Common or Trade Name

Common name is D-allulose, D-psicose, or pseudo-fructose.

1.C. Applicable Conditions of Use of the Notified Substance

1.C.1. Foods in Which the Substance is to be Used

Intended use and use levels of SamYang Corp.'s D-allulose are the same as GRN 693 which has been adopted from GRN 498 and GRN 400. SamYang Corp. proposes to use D-allulose as a sugar substitute in selected low calorie, reduced calorie, or sugar-free foods including bakery products; beverages; cereals; chewing gums; confections and frostings; frozen dairy desserts; yogurt and frozen yogurt; dressings for salads; gelatins, pudding and fillings; hard and soft candies; jams and jellies; sugar; sugar substitutes; sweet sauces and syrups; and fat-based creams. Samyang Corp. does not intend to use D-allulose as a component of infant formula or in foods under the USDA's jurisdiction such as meat, poultry, or egg products.

1.C.2. Levels of Use in Such Foods

As shown in Table 1, SamYang Corp. proposes to use D-allulose as a sugar substitute in food applications at use levels ranging from 2 to 100%.

Table 1. Intended Use and Maximum Use Levels of D-Allulose, % (w/w)

Food category	Maximum use levels, % (w/w)
Bakery products (rolls, cakes, pastries, cakes, low calorie or dietetics)	10
Beverages (non-alcoholic), low calorie, reduced calorie, sugar-free	3.5
Cereals, regular	2
Cereals, low calorie, reduced calorie, sugar-free	5
Chewing gum	50
Confections and frostings	5
Frozen dairy desserts (ice cream, soft serve, sorbet), low calorie, reduced calorie, sugar-free	5
Yogurt and frozen yogurt, low calorie, reduced calorie, sugar-free	5

Dressings for salads	5
Gelatins, pudding and fillings, low calorie, reduced calorie, sugar-free	10
Hard candies, low calorie, reduced calorie, sugar-free	50
Soft candies, low calorie, reduced calorie, sugar-free	25
Jams and jellies	10
Sugar	10
Sugar substitutes	100
Sweet sauces and syrups, low calorie, reduced calorie, sugar-free	10
Fat-based cream (used in modified fat/calorie cookies, cakes, pastries, and pie)	5

*Maximum use levels of GRN 693 should have been 10% for bakery products.

1.C.3. Purpose for Which the Substance is Used

The substance will be used as a sugar substitute.

1.C.4. Description of the Population Expected to Consume the Substance

The population expected to consume the substance consists of members of the general population who consume at least one of the products described above.

1.D. Basis for the GRAS Determination: Through scientific procedures.

1.E. Availability of Information

The data and information that serve as the basis for this GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of NutraSource, Inc.

1.F. Availability of FOIA Exemption

None of the data and information in Parts 2 through 7 of this GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. §552.

1.G. Certification

We certify that, to the best of our knowledge, our GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, available and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

(b) (6)

12/15/2017

Name; Chong-Jin Park, Ph.D. Date

Title: Team leader

Please address correspondence to

Susan S. Cho, Ph.D.

NutraSource, Inc., 6309 Morning Dew Ct, Clarksville, MD 21029

Susanscho1@yahoo.com; 410-531-3336 (O); 301-875-6454 (MP)

PART 2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECTS OF THE NOTIFIED SUBSTANCE

2.A. Scientific Information About the Identity of the Notified Substance

2.A.1. Scientific Information Sufficient to Identify the Biological Source

2.A.1.1. Common Name
D-allulose, D-psicose, or pseudo-fructose

2.A.1.2. Chemical Name
D-ribo-2-ketohexose

2.A.1.3. Chemical Abstract Service (CAS) Registry Number
551-68-8

2.A. 1.4. Empirical Formula
Molecular formula, $C_6H_{12}O_6$

2.A.1.5. Molecular Weight
180.16

2.A.1.6. Structural Formula
Figure 1 shows the structure of D-allulose.

Chemical structure of D-allulose is shown in Figure 1.

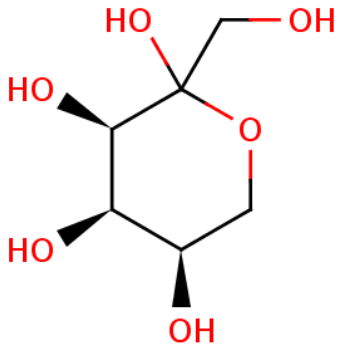

Figure 1. Chemical Structure of D-Allulose

2.A.1.7. Background
D-allulose is a monosaccharide, an epimer of D-fructose isomerized at C-3 (Karabinos, 1952). D-allulose has 70% of the sweetness of sucrose and has a higher solubility that makes it easy to use for food processing. Based on the results of the plot of breath hydrogen concentration vs. calories ingested, the energy value of D-allulose was predicted to be less than 0.2 kcal/g (Iida et al., 2010). Thus, it belongs to the non-digestible carbohydrate category. It is odorless, white or

almost white, and non-hygroscopic. D-allulose is a naturally occurring monosaccharide present in small quantities in food products.

Standards of Identity

In the notice, SamYang Corp. states its intention to use D-allulose in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

2.A.2. Potential Toxicants in the Source of the Notified Substance

No toxicant production is expected in the manufacture of D-allulose. The final product is highly purified through several steps during production. Further, the enzymatic conversion of D-fructose to D-allulose is an enzymatic reaction that occurs in nature, with no known toxicant production.

2.A.3. Particle Size

NLT 90% passes through a 40 mesh screen.

2.B. Method of Manufacture

D-allulose is manufactured from fructose in aqueous solution by enzymatic epimerization in the presence of magnesium chloride. The enzyme used is an immobilized D-allulose-3-epimerase, which converts fructose to D-allulose. Compared to those described in previous GRAS notices, SamYang Corp. employs a unique immobilized enzyme system described below. The enzyme system has been proven safe.

Current notice – non genetically modified organism (non-GMO) production microorganism

The neutralized fructose syrup is passed into an immobilized cell system (calcium alginate gel bead) with non-GMO *Microbacterium foliorum* (non-viable cell) having D-allulose 3-epimerase.

Differences in enzyme systems described in various GRNs

GRN 693 - SamYang Corp.

The neutralized fructose syrup is passed into an immobilized cell system (calcium alginate gel bead) with recombinant *Corynebacterium glutamicum* (non-viable cell) harboring D-allulose 3-epimerase from *Clostridium scindens*.

GRN 400 - CJ CheilJedang

An immobilized cell system (calcium alginate gel bead with recombinant *Corynebacterium glutamicum* (non-viable cell) harboring D-allulose 3-epimerase originated from *Agrobacterium tumefaciens.*

GRN 498 - Matsutani

D-allulose 3-epimerase is from recombinant *Escherichia coli* (K12) (non-viable cell) or *Streptomyces violaceoruber* harboring D-allulose 3-epimerase that originated from *Arthrobacter globiformis or Arthrobacter globiformis* itself.

SamYang's Manufacturing Process

D-allulose is manufactured from fructose in an aqueous solution by enzymatic epimerization in the presence of manganese chloride or calcium chloride.

1. The fructose syrup (≥75% solids concentration) is diluted with clean water (>50% solids concentration) in a reception tank and then stored in a stock tank.
2. The neutralized fructose syrup is passed into an immobilized cell system (calcium alginate gel bead with non-GMO *Microbacterium foliorum* (SYG27B-MF) cell possessing D-allulose-3-epimerase activity. The fructose then is converted to D-allulose at 50℃.
3. For decolorization and desalting, the D-allulose solution is mixed with active carbon in a stirred tank reactor. The liquid undergoes pressure filtration to clarify it, and it is treated through an ion exchange process (i.e., a cation column with strongly acidic cation exchange resin; an anion column with intermediate basic anion exchange resin; and a mixed bed column that has a combination of both strongly acidic and strongly basic resins) to remove any impurities (e.g. calcium, manganese, chloride, and other ionic components, including amino acids, peptides, and proteins).
4. Following ion exchange purification, the D-allulose solution is concentrated with an evaporator to produce allulose syrup ≥10%.
5. This concentrated syrup is pumped into a separation chromatography system to separate D-allulose from other sugars (i.e., fructose).
6. Using an evaporator, the solution is concentrated to the final density of ≥65 ºBx to produce allulose syrup ≥90%.
7. The final concentrated product is pumped into a batch continuous crystallizer.
8. The crystalline D-allulose is separated by basket centrifugation, washed by spraying distilled water, and finally dried in a dryer to give the final D-allulose concentration of ≥98%.

Table 2. List of Raw Materials and Processing Aids

No	Materials	CAS No.
1.	Fructose syrup	57-48-7
2.	Manganese chloride	7773-01-5
3.	Calcium chloride	7440-70-2
4.	Activated carbon	7440-44-0

Quality assurance procedure:

SamYang Corp.'s D-allulose is manufactured under current Good Manufacturing Practices (cGMP) using common food industry materials and processes in accordance with the applicable parts of 21 CFR, part 110 of the Code of Federal Regulations. SamYang Corp. utilizes a Hazard Analysis and Critical Control Point (HACCP)-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications. All processing aids used in the manufacturing process are food grade. Process tanks and lines are cleaned with sodium hydroxide and hydrogen peroxide following standard procedures common to the dairy industry. The ion exchange resins used in the manufacturing process are food grade

and comply with 21 CFR 173.25. A flow diagram of the manufacturing process is presented in Figure 2.

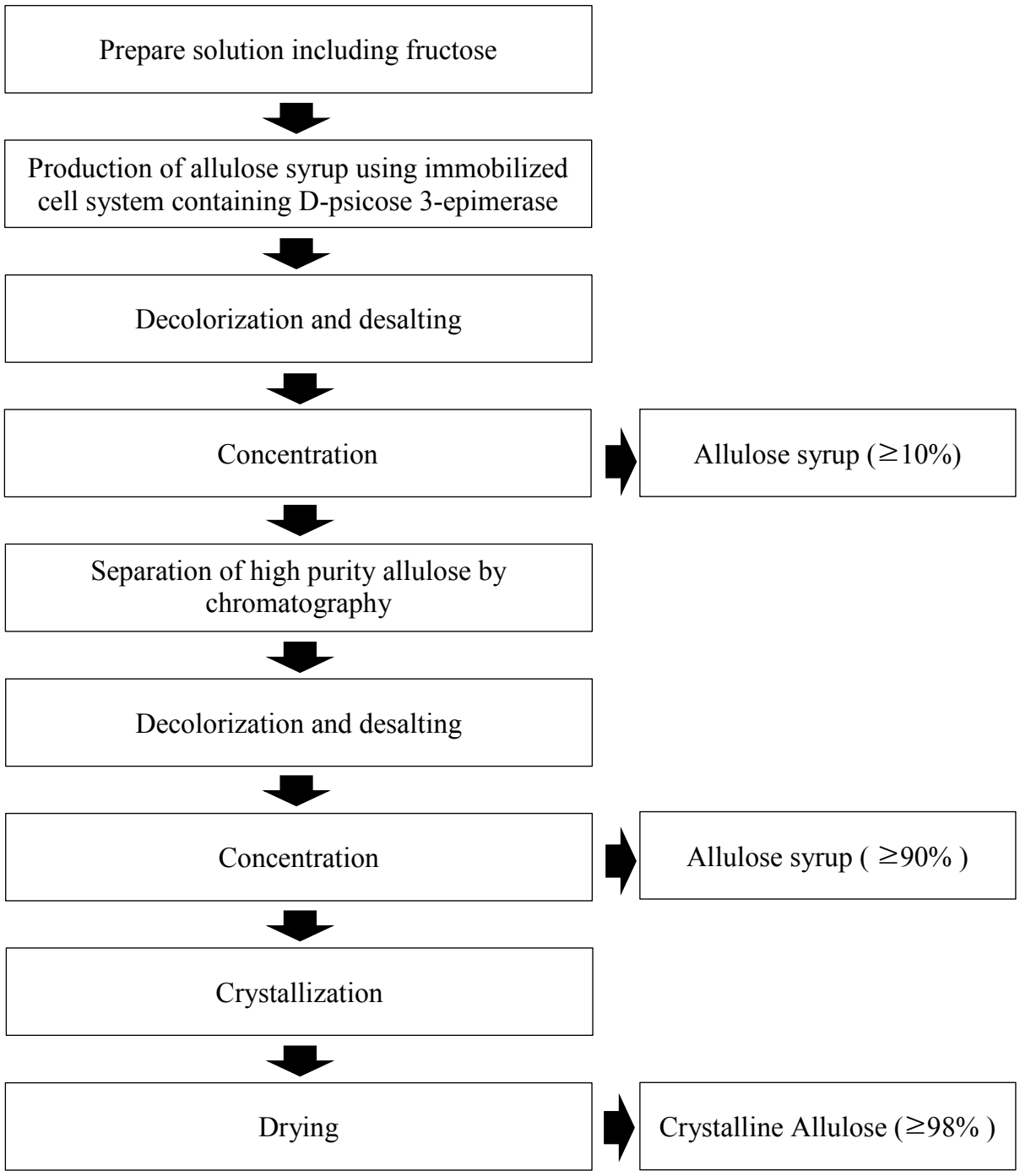

Figure 2. Flow Diagram of Manufacturing Process

2.C. Composition and Specifications of D-Allulose

As shown in Tables 3-1 to 3-3, the only differences in composition and specification are found in the concentrations of D-allulose, excipients (glucose, fructose, and oligosaccharides), and moisture. Specifications for microbial and heavy metal content are the same for powder and liquid forms.

Table 3-1. Specifications and Analytical Values of Product 1 (D-allulose Syrup)

	Specificatio	Analytical	COA	COA	COA
Appearance	Clear yellow liquid	Visual	Clear yellow liquid		
Odor	No odor		No odor	No odor	No odor
Allulose, %*	≥10	HPLC	14.0	13.9	13.8
Fructose,	≤45	HPLC	44.6	44.4	44.3
Glucose, %	≤38	HPLC	36.2	36.5	36.1
Oligosaccharides, %	≤7	HPLC	5.2	5.2	5.8
Moisture	≤35	AOAC 941.14	24.3	24.3	24.3
Brix	≥65	Brix meter	75.7	75.7	75.8
pH	3.0 – 7.0	pH meter	4.5	4.5	4.5
Ash, %, wt/wt	≤0.5	AOAC 900.02	0	0	0.01
Pb, ppm	≤0.3	AOAC 2015.01	0.0039	0.0019	0.0203
As, ppm	≤0.3	AOAC 2015.01	0.0022	0.0019	0.0010
Cd, ppm	≤0.3	AOAC 2015.01	0.0018	0.0013	0.0030
Total plate count, CFU/g	≤1,000	AOAC 2002.07	Negative	10	20
Coliforms	Negative	AOAC 991.14	Negative	Negative	Negative
Salmonella	Negative	AOAC 989.14	Negative	Negative	Negative
Staphylococcus aureus	Negative	AOAC 987.09	Negative	Negative	Negative

*Dry wt. basis, wt/wt; CFU=colony forming units.

Table 3-2. Specifications and Analytical Values of Product 2 (D-allulose Syrup)

Composition	Specification	Analytical Method	COA (1 lot)	COA (2 lot)	COA (3 lot)
Appearance	Clear yellow liquid	Visual	Clear yellow liquid		
Odor	No odor		No odor	No odor	No odor
Allulose*, %	≥90	HPLC	94.7	96.3	96.6
Moisture, %	≤35	AOAC 941.14	25	25	25
Brix	≥65	Brix meter	75	75	75
pH	3.0 – 7.0	pH meter	4.0	4.0	4.0
Ash, %	≤0.5	AOAC 900.02	0.00	0.00	0.00
Pb, ppm	≤0.3	AOAC 2015.01	0.0024	0.0021	0.0028
As, ppm	≤0.3	AOAC 2015.01	0.0011	0.0006	0.0018

Cd, ppm	≤0.3	AOAC 2015.01	0.0022	0.0012	0.0014
Total plate count, CFU/g	≤1,000	AOAC 2002.07	50	100	110
Coliforms	Negative	AOAC 991.14	Negative	Negative	Negative
Salmonella	Negative	AOAC 989.14	Negative	Negative	Negative
Staphylococcus aureus	Negative	AOAC 987.09	Negative	Negative	Negative

*Dry wt. basis, wt/wt; CFU=colony forming units.

Table 3-3. Specifications and Analytical Values of Product 3 (Crystalline D-allulose, ≥98%)

Composition					
Appearance	Powder	Visual	Powder	Powder	Powder
Odor	No odor		No odor	No odor	No odor
Allulose*, %	≥98	HPLC	99.2	99.4	99.3
Moisture, %	≤2	AOAC 941.14	0.15	0.16	0.14
Ash, %	≤0.1	AOAC 900.02	0.00	0.00	0.00
Pb, ppm	≤0.3	AOAC 2015.01	0.0065	0.0054	0.0062
As, ppm	≤0.3	AOAC 2015.01	0.0027	0.0059	0.0017
Cd, ppm	≤0.3	AOAC 2015.01	0.0014	0.0016	0.0011
Total plate count, CFU/g	≤1,000	AOAC 2002.07	150	30	50
Coliforms	Negative	AOAC 991.14	Negative	Negative	Negative
Salmonella	Negative	AOAC 989.14	Negative	Negative	Negative
Staphylococcus aureus	Negative	AOAC 987.09	Negative	Negative	Negative

*Dry wt. basis, wt/wt; CFU=colony forming units.

PART 3. DIETARY EXPOSURE

3.A. Exposure Estimates for D-Allulose Under the Intended Use

The intended use of D-allulose is in the same food products and at the same use levels to those described in GRN 693. Intended use levels of GRN 693 were adopted from GRNs 400 and 498. The results of the EDI assessment are summarized in the two tables below (Tables 4-1 and 4-2). The first table presents the results of the mean of the population as well as the 90th percentile in g/day (Table 4-1), and the second in g/kg bw/day (Table 4-2). The mean and 90th percentile EDIs of all users were 11.0 and 30.0 g/person/day, respectively. All users had the EDIs equal to or below 0.5 g/kg bw/day. These results reveal an average maximum exposure would occur in males greater than 19 years of age, with a 90th percentile value of 36.3 g/day or 0.39 g/kg bw/day. On a body weight basis, children aged 2-12 years had shown the highest 90th percentile EDI at 0.50 g/kg bw/day.

These estimates are highly amplified since it is not likely that D-allulose will be used at maximum levels for all food categories under the intended uses. Also, food wastes should be considered. Overall, intended use will result in EDIs at levels significantly below those associated with any potential side effects.

Table 4-1. Maximum EDIs of D-Allulose, g/day * (Assuming All the Foods will be Used at the Maximum Use Levels)

		Per User (g/day)		Per Capita (g/day)	
Population	N-user*	Mean	90th Percentile	Mean	90th Percentile
U.S. 2+ y	13,455	11.0	30.0	8.6	24.8
Infants < 2 y	536	0.8	2.6	1.7	4.1
Children 2-12 y	3,223	5.2	14.2	4.1	12.0
Adolescents 13-18 y	1,283	7.6	16.7	5.1	14.6
Males 19+ y	4,178	13.0	36.3	9.8	29.0
Females 19+ y	4,771	12.7	32.6	10.0	29.3

* Based on NHANES 2007-2010. U.S.= United States

Table 4-2. Maximum EDIs of D-Allulose, g/kg bw/day (Assuming All the Foods will be Used at the Maximum Use Levels)

		Per User (g/kg bw/day)		Per Capita (g/kg bw/day)	
Population	N-user*	Mean	90th Percentile	Mean	90th Percentile
US 2+ y	13,455	0.16	0.42	0.12	0.35
Infants < 2 y	536	0.08	0.24	0.15	0.42
Children 2-12 y	3,223	0.19	0.50	0.15	0.42
Adolescents 13-18 y	1,283	0.12	0.29	0.08	0.24
Males 19+ y	4,178	0.14	0.39	0.11	0.31
Females 19+ y	4,771	0.16	0.44	0.13	0.38

* Based on NHANES 2007-2010. BW=body weight.

3.B. Food Sources of D-Allulose

As shown in Table 5, D-allulose is a naturally occurring monosaccharide present in small quantities in food products, particularly in selected bakery products, sweets, and fruits (Oshima et al., 2006).

Table 5. D-Allulose Content in Foods (adopted from Oshima et al., 2006)

Item	mg/100 g food
Bakery products	
Sponge cake	11.0
Corn-snack	47.0
Rice cracker	27.3
Cookie	26.7
Brown sugar drop	76.5
Fried dough cake	95.6
Chocolate-chip cookie	6.4
Cereal	2.2
Dishes	
Fish broiled with soy	39.1
Simmered dishes of dried radish strips	8.1
Fermented soybeans	7.8
Seasonings and beverages	
Caramel sauce	83.0
Brown sugar	71.1
Meat sauce	15.8
Demiglace	16.3
Maple syrup	57.9
Ketchup	39.8
Worcester sauce	130.6
Coke	38.3
Coffee	0.5
Fruit juice	21.5
Tomato juice	2.4
Fruits	
Dried fig	29.6
Dried kiwi fruit	9.4
Raisin	38.7
Canned peaches	1.5
Can of mandarin oranges	8.4
Canned cherries	2.0

3.C. Estimated Daily Intakes (EDIs) of Naturally Occurring D-Allulose from the Diet

The D-allulose level in each food is not listed in the USDA food composition tables or the National Health and Nutrition Examination Survey (NHANES) databases. Using the dietary

content of D-allulose available from the studies of Oshima et al. (2006; Table 5), the EDIs from the diet were estimated. The mean and 90th percentile EDIs of users are 94.8 and 260.7 mg D-allulose/person/day. These values are the same as those described in GRN 693. These values are comparable to the EDI value of 206 mg/person/day, which was reported by Oshima et al. (2006) by assuming a daily diet consisting of fruit, cereal, fruit juice, Bolognese spaghetti, crème caramel, coke, hamburger, and fruit cocktail.

3.C.1. EDI of Other Components Under the Intended Use

Two D-allulose syrup products (Products 1 and 2) contain other nutrients such as fructose and glucose. Glucose is subjected to 21CFR 184.1277and 168.120. Fructose (in the form of high fructose corn syrup) is subjected to 21CFR 184.1866. Thus, we have not calculated the EDIs of these nutrients from the diet.

Summary of Consumption Data

Among consumers in the total population, the mean and 90th percentile all-user intakes of D-allulose were determined to be 11.0 and 30.0 g/person/day, respectively, under the intended use when the 2011-2014 NHANES dataset was used for calculation of EDIs. Males older than 19 years of age would have the highest 90th percentile intake among the various age/gender groups, with the 90th percentile value of 36.3 g/person/day in all-users. On a body weight basis, children aged 2-12 years had the highest 90th percentile EDI at 0.5 g/kg bw/day in all-users. Compared to EDIs under the intended use, exposure to D-allulose from the diet is negligible: the mean and 90th percentile EDIs from the diet were estimated to be 94.8 and 260.7 mg D-allulose/person/day in all users.

The EDI assessments are based on the assumption that Samyang Corp.'s D-allulose will replace currently marketed D-allulose. Thus, cumulative exposures are not expected to change. In addition, the EDIs presented in this notice are highly amplified estimates since it is not likely that D-allulose will be used at maximum levels for all food categories under the intended uses. Also, food wastes should be considered. In addition, short-term surveys, such as the typical 2-day dietary surveys, may overestimate the consumption of food products that are consumed relatively infrequently.

PART 4. SELF-LIMITING LEVELS OF USE

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

PART 5. HISTORY OF CONSUMPTION OF THE SUBSTANCE FOR FOOD USE BY A SIGNIFICANT NUMBER OF CONSUMERS (OR ANIMALS IN THE CASE OF ANIMAL FOOD) PRIOR TO JANUARY 1, 1958

Not applicable.

PART 6. BASIS FOR THE CONCLUSION OF GRAS STATUS

6.A. Current Regulatory Status

The FDA has issued 'no question' letters for three GRAS notices related to food uses of D-allulose (GRN 400 submitted by CJ CheilJedang, Inc., 2011; GRN 498 submitted by Matsutani Chemical, 2014; GRN 693 submitted by SamYang Corp). In these GRAS notices, toxicity-related studies on D-allulose from the literature were presented that support the safety of use of D-allulose. The FDA did not question the acceptability and suitability of these studies to establish the safety of D-allulose for the proposed food uses. The FDA did not have questions on the summary of safety, concluding that D-allulose intake of less than 0.5-0.6 g/kg bw/day is safe. Table 6 summarizes previous GRAS notices and the current notice for D-allulose.

Table 6. Summary of Previous and Current GRAS Notices

GRN	Company	Production microorganism harboring enzyme*	Intended use	EDI, 90th pctl for all users
Current notice	Samyang Corp.	Non-GMO *Microbacterium foliorum*	As a sugar substitute in food applications at use levels ranging from 2 to 100%.	30 g/person/day or 0.42 g/kg bw/day
693	Samyang Corp.	GMO *Corynebacterium glutamicum*		
400	CJ CehilJedang	GMO *Corynebacterium glutamicum*	As a sugar substitute in foods at use levels ranging from 2 to 10%.	28.5 g/person/day or 0.36 g/kg bw/day
498	Matsutani	GMO *Streptomyces violaceoruber*	As a sugar substitute in food applications at use levels ranging from 2 to 100%.	24.8 g/person/day or 0.33 g/kg bw/day

*Enzyme= D-allulose 3-epimerase; bw= body weight; GRAS= generally recognized as safe; pctl=percentile.

The pertinent information is available as indicated below:
GRN 400: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=400.
GRN 498: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=498.
GRN 693: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=693.

6.B. Intended Technical Effects

D-allulose will be used as a food ingredient for low calorie and/or dietetic foods due to its technological properties (e.g., functions as a sweetener and humectant) and nutritional benefits (such as low calorie and glycemic control).

6.C. Review of Safety Data

As noted above, the FDA has had no question on three GRAS notices related to food uses of D-allulose. The FDA did not have questions on the summary of safety, concluding that D-allulose intake up to 0.5 - 0.6 g/kg bw/day is safe. Since the specifications for the liquid and powder forms of D-allulose in this notice are similar to those described in previous GRAS notices, the metabolism and safety data and other pertinent information discussed in GRN 400, 498, and 693 are applicable to the safety of D-allulose in this GRAS notice. The information is hereby incorporated by reference in these documents and will not be discussed in detail.

We have focused on the review of the literature that has been published since the FDA's review of GRNs 400, 498, and 693 (GRN 400, FDA, 2012; GRN 498, FDA, 2014; GRN 693, FDA, 2017), i.e., the papers published between December 2016 and November 2017. Since FDA's last review in 2017 (GRN 693) covering the papers published until November 2016, one animal toxicity study (Nishii et al., 2017), two animal efficacy studies (Nagata et al., 2017; Nishii et al., 2016b), and one human clinical study (Kimura et al., 2017) were published. Findings from these studies were not inconsistent with the agency's prior decision. In our review, we have excluded the studies that tested a mixture of rare sugars such as a mixture of D-allulose, D-tagatose, D-sorbose, etc.

6.C.1. Metabolism

Since the FDA's review in 2017, no new metabolism study has been published. Following oral administration, D-allulose is partly absorbed in the digestive tract and enters the bloodstream. The maximum blood concentration (48.5±15.6 μg/g) was observed at 1 hour. Excretion via urine was 20% within 1 hour and 33% within 2 hours (Tsukamoto et al., 2014). Accumulation in organs was detected only in the liver. Following intravenous administration, blood concentration of D-allulose was decreased with the half-life of 57 minutes, and the excretion via urine reached almost 50% within 1 hour. Seven days after the single-dose oral administration, the remaining amount in the whole body was less than 1%.

Previously reviewed studies reported that about 98% of intravenously administered D-allulose is excreted in the urine within 6 h (Whistler et al., 1974). When orally ingested, urinary excretion of unchanged D-allulose ranged from 11 to 25% (Matsuo et al., 2003). The data indicate that D-allulose absorbed in the small intestine may pass into the bloodstream and be excreted in the urine without being significantly metabolized (Matsuo et al., 2003). Unabsorbed D-allulose is fermented to short chain fatty acids (SCFA) by intestinal microflora in the colon (Noda and Oh, 1992) or is excreted in the feces (Matsuo, 2004).

6.C.2. Animal Toxicity Studies

Since the FDA's last review of D-allulose in 2016-2017 (GRN 693, U.S. FDA, 2017, respectively), one 90-day safety study in dogs has been published (Nishii et al., 2017). This study reported that D-allulose given at a daily dose of 200 mg/kg bw for 12 weeks caused no harmful effects in dogs. In addition, there is one unpublished study of SamYang Corp's D-allulose produced by using a non-GMO microorganism, *M. foliorum* (Table 7).

6.C.2.1. Toxicity Studies First Reviewed in This GRAS Notice

A Single-Dose Oral Toxicity Study of SamYang Corp's D-Allulose (Using Non-GMO Production Microorganism, *M. foliorum*) in Rats

Kim (2015) evaluated acute toxicity of D-allulose after a single day oral administration in rats. In this study, D-allulose was administered to 5 eight-week old Sprague-Dawley rats by oral gavage at a single day dose of 0 or 5 g/kg bw and observed for 14 days to monitor changes in body weights and clinical signs. At the end of the study, animals were sacrificed and gross necropsy also was done. No animal died during the 14 days observation period and no clinical signs of abnormality were observed at any dose level. Furthermore, no significant differences in mean body weights were found among the test and control groups. No treatment-related abnormalities were observed upon macroscopic examinations. The exception was watery diarrhea which was observed in 4 males and 4 females of the test group within one hour after administration. On the same day, a transient watery diarrhea was observed in all of the animals of the test group within six hours after administration. The researcher concluded that the lethal dose (LD_{50}) of D-allulose was far above 5 g/kg bw, the highest does tested.

Subchronic Safety Study in Dogs

Nishii et al. (2017) studied the safety and biological effects of D-allulose in healthy dogs. For 12 weeks, the dogs were administered D-allulose (0.2 g/kg bw) or placebo daily. Administration of D-allulose at the dose rate of 200 mg/kg/day was well tolerated in dogs. D-Allulose administration did not influence clinical signs, body weight, hematological or blood biochemical indices, except for total cholesterol concentrations which were decreased by 24% after 12 weeks of the test period. Blood biochemical tests included liver function enzymes (alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase), lipid and glucose metabolism profile, urea nitrogen, bilirubin, and electrolytes. Authors concluded that administration of D-allulose caused no harmful effects in dogs.

6.C.2.2. Animal Toxicity Studies Reviewed in Previous GRAS Notices

Previous reviews (GRN 693 - pages 22-23; GRN 498 – pages 11-12; GRN 400 – pages 10-15) included the LD_{50} value of D-allulose in rats at 15.8-16.3 g/kg bw (Matsuo et al., 2002). Subacute toxicity studies (up to 34 days) in rats showed that D-allulose concentration of up to 20% of the diet did not show adverse effects (Table 8; Matsuo et al., 2002). A 90 day subchronic toxicity study reported the no observed adverse effect level (NOAEL) for D-allulose as 3% of the diet, the highest level tested (Matsuo et al., 2012). A 12-18 month chronic toxicity study showed that D-allulose at the dose of 3% D-allulose in the diet (or 1,280 mg/kg bw/day), the highest level tested, did not show adverse effects (Yagi and Matsuo, 2009).

Table 7. Summary of Animal Toxicity Studies

Species	Dosage	Duration	Primary endpoints and NOAEL	Reference
Unpublished Study of SamYang's D-allulose Produced by Non-GMO *M. foliorum*				
Rats, SD	0 and 5 g/kg bw	Single dose, 14 d observation	LD_{50}>>5 g/kg bw	Kim, 2015
Published Study First Reviewed in This GRAS Notice				
Dogs, beagle	0 or 200 mg/kg bw	12 weeks	Clinical biochemistry, hematology, body weight NOAEL- 200 mg/kg bw/d	Nishii et al., 2017
Studies Reviewed in Previous GRAS Notices 400, 498, and 693				
Dogs	1 and 4 g/kg bw	Single dose	Acute toxicity-food intake and selected clinical chemistry	Nishii et al., 2016a
Male rats	8, 11, 14, 17, and 20 g/kg bw (D-allulose in water)	Single dose	Acute toxicity- LD_{50}, 16.3 g/kg bw	Matsuo et al., 2002
Young rats	10, 20, 30, and 40% in the diet	34 days	Feed intake, wt. gain, and organ wt.; NOAEL-up to 20% in the diet (corresponding to 10,000 mg/kg bw/day)	Matsuo et al., 2002
Male Wistar rats	3% in the diet	90 days	Feed intake, wt gain, organ wt., serum biochemistry, hematology, and histology; NOAEL- 3% in diet, the highest level tested	Matsuo et al., 2012
36 Male rats, Wistar	3% in the diet or 1,280 mg/kg bw/d (control, 3% sucrose)	12-18 months	Feed and energy intakes, wt. gain, organ wt., digestive tract size, serum biochemistry, hematology, and histology; NOAEL- 1,280 mg/kg bw/day, the highest level tested	Yagi and Matsuo, 2009

bw= body weight; NOAEL= no observed adverse effect level; wt= weight.

Conclusion: Based on these studies, for purposes of this evaluation, a NOAEL of over 1,280 mg/kg bw/day, the highest level tested, was chosen for D-allulose. D-allulose, like other monosaccharides, belongs to the group that has the lowest toxicity rating and is classified as an ordinary carbohydrate substance. Thus, the use of D-allulose in foods and beverages is not expected to pose a safety concern.

6.C.3. Animal Efficacy Studies Reporting No Adverse Effects of D-Allulose

Since the FDA's last review of D-allulose (GRNs 400, 498, and 693; U.S. FDA, 2012 2014, and 2017, respectively), two animal efficacy studies (Nishii et al., 2016b; Ochiai et al.,

2017) were published based on the repeat dose administration of D-allulose at high dietary concentrations for long durations (Table 9). No studies reported results inconsistent with the FDA's prior reviews of 2012-2017. Although these studies were designed to investigate the efficacy of D-allulose on various health parameters, several safety-related endpoints were obtained during the experiments. Therefore, these studies are reviewed below as additional supporting information.

6.C.3.1. Animal Efficacy Studies First Reviewed in This GRAS Notice

The following two animal efficacy studies were published between December 2016 and November 2017. In our review, we have excluded the studies that tested a mixture of rare sugars such as D-allulose, D-tagatose, D-sorbose, etc.

Nagata et al. (2017) determined if and how D-allulose modulates lipid metabolism in rats. After feeding D-allulose (3% of diet for 4 weeks) to rats, lipid metabolism outcomes were determined. No diet-related effects were observed on body weight or food intake. D-alluose lowered lipogenic enzyme activity by 30-50% and non-significantly decreased fecal fatty acid excretion by 22%.

Nishii et al. (2016b) evaluated the effects of D-allulose on glucose metabolism in healthy dogs. A single dose of D-allulose at a dose of 0.2 g/kg bw was orally administered to dogs after an overnight fast. Blood samples were taken from the cephalic vein before and 30, 60, 90, and 120 min after administration of sugars for measurements of glucose and insulin concentrations. No adverse effects of D-alluose were reported on measured outcomes.

6.C.3.2. Animal Efficacy Studies Reviewed in Previous GRAS Notices

Previous GRAS notices (GRN 693 – pages 23-27; GRN 498 – pages 12-14; GRN 400 - pages 15-17) indicated that D-allulose at the level of up to 5% in the diet (corresponding to up to 2,500 mg/kg bw/day) did not cause any adverse effects on food efficiency, glucose metabolism, lipid metabolism, inflammatory biomarkers, body fat accumulation, and/or histopatholgical parameters (Baek et al., 2010; Chung et al., 2012a; Han et al., 2016; Hossain et al., 2012, 2015; Itoh et al., 2015; Matsuo et al., 2001a, 2001b; Matsuo and Izumori, 2004, 2006, 2009; Nagata et al., 2015; Nishi et al., 2016 b; Ochiai et al., 2013, 2014).

Animal efficacy studies are summarized in Table 8. None of the animal efficacy studies reported adverse effects of D-allulose. For these 'pivotal' studies, the dose levels represent the maximum doses administered, rather than absolute safety endpoints.

Table 8. Animal Efficacy Studies Reporting No Adverse Effects of D-Allulose

Species	Dosage	Length	Primary endpoints	Reference
Studies First Reviewed in This GRAS Notice				
Rat	3% of diet	4 weeks	Liver enzyme activity (cytosol fatty acid synthase, glucose-6-phosphate dehydrogenase, malic enzyme, microsomal phosphatidate phosphohydrolase, and mitochondrial carnitine palmitoyltransferase	Nagata et al., 2017

			mitochondrial carnitine palmitoyltransferase), serum and tissue biochemical analysis (insulin, leptin, lipid profile, and free fatty acid oxidation), fecal lipids, and gene expression of enzymes and protein involved in lipid metabolism	
Dogs	0.2 g/kg bw	Single dose	Blood glucose and insulin parameters	Nishii et al., 2016b
Studies Reviewed in GRN 693				
Mice	5% of high fat diet	16 weeks	Body weight, plasma concentrations of leptin and resistin, plasma and hepatic levels of lipids, and fecal excretion of lipids	Han et al., 2016
Young male Wistar rats	5% of high sucrose diet or control diet	8 weeks	Feed intake, weight gain, clinical chemistry, energy expenditure, and body fat accumulation	Ochiai et al., 2014
Diabetic rats	5% of diet	60 weeks	Body weight gain, glucose metabolism, inflammatory biomarkers, and abdominal fat deposition	Hossain et al., 2015
Rat, Sprague Dawley	3% of diet	4 weeks	Lipid metabolism (serum and liver lipid levels, liver enzyme activity, and gene expression), body weight	Nagata et al., 2015
Mice (ob/ob and wild type C57BL/6J)	0, 2.5, or 5% of diet	15 weeks	Body and fat weights, liver weights, and hepatic steatosis	Itoh et al., 2015
Studies Referenced in GRNs 400 and 498				
Rat, Sprague-Dawley	5% of high fat diet	8 weeks	Feed intake, weight gain, liver weight, visceral fat mass, blood lipid profile	Chung et al., 2012a
Male Wistar rats	5% of high sucrose diet or high starch diet	8 weeks	Body weight, food intakes, organ weight, serum clinical chemistry, liver triglycerides, carbohydrates and glycogen, and body fat	Ochiai et al., 2013
Diabetic rats	5% of diet	13 weeks	Body weight, glucose metabolism, inflammatory biomarkers, and abdominal fat deposition	Hossain et al., 2012
Male mice	0.2 g/kg bw/d	4 weeks	Glycemic responses, insulin release, and blood lipid profiles, 0.2 g/kg bw/day	Baek et al., 2010
24 Male rats, Wistar	5% in the high (25%) and low fat (5%) diets	16 weeks	Body weight, energy intake, body fat, organ wt., glucose tolerance, serum adipocytokine concentrations (adiponectin, tumor necrosis factor alpha, leptin), and liver glycogen and	Matsuo and Izumori, 2004

			triglycerides.	
Male rat	5% in the diet	3 weeks	Body fat and lipid metabolism	Matsuo et al., 2001a
Male rat	5% in the diet	4 weeks	Body fat and lipid metabolism	Matsuo et al., 2001b
Male rat	5% in the diet	8 weeks	Body fat and glycemic responses	Matsuo and Izumori, 2006
Male rat	2,000 mg/kg bw	Single dose	Body fat and glycemic responses	Matsuo and Izumori, 2009

bw= body weight; d= day

6.C.4. Human Clinical Studies

Since the FDA's last review of D-allulose in 2016-2017 (GRNs 400, 498, and 693, one human study (Kimura et al., 2017) has been published.

6.C.4.1. A Human Clinical Study First Reviewed in This GRAS Notice

Kimura et al. (2017) examined the effects of a single ingestion of d-allulose on postprandial energy metabolism in healthy participants. Thirteen healthy men and women (mean age of 35.7 ± 2.1 years and body mass index 20.9 ± 0.7 kg/m^2) were randomized in a crossover design with a one week washout period. At 30 min after taking a single dose of 5 g D-allulose or 10 mg aspartame without any sugar as a control, overnight-fasted participants ingested a standardized meal, and energy metabolism was evaluated by a breath-by-breath method. During the experiment, blood was collected and biochemical parameters such as plasma glucose were analyzed. No adverse effects of D-allulose were noted on measured outcomes.

6.C.4.2. Human Clinical Studies Reviewed in Previous GRAS Notices

GRNs 693 (page 27), 498 (page 14), and 400 (pages 20-21) reported that several human clinical studies found no adverse effects of D-allulose (Table 9; Hayashi et al., 2010; Iida et al., 2007, 2008, 2010). Like non-digestible oligosaccharides and fiber ingredients, the only known side effect of D-allulose is gastrointestinal discomfort when ingested in large quantities. Even if gastrointestinal discomfort is noted when consumed in large quantities of D-allulose, it is not considered to be of toxicological significance since this type of symptom is usually transient and is often associated with ingestion of non-digestible carbohydrates including dietary fiber (IOM, 2002). A clinical study showed that the maximum tolerable levels in humans were 0.5 g/kg bw/day for males and 0.6 g/kg bw/day for females, with the mean value of 0.55 g/kg bw/day (Iida et al., 2007).

Conclusion: Based on these studies, for purposes of this evaluation, the maximum tolerable levels of 0.5 g/kg bw/day for males and 0.6 g/kg bw/day for females, with the mean value of 0.55 g/kg bw/day, were chosen for D-allulose (Iida et al., 2007). These dosages correspond to 33.0-36.0 g/day for an adult weighing 60 kg.

Table 9. Summary of Human Clinical Studies

Dosage	Length	Measurements	Reference
Study Reviewed the First time in This Notice			
5 g D-allulose (>99% purity)	Single dose	Energy metabolism, blood biochemistry (lipid and glucose metabolism)	Kimura et al., 2017
Studies Referenced in GRNs 400, 498, and 693			
Up to 0.9 g/kg bw/d	6 days	No gastrointestinal symptoms up to 0.5 - 0.6 g/kg bw/d	Iida et al., 2007
15 g/d (5 g in tea, three times a day)	12 weeks	Positive impact on glycemic responses; no adverse effects were noted.	Hayashi et al., 2010
7.5 g in beverage	Single dose	Positive impact on glycemic and insulinemic responses; no adverse effects were noted.	Iida et al., 2008
Up to 340 mg/kg bw in beverage	Single dose	Metabolism study; no adverse effects were noted.	Iida et al., 2010

bw= body weight; d=day

6.C.5. Safety of Production Organism

M. foliorum SYG27B-MF was identified by 16S ribosomal (r) DNA sequence analysis and a morphological examination. Morphology: A Gram-positive, aerobic, light yellow color and rod-shaped bacterium (Figure 3).

Taxonomic Classification of SYG27B-MF:
Kingdom: *Bacteria*
Phylum: *Actinobacteria*
Oder: *Actinomycetales*
Suborder: *Micrococcineae*
Family: *Microbacteriaseae*
Genus: *Microbacterium*
Species: *M. foliorum*

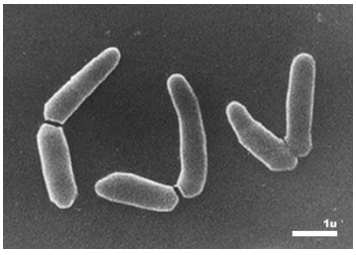

Figure 3. Morphology of *M. foliorum* SYG27B-MF

This strain was subjected to 16S ribosomal RNA sequence analysis. Ribosomal RNA sequence, especially 16S ribosomal RNA, is the best single target for defining phylogenetic relationships among bacteria. This genetic information provides a phylogenetic framework and basis for modern microbial taxonomy (Ludwig and Klenk, 2001). For the delineation of microorganisms at species level, 97% of 16S ribosomal RNA similarity is commonly applied as

conservative threshold in microbial phylogeny. Genomic sequences of the 16S rRNA gene present in *Microbacterium foliorum* SYG27B (SYG27B-MF were compared with those of a reference strain, *Microbacterium foliorum* DSM 12966. It was shown that 16S rRNA genes of both strains have a 99.45% homology in genomic sequences. Based on these findings, the isolated microorganism was named *M. foliorum* SYG27B(SYG27B-MF) and was deposited at the Korean Culture Center of Microorganism (KCCM), the nationally recognized gene bank. Its accession number is KCCM 11774P.

The non-GMO production organism utilized in the manufacture of D-allulose is not mutagenic, genotoxic, or toxic. An acute toxicity study showed that a single dose of 3 g/kg bw did not cause any treatment-related abnormalities in Sprague-Dawley rats. The LD_{50} was determined to be far above 3 g/kg bw. A 90 day subchronic toxicity study determined the NOAEL of *M. foliorum* SYG27B-MF as 2,000 mg/kg bw/day, the highest level tested.

6.C.5.1. A Single-Dose Oral Gavage Toxicity Study of *M. foliorum* SYG27B-MF in Rats

The acute oral toxicities of *M. foliorum* SYG27B-MF were studied in 8-week old Sprague-Dawley (SD) rats (n=5/group) (Lee, 2016a). Test substance was administered by oral gavage at a single dose of 0 or 3 g/kg bw. Animals were observed for fourteen days to monitor changes in body weight and clinical signs. At the end of the study, animals were sacrificed and major organs were macroscopically examined.

No animal died during the 14-day observation period and no abnormal clinical signs were observed at any dose level. No significant difference in mean body weight was found among the test and control groups. No treatment-related abnormalities were observed in macroscopic examinations. The author concluded that lethal dose (LD_{50}) of *M. foliorum* SYG27B-MF was well above 3 g/kg bw, the highest dose tested.

6.C.5.2. Subchronic Toxicity Study of *M. foliorum* SYG27B-MF in Rats

Ninety-day, repeated oral dose studies were conducted to evaluate the oral toxicities of *M. foliorum* SYG27B-MF in male and female SD rats. The test substance was orally administered to 6-week-old Sprague-Dawley rats (10 male and 10 female rats) at a daily dose of 0, 500, 1,000, or 2,000 mg/kg bw for 90 days (Lee, 2016b). Toxicity parameters included general symptoms, body weights, feed intakes, urinalysis, electrolyte, hematology, and blood biochemistry. In addition, eye test, organ weights, gross necropsy examination, and histopathological examination were performed.

None of the animals died during the period of administration, and no treatment-related abnormalities were noted in any parameters tested. Based on the results of the 90-day repeated toxicity test of SYG27B-MF, the NOAEL was determined to be 2,000 mg/kg/day, the highest level tested, in both male and female rats.

6.C.5.3. *In Vivo* Micronucleus Test of *M. foliorum* SYG27B-MF in Mice

M. foliorum SYG27B-MF was tested for its ability to induce micronuclei in polychromatic erythrocytes (PCE) of the bone marrow of treated Imprinting Control Region (ICR) mice according to the OECD Guidelines. The doses used in the study were 0 (solvent control), 500, 1,000, and 2,000 mg/kg bw (Kim, 2016a). The 25 mice, aged 8 weeks (weighing

34.9 ~ 36.8 g), were treated by oral gavage with *M. foliorum* SYG27B-MF dissolved in saline over 2 consecutive days. Mitomycin C (2 mg/kg bw) was administered as a positive control. Animals were observed for clinical signs and mortality for 24 hours post-dosing. All doses were well tolerated and no clinical signs were observed. Bone marrow cells were collected at 24 hours after dosing and evaluated the frequency of micronuclei.

No statistically significant increases in the incidence of micronucleated polychromatic erythrocytes (MNPCE) in PCE were observed in any test substance groups compared with the negative control group. A significant increase in the incidence of MNPCE in PCE was observed in the positive control group compared with the negative control group. Body weights of mice were comparable among the groups before and after the treatment with the test substance. The data suggest that *Microbacterium foliorum* SYG27B-MF did not induce chromosomal aberrations and is non-clastogenic in either the presence or absence of metabolic activation.

6.C.5.4. Bacterial Reverse Mutation Test of *M. foliorum* SYG27B-MF

In vitro bacterial mutagenicity assays were performed to evaluate the mutagenic potential of *Microbacterium foliorum* SYG27B-MF in 4 strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and 1 strain of *E. coli* WP2*uvrA* (pKM101) in the absence and presence of metabolic activation (Kim 2016b). The test item was prepared by suspending in sterile distilled water. For the concentration determination test, 5 different concentrations (0, 61.7, 185, 556, 1,670, 5,000 ug/plate) were set. As a result of the concentration determination test, no increase in the number of reverse mutation colonies or overt cytotoxicity was observed for any of the strains regarding all concentrations.

In the main test, no reproducible increase in the number of colonies was noted in the presence and the absence of a metabolic activator in all five strains (*Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, and *Escherichia coli* WP 2uvrA) (up to 5,000 ug/plate). In addition, no dose-response relationship was observed. The frequency of colony formation by reverse mutation in the positive control group was 2-10 times ($P<0.05$) higher than the solvent control group, in the absence or the presence of a metabolic activation; thus, the conditions in this study were considered appropriate.

The data show that the test substance, *Microbacterium foliorum* SYG27B-MF, was not mutagenic under the conditions used in this study.

6.C.5.5. *In Vitro* Mammalian Chromosome Aberration Test of *M. foliorum* SYG27B-MF

The cytotoxicity of *Microbacterium foliorum* SYG27B-MF and its potential to induce chromosomal aberrations were assessed in Chinese hamster ovarian fibroblasts (CHO-K1 cell) in the presence or absence of metabolic activation. The test substance was prepared by suspending it in sterile distilled water.

In a dose range test, 7 different concentrations (80, 160, 320, 630, 1,250, 2,500, and 5,000 ug/mL) of the test substance were used to measure the inhibition of cell proliferation (as measured by cell number per concentration, and the rate of inhibition of proliferation). The inhibition of cell proliferation by more than 50% was not found in any of the concentrations in the presence and the absence of S9 (rat liver homogenate) at 6 and 24 h. Therefore, in the main

experiment, three test concentrations of 1,250, 2,500, and 5,000 ug/mL were chosen. All three test groups did not show significant differences in the numerical or structural chromosomal anomalies compared to the solvent control. Positive controls (cyclophosphamide monohydrate and Mitomycin C) showed significant differences in numerical and structural chromosomal anomalies; thus, the conditions in this study were considered appropriate.

The author concluded that the test substance, *Microbacterium foliorum* (SYG27B-MF) did not cause numerical or structural chromosomal anomalies in these experimental conditions.

6.D. Safety Determination

Numerous human and animal studies have reported benefits of D-allulose with no major adverse effects. Samyang Corp.'s D-allulose is manufactured under cGMP using common food industry materials and processes. Samyang Corp. uses a HACCP-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications. There is broad-based and widely disseminated knowledge concerning the chemistry of D-allulose. This GRAS determination is based on the data and information generally available and consented opinion about the safety of D-allulose. The literature indicates that D-allulose offers consumers benefits without adverse effects.

The following safety evaluation fully considers the composition, intake, nutritional, microbiological, and toxicological properties of D-allulose as well as appropriate corroborative data.

1. Analytical data from multiple lots indicate that D-allulose complies reliably with the established food-grade product specifications and meets all applicable purity standards.

2. Intended use and use levels are the same as those described in GRN 693. Samyang Corp.'s D-allulose will be used as a sugar substitute and/or as a flavor modifier in food applications at use levels ranging from 2 to 100% in: selected bakery products (rolls, cakes, pastries, cakes, low calorie or dietetics), beverages (non-alcoholic, low or reduced calorie, sugar free); cereals; chewing gums; confections and frostings; frozen dairy desserts (ice cream, soft serve, sorbet; low calorie, reduced calorie, sugar-free); yogurt and frozen yogurt (low calorie, reduced calorie, sugar-free); dressings for salads; gelatins, pudding and fillings (low calorie, reduced calorie, sugar-free); hard and soft candies (low calorie, reduced calorie, sugar-free); jams and jellies; sugar; sugar substitutes; sweet sauces and syrups (low calorie, reduced calorie, sugar-free); and fat based cream.

3. The LD_{50} value of D-allulose in rats is 15.8-16.3 g/kg. A chronic toxicity study in rats showed that D-allulose at a dose of 1,280 mg/kg bw/day, the maximum level tested, did not show adverse effects. A 90 day subchronic toxicity study in rats reported the NOAEL for D-allulose as 3% of the diet, the highest level tested. A 90 day subchronic toxicity study in beagle dogs found that 200 mg/kg bw/day, the highest level tested, was well tolerated with no side effects.

4. An acute toxicity study of SamYang's D-allulose, produced by using a non-GMO production microorganism, was found to be much higher than 5 g/kg bw, the highest level tested.

5. A human clinical study showed that the maximum tolerable levels in humans were 0.5 g/kg bw/day for males and 0.6 g/kg bw/day for females. The only side effect of non-digestible carbohydrates, including D-allulose, is gastrointestinal discomfort when ingested in large quantities. This type of symptom is usually transient and is not considered to be of toxicological significance (IOM, 2002).

6. The proposed food use results in exposure at levels below those associated with any adverse effects. The EDI assessments are based on the assumption that Samyang Corp.'s D-allulose will replace currently marketed D-allulose. Thus, cumulative exposures are not expected. In addition, the EDIs presented in this notice are highly amplified estimates.

7. In the previous GRAS notices (GRNs 400, 498, and 693) to the FDA, the safety of D-allulose has been established in animal toxicity studies and mutagenicity studies, and is further supported by human clinical studies.

8. Safety of the non-GMO production microorganism, *Microbacterium foliorum*, has been fully proven through a battery of toxicity studies. *Microbacterium foliorum* was found to be not mutagenic or genotoxic. In a 90 day subchronic toxicity study in rats, the NOAEL of *Microbacterium foliorum* was determined to be over 2,000 mg/kg bw/day, the highest level tested.

6.E. Conclusions and General Recognition of the Safety of D-allulose

6.E.1. Common Knowledge Element of the GRAS Determination

D-allulose has been safely used as a food ingredient around the world for a decade. As a result, a number of comprehensive reviews of the safety of D-allulose have been published (Chung et al., 2012b). In addition, the FDA has had no question on three GRAS notices related to the safety of D-allulose (GRN 400, FDA 2012; GRN 498, FDA, 2014; GRN 693, FDA, 2017).

6.E.2. Technical Element of the GRAS Determination (Safety Determination)

Numerous human and animal studies have reported benefits of D-allulose with no major adverse effects. Samyang Corp. uses a HACCP-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications and, thus, are manufactured consistent with cGMP for food (21 CFR Part 110 and Part 117 Subpart B). The raw materials and processing aids used in the manufacturing process are food grade and/or commonly used in fermentation and food manufacturing processes. There is broad-based and widely disseminated knowledge concerning the chemistry of D-allulose. The literature indicates that D-allulose offers consumers benefits without adverse effects. In addition, the intended uses of D-allulose have been determined to be safe though scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the "technical" element of the GRAS determination.

SamYang Corp. concluded that these uses of D-allulose, produced using a non-GMO production microorganism, *Microbacterium foliorum*, is GRAS based on scientific procedures, and that other experts qualified to assess the safety of foods and food additives would concur with these conclusions. Therefore, not only is the proposed use of D-allulose safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also Generally Recognized as Safe (GRAS) according to Title 21 Code of Federal Regulations (21 CFR). Recent reviews of the scientific literature revealed no potential adverse health concerns.

6.F. Discussion of Information Inconsistent with GRAS Determination

We are not aware of information that would be considered inconsistent with the finding that the proposed use of D-allulose preparations in foods and beverages, meeting appropriate specifications and used according to cGMP, is GRAS.

PART 7. DATA AND INFORMATION ARE GENERALLY AVAILABLE

7.A. Data and Information are Generally Available

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FDA. 2012. GRN 400. D-psicose, filed by CJ CheilJedang.

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7.B. Data and Information are Not Generally Available

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Kim 2015. A single-dose oral gavage toxicity study of D-allulose in rats

Microbacterium foliorum
Kim MH. 2016a. In vivo mouse micronuceleus test of SYG27B-MF
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