GRAS Associates, LLC
11810 Grand Park Ave
Suite 500
North Bethesda, MD 20852
T: 519.341.3667 | F: 888.531.3466
www.gras-associates.com

July 21, 2021

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

Attention: Dr. Susan Carlson
Re: GRAS Notice – *Allulose*

Dear Dr. Carlson:

GRAS Associates, LLC, acting as the Agent for Blue California, is submitting for FDA review Form 3667 and the enclosed CD, free of viruses, containing a GRAS Notice for *Allulose* produced by enzymatic bioconversion from D-fructose. Along with Blue California's determination of safety, an Expert Panel of qualified persons was assembled to assess the composite safety information of the subject substance with the intended use as a sugar substitute/sweetener in a variety of applications detailed in Part 3.A.2 of the GRAS dossier. The proposed uses do not include infant formulas or meat and poultry products. The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

If additional information or clarification is needed as you and your colleagues proceed with the review, please feel free to contact me via telephone or email.

We look forward to your feedback.

Sincerely,

William J. Rowe, President
Agent for Blue California
GRAS Associates, LLC
11810 Grand Park Ave
Suite 500
North Bethesda, MD 20852
wrowe@nutrasource.ca
Enclosure: GRAS Notice for Blue California – *Allulose*

GRAS Notification

of

Allulose

Food Usage Conditions for General Recognition of Safety

on behalf of

Blue California

30111 Tomas
Rancho Santa Margarita, CA 92688

7/21/21

TABLE OF CONTENTS

FIGURES

TABLES

FOREWORD

Blue California based our Generally Recognized as Safe (GRAS) assessment of Allulose, also known as “D-allulose” and “D-psicose”, primarily on the composite safety information, i.e., scientific procedures with corroboration from history of use. The safety/toxicity of Allulose, history of use of Allulose, and compositional details, specifications, and method of preparation of the subject ingredient were reviewed. In addition, a search of the scientific and regulatory literature was conducted through April 2021, with particular attention paid to adverse reports, as well as those that supported conclusions of safety. Those references that were deemed pertinent to this review are listed in Part 7. The composite safety/toxicity studies, in concert with dietary exposure information, ultimately provide the specific scientific foundation for the GRAS conclusion.

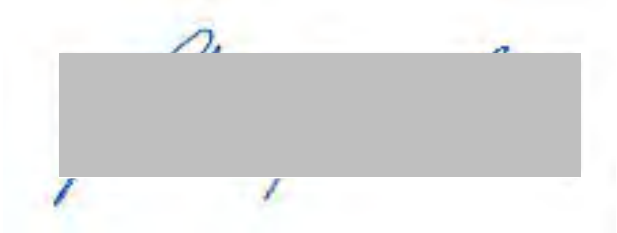
At Blue California’s request, GRAS Associates, LLC (“GA”) convened an Expert Panel to complete an independent safety evaluation of Blue California’s Allulose product. Blue California manufactures Allulose via enzymatic bioconversion from D-fructose using an enzyme from *Escherichia coli* (*E. coli*). The purpose of the evaluation is to ascertain whether Blue California’s Allulose is generally recognized as safe, i.e., GRAS, under the intended conditions of use. In addition, Blue California has asked GA to act as Agent for the submission of this GRAS notification.

PART 1. SIGNED STATEMENTS AND CERTIFICATION

A. Basis of Exclusion from the Requirement for Premarket Approval Pursuant to 21 CFR 170

Blue California has concluded that our Allulose preparation, also referred to as “D-allulose”, and which meets the specifications described below, is GRAS in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic Act (FD&C Act). This determination was made in concert with an appropriately convened panel of experts who are qualified by scientific training and experience. The GRAS determination is based on scientific procedures as described in the following sections. The evaluation accurately reflects the intended conditions of food use for the designated Allulose preparation.

Signed:



[1] See 81 FR 54960, 17 August 2016. Accessible at: https://www.gpo.gov/fdsys/pkg/FR-2016-08-17/pdf/2016-19164.pdf (Accessed 5/20/21)

Agent for Blue California

William J. Rowe
President
GRAS Associates, LLC
11810 Grand Park Ave
Suite 500
North Bethesda, MD 20852

Date: July 21, 2021

B. Name and Address of Responsible Parties

Blue California
30111 Tomas
Rancho Santa Margarita, CA 92688

As the Responsible Party, Blue California accepts responsibility for the GRAS conclusion that has been made for our Allulose ingredient as described in the subject safety evaluation; consequently, the Allulose ingredient having an acceptable composition which meets the conditions described herein, is not subject to premarket approval requirements for food ingredients.

C. Common Name and Identity of Notified Substance

The common name of the ingredient to be used on food labels is “Allulose” and Blue California also plans to market this product as Allulose. Allulose is also known as “D-allulose” and “D-psicose.”

D. Conditions of Intended Use in Food

Blue California’s Allulose is intended for use as a sugar substitute/sweetener in a variety of applications as detailed in Part 3.A.2 Table 5 at levels determined by current good manufacturing practices (CGMP). The proposed uses do not include meat and poultry products or infant formulas.

E. Basis for GRAS Conclusion

Pursuant to 21 CFR 170.30(a) and (b)[2], Blue California’s Allulose preparation has been concluded to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

Allulose is not subject to premarket approval requirements of the FD&C Act based on Blue California’s conclusion that the substance is GRAS under the conditions of its intended food use.

Blue California certifies, to the best of our knowledge, that this GRAS notice is a complete, representative, and balanced assessment that includes all relevant information, both favorable and

[2] See 21 CFR 170.30(a) and (b). Accessible at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=170.30 (Accessed 5/20/21)

unfavorable, available and pertinent to the evaluation of the safety and GRAS status of Allulose. This safety evaluation included a comprehensive search of the literature published through April 2021.

F. Availability of Information

The data and information that serve as the bases for this GRAS Notice will be maintained at the offices of Blue California, located at 30111 Tomas, Rancho Santa Margarita, CA 92688, and will be made available during customary business hours.

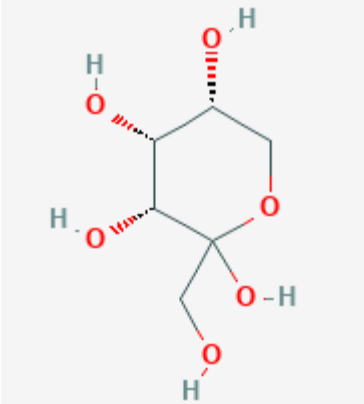
Blue California certifies that no data or information contained herein are exempt from disclosure under the Freedom of Information Act (FOIA). No non-public, safety-related data were used by the Expert Panel to reach a GRAS conclusion.

PART 2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT

A. Chemical Identity of Ingredient

A C-3 epimer of D-fructose, allulose[3] is low in energy and has a sweetness of approximately 70% of that of sucrose (Oshima et al., 2006). Allulose is a ketohexose naturally present at low levels in processed cane and beet molasses (Binkley, 1963), and wheat (Hough and Stacey, 1963; Tsukamoto, 2014). It is also formed from fructose or foods that contain fructose, such as fruit juice, fruit cereal, and Worcestershire sauce during the cooking process (Chung et al., 2012). Trace levels of allulose have been found in human urine (Strecker et al., 1965) and human skin. The structure of allulose is shown in Figure 1.

Figure 1. Structure of Allulose[a]



[a] From PubChem.[4]

[3] The synonyms “allulose”, “psicose”, “D-allulose”, and “D-psicose” are often used interchangeably in the published literature. For consistency, “allulose” will be used throughout this dossier to describe the compound in general and “Allulose” will be used to describe Blue California’s high purity allulose preparation.
[4] Accessible at: https://pubchem.ncbi.nlm.nih.gov/compound/441036#section=2D-Structure (Access date July 13, 2021)

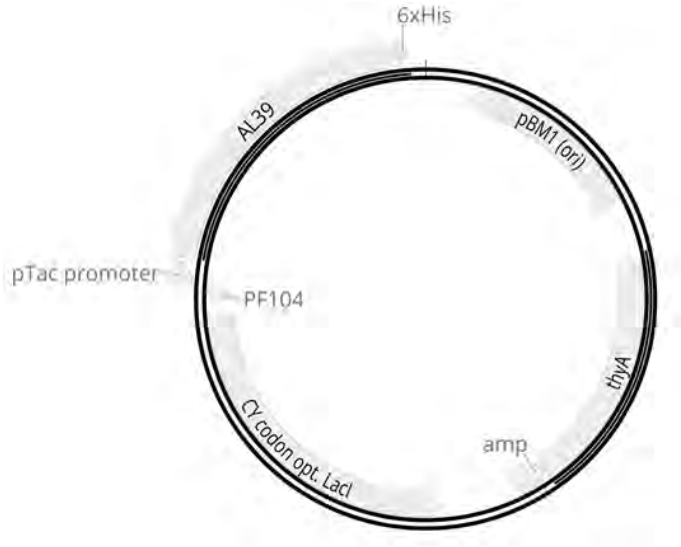
Common or Usual Name:	Allulose
Chemical Name:	D-Ribo-2-hexulose, (hydroxymethyl)tetrahydropyran-2,3,4,5-tetrol
Synonyms:	D-Psicose, D-Allulose, D-Altrulose, D-Pseudofructose, D-Erythro-hexulose, Psicopyranose, D-Psicopyranose, D-Ribo-2-ketohexulose, Psicopyranoside
CAS Number:	551-68-8
Molecular Formula:	$C_6H_{12}O_6$
Molecular Weight (MW):	180.16 g per mole

B. Manufacturing Processes

Blue California manufactures its high purity Allulose in an enzymatic bioconversion process, using D-allulose 3-epimerase (DAE), to produce allulose from D-fructose. The biosynthesis pathway process involves production in and extraction of DAE from *E. coli*, and subsequent bioconversion of D-fructose to allulose by the enzyme.

The fragment coding D-allulose 3-epimerase (also referred to as D-psicose 3-epimerase), derived from *Thermoclostridium caenicola,* with a C-terminal Hisx6 tag fusion enzyme was inserted into *E. coli* expression construct through golden gate cloning strategy. The sequence coding DAE enzyme was expressed under the control of the pTAC promoter. The generated *E. coli* expression construct was transformed into *E. coli* K-12 strain for DAE enzyme production. The expression construct map for the production of DAE is shown in Figure 2.

Figure 2. DAE Expression Construct



Most *E. coli* are harmless and are important components of the healthy human intestinal tract. The microbe is gram-negative, non-spore forming, facultative anaerobe, is nonpathogenic and nontoxigenic, and has a long history of safe industrial use. The *E. coli* K12 strain is the most commonly used industrial strain, and has GRAS status [21 CFR 170.36 (62 FR 18938; April 17, 1997)].

E. coli K12 is not considered a human, animal or plant pathogen, nor is it toxicogenic (EPA, 1997a). It has a history of safe use in the production of specialty chemicals and human drugs and was exempted from the U.S. Environmental Protection Agency (EPA) review under the Toxic Substance Control Act (EPA, 1997b). In addition, *E. coli* K12 derivatives have been used in the production of GRAS notified food ingredients, *e.g.,* α-cyclodextrin (GRN 000155), L-leucine (GRN 000308), and lycopene (GRN 000299). *E. coli* K12 JM109 strain, which is used to manufacture allulose, is expected to be non-pathogenic and non-toxigenic. The production organism is not known to produce any toxic amines (Appendix 1).

The manufacturing process for Blue California's Allulose is summarized in Figure 3.

1. Fermentation Process

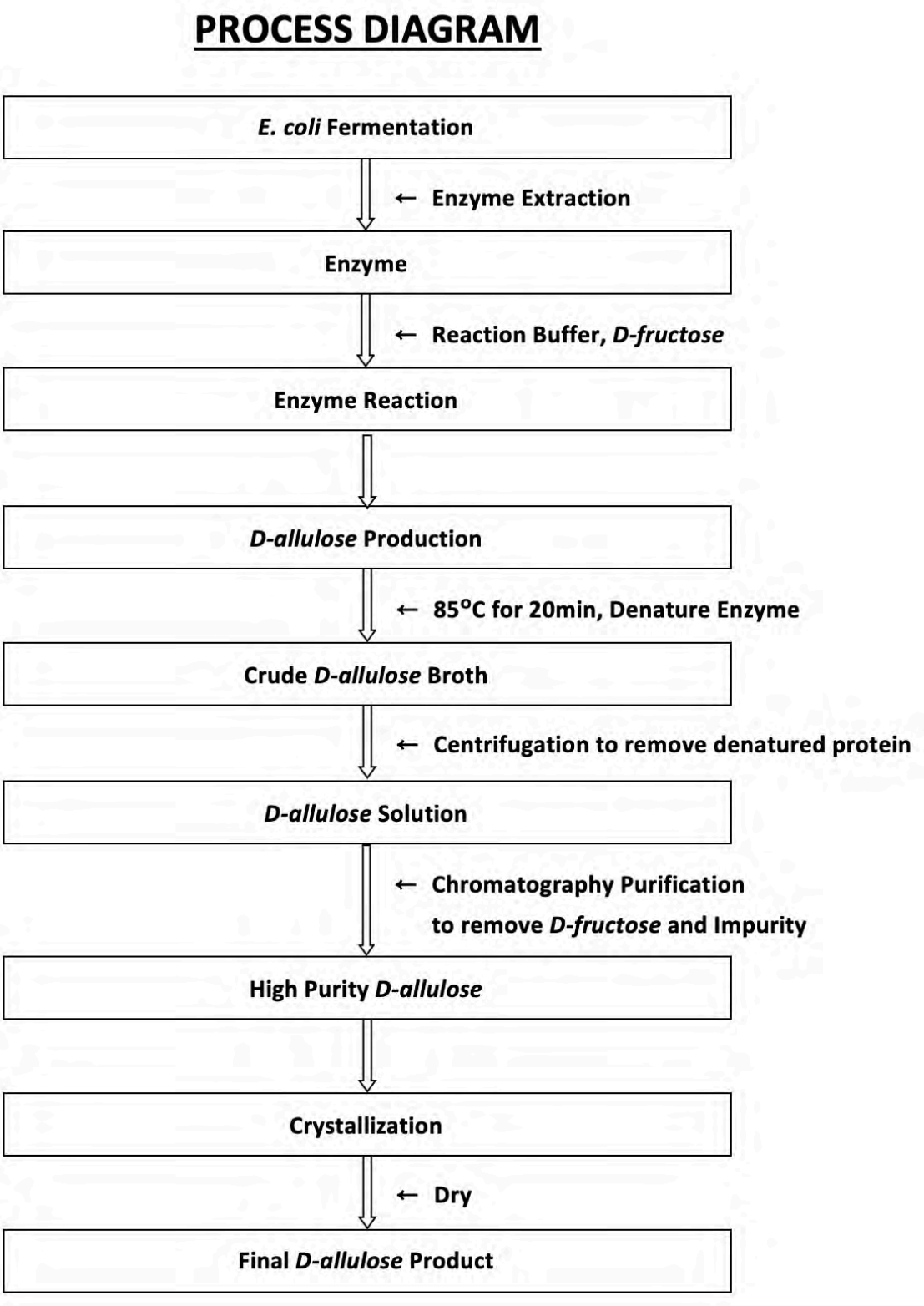
The glycerol stocks of *E. coli* JM109 strain carrying the gene for D-allulose 3-epimerase that converts D-fructose to allulose are removed from a -70°C freezer, thawed to room temperature and grown in 50 mL Luria Broth (LB) culture seed media at 37°C. After 16 hours, the growing Seed Culture 1 is transferred to 2-L LB culture seed media as Seed Culture 2. When the cells read OD_{600} = 5, they are transferred to 500-L fermenters[5]. This level 3 Seed Culture is then transferred to a 5-ton production fermenter.

The *E. coli* cells are cultured for 24 hours and harvested along with the plasmid by centrifugation. The cells are then passed through a homogenizer. The homogenized mixtures are separated by another centrifugation step and the supernatant is passed over a column, which binds the enzymes. The enzymes are subsequently eluted and are ready for bioconversion.

For the catalytic reaction needed to convert D-fructose to allulose, the enzymes are mixed in the reaction buffer in a large 60-ton reaction tank with slow agitation. The D-fructose substrate is fed into the tank and the reaction is allowed to proceed for 12 hours. The reaction mixture is then heated to 85°C for 20 minutes to denature the enzymes in the supernatant, which is then removed for down-stream processing.

[5] Blue California uses older, larger cells to perform the measurement.

Figure 3. Flow Chart of Blue California's Allulose Manufacturing Process



2. Extraction and Purification

The crude allulose broth is centrifuged/filtered to remove denatured protein and the clarified liquid is passed through a column filled with active carbon to remove certain ions and colored materials. The resulting allulose solution is then treated through an ion exchange process with a cationic exchange resin and an anion exchange resin to remove impurities. The treated allulose solution is then subjected to a separation chromatography system to separate allulose from the substrate, fructose. The purified allulose solution is concentrated into a high-purity allulose preparation prior to crystallization from an ethanol/water mixture. The crystalline allulose is subsequently collected and dried in a rotary dryer.

The absence of plasmid in the finished product is confirmed by polymerase chain reaction (PCR) analysis.

All raw materials, processing aids, and additives used to manufacture Blue California's Allulose are food-grade ingredients permitted by U.S. regulations or have previously been determined to be GRAS for their respective uses, as detailed in Appendix 2. Blue California's Allulose is produced in accordance with FDA's CGMP principles. CGMP certification is provided in Appendix 3.

C. Product Specifications

1. Specifications for Allulose

There are no established standardized specifications for allulose per JECFA/FCC/21 CFR. Therefore, specifications for Blue California's Allulose were developed based on the review of specifications for allulose in GRNs 693 and 828, which received "no questions" responses from FDA. The specifications for Blue California's Allulose are compared to those for GRN 693 and 828 in Table 1. Blue California notes that the chemical composition and specifications of our Allulose is of equivalent quality to the allulose preparations described in GRNs 693 and 828. Some parameters established by other manufacturers, such as specifications for protein and fat, do not alter the conclusion that Blue California's Allulose is substantially equivalent to the allulose preparations described in GRNs 693 and 828.

Table 1. Blue California's Specifications for Allulose Compared to Specifications for Allulose in GRNs 693 and 828

Physical and Chemical Parameters	Specifications for Blue California's Allulose		Specifications for Allulose in GRN 693		Specifications for D-Allulose in GRN 828	
	Specification	Method	Specification	Method	Specification	Method
Appearance	Off white to white powder	Visual	Powder	Visual	White Powder	Visual
Odor	Characteristic	Olfactory	No odor	NS	No odor	NS
Foreign Matter	Absent	Visual	NS	NS	NS	NS

Physical and Chemical Parameters	Specifications for Blue California's Allulose		Specifications for Allulose in GRN 693		Specifications for D-Allulose in GRN 828	
	Specification	Method	Specification	Method	Specification	Method
Taste	Characteristic	Gustatory	NS	NS	NS	NS
D-allulose (%wt/wt)	≥97	HPLC	≥98	HPLC	≥98	HPLC
Loss on drying (%)	≤5	USP 34	≤2[a]	AOAC 941.14	≤2[a]	AOAC 941.14
pH	3.0-7.0	USP 34	3.0 - 7.0	pH meter	NS	NS
Protein (% wt/wt)	NS	NS	NS	NS	≤1	AOAC 945.23
Fat (% wt/wt)	NS	NS	NS	NS	≤1	AOAC 920.39
Ash (%wt/wt)	≤0.5%	USP 34	≤0.1	AOAC 900.02	≤0.1	AOAC 900.02
Residual Ethanol	<1,000 ppm	USP 34	NS	NS	NS	NS
Methanol (µg/g)	<200 ppm	USP 34	NS	NS	NS	NS
Heavy Metals (ppm)	<10	USP 34	NS	NS	NS	NS
Lead (ppm)	<0.5	ICP-MS	≤0.5	AOAC 2015.01	≤0.5	AOAC 2015.01
Mercury (ppm)	<0.5	ICP-MS	NS	NS	NS	NS
Cadmium (ppm)	<0.5	ICP-MS	≤0.5	AOAC 2015.01	≤0.5	AOAC 2015.01
Arsenic (ppm)	<0.5	ICP-MS	≤0.5	AOAC 2015.01	≤0.5	AOAC 2015.01
Total Plate Count (CFU/g)	<1,000	AOAC 988.18	≤1,000	AOAC 2002.07	≤1,000	AOAC 2002.07
Total Coliforms (CFU/g)	<100	AOAC 991.14	Negative	AOAC 991.14	Negative	AOAC 991.14
Yeast and Molds	<100 (CFU/g)	AOAC 997.02	NS	NS	Negative (MPN/g)	AOAC 997.02
E. coli (CFU/g)	Negative	AOAC 991.14	NS	NS	NS	NS
Salmonella	Negative	AOAC 2004.3	Negative	AOAC 989.14	Negative (CFU/25 g)	AOAC 989.14
Staphylococcus aureus	NS	NS	Negative	AOAC 987.09	Negative (CFU/g)	AOAC 987.09

[a] as moisture (%wt/wt)
AOAC – Association of Official Agricultural Chemists; CFU – colony forming unit; g – gram; HPLC – high performance liquid chromatography; ICP-MS – inductively coupled plasma-mass spectrometry; mL – milliliter; MPN – Most Probably Number; NS – not specified; ppm – parts per million; µg – microgram; USP – United States Pharmacopoeia; wt – weight

2. Nutritional Profile for Blue California's Allulose

The nutritional profile for Blue California's Allulose is shown in Table 2.

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Table 2. Nutritional Profile for Blue California's Allulose

Attributes	Methods	Results
Protein-Combustion	AOAC 992.23	0.62%
Ash	AOAC 945.46	<0.04%
Calories, Calculated	Atwater Factors	428 kcal/100 g
Carbohydrates (Calculated)	Calculation	93.27%
Crude Fat (By Acid Hydrolysis)	AOAC	5.83%
Moisture (By Vacuum Oven)	AOAC	0.28%

AOAC – Association of Official Agricultural Chemists; g – gram; kcal – kilocalories

3. Specifications for Blue California's Allulose Preparation and Supporting Methods

Results of analyses performed by Blue California demonstrate that five representative, non-consecutive production batches meet the designated specifications, as shown in Table 3. Certificates of Analysis for 5 lots of Blue California's Allulose are found in Appendix 4. Validation Reports and Protein Assay Reports are found in in Appendix 5 and Appendix 6, respectively. The collection of these reports demonstrates that the substance is well-characterized and meets the established purity criteria.

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Table 3. Specifications for Blue California's Allulose

Physical & Chemical Parameters	Blue California's Specifications for Allulose	Representative Lots of Allulose				
		Lot # 833-20180925	Lot # 833-20181109	Lot # 833-20190123	Lot # 833-20190411	Lot # 833-20190617
Appearance	Off white to white powder	Pass	Pass	Pass	Pass	Pass
Odor	Characteristic	Pass	Pass	Pass	Pass	Pass
Foreign Matter	Absent	Pass	Pass	Pass	Pass	Pass
Taste	Characteristic	Pass	Pass	Pass	Pass	Pass
D-Allulose (% wt/wt, dry basis)	97	98	97.8	97.2	99.8	98.9
Loss on Drying (%)	≤5	3.20	3.10	3.10	3.20	3.30
Ash	<0.5	Pass	Pass	Pass	Pass	Pass
pH	3-7	Pass	Pass	Pass	Pass	Pass
Residual Ethanol (ppm)	<1,000	<200	<200	<200	<200	<200
Residual Methanol (ppm)	<200	<100	<100	<100	<100	<100
Heavy Metals (ppm)	<10	Pass	Pass	Pass	Pass	Pass
Arsenic (ppm)	<0.5	<0.02	<0.02	<0.02	<0.02	<0.02
Lead (ppm)	<0.5	<0.02	<0.02	<0.02	<0.02	<0.02
Mercury (ppm)	<0.5	<0.01	<0.01	<0.01	<0.01	<0.01
Cadmium (ppm)	<0.5	<0.01	<0.01	<0.01	<0.01	<0.01
Total Plate Count (CFU/g, max)	≤1,000	<1,000	<1,000	<1,000	<1,000	<1,000
Total Coliform (CFU/g)	<100	<3	<3	<3	<3	<3
Yeast and Molds (CFU/g)	<100	<10	<10	<10	<10	<10
E. coli (in 10 g)	Negative	Negative	Negative	Negative	Negative	Negative
Salmonella spp. (in 25 g)	Negative	Negative	Negative	Negative	Negative	Negative

CFU – colony forming unit; g – gram; ppm – parts per million; wt – weight

D. Physical or Technical Effect

Allulose will be added 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) in conventional foods.

E. Stability

1. Stability Data for Allulose

GRN 893 reported that crystalline allulose was stable for up to 30 months and that liquid allulose is stable under conditions of 4°C, 25°C, and 35°C through the end of the product's shelf life for up to 9 months.

2. Stability Data for Blue California's Allulose

Blue California has conducted an accelerated stability study on our Allulose preparation. The study was conducted on 5 non-consecutive lots of Allulose for 6 months under storage condition of 40°C ± 2°C and 75% ± 5% relative humidity (RH) (See Appendix 7). The data show that Blue California's Allulose is stable under the described conditions.

PART 3. DIETARY EXPOSURE

A. Estimate of Dietary Exposure to Allulose

1. Estimated Background Intake of Allulose from the Diet

Allulose occurs naturally in small amounts in the diet. It is present in bakery products, sweets, and fruits (FDA, 2017b; Oshima et al., 2006). The allulose content in certain foods is listed in Table 4. The mean and 90th percentile Estimated Daily Intakes (EDIs) of naturally occurring allulose reported in GRN 693 were 94.8 and 260.7 mg of allulose per person per day (FDA, 2017b).

Table 4. Occurrence of Allulose in the Diet

Food	mg Allulose/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

Food	mg Allulose/100 g Food
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
Worcestershire sauce	130.6
Coke	38.3
Coffee	0.5
Fruit juice	21.5
Tomato juice	2.4
Fruits	
Dried fig	29.6
Dried kiwi	9.4
Raisin	38.7
Canned peaches	1.5
Canned mandarin oranges	8.4
Canned cherries	2.0

[a] Adapted from Oshima (2006) and FDA (2017b).

2. Estimated Dietary Intakes of Allulose from Intended Use in Foods

Blue California's Allulose preparation is intended to be used as a sweetener in select foods and beverages and is not intended for use in infant formulas or meat and poultry. The amounts of Blue California's Allulose to be added to foods will not exceed the amounts reasonably required to accomplish the intended technical effect in foods. The proposed uses and use levels of allulose described in GRAS Notices that have received "no questions" letters from FDA through May 24, 2021 are compared to the proposed uses and use levels for Blue California's Allulose in Table 5. It should be noted that the intended use for Blue California's Allulose is as a substitute for existent uses of allulose, as well as proposed expanded uses in: grain based cereal and protein bars; low-sugar, reduced-sugar, and diet fruit juices; and low- and reduced-calorie alcoholic beverages.

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Table 5. Proposed Uses and Use Levels of Allulose

Food Category	GRN 400	GRN 498	GRN 693 (w/w)	GRN 828	Blue California's Allulose
Bakery products (rolls, cakes, pies, pastries, and cookies) rolls, cakes, pastries, cakes, low calorie or dietetic	10%	NS	10%*	10%	10%
Beverages (non-alcoholic) low calorie, reduced calorie, sugar-free	2.1%	3.5%	3.5%	3.5%	3.5%
Cereals Regular cereals, low calories, reduced sugar, sugar-free	10% -- --	-- 2% 5%	-- 2% 5%	-- 2% 5%	-- 2% 5%
Chewing gum	50%	50%	50%	50%	50%
Confections and frostings	NS	5%	5%	5%	5%
Frozen dairy desserts (ice cream, soft serve, sorbet: low calorie, reduced calorie, sugar free)	5%	5%	5%	5%	5%
Yogurt (regular and frozen), low calorie, reduced calorie, sugar free	5%	5%	5%	5%	5%
Dressings for salads	NS	5%	5%	5%	5%
Gelatins, pudding, and fillings; low calorie, reduced calorie, sugar free	NS	10%	10%	10%	10%
Hard candies Hard candies (including pressed candy, mints)	70%	50%	50%	50%	50%
Soft candies (non-chocolate, plain chocolate, chocolate coated) (low calorie, reduced calorie, sugar free)	25%	25%	25%	25%	25%
Jams and jellies	NS	10%	10%	10%	10%
Sugar	NS	10%	10%	10%	10%
Sugar substitutes	100%	100%	100%	100%	100%
Sweet sauces and syrups low calorie, reduced calorie and sugar free	NS	10%	10%	10%	10%
Fat based creams	10%	NS	5%	5%	10%
Coffee mix	30%	NS	NS	NS	30%
Grain based cereal bars, protein bars	NS	NS	NS	NS	15%
Fruit Juices (low/reduced sugar, diet, low/reduced kcal only)	NS	NS	NS	NS	5%
Alcoholic beverages (pre-mixed cocktails, wine coolers, and malt beverages) (low/reduced kcal only)	NS	NS	NS	NS	3.5%
Medical foods	15%	NS	NS	NS	NS

* = GRN 828 states that these values were accidentally noted as 10-100% in GRN 693

NS – not specified

B. Estimate of Dietary Exposure to the Substance

1. Estimated Dietary Intakes (EDIs) of Allulose From Intended Use in Foods

It is currently impossible to determine the actual intake levels of allulose from commercial applications as no publicly available consumption data are available. However, this is not a concern since the use of Blue California's Allulose is expected to be a substitute for equivalent products already in the marketplace.

For exposure estimates for allulose under the intended uses, the National Nutrition and Health Examination Survey (NHANES) 2015-2018 dietary data were used after exclusions for pregnant or lactating females and unreliable data. SAS 9.4 along with strata, primary sampling units (PSUs), and day 2 dietary weights were used for analyses.[6]

Intake of allulose was examined for intended use within NHANES food codes. Intake was calculated as the average of day 1 and day 2 intakes. The sample population was limited to subjects with both day 1 and day 2 dietary data. Intake was reported in g per day and in g per kg body weight per day. The estimated mean and 90th percentiles are given for the total population and for consumers only.

The results of the EDI assessment under the intended uses are summarized in Table 6 and Table 7. Table 6 shows the results of the mean and the 90th percentile intakes in g per day and mg per kg body weight (bw) per day for all-users and Table 7 shows the mean and the 90th percentile intakes in g per day and mg per kg bw per day for the total population. The mean and 90th percentile EDIs of all users aged 2 years and older were 8.6 and 19.1 g per person per day, respectively. All users aged 2 to 99 years had EDIs equal to or below 0.30 g per kg bw per day. These results reveal an average maximum exposure would occur in males 19 years of age or older, with a 90th percentile value of 30.4 g per day or 0.33 g per kg bw per day. On a body weight basis, children ages 2-5 years had the highest 90th percentile EDI at 0.50 g per kg bw per day.

Table 6. Maximum EDIs of Allulose Based on NHANES [2015-2018] Survey Data (All Users)

Age/gender group	N of Users	% Users	g/person/day		g/kg bw/day	
			Mean	90th percentile	Mean	90th percentile
2-5 y	936	94.22	3.8±0.2	8.6±0.5	0.22±0.01	0.50±0.03
6-12 y	1,617	93.07	4.9±0.3	11.3±0.8	0.15±0.01	0.34±0.02
13-18 y, M+F	1,171	82.77	4.6±0.3	10.6±0.8	0.07±0.00	0.18±0.01
13-18 y, M	567	80.54	4.6±0.3	11.0±0.7	0.07±0.00	0.18±0.01
13-18 y, F	604	84.99	4.5±0.3	10.1±1.5	0.08±0.01	0.17±0.03
19-99 y, M+F	7,474	90.15	9.8±0.4	22.8±1.9	0.12±0.00	0.27±0.01

[6] The analyses for exposure estimates for allulose under the intended uses were performed on September 14, 2020, by AceOne RS, Inc. The full report is on file at Blue California's offices in Rancho Santa Margarita, California.

Age/gender group	N of Users	% Users	g/person/day		g/kg bw/day	
			Mean	90th percentile	Mean	90th percentile
19-99 y, M	3,568	88.73	12.1±0.6	30.4±1.7	0.14±0.01	0.33±0.03
19-99 y, F	3,906	91.53	7.7±0.3	18.0±0.9	0.10±0.00	0.24±0.01
2-99 y, M+F	11,198	90.02	8.6±0.3	19.1±1.0	0.12±0.00	0.30±0.01

bw – body weight; F – female; g = grams; kg – kilogram; M – male; N – number; y – years

Table 7. Maximum EDIs of Allulose Based on NHANES [2015-2018] Survey Data for the Total Population)

Age/gender group	N of Users	% Users	g/person/day		g/kg bw/day	
			Mean	90th percentile	Mean	90th percentile
2-5 y	999	100	3.6±0.2	8.5±0.4	0.21±0.01	0.50±0.02
6-12 y	1,744	100	4.5±0.2	10.7±0.7	0.14±0.01	0.33±0.02
13-18 y, M+F	1,433	100	3.8±0.2	9.8±0.7	0.06±0.00	0.16±0.01
13-18 y, M	720	100	3.7±0.2	10.1±0.7	0.06±0.00	0.16±0.01
13-18 y, F	713	100	3.8±0.3	9.6±1.1	0.06±0.01	0.16±0.02
19-99 y, M+F	8,374	100	8.9±0.4	21.4±1.7	0.11±0.00	0.26±0.01
19-99 y, M	4,080	100	10.8±0.5	28.3±1.8	0.12±0.01	0.30±0.02
19-99 y, F	4,294	100	7.0±0.3	17.3±1.0	0.09±0.00	0.23±0.01
2-99 y, M+F	12,550	100	7.8±0.3	18.2±0.7	0.11±0.00	0.27±0.01

bw – body weight; F – female; g = grams; kg – kilogram; M – male; N – number; y – years

As reported in GRN 828, the mean and 90th percentile EDI of allulose in the total population aged 2 years and older, based upon the 2011-2014 NHANES dataset, was 11.0 and 30.0 g per person per day. These estimated intakes are higher than the corresponding mean and 90th percentile EDIs determined by Blue California of 8.6 and 19.1 g per person per day, respectively, determined using more recent NHANES survey data. GRN 828 notes that “males older than 19 years of age would have the highest 90th percentile intake among user groups, with the 90th percentile value of 36.3 g per person per day in all-uses.” Blue California’s intake assessment data also indicates that exposure would be greatest for males 19 years of age or older, with a lower estimated 90th percentile value of 30.4 g per person per day. It should be noted that Blue California’s lower EDI estimates were obtained for the intended use as a substitute for existent uses of allulose, as well as proposed expanded uses in: grain based cereal and protein bars; low-sugar, reduced-sugar, and diet fruit juices; and low- and reduced-calorie alcoholic beverages. On a body weight basis, both GRN 828 and Blue California note that children ages 2-5 years have the highest 90th percentile EDI, calculated to be 0.5 g per kg bw per day in both intake assessments.

Furthermore, Blue California's EDI estimates are highly amplified since it is not likely that allulose will be used at the maximum levels for all food categories under the intended uses. 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. Even if some consumers consumed full servings from all categories on a given day, it is highly unlikely that they would do so 365 days per year.

C. Estimated Dietary Exposure to Any Other Substance That is Expected to be Formed in or on Food

This section is not applicable to Blue California's Allulose as it would be chemically stable under the proposed conditions of use.

D. Dietary Exposure to Contaminants, Byproducts, and Bioactives

No concerns regarding dietary exposure to contaminants, byproducts, or bioactives have been raised by FDA upon review of previous GRAS Notices for allulose preparations.

PART 4. SELF-LIMITING LEVELS OF USE

There are no published data on a self-limiting level for allulose.

PART 5. EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

The statutory basis for Blue California's conclusion of GRAS status of Allulose in this document is not based on common use in food before 1958. The GRAS conclusion is based on scientific procedures.

PART 6. NARRATIVE

A. Summary of Regulatory History

1. U.S. Regulatory History

A search of FDA's GRAS Notice Inventory website[7] using the search terms "D-allulose", "D-psicose", "allulose", and "psicose" identified a number of GRAS Notices submitted to FDA. As of May 24, 2021, FDA has filed four GRAS notices relating allulose, which have received "no questions" responses. In addition, three other GRAS notices were filed and subsequently ceased evaluation by FDA at the notifier's request due to submission issues. The GRAS submissions are summarized in Table 8.

[7] GRAS Notice Inventory. Available online at: https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices (accessed on July 13, 2021)

Table 8. Summary of Allulose Submissions in FDA's GRAS Notice Inventory

GRN # / Closure Date	Intended Use	Use Rate	Company (Year)	FDA Response
400 / June 18, 2012	As a sugar substitute in rolls, cakes, pies, pastries, and cookies, dietetic or low calories; chewing gum; fat-based cream used in modified fat/calorie cookies, cakes, and pastries; hard candies, low calorie (including pressed candy, mints); frozen dairy desserts (regular ice cream, soft serve, sorbet), low calorie; carbonated beverages, low calorie; non-carbonated beverages, reduced and low calorie; soft candies, low-calorie (non-chocolate, plain chocolate, chocolate coated); sugar substitutes (carrier); yogurt (regular and frozen), low calorie; medical foods; ready-to-eat cereals (< 5 percent sugar); coffee mix	2.1–100%	CJ Cheiljedang (2011)	FDA has no questions FDA (2012)
498 / June 12, 2014	Chewing gum; confections and frostings; dressings for salads; jams & jellies; sugar; sugar substitutes (carrier), and various low-calorie or dietetic foods including low-calorie, reduced-calorie, sugar-free beverages (non-alcoholic) low calorie, reduced calorie, sugar free; cereals (regular, low calorie, reduced calorie, sugar-free); frozen dairy desserts (ice cream, soft serve, sorbet) low calorie, reduced calorie, sugar-free; yogurt and frozen yogurt, low calorie, reduced calorie, sugar-free; gelatins, pudding and fillings, low calorie, reduced calorie, sugar-free; hard candies, low calorie, reduced calorie, sugar-free; soft candies, ;low calorie, reduced calorie, sugar-free: and sweet sauces & syrups, low calorie, reduced calorie, sugar-free	2–100%	Matustani Chemical Industry Company (2013)	FDA has no questions FDA (2014)
647 / October 11, 2016	Baked products (bread, muffin, cake and cookies), dietetic or low calorie; baked products (pastries); alcohol beverages, reduced calorie; soft drinks, cola type, low or reduced calorie; soft drinks, pepper type, low or reduced calorie; fruit juice drinks, low or reduced calorie; fruit flavored drinks, low or reduced calorie; yogurt, low or reduced calorie; hard candy, low or reduced calorie; soft candy, low or reduced calorie; chocolate, low or reduced calorie; chewing gum; coffee mix; sauce, low or reduced calorie; fat-based cream used in modified fat/calorie cookies, cakes, pastries, pie; sugar substitutes; nutrition bars (meal replacement bars, protein bars, and energy bars); meal replacement shakes, liquid; and medical foods	1 to 100%	Samyang Corporation (2016)	At the notifier's request, FDA ceased to evaluate this notice FDA (2016)
693 / August 28, 2017	Bakery products (rolls, cakes, pastries, cakes, low calorie or dietetics); beverages (non-alcoholic), low calorie, reduced calorie, sugar-free; cereals, regular cereals, low calorie, reduced calorie, sugar-free; chewing gum; confections and frostings; frozen dairy desserts (ice cream, soft serve, sorbet),	2–100%	SamYang Corporation (2017a)	FDA has no questions FDA (2017a)

GRN # / Closure Date	Intended Use	Use Rate	Company (Year)	FDA Response
	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 candies, low calorie, reduced calorie, sugar-free, soft candies, low calorie, reduced calorie, sugar-free; jams and jellies; sugar; sugar substitutes; sweet sauces and syrups, low calorie, reduced calorie, sugar-free; fat based cream (used in modified fat/calorie cookies, cakes, pastries, and pie)			
755 / May 10, 2018	For use as a sugar substitute in bakery products (rolls, cakes, pastries, cakes, low calorie or dietetics); beverages (non-alcoholic), low calorie, reduced calorie, sugar-free; cereals, regular cereals, low calorie, reduced calorie, sugar-free; chewing gum; 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 candies, low calorie, reduced calorie, sugar-free; soft candies, low calorie, reduced calorie, sugar-free; jams and jellies; sugar; sugar substitutes; sweet sauces and syrups, low calorie, reduced calorie, sugar-free; fat-based cream (used in modified fat/calorie cookies cakes, pastries and pies)	2–100%	Samyang Corporation (2017b)	At the notifier's request, FDA ceased to evaluate this notice FDA (2018)
828 / March 2, 2020	Bakery products -rolls, pastries, cakes (low calorie or dietetics); beverages - non-alcoholic (low-and reduced-calorie, sugar-free); cereals, regular; cereals (low-and reduced calorie, sugar-free); chewing gum; 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, puddings and fillings (low and reduced calorie, sugar free); hard candies (low and reduced calorie, sugar free); soft candies (low and reduced calorie, sugar free); jams and jellies; sugar; sugar substitutes, sweet sauces and syrups (low and reduced calorie, sugar free); fat-based cream (used in modified fat/calorie cookies, cakes, pastries and pie)	2–100 percent	Samyang Corporation (2018)	FDA has no questions. (FDA, 2020a)
GRN 893 / June 5, 2020	Intended for use as a sweetener in alcoholic beverages, meat and poultry products, grain-based cereal bars, dried cranberries, and pre-sweetened cereals	At levels ranging from 2 to 25 percent of the finished food	Tate & Lyle (2019)	At the notifier's request, FDA ceased to evaluate this notice. (FDA, 2020b)

In October 2020, FDA issued a guidance document that indicated that they intend to exercise enforcement discretion for excluding allulose from the amount of "Total Sugars" and "Added Sugars" declared on the label and for the use of a general factor of 0.4 calories per gram for allulose to determine "Calories" on the Nutrition and Supplement Facts Labels pending review of the issues in a rulemaking.[8]

The search of FDA's GRAS Notice Inventory using the term "allulose" revealed that GRN 624 for D-allulose 3-epimerase from *Arthrobacter globiformis* M30 produced in *E. coli* received a "no questions" response from FDA.

2. Canadian Regulatory History

Health Canada lists allulose in the Natural Health Products Ingredient Database as a sweetening agent[9]. Allulose is not listed in the list of permitted sweeteners.[10]

3. European Regulatory History

In 2018, an application was submitted by Petiva Europe SA in 2019 to include allulose in the Union list of novel foods. In April 2018, CJ-Tereos Sweeteners Europe SAS, submitted an application to support the approval allulose as a Novel Food Ingredient in the European Union (EU). In 2019, Tate & Lyle Ingredients France SAS, submitted an application to market allulose as a novel food for use as a low-calorie sweetener in food and beverage products in the EU. No updates on the status of these applications were available as of May 2021.[11]

The enzyme D-allulose-3-epimerase from *Arthrobacter globiformis* expressed in *E. coli* was evaluated by the Joint Expert Committee on Food Additives (JECFA). An acceptable daily intake (ADI) was not specified at the 89th JECFA meeting.[12]

4. United Kingdom Regulatory History

No information was returned following a search of the Food Standards Agency website using the terms "allulose" and "psicose."

5. Danish Veterinary and Food Administration (DVFA)

No information was returned following a search of the Danish Veterinary and Food Administration using the terms "allulose" and "psicose."

[8] Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-declaration-allulose-and-calories-allulose-nutrition-and-supplement-facts-labelsl (Accessed May 24, 2021)
[9] Available at: http://webprod.hc-sc.gc.ca/nhpid-bdipsn/pfReq.do?id=85&lang=eng (Accessed May 24, 2021)
[10] Available at: https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/food-additives/lists-permitted/9-sweeteners.html (Accessed May 24, 2021)
[11] Available at: https://ec.europa.eu/food/safety/novel_food/authorisations/summary-applications-and-notifications_en (Accessed May 24, 2021)
[12] Available at: https://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=6508 (Accessed May 24, 2021)

6. Spanish Agency for Food Safety and Nutrition (AESAN)

No information was returned following a search of the Agency for Food Safety and Nutrition's website using the terms "allulose" and "psicose."

7. Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM)

No results were returned following a search of the Norwegian Scientific Committee for Food Safety website using the terms "allulose" and "psicose."

8. Korean Regulatory History

In 2018, the Ministry of Food and Drug Safety of Korea canceled the temporary requirements for allulose. Allulose is now registered in the Korean Food Standards Codex[13].

9. Japanese Regulatory History

Allulose has been available for commercial use in Japan since 2011. In February 2016, the Food Safety Commission of Japan designated allulose as a Food for Specified Health Uses (FS/97/2016). As of January 15, 2021, the Japan Ministry of Health and Welfare's List of Existing Food Additives includes psicose epimerase[14].

10. Australia and New Zealand Regulatory History

No information was identified following a search of the Food Standards Australia New Zealand (FSANZ) website.

B. Discussion of Safety of Allulose

FDA has responded with "no questions" following the submission of four of a total of seven GRAS Notices on allulose that have been submitted to date (FDA, 2012; FDA, 2014; FDA, 2017a; FDA, 2020a). As noted in Part 2.C.1., the chemical composition and specifications for Blue California's Allulose is substantially equivalent to the allulose preparations described in GRNs 693 and 828. Therefore, the safety data discussed in GRNs 693 and 828, as well as the safety data reported and published for allulose in the published literature, are pertinent to the safety conclusion for Blue California's Allulose. The intended uses and intended use levels for Blue California's Allulose are similar to those found GRNs 400, 498, 693, and 828, with additional proposed uses in grain based cereal and protein bars; low-sugar, reduced-sugar, and diet fruit juices; and low- and reduced-calorie alcoholic beverages. As discussed in Part 3.B.1, the expanded proposed uses do not result in EDIs greater than those presented in GRN 828 based on the most recent NHANES datasets. In addition,

[13] Available at: https://mfds.go.kr/eng/brd/m_60/view.do?seq=73753 (Accessed May 25, 2021)
[14] Available at: https://www.ffcr.or.jp/en/tenka/list-of-designated-additives/list-of-designated-additives.html (Accessed June 3, 2021).

FDA conducted a scientific review of the evidence on the metabolism, caloric value, glycemic response and cariogenic potential of allulose.

A literature search covering the time period from 2017 to April 2021 was conducted in PubMed and Google Scholar using the search terms “D-allulose”, “allulose”, “D-psicose”, or “psicose.” These searches identified a reproductive study in rats (Kim et al., 2019), and efficacy study (Ochiai et al., 2017), and multiple new reports on human clinical trials (Noronha et al., 2018a; Tanaka et al., 2020; Tanaka et al., 2019). In addition, GRN 828 cites an unpublished acute toxicity study in male and female Sprague-Dawley rats (FDA, 2020b).

1. Absorption, Distribution, Metabolism & Excretion (ADME) Studies

After oral administration, allulose undergoes absorption in the small intestine and enters the blood stream. According to Tsukamoto et al., 2014, the maximum concentration of allulose in blood of rats was measured at one hour. Urinary excretion was at 20% within one hour and at 33% within three hours. Matsuo (2003) also conducted a study in rats and found that. In addition, a human study reported that allulose was absorbed but was not metabolized (Williamson et al., 2014).

No new studies were identified regarding metabolism of allulose other than those described in previous GRAS Notices; therefore, the metabolism of allulose will not be discussed in detail herein. A few key studies on the metabolism of allulose are summarized below.

a. Animal Studies

A key study by Tsukamoto (2014), discussed in detail in GRN 693, confirms data from earlier studies that, following oral administration in laboratory rats, allulose is absorbed in the small intestine and then rapidly excreted in urine. Radioactive allulose (100 mg per kg bw) was administered orally by gavage and intravenously to male Wistar rats (n=10), and concentrations of allulose in the blood, urine, liver, kidney, lung, thymus, spleen, heart, brain, skin, muscle, stomach, small intestine, cecum, large intestine and the gastrointestinal contents were subsequently determined at 10, 30, 60, and 120 minutes post-administration. Following oral dosing, allulose rapidly entered the bloodstream with the maximum concentration (48.5±15.6 µg per g) observed at one hour after dosing. Excretion in the urine was at 20% within one hour and at 33% within two hours. The liver was the only organ where accumulation was noted. At seven days after a single oral dose, the remaining amounts of allulose observed in the body were less than 1%. Following intravenous administration, blood concentration had a half-life of 57 minutes with the excretion in urine at almost 50% within one hour. As with oral administration, accumulation was only observed in the liver.

Matsuo (2003) investigated the metabolism of allulose in a series of studies. In the first study, 6-week-old male Wistar rats (n=58) were orally administered a single dose of 5 mg per kg bw of allulose. Urine and feces samples were collected at 24-hour intervals for the first 72 hours of the study. The animals had *ad libitum* access to food during the collection period. At 24 hours, allulose was present in urine at 11-15% of the initial dosage and in feces at 8-13% of the initial dosage. No

allulose was detected in the urine or feces samples collected at 24-48 hours and 48-72 hours after dosing.

In a second study, Matsuo (2003) evaluated the absorption of allulose from the gastrointestinal tract of 5-week-old male Wistar rats (n=18) following a single oral dose (5 mg per kg). Animals were fed a standard diet *ad libitum* for one week prior to dosing and then euthanized at 1, 3, and 7 hours post dose (n=6 per timepoint). Following euthanasia at each timepoint, blood was collected, and the serum extracted to evaluate allulose levels. Residual food in the stomach, small intestine and cecum was also collected at each timepoint and evaluated for allulose levels. Allulose levels decreased rapidly in the blood at one hour after administration. In the stomach, allulose levels were higher at one hour (26 – 37% of initial dose) after dosing than at three hours (0.4 – 0.6% of initial dose) after dosing, while none was detected at seven hours after administration. In the small intestine, allulose levels were 6 – 10%, 2 – 3%, and 1 – 3% of the initial dose at 1, 3, and 7 hours, respectively. The allulose levels in the cecum at 1, 3, and 7 hours was 0%, 11 – 18%, and 10 – 19%, respectively.

In an additional study by the same authors, twenty-six 3-week-old male Wistar rats were randomized into four groups and fed a basal diet until they were four weeks old (Matsuo, 2003). Rats were then fed a synthetic high carbohydrate diet that contained 5% corn oil, 0, 10, 20, or 30% allulose, and 65, 55, 45, or 35% corn starch. Each group was given *ad libitum* access to the allulose containing diets and water for 34 days, after which the rats were fasted overnight and euthanized. The cecum was removed immediately, and residual food collected, cecal weight, surface area and cecal content weight was measured. Body weight gain, food intake and food efficiency were evaluated throughout the study, and all decreased with increasing amounts of allulose in the diets. Cecal weight and surface area increased with increasing levels of allulose in the diet as did the short chain fatty acids (SCFA), and acetic, propionic and butyric acid. Cecal density did not differ between groups. The authors of these studies concluded that allulose is partly absorbable in the digestive tract and is excreted in the urine and feces, and that allulose is a fermentable saccharide as evidenced by the SCFAs produced in the cecum.

b. Human Studies

Williamson et al. (2014) (abstract only) investigated the mass balance recovery of allulose[15]. Eight healthy men were fed a light breakfast and were orally administered 776 nCi [$_{14}$C(U)] of allulose (99% purity) in a beverage that included 15 g of unlabeled sweetener. Samples of blood, urine, feces, and expired air were collected at baseline and at multiple time points up to 168 hours post dosing. The maximum plasma concentration (C_{max}) was observed at approximately 1.5 hours following administration. The study reported that most of the radiotracer remained intact (80.3% in plasma, 83.6% urine, and 16% fecal) and the ^{14}C rare sugar was the most abundant ^{14}C-labeled compound in the plasma and excreta. This study demonstrated that radio-labeled allulose was absorbed but was not metabolized.

[15] This sugar was not defined as allulose in the abstract; however, the rare sugar was identified as allulose in the following document: https://www.tateandlyle.com/sites/default/files/2017-12/tate-lyle-sweetener-brochure-2017%20%281%29_2.pdf (Accessed June 3, 2021)

2. Toxicity Studies

a. Acute, Subchronic, and Chronic Toxicity Studies

In an acute oral administration study by Matsuo et al. (2002), male Wistar rats were give single doses of 8, 11, 14, 17, or 20 g per kg bw allulose. All rats displayed diarrhea within 24 hours of allulose administration. Three of eight rats in the 14 g per kg bw group, three of eight rats in the 17 g per kg bw group, and all eight rats in the 20 g per kg bw group died within 48 hours of administration. Deceased rats in the 17 and 20 g per kg bw dose groups displayed bleeding in the mucous layers of the stomach or small intestine. Based on these observations, Matsuo et al. determined that the LD_{50} of allulose is 16.3 g per kg bw and 15.8 g per kg bw using the Behrens-Karber and Litchfield-Wilcoxon methods, respectively. The authors also conducted a 34-day subchronic feeding study in rats with levels of allulose at 0, 10, 20, 30, and 40% allulose in the diet. One rat out of seven fed the 30% diet and five rats out of seven fed the 40% diets died during the experimental period. The authors noted only that high consumption of allulose “appeared harmful to the intestinal tract.” Rats fed the 20%, 30%, and 40% diets had diarrhea for the first eight days and body weight gain was more suppressed by feeding the higher levels of allulose. Food intake and food efficiency were lower in the rats fed the higher allulose diets and carcass fat content and percentage of carcass fat decreased significantly with increasing allulose levels in the diet. The weights of the heart, spleen and abdominal adipose tissue were smaller in rats fed the higher concentrations but cecal weight increased with increasing allulose concentrations and cecal hypertrophy was observed in those fed 10 – 40% allulose.

Nishii et al. (2017) conducted a study in which healthy dogs were given 200 mg per kg bw per day of allulose orally for 12 weeks. Exposure to allulose did not cause any adverse clinical signs or changes in hematological and biochemical endpoints except for lipids. There was no adverse effect on body weight noted. No cumulative effects on glucose metabolism were reported. The authors concluded that long-term dosing with allulose caused no harmful effects in dogs.

Nishii et al. (2016b) conducted a study in which healthy dogs were given a single acute oral dose of either a placebo or allulose at 1 or 4 g per kg. Some transient clinical signs were noted following the 4 g per kg dose and clinical pathology changes were noted in both allulose groups. The authors concluded that a single oral dose of allulose up to 4 g per kg bw did not show severe toxicity in dogs.

In a chronic study performed by Yagi and Matsuo (2009), male Wistar rats were fed a diet containing 3% allulose for 18 months. The authors concluded that no adverse effects were noted when rats were exposed to 3% allulose in the diet, equivalent to 1.28 g per kg bw per day, for up to 18 months.

A study described in GRN 828 reported that 5-week-old male and female Sprague Dawley rats were given a single dose of 0 or 5 mg per kg bw per day of allulose and were then observed for 14 days. The authors concluded that the median lethal dose (LD_{50}) was higher than 5 g per kg bw, the highest dose tested, which does not alter the LD_{50} conclusion that was previously reported in the published literature.

Study details of the acute, subchronic, and chronic toxicity studies identified in GRN 693 and one additional study identified, are presented in Table 9.

Table 9. Summary of Pre-Clinical Safety Studies for Allulose

Study Setup and Details	Pre-Clinical Study Details and Results	Reference
Study Design: *In vivo* Study Length: 90 days Animals: n = 32; male and female (n=4/sex/group) Dose/Concentration: 0, 1,250, 2,500, 5,000 mg/kg bw Delivery/Vehicle: gavage/distilled water Frequency: daily	Outcome Measurements: • OECD guideline 408 compliant • Allulose was manufactured from an aqueous solution of fructose via enzymatic epimerization using a non-GMO *M. foliorum* SYG27B-MF • Animals were observed once daily for clinical signs of toxicity, twice daily for mortality, and weighed on a weekly basis. The eyes of all animals in the study were examined prior to dosing and then again during the last week of dosing • Food consumption was measured every 7 days for the first 13 weeks and then every 6 days, the average intake per day was calculated. • A complete urinalysis was performed on 5 rats per group after week 13 of dosing. • At the end of the dosing period, all animals were anesthetized, and blood collected for a complete blood count and a blood chemistry evaluation. Animals were then exsanguinated and underwent a complete gross necropsy. • Select organs and tissues were weighed and included the ovaries, adrenal glands, pituitary gland, prostate gland, testes, epididymides, spleen, kidneys, heart, lungs, brain and liver. The following tissues and organs were collected, fixed and prepared for histopathological examination: testes, epididymides, prostate gland, ovaries, uterus, vagina, urinary bladder, spleen, stomach, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, mesenteric lymph nodes, adrenal glands, kidneys, liver, femurs, submandibular lymph nodes, salivary glands, sternum, thymus, heart, lungs, aorta, spinal cord, tongue, trachea, esophagus, thyroid gland, eyes, Harderian gland, brain, pituitary gland and skin/mammary gland. • Blood was evaluated for white and red blood cell numbers, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, red cell distribution width, hemoglobin distribution width, reticulocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils and large unstained cells. Serum was collected and evaluated for the following parameters: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, glucose, albumin, bilirubin, triglycerides, albumin/globulin ratio, inorganic phosphorus, electrolytes and calcium. Prothrombin time and activated partial thromboplastin time were also evaluated.	An et al. (2019)

Study Setup and Details	Pre-Clinical Study Details and Results	Reference
	Results and Significance: • No mortalities were reported, and no obvious adverse clinical signs were seen in either sex. • There was no significant difference in body weight between the treated and control animals except for the high dose males. The body weight significantly decreased by 11.9% as compared to controls. There were no differences in food consumption. • No allulose related changes in any of the hematology or clinical chemistry parameters were noted. • No allulose related abnormalities were noted at gross necropsy or histopathological examination. Significant changes were observed in the absolute weight of the thymus in the males (decreased), and the liver and kidneys in females (increased). • The authors concluded that the NOAEL of allulose in both male and female rats was determined to be 5,000 mg per kg per day.	
Study Design: *In vivo* Study Length: 144 hours for each dose Animals: n = 6 dogs (1 neutered male; 5 spayed females) Dose/Concentration: 1 and 4 g/kg bw Delivery/Vehicle: oral with plastic syringe/water Frequency: single dose/2 dosage levels each; Latin square design with 7 intervals of at least 7 days between	Outcome Measurements: • Blood samples collected before dosing and at 20 minutes, 40 minutes, 1, 2, 4, 8, 12, 24, 48, 96, and 144 hours after dosing. • Plasma concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, urea nitrogen, creatinine, total protein, albumin, total cholesterol and triglyceride were determined at the 0, 4, 8, 12, 24, 48, 96, and 144-hour timepoints. • Plasma insulin concentrations were determined at 0, 20 minutes, 40 minutes, 1, and 2 hours after dosing • Plasma concentrations of glucose, total calcium, inorganic phosphorus, sodium, potassium, and chlorine were determined at 0, 20 minutes, 40 minutes, 1, 2, 4, 8, 12, 24, 48, 96, and 144-hour timepoints. • Dogs were fed at 12 hours after dosing and then twice daily thereafter Results and Significance: • One dog was vomiting shortly after dosing with 4 g/kg of allulose and was removed from the study. The remaining 5 animals in the group did not exhibit vomiting but did experience transient diarrhea between 2 and 24 hours after dosing. Two dogs exhibited transient nausea within 1 hour of dosing with 1 g/kg allulose. No other adverse clinical signs were noted, dogs were active and had good appetite throughout the rest of the study period. • Physiological Findings: Blood glucose concentrations decreased slightly at 2 hours after dosing of both 1 and 4 g/kg bw allulose. Plasma alkaline phosphatase activities showed were mildly increased in a dose dependent manner between 12 and 48 hours after allulose administration. Plasma inorganic phosphorus was mildly decreased,	FDA (2017b); Nishii et al. (2016b)

Study Setup and Details	Pre-Clinical Study Details and Results	Reference
	which was followed by a transient increase within 12 hours and the concentration at the 8-hour timepoint in the dogs that received 4 g/kg bw allulose was significantly higher when compared to the control dogs. There were no significant differences found in any other parameters between the dose rates. • The transient adverse gastrointestinal effects noted were not considered to be signs of serious toxicity but were assumed to be due to a rise in the enteric osmotic pressure caused by the administration of allulose. The authors did not consider the drop in blood glucose levels to be significant enough to be considered significant hypoglycemia. The authors noted that the increase in plasma ALP activity was mild and transient without a significant rise in other hepatic enzymes and was therefore not considered a serious toxic effect. The pattern of change in the plasma inorganic phosphorus concentration was similar to normal diurnal patterns found in dogs but the authors concluded that the administration of allulose may mildly exaggerate the pattern in dogs but did not consider this a serious sign of toxicity. • The authors concluded that a single oral dose of allulose up to 4 g/kg bw did not show severe toxicity in dogs.	
Study Design: *In vivo* Study Length: 14 days Animals: n = 40 male Wistar rats (n=8/group); 4 weeks old Dose/Concentration: 8, 11, 14, 17, and 20 g/kg bw Delivery/Vehicle: gavage; water Frequency: once	Outcome Measurements: • Rats were observed daily for clinical signs and mortality • Animals were fasted for 12 hours prior to dosing and for 4 hours after, then had *ad libitum* access to certified diet and water. • LD_{50} was calculated from the mortality using the Behrens-Karber method and Litchfield-Wilcoxon method Results and Significance: • All rats had diarrhea at 1-24 hours after dosing with allulose with the animals in the 17 and 20 g/kg bw groups becoming very weak. • Three rats in the 14 g/kg bw, three rats in the 17 g/kg bw, and all rats in the 20 g/kg bw group died in the first 48 hours following dosing. All rats that died in the 17 and 20 g/kg bw groups exhibited bleeding in the mucous layers of the stomach and small intestine. • No mortalities were noted after 48 hours and all surviving rats were normal after day 3 • The calculated LD_{50} was 16.3 g/kg bw and 15.8 g/kg bw by the Behrens-Karber and Litchfield-Wilcoxon methods, respectively.	Matsuo et al. (2002)
Study Design: *In vivo* Study Length: 34 days Animals: n = 30 male Wistar rats (n=7/group); 4 weeks old Dose/Concentration: 0, 10, 20, 30, and 40%	Outcome Measurements: • Body weight gain and food intake were recorded daily • The number of rats with diarrhea were noted • Following euthanasia on day 34, blood was collected, and the serum was analyzed for glucose and triacylglycerol.	Matsuo et al. (2002)

Study Setup and Details	Pre-Clinical Study Details and Results	Reference
Delivery/Vehicle: diet Frequency: daily in the diet	• The liver, heart, spleen, kidneys, cecum and intra-abdominal adipose tissues were removed immediately following euthanasia and weighed • The remaining organs and tissues were removed, and the carcass stored at -20°C until analysis of carcass composition. • Total liver lipid and liver triacylglycerol were determined as well as carcass fat and protein. Results and Significance: • One rat in the 30% group and five rats in the 40% group died during the study • Rats in the 20, 30, and 40% diet groups had diarrhea for the first 8 days. • Body weight gain decreased as the level of allulose in the diet increased. A significant difference in weight gain was noted between the 0, 10, 20, and 30% allulose groups. • Food intake and food efficiency were lower in rats fed higher allulose levels • Carcass fat content and percentage of carcass fat decreased significantly with increasing allulose levels in the diet. Carcass protein content decreased as the level of allulose in the diet increased; the level was significantly higher in the 0 and 10% groups as compared to the 20 and 30% groups. • The weights of heart, spleen and abdominal adipose tissue were decreased as the levels of allulose increased in the diet. Cecal weights increased as the level of allulose increased in the diet and cecal hypertrophy was observed in the rats fed diets with 10 – 40% allulose. • The authors concluded that feeding diets extremely high in allulose is harmful to the intestinal tract.	
Study Design: *In vivo* Study Length: 12 weeks Animals: n = 10 beagle dogs (1 neutered male + 4 spayed females/group) Dose/Concentration: 0.2 g/kg Delivery/Vehicle: oral/vehicle not specified Frequency: daily	Outcome Measurements: • Animals were fed a maintenance diet and had free access to water • Control group received water rather than the allulose solution • Food consumption, feces characteristics, activity and clinical signs were recorded daily. Body weight was measured at 0, 2, 4, 8, and 12 weeks. Blood samples were collected before the study started and during week 12 for complete blood counts and the following biochemical analyses: ALT, AST, ALP, total bilirubin, urea nitrogen, creatinine, total protein, albumin, total cholesterol, triglyceride, total calcium, inorganic phosphorus, sodium, potassium, and chlorine concentrations. • An intravenous glucose tolerance test was conducted before the start of dosing and one day after the last allulose dose. A 50% glucose solution was injected intravenously at a rate of 0.5 g glucose/kg bw and blood samples collected at 0, 5, 10, 15, 30, and 60 minutes after for measurement of glucose and insulin concentrations.	Nishii et al. (2017)

Study Setup and Details	Pre-Clinical Study Details and Results	Reference
	Results and Significance: • During the experimental period, all dogs had a normal appetite, normal feces, and were active with no adverse clinical signs. Body weights were stable in both groups and there was no significant difference between controls and experimental groups. • Allulose administration did not cause clinical signs, body weight or changes in the hematological or biochemical levels, with the exception of significantly decreasing the total cholesterol. • Plasma glucose and insulin concentrations in the glucose tolerance test were not significantly different between groups. The authors concluded that this was evidence that allulose did not have cumulative effects on glucose metabolism in healthy dogs. • The authors concluded that long-term administration of allulose caused no harmful effects in healthy dogs.	
Study Design: *In vivo* Study Length: 90-days Animals: n = 20 male Wistar rats (n=10/group); 4 weeks old Dose/Concentration: 3% in the diet Delivery/Vehicle: diet Frequency: daily	Outcome Measurements: • Sucrose was used as a control • Animals had free access to the control or allulose-containing diets and water for 90-days • At the end of the dosing period, animals were euthanized, and blood collected. The brain, heart, lungs, liver, pancreas, kidneys, adrenal glands, spleen, testicles, intra-abdominal adipose tissues and muscle tissues were removed. The stomach, small intestine, large intestine and cecum were also removed and weighed. Pieces of liver, kidneys and jejunum were placed in 10% neutral buffered formalin and examined histologically. The small and large intestine length, surface area and cecal content weight were measured. • The following hematological and clinical chemistry parameters were evaluated: white blood cell and red blood cell count, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelet, total protein, albumin/globulin ratio, albumin, globulin, AST, uric acid, blood urea nitrogen, creatine, calcium, iron, cholesterol, triglycerides, glucose and free fatty acid. Results and Significance: • Final body and tissue weights, food intake, and digestive tract size did not differ between control and allulose groups. • Actual allulose ingestion was 1.67 g/kg bw per day • Mean liver and kidney weights were significantly higher in the allulose group as compared to the sucrose group. No other tissue weight differences were noted. • Total protein, albumin, white blood cell and red blood cell count, mean cell hemoglobin concentration, and platelet count were all significantly higher and the uric acid, mean cell volume and mean cell hemoglobin	Matsuo et al. (2012); FDA (2017b)

Study Setup and Details	Pre-Clinical Study Details and Results	Reference
	were significantly lower in the allulose group as compared to the sucrose group. These were not considered toxicologically significant. • No histological differences were noted between groups in the liver and kidneys. • The authors concluded that there were no adverse effects found following the consumption of a diet containing 3% allulose for 90 days	
Study Design: *In vivo* Study Length: 12-18 months Animals: n = 36 male Wistar rats (n=18/group); 4 weeks old Dose/Concentration: 1.28 g/kg bw per day; 3% in the diet Delivery/Vehicle: diet Frequency: daily	Outcome Measurements: • Control diet contained 3% sucrose (actual dose consumed was 1.22 g/kg bw per day) • Animals had *ad libitum* access to the diets and water • Body weight and feed consumption was recorded during the study, but the frequency was not specified • At the end of 12 months, 8 animals/group were fasted for 4.5 hours, anesthetized and blood was collected for hematological and clinical chemistry analysis. Samples were analyzed for platelet count, hemoglobin, erythrocyte count, leukocyte count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, glucose, insulin, triglycerides, free fatty acids, total cholesterol, AST, ALT, total bilirubin, direct bilirubin, indirect bilirubin, creatinine, urea nitrogen, uric acid, albumin, total protein, ratio of albumin and globulin, lipid peroxide, calcium and iron. The remaining animals (10/group) underwent the same procedures at the end of 18 months • The brain, heart, lungs, liver, pancreas, kidneys, adrenals, spleen, testicles, intra-abdominal adipose tissues and muscle tissues were quickly removed and weighed following exsanguination. Sections of the liver and kidney were placed in 10% neutral buffered formalin for histopathological examination. Results and Significance: • Final body weight, weight gain, and energy intake did not differ between the allulose and sucrose groups at the end of 12 months. Body weight and body weight gain and intra-abdominal adipose tissue weight was significantly reduced in the rats on the allulose-containing diet as compared to those on the sucrose containing diet at 18 months • No toxicologically significant differences were found in any of the hematological or clinical chemistry parameters evaluated • Relative liver and kidney weights were significantly higher in the allulose group as compared to the sucrose group, but no gross pathological findings were evident which correlated with hypertrophy of the liver or kidney. No toxicologically significant histopathological lesions were noted in the liver or kidneys.	Yagi and Matsuo (2009); FDA (2017b)

Study Setup and Details	Pre-Clinical Study Details and Results	Reference
	• The authors concluded that no adverse effects were noted in the current study when rats were exposed to 3% allulose in the diet for up to 18 months	

bw – body weight; g – grams; kg – kilograms

Blue California has reviewed these studies and concludes that, while adverse effects on the intestinal tract were noted in Matsuo et al. (2002), the doses utilized in the acute study (ranging from 8 to 20 g per kg bw per day) were much higher than those anticipated from the intended use of Blue California's Allulose, where the highest 90th percentile EDI for any population group is determined to be 0.5 g per kg bw per day. The other studies reviewed show that oral allulose is well tolerated.

b. Reproductive and Developmental Toxicity Studies

Reproductive and development studies are important for GRAS assessments because the concept of GRAS presumes safety for the general population. No reproductive or developmental studies were reviewed in the previous GRNs (GRN 400, 498, 693 and 828). One new study was found in the literature.

Kim et al. (2019) investigated the reproductive toxicity of allulose in rats in a one generation study that was conducted in accordance with OECD Test Guideline 415. The females were continuously dosed from two weeks prior to mating until day 21 of lactation and males were dosed for the ten weeks prior to mating. Animals were exposed to either 0, 500, 1,000, or 2,000 mg allulose per kg bw per day. Animals were observed daily, and the males were euthanized when the mating period was completed. Females were allowed to give birth and rear their young until weaning at lactation day 21. All euthanized animals underwent a gross necropsy and special attention was given to the reproductive tissues. The ovaries, uterus, cervix, vagina, testes, epididymides, seminal vesicles, prostate, coagulating gland, and pituitary gland were all fixed, prepared for histopathological examination, and then evaluated. On the day of birth [postnatal day (PND) 0], the pups were examined, and the number of live and stillborn pups was recorded. The live pups were then sexed, weighed, and underwent an external examination. The pups were then examined daily on PND 4, 7, 14 and 21. On PND 4, litters were reduced to 4 males and 4 females when possible. Any deceased pups were evaluated for any structural or pathological findings. On PNDs 0, 3, 6, 9, 12, 15, 18, and 21, the body weights of the F_1 pups were measured and physical findings were assessed for one random male and female in each litter.

There was no evidence of toxicity or mortality linked with treatment and there were no significant differences in the body weights in rats of any sex or at any time relative to the mating period. There were no treatment related effects on pregnancy rates, implantation, length of pregnancy, gender ratios, viability and lactation indexes, prenatal death rates, or the number of live young at birth. There were no treatment related changes identified at gross necropsy, with organ weights or with histopathological examination in the study rats. The body weights of the F_1 pups from the treated

parents were slightly higher up to day 9, but the authors reported the “changes were small, with no obvious dose dependence.” No malformed pups were observed in any group. The authors concluded that the No Observed Adverse Effects Level (NOAEL) for both the parental generation and the offspring was equal to or greater than 2,000 mg per kg bw per day, the highest dose tested.

c. Genotoxicity/Mutagenicity Studies

No new studies were identified in the literature search since GRN 693 or GRN 828 were submitted to FDA. No genotoxicity or mutagenicity studies were reviewed in GRN 693 or GRN 828, but some studies were reviewed in GRN 400. Genotoxicity studies reviewed in GRN 400 included an Ames test, a micronucleus test and a chromosomal aberration test. No mutagenic potential for allulose was observed at levels up to 5,000 µg per plate in the Ames study and no significant increase in micronucleated polychromatic erythrocytes were noted at concentrations up to 2,000 mg per kg per day of allulose in a micronucleus test. In the chromosomal aberration test reported in GRN 400, allulose did not induce an increase in the number of chromosomal aberrations at a dosage of 1,800 µg per mL.

Blue California has reviewed these studies and concludes our material is substantively similar to the allulose preparations described in GRN 693 and GRN 828, and that the results of the genotoxicity and mutagenicity studies detailed in the previous GRNs are relevant to the safety conclusion of Blue California’s Allulose.

3. Carcinogenicity

No carcinogenicity studies were reviewed in GRN 693 or 828, but three studies were reviewed in GRN 400 that demonstrated allulose is not carcinogenic. Furthermore, no new studies were identified in a literature search since GRN 693 and 828 were submitted to FDA.

4. *In vitro* Studies with Allulose

No *in vitro* studies for allulose were found in the published literature and none were included in previous GRAS Notices submitted to FDA.

5. Animal Efficacy Studies with Allulose

Studies on the efficacy of allulose did not assess safety but provide supportive evidence. Ochiai et al. (2017) assessed the anti-obesity effect of a “rare sugar” syrup that contained 5.62% allulose and a modified glucose syrup that contained allulose at 12.83% of the diet rats. Because allulose was present at a low level in both syrups, the study was not considered to have a significant bearing on safety.

Details of recent studies conducted by Choi et al. (2018) and Nishii et al. (2016b) were found to be relevant, and details about these studies as well as those discussed in related allulose GRNs are presented in Table 10.

Table 10. Summary of Animal Efficacy Studies for Allulose

Study Setup and Details	Animal Efficacy Study Details and Results	Reference
Study Design: *In vivo* Study Length: 12 weeks Animals: n = 70; male C57BL/6J mice Dose/Concentration: 3% allulose in the diet Delivery/Vehicle: Diet Frequency: daily	Outcome Measurements: • Mice were fed a normal diet for 16 weeks and then a high-fat diet (HFD) for 4 weeks to induce obesity. Following this, the mice were divided into seven groups (n=10/group) and fed: 1) HFD; 2) HFD with *Lactobacillus sakei*; 3) HFD with *Leuconostoc kimchi*; 4) 3% allulose with HFD; 5) HFD with allulose and *Lactobacillus sakei*; 6) HFD with allulose and *Leuconostoc kimchi*; or 7) HFD with allulose, *Lactobacillus sakei,* and *Leuconostoc kimchi*; all for 12 weeks. • The body weights were evaluated throughout the experimental period and used to determine the feed efficiency ratio throughout the experimental period. • At the end of 12 weeks, the animals were euthanized following a 16 hour fast, blood was collected, and the liver and adipose tissue were removed and stored for analysis. • Plasma triglycerides, total cholesterol, high-density lipoprotein cholesterol, glutamic oxaloacetic transaminase (AST) and glutamic pyruvic transaminase (ALT) levels, plasma apolipoprotein AI and apolipoprotein B, plasma free fatty acid, plasma adipokines and cytokines were measured. • Hepatic lipid content including triglycerides, cholesterol and fatty acid contents, were determined. Hepatic lipid-regulating enzyme activities were also evaluated. The livers were examined histopathologically. Results and Significance: • The authors concluded that this study demonstrated that the symbiotic mixture with allulose was more effective in suppressing diet-induced obesity and its complications via the regulation of lipid metabolism than either the probiotics or allulose alone. They suggest that this may mean the allulose acts as a prebiotic for the two probiotics tested. Safety Measurements/Adverse Events Reported: • No specific safety outcomes were reported in this study.	Choi et al. (2018)
Study Design: *In vivo* Study Length: 4 weeks Animals: n = 30; Sprague-Dawley rats (n=6/group) Dose/Concentration: control diet or diet containing 3% allulose, D-tagatose or D-sorbose Delivery/Vehicle: diet Frequency: daily	Outcome Measurements: • Body weight and food intake were determined and used to calculate body weight gain and food efficiency ratio. • Animals were then euthanized without fasting. The liver and mesenteric, perirenal and epididymal adipose tissue were weighed and stored at -80°C prior to tissue lipid, enzyme or gene expression analysis. The small intestine was collected and flushed with ice-cold saline. The jejunum and ileum were isolated and the jejunum was used for real-time quantitative PCR analysis. Serum was collected to determine lipid levels and feces were collected for 2 days prior to euthanasia and analyzed for lipid excretion	Nagata et al. (2018)

Study Setup and Details	Animal Efficacy Study Details and Results	Reference
	Results and Significance: • No differences in body weight gain, food efficiency and liver weight were reported between groups. No difference in food intake was noted, which the authors concluded to mean there was no differences in caloric intake. • Hepatic lipogenic enzyme activity was lower for animals who consumed diets supplemented with allulose and D-sorbose but increased for animals that consumed diets supplemented with D-tagatose. Fecal fatty acid excretion was not significantly decreased by allulose. There was a trend towards reduced adipose tissue weight observed in the rare sugars' groups. Allulose tended to down-regulate the gene expression of cholesterol metabolism-related liver proteins. Safety Measurements/Adverse Events Reported: • No effects on body weight or food efficiency were noted. There was no reporting on any other safety measurements or any reports of mortalities. • The average feed intake in the allulose group was 24.5±0.88g/day; therefore, approximately 0.74g of allulose was ingested/mouse/day.	
Study Design: *In vivo* Study Length: 16 weeks Animals: n = 60; male C57BL/6J mice (n=10/group) Dose/Concentration: 5% in the diet	Outcome Measurements: • Groups included in the study were control, high fat diet, 5% allulose, 5% erythritol, 5% D-glucose, and 5% D-fructose. The allulose, erythritol, D-glucose, and D-fructose were substituted for sucrose in the high fat diet to make the test diets. All animals were given isocaloric diets based on the energy intake of the allulose fed groups • Food intake was recorded daily, and body weights were collected every two weeks • Plasma, hepatic and fecal lipid profiles (triglycerides, high density lipoprotein cholesterol and total cholesterol) were determined for all animals at the end of the 16-week experimental period • Plasma leptin, resistin and adiponectin were determined at the end of the experimental period • The liver and epididymal white adipose tissue were collected from all animals at the end of the experimental period and fixed in 10% formalin for histopathological evaluation. Results and Significance • Body weights and the fat-pad mass in the allulose group were lower than that in the control group with a decrease in plasma leptin and resistin concentrations. • Allulose lowered plasma and hepatic lipids but elevated fecal lipids with a decrease in mRNA expression of CD36, ApoB48, FATP4 in the small intestine • Both liver fatty acid synthase and β-oxidation were downgraded in the liver by allulose to the level of that in the normal group but in the	Han et al. (2016)

Study Setup and Details	Animal Efficacy Study Details and Results	Reference
	epididymal white adipose tissue, fatty acid synthase was decreased while β-oxidation activity was enhanced • The authors concluded that 5% dietary allulose led to the normalization of the metabolic status of diet-induced obesity by altering lipid-regulation enzyme activities and their gene expression levels as well as fecal lipids Safety Measurements/Adverse Events Reported: • No specific adverse events were reported.	
Study Design: *In vivo* Study Length: 8 weeks Animals: n = 31; male Wistar rats Dose/Concentration: 5% allulose in the diet Delivery/Vehicle: Diet Frequency: Daily	Outcome Measurements: • Base diet was a high sucrose diet; control diet had 5% added cellulose and the experimental diet had 5% added allulose (n=10). The cellulose group (n=21) was again divided into two groups: one fed the cellulose diet *ad libitum* (n=11) and a second group that was pair fed the cellulose + allulose diets (n=10). • Body weight and dietary intake were monitored daily. • Rats in both the cellulose + allulose and the allulose groups consumed equal amounts of the metabolizable energy during the experimental period • Between weeks 5 and 7, energy expenditure was measured • At the end of the experimental period, the rats were fasted for 4 hours, euthanized, and blood was collected and the serum harvested. Heart, liver, kidney, abdominal adipose tissues, brown adipose tissue, and muscles were rapidly removed, weighed, and stored at -80°C until analyzed. Results and Significance: • The resting energy expenditure during darkness and the lipoprotein lipase activity in the soleus muscle were significantly higher in the allulose group than in the cellulose + allulose group • Serum levels of glucose, leptin and adiponectin were significantly lower in the allulose group as compared with the cellulose + allulose group. • The glucose-6-phosphate dehydrogenase activities in the liver and perirenal adipose tissue and body fat accumulation were significantly lower in the allulose group as compared with the cellulose + allulose group. • The authors concluded that the anti-obesity effects of allulose could be induced by suppressing lipogenic enzyme activity and by increasing energy expenditure in a high sucrose induced obese rat model. Safety Measurements/Adverse Events Reported: • No specific adverse events were reported.	Ochiai et al. (2014)
Study Design: *In vivo* Study Length: 4 weeks	Outcome Measurements: • Experiment 1: Rats were provided *ad libitum* a diet with 3% allulose or without allulose (control) for 4 weeks and then five to six animals were euthanized every 6 hours for 4 timepoints. Blood was collected and the	Nagata et al. (2015)

Study Setup and Details	Animal Efficacy Study Details and Results	Reference
Animals: Exp 1: n = 48 (n=24/group); Sprague-Dawley rats Exp 2: n = 16 (n=8/group); Sprague-Dawley rats Dose/Concentration: 3% allulose in the diet Delivery/Vehicle: diet Frequency: daily	liver, soleus muscle and adipose tissue collected. Intrascapular brown adipose tissue was also collected for enzyme activity measurement. The small intestine was collected. Serum glucose and lipid levels, serum insulin and leptin levels, and the cholesterol, phospholipid and triglyceride levels in the liver were measured. The activity of lipid metabolism-related enzymes in the liver and brown adipose tissue were also measured. Gene expression of enzymes and proteins involved in lipid metabolism in the liver, jejunum soleus muscle and mesenteric adipose tissue was measured. • Experiment 2: Rats were fed the appropriate diet (3% allulose or control) for 4 weeks and then the 24-hour energy expenditure was measured. Rats were placed in a metabolic chamber and maintained on the appropriate diet. Energy expenditure and oxidation of carbohydrate and fat were measured over a 24-hour period. Results and Significance: • In the first experiment, rats fed allulose had significantly lower serum insulin and leptin levels as well as liver enzyme activity involved in lipogenesis. Gene expression of a transcriptional modulator of fatty acid oxidation was enhanced. • In the second experiment, rats fed the allulose diet had significantly lower body weights and food intake as compared with controls. The rats in the allulose group had significantly higher energy expenditure during the light period and fat oxidation in the dark period, as compared with controls and carbohydrate oxidation was lower. • The authors concluded that allulose decreased lipogenesis, increased fatty acid oxidation and enhanced 24-hour energy expenditure, which could demonstrate a potential for weight management. Safety Measurements/Adverse Events Reported: • No specific safety endpoints were included in the study and no reports of adverse events were noted.	
Study Design: *In vivo* Study Length: Single dose Animals: n = 7; dogs (one male; five females used in both experiments and one additional male for the oral administration study) Dose/Concentration: 0.2 g/kg bw allulose Delivery/Vehicle: oral Frequency: once	Outcome Measurements: • The same dogs were used for all experiments with a minimum of a 1-week washout period between studies • Dogs were determined to be healthy prior to dosing. All dogs were fasted overnight with free access to water. • Oral study: Seven dogs were administered a 50% glucose (2.0 g/kg bw) solution or a 50% maltose (2.0 g/kg bw) solution, with oral allulose (0.2 g/kg bw) or the equivalent water dose. Blood samples were collected before dosing and at 30, 60, 90, and 120 minutes after dosing for determination of plasma glucose and insulin concentrations. • Intravenous study: The same dose rate was used as in the oral study. Six dogs were given an oral allulose solution (0.2 g/kg bw) or the equivalent volume of water 60 minutes before the intravenous	(Nishii et al., 2016a)

Study Setup and Details	Animal Efficacy Study Details and Results	Reference
	administration of 50% glucose (0.5 g/kg bw). Blood samples were collected before dosing and at 5, 10, 15, 30, and 60 minutes after the glucose dose, for the determination of plasma glucose and insulin concentrations. • Feeding study: Six dogs were fed a commercial maintenance dry food and given allulose (0.2 g/kg bw) or water. Blood samples were taken before feeding and at 1, 2, 3, 4, 6, and 8 hours after feeding for determination of glucose and insulin concentrations. Results and Significance: • Oral study: Oral dosing with glucose or maltose increased plasma glucose and insulin levels. Administration of allulose after dosing with glucose or maltose significantly diminished the rise in plasma glucose. The concentration of plasma insulin was significantly lower as well. The area under the curves (AUCs) for plasma glucose and insulin concentrations after oral dosing with glucose and maltose were significantly lower in the allulose group as compared with the water control group. • Intravenous study: The concentration of plasma glucose was lower at 5, 10, and 15 minutes after intravenous dosing with glucose when allulose was also given. There was no significant difference in the plasma insulin levels between the control and allulose groups. • Feeding study: After feeding, the level of plasma insulin increased while the level of plasma glucose did not fluctuate. Oral administration of allulose did not alter these parameters. Safety Measurements/Adverse Events Reported: • No specific safety outcomes were reported in this study.	
Study Design: *In vivo* Study Length: 60 weeks Animals: n = 20 (n=10/group); Otsuka Long-Evans Tokushima Fatty (OLETF) rats Dose/Concentration: 0 or 5% allulose Delivery/Vehicle: tap water Frequency: daily/*ad libitum*	Outcome Measurements: • Animals were divided into two groups: control receiving tap water only and treatment group receiving 5% allulose in the water • Body weights were measured daily • Food and water intake were determined for three consecutive days each week and the average rat/day intake was calculated • Periodic fasting and postprandial blood glucose levels were measured; plasma was also collected. Rats were fasted for 12 hours for an oral glucose tolerance test. Additional blood was collected for plasma, which was tested for insulin, total cholesterol, triglycerides, and high- and low-density lipoproteins. • At the end of the experimental period, animals were fasted for 12 hours, anesthetized, blood collected, and organs/tissues removed. Abdominal fat was collected from the epididymal, retroperitoneal and mesenteric areas and weighed. Serum was analyzed for glutathione, IL-6, tissue necrosis factor alpha, leptin and adiponectin levels. Total fat mass, fat-free body mass and body mass index were estimated. To measure	Hossain (2015)

Study Setup and Details	Animal Efficacy Study Details and Results	Reference
	inflammatory profile, the pancreas and adipose tissues were fixed in formalin and prepared for histopathological evaluation. Results and Significance: • Allulose prevented the start and progression of type II diabetes until week 60 by maintaining blood glucose levels, decreasing body weight gain and the control of postprandial hyperglycemia as compared with control rats. The improvement of glycemic control was accompanied by the maintenance of plasma insulin levels and preservation of pancreatic beta cells with a reduction in inflammatory markers. • Body fat accumulation was significantly lower in the treatment group. • The authors concluded that allulose could be beneficial in the prevention and control of obesity and hyperglycemia. Safety Measurements/Adverse Events Reported: • No specific safety endpoints were included in this study and no adverse effects were reported.	
Study Design: *In vivo* Study Length: 13 weeks Animals: n = 45 Otsuka Long-Evans Tokushima Fatty (OLETF) rats and non-diabetic Long-Evans Tokushima Otsua (LETO) as controls (n=15) Dose/Concentration: 5% allulose in water Delivery/Vehicle: drinking water Frequency: daily	Outcome Measurements: • Treated OLETF rats were fed with 5% allulose (n=15) or 5% D-glucose (n=15) supplemented drinking water, and only water (n=15) in the control for 13 weeks. A non-diabetic Long-Evans Tokushima Otsuka (LETO), given water only served as a counter control of OLETF. • Animals were allowed free access to water and food, and food intake for 3 consecutive days each week was measured to calculate the average of g/100g body weight consumption and amount of water consumption was also calculated. • Multiple measurements of obesity, characterization of glucose metabolism and inflammatory profile were also evaluated Results and Significance: • Consumption of allulose significantly attenuated progressive beta-islet fibrosis and preserved the islets. • Allulose significantly reduced increase in body weight and abdominal fat deposition. The oral glucose tolerance test showed a reduced blood glucose level which suggests the improvement of insulin resistance. • The authors concluded that the data suggested that allulose protected and preserved pancreatic beta-islets through the maintenance of hyperglycemia and the prevention of fat accumulation in OLETF rats. Safety Measurements/Adverse Events Reported: • No behavior changes were observed during the study, weight gain tended to be lower in the allulose treated group and food intake was lower during the first weeks but was then not different from other groups.	Hossain et al. (2012)

Study Setup and Details	Animal Efficacy Study Details and Results	Reference
Study Design: *In vivo* Study Length: 15 weeks Animals: n = not specified; mice – Lep^{ob}/Lep^{ob} and C57BL/6J wildtype Dose/Concentration: 0, 2.5, or 5% in the diet Delivery/Vehicle: diet Frequency: daily	Outcome Measurements: • This study investigated the benefits of dietary supplementation of allulose in inherited leptin-deficient mice with severe obesity. • Animals were allowed free access to both food and water with intake measurements and body weights determined weekly. • Body composition was assessed *in vivo* and then mice were euthanized, the abdominal visceral fat and the liver and kidneys excised, and the wet weight was measured. • Hepatic steatosis and abdominal visceral fat were evaluated using magnetic resonance imaging (MRI). The liver was examined histologically for changes in hepatic steatosis. Results and Significance: • The subchronic ingestion in the *ob/ob* mice significantly decreased body and liver weights. The loss of body weight was linked with the reduction of total fat mass including abdominal visceral fat but not fat-free body mass, including muscle. In addition, ingestion of allulose improved hepatic steatosis in the *ob/ob* mice. • None of these parameters were influenced by ingestion of allulose in the wildtype mice. • The authors concluded that allulose may be useful as a supplement for preventing and improving obesity and obesity-related disorders. Safety Measurements/Adverse Events Reported: • No specific safety endpoints were included in this study and no adverse effects were reported.	Itoh et al. (2015)

6. Reviews

Chung et al. (2012) reviewed the properties, absorption, and excretion of allulose, as well as its biological production, function, and safety. The study reported that although extremely high levels of allulose (> 20% of the diet for 34 days) may prove harmful to rats and induce diarrhea (Matsuo et al., 2002), chronic intake of allulose at 3% of the diets of young rats for 12 to 18 months was well tolerated (Yagi and Matsuo, 2009). Chung et al. noted that "the dosage selected by Matsuo et al. (2002) was likely too high because their observation is contradicted" by more recent studies. The authors also reported that ingestion of 5 g of allulose with meals for 12 weeks did not result in toxicity issues with respect to hepatic function and physical symptoms in healthy humans with normal blood glucose levels (Hayashi et al., 2010). It was also noted that doses of allulose below 0.5 g per kg bw for males and 0.6 g per kg bw for females did not induce diarrhea (Iida et al., 2007). In addition, the LD_{50} value for allulose is 16 g per kg in rats and is similar to the LD_{50} of D-sucralose for mice (16 g per kg) and rats (10 g per kg) (Goldsmith, 2000). In summary, the review reported that allulose is

generally considered to be safe but noted that additional studies should be conducted about the upper safety level of allulose.

7. Human Studies and Experience

A number of human studies were reviewed in other GRNs for allulose. A study discussed in GRN 693, Iida et al. (2007) indicated that allulose is safe to ingest at 0.5 – 0.6 g per kg bw as a single dose, while a more recent gastrointestinal tolerance study by Han et al. (2018a) recommended that the maximum single dose of allulose should be 0.4 g per kg bw and that the maximum total daily intake of allulose be 0.9 g per kg bw. These recommendations were based on incidences of severe nausea, abdominal pain, headache, anorexia, and diarrheal symptoms when the total daily intake of allulose was gradually increased to 1.0 g per kg bw. These symptoms of gastrointestinal discomfort are transient and generally not considered to be of toxicological significance.

a. Clinical Trials

Numerous clinical trials have been conducted on allulose for various health related endpoints. While these studies were not designed with safety-related endpoints, they are summarized in Table 11 to outline the relevant study details in order to assess tolerability and safety. Blue California has reviewed the data and agrees with the safety conclusions of these studies.

Table 11. Summary of Clinical Studies

Study Setup and Details	Human Study Results, Significance, Safety	Reference
Study Design: Randomized, double-blinded, placebo-controlled trial Study Length: 52 weeks total; 48 weeks consumption and 4-week post-consumption observation period Subjects: Men and women with high LDL-C levels ranging from 120-159 mg/dL, fasting blood glucose ranging from 100-125 mg/dL, or hemoglobin HbA1c levels of 6.0-6.5%; ages 20-65 years, Dose, Delivery, and Frequency: Placebo – no allulose (n=28); low dose - 5 g/day allulose (n=27), and high dose: 15 g/day allulose (n=27). Allulose was	Outcome Measurements • Physical examination, blood biochemical marker analysis, and urine analysis (protein, lipid, saccharide, electrolyte, hepatic function and renal function), hematological parameters, urine analysis were performed at each examination. • On examination day, fasting morning urine and blood were collected, physical measurements were taken. Routine blood biochemical marker analysis, hematological parameter measurements and urine analysis were performed at each examination timepoint. Additional endpoints were evaluated at various timepoints as detailed in the publication including the absolute risk of atherosclerotic cardiovascular disease (ASCVD) which was calculated and divided into three groups (low risk, moderate risk, and high risk). • Examinations were performed 4 weeks prior to the start of consumption, on the first day of consumption, and then on week 8, 16, 24, 32, 40, and 48 after starting consumption. • A 75 g oral glucose tolerance test was performed on the first day of the consumption period and at 48 weeks after starting consumption	Tanaka et al. (2020)

Study Setup and Details	Human Study Results, Significance, Safety	Reference
administered as a beverage 30 min. before breakfast	Results and Significance • No significant increase in total cholesterol and LDL-C for the allulose group in comparison with the placebo group, no change in risk factors for atherosclerotic cardiovascular disease • No clinical issues for other parameters • Small declines of DAST, DALT, DALP, and Dg-GTP observed in the allulose groups compared to placebo were not of toxicological significance. • Changes in fatty liver significantly improved in the allulose groups compared to placebo. Safety Measurements/Adverse Events Reported: • Adverse events were observed during the dosing period: 42 episodes in 19 subjects in the placebo group, 34 episodes in 22 subjects in the 5 g/day group, and 38 episodes in 21 subjects in the 15 g/day group were observed, respectively. No significant differences in the incidence of adverse events were found between the placebo and treatment groups. The principal physician determined none of the adverse events were treatment-related. No increase in ASCVD risks was reported. The authors concluded that allulose consumption is safe for long-term intake up to a year, and allulose may be effective for improving hepatic functions and glucose metabolism.	
Study Design: Open trial Study Length: 16 weeks (12-week consumption period followed by a 4-week observation period) Subjects: n=18; 9 men and 9 women; 12 subjects had borderline type 2 diabetes and 6 had Type 2 diabetes; ages 20-75 years Dose, Delivery, and Frequency: 5 g/subject three times daily with meals	Outcome Measurements: • General physical parameters included height, body weight, body mass index, body fat percentage, waist circumference, systolic blood pressure, diastolic blood pressure and pulse. Biochemical, hematological, and general urine analysis parameters were also evaluated. Living habits were also recorded. • The authors note that there are limitations with this study – small sample size and influencing factors could not be excluded because of study design (open study/no control group). Results and Significance: • One woman was removed from the study because she was found to be ineligible after 2 weeks. One man was dropped from the study because he had increased hepatic markers (total bilirubin, direct bilirubin, AST, ALT, and γ-GTP). • There were improvements in uric acid and B2-microglobulin values over time. • There were significant increases in total cholesterol and LDL-C compared with the first day of consumption; however, they were short term and attributed to seasonal variation and not considered to be a serious issue.	Tanaka et al. (2019)

Study Setup and Details	Human Study Results, Significance, Safety	Reference
	• There were significant improvements in hepatic functions (γ-GTP and ALP • There were no significant changes in urine values. Positive urine protein and occult blood were not related to changes in renal blood parameters and were therefore not thought to be related to allulose intake Safety Measurements/Adverse Events Reported: • No serious clinical symptoms occurred. Twenty-nine adverse events were reported by 10 subjects and, of them, only constipation was thought to potentially be related to allulose consumption.	
Study Design: randomized double-blind, placebo-controlled trial Study Length: 12 weeks Subjects: n=121 (n=48/group); male and females with a BMI ≥23 kg/m^2 (20-40 years) Dose, Delivery, and Frequency: low dose = 4 g/subject twice daily and high dose = 7 g/subject twice daily; given as a grapefruit flavored non-carbonated drink	Outcome Measurements: • Sucralose was included as the placebo control • Parameters for body composition, nutrient intake, computed tomography scan and plasma lipid profiles were assessed. Results and Significance: • There was no significant difference in nutrient intake, plasma lipid profiles, markers of liver and kidney function and major inflammation markers between groups. Safety Measurements/Adverse Events Reported: • The authors did not report that adverse events were monitored.	Han et al. (2018b)
Study Design: non-randomized control trial Study Length: Experiment 1: 11 acute exposures separated by 1 week washouts; Experiment 2: 6 days Subjects: Experiment 1: n=30 healthy men (n=15) and women (n=15) with a BMI of ≤23 kg/m^2 (21-30 yrs). Experiment 2: n=19 healthy men (n=10) and women (n=9) Dose, Delivery, and Frequency: dose gradually increased in steps; given as a grape-flavored non-carbonated drink.	Outcome Measurements: • Two single-group open studies were done, with a separation of 7 days • Experiment 1: the dose gradually increased in steps of 0.1 g/kg bw, with a 1-week washout between doses, to a dose of 0.6 g/kg bw during week 11, to identify the maximum single dose for occasional ingestion. Each dose level was consumed once daily for 7 days • When the maximum dose for occasional consumption was identified in Experiment 1, Experiment 2 was conducted to determine the maximum total daily intake for regular ingestion. The subjects consumed increasing doses of allulose each day. • For both studies, the subjects were asked to record the incidences and magnitudes of the gastrointestinal (GI) responses for the 24-hour period following the consumption of the test products. Results and Significance: • In Experiment 1, no severe diarrhea or GI symptoms were noted until a dose of 0.4 g/kg. Severe symptoms of diarrhea were noted at 0.5 g/kg bw.	Han et al. (2018a)

Study Setup and Details	Human Study Results, Significance, Safety	Reference
	• In Experiment 2, increasing the total daily allulose intake gradually to 1.0 g/kg bw for regular ingestion resulted in incidences of severe nausea, abdominal pain, headache, anorexia, and diarrhea. • The authors concluded that the maximum single dose and maximum total daily intake of allulose should be 0.4 g/kg bw and 0.9 g/kg bw, respectively. Safety Measurements/Adverse Events Reported: • GI effects were observed during these studies.	
Study Design: double-blind, multiple crossover, randomized, controlled, acute feeding, equivalence trial Study Length: multiple crossover; 1 week wash out Subjects: n=24 male and female subjects (ages 18-75 yrs) with type II diabetes Dose, Delivery, and Frequency: single dose of 0, 5, or 10 g allulose or fructose added to a 75 g glucose drink	Outcome Measurements: • Each participant was randomly assigned to six treatments separated by ≥1-week washout period. • A standard oral glucose tolerance test protocol was followed and blood samples were collected 30 minutes before dosing and at 0, 30, 60, 90, and 120 minutes post dosing. • The main outcome measured was the plasma glucose incremental area under the curve. Results and Significance • Allulose significantly reduced plasma glucose incremental area under the curve by 8% at 10 g dose with a linear dose response. Safety Measurements/Adverse Events Reported: • It was reported that most participants tolerated the treatments well and there were no specific reports of adverse effects with allulose.	Noronha et al. (2018b) and Braunstein et al. (2018)
Study Design: randomized, single-blind crossover design with a 1-week washout period between treatments Study Length: acute Subjects: n=13 healthy male and female subjects (mean age 35.7±2.1 years) Dose, Delivery, and Frequency: 5 g allulose, or 10 mg of aspartate once per subject	Outcome Measurements: • At 30 minutes after taking 5 g of allulose or 10 mg aspartate without sugar as a control, the overnight-fasted subjects ingested a standardized meal. The energy metabolism was evaluated by a breath-by-breath method. Blood was collected during the experiment and biochemical parameters were analyzed. Safety Measurements/Adverse Events Reported: • No adverse effects were reported.	Kimura et al. (2017)
Study Design: randomized double-blind, placebo-controlled parallel-group Study Length: single dose Subjects: n=26 randomly assigned to two groups; healthy male and female subjects with fasting blood glucose levels between 100 - 126 mg/dL	Outcome Measurements: • Meal-loading experiment, single meal with a one-week washout period • Each subject was given tea with test or control substance (aspartame) and a standard meal. After the one week they were given another sample of tea with the same standard meal. They were not allowed to eat or drink anything else until the next day.	Hayashi et al. (2010)

Study Setup and Details	Human Study Results, Significance, Safety	Reference
Dose, Delivery, and Frequency: 5 g allulose in tea three times daily with a meal	• Fasting blood was collected within 1 hour prior to the meal and then blood was collected at 30, 60, 90, and 120 minutes after the meal. Blood glucose level and insulin level were evaluated. Safety Measurements/Adverse Events Reported: • Adverse events were not monitored in the single dose portion of the study.	
Study Design: randomized double-blind, parallel group Study Length: 12 weeks Subjects: n=18 randomized to 2 groups; healthy male and female subjects with fasting blood glucose levels below 110 mg/dL Dose, Delivery, and Frequency: 5 g allulose in tea three times daily with a meal	Outcome Measurements: • The study involved a 2-week observation period before starting the treatment and a 4-week observation period following the 12-week treatment period. Each subject consumed 5 g of either allulose or aspartame, three times daily, for 12 weeks. • Fasting morning urine and blood were collected 2 weeks before treatment, on the first day of treatment, 2, 4, 8 and 12 weeks after start of the treatment and 4 weeks after completing the treatment. • Physical examinations (height, body weight, body mass index, body fat percentage, waist circumference, systolic blood pressure, diastolic blood pressure and pulse rate), blood sample analysis (total protein, albumin, albumin, globulin ratio, total bilirubin, direct bilirubin, indirect bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactase dehydrogenase, gamma glutamyl transpeptidase, cholinesterase, creatine phosphokinase, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, remnant-like particle cholesterol, triglyceride, free fatty acid, phospholipids, urea nitrogen, uric acid, creatinine, sodium, potassium, chlorine, calcium, inorganic phosphate, magnesium, serum amylase, glucose, insulin, glycoalbumin, white blood cells, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and platelets), urine analysis (protein, glucose, urobilinogen, specific gravity and occult blood) were measured. Interviews were conducted on each examination day. • Body weight and percentage of body fat were determined, as well as body mass index two weeks prior to the experiment. Results and Significance: • No abnormal effects or clinical problems were noted during the continuous ingestion of 15 g allulose/day for 12 weeks. Safety Measurements/Adverse Events Reported: • Subjects were examined throughout the study. One male in the test group had moderate symptoms in the lower digestive tract (diarrhea, borborygmus, and increased defecation frequency) starting at 4 weeks of treatment. One female in the control group	Hayashi et al. (2010)

Study Setup and Details	Human Study Results, Significance, Safety	Reference
	had moderate borborygmus and flatus throughout the treatment period. The frequency of adverse events was the same in both treatment groups.	
Study Design: Crossover Study Length: acute Subjects: n=21 healthy male and female subjects Dose, Delivery, and Frequency: Study 1: allulose 0.35 g/kg bw once (20 g per subject); Study 2 and 3: 20, 10, or 5 g per subject; Study 4: 15 g allulose per subject per day	Outcome Measurements: • Study 1: six subjects participated, they ingested either starch hydrolysate, allulose, or water alone at intervals of at least 1 week. Subjects consumed an evening meal the day before the study and then did not consume any food or drink other than water from then to the completion of measurements. Respiratory exchange was measured shortly after ingestion for 180 minutes. Urine was collected at the end of the measurement. • Studies 2 and 3: fourteen subjects participated in these two studies; Fructooligosaccharides (FOS) was used as a positive control. Subjects ingested either 20, 10, or 5 g of allulose or FOS and no ingestion was used as a negative control. Each measurement was randomly performed at intervals of at least one week. Standard meals were given during the study at 4 and 8 hours after test sample ingestion. End expiratory gas was collected at 1-hour intervals for 10 hours. Urine was collected for 48 hours. • Study 4: eight subjects participated and ingested 5 g of allulose three times daily for 8 weeks. End-expiratory gas collection was collected on the first and last day of ingestion where they ingested 15 g of allulose before collection. Results and Significance: • Based on the results of the plot of breath hydrogen concentration versus the calories ingested, the energy value of allulose was expected to be less than 1.6 kJ/g. Incremental allulose fermentability subsequent to an adaptation period was not observed. Safety Measurements/Adverse Events Reported: • The authors did not report that adverse events were monitored.	Iida et al. (2010)
Study Design: crossover; 1-week washout Study Length: acute Subjects: n=20; healthy male and female subjects (ages 20-39 years) with fasting plasma glucose of 100 mg/100 mL or less Dose, Delivery, and Frequency: single dose of 7.5 g allulose	Outcome Measurements: • Subjects consumed one of five test beverages: 7.5 g allulose alone, 75 g maltodextrin alone, 75 g maltodextrin with either 2.5, 5, or 7.5 g allulose, with a 1-week washout period between. The order of intake was randomly assigned. • The subjects were fasted for 12 hours, blood was collected, and then the subjects consumed the test beverage. Blood was again collected at 30, 60, 90, and 120 minutes after intake. Plasma glucose was determined for each timepoint. Safety Measurements/Adverse Events Reported: • The authors did not report that adverse events were monitored.	Iida (2008)

Study Setup and Details	Human Study Results, Significance, Safety	Reference
Study Design: Microtracer study **Study Length:** one week **Subjects:** 8 healthy men **Dose, Delivery, and Frequency:** Single oral dose of 776nCi[14C(U)} rare sugar ^{14}C radio labeled tracer[1] in a beverage	Outcome Measurements: • Subjects consumed a beverage containing 15 g of 776nCi [14C(U)-rare sugar (99% purity). • Blood, urine, fecal, and expired air samples collected at baseline and at multiple points during the study through 168 h. • Plasma C_{max} occurred approximately 1.5 hours after dosing. • Urinary total radioactivity was 48.02-90.25% of the dose over the collection period. • Fecal total radioactivity was 1.79-5.65% of the dose. • Expired air radioactivity detection over 6 hours was negligible. • Results indicate the ^{14}C-rare sugar is absorbed but not metabolized	Williamson et al. (2014) (Abstract only)

[1] This sugar was not defined as allulose in the abstract; however, the rare sugar was identified as allulose in the following document: https://www.tateandlyle.com/sites/default/files/2017-12/tate-lyle-sweetener-brochure-2017%20%281%29_2.pdf

C. GRAS Criteria

FDA defines "safe" or "safety" as it applies to food ingredients as:

> "…reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use."[16]

Amplification is provided in that the conclusion of safety is to include probable consumption of the substance in question, the cumulative effect of the substance and appropriate safety factors. It is FDA's operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that:

> "…General recognition of safety requires common knowledge, throughout the expert scientific community knowledgeable about the safety of substances directly or indirectly added to food, that there is reasonable certainty that the substance is not harmful under the conditions of its intended use."
>
> "'Common knowledge' can be based on either "scientific procedures" or on experience based on common use of a substance in food prior to January 1, 1958."[17]

[16] See 21 CFR 170.3 (e)(i) and 81 FR 54959 Available at: https://www.federalregister.gov/documents/2016/08/17/2016-19164/substances-generally-recognized-as-safe (Accessed on 9/8/18).
[17] See 81 FR 54959 Available at: https://www.federalregister.gov/documents/2016/08/17/2016-19164/substances-generally-recognized-as-safe (Accessed on 8/26/19).

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called "common knowledge element," in terms of the two following component elements:[18]

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and
- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as JECFA and the National Academy of Sciences.

General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive. General recognition of safety through scientific procedures shall be based upon the application of generally available and accepted scientific data, information, or methods, which ordinarily are published, as well as the application of scientific principles, and may be corroborated by the application of unpublished scientific data, information, or methods.

The apparent imprecision of the terms "appreciable," "at the time," and "reasonable certainty" demonstrates that the FDA recognizes the impossibility of providing absolute safety in this or any other area (Lu, 1988; Renwick, 1990; Rulis and Levitt, 2009).

As noted below, this safety assessment to ascertain GRAS status for the specified food uses meets FDA criteria for reasonable certainty of no harm by considering both the technical and common knowledge elements.

D. Expert Panel Findings on Safety of Blue California's Allulose

An evaluation of the safety and GRAS status of the intended use of Blue California's Allulose has been conducted by an Expert Panel convened by GRAS Associates; the Panel consisted of Margitta Dziwenka, DVM, DABT; Michael Falk, Ph.D.; and Katrina Emmel, Ph.D., as Panel Chair. The Expert Panel reviewed Blue California's dossier as well as other publicly available information available to them. The individuals who served as Expert Panelists are qualified to evaluate the safety of foods and food ingredients by merit of scientific training and experience.

The GRAS Expert Panel report is provided in Appendix 8.

[18] See Footnote 1.

E. Common Knowledge Elements for GRAS Conclusions

The first common knowledge element for a GRAS conclusion requires that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing studies published in peer-reviewed scientific journals. The second common knowledge element for a GRAS conclusion requires that consensus exists within the broader scientific community.

1. Public Availability of Scientific Information

With regard to the safety documentation, the key data evaluated to establish safety is published in the scientific literature. In addition, FDA has reviewed GRAS notices that describe the scientific basis for the conclusion of the GRAS status of allulose with similar proposed uses and use levels and has responded with “no questions”. There are publicly available GRAS notices and “no questions” responses from FDA to these GRAS notices on FDA’s website.

2. Scientific Consensus

The second common knowledge element for a GRAS conclusion requires that there be a basis to conclude that consensus exists among qualified scientists about the safety of the substance for its intended use.

The enzyme used in the manufacturing of Blue California’s Allulose is manufactured from a strain of *E. coli* K12 that is expected to be non-toxigenic and non-pathogenic and is not known to produce biogenic amines. Analysis of the finished product confirms the absence of residual enzyme in the finished product. Blue California affirms that Allulose is manufactured under CGMP conditions with raw materials and processing aids that meet the appropriate food grade regulations. Blue California has established sufficient rigorous product specifications and has demonstrated batch-to-batch consistency against these specifications. In addition, the stability of Blue California’s Allulose has been demonstrated in studies conducted on five lots of Allulose for 6 months under storage conditions of 40°C±2°C and 75%± 5% RH.

Blue California notes that the chemical composition and specifications of our Allulose is of equivalent quality to the allulose preparations described in GRNs 693 and 828. Some parameters established by other manufacturers, such as specifications for protein and fat, do not alter the conclusion that Blue California’s Allulose is substantially equivalent to the allulose preparations described in GRNs 693 and 828, which received “no questions” letters from FDA.

In addition, Blue California has reviewed safety information about allulose, including information about the absorption, metabolism, distribution and excretion of allulose as well as *in vitro,* acute, subacute, subchronic, chronic, and reproductive toxicity studies of allulose. The LD_{50} of allulose ranges from 15.8 to 16.3 g per kg in rats. A subchronic toxicity study in which rats were administered allulose for 90 days reported that the NOAEL was 3% of the diet, which is equivalent to 1.67 g per kg bw per day and was the highest dose tested (Matsuo et al., 2012). A subchronic toxicity study in Sprague Dawley

rats reported a NOAEL of 5,000 mg per kg bw per day (An et al., 2019). A reproductive toxicity study reported that the NOAEL for parental animals and their offspring was 2,000 mg per kg bw, the highest dose tested (Kim et al., 2019). A chronic toxicity study in rats reported that the NOAEL for allulose was 1,280 mg per kg bw per day (Yagi and Matsuo, 2009).

Multiple human studies demonstrate the safety and lack of adverse events in humans at intake levels up to 15 g per day for 12 weeks (Han et al., 2018b; Hayashi et al., 2010; Tanaka et al., 2019) and 48 weeks (Tanaka et al., 2020), respectively.

Using 2015-2018 NHANES data, Blue California determined that the EDI for individuals ages 2 years or greater is 8.6 g per person per day at the mean and 19.1 g per person per day at the 90th percentile, based on the proposed uses and use levels detailed in Part 3.A.2. On a mg per kg bw per day basis, for an individual weighing 70 kg, this intake is equivalent to 0.12 g per kg bw per day for an individual at the mean and 0.27 mg per kg bw per day for an individual in the 90th percentile. The maximum tolerable single dose level of allulose in humans was reported to be 0.5 g per kg bw for males and 0.6 g per kg bw for females (Iida et al., 2007), which is higher than the calculated EDIs determined for Blue California's Allulose. Furthermore, Han et al. (2018a) concluded that the maximum single dose of allulose and the maximum daily intake of allulose should be 0.4 g per kg bw and 0.9 g per kg bw per day, respectively. Blue California notes that the highest 90th percentile estimated daily intake for Allulose for any subpopulation is 0.50 g per kg bw per day, which is well-below the maximum daily intake reported by Han et al. (2018a). FDA has previously determined that such exposures to allulose resulting from the similar uses and use levels are GRAS. Exposure from consuming doses of allulose that occur naturally in foods is negligible. Blue California's Allulose would be expected to be used in place of other allulose products that are currently on the market.

The proposed uses and use levels of Blue California's Allulose are similar to those that have been proposed in GRAS notices which have received "no questions" responses from FDA, with the addition of proposed uses in grain based cereal and protein bars; low-sugar, reduced-sugar, and diet fruit juices; and low- and reduced-calorie alcoholic beverages. While Blue California proposes expanded uses, it should be noted that the resulting EDIs determined using 2015-2018 NHANES data are lower than those presented in GRN 828, the most recent GRAS Notice to receive a "no questions" letter from FDA. A number of well-respected regulatory agencies, including FDA and Health Canada, as well as numerous well-respected individual scientists, have concluded that allulose is safe for human consumption at levels similar to those proposed for Blue California's Allulose.

In summary, a compelling case can be made that scientific consensus exists regarding the safety of allulose. Based on the information reviewed herein, Blue California has concluded that our Allulose preparation is generally recognized as safe for the proposed uses at the proposed use levels for the specified food applications and maintains that well-qualified scientists would concur.

F. Conclusion

In consideration of the aggregate safety information available on allulose, Blue California concludes that Allulose as defined in the subject notification and produced in accordance with FDA Current Good Manufacturing Practices, is safe for use as a sugar substitute or sweetener, in foods other than infant formulas or meat and poultry products. The dietary levels from anticipated food consumption are not likely to exceed the ADI when Allulose is used as proposed in this notification.

This declaration has been made in accordance with FDA's standard for food ingredient safety, i.e., reasonable certainty of no harm under the intended conditions of use as described in this dossier and, therefore, Blue California's Allulose is generally recognized as safe (GRAS) within the meaning of the Food, Drug, and Cosmetic Act.

PART 7. LIST OF SUPPORTING DATA AND INFORMATION IN THE GRAS NOTICE

A. References

1. List of Acronyms

µg	Microgram
ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism and Excretion
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AOAC	Association of Official Agricultural Chemists
AST	Aspartate aminotransferase
AUCs	Area under the curves
bw	Body weight
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
CGMP	Current Good Manufacturing Practice
C_{max}	Maximum plasma concentration
DAE	D-allulose 3-epimerase
dL	Deciliter
EDIs	Estimated Daily Intakes
EPA	Environmental Protection Agency
EU	European Union
F	Female
FD&C Act	Federal Food Drug and Cosmetics Act
FOIA	Freedom of Information Act
FOS	Fructooligosaccharide
FSANZ	Food Standards Australia New Zealand
g	gram
GI	Gastrointestinal
GRAS	Generally Recognized as Safe
HPLC	High Performance Liquid Chromatography
ICP-MS	Inductively coupled plasma-mass spectrometry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kcal	Kilocalories
kg	kilogram
kJ	Kilojoules
LB	Luria Broth
LD_{50}	Median (50%) lethal dose
LETO	Long-Evans Tokushima Otsua
M	Male
mL	milliliter
MPN	Most probable number
MRI	Magnetic resonance imaging
MW	Molecular weight
N	Number
NHANES	National Nutrition and Health Examination Survey
NOAEL	No Observed Adverse Effects Level
NS	Not specified
OLETF	Otsuka Long-Evans Tokushima Fatty
PCR	Polymerase chain reaction

ppm	parts per million
PSUs	Primary Sampling Units
RH	Relative humidity
SCFA	Short chain fatty acids
U.S.	United States
USP	United States Pharmacopoeia
wt	weight
y	years

2. References

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B. Appendices

Appendix 1 Manufacturing Declaration

blue california

30111 Tomas
Rancho Santa Margarita, CA 92688
T: 949.635.1990 F: 949.635.1986

May 19, 2021

Manufacturing Declaration

We hereby declare that the strain of E coli used in the enzyme production of Allulose manufacturing process does not produce any toxic amines.

We certify this to be true to the best of our knowledge.

Sincerely,

Hadi Omrani

Hadi Omrani
Director, Technical and Regulatory Affairs

Appendix 2 Raw Materials, Processing Aids, and Additives Used to Manufacture Allulose

Name	CAS #	Function	Grade
Sodium phosphate, monobasic	7558-80-7	Fermentation	Food grade
Sodium phosphate, dibasic	7558-79-4	Fermentation	Food grade
NaCl, non-iodized	7647-14-5	Fermentation	Food grade
NaOH	1310-73-2	Fermentation Extraction	Food grade
Phosphoric acid	7664-38-2	Fermentation	Food grade
Yeast extract	--	Fermentation	Food grade
Yeast peptone	--	Fermentation	Food grade
Glycerin	56-81-5	Fermentation	Food grade
Trizma® base	77-86-1	Bioconversion	--
HCl	7647-01-0	Bioconversion Extraction	Food grade
Manganese sulfate	15244-36-7	Bioconversion	Food grade
D-fructose	57-48-7	Bioconversion	Food grade
Ethanol	64-17-5	Extraction	Food grade
Calcium chloride	10043-52-4	Extraction	Food grade
Calcium acetate	62-54-4	Extraction	Food grade

Appendix 2.1 Sodium Phosphate, Monobasic

Certificate of Analysis Page 1 of 1

Fisher Scientific

1 Reagent Lane
Fairlawn, NJ 07410
201.796.7100 tel
201.796.1329 fax

Certificate of Analysis

Fisher Scientific's Quality System has been found to conform to Quality Management System Standard ISO9001:2008 standard by DNV Certificate number CERT-08052-2006-AQ-HOU-ANAB

This is to certify that units of the above mentioned lot number were tested and found to comply with the specifications of the grade listed. Certain data have been supplied by third parties. Fisher Scientific expressly disclaims all warranties, expressed or implied, including the implied warranties of merchantability and fitness for a particular purpose. Certain products (USP/FCC/NF/EP/BP/JP grades) are sold for use in food, drug, or medical device manufacturing. Fisher does not claim regulatory coverage under 21 CFR nor maintain DMF's with the FDA. The following are the actual analytical results obtained:

Catalog Number	**BP329**	**Mfg. Date**	**4/6/2010**
Lot Number	**100393**		
Description	**SODIUM PHOSPHATE, MONOBASIC, ANHYDROUS**		
Country of Origin	**Israel**		
Chemical Origin	**Inorganic-non animal**		
BSE/TSE Comment	No animal products are used as starting raw material ingredients, or used in processing, including lubricants, processing aids, or any other material that might migrate to the finished product.		

Result name	Units	Specifications	Test Value
APPEARANCE		REPORT	Colorless to white crystals
ASSAY	%	>= 99	100.1
HEAVY METALS (as Pb)	%	<= 0.001	<0.0010
IDENTIFICATION	PASS/FAIL	= PASS TEST	PASS TEST
INSOLUBLE MATTER	%	<= 0.03	0.010
MOISTURE CONTENT	%	<= 0.5	0.10
pH OF A 1 MOLAR SOLN		Inclusive Between 4.0 6.0	4.1

Lab Manager Fairlawn

Note: The data listed is valid for all package sizes of this lot of this product, expressed as a extension of this catalog number listed above. If there are any questions with this certificate, please call Chemical Services at (800) 227-6701.

Appendix 2.2 Sodium Phosphate, Dibasic

SIGMA-ALDRICH sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:
Sodium phosphate dibasic – for molecular biology, ≥98.5% (titration)

Na_2HPO_4

Product Number:	**S3264**
Batch Number:	**SLBT7509**
Brand:	SIGMA
CAS Number:	7558-79-4
MDL Number:	MFCD00003496
Formula:	HNa2O4P
Formula Weight:	141.96 g/mol
Quality Release Date:	13 APR 2017
Recommended Retest Date:	APR 2020

Test	Specification	Result
Appearance (Color)	White	White
Appearance (Form)	Powder	Powder
Solubility (Color)	Colorless	Colorless
Solubility (Turbidity) 100 mg/mL, H2O	Clear	Clear
Loss on Drying	≤ 0.1 %	0.0 %
Chloride (Cl)	≤ 40 ppm	20 ppm
Iron (Fe)	≤ 20 ppm	20 ppm
Heavy Metals (as Lead)	≤ 10 ppm	10 ppm
Titration with HCl	≥ 98.5 %	99.6 %
DNase, Exonuclease Detection	None Detected	None Detected
RNase Detection	None Detected	None Detected
NICKase,Endonuclease Detection	None Detected	None Detected
Protease Detection	None Detected	None Detected

Rodney Burbach, Manager
Analytical Services
St. Louis, Missouri US

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Version Number: 1 Page 1 of 1

Appendix 2.3 Sodium Chloride, Non-ionized

Test Report

(2015) Commission Checked No. 4

Product Name: Non-iodized refined salt
Specifications and Model: N/A
Trademark: N/A
Trust Unit: Zhongyan Dongxing Yanhua Co., Ltd.
Manufacture: Zhongyan Dongxing Yanhua Co., Ltd.
Test Category: Commissioned inspection

QUALITY SUPERVISION INSPECTION CENTER OF NATIONAL LIGHT INDUSTRY WELL MINERAL SALT ADMINISTRATION

1

Description

1. Entrusted inspection is only responsible for the sample.
2. This Inspection Report is invalid if no official seal of the inspection unit.
3. The copy of this Inspection Report is invalid if no official seal of the re-stamped inspection unit.
4. Altered "Inspection Report" is invalid.
5. If there is any objection to the Inspection Report, please submit written opinions to the inspection unit within 15 days from the date of receipt of the Inspection Report, and shall be deemed to recognize the Inspection Report.
6. If no preparation, inspection, review, and approval of the signature, the Inspection Report is invalid.
7. If no objection to the Inspection Report within one month after receipt, the sample should be taken back, otherwise it will be dealt with in accordance with the relevant provisions.

Brief Introduction of Quality Supervision and Testing Center of National Light Industry Well Salt

The Center has passed the China National Accreditation Board for accreditation of Conformity Assessment Laboratory and Food Inspection Agency. The laboratory is in good condition and well equipped, mainly to carry out salt products, food, chemical products, food additives, and feed additives testing, but also bear the quality supervision and inspection, revision of national standards, industry standards and test methods of research, testing personnel technical training, and technical advice business.

Address: No. 11 Dongxing Temple, Zigong City, Sichuan Province
Zip code: 643000
Tel: (0813) 8104587
Fax: (0813) 8207279

QUALITY SUPERVISION INSPECTION CENTER OF NATIONAL LIGHT INDUSTRY WELL MINERAL SALT ADMINISTRATION

Test Report

Product Name	Non-iodized refined salt	Trademark	N/A
Trust Unit	Zhongyan Dongxing Yanhua Co., Ltd.	Specifications and Model	N/A
Address	Dindyuan Salt Mine, Dingyuan County, Chuzhou City, Anhui Province	Sampling Batch	80t
Zip Code	N/A	Sample Amount	500g
Product Unit	Zhongyan Dongxing Yanhua Co., Ltd.	Sample Grade	N/A
Sampling Date and Site	N/A	Sent Date	01/07/2015
Production Date / Lot Number	2015.01.05	Sent By	Sufang Chen
Test Date	01/13/2015	Test Category	Commissioned inspection
Test Standard(s)	GB5461-2000 GB/T5009.15-2003 GB/T5009.17-2003	Environment	11°C
Sample Reception Description	Mailed, plastic bag packaging, packaging intact, the sample is white granular solid.		
Test Conclusion	Based on GB 5461-2000 and GB2762-2012, the sample meets the requirement of non-iodized refined edible salt excellent grade. (Stamp) Date of Issue: 01/20/2015		
Remarks	All information related to the sample, except the inspection result, is provided by the client, who is responsible for the authenticity of the information provided.		

Approver: Wenjie Lei Examiner: Shuying Fu Major Tester: Qian Tan
Prepared by: Zhiyong Chen

QUALITY SUPERVISION INSPECTION CENTER OF NATIONAL LIGHT INDUSTRY WELL MINERAL SALT ADMINISTRATION

Test Report

Test Items	Specification	Test Results	Evaluation
Level of whiteness, degree	>= 80	88	Pass
Granularity (0.15 – 0.85) mm, %	>= 85	99	Pass
NaCl, %	>= 99.10	99.45	Pass
Moisture, %	<= 0.30	< 0.01	Pass
Water-insoluble, %	<= 0.05	< 0.01	Pass
As, mg/kg	<= 0.5	< 0.5	Pass
Pb, mg/kg	<= 2.0	< 2.0	Pass
Cd, mg/kg	<= 0.5	< 0.005	Pass
Total Hg, mg/kg	<= 0.1	< 0.025	Pass
Ba, mg/kg	<= 15.0	< 15.0	Pass
$[Fe(CN)_6]^{4-}$, mg/kg	<= 10.0	4.8	Pass
I, mg/kg	< 5	0.1	Pass
Sensation: white, taste salty, no strange smell, no obvious foreign substance that is not related to salt.	Meet the requirements	Meet the requirements	Pass

Blank Below

Appendix 2.4 Sodium Hydroxide

Binzhou Product Quality Supervision and Inspection Institute

Test Report

No. H2017- W-10002 Page 1 of 1

Sample Name		Food Additive NaOH	Specifications & Model	98.0-100.5%	Registered Trademark	/
Consignor		Befar Group Co., Ltd.			Test Type	Consigned Inspection
Producer		Befar Group Co., Ltd.			Sample Grade	Qualified product
Sampling Site		/			Consigned Person	Tian Yuhong
Sampling Base		/	Quantity of Sample	1,000 g	Date of Collection	February 7, 2017
Inspection Requirements		Total alkali, sodium carbonate			Batch No. or Date of Production	20170125B January 25, 2017
Test Standard(s)		GB 1886. 20-2016			Sample State	Solid
Test Content	Serial No.	Test Item	Standard Requirements		Test Result	Individual Judge
	1	Color	White or almost white		White	Qualified
	2	Status	Solid		Solid	Qualified
	3	Total alkali (measuring in NaOH), ω/%	98.0-100.5		99.1	Qualified
	4	Sodium carbonate (measuring in Na_2CO_3), ω/%	≤2.0		0.6	Qualified
	5	As, mg/kg	≤3.0		<0.01	Qualified
	6	Heavy metal (measuring in Pb), mg/kg	≤5		<5	Qualified
	7	Insoluble substance and organic impurity	Pass		Pass	Qualified
	8	Hg, mg/kg	≤0.1		0. 002	Qualified
Test Conclusion		Upon testing, the sample has met the standard requirements of the test items. Signature Date: February 16, 2017 *(Special Seal of Testing and Inspection of Binzhou Product Quality Supervision and Inspection Institute)*				
Notes		1. "/" means no content. 2. The sample information is provided by the entrusting party and thus the entrusting party shall be responsible for the authenticity of such information.				

Approver: *(Signature of Liu Xitao)*, February 16, 2017)
Reviewer: *(Signature of Tao Gongting)*, February 16, 2017
Chief Inspector: *(Signature of Wu Linmen)*, February 16, 2017

申明

Statement

敬启者，

To whom it may concern,

我公司供应的液体氢氧化钠符合 Food Chemicals Codex (FCC), 3d Ed. (1981)要求，特此申明。

We hereby declare that this ingredient, Sodium Hydroxide Solutions meets the specification of the Food Chemicals Codex (FCC), 3d Ed. (1981).

合肥德泽化工科技有限公司

2021.06.17

Appendix 2.5 Phosphoric Acid

Jiangsu ChengXing Phosph-Chemicals Co.

Inspection Report

CERTIFICATE OF ANALYSIS

Product Name	85% Phosphoric Acid		Product Performance Standards	GB/T 2091-2008	
Production Batch	17020702	Production Date	2017.02.07	Package	500 ml

Sensory requirements

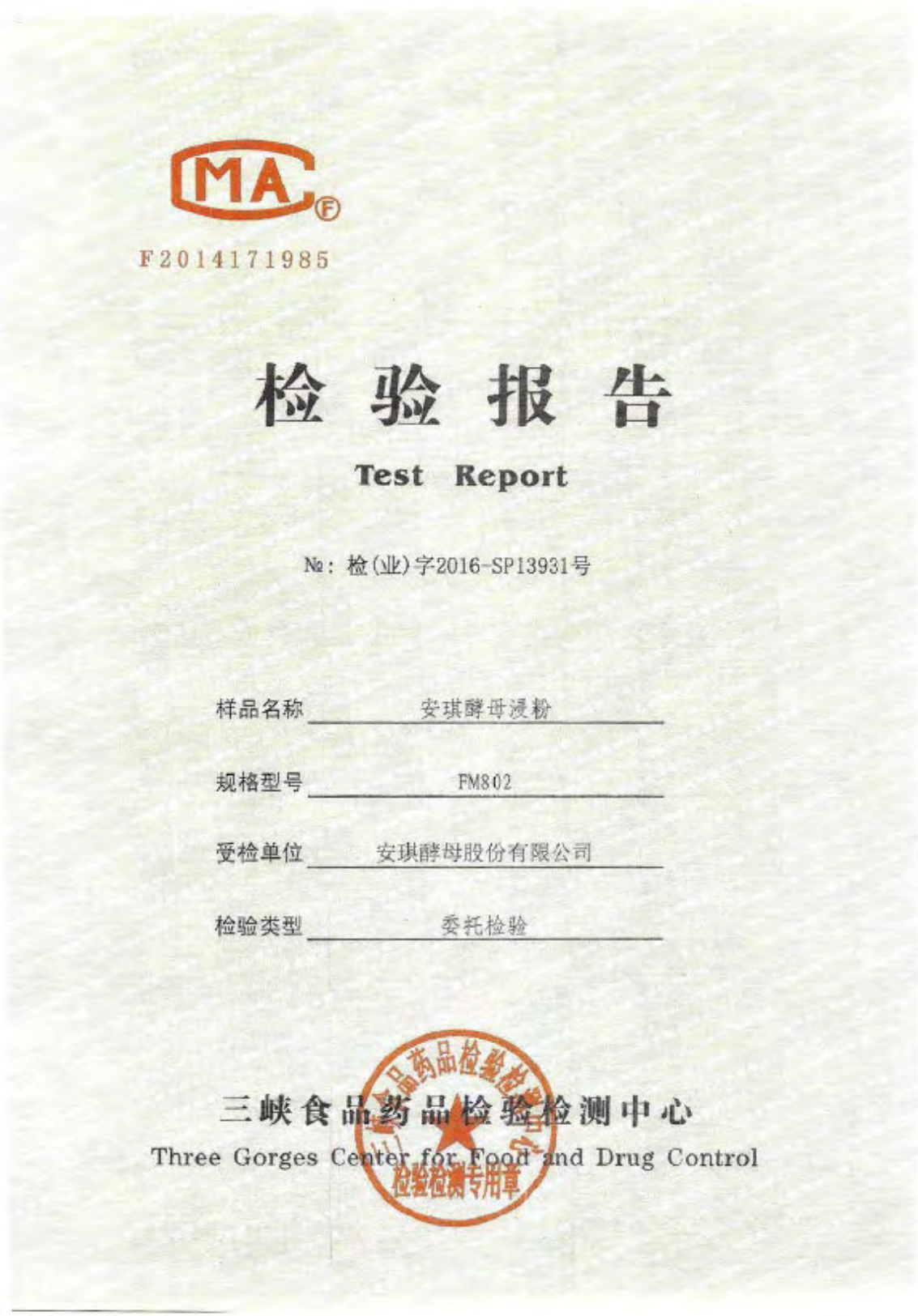
Test Items	Claim	Testing Method	Test Value	Determinatio
Color	Color Hazen ≤ 20	UV/VIS Spectrophotometer, VISUAL	< 20	PASS
Appearance	Transparent, light thick liquid		Liquid	PASS

Quality Index

Project	Index	Test Value	Test Based On	Determination
Phosphoric Acid（H_3PO_4）, %	> 85.0	85.5	GB/T 2091-2008	PASS
Chloride（to Cl meter）, %	< 0.0005	< 0.0005	GB/T 2091-2008	PASS
Sulfate (to SO_4 meter), %	< 0.003	< 0.003	GB/T 2091-2008	PASS
Iron, (Fe), %	< 0.002	< 0.002	GB/T 2091-2008	PASS
Arsenic (AS), %	< 0.0001	< 0.0001	GB/T 2091-2008	PASS
Heavy Metal (to Pb meter), %	< 0.001	< 0.001	GB/T 2091-2008	PASS
Test Result	In Compliance with GB/T 2091-2008《Phosphoric Acid》requirement.			

Inspector : Juan Di Reviewer : Yeqing Du

Appendix 2.6 Yeast Extract



CMA
F2014171985

检 验 报 告

Test Report

№: 检(业)字2016-SP13931号

样品名称 安琪酵母浸粉

规格型号 FM802

受检单位 安琪酵母股份有限公司

检验类型 委托检验

三峡食品药品检验检测中心

Three Gorges Center for Food and Drug Control

三峡食品药品检验检测中心
检 验 报 告

№：检(业)字2016-SP13931号 共 2 页 第 1 页

样品名称	安琪酵母浸粉	规格型号	FM802
样品等级	———	商标（标称）	安琪
受检单位名称	安琪酵母股份有限公司	受检单位地址	———
委托单位名称	安琪酵母股份有限公司	检验类型	委托检验
生产单位（标称）	安琪酵母股份有限公司	生产日期或批号	20161103039
抽样人员	———	委托人员	罗必英
抽样地点	———	抽样日期	———
样品数量	500g×2	到样日期	2016-12-21
样品基数	———	检验日期	2016-12-22~2017-01-13
检测项目	详见附页	样品描述	样品正常，符合检测要求
检验依据判定原则	Q/YB 2147S-2016		
检验结论	该样品所检指标符合Q/YB 2147S-2016 标准要求。 检验专用章 签发日期：2017-1-16		
备注	———		

批准： [redacted] 审核： [redacted] 主检： [redacted]

三峡食品药品检验检测中心
检 验 结 果

№.检(业)字2016-SP13931号 共 2 页 第 2 页

序号	检验项目		单位	标准（技术）要求	实测结果	单项结论
1	感官要求	色泽	——	黄色至淡黄色	黄色	合格
		性状	——	粉状	粉状	合格
		气、滋味	——	具有酵母浸出物所特有的气味，无腐败异臭	具有该产品特有的气味和滋味	合格
		杂质	——	无肉眼可见外来杂质	无肉眼可见杂质	合格
2	总氮(以干基计)		%	≥9.0	12.0	合格
3	氨基氮(以干基计)		%	≥3.0	5.2	合格
4	水分		%	≤6.0	3.8	合格
5	灰分		%	≤15.0	9.8	合格
6	pH值（2%水溶液）		——	5.3~7.2	5.6	合格
7	铅		mg/kg	≤1.0	0.11	合格
8	总砷(以As计)		mg/kg	≤0.5	0.12	合格
9	菌落总数		CFU/g	≤50000	1600	合格
10	大肠菌群		MPN/g	≤0.3	<0.3	合格
11	致病菌	沙门氏菌	/25g	不得检出	未检出，/25g	合格
		金黄色葡萄球菌	/25g	不得检出	未检出，/25g	合格

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Test Report

No.2016-SO13931

Product Name:	Angel Yeast Extract Powder
Specification:	FM802
Requestor:	Angel Yeast Co., Ltd
Test type:	Analysis Request

Three Gorges Center for Food and Drug Control

Test Report

No: 2016-SP13931 Total 2 Pages / Page 1

Sample Name	Angel Yeast Extract Powder	Specification Type	FM802
Sample Grade	-	Trademark	Angel
Test Unit Name	Angel Yeast Co., Ltd	Test Unit Address	-
Requestor Name	Angel Yeast Co., Ltd	Test Type	Analysis Request
Manufacturer (trademark)	Angel Yeast Co., Ltd	Manufacturing Date or Batch Number	2016110303B9
Sampling Personnel	-	Requestor	Ruo, Biying
Sampling Location		Sampling Date	
Sampling Quantity	500g x 2	Received Date	2016-12-21
Sampling Base		Testing Date	2016-12-22~ 2017-01-13
Test Item	See attached	Sample Description	Normal, requirements met
Test Compliance	Q/YB2147S-2016		
Test Conclusion	Sample meets the standard of Q/YB2147S-2016. Inspection Stamp Approval Date:		
Notes			

Approved by: Reviewed by: Analyzed by:

Three Gorges Center for Food and Drug Control

Test Results

No. 2016-SP13931 Total 2 Pages / Page 2

No.	Test Items		Units	Standard/Technical Requirements	Test Results	Evaluation
1	Appearance	Color		Yellow to light yellow	Yellow	PASS
		Traits		Powder	Powder	PASS
		Odor, taste		Standard odor of yeast extract, no corrupt smell	Standard odor and taste of item tested	PASS
		Impurities		No visible impurities	No visible impurities	PASS
2	Total Nitrogen (dry basis)		%	≥9.0	12	PASS
3	Amino Nitrogen (dry basis)		%	≥3.0	5.2	PASS
4	Moisture Content		%	≤6.0	3.8	PASS
5	Ash		%	≤15.0	9.8	PASS
6	pH (2% solution)			5.3~7.2	5.6	PASS
7	Lead (based on Pb)		mg/kg	≤2	<0.1	PASS
8	Arsenic (based on As)		mg/kg	≤2	0.13	PASS
9	Total Number of Colonies		cfu/g	≤50000	1600	PASS
10	Coliforms		MPN/g	≤0.3	<0.3	PASS
11	Pathogens	Staphylococcus aureus	mg/L	Negative	Negative, /25g	PASS
		Salmonella		Negative	Negative, /25g	PASS
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Appendix 2.7 Yeast Peptone

CMA
F2014171985

检 验 报 告

Test Report

№: 检(业)字2016-SP13935号

样品名称 安琪酵母蛋白胨（酵母浸出物）

规格型号 粉状

受检单位 安琪酵母股份有限公司

检验类型 委托检验

三峡食品药品检验检测中心

Three Gorges Center for Food and Drug Control

三峡食品药品检验检测中心
检 验 报 告

No：检(业)字2016-SP13935号 共 2 页 第 1 页

样品名称	安琪酵母蛋白胨（酵母浸出物）	规格型号	粉状
样品等级	———	商标（标称）	安琪
受检单位名称	安琪酵母股份有限公司	受检单位地址	———
委托单位名称	安琪酵母股份有限公司	检验类别	委托检验
生产单位（标称）	安琪酵母股份有限公司	生产日期或批号	20161123028
抽样人员	———	委托人员	罗必英
抽样地点	———	抽样日期	———
样品数量	500g×2	到样日期	2016-12-21
样品盏数	———	检验日期	2016-12-22～2017-01-19
检测项目	详见附页	样品前述	样品正常，符合检测要求
检验依据判定原则	Q/YB 2187S-2015		
检验结论	该样品所检指标符合Q/YB 2187S-2015标准要求。 检验专用章 签发日期：2017-1-20		
备注	———		

批准： 审核： 主检：

三峡食品药品检验检测中心
检 验 结 果

No:检(油)字2016-SP13925号 共 9 页 第 2 页

序号	检验项目		单位	标准（技术）要求	实测结果	单项结论
1	色泽		——	淡黄色至浅棕色	黄色	合格
2	气味		——	具有酵母蛋白胨应有的气味	无异味	合格
3	外观		——	粉末或者块	粉末	合格
4	杂质		——	无肉眼可见的外来杂质	无肉眼可见异物	合格
5	总氮(以干基计)		%	≥8.0	11.8	合格
6	氨基酸态氮(以干基计)		%	≥1.5	3.3	合格
7	水分		%	≤6.0	3.8	合格
8	灰分		%	≤15.0	9.0	合格
9	氯化钠		%	≤2.0	0.5	合格
10	pH		——	5.3~7.3	5.8	合格
11	铅（以Pb计）		mg/kg	≤2	<0.1	合格
12	总砷（以As计）		mg/kg	≤2	0.13	合格
13	菌落总数		cfu/g	≤50000	4200	合格
14	大肠菌群		MPN/g	≤0.3	<0.3	合格
15	致病菌	金黄色葡萄球菌	/25g	不得检出	未检出，/25g	合格
		沙门氏菌	/25g	不得检出	未检出，/25g	合格

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Test Report

No.2016-SO13935

Product Name:	Angel Yeast Peptone (Yeast Extract)
Specification:	Powder
Requestor:	Angel Yeast Co., Ltd
Test type:	Analysis Reques

Three Gorges Center for Food and Drug Control

Test Report

No. 2016SP13935 Total 2 Pages / Page 1

Sample Name	Angel Yeast Peptone (Yeast Extract)	Specification Type	Powder
Sample Grade	-	Trademark	Angel
Test Unit Name	Angel Yeast Co., Ltd	Test Unit Address	-
Requestor Name	Angel Yeast Co., Ltd	Test Type	Analysis Request
Manufacturer (trademark)	Angel Yeast Co., Ltd	Manufacturing Date or Batch Number	20161230288
Sampling Personnel	-	Requestor	Ruo, Biying
Sampling Location		Sampling Date	
Sampling Quantity	500g x 2	Received Date	2016-12-21
Sampling Base		Testing Date	2016-12-22~ 2017-01-19
Test Item	See attached	Sample Description	Normal, requirements met
Test Compliance	Q/YB2187S-2015		
Test Conclusion	Sample meets the standard of Q/YB2187S-2015. Inspection Stamp Approval Date:		
Notes			

Approved by: Reviewed by: Analyzed by:

Three Gorges Center for Food and Drug Control

Test Results

No. 2016SP13935 Total 2 Pages / Page 2

No.	Test Items		Units	Standard/Technical Requirements	Test Results	Evaulation
1	Color			Light yellow to light brown	Yellow	PASS
2	Odor			Standard odor of yeast peptone	No odor	PASS
3	Appearance			Powder or piste	Powder	PASS
4	Impurities			No visible imprities	No visible impurities	PASS
5	Total Nitrogen (dry basis)		%	≥8.0	11.8	PASS
6	Amino Nitrogen (dry basis)		%	≥1.5	3.3	PASS
7	Moisture Content		%	≤6.0	3.8	PASS
8	Ash		%	≤15.0	9.0	PASS
9	Sodium Chloride		%	≤2.0	0.5	PASS
10	pH			5.3~7.2	5.8	PASS
11	Lead (based on Pb)		mg/kg	≤2	<0.1	PASS
12	Arsenic (based on As)		mg/kg	≤2	0.13	PASS
13	Total Number of Colonies		cfu/g	≤50000	4200	PASS
14	Coliforms		MPN/g	≤0.3	<0.3	PASS
15	Pathogens	Staphylococcus aureus	mg/L	Negative	Negative, /25g	PASS
		Salmonella		Negative	Negative, /25g	PASS
Blank Below						

Appendix 2.8 Glycerin

中华人民共和国出入境检验检疫

入境货物检验检疫证明

编号 160000021960540I

收货人	厦门方盛华进出口贸易有限公司XIAMEN FANGSHENGHUA IMPORT AND EXPORT TRADE CO.,LTD.		
发货人	***PROCTER AND GAMBLE INTERNATIONAL OPERATIONA SA SINGAPORE BRANCH		
品 名	甘油	报检数/重量	**40000千克
包装种类及数量	**160桶	输出国家或地区	马来西亚
合同号	SY-160928	标记及号码 NIL	
提/运单号	MYKSPKGS15763700		
入境口岸	黄岛		
入境日期	2016年12月07日		

证明

品名	品牌	原产国	规格	数量	生产日期
甘油	无	马来西亚	250KG/桶	160桶	2016.10.31/2016.11.02

该批食品添加剂按照GB29950-2013检验检疫监督管理，准予进口。

****

签字: [redacted] 日期: 2016 年 12 月 20 日

检验检疫专用章 (1)

备注

****

[5-1(2001.1.1)-1] ① 货主收执

AA6565589

Entry-Exit Inspection and Quarantine of the People's Republic of China

Inspection and Quarantine Certificate of Import and Export goods

No.116000002196054001

Consignee: Xiamen Fangshenghua Import and Export Trade Co., LTD.

Consignor: Proctor and Gamble International Operations SA Singapore Branch

Item Name: Glycerin Net Weight: 40,000kg

Number and Kind of Packages: 160 Drums Country/Place of Export: Malaysia

Contract No: SY-160928 Marks and Number: NIL

Bill of Lading No: NYKSPKGS15763700

Port of Entry: Huangdao

Entry Date: December 07, 2016

Certification

Item Description	Brand	Place of Origin	Specification	Quantity	Date of Manufacturing
Glycerin	None	Malaysia	250KG/Drum	160 Drums	2016.10.31/ 2016.11.22

This batch of food additive is approved for import in accordance to GB29950-2013 inspection and quarantine supervision.

Signature: Date: December 20, 2016

Notes:

(1) For Consignee

Appendix 2.9 Trizma® Base

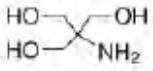
SIGMA-ALDRICH sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:
Trizma® base - anhydrous, free-flowing, Redi-Dri™, ≥99.9%

Product Number:	**RDD008**
Batch Number:	**SLBK9274V**
Brand:	SIGMA
CAS Number:	77-86-1
Formula:	C4H11NO3
Formula Weight:	121.14 g/mol
Quality Release Date:	27 JUN 2014



Test	Specification	Result
Appearance (Color)	White	White
Appearance (Form)	Crystalline Powder	Crystalline Powder
Solubility (Color)	Colorless	Colorless
Solubility (Turbidity) 200 g plus 300 mL of H2O	Clear	Clear
Water (by Karl Fischer)	≤ 0.2 %	0.1 %
Infrared spectrum	Conforms to Structure	Conforms
A290 UV absorbance 40% (W/W)	≤ 0.05	0.02
Heavy Metal as Lead	<= 2 ppm	<= 2 ppm
Iron (Fe) ICP atomic emission	≤ 1 ppm	< 1 ppm
Initial Melting Point	≥ 168 °C	169 °C
Final Melting Point	≤ 172 °C	172 °C
Titration with H2SO4	≥ 99.9 %	100.0 %

[redacted]

Rodney Burbach, Manager
Analytical Services
St. Louis, Missouri US

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Version Number: 2 Page 1 of 1

Appendix 2.10 Hydrochloric Acid

Certificate of Analysis

Product Name: HYDROCHLORIC ACID, 37%, REAGENT (ACS)

Item #: 625

Lot #: 18303026

Certified Values:

Specifications (Max Limits or as Specified)	Pass/Fail	Numerical Results
Assay (as HCl) (%) 36.5 - 38.0 %	Pass	37.6
Appearance	Pass	Conforms
Color (APHA) 10 Max	Pass	< 10
Residue after ignition 0.0005 % Max	Pass	< 0.0005
Bromide 0.005 % Max	Pass	< 0.005
Sulfate 0.0001 % Max	Pass	< 0.0001
Sulfite 0.0001 % Max	Pass	< 0.0001
Free chlorine (%) 0.0001 % Max	Pass	< 0.0001
Ammonium 0.0003 % Max	Pass	< 0.0003
Arsenic 0.000001 % Max	Pass	< 0.000001
Heavy Metals (by ICP-OES) 0.0001 % Max	Pass	< 0.0001
Iron 0.00002 % Max	Pass	< 0.00002

Comments

Certificate Create By: Jacob Watson — **Signature on File**

Certifying Officer: Jacob Watson — **Signature on File**

Best by: August 2, 2023

Certificate of Analysis

GFS CHEMICALS

Product Name:	HYDROCHLORIC ACID, 37%, REAGENT (ACS)
Item #:	625
Lot #:	18303026

Certified Values:

Not for direct use in food,cosmetics,finished pharmaceuticals or drug products.Supplier is not responsible for compliance with FDA Current Good Manufacturing Practice(cGMP) requirements,including without limitation those for finished drug products in 21 C.F.R Parts 210 and 211.Consult warrantly limitations at
www.gfschemicals.com/statics/documents/aboutus/termsandconditions.html
For resale by GFS authorized distributors only.

GFS Chemicals,Inc.P.O. Box 245 Powell, OH 43065 * Signed Orig. Doc. On File
1-800-858-9682 (U.S. and Canada) 1-740-881-550(International) 1-70-881-5989(Fax)

18303026

Appendix 2.11 Manganese Sulfate

Jiangsu Kelundo Food Ingredients Co., Ltd.

Inspection Report

CERTIFICATE OF ANALYSIS

Product Name	Manganese Sulfate		Product Performance Standards	GB 29208-2012	
Production Batch	21043001	Production Date	2021.04.30	Package	0.5 T

Sensory requirements

Test Items	Claim	Testing Method	Test Value	Determination
Color	Light pink	Take an appropriate amount of sample and place it in a 50mL beaker Medium, observe	Light pink	PASS
Organization Status	Granules or Powder	the color and composition under natural light Weaving state	Granule	PASS

Quality Index

Project	Index	Test Value	Test Based On	Determination
Manganese sulfate (MnSO4•H2O) Content, w/%	98.0~102.0	98.85	Appendix A in A.4	PASS
Lead (Pb) /mg/kg ≤	4	<4	GB/T 5009.76	PASS
Arsenic (As) /mg/kg ≤	3	<3	Appendix A in A.5	PASS
Selenium (Se) /mg/kg ≤	30	<30	Appendix A in A.6	PASS
Ignition Loss , w/%	10.0~13.0	11.60	Appendix A in A.7	PASS
Test Result	Meets the GB 29208-2012 《Food Additives Manganese Sulfate 》 Claim。			

Inspector : Shi Jinming

Reviewer : Xu Guangsheng

Appendix 2.12 D-Fructose

苏州天可贸易有限公司
Suzhou Tiango Trading Co., LTD
Tel: 13776026956 fax:0512-66566729
E-maill:tkding@163.com 网址：www.tktrd.cn

CERTIFICATION OF ANALYSIS

Product Name	**D-(-)Fructose**
Quantity	5kg
Batch number	0804K2020
Date of production	2020.08.02
Date of Analysis	2020.08.02
Formula	C6H1206
Molecular Weight	180.16
Specification	For Molecular Biology

Items	Requirements	Results
Apperarance	White crystalline powder	Complies
Assay (HPLC)	≥99%	≥99%
Loss on drying	<0.5%	0.05%
Sulphate ASH	<0.05%	0.01%
Chloride	≤0.1%	<0.1%

QC Dept.Manager: zhanghua

Checker: xujia

苏州天可贸易有限公司 质检专用章

申明

Statement

敬启者，

To whom it may concern,

我公司供应的果糖符合 Food Chemicals Codex (FCC), 3d Ed. (1981)要求，特此申明。

We hereby declare that this ingredient, Fructose meets the specification of the Food Chemicals Codex (FCC), 3d Ed. (1981).

苏州天可贸易有限公司

2021.06.17

Appendix 2.13 Ethanol

告 20172300017

No: 20172300017 (2)

CMA 20151000722

CAL 2015苏质检验字072号

TCT

检验报告

TEST REPORT

太仓市检验检测中心

Taicang Inspection and Testing Center

太仓市检验检测中心

检 验 报 告 20172300017

Test Report

No: 20172300017 (2)

检验类别: 委托送样检验 共 2 页 第 1 页

样品编号 Serial Number	20172300017	规格型号 Specification Type	散装
产品名称 Product Name	食用酒精	商标 Trademark	*
委托单位名称\地址\电话\邮编 Consigner\Address\Tel\Post Code	太仓新太酒精有限公司 太仓港港口开发区协鑫路 2 号\0512-53524458\215400		
生产单位名称\地址\电话\邮编 Manufacturer\Address\Tel\Post Code	太仓新太酒精有限公司 太仓港港口开发区协鑫路 2 号\0512-53524458\215400		
样品数量(n) Sum of Sample(n)	2 瓶	生产日期/批号 Producing Date/Batch No.	-/17010312
样品等级 Sample Grade	特级	到样日期 Sampling Date	2017-01-17
样品状态描述 Sample Description	符合检验要求		
检验日期 Date of Test	2017-01-17～2017-02-03	检验地点 Test Place	太仓市检验检测中心
检验依据 Test Standard(s)	GB 10343-2008 《食用酒精》 GB/T 394.2-2008 《酒精通用分析方法》		
检验结论 Test Conclusion	经送样检验，所检项目符合 GB 10343-2008 标准和 GB/T 394.2-2008 标准要求。 (盖章) 签发日期: 2017-02-06 Signature Date		
检验说明 Test Explain	此栏空白。		

审 签: Approved By [redacted] 校 核: Checked by [redacted] 编 制: Tested by [redacted]

地址: 江苏省太仓市城厢镇东亭南路55号 电话: 0512-53542648/82786000-8908 传真: 0512-53541808 电子邮箱: tctjszlb@163.com

太仓市检验检测中心
Taicang Inspection and Testing Center

检验结果

No: 201722000617

Test Results

共 2 页 第 2 页

序号 Serial	检验项目 Test Items		单位 Units	技术要求 Technical Requirements	检验结果 Test Results	单项评价 Individual Judge
1	感官指标	外观	—	无色透明	无色透明	合格
		气味	—	具有乙醇固有的香气，香气纯正	具有乙醇固有的香气，香气纯正	
		口味	—	纯净，微甜	纯净，微甜	
2	色度		号	≤10	5	合格
3	乙醇% (V/V) (20℃)		—	≥96.0	96.9	合格
4	硫酸试验 (照管单位)		—	≤10	5	合格
5	氧化时间		min	≥40	50	合格
6	酸 (以乙酸计)		mg/L	≤7	4	合格
7	醛		mg/L	≤1	0.4	合格
8	甲醇		mg/L	≤2	未检出（检出限 0.5 mg/L）	合格
9	正丙醇		mg/L	≤2	未检出（检出限 0.5 mg/L）	合格
10	异丁醇+异戊醇		mg/L	≤1	未检出（检出限 0.5 mg/L）	合格
11	酯 (以乙酸乙酯计)		mg/L	≤10	6	合格
12	重金属 (以Pb计)		mg/L	≤1	<1	合格
13	不挥发物		mg/L	≤10	2	合格
14	氰化物		mg/L	≤5	未检出（检出限 0.2 mg/L）	合格

（仅对来样负责）
（Only responsible for the submitted samples）

地址：江苏省太仓市城厢镇东亭南路55号 电话：0512-53542848/82786000-8908 传真：0512-53541808 电子邮箱：tctcjsdb@163.com

Test Report

No: 2017230017 (2)

Test Kind: Sample Analysis Request Total 2 Pages / Page 1

Serial Number	20172300017	Specification Type	Bulk
Product Name	Edible Alcohol	Trademark	
Consigner/Address/Tel/Post Code	Taicang Xintai Jiujing Limited Company 2 Xie Xin Road , Jiangsu Province, Taicang City Port Development Zone/0512-53524458/215400		
Manufacturer/Address/Tel/Post Code	Taicang Xintai Jiujing Limited Company 2 Xie Xin Road , Jiangsu Province, Taicang City Port Development Zone/0512-53524458/215400		
Sum of Samples (n)	2 Bottles	Producing Date/ Batch No.	-/17010312
Sample Grade	Premium	Sampling Date	2017-01-17
Sample Description	Analysis requirements met		
Date of Test	2017-01-17~ 2017-02-03	Test Place	Taicang Inspection and Testing Center
Test Standard(s)	GB 10343-2008 Ediable Alcohol GB/T 394.2-2008 General Alcohol Analysis Method		
Test Conclusion	After analysis, item meets the standard of GB 10343-2008 and GB/T 394.2-2008.		
Test Explain	Blank		

Test Results

No: 20172300017

Total 2 Pages / Page 2

Serial	Test Items		Units	Technical Requirements	Test Results	Individual Judge
1	Appearance	Color		Clear	Clear	PASS
		Odor		Inherent ethanol pure aroma	Inherent ethanol pure aroma	PASS
		Taste		Pure, slightly sweet	Pure, slightly sweet	PASS
2	Chroma		No.	≤10	5	PASS
3	Ethanol % (V/V) (20°C)			≥96.0	96.9	PASS
4	Sulfuric Acid Test (Black Unit)			≤10	5	PASS
5	Oxidation Time		min	≤40	50	PASS
6	Acid (based on acetic acid)		mg/L	≤7	4	PASS
7	Aldehyde		mg/L	≤1	0.4	PASS
8	Methanol		mg/L	≤2	4	PASS
9	N-propanol		mg/L	≤2	Negative (Detection limit of 0.5 mg/L)	PASS
10	Isobutanol / isoamyl alcohol		mg/L	≤1	Negative (Detection limit of 0.5 mg/L)	PASS
11	Ester (based on ethyl acetate)		mg/L	≤10	Negative (Detection limit of 0.5 mg/L)	PASS
12	Heavy Metal		mg/L	≤1	<1	PASS
13	Non-volatile matter		mg/L	≤10	2	PASS
14	Cyanide		mg/L	≤5	Negative (Detection limit of 0.2 mg/L)	PASS

Only Responsible for the Submitted Samples

Address: 55 S. Dongting Rd, ChenXiang Town, Taicang City, Jiangsu Province

Tel: Fax: Email:

申明

Statement

敬启者，

To whom it may concern,

我公司供应的乙醇符合 Food Chemicals Codex (FCC), 3d Ed. (1981)要求，特此申明。

We hereby declare that this ingredient, Ethyl Alcohol meets the specification of the Food Chemicals Codex (FCC), 3d Ed. (1981).

南京盛虎和化工有限公司

2021.06.17

Appendix 2.14 Calcium Chloride

Jiangsu Kelundo Food Ingredients Co., Ltd.

Inspection Report
CERTIFICATE OF ANALYSIS

Product Name	Calcium chloride (Anhydrous)		Product Performance Standards	GB 1886.45-2016	
Production Batch	21040301	Production Date	2021.04.03	Package	5 T

Sensory requirements

Test Items	Claim	Testing Method	Test Value	Determination
Color	White, off-white or light yellow	Take an appropriate amount of sample and place it in a 50mL beaker Medium, observe the color and composition under natural light Weaving state	White	PASS
Organization Status	Needle crystal or powder		Granule	PASS

Quality Index

Project	Index	Test Value	Test Based On	Determination
Calcium chloride (To $CaCl_2$ meter) w/%≥	93.0	93.21	Appendix A in A.4	PASS
Free Base [Ca(OH)2], w/% ≤	0.25	0.11	Appendix A in A.5	PASS
Magnesium and alkali metal salts, w/% ≤	5.0	3.54	Appendix A in A.6	PASS
Heavy Metal (To Pb meter) /mg/kg ≤	20	<20	GB 5009.74	PASS
Lead (Pb) /mg/kg ≤	5	<5	GB 5009.75	PASS
Arsenic (As) /mg/kg ≤	3	<3	GB 5009.76	PASS
Fluorine (F), w/% ≤	0.004	0.0019	Appendix A in A.7	PASS
Test Result	Meets the GB 1886.45-2016《Food Additives Calcium Chloride》Claim。			

Inspector : Wang Tiantian Reviewer : Xu Guangsheng

申明

Statement

敬启者，

To whom it may concern,

我公司供应的氯化钙符合 Food Chemicals Codex (FCC), 3d Ed. (1981)要求，特此申明。

We hereby declare that this ingredient, Calcium Chloride meets the specification of the Food Chemicals Codex (FCC), 3d Ed. (1981).

江苏科伦多食品配料有限公司

2021.06.17

Appendix 2.15 Calcium Acetate

Jiangsu Kelundo Food Ingredients Co., Ltd.

Inspection Report

CERTIFICATE OF ANALYSIS

Product Name	Calcium acetate		Product Performance Standards	GB 15572-1995	
Production Batch	17020401	Production Date	2017.02.04	Package	0.025T

Sensory requirements

Test Items	Claim	Testing Method	Test Value	Determination
Color	White	Take an appropriate amount of test sample and place it in a 50mL beaker Medium, observe the color and composition under natural light Weaving state	White	PASS
Organization Status	Needle crystal or powder		Powder	PASS

Quality Index

Project	Index	Test Value	Test Based On	Determination
Content (To $C_4H_6O_4Ca$ meter), w/%	98.0~102.0	99.21	GB 15572-1995	PASS
pH Value	6.0~8.0	7.30	GB 15572-1995	PASS
Sulfate, %≤	0.1	<0.1	GB 15572-1995	PASS
Chloride, %≤	0.05	<0.05	GB 15572-1995	PASS
Heavy Metal (To Pb meter), %≤	0.0025	<0.0025	GB 15572-1995	PASS
Arsenic (To As meter), %≤	0.0002	<0.0002	GB 15572-1995	PASS
Magnesium Salt & Alkali Metal Salt, %≤	1.0	<1.0	GB 15572-1995	PASS
Barium Salt	Compliance	PASS	GB 15572-1995	PASS
Moisture, %≤	7	4.32	GB 15572-1995	PASS
Fluoride, %≤	0.005	<0.005	GB 15572-1995	PASS
Test Result	Meets the GB 15572-1995 《Food Additives Calcium Acetate》 Claim.			

Inspector : Huang Yalin Reviewer : Xu Guangsheng

申明

Statement

敬启者，

To whom it may concern,

我公司供应的乙酸钙符合 Food Chemicals Codex (FCC), 3d Ed. (1981)要求，特此申明。

We hereby declare that this ingredient, Calcium Acetate meets the specification of the Food Chemicals Codex (FCC), 3d Ed. (1981).

江苏科伦多食品配料有限公司

2021.06.17

Appendix 3 Documentation for CGMP for Allulose

A Perfect Blend of Science and Nature

March 11, 2020

CERTIFICATE OF FOOD GRADE AND CONTINUING GUARANTEE

Product Name: **Allulose 97%**

Blue California hereby certifies that **Allulose 97%** is produced and stored under strict GMP quality requirements. The product is produced in a manufacturing facility that is certified by BRC- Global Standard for Food Safety.

We further certify our product is not adulterated or misbranded within the meaning of the United States Federal Food, Drug, and Cosmetic Act, or any amendment thereto.
Furthermore, our product has been tested to meet the necessary requirements for use in human food.

Additionally, Blue California is in compliance with section 404/405 of the FD&C Act and any regulation mandated by Interstate Commerce Act.

Regards,

Hadi Omrani
Hadi Omrani
Director, Technical and Regulatory Affairs

Corporate Headquarters
30111 Tomas, Rancho Santa Margarita, CA 92688 **Tel:** 949-635-1990 **Fax:** 949-635-1986
Website: www.bluecal-ingredients.com

Appendix 4 Certificates of Analysis for Multiple Lots of Blue California's Allulose

Appendix 4.1 Allulose Lot 833-20180925

Blue California®
30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

CERTIFICATE OF ANALYSIS

Product: Allulose 97%

Lot No:	**833-20180925**	**Original Manufacturer:**	**Blue California**
Date of Manufacturing:	**September 28-2018**	**Expiration/Re-test date:**	**September 11-2020**
QC acceptance date:	**October 16-2019**	**Country of Origin of Raw Material:**	**China**

This product has NOT been treated by Irradiation or ETO

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	Off white to white powder	VISUAL	PASS
FOREIGN MATTER	ABSENT	VISUAL	PASS
ODOR	CHARACTERISTIC	OLFACTORY	PASS
TASTE	CHARACTERISTIC	GUSTATORY	PASS
D-ALLULOSE	**≥ 97%**	**HPLC**	**98% (dry-basis)**
LOSS ON DRYING	≤ 5%	USP 34	3.20%
HEAVY METALS	< 10 ppm	USP 34	PASS
ARSENIC	< 0.5 ppm	ICP-MS	< 0.02 ppm
CADMIUM	< 0.5 ppm	ICP-MS	< 0.01 ppm
LEAD	< 0.5 ppm	ICP-MS	< 0.02 ppm
MERCURY	< 0.5 ppm	ICP-MS	< 0.01 ppm
ETHANOL	< 1,000 ppm	USP 34	< 200 ppm
METHANOL	< 200 ppm	USP 34	< 100 ppm
ASH	< 0.5	USP 34	PASS
PH	3-7	USP 34	PASS
TOTAL PLATE COUNT	≤ 1,000 cfu/gm	AOAC	< 1,000 cfu/gm
TOTAL COLIFORM	< 100 cfu/gm	AOAC	< 3 cfu/gm
YEAST AND MOLDS	< 100 cfu/gm	AOAC	< 10 cfu/gm
E. COLI:	NEGATIVE	AOAC	NEGATIVE
SALMONELLA	NEGATIVE	AOAC	NEGATIVE
SHELF LIFE	2 YEARS	HPLC	PASS

Approved by: X.Y.Mao (QC Manager) Revision date: 06-17-2020

Appendix 4.2 Allulose Lot 833-20181109

Blue California®
30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

CERTIFICATE OF ANALYSIS

Product: Allulose 97%

Lot No: **833-20181109** **Original Manufacturer:** **Blue California**
Date of Manufacturing: **November 09-2018** **Expiration/Re-test date:** **September 09-2020**
QC acceptance date: December 10-2018 Country of Origin of Raw Material: China This product has NOT been treated by Irradiation or ETO

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	Off white to white powder	VISUAL	PASS
FOREIGN MATTER	ABSENT	VISUAL	PASS
ODOR	CHARACTERISTIC	OLFACTORY	PASS
TASTE	CHARACTERISTIC	GUSTATORY	PASS
D-ALLULOSE	**> 97%**	**HPLC**	**97.8% (dry-basis)**
LOSS ON DRYING	< 5%	USP 34	3.10%
HEAVY METALS	< 10 ppm	USP 34	PASS
ARSENIC	< 0.5 ppm	ICP-MS	< 0.02 ppm
CADMIUM	< 0.5 ppm	ICP-MS	< 0.01 ppm
LEAD	< 0.5 ppm	ICP-MS	< 0.02 ppm
MERCURY	< 0.5 ppm	ICP-MS	< 0.01 ppm
ETHANOL	< 1,000 ppm	USP 34	< 200 ppm
METHANOL	< 200 ppm	USP 34	< 100 ppm
ASH	< 0.5	USP 34	PASS
PH	3-7	USP 34	PASS
TOTAL PLATE COUNT	< 1,000 cfu/gm	AOAC	< 1,000 cfu/gm
TOTAL COLIFORM	< 100 cfu/gm	AOAC	< 3 cfu/gm
YEAST AND MOLDS	< 100 cfu/gm	AOAC	< 10 cfu/gm
E. COLI:	NEGATIVE	AOAC	NEGATIVE
SALMONELLA	NEGATIVE	AOAC	NEGATIVE
SHELF LIFE	2 YEARS	HPLC	PASS

Approved by: X.Y.Mao (QC Manager) Revision date: 06-17-2020

Appendix 4.3 Allulose Lot 833-20190123

Blue California®
30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

CERTIFICATE OF ANALYSIS

Product: Allulose 97%

Lot No:	**833-20190123**	**Original Manufacturer:**	**Blue California**
Date of Manufacturing:	**January 23-2019**	**Expiration/Re-test date:**	**January 23-2021**

QC acceptance date: February 02-2019 Country of Origin of Raw Material: China This product has NOT been treated by Irradiation or ETO

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	Off white to white powder	VISUAL	PASS
FOREIGN MATTER	ABSENT	VISUAL	PASS
ODOR	CHARACTERISTIC	OLFACTORY	PASS
TASTE	CHARACTERISTIC	GUSTATORY	PASS
D-ALLULOSE	**> 97%**	**HPLC**	**97.2% (dry-basis)**
LOSS ON DRYING	< 5%	USP 34	3.10%
HEAVY METALS	< 10 ppm	USP 34	PASS
ARSENIC	< 0.5 ppm	ICP-MS	< 0.02 ppm
CADMIUM	< 0.5 ppm	ICP-MS	< 0.01 ppm
LEAD	< 0.5 ppm	ICP-MS	< 0.02 ppm
MERCURY	< 0.5 ppm	ICP-MS	< 0.01 ppm
ETHANOL	< 1,000 ppm	USP 34	< 200 ppm
METHANOL	< 200 ppm	USP 34	< 100 ppm
ASH	< 0.5	USP 34	PASS
PH	3-7	USP 34	PASS
TOTAL PLATE COUNT	< 1,000 cfu/gm	AOAC	< 1,000 cfu/gm
TOTAL COLIFORM	< 100 cfu/gm	AOAC	< 3 cfu/gm
YEAST AND MOLDS	< 100 cfu/gm	AOAC	< 10 cfu/gm
E. COLI:	NEGATIVE	AOAC	NEGATIVE
SALMONELLA	NEGATIVE	AOAC	NEGATIVE
SHELF LIFE	2 YEARS	HPLC	PASS

Approved by: X.Y.Mao (QC Manager) Revision date: 06-17-2020

Appendix 4.4 Allulose Lot 833-20190411

Blue California®
30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

CERTIFICATE OF ANALYSIS

Product: Allulose 97%

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	Off white to white powder	VISUAL	PASS
FOREIGN MATTER	ABSENT	VISUAL	PASS
ODOR	CHARACTERISTIC	OLFACTORY	PASS
TASTE	CHARACTERISTIC	GUSTATORY	PASS
D-ALLULOSE	**> 97%**	**HPLC**	**99.8% (dry-basis)**
LOSS ON DRYING	< 5%	USP 34	3.20%
HEAVY METALS	< 10 ppm	USP 34	PASS
ARSENIC	< 0.5 ppm	ICP-MS	< 0.02 ppm
CADMIUM	< 0.5 ppm	ICP-MS	< 0.01 ppm
LEAD	< 0.5 ppm	ICP-MS	< 0.02 ppm
MERCURY	< 0.5 ppm	ICP-MS	< 0.01 ppm
ETHANOL	< 1,000 ppm	USP 34	< 200 ppm
METHANOL	< 200 ppm	USP 34	< 100 ppm
ASH	< 0.5	USP 34	PASS
PH	3-7	USP 34	PASS
TOTAL PLATE COUNT	< 1,000 cfu/gm	AOAC	< 1,000 cfu/gm
TOTAL COLIFORM	< 100 cfu/gm	AOAC	< 3 cfu/gm
YEAST AND MOLDS	< 100 cfu/gm	AOAC	< 10 cfu/gm
E. COLI:	NEGATIVE	AOAC	NEGATIVE
SALMONELLA	NEGATIVE	AOAC	NEGATIVE
SHELF LIFE	2 YEARS	HPLC	PASS

Lot No: 833-20190411 Original Manufacturer: Blue California Date of Manufacturing: April 11-2019 Expiration/Re-test date: April 11-2021 QC acceptance date: April 16-2019 Country of Origin of Raw Material: China This product has NOT been treated by Irradiation or ETO

Approved by: X.Y.Mao (QC Manager) Revision date: 06-17-2020

Appendix 4.5 Allulose Lot 833-20190617

Blue California®
30111 Tomas
Rancho Santa Margarita, CA 92688
Tel: 949.635.1990
Fax: 949.635.1988

CERTIFICATE OF ANALYSIS

Product: Allulose 97%

ATTRIBUTES	SPECIFICATION	METHODS	RESULTS
APPEARANCE	Off white to white powder	VISUAL	PASS
FOREIGN MATTER	ABSENT	VISUAL	PASS
ODOR	CHARACTERISTIC	OLFACTORY	PASS
TASTE	CHARACTERISTIC	GUSTATORY	PASS
D-ALLULOSE	**> 97%**	**HPLC**	**98.9% (dry-basis)**
LOSS ON DRYING	< 5%	USP 34	3.30%
HEAVY METALS	< 10 ppm	USP 34	PASS
ARSENIC	< 0.5 ppm	ICP-MS	< 0.02 ppm
CADMIUM	< 0.5 ppm	ICP-MS	< 0.01 ppm
LEAD	< 0.5 ppm	ICP-MS	< 0.02 ppm
MERCURY	< 0.5 ppm	ICP-MS	< 0.01 ppm
ETHANOL	< 1,000 ppm	USP 34	< 200 ppm
METHANOL	< 200 ppm	USP 34	< 100 ppm
ASH	< 0.5	USP 34	PASS
PH	3-7	USP 34	PASS
TOTAL PLATE COUNT	< 1,000 cfu/gm	AOAC	< 1,000 cfu/gm
TOTAL COLIFORM	< 100 cfu/gm	AOAC	< 3 cfu/gm
YEAST AND MOLDS	< 100 cfu/gm	AOAC	< 10 cfu/gm
E. COLI:	NEGATIVE	AOAC	NEGATIVE
SALMONELLA	NEGATIVE	AOAC	NEGATIVE
SHELF LIFE	2 YEARS	HPLC	PASS

Lot No: 833-20190617 Original Manufacturer: Blue California Date of Manufacturing: June 17-2019 Expiration/Re-test date: June 17-2021 QC acceptance date: July 02-2019 Country of Origin of Raw Material: China This product has NOT been treated by Irradiation or ETO

Approved by: X.Y.Mao (QC Manager) Revision date: 06-17-2020

eurofins | Supplement Analysis Center

Eurofins Scientific Inc.
Supplement Analysis Center
1365 Redwood Way
Petaluma, CA 94954
Tel.+1 707 792 7300

October 10, 2019

Hadi Omrani
Blue California Co.
30111 Tomas
Rancho Santa Margarita, CA 92688

CERTIFICATE OF ANALYSIS
AR-19-KK-012776-01
Batch #: EUCAPE-00116045

Sample Identification:
Sample #: 740-2019-08300059
Description: Allulose Food Grade Ingredient, Powder, Lot# 833-20180925
Condition: Acceptable
Date Received: August 30, 2019

KK04H: Validation of method for a Nutritional Supplement
Method Reference: N/A
Completed: 10/10/2019

	Result	Theoretical Level
See Validation Report	DONE	

KK987: Special Analysis (R&D)
Method Reference: N/A
Completed: 10/10/2019

	Result	Theoretical Level
Allulose (dry-basis)	98.0 % (w/w)	>95 (dry-basis) % (w/w)

Appendix 5 Validation Report

Please refer to the Appendix 5 report, provided as a separate file:

Validation Report of Allulose – Blue California.pdf

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Appendix 6 Protein Assay Reports

Certificate Issued To: Blue California 30111 Tomas Rancho Santa Margarita, CA 92688 Phone:949-635-1990 Fax: 949-635-1986	Work performed at: **International RINP, Inc.** 23151 Verdugo Dr., Suite 101 Laguna Hills, CA 92653 Phone: (949) 916-0780 FAX: (949) 916-2820 E-mail: rinp1@live.com Website:www.internationalrinp.com **FDA Registration No. 18174842550**

Certificate of Analysis: Determination of Protein in Allulose 97% by UV Method (BCA Method)

Company Name:	Blue California
Sample Description:	Allulose 97%
Received Date:	03-04-21
Lot Number:	833-20201015
Lab Number:	L#17560

The Analysis Results

Sample	Lab#	Analyte	Limit of Detection	Target	Results
Allulose 97%	L#17560	Protein	5 µg/ml (5 ppm)	N/A	Not Detected

Analyzed by:	[redacted]		
Approved by:	[redacted] Hongyan Wang, President/PhD	Report Date:	03-09-2021

Certificate Issued To: Blue California 30111 Tomas Rancho Santa Margarita, CA 92688 Phone:949-635-1990 Fax: 949-635-1986	Work performed at: **International RINP, Inc.** 23151 Verdugo Dr., Suite 101 Laguna Hills, CA 92653 Phone: (949) 916-0780 FAX: (949) 916-2820 E-mail: rinp1@live.com Website:www.internationalrinp.com **FDA Registration No. 18174842550**

Certificate of Analysis: Determination of Protein in Allulose 97% by UV Method (BCA Method)

Company Name:	Blue California
Sample Description:	Allulose 97%
Received Date:	03-04-21
Lot Number:	833-20200512
Lab Number:	L#17561

The Analysis Results

Sample	Lab#	Analyte	Limit of Detection	Target	Results
Allulose 97%	L#17561	Protein	5 µg/ml (5 ppm)	N/A	Not Detected

Analyzed by:	[redacted]		
Approved by:	[redacted] Hongyan Wang, President/PhD	Report Date:	03-09-2021

Certificate Issued To: Blue California 30111 Tomas Rancho Santa Margarita, CA 92688 Phone:949-635-1990 Fax: 949-635-1986	Work performed at: **International RINP, Inc.** 23151 Verdugo Dr., Suite 101 Laguna Hills, CA 92653 Phone: (949) 916-0780 FAX: (949) 916-2820 E-mail: rinp1@live.com Website:www.internationalrinp.com **FDA Registration No. 18174842550**

Certificate of Analysis: Determination of Protein in Allulose 97% by UV Method (BCA Method)

Company Name:	Blue California
Sample Description:	Allulose 97%
Received Date:	03-04-21
Lot Number:	833-20200924
Lab Number:	L#17562

The Analysis Results

Sample	Lab#	Analyte	Limit of Detection	Target	Results
Allulose 97%	L#17562	Protein	5 µg/ml (5 ppm)	N/A	Not Detected

Analyzed by:	[redacted]		
Approved by:	[redacted] Hongyan Wang, President/PhD	Report Date:	03-09-2021

Appendix 7 Stability

Product Name: Allulose 97%

Batch No.: 833-20190411, 833-20180925, 833-20181109, 833-20190123 and 833-20190617

Observation Method: Accelerated stability test (0, 1,2,3,4, 5, 6 months) Storage condition: 40℃±2℃/ 75% RH ±5%

Item / Time (month)		Appearance	Moisture (%)	Allulose (HPLC) (%)
833-20190411	0	Off White to White Powder	3.46	99.5
	1	Off White to White Powder	3.41	99.5
	2	Off White to White Powder	3.41	98.8
	3	Off White to White Powder	3.40	98.2
	4	Off White to White Powder	3.40	98.2
	5	Off White to White Powder	3.38	98.5
	6	Off White to White Powder	3.30	97.9
	average	Off White to White Powder	**3.39**	**98.65**
833-20180925	0	Off White to White Powder	3.01	98.2
	1	Off White to White Powder	3.11	98
	2	Off White to White Powder	2.85	98
	3	Off White to White Powder	2.93	97.8
	4	Off White to White Powder	2.90	97.9
	5	Off White to White Powder	2.78	96.5
	6	Off White to White Powder	2.77	96.5
	average	Off White to White Powder	**2.90**	**97.55**

833-20181109	0	Off White to White Powder	2.33	98
	1	Off White to White Powder	2.58	97.8
	2	Off White to White Powder	2.62	97.8
	3	Off White to White Powder	2.66	97.5
	4	Off White to White Powder	2.63	97.5
	5	Off White to White Powder	2.71	97.6
	6	Off White to White Powder	2.72	97.2
	average	Off White to White Powder	**2.82**	**97.62**

Appendix 8 GRAS Associates Expert Panel Report

GRAS ASSOCIATES, LLC

11810 Grand Park Ave
Suite 500
North Bethesda, MD 20852

THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF THE PROPOSED USES OF BLUE CALIFORNIA'S ALLULOSE

July 21, 2021

Foreword

An independent panel of experts ("Expert Panel") was convened by GRAS Associates, LLC on behalf of their client, Blue California, to evaluate the safety and Generally Recognized as Safe (GRAS) status of Blue California's proposed uses of allulose in conventional foods. The members of this Expert Panel† are qualified to serve in this capacity by their scientific training and experience in the safety of food and food ingredients.

Discussion

A significant amount of safety information related to the consumption of allulose is generally available, and has been discussed in Part 6 of Blue California's allulose GRAS dossier. First, there is a history of safe consumption of allulose when used as an ingredient in food products in the US, Japan, and Korea. Second, a number of experimental studies have investigated the safety of allulose. The composite evidence from historical safe consumption and experimental studies demonstrates the safety of allulose preparations for human food consumption.

Blue California's manufacturing process utilizes D-allulose 3-epimerase (DAE) derived from *Thermoclostridium caenicola* and produced by *E. coli* K-12 to manufacture allulose from neutralized fructose syrup. *E. coli* K-12 is a gram-negative, non-spore forming, facultative anaerobe, is nonpathogenic and nontoxigenic, and has a long history of safe industrial use. Blue California has confirmed the absence of residual protein in the finished product, as demonstrated by polymerase chain reaction (PCR) assay results provided in Appendix 6 of their GRAS dossier.

Blue California states that their allulose is manufactured under Current Good Manufacturing Practices (CGMP) and it has been demonstrated that their manufacturing process consistently yields a reproducible product, as detailed in Table 3 of Blue California's GRAS dossier. The specifications for allulose are consistent with industry-established parameters and values, and are

† Dr. Emmel, Chair of the Expert Panel, is a chemist with substantial food safety experience in addressing steviol glycosides and other food ingredients. Dr. Dziwenka holds a Doctor of Veterinary Medicine degree from the University of Saskatchewan and is a Diplomat with the American Board of Toxicology. She has over 23 years' experience as a practicing veterinarian and 20 years of experience in research, preclinical regulatory toxicology, and safety evaluation in food and animal feed additives and GRAS dossier preparation. Dr. Falk is an independent consultant with over 22 years of experience in reviewing food safety issues, GRAS reviews, and new dietary ingredient notifications at the Life Science Research Office (LSRO) and LSRO Solutions. All three panelists have extensive technical backgrounds in the evaluation of food ingredient safety and in participating in deliberations of GRAS Expert Panels.

appropriate and sufficient for an ingredient intended for human consumption. Furthermore, the specifications for Blue California's allulose are substantially equivalent to those described in GRNs 693 and 828, respectively, which received "no questions" letters from FDA. Therefore, the safety data presented in GRNs 693 and 828, as well as in the published literature, are applicable to the GRAS conclusion for Blue California's Allulose preparation.

The majority of studies reviewed on allulose (syn. D-psicose) have been discussed in detail in previous GRAS Notices (GRNs) that received "no questions" letters from FDA: GRN 400, GRN 498, GRN 693, and GRN 828.

The key pharmacokinetic data presented by Tsukamoto (2014) establish that allulose is absorbed in the small intestine and then rapidly excreted in the urine. The liver was noted to be the only organ with allulose accumulation following oral and intravenous administration. No pathology linked to liver concentration has been reported. Rapid excretion of allulose was confirmed by Matsuo (2003), who concluded that allulose is partially absorbed in the digestive tract and rapidly excreted in the urine and feces. In addition, the presence of short chain fatty acids in the cecum indicates that allulose is a fermentable saccharide.

The median lethal dose (LD_{50}) of allulose was determined to be 16.3 g per kg body weight (bw) in rats (Matsuo, 2002). A 90-day repeat dose study conducted by An et al. (2019) in male and female Sprague-Dawley rats determined the No Observed Adverse Effect Level (NOAEL) for allulose to be equal to or greater than 5,000 mg per kg bw per day, the highest dose tested. Previous studies by Yagi and Matsuo (2009) and Matsuo et al. (2012) determined the NOAEL of allulose to be 3% of the diet, the highest dose tested (equivalent to 1,280 mg D-allulose per kg bw day), in male Wistar rats.

Allulose was not observed to cause any adverse effects in healthy dogs when administered as a single dose of 1 or 4 g per kg bw or administered at 200 mg per kg bw per day for 12 weeks (Nishii et al., 2016, 2017).

Kim et al. (2019) investigated the reproductive toxicity of allulose in rats (strain unspecified). No treatment-linked toxicity, mortality, or adverse effects on reproduction were observed. The authors determined the NOAEL to be equal to or greater than 2,000 mg per kg bw per day for the parental and offspring generations.

As reviewed in GRN 400, genotoxicity and mutagenicity studies indicate that allulose is not mutagenic at levels of up to 5,000 µg per plate (Ames study), no significant increase in micronucleated polychromatic erythrocytes was noted at levels of up to 2,000 mg per kg per day (micronucleus test), and no significant increase in the number of chromosomal aberrations was observed at 1,800 µg per mL.

Numerous studies did not detect any adverse effects in humans with doses as much as 15 g total intake for as many as 48 weeks. Products with as much as 30.0 g per person per day allulose, equivalent to 0.4 g per kg bw per day allulose for 70 kg individual, have been in commerce for as many as four years without any causal connections to adverse health effects. Furthermore, Han et

al. (2018) recently recommended a maximum single dose of 0.4 g per kg bw allulose and a maximum total daily intake of 0.9 g per kg bw of allulose, as transient gastrointestinal discomfort, including severe nausea, abdominal pain, headache, anorexia, and diarrhea, is reported when total daily intake approaches 1.0 g per kg bw.

Blue California states in their GRAS dossier that their D-allulose preparation is intended to be used as a sweetener in the food products and at use levels to those presented in Table 5 of their GRAS dossier. Blue California determined the mean and 90th percentile estimated daily intake (EDI) of all users aged 2 years and older of their D-allulose to be 8.6 and 19.1 g per person per day, respectively. All users aged 2 to 99 years had EDIs equal to or below 0.30 g per kg bw per day. The average maximum exposure was estimated in males 19 years of age or older, with a 90th percentile value of 30.4 g per day or 0.33 g per kg bw per day. On a body weight basis, children ages 2-5 years had the highest 90th percentile EDI, at 0.50 g per kg bw per day.

The Expert Panel notes that even with the additional proposed uses, the EDIs are well below the maximum single dose and maximum total daily intake levels recommended by Han et al. (2018). Blue California's Allulose preparation is expected to replace products currently on the market and the additional proposed uses in grain based cereal and protein bars, low-sugar, reduced-sugar, and diet fruit juices, and low- and reduced-calorie alcoholic beverages are not expected to considerably alter exposure. The estimate for cumulative exposure from all uses determined with 2015-2018 NHANES intake data was found to be lower than the EDIs in GRN 828. In addition, the Expert Panel agrees with Blue California's assessment that it is unlikely that allulose will be used at the maximum levels in all food categories and that a consumer would ingest products from all categories on a daily basis. Therefore, allulose preparations are expected to be safe within established allowable limits.

Conclusion

In summary, sufficient qualitative and quantitative scientific evidence in the composite is available to support the safety-in-use of Blue California's allulose given the following conditions:

- Blue California's allulose continues to meet the designated specifications;
- The proposed uses and use levels of Blue California's allulose remain unchanged; and
- Blue California's allulose is produced in accordance with Current Good Manufacturing Practices (CGMPs) using raw materials and processing aids that comply with applicable US federal regulations and are of appropriate purity for food manufacturing purposes.

The Expert Panel critically reviewed the data provided by Blue California for their allulose, as well as publicly available published information obtained from peer-reviewed journals and other safety assessments prepared by other Expert Panels and well-respected international regulatory bodies.

The ingestion of Blue California's allulose from the intended uses results in intakes that are expected to be safe within the limits of established historical use and published safety studies. Furthermore, Blue California determined that the EDI for individuals 2 years or older is 8.6 g per

person per day at the mean and 19.1 g per person per day at the 90th percentile, which is lower than the EDIs reported in GRN 828 of 11.0 and 30.0 g per person per day for the mean and 90th percentile, respectively.

On the basis of scientific principles, the Expert Panel unanimously concludes that the proposed uses of Blue California's allulose preparation, manufactured under GMP standards using raw materials and processing aids in compliance with applicable US federal regulations and as described in Part 2.B. of Blue California's GRAS dossier, and declared within the subject assessment meets the FDA definition of safety in that there is "reasonable certainty of no harm under the intended conditions of use" as described herein, and Blue California's allulose preparation is generally recognized as safe (GRAS).

Margitta Dziwenka DVM, DABT

Michael Falk, Ph.D.

Katrina V. Emmel, Ph.D.
Panel Chair

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Yagi, K. and Matsuo, T. (2009) 'The study on long-term toxicity of d-psicose in rats', J Clin Biochem Nutr, 45(3), pp. 271-7.

END

END

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE

Form Approved: OMB No. ; Expiration Date:
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
001024	07/21/2021
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740-3835.

PART I – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3a. For New Submissions Only: Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: N/A

3b. For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

PART II – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position
Hadi Omrani	Director-Technical & Regulatory Affairs

Company *(if applicable)*: Blue California

Mailing Address *(number and street)*: 30111 Tomas

City	State or Province	Zip Code/Postal Code	Country
Rancho Santa Margarita	California	92688	United States of America

Telephone Number	Fax Number	E-Mail Address
949-635-1990 X131	949-635-1984	hadi@bluecal-ingredients.com

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position
William J. Rowe	President

Company *(if applicable)*: GRAS Assocaites

Mailing Address *(number and street)*: 11810 Grand Park Ave., Suite 500

City	State or Province	Zip Code/Postal Code	Country
North Bethesda	Maryland	20852	United States of America

Telephone Number	Fax Number	E-Mail Address
519-341-3367	888-531-3466	wrowe@nutrasource.ca

PART III – GENERAL ADMINISTRATIVE INFORMATION

1. Name of Substance

Alluose (D-allulose, D-psicose, psicose)

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☒ Electronic files on physical media with paper signature page

☐ Paper

If applicable give number and type of physical media

_____

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in FDA's files by reference? *(Check one)*

☐ Yes *(Proceed to Item 5)* ☒ No *(Proceed to Item 6)*

5. The submission incorporates by reference information from a previous submission to FDA as indicated below *(Check all that apply)*

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for determination of GRAS status *(Check one)*

☒ Scientific Procedures *(21 CFR 170.30(b))* ☐ Experience based on common use in food *(21 CFR 170.30(c))*

7. Does the submission (including information that you are incorporating by reference) contain information that you view as trade secret or as confidential commercial or financial information?

☐ Yes *(Proceed to Item 8)*

☒ No *(Proceed to Part IV)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, see attached Designation of Confidential Information

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

PART IV – INTENDED USE

1. Describe the intended use of the notified substance including the foods in which the substance will be used, the levels of use in such foods, the purpose for which the substance will be used, and any special population that will consume the substance *(e.g., when a substance would be an ingredient in infant formula, identify infants as a special population).*

Blue California's Allulose is intended for use as a sugar substitute/sweetener in a variety of applications as detailed in Part 3.A.2. Table 5 of the GRAS dossier at levels determined by current good manufacturing practices (CGMP). The proposed uses do not include meat and poultry products or infant formulas.

2. Does the intended use of the notified substance include any use in meat, meat food product, poultry product, or egg product? *(Check one)*

☐ Yes ☒ No

PART V – IDENTITY

1. Information about the Identity of the Substance

	Name of Substance[1]	Registry Used *(CAS, EC)*	Registry No.[2]	Biological Source *(if applicable)*	Substance Category *(FOR FDA USE ONLY)*
1	Allulose	CAS	551-68-8	Biosynthesized with D-allulose 3-epimerase	
2					
3					

[1] Include chemical name or common name. Put synonyms *(whether chemical name, other scientific name, or common name)* for each respective item (1 - 3) in Item 3 of Part V *(synonyms)*

[2] Registry used e.g., CAS *(Chemical Abstracts Service)* and EC *(Refers to Enzyme Commission of the International Union of Biochemistry (IUB), now carried out by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB))*

2. Description

Provide additional information to identify the notified substance*(s)*, which may include chemical formula*(s)*, empirical formula*(s)*, structural formula*(s)*, quantitative composition, characteristic properties *(such as molecular weight(s)),* and general composition of the substance. For substances from biological sources, you should include scientific information sufficient to identify the source *(e.g., genus, species, variety, strain, part of a plant source (such as roots or leaves), and organ or tissue of an animal source)*, and include any known toxicants that could be in the source.

Molecular formula: C6H12O6
Chemical name: D-Ribo-2-hexulose
Molecular weight: 180.16 g per mole

Allulose is synthesized from D-fructose in an enzymatic bioconversion process by D-allulose 3-epimerase produced by a strain of *E. coli* K-12. The resulting product is purified to yield a > 97% allulose (%wt/wt) finished product.

There are no known toxicants.

3. Synonyms

Provide as available or relevant:

1	D-psicose or psicose
2	D-Altrulose
3	D-erythro-hexulose

PART VI – OTHER ELEMENTS IN YOUR GRAS NOTICE

(check list to help ensure your submission is complete – check all that apply)

- ☒ Any additional information about identity not covered in Part V of this form
- ☒ Method of Manufacture
- ☒ Specifications for food-grade material
- ☒ Information about dietary exposure
- ☒ Information about any self-limiting levels of use *(which may include a statement that the intended use of the notified substance is not-self-limiting)*
- ☒ Use in food before 1958 *(which may include a statement that there is no information about use of the notified substance in food prior to 1958)*
- ☒ Comprehensive discussion of the basis for the determination of GRAS status
- ☒ Bibliography

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☒ Yes ☐ No

Did you include this other information in the list of attachments?

☒ Yes ☐ No

PART VII – SIGNATURE

1. The undersigned is informing FDA that Blue California *(name of notifier)*

has concluded that the intended use*(s)* of Alluose (D-allulose, D-psicose, psicose) *(name of notified substance)*

described on this form, as discussed in the attached notice, is (are) exempt from the premarket approval requirements of section 409 of the Federal Food, Drug, and Cosmetic Act because the intended use(s) is (are) generally recognized as safe.

2. ☒ Blue California *(name of notifier)* agrees to make the data and information that are the basis for the determination of GRAS status available to FDA if FDA asks to see them.

Blue California *(name of notifier)* agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so.

30111 Tomas, Rancho Santa Margarita, A 92688 *(address of notifier or other location)*

Blue California *(name of notifier)* agrees to send these data and information to FDA if FDA asks to do so.

OR

☐ The complete record that supports the determination of GRAS status is available to FDA in the submitted notice and in GRP No. ---------- *(GRAS Affirmation Petition No.)*

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
Katrina Emmel Digitally signed by Katrina Emmel Date: 2021.07.21 10:18:06 -07'00'	Katrina Emmel on behalf of William J. Rowe, President	7/21/2021

PART VIII – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Appendices 1-4 and 6-8 in the body of the dossier	
	Appendix 5 as a separate file on the CD	