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November 20, 2018

Dr. Paulette 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

#828

Subject: GRAS Notification – D-allulose (D-psicose)

Dear Dr. Gaynor,

On behalf of Samyang Corp., we are submitting a GRAS notification for D-allulose as a food ingredient. The enclosed document provides notice of a claim that the food ingredient, D-allulose described in the enclosed notification is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be generally recognized as safe (GRAS), based on scientific procedures, for addition to foods. 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 and a CD Rom containing electronic files 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,

[redacted]

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

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

On behalf of Samyang Corp.

Prepared by: NutraSource, Inc.
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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.
Address: 295 Pangyo-ro, Bundang-gu, Seongnam-si, Gyeonggi-do, Korea

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

The 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 (rolls, cakes, pastries, cakes, low calorie, or dietetics); non-alcoholic beverages (low- or reduced-calorie, sugar-free); cereals (regular, low- and reduced-calorie, sugar-free); chewing gums; confections and frostings; frozen dairy desserts (ice cream, soft serve, sorbet; low- and reduced-calorie, sugar-free); yogurt and frozen yogurt (low- and reduced-calorie, sugar-free); dressings for salads; gelatins, pudding, and fillings (low- and reduced-calorie, sugar-free); hard and soft candies (low- and reduced-calorie, sugar-free); jams and jellies; sugar; sugar substitutes; sweet sauces and syrups (low- and reduced-calorie, sugar-free); 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- and reduced-calorie, sugar-free	3.5
Cereals, regular	2
Cereals; low- and reduced calorie, sugar-free	5
Chewing gum	50

Confections and frostings	5
Frozen dairy desserts (ice cream, soft serve, sorbet; low- and reduced-calorie, sugar-free)	5
Yogurt and frozen yogurt; low- and reduced-calorie, sugar-free	5
Dressings for salads	5
Gelatins, pudding and fillings; low- and reduced-calorie, sugar-free	10
Hard candies; low- and reduced-calorie, sugar-free	50
Soft candies; low- and reduced-calorie, sugar-free	25
Jams and jellies	10
Sugar	10
Sugar substitutes	100
Sweet sauces and syrups; low- and reduced- calorie, sugar-free	10
Fat-based cream (used in modified fat/calorie cookies, cakes, pastries, and pie)	5

In GRN 693, the level was accidently noted as 10-100%, instead of 10%. EDI calculations were based on 10%.

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.

11/20/2018

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

Title: Vice President

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)

1.I. FSIS/USDA Statement

Samyang does not intend to add D-allulose to any meat and/or poultry products that come under USDA jurisdiction. Therefore, 21 CFR 170.270 does not apply.

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 chemical structure of D-allulose.

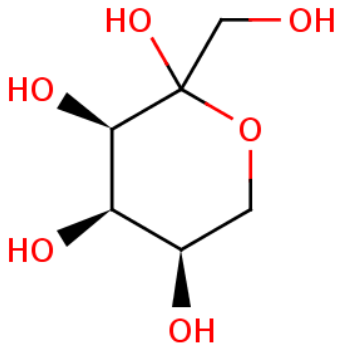


Figure 1. Chemical Structure of D-Allulose

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 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.

Absence of Host Organism and Enzyme Residues

Any residual microorganism used in the immobilized cell system, if there are any, is efficiently removed by the purification step. The absence of the microorganism and residual protein in the ingredient is also supported by the analysis of residual DNA in batches of the final ingredient. The absence of residual DNA from the microorganism is confirmed by validated PCR methods.

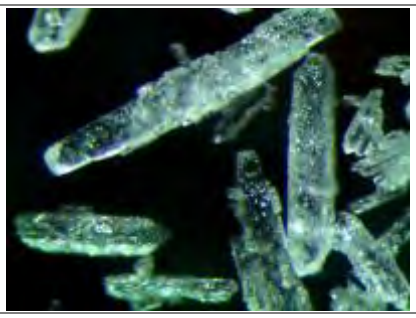
Absence of biogenic amine production

The D-allulose samples were analyzed for residual biogenic amines. Briefly, determination of histamine, putrescine, cadaverine, spermidine, and spermine was carried out by high-performance liquid chromatography (HPLC). No biogenic amines were detected from any of the D-allulose samples.

2.A.3. Particle Size

NLT 90% passes through a 40 mesh screen. Table 2 shows the particle size and shape of D-allulose. Particle size was measured using Mastersizer 2000 (Malvern Panalytical). Samyang's D-allulose has a particle size of 50-500 um.

Table 2. Particle Size and Shape of Samyang's D-allulose

Condition	Sample
Purity (%)	**Allulose 99.7%**
Particle size	**50~500 ㎛**
Crystal particle (x100)	

2.B. Method of Manufacture

D-allulose is manufactured from fructose in an aqueous solution by enzymatic epimerization in the presence of manganese sulfate or magnesium sulfate. The enzyme used is an immobilized *Microbacterium foliorum* harboring 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 sulfate or magnesium sulfate. The enzyme used is an immobilized *Microbacterium foliorum* harboring D-allulose-3-epimerase, which converts D-fructose to D-allulose.

1. The D-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, non-viable *Microbacterium foliorum* (SYG27B-MF) possessing D-allulose-3-epimerase activity. The D-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, magnesium, chloride, sulfate, other ionic components, 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 ≥65ºBx 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 3 presents the list of raw materials and processing aids and their CAS numbers.

Table 3. List of Raw Materials and Processing Aids

No	Materials	CAS No.
1.	Fructose syrup	57-48-7
2.	Manganese sulfate	7785-87-7
3.	Magnesium sulfate	7487-88-9
4.	Activated carbon	7440-44-0

Quality assurance procedure:

Samyang Corp.'s D-allulose is manufactured under Good Manufacturing Practices (GMP) 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 the principles of Hazard Analysis and Critical Control Point (HACCP) in the 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.

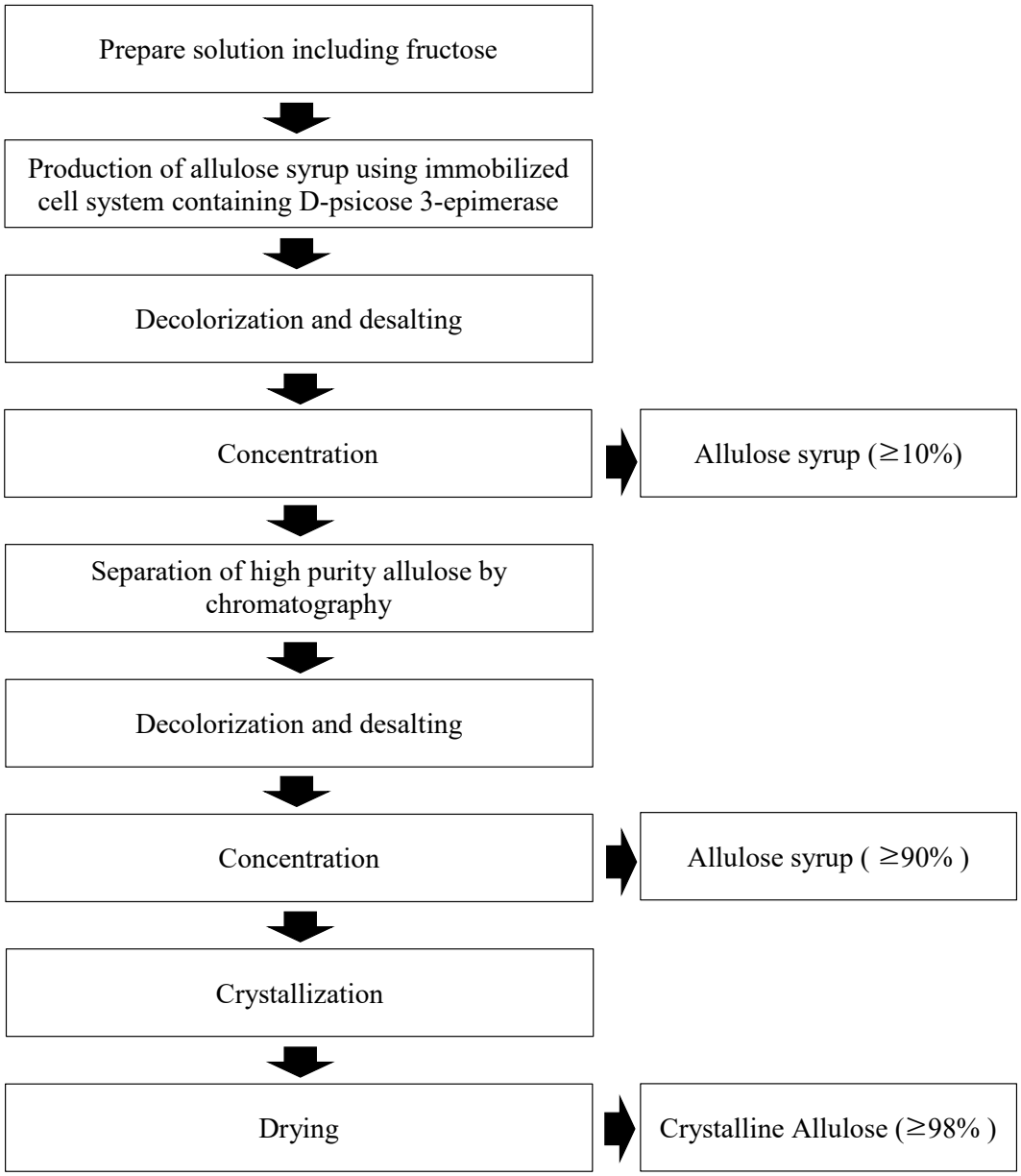


Figure 2. Flow Diagram of Manufacturing Process

2.C. Composition and Specifications of D-Allulose

As shown in Tables 4-1 to 4-3, the only differences in composition and specification are found in the concentrations of D-allulose, other carbohydrates (glucose, fructose, and oligosaccharides), and moisture. The crystalline D-allulose powder is ≥ 98% pure on a dry weight basis, as measured by high performance liquid chromatography (HPLC; Tables 4-1 and 5). Specifications for microbial and heavy metal contents are the same for powder and liquid forms (Table 5). Analysis of 5 non-consecutive batches of D-allulose ingredients demonstrated that the manufacturing process produces consistent products that are in compliance with the established specifications.

Table 4-1. Composition of Product 1 (≥98% Crystalline D-Allulose)

Composition	COA (1 lot)	COA (2 lot)	COA (3 lot)	COA (4 lot)	COA (5 lot)	Mean
Appearance	Powder	Powder	Powder	Powder	Powder	Powder
Odor	No odor	No odor	No odor	No odor	No odor	No odor
Allulose*, %	99.93	99.59	99.80	99.70	99.79	99.76
Moisture, %	0.15	0.16	0.14	0.14	0.15	0.15
Protein, %,	0.12	0.16	0.09	0.08	0.12	0.11
Fat, %	0.14	0.18	0.28	0.08	0.19	0.17
Ash, %	0.00	0.00	0.00	0.00	0.00	0.00
Pb, ppm	0.0065	0.0054	0.0062	0.0134	0.0192	0.0101
As, ppm	0.0027	0.0059	0.0017	0.0030	0.0003	0.0027
Cd, ppm	0.0014	0.0016	0.0011	0.0004	0.0006	0.0010
Total plate count, CFU/g	0	0	0	0	0	0
Coliforms, CFU/g	negative	negative	negative	negative	negative	negative
Salmonella, CFU/25 g	negative	negative	negative	negative	negative	negative
Staphylococcus aureus, CFU/g	negative	negative	negative	negative	negative	negative
Molds & Yeasts, MPN/g	Yeast 0 Mold 0	Yeast 0 Mold 0	Yeast 0 Mold 0	Yeast 0 Mold 0	Yeast 0 Mold 0	negative

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

Table 4-2. Analytical Values of Product 2 (≥90% D-Allulose Syrup)

Composition	COA (1 lot)	COA (2 lot)	COA (3 lot)	COA (4 lot)	COA (5 lot)	Mean
Appearance	Clear yellow liquid	Clear yellow liquid	Clear yellow liquid	Clear yellow liquid	Clear yellow liquid	Clear yellow liquid
Odor	No odor	No odor	No odor	No odor	No odor	No odor
Allulose*, %	93.38	93.12	93.12	97.78	97.26	94.93
Moisture, %	30	30	30	30	30	30

Fructose, %, wt/wt	2.36	2.68	2.68	1.01	1.00	1.95
Glucose, %, wt/wt	0	0	0	0.27	0	0.054
Oligosaccharide, %, wt/wt	0	0	0	0.43	0.32	0.15
Protein, %	0.06	0.06	0.05	0.12	0.10	0.08
Fat, %	0.07	0.17	0.12	0.25	0.09	0.14
Brix	70	70	70	70	70	70
pH	4.0	4.0	4.0	4.0	4.0	4.0
Ash, %	0.00	0.00	0.00	0.00	0.00	0.00
Pb, mg/kg	0.0024	0.0021	0.0028	0.0039	0.0046	0.0032
As, mg/kg	0.0011	0.0006	0.0018	0.0012	0.0009	0.0011
Cd, mg/kg	0.0022	0.0012	0.0014	0.0003	0.0003	0.0011
Total plate count, CFU/g	5	10	0	5	0	4
Coliforms, CFU/g	negative	negative	negative	negative	-	negative
Salmonella, CFU/25 g	negative	negative	negative	negative	negative	negative
Staphylococcus aureus, CFU/g	negative	negative	negative	negative	negative	negative
Molds & Yeasts, MPN/g	Yeast 0 Mold 0	Yeast 0 Mold 0	Yeast 0 Mold 0	Yeast 0 Mold 0	Yeast 0 Mold 0	negative

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

Table 4-3. Composition of Product 3 (≥10% D-Allulose Syrup)

Composition	COA (1 lot)	COA (2 lot)	COA (3 lot)	COA (4 lot)	COA (5 lot)	Mean
Appearance	Clear yellow liquid	Clear yellow liquid	Clear yellow liquid	Clear yellow liquid	Clear yellow liquid	Clear yellow liquid
Odor	No odor	No odor	No odor	No odor	No odor	No odor
Allulose*, %,	15.11	15.74	15.67	14.01	14.56	15.02
Fructose, %,	44.49	42.56	42.43	41.94	42.91	42.87
Glucose, %,	36.2	36.5	36.1	37.4	36.1	36.46
Oligosaccharide, %	5.2	5.2	5.8	6.65	6.43	5.86
Moisture, %	24.3	24.3	24.2	24.2	24.2	24.25
Protein, %	0.05	0.02	0.00	0.20	0.21	0.10
Fat, %	0.22	0.32	0.19	0.27	0.05	0.21
Brix	75.7	75.7	75.8	75.8	75.8	75.8
pH	4.5	4.5	4.5	4.5	4.5	4.5

Ash, %	0.00	0.00	0.01	0.00	0.00	0.00
Pb, mg/kg	0.0039	0.0019	0.0203	0.0079	0.0056	0.0079
As, mg/kg	0.0022	0.0019	0.0010	0.0011	0.0021	0.0017
Cd, mg/kg	0.0018	0.0013	0.0030	0.0003	0.0003	0.0013
Total plate count, CFU/g	5	0	15	5	15	8
Coliforms, CFU/g	negative	negative	negative	negative	negative	negative
Salmonella, CFU/25 g	negative	negative	negative	negative	negative	negative
Staphylococcus aureus, CFU/g	negative	negative	negative	negative	negative	negative
Molds & Yeasts MPN/g	Yeast 0 Mold 0	Yeast 0 Mold 0	Yeast 0 Mold 0	Yeast 0 Mold 0	Yeast 0 Mold 0	negative

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

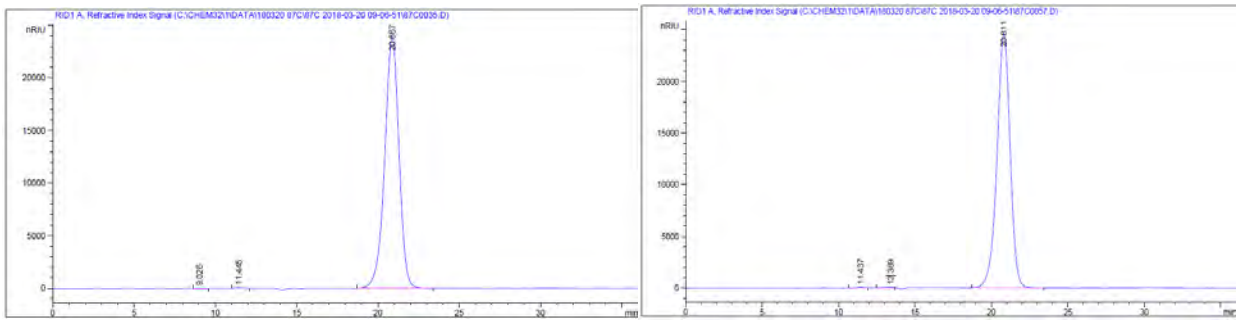
Table 5. Specifications of Crystalline D-Allulose and D-Allulose Syrups

Parameter	≥98% Crystalline D-allulose	≥90% D-allulose syrup	≥10% D-allulose syrup	Analytical Method	Limit of detection
Appearance	White powder	Clear yellow liquid	Clear yellow liquid	Visual	
Odor	No odor	No odor	No odor		
Allulose*, %, wt/wt	≥98	≥90	≥10	HPLC	0.01
Moisture	≤2	≤65	≤35	AOAC 941.14	0.01
Protein, %, wt/wt	≤1	≤1	≤1	AOAC 945.23	0.01
Fat, %, wt/wt	≤1	≤1	≤1	AOAC 920.39	0.01
Brix	-	≥65	≥65	Brix meter	0.1
pH	-	3.0 – 7.0	3.0 – 7.0	pH meter	1~14
Ash, %, wt/wt	≤0.1	≤0.5	≤0.5	AOAC 900.02	0.01
Pb, ppm	≤0.5	≤0.5	≤0.5	AOAC 2015.01	0.0001
As, ppm	≤0.5	≤0.5	≤0.5	AOAC 2015.01	0.0001
Cd, ppm	≤0.5	≤0.5	≤0.5	AOAC 2015.01	0.0001
Total plate count, CFU/g	≤1,000	≤1,000	≤1,000	AOAC 2002.07	1
Coliforms, CFU/g	negative	negative	negative	AOAC 991.14	1
Salmonella, CFU/25 g	negative	negative	negative	AOAC 989.14	1
Staphylococcus aureus, CFU/g	negative	negative	negative	AOAC 987.09	1
Molds & Yeasts, MPN/g	negative	negative	negative	AOAC 997.02	1

*Dry wt. basis; CFU=colony forming units

2.D. Identification Methods

The HPLC analyses confirmed that the D-allulose manufactured by Samyang is chemically and structurally identical to those of the standard D-allulose (Carbosynth). Figure 3 shows HPLC chromatograms of the standard and Samyang's D-allulose. The HPLC analyses were conducted using Refractive index (RI) detector and a mobile system containing deionized water (100%) at a flow rate of 0.6 mL/min, using a Bio-Rad Carbohydrate Amine® HPX-87C, 300 mm×7.8 mm (Catalog #125-0095 or equivalent) at 364 psi (26 kg/cm2)/80℃. Both Carbosynth's standard and Samyang's D-allulose crystalline powder produced identical peaks.



a) Standard b) Samyang's

Figure 3. HPLC Chromatograms: Standard and Samyang's D-allulose

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 as those described in GRN 693. The results of the EDI assessment are summarized in Tables 6-1 and 6-2. Table 6-1 presents the results of the mean of the population as well as the 90th percentile in g/day, and Table 6-2 in g/kg bw/day. The mean and 90th percentile EDIs of all users aged 2 years and older were 11.0 and 30.0 g/person/day, respectively. All users had EDIs equal to or below 0.5 g/kg bw/day. These results reveal an average maximum exposure would occur in males older 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 the maximum levels for all food categories under the intended uses. Also, food wastes should be considered. Overall, the intended use will result in EDIs at levels significantly below those associated with any potential side effects.

Table 6-1. 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 2011-2014. U.S.= United States

Table 6-2. 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 2011-2014. BW=body weight.

3.B. Food Sources of D-Allulose

As shown in Table 7, D-allulose is a naturally occurring monosaccharide present in small quantities in food products, particularly in selected bakery products, sweets, sauces, and fruits (Oshima et al., 2006).

Table 7. 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 8), 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 184.1277 and 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 aged 2 years and older, the mean and 90th percentile of 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 to calculate the 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 the 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 studies on D-allulose from the literature were presented supporting 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 8 summarizes the previous GRAS notices and the current notice for D-allulose.

Table 8. 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 Cheiljedang	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* or *E. coli* K12	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.

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 had no question on three GRAS notices related to the 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, 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 to these documents and will not be discussed in detail.

We have focused the review on 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., papers published between December 2016 and October 2018. Since FDA's last review in 2017 (GRN 693) covering papers published until November 2016, one animal toxicity study (Nishii et al., 2017), four animal efficacy studies (Kim et al., 2017; Hayakawa et al. 2018; Iwasaki et al., 2018; Nagata et al., 2018), and four human clinical studies (Braunstein et al., 2018; Han et al., 2018; Kimura et al., 2017; Noronha et al., 2018) were published. Findings from these studies were consistent with the agency's prior decision. In our review, we have excluded 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 a 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 hours (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), 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 using a non-GMO microorganism, *M. foliorum* (Table 9).

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

In order to examine the acute toxicity of D-allulose, a single dose of 0 or 5 g/kg bw was administered to five 8-week-old (at the onset of administration) male and female Sprague-Dawley rats (Lee, 2017). The death rate, general symptoms, weight changes, and necropsy findings for 14 days after administration were observed, and the results are as follows:

1) No animal death occurred during the 14-day observation period.
2) Watery diarrhea 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.
3) Regarding weight changes, there was no significant difference between the test and control groups of both sexes.
4) No unusual abnormalities were found in necropsies.

The data suggest that the mean lethal dose (LD_{50}) is much higher than 5 g/kg bw, the highest level tested.

Subchronic Safety Study of Another Source of D-allulose 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, and 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 adverse 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 as 15.8-16.3 g/kg bw (Matsuo et al., 2002). An acute toxicity study in dog also reported no adverse effects of D-allulose (Nishii et al., 2016). 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 9; 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% in the diet (or 1,280 mg/kg bw/day), the highest level tested, did not show adverse effects (Yagi and Matsuo, 2009).

Table 9. 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	Lee, 2017
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 the chronic toxicity study (12-18 months), 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), four animal efficacy studies (Kim et al., 2017; Hayakawa et al. 2018; Iwasaki et al., 2018; Nagata et al., 2018) were published based on the repeated dose administration of D-allulose at high dietary concentrations for long durations (Table 10). 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 four animal efficacy studies were published between December 2016 and August 2018 (Table 10). In our review, we have excluded studies that tested a mixture of rare sugars such as D-allulose, D-tagatose, D-sorbose, etc.

Kim et al. (2017) identified target genes in adipose tissue affected by Samyang's D-allulose by transcriptomic analysis and provided mechanistic explanations for the anti-adipogenic effect. C57BL/6J *ob/ob* mice were fed with a control or 5% D-allulose diet for 12 weeks. D-allulose decreased the final body weight (55.77 vs. 50.99 g, $p<0.05$), adipose tissue mass (15.31 vs 13.91% of bw, $p<0.05$), adipocyte size (volume: ~900000 vs. ~610000 μm^3, $p<0.01$), and serum total cholesterol levels (376.93 vs. 293.41 mg/dL, $p<0.05$). The authors suggested that *Fos*, *Mmp3*, *Fgf21*, and *Abcd2* might be the key target genes associated with D-allulose-induced changes in lipid metabolism and subsequent chronic inflammatory responses. No adverse effects of D-allulose were reported.

Hayakawa et al. (2018) examined whether a single oral dose administration of allulose could stimulate glucagon-like peptide 1 (GLP-1) secretion in rats and investigated the underlying mechanism. Oral, but not intraperitoneal, administration of allulose (0.5-2.0 g/kg bw) elevated plasma GLP-1 levels for more than 2 hours in a dose-dependent manner (at 30 min: 14 vs 28 p/M, $p<0.05$; at 60 min: 12 vs 40 p/M, $p<0.05$). No adverse effects of D-allulose were reported.

Iwasaki et al. (2018) examined the effects of D-allulose (single dose) on feeding behavior and glucose metabolism (glucose tolerance test [GTT], insulin tolerance test [ITT], and pyruvate tolerance test [PTT]). The oral administration of D-allulose (D-psicose) induced GLP-1 release, activated vagal afferent signaling, reduces food intake, and promotes glucose tolerance in healthy and obese-diabetic animal models. No adverse effects of D-allulose were reported.

Nagata et al. (2018) determined if and how D-allulose modulates lipid metabolism in rats. After feeding 3% D-allulose diet for 4 weeks to rats, lipid metabolism outcomes were determined. No diet-related effects were observed on body weight or food intake. D-allulose lowered lipogenic enzyme activity by 30-50% and non-significantly decreased fecal fatty acid excretion by 22%. No adverse effects of D-allulose were reported.

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 histopathological parameters (Table 10; 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; Ochiai et al., 2013, 2014).

Animal efficacy studies are summarized in Table 10. 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 the absolute safety endpoints.

Table 10. Animal Efficacy Studies Reporting No Adverse Effects of D-Allulose

Species	Dosage	Length	Primary endpoints	Reference
Studies First Reviewed in This GRAS Notice				
C57BL/6J mice	5% in diet (Samyang's D-allulose)	12 wk	Positive impact on final bw, adipose tissue mass, adipocyte size, and serum total cholesterol concentration	Kim et al., 2017
SD rats	3% in AIN-93G diet	4 wk	Positive impact on hepatic lipogenic enzyme activity; no effect on bw and food intake	Nagata et al., 2018
SD rats	0.5-2.0 g/kg in 10 mL/kg	Single dose	Positive impact on plasma GLP-1	Hayakawa et al., 2018
C57BL/6J mice	0.3, 1, or 3 g/kg bw	Single dose	Positive impact on GLP-1 release, vagal afferent signaling, food intake, and glucose intake	Iwasaki et al., 2018
Studies Reviewed in GRN 693				
Mice	5% of high fat diet	16 wk	Positive impact on bw and fat-pad mass; ↓ plasma leptin and resistin, ↓ plasma and hepatic lipids; ↑ fecal lipids, ↓ hepatic activities of fatty acid synthase and β-oxidation; ↓ fatty acid synthase in white adipose tissue and ↑ β-oxidation activity	Han et al., 2016
Young male Wistar rats	5% of high sucrose diet or control diet	8 wk	Positive impact on serum glucose, leptin, and adiponectin; glucose-6-phosphate dehydrogenase activities in liver and perirenal adipose tissue; and body fat accumulation	Ochiai et al., 2014
Diabetic rats	5% of diet	60 wk	Positive impact on bw gain, HbA_{1c}, pancreatic β-cells, inflammatory markers, and body fat accumulation	Hossain et al., 2015

Rat, Sprague Dawley	3% of diet	4 wk	Positive impact on serum insulin and leptin, hepatic enzyme activities involved in lipogenesis, bw, food intake, energy expenditure, and fat and carbohydrate oxidation	Nagata et al., 2015
Mice (ob/ob and wild type C57BL/6J)	0, 2.5, or 5% of diet	15 wk	Positive impact on body and liver weights, total fat mass (abdominal visceral fat), and hepatic steatosis	Itoh et al., 2015
Studies Referenced in GRNs 400 and 498				
Rat, Sprague-Dawley	5% of high fat diet	8 wk	Positive impact on weight gain, food efficiency ratio, fat accumulation, differentiation of mesenchymal stem cell; no effect on serum cholesterol/HDL-C and LDL-C/HDL-C ratios	Chung et al., 2012a
Male Wistar rats	5% of high sucrose diet or high starch diet	8 wk	Positive impact on food efficiency, carcass fat percentage, abdominal fat accumulation, and bw gain	Ochiai et al., 2013
Diabetic rats	5% of diet	13 wk	Positive impact on progressive β-islet fibrosis, preserved islets, bw, abdominal fat deposition, and blood glucose	Hossain et al., 2012
Male mice	0.2 g/kg bw/d	4 wk	Positive impact on weight gain, glucose tolerance, AUC for glucose, LDL-C/HDL-C ratio, TG, and TC; no effect on serum insulin	Baek et al., 2010
24 Male rats, Wistar	5% in the high (25%) and low fat (5%) diets	16 wk	No effect on glucose tolerance	Matsuo and Izumori, 2004
Male rat	5% in the diet	3 wk	Positive impact on abdominal adipose tissue weight, hepatic fatty acid synthase and glucose-6-phosphate dehydrogenase activities	Matsuo et al., 2001a
Male rat	5% in the diet	4 wk	Positive impact on abdominal adipose tissue weight, hepatic fatty acid synthase and glucose-6-phosphate dehydrogenase activities	Matsuo et al., 2001b
Male rat	5% in the diet	8 wk	Positive impact on plasma glucose induced by sucrose or maltose	Matsuo and Izumori, 2006
Male rat	2,000 mg/kg bw	Single dose	Positive impact on plasma glucose induced by sucrose or maltose	Matsuo and

				Izumori, 2009

bw= body weight; GLP-1= glucagon-like peptide 1; HDL-C=high density lipoprotein cholesterol; LDL-C= low density lipoprotein cholesterol: TC= total cholesterol; TG= triglyceride; wk= weeks.

6.C.4. Human Clinical Studies

Since the FDA's last review of D-allulose in 2016-2017 (GRNs 400, 498, and 693), four human studies (Table 11; Braunstein et al., 2018; Han et al., 2018; Kimura et al., 2017; Noronha et al., 2018) have been published.

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

Han et al. (2018) performed a 12 week intervention preliminary study with 121 adults (aged 20-40 years, BMI ≥ 23 kg/m^2) in a randomized controlled trial (parallel design) involving placebo control (sucralose, 0.012 g x 2 times/day), low D-allulose (4 g x 2 times/day), and high D-allulose (7 g x 2 times/day) groups. Measurements included anthropometric measurements, blood pressure, physical examinations, glucose metabolism indicators (fasting blood glucose, plasma glycosylated hemoglobin [HbA1c]), plasma lipid profiles (total cholesterol, HDL-cholesterol, apolipoprotein A-1 [Apo A-1], and lipoprotein-associated phospholipase A2 [Lp-PLA2]), plasma levels of adiponectin, leptin, insulin, ghrelin, gastric inhibitory polypeptide (GIP), plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor-alpha (TNF- alpha), total bilirubin and monocyte chemoattractant protein 1 (MCP-1), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and gamma-glutamyltransferase (gamma-GTP). No treatment-related adverse events were reported.

Braunstein et al. (2018) conducted an acute randomized, crossover, equivalence trial to assess the effect of small single doses of fructose and allulose on postprandial blood glucose regulation in response to a 75 g-oral glucose tolerance test (75 g-OGTT) in healthy adults. Each participant randomly received six treatments, separated by a minimum one-week washout. Treatments consisted of a 75 g-OGTT with the addition of fructose or allulose at 0 g (control), 5 g or 10 g. Small doses of fructose or allulose did not show a significant effect on plasma glucose incremental area under the curve (iAUC) or other secondary markers of postprandial blood glucose regulation in response to a 75 g-OGTT in healthy individuals. No adverse effects of D-allulose were reported.

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. Additionally, plasma concentrations of glucose, insulin, free fatty acid insulin, total cholesterol, and triacylglycerol were measured. No adverse effects of D-allulose were reported.

Noronha et al. (2018) assessed and compared the effect of small doses of fructose and allulose on postprandial blood glucose regulation in type 2 diabetes. A double-blind, multiple-crossover, randomized, controlled, acute feeding, equivalence trial in 24 participants with type 2 diabetes was conducted. Each participant was randomly assigned six treatments separated by ≥1-week washouts. Treatments consisted of fructose or allulose at 0 g (control), 5 g or 10 g added to a 75 g glucose solution. A standard 75 g oral glucose tolerance test protocol was followed with blood samples at -30, 0, 30, 60, 90, and 120 minutes. There was no effect of fructose at any dose. D-allulose had a positive impact on plasma glucose iAUC, but the values were within specified equivalence margins of ±20%. No adverse effects of D-allulose were reported.

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 11; 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).

Table 11. Summary of Human Clinical Studies

Dosage	Length	Measured Outcomes	Reference
Study Reviewed the First time in This Notice			
8 or 14 g in 30 mL beverage, divided into 2 doses	12 wk	Positive impact on BMI and total abdominal and subcutaneous fat areas; no significant difference in glucose metabolism indicators, plasma lipid profile, markers of liver and kidney function, and major inflammation markers; no treatment-related adverse effects were reported.	Han et al., 2018
0, 5, or 10 g in drinks	Single dose	No significant effect on plasma glucose iAUC or other secondary markers of postprandial blood glucose regulation; no treatment-related adverse effects were reported.	Braunstein et al., 2018
5 g D-allulose (>99% purity)	Single dose	Positive impact on AUC of fat oxidation and of carbohydrate oxidation, plasma glucose, and free fatty acids; no effect on insulin, TC, or TAG; no treatment-related adverse effects were reported.	Kimura et al., 2017

0, 5 or 10 g allulose	Single dose	Positive impact on plasma glucose iAUC, but the values were within specified equivalence margins of ±20%; no treatment-related adverse effects were reported	Noronha et al., 2018
Studies Referenced in GRNs 400, 498, and 693			
Up to 0.9 g/kg bw/d	6 d	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 wk	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

AUC= area under the curve; bw= body weight; d=days; iAUC= incremental area under the curve; TAG= triacylglycerol; TC= total cholesterol; wk= weeks.

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.

6.C.5. Safety of Production Organism

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

In order to examine the death rate and toxicity caused by a single oral dose of SYG27B-MF, the test substance, a single dose of 0 or 3,000 mg/kg was administered to five 8-week-old (at the onset of administration) male and female Specific Pathogen Free (SPF) Sprague-Dawley rats (Kim et al., 2018). Death rate, general symptoms, weight changes, and necropsy findings for 14 days after administration were observed, and the results are as follows:

1) No animal death was observed during the entire test period.
2) No significant differences in body weights were noted between the test and control group in both sexes.
3) No unusual abnormalities were found in the necropsies.

Accordingly, the mean lethal dose (LD_{50}) of rats for the test substance is presumed to exceed 3,000 mg/kg bw.

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

This test evaluated the toxicity of repeated oral administration of the test substance SYG27B-MF (Kim et al., 2018). The test substance was orally administered to 6-week-old SPF Sprague-Dawley rats (10 male and 10 female rats) at a daily dose of 0, 500, 1,000, or 2,000 mg/kg for 90 days. 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 there were no treatment-related abnormalities 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 (corresponding to 3.4 x10^{16} CFU/kg bw/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

This study evaluated the mutagenic potential of *M. foliorum* SYG27B-MF in a micronucleus test using bone marrow cells of ICR mice according to the OECD Guidelines (Kim et al., 2018). The doses used in the study were 0 (solvent control), 500, 1,000, and 2,000 mg/kg bw. 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.

Regarding the comparison of polychromatic erythrocytes (PCE), there was no significant difference between the negative control group and the test group ($p>0.05$). Also, when comparing the average frequency of micronucleated polychromatic erythrocyte (MNPCE) in 2,000 polychromatic erythrocytes per animal, there was no significant difference between the negative control group and the test group, and no dose-response relationship was noted. In this study, the average frequency of micronucleus formation polychromatic erythrocytes was significantly higher in the positive control group compared to the negative control group; thus, the test conditions were appropriate for seeing the frequency of micronucleus formation in the test material. The data suggest that *M. foliorum* SYG27B-MF does not cause formation of micronucleus in mouse bone marrow cells under the test conditions.

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

In order to test the mutagenicity of *M. foliorum* SYG27B-MF, a reverse mutation test was conducted with *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, and *Escherichia coli* WP 2uvrA in the presence or absence of the metabolic activation (Kim et al., 2018). The test item was prepared by suspending it in sterile distilled water. For the concentration determination test, 5 different concentrations (0, 61.7, 185, 556, 1,670, and 5,000 ㎍/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 the metabolic activator in all five strains (*Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, and *Escherichia coli* WP 2uvrA). 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 presence of the metabolic activator; thus, the authors concluded that the test conditions and conclusion were appropriate.

The data show that the test substance, *M. foliorum* SYG27B-MF, did not cause reverse mutation under the conditions used in this study.

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

This study was conducted in Chinese hamster ovarian fibroblasts (CHO-K1 cell) in order to evaluate the genotoxicity of *M. foliorum* SYG27B-MF (OECD Guidelines) (Kim et al., 2018). The test substance was prepared by suspending it in sterile distilled water.

In a dose range finding test, 8 different concentrations (0, 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. Results of the cell proliferation inhibition test showed that the inhibition of cell proliferation by more than 50% was not seen in any of the concentrations, including the highest concentration of 5,000 ug/mL in the presence and absence of S9 (rat liver homogenate) at 6 and 24 hours. 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 a significant difference in the numerical or structural chromosomal anomalies compared to the solvent control. Positive controls showed significant differences in numerical and structural chromosomal anomalies; thus, the conditions in this study were considered appropriate.

The data led to a conclusion that the test substance, *Microbacterium foliorum* SYG27B-MF, does not cause numerical or structural chromosomal anomalies under the test 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 GMP 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, and 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. In addition, no residual production microorganism and biogenic amines were detected from Samyang's D-allulose.

2. The 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.

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 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 GMP 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.

We 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). We are not aware of any information that would be inconsistent with the finding that the proposed use of D-allulose, meeting appropriate specifications, is GRAS. 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, is GRAS.

PART 7. DATA AND INFORMATION ARE GENERALLY AVAILABLE

7.A. Data and Information are Generally Available

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7.B. Data and Information are Not Generally Available

Reports issued by Lee, 2017.
A single-dose oral gavage toxicity study of D-allulose in rats

APPENDIX A. CERTIFICATE OF ANALYSIS

Please see pdf files in the attached.

From: Susan S Cho
To: Bonnette, Richard
Subject: Re: GRN 828 D- psicose or D-allulose
Date: Friday, November 01, 2019 10:33:52 AM

Dear Mr. Bonnette,

Thank you for your review. *Microbacterium foliorum* is not known to be pathogenic. It is present in dairy products and fermented Korean foods (Hone et al., 2012; Quigley, 2011) One species of *Microbacterium, Microbacterium gubbeenense,* is included in the 2012 International Dairy Federation (IDF)/The European Food & Feed Cultures Association (EFFCA) inventory (Bulletin of the IDF, 2012 - page 15). The genus *Microbacterium* has been used to produce fructooligosaccharides (Ohja et al., 2016) and xanthooligosaccharides (Yang et al., 2016).

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I hope this answers your question. Thank you again. Have a nice day!

Sincerely,
Susan
Susan Cho, Ph.D.
NutraSource, Inc. 6309 Morning Dew Ct Clarksville, MD 21029 +1-410-531-3336 (O) +1-301-875-6454 (C)

On Friday, November 1, 2019, 08:30:25 AM EDT, Bonnette, Richard <richard.bonnette@fda.hhs.gov> wrote:

Hello Dr. Cho,

I hope things are going well with you. The review team does have a quick request regarding GRN 828 (D-psicose). For our administrative record, can you please include a brief statement about whether or not the production organism (*Microbacterium foliorum*) is known to be pathogenic? Also, is the organism “banked” in any of the usual repositories (like ECGC)? These are more administrative details that we typically discuss in our response documents and just need to confirm the situation.

Thanks,

Richard

From: Susan S Cho <susanscho1@yahoo.com>
Sent: Tuesday, September 17, 2019 9:26 AM
To: Bonnette, Richard <Richard.Bonnette@fda.hhs.gov>
Subject: GRN 828 D- psicose or D-allulose

Dear Mr. Bonnette,

How are you? I was wondering about the status of D-psicose or D-allulose GRAS notice.
I remember our notice as GRN 828, but my memory may be incorrect. In any case, I would appreciate it if you would let me know the status of D-pdicose GRAS notice. Thank you. Have a nice day!

Sincerely,

Susan

Susan Cho

NutraSource

301-875-6454