FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

8 November 2023
269-23

Supporting document 1

Technical and Risk assessment – Application A1247

D-allulose as a novel food

Executive summary

FSANZ has assessed an application from Samyang Corporation (Samyang) to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of D-allulose as a novel food. D-allulose is intended to be used as a low-energy substitute for conventional sugar ingredients, particularly sucrose. Samyang's D-allulose is manufactured by enzymatic epimerisation of D-fructose. The application also requests approval of the D-psicose-3-epimerase contained in the organism *Microbacterium foliorum* to be used as a processing aid.

Samyang have provided assurance of their ability to produce D-allulose suitable for use in foods as a sugar replacer and which is consistent with specifications set out in scientific literature.

Most (80%) of an oral dose of D-allulose is rapidly absorbed from the small intestine, and rapidly excreted in the urine. There is some metabolism of D-allulose by microbiota in the large intestine, but it appears that most D-allulose that reaches the large intestine is excreted unchanged in the faeces. D-allulose is of very low acute and subchronic (90 day) toxicity in rats. Results of genotoxicity assays were negative, and D-allulose was not associated with carcinogenicity or with adverse reproductive or developmental effects in rats. Laxative effects, attributed to the osmotic effect of D-allulose that is not absorbed from the gastrointestinal tract, have been observed in laboratory animals and in humans. The lowest single dose of D-allulose associated with gastrointestinal effects in humans was 0.4 g/kg bw and to avoid laxation, repeated intake should not exceed a total consumption of 0.9 g/kg bw/day.

No public health or safety concerns were identified in relation to the use of *M. foliorum* in the production of D-psicose-3-epimerase. It is neither pathogenic nor toxigenic. D-psicose 3-epimerase has a five-year history of safe use for the production of D-allulose, and the applicant has provided analytical evidence that there is negligible likelihood of consumer exposure to the production organism, the intact enzyme, or residues from the enzyme. No significant homology was found with any known toxins or allergens.

Dietary intake assessments were conducted to estimate dietary intake of the added and naturally occurring D-allulose. Estimated mean and high chronic dietary intakes of added D-allulose based on maximum use levels requested in the application ranged between 150 and 730 mg/kg bw/day across the Australian and New Zealand population groups and scenarios

assessed. Estimated chronic dietary intakes from naturally occurring sources of D-allulose were very low compared to intakes from added sources (≤11 mg/kg bw/day).

A short-term dietary intake assessment identified a number of food categories from which the intake of around 10% of high consumers exceeded the level of D-allulose that causes a laxative effect based on the maximum use levels provided in the application. A further assessment was then undertaken to determine what use levels would result in intakes not exceeding the level that causes a laxative effect based on normal food consumption amounts when consumed as one food containing D-allulose per eating occasion. This resulted in lower concentration levels compared to the maximum use levels proposed in the application for some foods.

Although D-psicose-3-epimerase enzyme is used in the D-allulose manufacturing process, analytical results confirmed that the enzyme will not be present in D-allulose. Hence, FSANZ has not undertaken a dietary exposure assessment for the enzyme, which is consistent with similar international assessments.

FSANZ considered the available evidence for calculation of the components of the equation for metabolisable energy of D-allulose. It is concluded that:

Gross energy (GE) = 15.7 kJ/g
Urinary energy (UE) = 80% of gross energy
Fecal energy (FE) = 3% of gross energy
Gaseous energy (GaE) = 5% of gross energy
Surface area energy (SE) = 0% of gross energy.

No evidence was identified to indicate that D-allulose consumption would affect the absorption of other nutrients. It is concluded that there is no toxicological risk to public health and safety from consumption of D-allulose in food, or from the use of D-psicose 3-epimerase in the production of D-allulose. An Acceptable Daily Intake (ADI) “not specified” is appropriate for both D-allulose and D-psicose 3-epimerase.

Based on previous assessments of rare sugars and polyols that may induce osmotic laxation, a recommendation against consuming a high single dose of D-allulose may be appropriate. The lowest dosage of D-allulose associated with gastrointestinal symptoms is 0.4 g/kg bw (400 mg/kg bw; 28 g for a 70 kg adult) as a single dose. High consumption of some food categories was estimated to result in dietary intakes of D-allulose above this level at the proposed maximum use levels in the application. Further assessment showed that lower concentrations of D-allulose for some food classes could result in intakes that do not exceed the level that causes laxative effects if eaten without consuming other foods containing D-allulose in the same eating occasion.

No public health or safety concerns were identified in the microbiological safety assessment of D-allulose and healthy adults. D-allulose intakes for chronic human feeding trials (≥8 weeks duration) were similar to the estimated dietary intakes for single day of consumption. Exclusion criteria for the human feeding studies did not include sub-populations such as diabetics, which may be a potentially sensitive sub-population. The high percentage of absorption of untransformed D-allulose in the small intestine through the kidneys to urine does flag potential issues with urinary tract infections. Uropathogenic bacteria such as *Klebsiella pneumoniae* which are able to metabolise D-allulose. The microbiological safety of subpopulations consuming D-allulose has not been established. FSANZ will continue to monitor the emerging literature on any potential microbiological risks associated with D-allulose consumption.

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1 Introduction

The applicant, Samyang Corporation (Samyang), is seeking permission for D-allulose as a novel food. D-allulose is intended to be used as a low-energy substitute for conventional sugar ingredients, particularly sucrose. Samyang's D-allulose is manufactured by enzymatic epimerisation of D-fructose. The application also requests approval of the D-psicose-3-epimerase contained in the organism *Microbacterium foliorum* to be used as a processing aid.

2 Food technology assessment

2.1 Objectives of the food technology assessment

The food technology assessment evaluated information on the identity and properties of the D-allulose and its use and stability in the various foods proposed by the applicant. The assessment also considered the manufacturing process of both the D-allulose and also the identity and properties of the D-psicose-3-epimerase enzyme.

2.2 D-allulose

D-allulose, also widely known as D-psicose, is an epimer[1] of D-fructose and a natural monosaccharide but rarely occurs in nature. It is reported to be increasingly being used by food and beverage manufacturers as a low-calorie sweetener. D-fructose can be converted to D-allulose by the enzyme D-tagatose 3-epimerase, which has allowed for mass production of allulose (Hossain et al. 2015, Mu et al. 2012). D-allulose is found naturally in trace amounts various fruits and has similar physical properties to those of regular sucrose, when used in the food industry such as bulking agent, providing mouthfeel, browning capability, and depression of freeze point. This makes it suitable as a sugar replacement in many food products, including ice cream.

D-allulose is 70% as sweet as sucrose, but only contains around 0.8 kJ/g compared to 17 kJ for sucrose (Mooradian et al 2017, Hossain et al (2015 b)). It provides the same or improved functional properties of conventional sugar ingredients including high solubility and antioxidant activity. First identified in wheat in the 1940s, allulose is naturally present in various fruits in small quantities and other foods, such as bakery products, seasonings and fermented sauces.

Samyang determined from stability studies that D-allulose crystalline powder and syrups are stable under typical storage conditions. Their D-allulose, contained in a cereal food matrix was stable for 85 days at accelerated storage conditions. Samyang expect D-allulose to exhibit similar stability in foods as other simple monosaccharides such as D-fructose and glucose.

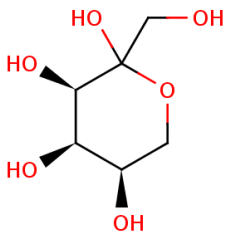
[1] In stereochemistry, an epimer is one of a pair of diastereomers. The two epimers have opposite configuration at only one stereogenic center out of at least two. All other stereogenic centers in the molecules are the same in each. Epimerization is the interconversion of one epimer to the other epimer.

Compared to intense sweeteners, D-allulose provides greater bulking and sensory properties in foods. D-allulose provides improved properties when combined with egg white proteins (foaming, cross-linking and browning) compared to sucrose, glucose and fructose. As a replacement for sucrose and fructose in custard pudding desserts, it provides higher antioxidant activity and strong gelling activity, according to Samyang and supported by scientific literature (Daniells 2008, Sun et al. 2008).

2.2.1 Identity of D-allulose

D-allulose is a monosaccharide and a ketohexose (PubChem 2004) and a C3 epimer of D-fructose (Hu et al. 2021).

Table 1 Identity

Generic common names:	D-allulose, D-psicose
IUPAC name:	(3R,4R,5R)-1,3,4,5,6-Pentahydroxyhexan-2-one
Other names:	DL-psicose, D-ribo-2-hexulose, D-ribo-2-ketohexose, Pseudo-fructose, Erythrohexulose,
CAS Registry ID:	551-68-8
Molecular formula:	$C_6H_{12}O_6$
Molecular Weight:	180.16
Melting point:	114-115 °C
Specific optical rotation:	+4.7 (Crystal)
Solubility in water:	324.2 g/100 mL @ 25°C
Specific gravity:	1.35 g/cm^3, 74% solution
Particle size:	50-500 micrometres.
Chemical Structure of D-allulose:	

There are relevant international specifications in the primary and secondary sources listed in section S3—2 of Schedule 3 of the Code. Food Chemicals Codex (FCC 2020) and the Merck Index, 15th Edition, (O'Neil et al 2013) have specifications for Allulose and D-psicose respectively.

2.2.2 Method of Manufacture

Samyang's D-allulose is manufactured by conversion of D-fructose in an aqueous solution by enzymatic epimerisation in the presence of manganese ions or magnesium ions. The enzyme used is an immobilized D-psicose 3-epimerase expressed in the microorganism *M. foliorum* (SYG27B-MF), which converts the fructose to D-allulose. Samyang employs a unique immobilized enzyme system described below.

A 75º Brix fructose syrup is diluted with potable water to a 50º Brix concentration before being passed through a food grade immobilized cell system utilising a calcium alginate gel bead entrapped with a non-GMO *M. foliorum* (SYG27B-MF) cell possessing D-psicose 3-epimerase activity. The D-fructose is then converted to D-allulose at 50ºC in the presence of manganese ions, which promote the enzymatic epimerization process. Samyang uses manganese sulphate as a source of manganese.

The D-allulose ingredients are treated with activated carbon and clay in the decolourisation and desalting step, primarily to adsorb pigments, but also to reduce the any oxidation products, such as trace metals, phosphorus and, to a lesser extent, sulphur compounds. The liquid then undergoes pressure filtration for clarification, before being treated through an ion exchange process. This process employs a cation column with strongly acidic cation exchange resin, an anion column with intermediate basic anion exchange resin and a mixed bed column that has a combination of both strongly acidic and strongly basic resins. This process removes any impurities such as calcium, manganese, chloride, and other ionic components, including amino acids, peptides, and proteins.

Following ion exchange purification, the D-allulose solution is concentrated with an evaporator to produce an 8% allulose syrup (D-allulose Syrup L) on an as-is basis. This concentrated syrup is pumped into a separation chromatography system to separate D-allulose from other sugars, particularly fructose. By evaporation, the solution is concentrated to the final density of 68º Brix to produce a 62% allulose syrup (D-allulose Syrup H) on an as-is basis.

The final concentrated product is pumped into a crystallizer, where the crystalline D-allulose is separated by basket centrifugation, washed by spraying distilled water, and finally dried to give the final D-allulose concentration of 98% (Crystalline D-allulose).

Samyang's manufacturing plant was designed specifically for D-allulose and uses unit processes such as neutralisation, bleaching and deodorizing that are standard in the edible carbohydrate industry, providing a final D-allulose ingredient suitable for use in foods. The manufacturing plant operates in accordance with standard GMP, under International Standards Organisation (ISO) 9001:2000 and Hazard Analysis and Critical Control Point (HACCP) certification.

Samyang's D-allulose manufacturing process adequately demonstrates that D-psicose 3-epimerase enzyme, characterised below, produces a D-allulose which meets the internal target specification on a consistent basis, for both the syrup and powder varieties.

Furthermore, Samyang provided evidence that the D-psicose 3-epimerase enzyme is not present in its final D-allulose and subsequently not present in D-allulose when sold as a food, or used as an ingredient in other foods.

Samyang produces both syrup and powder varieties of D-allulose, with those relevant to this application being shown in Table 2. During the assessment period, the brand name changed from those included in the application for the three Samyang D allulose products:

- Nexweet Crystalline Allulose, previously Crystalline D-allulose (Product 1)
- Nexweet Allulose 95L, previously D-allulose Syrup H (Product 2)
- Nexweet Allulose 10L, previously D-alluose Syrup L (Product 3).

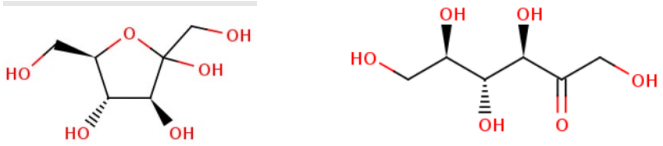
Table 2 Specifications for D-allulose crystalline D allulose and syrups 95L and 10L

Composition	**Specification**		
Product name	**Nexweet Crystalline Allulose**	**Nexweet Allulose 95L**	**Nexweet Allulose 10L**
Appearance	Powder	Clear yellow liquid	
Odour	No odour	No odour	No odour
D-allulose, g/100g, dry wt. basis	≥98	≥90	≥10
D-allulose, g/100g, as-is basis	≥98	≥62	≥8
Moisture, g/100g	≤2	≤32	≤25
Brix	NA	≥68	≥75
pH	NA	3.0 – 7.0	3.0 – 7.0
Ash, g/100g	≤0.1	≤0.1	≤0.1
Pb, mg/kg	≤0.1	≤0.1	≤0.1
As, mg/kg	≤0.1	≤0.1	≤0.1
Cd, mg/kg	≤0.1	≤0.1	≤0.1
Hg, mg/kg	≤0.1	≤0.1	≤0.1
Total plate count, CFU/g	≤1,000	≤1,000	≤1,000
Coliforms, CFU/g	ND	ND	ND
Salmonella, CFU/25g	ND	ND	ND
Staphylococcus aureus, CFU/g	ND	ND	ND

2.3 Characterisation of the D-psicose 3-epimerase enzyme

2.3.1 Identity

Table 3 Identity of D-psicose 3-epimerase

Generic common name:	D-psicose 3-epimerase
Systematic name:	D-allulose 3-epimerase
Other names:	D-allulose 3-epimerase, DPEase, L-ribulose 3-epimerase; ketose 3-epimerase, Psicose epimerase, Allulose epimerase
EC number:	5.1.3.30
Reaction: ***(from the enzyme database BRENDA[2])***	Epimerization of D-fructose at the C3 position to D-allulose and vice-versa  D-fructose = D-psicose

FSANZ considers D-psicose 3-epimerase enzyme expressed in *Microbacterium foliorum* (SYG27B-MF) as a processing aid in the context of the Code. D-psicose 3-epimerase from *Microbacterium foliorum* (SYG27B-MF), is not currently permitted for use as a processing aid in the Code.

The activity of D-psicose 3-epimerase is ≥1400 U, where 'U' is the amount (mM) of allulose that is converted from D-fructose per gram of cells or enzymes. The optimal pH for enzyme activity is 6.5 at a temperature of 60°C. The enzymatic epimerisation of D-fructose to D-allulose can be promoted in the presence of manganese ions (Mn(2+)). Samyang uses manganese sulphate as a source of manganese to promote the epimerisation process.

2.3.2 Production of the enzyme

The manufacture of the processing aid, D-psicose-3-epimerase, including the raw materials and ingredients used, cultivation of *M. foliorum* SYG27B-MF and preparation of the immobilisation bead for D-allulose production was provided in detail in within a CCI Annex supplied by Samyang. The enzyme is produced by submerged fermentation which is a common method for producing food enzymes.

[2] RENDA Comprehensive Enzyme Information System. https://www.brenda-enzymes.org/enzyme.php?ecno=5.1.3.30&Suchword=&reference=&UniProtAcc=&organism%5B%5D=

2.3.3 Specifications

There are international general specifications for enzyme preparations used in the production of food. These have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in its Compendium of Food Additive Specifications (FAO/WHO 2006) and in the Food Chemicals Codex (FCC) (USPC, 2020). These specifications are included in earlier publications of the primary sources listed in section S3—2 of Schedule 3 of the Code, and enzymes used as a processing aid must meet either of these specifications. In addition, under JECFA, enzyme preparations must meet the specifications criteria contained in the individual monographs. In the case of D-psicose 3-epimerase, there is no individual monograph.[3]

Samyang Corporation's D-psicose 3-epimerase enzyme is not a purified enzyme preparation. The enzyme is naturally present in *M. foliorum* SYG27B. Specifications for Samyang Corporation's *M. foliorum* SYG27B harbouring the enzyme D-psicose 3-epimerase meet the JECFA specifications for enzyme preparations. A specification for *M. foliorum* SYG27B harbouring D-psicose 3-epimerase, including enzyme activity was provided as well as results from five non-consecutive representative batches, shown in Table 4 below.

Table 4 Comparison of Samyang Corporation's D-psicose 3-epimerase enzyme compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes

Analysis	***Analysis provided by manufacturer****	***Specifications***		
		JECFA (2006)	***Food Chemicals Codex (FCC, 2020)***	***Australia New Zealand Food Standards Code (section S3—4)***
Lead (mg/kg)	Not detected (LOD* 0.05)	≤ 5	≤ 5	≤2
Arsenic (mg/kg)	Not detected (LOD 0.05)	0.05	-	≤1
Cadmium (mg/kg)	Not detected (LOD 0.04)	0.04	-	≤1
Mercury (mg/kg)	Not detected (LOD 0.05)	50	-	≤1
***Listeria monocytogenes* (in 25 g)**	Negative	-	-	-
***Salmonella* (in 25 g)**	Negative	Absent	Negative	-
***E. coli* (in 25 g)**	0	Absent	-	-
Mould & yeast plate count (CFU/g)	0 (Target ≤ 20)	-	-	-
Total plate count (CFU/g)	0 (Target ≤ 100)	-	-	-

*LOD = Limit of Detection

[3] For the functional use 'enzyme preparation', the JECFA database can be searched for individual monographs: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/

Based on the above results, the enzyme preparation meets international and Code specifications for enzymes used in food production.

2.4 Technological purpose of the enzyme

D-psicose 3-epimerase (also known as D-allulose-3-epimerase), is used exclusively for the production of D-allulose, having the specific technological function of optimising the conversion process of D-fructose to D-allulose.

The enzyme and the *M. foliorum* organism harbouring the enzyme is not present in final D-allulose products that are sold as food or incorporated into foods as ingredients. Samyang has conducted testing to ensure no *M. foliorum* is present in commercial D-allulose products (in both the syrups and powder form). No colony forming units were detected after 6 days of incubation of the syrup and powder products; nor was any DNA of *M. foliorum* detected in commercial D-allulose products after applying a primer that specifically acts on the production strain.

The stated technological purpose of the D-psicose 3-epimerase enzyme is supported by scientific literature (Patel et al. 2021, Walch 2020).

2.5 Food technology conclusion

D-allulose:
Samyang have provided assurance of their ability to produce a final D-allulose ingredient suitable for use in foods as a sugar replacer and which is consistent with specifications set out in scientific literature. Their manufacturing plant operates in accordance with standard GMP, under International Standards Organisation (ISO) 9001:2000 and Hazard Analysis and Critical Control Point (HACCP) certification and are operated to comply with a strict environmental protection code.

Stability studies provide assurance that the D-allulose crystalline powder and syrups are stable under typical storage conditions as well as when contained in a food matrix typical of the proposed end use.

D-psicose 3-epimerase enzyme:
FSANZ concludes that the proposed use of D-psicose 3-epimerase as an enzyme used exclusively for the production of D-allulose is justified. FSANZ concludes that the evidence presented to support the proposed use provides adequate assurance that the use of the enzyme, in the form and requested amount (i.e. at a level consistent with GMP) is technologically justified and has been demonstrated to be effective in achieving its stated purpose.

D-psicose 3-epimerase performs its technological purpose during the production of D-allulose and is not performing a technological purpose in the final food. It is therefore appropriately categorised as a processing aid as defined in the Code. Samyang also provided evidence that the D-psicose 3-epimerase enzyme is not present in its final D-allulose and subsequently not present in D-allulose when sold as a food, or used as an ingredient in other foods.

There are relevant identity and purity specifications for the enzyme in the Code and the applicant provided evidence that the enzyme meets these specifications.

3 Safety assessment

Some information relevant to this section is Confidential Commercial Information (CCI), so full details cannot be provided in this public report.

3.1 Objectives for safety assessment

The objectives of this safety assessment are to

- Evaluate any potential public health and safety concerns that may arise from the use of D-allulose as a low-energy substitute for sugars, particularly sucrose
- Ascertain the safety of *Microbacterium foliorum*.
- Evaluate any potential public health and safety concerns associated with the use of the enzyme D-psicose 3-epimerase in the production of D-allulose.

3.2 Hazard assessment of D-allulose

3.2.1 Toxicokinetics of D-allulose

The kinetics of D-allulose have been investigated *in vitro*, and in studies in rats and human beings.

3.2.1.1 Absorption

D-allulose is highly stable in simulated gastric fluid (SGF) and in fasted-state simulated intestinal fluid[4] (FaSSIG). D-allulose, 250 µg, was incubated in the respective fluids and aliquots were collected at 0, 60 and 120 min from the SGF assay and at 0, 60, 120, and 240 min from the FaSSIG assay. Approximately 98% of the D-allulose remained about 120 min incubation in SGF, and approximately 100% remained after 240 min incubation in FaSSIG (Maeng et al 2019).

Radiolabelled D-allulose was used to investigate the kinetics of D-allulose in a study by Tsukamoto et al (2014). Wistar rats were fasted for 24 hours before D-allulose, 100 mg/kg bw, was administered either intravenously or by oral gavage. Cohorts of rats were killed 10, 30, 60 and 120 minutes after administration, and gastrointestinal contents were collected. Following oral gavage, radiolabel was first noted in the urine at 30 min, indicating rapid absorption of some D-allulose. The level of radiolabel in blood was highest at 60 min post-gavage.

Matsuo et al (2003) investigated the kinetics of D-allulose in male Wistar rats. In the first of three experiments, fasted rats were gavaged with D-allulose at 5 g/kg bw, and urine and faeces were collected at 24-hour intervals for up to 72 h. In samples collected at 24 h, 11-15% of the original dosage was measured in urine, and 8-13% was measured in faeces. No D-allulose was found in urine or faeces excreted between 24 to 48 hours, or in urine or faeces excreted between 48 and 72 hours. In the second experiment, fasted rats were gavaged with D-allulose at 5 g/kg bw, and six rats/timepoint were decapitated at 1 h, 3 h, and 7 h after D-allulose administration. D-allulose was measured in serum, stomach contents,

[4] Contents of commercial fasted-state and fed-state simulated intestinal fluids vary, but fasted-state simulated intestinal fluid typically contains less taurocholate, phospholipid, sodium chloride, and sodium hydroxide, and has higher pH, lower osmolality and lower buffer capacity than fed-state simulated intestinal fluid.

small intestinal contents and caecal contents. D-allulose decreased rapidly in serum but was still present at the 7 h timepoint. The concentration of D-allulose in stomach contents was 26-37% of the original dose at 1 h but only 0.4 to 0.6% at 3 h. D-allulose was not detected at 7 h. The fraction of the original dose present in the small intestinal contents was 6-10% at 1 h, 2-3% at 3 h and 1-3% at 7 h. D-allulose was not present in the caecum at 1 h, but caecal contents accounted for 10-19% of the original dose at 3 h and at 7 h. The results of these experiments indicate that D-allulose is only partially absorbed from the gastrointestinal tract. The third experiment is described in section 3.2.1.3.

As a result of a small series of studies of D-allulose in healthy volunteers, Iida et al (2010) estimated that approximately 80% of a dose of D-allulose is absorbed from the small intestine, and the remaining 20% passes to the large intestine. The first study was conducted on six healthy volunteers who fasted overnight prior to each intervention. The interventions were consumption of 100 mL of water, 0.35 g/kg bw starch hydrolysate, or 0.35 g/kg bw D-allulose. Interventions were separated by intervals of a week. Oxygen consumption and carbon dioxide production were measured in breath for 180 min, and urinary nitrogen was also measured. The measurements were used to calculate carbohydrate energy expenditure. Fourteen healthy volunteers participated in the second and third studies. Fructooligosaccharide (FOS) was used as the positive control substance. Interventions, separated by intervals of one week, were 0.08, 0.17 and 0.33 g/kg bw D-allulose, and the same dosages of FOS. All participants were provided with the same diets during the measurement periods, and parameters measured included breath hydrogen and urinary D-allulose. In the fourth and final study, eight participants consumed 5 g D-allulose three times a day with meals. In addition, participants consumed 15 g D-allulose once on the first and last days of the experiment. Breath hydrogen gas was assayed at pre-established intervals, including eight intervals after the 15 g D-allulose doses. The absorption estimates were derived from urinary D-allulose measurements. The authors noted that their estimates would vary with food consumption.

Transport of D-allulose across human enterocytes was investigated by Hishiike et al (2013) using a Caco-2 cell monolayer as the model. Permeation of D-allulose across the monolayer was unaffected by phlorizin, which inhibits the sugar transporter SGLT1, the predominant transporter of glucose into enterocytes. In contrast, permeation of D-allulose was significantly enhanced by addition of forskolin, an inducer of expression of GLUT5, the predominant transporter of fructose into enterocytes. The predominant sugar transporter of the basolateral membrane of enterocytes is GLUT2, which transports both D-glucose and D-fructose, as well as D-mannose and D-galactose. The rate of permeation of D-allulose was suppressed in the presence of glucose and fructose, suggesting that the three sugars are all transported from the enterocyte lumen to the lamina propria by GLUT2. Overall, the results support the conclusion that D-allulose is transported by the same transporters as D-fructose, and to the same extent.

Consistent with the *in vitro* findings of Hishiike et al (2013), Kishida et al (2019) found that when D-allulose and D-fructose were administered concurrently to rats by gavage, the absorption of D-allulose was delayed, and decreased overall, when compared to gavage administration of D-allulose alone. Partial inhibition of absorption of radiolabelled fructose by concurrent administration of D-allulose was also demonstrated. In rats in which expression of GLUT5 had been increased by the feeding of fructose, D-allulose absorption was dramatically higher than in rats that had not been fed fructose. These findings support the role of GLUT5 previously proposed by Hishiike et al (2013) and support partial competitive inhibition of the absorption of each sugar by the other.

3.2.1.2 Distribution

In their pharmacokinetic study with radiolabelled D-allulose administered to Wistar rats IV or by oral gavage, Tsukamoto et al (2014) found no evidence of accumulation of D-allulose in tissues other than the liver. They also conducted a whole-body autoradiography study in mice with radiolabelled D-allulose, administered at 100 mg/kg bw IV. In this study, high signals were observed in liver, kidney and urinary bladder. No signal was observed in the brain.

3.2.1.3 Metabolism

In contrast to fructose, which is extensively metabolised in hepatocytes, D-allulose is not degraded by human or rat hepatocytes *in vitro* (Maeng et al 2019).

Whistler (1974) found that administering radiolabelled D-allulose by oral gavage to rats resulted in approximately 20% of the radiolabel being excreted as carbon dioxide, whereas following intravenous (IV) administration, less than 1% was excreted as carbon dioxide. Furthermore, almost all the radiolabel found in the urine following IV administration was associated with D-allulose, whereas approximately 30% of the radiolabel found in the urine following gavage administration was associated with unidentified metabolites. These findings support the conclusion that a proportion of D-allulose administered orally is metabolised by microbial flora in the gastrointestinal tract of rats.

A 34-day dietary study in rats, the third of the three experiments conducted by Matsuo et al (2003), supports the conclusion that some of the D-allulose that is not absorbed into the systemic circulation is fermented in the large intestine. Juvenile male Wistar rats, 6/group, were provided with *ad libitum* access to a high-carbohydrate diet containing 0, 10, 20 or 30% D-allulose and 65, 55, 45 or 35% corn starch for 34 days, after which they were fasted overnight and killed by decapitation. Caecal weight, surface area and content weight were measured. D-allulose in the diet was associated with dose-related decreases, relative to the control group, in group mean values for bodyweight gain, food intake and food efficiency. There was a dose-related increase in group mean values for caecal weight and caecal surface area, compared to control values, although caecal density did not differ. There were dose-related increases in the short-chain fatty acids (SCFAs) acetic, propionic and butyric acid in the caecum, and these were positively correlated with increases in caecal weight and surface area.

Iida et al (2010) assessed the availability of D-allulose absorbed in the small intestine in six healthy volunteers by measurement of carbohydrate energy expenditure (CEE) using indirect calorimetry, after ingestion of 0.35 g/kg bw D-allulose. It was concluded that D-allulose was not metabolized to energy, because CEE did not increase within 3 h of D-allulose ingestion. Fourteen volunteers participated in a breath hydrogen gas study to evaluate D-allulose fermentation in the large intestine, using fructooligosaccharide (FOS) as a positive control because its available energy is known. Based on a plot of breath hydrogen concentration versus calories ingested, the energy value of D-allulose was estimated to be less than 1.6 kJ/g. This contrasts with FOS for which the energy value is 8.4 kJ/g. Eight subjects undertook a further study in which they consumed 5 g D-allulose three times a day with meals, to determine whether repeated consumption resulted in a change in D-allulose fermentation, but no significant difference in breath hydrogen concentration, relative to the acute exposure, was observed.

3.2.1.4 Excretion

Following intravenous administration to rats, D-allulose was eliminated with a mean half-life of 72.2 min and a systemic clearance of 15.8 mL/min/kg (Maeng et al 2019).

In their whole-body autoradiography study in mice dosed with radiolabelled D-allulose, Tsukamoto et al (2014) found that more than 99% of the radiolabel was excreted within 7 days, primarily via the renal route.

Iida et al (2010) measured urinary excretion of D-allulose in 14 healthy volunteers. The accumulated urinary excretion rate was approximately 70% for doses of D-allulose ≤ 0.34 g/kg bw.

3.2.1.5 Toxicokinetics summary

In summary, most of a dose of D-allulose is rapidly absorbed intact from the small intestine, by the same transporters as fructose, but rapidly excreted unchanged in the urine. There may be partial competitive inhibition of absorption of D-allulose and fructose if they are consumed together. Radiolabel associated with D-allulose does not accumulate in tissues other than the liver and does not appear to cross the blood-brain barrier. D-allulose absorbed from the small intestine does not appear to be converted to energy, and there appears to be little fermentation of the fraction (20%) of D-allulose that reaches the large intestine. Some of an oral dose of D-allulose is neither absorbed nor fermented, but excreted unchanged in the faeces.

3.2.2 Toxicology studies

There are several publications on the effects of administering D-allulose to laboratory animals, although most studies are efficacy studies rather than toxicology studies. Some information on toxicology can be obtained from these studies.

3.2.2.1 Acute and short-term toxicity studies in animals

Acute LD50 study of D-allulose in male Wistar rats. (Matsuo et al. 2002b). Regulatory status: Not GLP, no Guidelines specified.

The test article for this study was prepared in the testing laboratory from D-fructose, using immobilized D-tagatose 3-epimerase and diluted 50/50 with water. Dose analysis is not described. Male Wistar rats were assigned to five dose groups of 8 rats/group. It is not clear from the paper whether the rats were three or four weeks old when dosed, but they were 76 ± 2 g bw. They were fasted for 12 h prior to gavage with a bolus of D-allulose of 8, 11, 14, 17 or 20 g/kg bw. Four hours after dosing, rats were provided with *ad libitum* access to water and standard laboratory rat feed. Rats were then observed for 14 days for clinical abnormalities or mortality. All rats developed diarrhoea within the 24 h after dose administration. Physical weakness was observed in rats dosed with ≥ 17 g/kg bw. Death occurred to three rats in the 14 g/kg bw group, three rats in the 17 g/kg bw group and all eight rats in the 20 g/kg bw group. All deaths occurred within 48 h of dose administration. Calculated LD50 values were 16.3 g/kg by the Behrens-Karber method and 15.8 g/kg by the Litchfield-Wilcoxon method. Haemorrhages were found in the mucosa of stomach and small intestine on necropsy of rats that died in the 17 and 20 g/kg bw groups.

Four-week dietary study of D-allulose in Sprague-Dawley rats. (Nagata et al. 2015). Regulatory status: Not GLP, no Guidelines specified.

D-allulose, 97.5% pure, was purchased from a commercial supplier. Four-week-old Sprague-Dawley rats of unspecified sex were individually housed and acclimatised to standard laboratory environmental and husbandry conditions for a week. Rats were then assigned, 24/group, to two groups. One group was provided with a diet in which corn starch was a major source of carbohydrate, while the other group was provided with a diet in which 3% D-allulose replaced the equivalent amount of corn starch in the control diet. All other macro- and micronutrients in the two diets were the same. The rats were maintained on the diets *ad libitum* for four weeks. At the end of the study, rats were killed by decapitation, at four designated times (9:00 am, 3:00 pm, 9:00 pm and 3:00 am), and results were presented for each decapitation time. Blood was collected and processed to serum. Liver, soleus muscle, interscapular brown adipose tissue, jejunum, ileum and abdominal adipose stores were collected. Lipid levels were measured in serum and liver, and liver enzymes were measured in serum. Total RNA was extracted from liver, jejunum, soleus muscle and mesenteric adipose tissue, and real-time quantitative PCR was used to measure gene expression of enzymes and proteins involved in lipid metabolism.

Group mean values for bodyweight were moderately lower (approximately 6-7%) in the rats in the D-allulose group and the differences reached statistical significance, but food intake did not differ significantly between the groups on the two diets. Consumption of the D-allulose diet was associated with decreased group mean values for serum insulin, relative to control values, at all decapitation timepoints, and the difference was statistically significant at 9:00 am and 3:00 am. Group mean values for serum leptin, activity of glucose-6—phosphate dehydrogenase, and activity of malic enzyme were also consistently lower in rats fed D-allulose than in controls killed at the same timepoint. Consumption of the D-allulose diet was associated with relatively increased hepatic expression of peroxisome proliferator-activated receptor α (PPAR-α).

A further four-week experiment of similar design was conducted. In reporting this experiment, the authors specified that the rats were male. In this experiment, at the end of the four weeks, all rats were placed in metabolic chambers and energy expenditure was measured over 24 hours from oxygen consumption and carbon dioxide production. Analysis was by combined gas analyser and mass spectrometer.

At the time they were placed in metabolic chambers, the group mean values for bodyweight and food intake were significantly lower in rats that had been fed D-allulose than in rats fed the control diet. During the dark period, the group mean value for fat oxidation was significantly higher than that of rats fed the control diet, whereas the carbohydrate oxidation was significantly lower, and energy expenditure during the light period was significantly higher.

It is evident from these results that a diet containing 3% D-allulose is associated with significantly decreased bodyweight gain in Sprague-Dawley rats, compared to a control diet.

Thirty-four day dietary study of D-allulose in male Wistar rats. Matsuo et al. (2002b). Regulatory status: Not GLP, no Guidelines specified.

The test article for this study was prepared in the testing laboratory, as described above for the LD50 study that was reported in the same paper, and dose analysis is not described. Again, it is not clear whether the rats were 3 or 4 weeks old, because the abstract states that

they were 3 weeks old, but the methods section states that they were obtained at three weeks old and acclimatised for one week. Rats were assigned to five groups of seven rats/group. Diets contained 0, 10%, 20%, 30% or 40% D-allulose, with the corn starch component of the diet reduced as the D-allulose component increased. All other macro- and micronutrients in the diets were the same for all groups. Food and water were provided *ad libitum*. Other aspects of husbandry are not described. Mortality, clinical observations, bodyweight and food intake were recorded daily. After 34 d, the rats were fasted for 3 h and then killed by decapitation. Blood was collected and processed to serum, and weights of liver, heart, kidneys, spleen, caecum and intra-abdominal fat were recorded. All contents of the thoracic and abdominal cavities were removed, and the composition of remaining carcass assessed. Serum was analysed for glucose and triacylglycerol, and liver total lipid and triacylglycerol were measured.

Unscheduled death occurred in five rats in the 40% D-allulose group, and one rat in the 30% D-allulose group. Diarrhoea was observed during the first 8 days of the in-life phase in rats fed diets containing ≥ 20% D-allulose. All the D-allulose treatment groups had a group mean bodyweight gain that was significantly lower than that of the control group, and a dose-response relationship was evident up to 30% D-allulose. Group mean values for food intake and food efficiency likewise decreased, relative to the control group value, up to 30% D-allulose, with an apparent plateau thereafter, although since only two of the rats in the 40% D-allulose group survived, this apparent plateau may be artefact. Group mean values for carcass weight, carcass fat and carcass protein decreased in a dose-related manner from 10 to 40% dietary D-allulose, relative to the group mean value of the control group.

Organ weights are reported only as absolute values, with no organ-to-bodyweight or organ-to-brain weight values reported, and therefore cannot be fully interpreted. It is noteworthy that in contrast to other organ weights, the group mean value for caecal weight generally increased with D-allulose dose, compared to the control group. Group mean values for weights of epididymal, perirenal and mesenteric lipid stores all showed a dose-related decrease relative to the group mean value of the control group. Group mean serum glucose showed a slight decrease only at ≥30% dietary D-allulose. Group mean values for serum triacylglycerol and for liver triacylglycerol did not show a dose-response relationship.

The authors proposed that the diarrhoea observed in rats dosed with D-allulose was due to osmotic pressure from the D-allulose that was not absorbed and reached the large intestine.

Of toxicological relevance in this study are the findings that all dose levels (≥10% D-allulose) were associated with significantly decreased bodyweight gain, that dose levels ≥20% were associated with diarrhoea, and that dose levels ≥30% D-allulose were lethal to some rats in those groups.

Fifty-two day dietary study of D-allulose in obese Sprague-Dawley rats (Chung et al. 2012)
Regulatory status: Not GLP, no Guidelines specified.

The test article for this study was D-allulose purchased from a commercial source. The purity was not specified. Male Sprague-Dawley rats were obtained at seven weeks of age and acclimatised in individual housing to standard laboratory conditions of environment and husbandry for one week before being assigned to be fed either a normal diet or a high-fat diet. They were maintained on their respective diets for four weeks before assignment to experimental groups. In the first experiment, rats (10/group) were switched from the high-fat diet to normal diet, normal diet plus 5% sucrose, normal diet plus 5% erythritol, normal diet plus 2.5% D-allulose, or normal diet plus 5% D-allulose. All groups were maintained on their

assigned diets for 52 days. In the second experiment, the rats, 10/group, remained on the high-fat diet but three of the four groups were supplemented with sucrose, erythritol or D-allulose at 5% of the diet for 52 days. Food intake and bodyweight were recorded three times per week throughout the in-life phase. At the end of the study blood was collected under ether anaesthesia from all rats, and they were killed for collection and weighing of liver, brown adipose tissue, and abdominal adipose stores. Serum levels of total cholesterol, triglycerides and HDL-cholesterol were measured. The liver was examined by routine light microscopy.

In the first experiment, supplementation with D-allulose at 2.5% or 5% was associated with a significant decrease in group mean bodyweight gain, relative to that of the control group, the sucrose-supplemented group and the erythritol-supplemented group, although data were presented only as graphs, not as numerical values. A dose-response relationship between level of D-allulose and decreased bodyweight gain was evident. In the second experiment, supplementation with 5% D-allulose was associated with a dramatic 41% decrease in bodyweight gain, compared to control rats, whereas mean bodyweight gain for the erythritol-supplemented group was not significantly different to that of controls. Group mean total weight of white adipose tissue (epididymal, perirenal and retroperitoneal) was decreased by 38% in the 5% D-allulose group in the first experiment, compared to the control group, and brown adipose tissue was decreased 36%. Similar effects, although of smaller magnitude, were observed in the second experiment. Supplementation with D-allulose did not affect serum cholesterol/high-density lipoprotein (HDL)-C and low-density lipoprotein (LDL)-C/HDL-C ratios. The group mean liver weight in rats fed the normal diet with 5% D-allulose was higher than that of other groups, but without histological correlates. The authors concluded that D-allulose produces a marked decrease in weight gain and visceral fat in an established obesity mode.

From the toxicological perspective, decreased bodyweight gain can be an adverse effect and was observed at 2.5% dietary D-allulose. The magnitude of the change is uncertain in this study because the data were not presented in numerical form.

Four- and eight-week dietary studies of D-allulose in golden Syrian hamsters. (Kanasaki et al. 2019). Regulatory status: Not GLP, no Guidelines specified.

These studies were conducted using D-allulose, purity >99%, purchased from a commercial supplier. Male golden Syrian hamsters were purchased at 5 weeks of age and acclimatised to standard laboratory conditions for one week on a standard hamster diet that differed only in the inclusion of 3% w/w D-allulose for the treatment group. Hamsters were maintained on their assigned diet for up to eight weeks. For the second experiment, all hamsters were fed a high-fat diet for four weeks and then eight continued on the high-fat diet for four further weeks, and eight were placed on the high-fat diet modified to include 3% D-allulose for a further four weeks. At the end of the in-life phase of both experiments, hamsters were fasted for 5 h, then anesthetised for blood collection by cardiopuncture. Blood was processed to serum. Hamsters were killed and liver, kidneys and adipose tissue were removed, weighed and snap-frozen in liquid nitrogen, then stored at -30˚C for further analysis.

In the first experiment, maintenance on a diet containing 3% D-allulose had no effect on group mean values for final bodyweight, food consumption, weights of selected tissues, or serum levels of glucose and insulin, as compared to control values. Total serum triglyceride levels were comparable between the two groups, the D-allulose-fed hamsters had a significantly lower group mean value for serum HDL-cholesterol than the control hamsters, and significantly lower LDL/HDL ratio. Group mean values for hepatic levels of triglycerides

and phospholipids in the D-allulose fed hamsters compared to the controls. Serum activities of AST and ALT were comparable between the two groups.

In the second experiment, in which the high-fat diet was fed, there were no differences between the two groups in group mean values for bodyweight, food consumption, organ weights, serum glucose or serum insulin levels. Total cholesterol was comparable between the two groups, but group mean values for cholesterol in LDL and HDL, and LDL/HDL ratio, were lower for hamsters fed D-allulose than control hamsters. Dietary D-allulose had no apparent effect on triglyceride levels in lipoproteins, on hepatic glycogen, or serum AST activity. The group mean value for serum ALT activity was significantly lower for the treated hamsters than the controls.

None of these effects are considered adverse, and FSANZ concludes that dietary D-allulose at 3% w/w for up to eight weeks has no adverse effects on golden Syrian hamsters, which is in contrast to the findings in laboratory strains of rat.

Ninety-day dietary study of D-allulose in male Wistar rats (Matsuo et al 2012) Regulatory status: Not GLP, no Guidelines specified.

The test article for this study was D-allulose of unspecified purity, purchased from a commercial supplier. Rats were purchased at 3 weeks old and acclimatised to individual housing in standard laboratory conditions for one week before assignment to two groups of 10 rats/group. A standard rodent diet was supplemented with 3% w/w of either sucrose as the control or D-allulose. Food and water were provided *ad libitum* for 90 days. Rats were anaesthetised, bled, and killed after 90 days of treatment, and a limited list of organs and tissues were collected and weighed. These included brain, heart, lungs, liver, pancreas, kidneys, adrenals, spleen, testes, intraabdominal adipose tissues (epididymidal, perirenal and mesenteric), selected skeletal muscles (soleus, gastrocnemius and plantarius), stomach, small intestine, large intestine and caecum. The lengths of small and large intestines and the surface area of the caecum were measured, and the weight of the caecal contents was also recorded. Liver, kidneys and part of the jejunum were fixed for histopathology. Blood was used for standard haematology and clinical chemistry analyses.

Consumption of 5% w/w D-allulose had no effect on group mean values for terminal bodyweight, bodyweight gain, food intake, organ/tissue weight, or tissue surface area, when compared to consumption of 5% w/w sucrose. Group mean absolute weights of liver and kidneys were significantly higher (12.5% and 14.4% increase respectively) for the D-allulose group than for the sucrose group, but there were no correlates in serum chemistry or on microscopic examination of these tissues. Statistically significant differences in serum chemistry included higher group mean values for total protein (+3.5%) and albumin (+3.5%), and a lower group mean value for uric acid (-23.6%), in the D-allulose group than in the sucrose group. These differences were not considered to be adverse. The D-allulose group had higher group mean values for total white cell count (+43.7%), total red cell count (+4.5%), mean corpuscular haemoglobin concentration (+ .2%) and platelet count (+11%) and lower group mean values for mean corpuscular volume (-5.5%) and mean corpuscular haemoglobin (-4.8%). Although these differences are statistically significant, they are not considered to be biologically relevant.

The authors state that the rats consumed 1.67 g/kg bw/day D-allulose through the in-life phase, although the frequency at which bodyweights and food consumption were recorded is not specified.

The authors remarked that the results of this study are not consistent with their 2002 study. Based on the results of this longer study, they concluded that extended exposure to ≤3% D-allulose w/w in the diet was not associated with adverse effects. They noted that in contrast to their earlier studies using higher dose rates, 3% D-allulose in the diet does not appear to have any effect on the intestinal tract.

Ninety-day oral gavage toxicity study of D-allulose in Sprague Dawley rats (An et al. 2019) Regulatory status: GLP, conducted in compliance with OECD Guideline 408.

This study was conducted using as the test article the D-allulose, purity 98%, produced by *Microbacterium foliorum* that is the subject of the current application. The age of the rats at acquisition is not specified, but the initial bodyweights and the growth curves presented in the paper show that they were juveniles between 150 and 200 g at study start. They were pair-housed under standard laboratory environmental conditions. They were assigned, 10/sex/group, to groups gavaged daily with 0, 1250, 2500 or 5000 mg/kg bw/day, at a constant dose volume of 10 mL/kg bw. Water was used as the vehicle and control article. Stability of dose formulations was verified. Parameters measured included survival, clinical observations, bodyweights (weekly and terminal), food consumption, ophthalmologic findings (pre-study and pre-termination), clinical pathology (pre-termination; haematology, coagulation, serum chemistry, urinalysis), necropsy findings, absolute and relative organ weights, and histopathology.

All rats survived to scheduled kill and there were no test article-related effects on clinical observations, ophthalmologic findings, food consumption, haematology, urinalysis, gross necropsy findings, or microscopic findings. Bodyweights and bodyweight gains in females, and males dosed with ≤2500 mg/kg bw/day D-allulose were comparable to those of control animals of the same sex, but the group mean value for bodyweight of the 5000 mg/kg bw/day males at the end of study was 11.9% lower than that of the control male group. The growth curves of the rats are presented in graphical form rather than in numerical form and it is not possible to determine whether there was any corresponding, although not statistically significant, effect in 5000 mg/kg bw/day females. This was interpreted as a minor effect of D-allulose treatment. A small number of statistically significant differences between group mean values for clinical chemistry parameters of treated and control rats were considered to be spurious because values remained within the historical control range. An exception was an increase in alkaline phosphatase (ALP) in treated male rats which, although values remained within the historical range, showed a dose-response relationship. The group mean value for serum ALP of the 2500 mg/kg bw/day rats was 39.7% higher than that of the control group, and that of the 5000 mg/kg bw/day rats was 96.3% higher. However, all values remained within the historical control ranges. Group mean liver weight, relative to bodyweight, was increased in both sexes in the 5000 mg/kg bw/day group when compared to control values; by 15% in males and 18.5% in females. Group mean relative kidney weights were also significantly higher in 5000 mg/kg bw/day group compared to the control group. Kidneys were weighed separately. Relative group mean kidney weights of the 5000 mg/kg bw/day males were 17% and 16.1% higher than those of control males for left and right kidney respectively, and the corresponding values for 5000 mg/kg bw/day females were 16.9% and 17.9% respectively. In males, group mean relative weights of the left and right testes were 13.0% and 11.4%, respectively, greater than those of the control males. These differences were considered to be treatment-related but minor, because all values remained within historical control ranges.

The authors of the study concluded that the No Observed Adverse Effect Level is 5000 mg/kg bw/day. The effect on group mean bodyweight observed in the 5000 mg/kg bw/day males was considered to be a pharmacological rather than a toxic response. The increase in

liver weight had no histological correlates and the authors cite evidence that D-allulose can be exchanged for glycogen in the liver and that it inhibits lipogenesis in the liver. These two mechanisms could account for increased liver weight. Increases in relative weights of kidneys and testes were not addressed by the authors, but no histological correlates were reported for kidneys of either sex or for testes of 5000 mg/kg bw/day males, and all organ weight values remained within historical control ranges. FSANZ notes that relative organ weights of liver, kidney and testes may be expected to be elevated in animals with decreased bodyweight gain because the growth of these tissues tends to be conserved at the expense of other tissues such as adipose tissues.

Twelve-week drinking water toxicity study of D-allulose in Beagle dogs (Nishii et al 2017) Regulatory status: Not GLP, no Guidelines specified.

D-allulose, of unstated purity, used in this study was provided by a university research centre. Ten healthy dogs were divided into two groups of five dogs each, with groups matched for age, bodyweight and body condition score. There was one castrated male and four spayed females in each group. Throughout the in-life phase, dogs were provided with commercial dog food in a quantity appropriate to bodyweight, and *ad libitum* access to water. Once daily, with their food, dogs were provided with either water or an aqueous solution of D-allulose resulting in a dose of 0.2 g/kg bw/day. Food consumption and clinical observations were recorded daily. Bodyweights were recorded at 0, 2, 4, 8, and 12 weeks. Pre-study and at 12 weeks, blood was collected for haematology and clinical chemistry, and a glucose tolerance test was performed on each dog. All dogs remained healthy throughout the experiment with no treatment-related effects on food consumption, clinical observations, body weights, haematology, plasma glucose, plasma insulin, or clinical chemistry, with the exceptions that from week 2 until the end of study, dogs in the D-allulose group had a lower group mean plasma cholesterol level than control dogs, and group mean plasma triglyceride remained low in the D-allulose dogs throughout the study whereas it increased from week 2 in the control group. It was concluded that administration of D-allulose at 0.2 g/kg bw/day is well tolerated in dogs and shows some antihyperlipidaemic effects.

Sixteen-week dietary study of D-allulose in male C57BL/6J mice (Han et al. 2016) Regulatory status: Not GLP, no Guidelines specified.

Sugars used in this study, including D-allulose, were obtained from a commercial supplier. Mice were purchased at 4 weeks of age and acclimatized to standard laboratory environmental conditions for a week, after which they were randomized into six groups, 10/group. The groups were normal diet control (ND), high-fat (20% fat) diet control (HFD), 5% D-allulose (ALL), 5% erythritol (ERY), 5% D-glucose (GLU) and 5% D-fructose (FRU). In the diets of the ALL, ERY, GLU and FRU groups, the monosaccharide replaced sucrose in the high-fat diet on a w/w basis. The ERY, GLU and FRU groups were pair-fed to the ALL group so that they had the same calorie intake. Mice were weighed every two weeks, and food intake was recorded daily. Blood collection, killing and post-mortem procedures are not described, but parameters measured included determination of plasma leptin, resistin, free fatty acids, phospholipids, apolipoprotein A1, apolipoprotein B, HDL cholesterol, triglyceride and total cholesterol; hepatic and faecal free fatty acids, triglyceride and cholesterol; as well as a range of parameters related to regulation of lipids. Of relevance to risk assessment were the findings that body weight of the ALL group was significantly lower than that of the HFD, ERY, GLU and FRU groups from Week 8 of study. The group mean terminal bodyweight of the ALL group was comparable to that of the ND group but was 76.5% that of the HFD group. Both white and brown adipose tissue stores were significantly lower in the ALL group than in the HFD group.

Several other studies in animal models were included in the Application but they either had limitations in design or reporting, or did not contribute additional information concerning the safety of D-allulose. An eight-week non-GLP dietary study of D-allulose in male Wistar rats by Ochiai et al. (2013) lacked information on the source of the D-allulose, but FSANZ notes that findings of decreased bodyweight gain and food efficiency at 5% dietary D-allulose were consistent with the findings of Nagata et al. (2015) summarized above. Another non-GLP dietary study of D-allulose in male Wistar rats by the same researchers (Ochiai et al 2014) specified the source of the D-allulose but did not provide additional information of relevance to risk assessment. Findings of a study of dietary D-allulose at 5% w/w conducted by Han et al (2020) were similar to those of their 2016 study described above, and they also found an alteration in the microbiome. Changes to the microbiome are not generally considered to be adverse effects if not associated with gross or microscopic lesions. Studies in animal models of human diseases, such as obese mice (Itoh et al 2015; Kim et al 2017), diabetic mice (Baek et al 2010) and diabetic rats (Hossain et al 2012) are of less relevance to risk assessment than studies in healthy animals, and did not reveal additional adverse effects. Studies of effects of 5% dietary D-allulose in male Wistar rats, of durations ranging from three to 16 weeks by Matsuo et al (2001a, 2001b) and Matsuo and Izumori (2004, 2006) do not contribute additional information of toxicological relevance not identified in the studies reviewed above.

3.2.2.2 Chronic toxicity and carcinogenicity studies in animals

Twelve- to eighteen-month dietary study of D-allulose in male Wistar rats (Yagi and Matsuo 2009) Regulatory status: Not GLP, no Guidelines specified.

D-allulose for this study was donated by a university laboratory, and the control article, sucrose, was purchased from a commercial source. Rats were obtained at 3 weeks of age, housed individually and acclimatized to the laboratory conditions for one week before being assigned to two groups, 18 rats/group. The control group was maintained on a diet containing 3% sucrose, and the treatment group was maintained on a diet containing 3% D-allulose. The diets were otherwise identical. Frequency of measurement of body weight and food consumption are not reported, although it is reported that rats ingested 1.28 g/kg bw/day D-allulose or 1.22 g/kg bw/day of sucrose. The authors concluded that energy intake in kcal/day did not differ between the two groups. Eight rats/group were killed after 12 months on the diet, and the remaining rats (10/group) were killed after 18 months on the diet. At scheduled termination, blood was collected under anaesthesia, and rats were then killed by exsanguination. Whole blood and serum were used for comprehensive haematology and clinical chemistry. Weights of brain, heart, lungs, liver, pancreas, kidneys, adrenals, spleen, testicles, intra-abdominal adipose tissues (epididymal, perirenal and mesenteric), muscle tissues (soleus, gastrocnemius and plantarius), stomach, small intestine, large intestine and caecum were recorded. Length of small and large intestine, caecal surface area and weight of caecal contents were also recorded. Preservation of tissues for histopathology was limited to liver and kidney.

Group mean values for terminal bodyweight and for bodyweight gain were not significantly different between the two groups at 12 months, but at 18 months, the group mean terminal bodyweight of the D-allulose group was significantly lower (14.6%) than that of the sucrose group, and the weight gain over the course of the study was also significantly lower (11.5%). Group mean values for weights, expressed relative to body weight, of liver and kidneys were significantly higher in the D-allulose group compared to the sucrose group, and at 18 months, the relative organ weights of brain, lungs, liver, pancreas and kidneys were all significantly higher for the D-allulose group than the sucrose group. Relative weight of intraabdominal

adipose tissue was lower in the D-allulose group than the sucrose group and this difference reached statistical significance in the 18-month cohort. The group mean value for caecal content weight was higher for rats fed D-allulose compared to rats fed sucrose, and this difference was statistically significant for the 18-month cohort. Group mean values for mean corpuscular haemoglobin at 12 months, and haemoglobin and mean corpuscular volume at 18 months, were significantly lower in the D-allulose group compared to the sucrose group, but the differences are negligible in biological terms. There were no significant differences between the two groups on microscopic examination of liver and kidneys at 12 months. Slight, localised lesions of fatty degeneration and hepatocellular fibrosis were observed in the livers of rats in the D-allulose group, but not the sucrose group, at 18 months. It was concluded that there were no significant adverse effects of chronic administration of 3% D-allulose in the diet. The authors considered that increased relative weights of brain and liver and kidneys was a non-adverse effect of D-allulose. Absolute weights were not reported, and FSANZ notes that relative weights of parenchymal organs are often elevated when bodyweight is decreased, because the mass of these organs is preferentially conserved.

Hossain et al (2015a, b) conducted a 60-week drinking water study of D-allulose, 5% w/v, in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, which are a model of Type 2 diabetes, while Lee et al. (2020) fed a diet supplemented with 5% w/w D-allulose to C57BL/KsJ-db/db mice for 16 weeks. These studies are of limited value to assessment of risk of D-allulose to the general population, because they were not conducted in healthy animals, but FSANZ notes that no adverse effects were observed, other than decreased weight gain in the mice.

3.2.2.3 Genotoxicity studies

Bacterial reverse mutation test of D-allulose (Catholic University of Daegu, 2018; unpublished report). Regulatory status: GLP; conducted in compliance with OECD Guideline 471

This test was conducted using D-allulose manufactured by the Applicant, although specifications were not provided. Sterile distilled water was used as the diluent and negative control. The bacterial strains used as the test systems were *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, and *Escherichia coli* WP2uvrA. All assays were performed in triplicate. The method used (plate incorporation or preincubation) is not clearly stated. For the initial concentration determination test, D-allulose concentrations of 0, 312, 625, 1250, 2500, and 5000 µg/plate) were used. The results of the concentration determination test showed no increase in the number of reverse mutation colonies at any concentration of D-allulose, and no overt cytotoxicity at any concentration. Concentrations selected for the definitive assays were therefore 0, 61.7, 185, 556, 1670, and 5000 µg /plate. The results of the definitive mutagenicity test showed no significant increase in the number of revertant colonies with any bacterial strain exposed to D-allulose, with or without the presence of S9 for metabolic activation.
Positive controls for both the concentration determination test and the definitive test were sodium azide, 9-aminoacridine, 4-nitroquinoline-N-oxide and 2-aminoanthracene, as appropriate for bacterial strain and presence or absence of S9 mix. The expected increases in colonies exposed to the positive controls were observed, confirming the validity of the findings.
It is evident from the reported results that D-allulose was not mutagenic under the conditions of the test.

Chromosomal aberration test of D-allulose (Catholic University of Daegu 2018; unpublished report). Regulatory status: GLP; conducted in compliance with OECD Guideline 473[5]

This study was conducted using D-allulose manufactured by the Applicant, although specifications are not included in the report provided to FSANZ. The vehicle/negative control article was sterile distilled water. The test system was Chinese hamster ovarian fibroblast (CHO-K1) cells.
It was established through a preliminary cell proliferation inhibition test that inhibition of cell proliferation by more than 50% was not seen at any concentration, including the highest concentration tested, of 5 mg/mL, with or without S9 mix for 6 hours, or without S9 mix for 24 hours. The concentrations selected for the definitive test were 0, 0.08, 1.25, and 5 mg/mL. Each concentration was tested in triplicate. Positive controls were cyclophosphamide for use in the presence of S9 mix, and mitomycin C for use in the absence of S9 mix.
No significant differences to negative control results in either numerical or structural chromosomal anomalies were observed at any of the concentrations of D-allulose, either in the 6-hour exposure with or without S9 mix, or the 24-hour exposure without S9 mix. The positive controls induced the expected significant increases in numerical and structural chromosomal anomalies compared to the negative control group, confirming the validity of the assay.
It was concluded that D-allulose does not cause numerical or structural chromosomal anomalies under these conditions.

In vivo *micronucleus test of D-allulose (Catholic University of Daegu, 2018; unpublished report). Regulatory status: GLP; conducted in compliance with OECD Guideline 474*

This test was conducted using D-allulose manufactured by the Applicant, although specifications were not provided. Sterile distilled water was used as the vehicle and negative control article. The test system comprised male ICR mice, 5/group. Mice (5 animal per group) were administered 0, 500, 1000, or 2000 mg/kg bw D-allulose by oral gavage, once daily for two days. A positive control group were administered mitomycin C at 2 mg/kg bw Mice were killed approximately 24 hours after the second dose and slides of femoral bone marrow were prepared for examination. There was no significant difference in the proportion of erythrocytes that were polychromatic erythrocytes, or in the frequency of micronucleated polychromatic erythrocytes, between the negative control and treatment groups. The positive control article induced a significant increase in micronuclei in polychromatic erythrocytes, confirming the validity of the assay.

A similar genotoxicity test battery (bacterial reverse mutation test, chromosomal aberration test and micronucleus test) was summarized in GRAS Notification 400, filed with the US FDA by CJ Cheiljedang, South Korea, for D-allulose from *Corynebacterium glutamicum.* No evidence of genotoxicity was reported, although original study reports were not included in the GRAS Notification.

[5] Incorrectly identified as 474 in the Compliance Statement, but correctly as 473 in the References

3.2.2.4 Reproductive and developmental toxicity studies in animals

One-generation reproductive and developmental study of D-allulose in Sprague Dawley rats (Kim 2019; unpublished report). Regulatory status: Not GLP; but conducted in compliance with OECD Guideline 415

This study was conducted using the Applicant's D-allulose, derived from *M. foliorum*. The test article was administered by oral gavage at doses of 0, 500, 1000 or 2000 mg/kg bw/day, at an unspecified dose volume. The vehicle/control article is not specified, and details of environmental conditions are also not specified. P (F0) generation females were dosed daily from 2 weeks prior to mating until Postnatal Day (PND) 21, while males were dosed daily for 10 weeks prior to mating. P generation rats showed no evidence of toxicity from treatment and there were no statistically significant effects on body weight or food consumption. Each dose group comprised 12 males and 25 females. The presence of a vaginal plug was considered to be Day 0 of gestation. Male rats were killed after the mating period was complete and P generation females were killed after weaning their pups on PND 21. All P generation rats were subject to gross necropsy. Liver, kidneys, spleen, heart, lungs and pituitary glands were weighed from all P generation rats. In addition, the testes, epididymides and accessory sex glands of the males, and the uterus and ovaries of females, were weighed. Organs were preserved for histopathology. There were no treatment related effects on clinical observations, bodyweights, bodyweight gains, food consumption, absolute or relative organ weights, precoital time, reproductive indices of either sex, implantation, pregnancy length, prenatal loss, live/dead ratio of pups, viability of pups, sex ratio of pups, lactation index, gross findings at necropsy or histopathological findings. Pups were weighed on PNDs 0, 3, 6. 9, 12, 15, 18 and 21. Pups of rats treated with D-allulose had slightly higher group mean bodyweights than pups of control rats from PND 1 to 9, but a dose-response relationship was not evident. Pups, 1/sex/litter, were subject to assessments of development including righting reflex on PND 2, cliff avoidance on PND 4, negative geotaxis in PND 10, and rota-rod performance on PND 21. No treatment-related effects on physical development of pups were observed. It was concluded that the No Observed Adverse Effect Level (NOAEL) for rats of both sexes in both generations was the highest dose tested, 2000 mg/kg bw/day.

Teratogenicity study of D-allulose in Sprague Dawley rats (Sa et al 2020; unpublished report). Regulatory status: Not GLP; conducted in compliance with Chinese Standard: GB 15193.14-2015

This study was conducted using the Applicant's D-allulose. Sprague Dawley rats (100 females and 60 males), maintained under standard laboratory conditions, were mated. Females were examined daily and the day of finding of a vaginal plug identified as Gestational Day (GD) 0. Mated females, 20/group, were dosed with D-allulose at 0, 1250, 2500, or 5000 mg/kg bw/day from GD 6 to 15, at a dose volume of 10 mL/kg bw. The vehicle/control article was water. Pregnant female rats were killed under anaesthesia on GD 20. Maternal parameters evaluated included survival to scheduled termination, clinical observations, bodyweight and bodyweight gain, number of corpora lutea, number of implantations and number of resorptions. Parameters evaluated in pups included weight, body length, tail length, live/dead ratio, gross malformation rate, visceral malformation rate and skeletal malformation rate. There were no treatment-related effects observed in any of the maternal or fetal parameters evaluated. It was concluded that D-allulose did not exhibit adverse maternal or developmental effects at maternal doses ≤ 5000 mg/kg bw/day.

3.2.2.5 Human tolerance studies

Acute tolerance study of D-allulose in healthy volunteers (Iida et al 2007). Regulatory status: Not GLP; no Guidelines specified.

The purpose of this study was to establish the maximum level of D-allulose that does not cause diarrhoea or other adverse gastrointestinal symptoms. The participants were healthy volunteers, five men and six women, all aged between 20 and 30, without a history of gastrointestinal diseases. The D-allulose was provided by a university laboratory, and had ≥98% purity. Participants consumed their normal diet and level of activity. They were asked to refrain from activity level, fluid consumption or food consumption in excess of their normal level during the study. Doses of D-allulose were consumed at 10 a.m.. The initial dose was 0.4 g/kg bw, and doses were increased by 0.1 g/kg bw, with one-week washouts between doses, up to the onset of diarrhoea or until a maximum of 0.9 g/kg bw was reached. Stool consistency and abdominal symptoms such as pain, constipation, borborygmi, distention, nausea etc. were recorded.

All subjects tolerated 0.4 and 0.5 g/kg bw without diarrhoea or other symptoms. One man developed diarrhoea at 0.6 mg/kg bw, two women developed diarrhoea at 0.7 g/kg/bw, and most of the rest of the participants developed diarrhoea at 0.8 g/kg bw. Two men tolerated 0.9 g/kg bw without developing diarrhoea. Overall, the mean dose causing diarrhoea was 0.55 g/kg bw. The mean dosage in grams was estimated as 33.3 g for men and 31.0 g for women. The onset of diarrhoea was usually around 2 hours, with the shortest latency being 40 minutes. Diarrhoea was associated with mild, transient symptoms including lower abdominal pain, borborygmi, distension, and in one participant, nausea. No abdominal symptoms were reported in the absence of diarrhoea.

Tolerance study of D-allulose in healthy volunteers (Han et al 2018b). Regulatory status: Not GLP; no Guidelines specified.

Thirty healthy volunteers (15/sex) were recruited for this study, which involved two experiments with a washout period of one week between them. D-allulose and the control article, sucrose, were provided in the form of green-coloured, grape-flavoured drinks provided by a commercial supplier.

The first experiment was designed to determine the single maximum dose of D-allulose for occasional consumption. A single dose of D-allulose was administered once every two weeks, after a standardised meal. The initial dose rate was 0.1 g/kg bw and was increased stepwise by 0.1 g/kg bw thereafter, up to a maximum of 0.6 g/kg bw (11th week) or until the participant developed diarrhoea or a marked increase in gastrointestinal symptoms. A similar schedule was followed using sucrose as a control article. Twenty-nine participants completed both the D-allulose schedule and the sucrose schedule.

The second experiment was designed to determine the maximum dose for regular ingestion of D-allulose. There were 19 (10m, 9f) participants. They visited the test centre up to four times/day at set intervals of 3 to 4 h, consuming 0.2 or 0.3 g/kg bw D-allulose at each visit, so that the dose level was 0.2 g/kg bw on Day 1 and increased by 0.1 g/kg bw each day, up to a maximum of 1.0 g/kg bw on Day 8. As for the first experiment, dosing of a participant would stop if severe diarrhoea or a significant increase in gastrointestinal symptoms occurred.

No cases of diarrhoea or increased gastrointestinal symptoms were noted on either occasional or regular consumption of D-allulose until a dose of 0.4 g/kg bw was reached. Diarrhoea became severe in some participants at 0.5 g/kg bw. Consuming D-allulose repeatedly over one day to a maximum daily dose of 1.0 g/kg bw was associated with severe symptoms including nausea, abdominal pain, headache, anorexia, and diarrhoea. The authors concluded that a single dose of D-allulose should not exceed 0.4 g/kg bw and repeated intake over one day should not exceed a total consumption of 0.9 g/kg bw/day.

Several studies in humans that were not tolerance studies, but which provide information on safety and tolerance in humans, were also reviewed. Findings relevant to safety and tolerance are briefly summarized here, in order of publication:

- Iida et al (2008) recruited 20 healthy volunteers (11m/9f) for a study to determine the effect of D-allulose on blood levels of glucose and insulin. With one-week washouts between treatments, volunteers consumed five test beverages; 7.5 g D-allulose, 75 g maltodextrin, 75 g maltodextrin + 2.5 g D-allulose, 75 g maltodextrin + 5.0 g D-allulose, or 75 g maltodextrin + 7.5 g D-allulose. D-allulose was supplied by a university laboratory, and when administered alone had no effect on blood glucose or insulin. There was a dose-related decrease in blood glucose response to maltodextrin when combined with ≥5 g D-allulose, compared to maltodextrin consumed alone. All volunteers completed the study and no adverse effects of D-allulose were reported.
- Hayashi et al (2010) investigated the effects of D-allulose on postprandial blood glucose in adult men and women. As part of these investigations, a treatment group (4/sex) took 5 g D-allulose three times daily, with meals, for 12 weeks, while a control group (4m/5f) took sucrose. As part of the study, comprehensive clinical pathology assessment (haematology, clinical chemistry, urine chemistry) was carried out prior to intervention, at the end of Weeks 2, 4, 8 and 12, and four weeks after the end of the intervention. There were no significant differences between control (sucrose) and treatment (D-allulose) groups in clinical pathology parameters or gastrointestinal symptoms.
- In order to investigate the anti-obesity effects of D-allulose, Kimura et al. (2017) conducted a randomized, single-blind crossover study in 13 healthy men and women, with a one-week washout period between interventions. The interventions were consumption, after overnight fasting, of 5 g D-allulose or 10 mg aspartame. Thirty minutes after an intervention, participants consumed a standardised meal, and energy metabolism was evaluated from expired breath. Blood was collected for analysis of plasma glucose, insulin, total cholesterol, triacylglycerol and free fatty acids. No adverse effects of either intervention were reported.
- A comparison of the effects of feeding either fructose or D-allulose on post-prandial blood glucose metabolism of healthy volunteers (Braunstein et al. 2018) was conducted by giving each volunteer six treatments in random order, with one-week washouts between treatments. Treatments consisted of a 75 g oral glucose tolerance test (OGTT) with the addition of fructose or allulose at 0, 5, or 10 g. There were no adverse events reported that could be causally associated with treatment.
- Han et al (2018a) conducted a randomized controlled trial in overweight volunteers, in which the placebo was sucralose (0.012 g, twice daily), and the interventions were D-allulose at 4 g twice daily and D-allulose at 7 g twice daily. At the start of the experiment there were 48 participants/group, but a small number were lost to follow-up, so that 121 participants completed the study. Participants consumed either the placebo or the treatment for 12 weeks. Parameters measured were anthropometric measurements including computed tomography (CT) scan, and plasma lipid profiles. No treatment-related adverse events were reported.

- To compare the effect of small doses of fructose and D-allulose on postprandial blood glucose regulation in people with type 2 diabetes, Noronha et al (2018) conducted a trial in 24 participants with type 2 diabetes. Each participant was randomly assigned six treatments separated by washouts of at least one week. Treatments comprised either fructose or D-allulose at 0, 5 or 10 g added to a 75-g glucose solution as part of an OGGT. No treatment-related adverse events were reported.
- Ninety volunteers with high plasma LDL-cholesterol levels took part in a 48-week trial to investigate the effects of D-allulose on plasma cholesterol. Participants, 30/group, who were blinded to the group they had been assigned to, consumed a beverage once daily which contained 0, 5 or 15 g D-allulose. No adverse effects of chronic consumption of ≤ 15 g D-allulose/day were observed, and there were no adverse events considered to be associated with D-allulose consumption. Beneficial effects on plasma liver enzyme activities, fatty liver score and glucose metabolism were observed (Tanaka et al 2020).
- Franchi et al (2021) conducted a randomized double-blind placebo-controlled crossover study in 30 subjects (16m/14f) without diabetes mellitus. Study participants were given a standard oral (50 g) sucrose load and randomized to simultaneously receive placebo (water) or escalating doses of D-allulose (2.5, 5.0, 7.5 or 10.0 g). Wash-out periods between interventions were 7 to 14 days. Plasma glucose and insulin levels were measured at five time points: before and at 30, 60, 90 and 120 min after ingestion. No adverse events associated with D-allulose consumption were reported.
- Teysseire et al (2022) conducted a randomized, controlled, double-blind, crossover study in which 18 participants (5 men, 13 women) received an intragastric administration of 25 g D-allulose, 50 g erythritol, or tap water, with or without 450 ppm lactisole, respectively, in six different sessions. The solutions were administered on an empty stomach and 13C-sodium acetate was added to all solutions to determine gastric emptying. At fixed time intervals, blood and breath samples were collected, and appetite-related sensations and gastrointestinal symptoms were assessed. D-allulose alone was associated with three reports of abdominal pain, three reports of nausea, two reports of diarrhoea, 11 reports of bowel sounds, three reports of bloating, four reports of eructation (belching), and three reports of flatulence. The severity in all cases was mild. D-allulose administered with lactisole was associated with four reports of abdominal pain, three reports of nausea, 13 reports of bowel sounds, three reports of bloating, four reports of eructation, and two reports of flatulence. Severity in all cases was mild.

FSANZ notes that some history of safe use of D-allulose may be inferred from use in other countries. D-allulose has been the subject of three GRAS Notifications to the USFDA, to which the US FDA responded with No Questions letters. The earliest No Questions letter was dated June 2012. D-allulose has been approved in Mexico as a non-caloric sweetener since 2017, is marketed in Japan without the need for regulatory approval and is also marketed in South Korea. No case reports of adverse effects of D-allulose in consumers were located by literature search.

3.2.2.6 Other studies

An unpublished kinetics study by Williamson et al (2014) in which a radiolabelled rare sugar was administered to eight healthy adult men, who were housed in a metabolic ward for one week was submitted to the US-FDA by Tate and Lyle as part of a GRAS notification for D-allulose. The written record of the study is an abstract from the 2014 Experimental Biology meeting, and the rare sugar is not named in the abstract. The abstract is therefore considered to be of uncertain value for the assessment of D-allulose.

3.2.4 Discussion concerning safety of D-allulose

Most (80%) of an oral dose of D-allulose is rapidly absorbed intact from the small intestine, by the same transporters as fructose. There may be competitive partial inhibition of absorption of D-allulose and fructose if they are consumed together. Following administration of radiolabelled D-allulose, radiation does not accumulate in tissues other than the liver and does not appear to cross the blood-brain barrier. Some of an oral dose of D-allulose is excreted unchanged in the faeces. D-allulose that is absorbed into the systemic circulation is rapidly excreted by the renal route.

D-allulose is of very low acute toxicity, with an acute oral LD50 in the rat of approximately 16 g/kg bw. A single bolus dose of D-allulose of ≥ 8 g/kg bw causes diarrhoea in rats. Dietary intake of D-allulose at ≥20% w/w may also cause diarrhoea in rats.

Several short-term studies of D-allulose in laboratory rodents have been reviewed. The rat is more sensitive to the laxative effects of D-allulose than dogs or Syrian hamsters. The 90-day oral gavage study by An et al. (2019) is regarded as the most reliable for the purpose of this hazard assessment, because the study was conducted under GLP conditions, in compliance with OECD Guideline 408, and using as the test article the D-allulose that is the subject of the application. This study identifies a NOAEL of 5000 mg/kg bw/day, the highest dose tested. At that dose, the group mean terminal bodyweight of the males was significantly lower (11.9% lower) than that of the control males, without histological correlates. The decrease in weight gain is regarded a pharmacological effect of D-allulose (i.e. reduced fructose absorption), rather than an adverse effect.

Dietary studies in laboratory rats show variable results, with significant decreases in bodyweight gain at 3% w/w D-allulose in the diet observed in some studies but not others. Dietary D-allulose at 3% w/w was sufficiently well tolerated that an 18-month chronic study in rats found no significant effects other than a decrease in terminal group mean bodyweight. There was no evidence of decreased latency or increased incidence of neoplasia, and a battery of genotoxicity studies (bacterial reverse mutation assay, chromosomal aberration study and micronucleus test) with the applicant's D-allulose yielded consistently negative results. A reproductive and developmental study in rats, using the applicant's D-allulose, found no adverse effects in rats of either sex in either the P or F1 generation at doses ≤ 2000 mg/kg bw/day, and a teratogenicity study, also conducted in rats and with the applicant's D-allulose, had no adverse maternal or developmental effects at maternal doses ≤ 5000 mg/kg bw/day.

Two human tolerance studies were reviewed. The lowest single dose of D-allulose associated with gastrointestinal effects was 0.4 g/kg bw (28 g in a 70 kg adult) and repeated intake should not exceed a total consumption of 0.9 g/kg bw/day (Han et al 2018b). The acute value identified by Han et al is considered more relevant to the current application than the lower 25 g threshold identified by Teysierre et al (2022) because Han et al administered D-allulose to subjects who had consumed food, whereas Teysierre et al administered D-allulose to subjects whose stomachs were empty. The current application is for use of D-allulose in food or beverage rather than on its own.

Overall, no adverse effects of D-allulose were identified in laboratory animals or human beings that are not attributable to osmotic laxation, or to its competitive inhibition of fructose absorption. The effects of D-allulose are similar to those of other rare sugars which FSANZ has previously assessed; trehalose (A453, FSANZ 2003) and D-tagatose (A472, FSANZ 2004). FSANZ found no evidence of toxicity of trehalose in animals, even at very high doses, and based the recommendation that consumers should not exceed a level of 33-50 g from a single exposure to trehalose in food on the potential for trehalose to cause osmotic laxation. Animal studies of D-tagatose did not identify any effects relevant to humans, and it was concluded that D-tagatose is safe for use in food for all individuals up to 15 g/day. Mild gastrointestinal effects may be observed in some individuals at higher dose levels. Similar properties (that is; negligible toxicity but osmotic laxative effect at high doses) are observed for several polyols (sugar alcohols) including sorbitol, mannitol, maltitol, xylitol, lactitol, and isomalt. All of these polyols have been evaluated and assigned Acceptable Daily Intakes of 'not specified' by JECFA in 1983.

It is concluded that there is no toxicological risk to public health and safety from consumption of D-allulose in food. The lowest dosage of D-allulose associated with gastrointestinal symptoms is 0.4 g/kg bw (400 mg/kg bw; 28 g for a 70 kg adult) as a single dose. Daily consumption of D-allulose should not exceed 0.9 g/kg bw (63 g for a 70 kg adult) within 24 h.

3.3 History of use and identification of *Microbacterium foliorum*

Microbacterium foliorum is a naturally occurring bacterium found in foods such as surface-ripened cheese, and fermented seafood and soybean paste (Deetae et al 2007; Guan et al 2011; Lee et al 2012). The German Committee for Biological Agents has classified *M. foliorum* as a risk group 1 bacteria[6]. This is the lowest risk category. It is improbable that bacteria in this category will cause an infectious disease in humans[7]. Therefore *M. foliorum* is not considered to be pathogenic to humans.

The source organism for D-allulose 3-epimerase is *M. foliorum* SYG27B-MF. This strain has not been genetically modified. The applicant has stated that the taxonomy of the source organism was confirmed as *M. foliorum*.

To confirm the absence of toxigenic genes, the whole genome sequence of *M. foliorum* SYG27B-MF was analysed. *M. foliorum* SYG27B-MF was compared to four well-known pathogens: *Escherchia coli, Enterococcus, Listeria* and *Staphylococcus aureus.* None of the toxic or pathogenic genes associated with those four pathogens were presence in the *M. foliorum* SYG27B-MF sequence. Also, VFDB[8] was utilised to compare virulence genes between *M. foliorum* SYG27B-MF and other closely related microorganisms. No virulence genes related to adherence factors, iron uptake, or toxin genes were identified for *M. foliorum*

[6] https://www.dsmz.de/collection/catalogue/details/culture/DSM-12966
[7] https://www.baua.de/EN/Topics/Work-design/Biological-agents/Classification.html
[8] VFDB: Virulence factor database http://www.mgc.ac.cn/VFs/main.htm

SYG27B-MF. Therefore *M. foliorum* SYG27B-MF is considered to be non-toxigenic. ResFinder[9] was utilised to detect the presence of antibiotic resistance genes. Analysis of the whole genome sequence of SYG27B-MF with Resfinder showed that antibiotic resistance genes were absent in *M. foliorum* SYG27B-MF.

During the manufacturing process, the source organism *M. foliorum* SYG27B-MF forms part of the immobilised cell system used to convert fructose to D-allulose. The D-allulose is then subject to several purification steps to produce allulose syrups and finally dried into crystalline D-allulose. *M. foliorum* SYG27B-MF was not detected in the D-allulose preparations, so would not enter the food chain. This was confirmed by analysis of five independent batches of D-allulose preparations (D-allulose syrups and crystalline powder), PCR analysis of DNA from *M. foliorum,* below the level of detection.

The applicant has stated that the genetic stability of the source organism is checked by routinely observing the morphology, growth and cell activity of *M. foliorum* SYG27B-MF across batches.

3.4 Hazard assessment of D-psicose-3-epimerase

3.4.1 History of use of the enzyme

Evidence of five years' history of safe use of the immobilized cell system in South Korea, to produce D-allulose, was provided by the applicant, including confidential data on volumes used.

3.4.2 Bioinformatics on the potential toxicity of the enzyme

In 2022, the applicant compared the amino acid sequence of D-psicose 3-epimerase with those of known toxin proteins in the UniProtKB/Swiss-Prot database. In addition, the amino acid sequence was entered into several online toxin prediction tools including NTXpred (http://crdd.osdd.net/raghava/ntxpred/), BTXpred (http://crdd.osdd.net/raghava/btxpred/), KNOTTIN (http://www.dsimb.inserm.fr/KNOTTIN/), CLANTOX (http://www.clantox.cs.huji.ac.il), CONOSERVER (http://www.conoserver.org) and ToxinPred (https://webs.iiitd.edu.in/raghava/toxinpred/index.html). No significant homology was found with any known toxins.

3.4.3 Bioinformatics on the potential allergenicity of the enzyme

The Applicant submitted results of a recent (<24 months) comparison of the complete amino acid sequence of D-psicose 3-epimerase to allergens in the FARRP allergen protein database[10] Version 21. Searches included a search for full-length alignments by FASTA, a sliding-window search for 80 amino acid alignments by FASTA, and a search for an 8 amino acid exact match. No significant homology was found between D-psicose 3-epimerase and any allergens in the database.

[9] ResFinder identifies acquired genes and/or finds chromosomal mutations mediating antimicrobial resistance in total or partial DNA sequence of bacteria https://cge.cbs.dtu.dk/services/ResFinder/
[10] http://allergenonline.org

3.4.4 Other considerations

To confirm that commercial preparations of D-allulose do not contain detectable amounts of residual proteins or peptides, the applicant conducted an analysis, using the Kjeldahl method, of allulose syrup L, allulose syrup H and allulose crystalline powder. The Kjeldahl method was conducted with reference to AOAC 945.23 and KFDA Food Code 8.2.1.3.1. Five separate batches of each allulose product were tested. For each batch, 1 g of the commercial D-allulose product was tested. In all batches protein was not detected (limit of detection (LOD): 0.1 mg-N/g).

The D-psicose 3-epimerase is not used as a free enzyme but remains located within the production organism *Microbacterium foliorum*. D-allulose is synthesized by passing a fructose solution through an immobilized cell system comprising *M. foliorum* (SYG27B-MF) entrapped by calcium alginate gel beads. After conversion of fructose to D-allulose the solution is subject to a series of separation and purification steps, including filtration and ion exchange processes, which remove peptides and proteins, among other impurities. The commercial D-allulose preparation does not contain the enzyme or viable *M. foliorum*, nor any DNA from *M. foliorum* (as described above in section 3.3). The likelihood of consumer exposure to the organism or the enzyme is considered to be negligible, and therefore toxicological studies of D-psicose 3-epimerase are not considered to be necessary, provided that the specified manufacturing process is used.

The specifications for this application include several microbiological parameters for D-psicose 3-epimerase. These other parameters are total plate count: ≤100 CFU/g; *Escherichia coli*: ≤10 CFU/g; *Listeria monocytogenes:* absence in 25 g; *Salmonella*: absence in 25 g; and mould & yeast plate count: ≤20 CFU/g. As D-psicose 3-epimerase is not present in the final D-allulose product, the microbiological parameters listed do not pose a risk to consumers.

3.4.5 Discussion concerning the safety of the enzyme

The enzyme D-psicose-3-epimerase has a five-year history of safe use for the production of D-allulose, and the applicant has provided evidence that there is negligible likelihood of consumer exposure to the production organism, DNA from the production organism, the enzyme, or residues from the enzyme. No significant homology was found with any known toxins or allergens. Toxicological studies of D-psicose-3-epimerase are not considered to be necessary.

3.5 Dietary intake/exposure assessments

3.5.1 Introduction and purpose

This application requested permission to add D-allulose to different food categories at maximum use levels ranging from 2 to 100% (w/w) (Table 6) as a low-energy substitute for conventional sugar ingredients, particularly sucrose. D-allulose is not intended to be included as an ingredient in infant formula products, formulated supplementary foods for young children and in raw commodity products such as fresh meat, fruit and vegetables. D-allulose is a naturally occurring rare sugar that is present in small quantities in various foods (Oshima *et al.* 2006). The applicant has proposed to manufacture D-allulose by an enzymatic conversion of fructose to D-allulose using *M. foliorum* SYG27B-MF containing D-psicose-3-epimerase (also called D-allulose-3-epimerase) enzyme. Hence, the purposes of the assessments are to consider dietary intake of the added and naturally occurring D-allulose, as well as dietary exposure to the D-psicose-3-epimerase enzyme.

3.5.2 Dietary intake assessment for D-allulose

3.5.2.1 Methodology

Dietary intake assessments require data on the concentration of the chemical of interest in the food requested and consumption data for the foods that have been collected through a national nutrition survey.

The dietary intake assessment was conducted to estimate the level of chronic and short-term dietary intake of D-allulose. Chronic dietary intake estimates are used to represent the long term, usually life-long, dietary intake for the population from the range of foods containing the chemical of interest. Short-term dietary intake estimates are used to represent the high food consumer and a high intake, from a single food or food category, from one meal or over one day. In the case of this application, the short-term intakes were used to determine whether there is a potential for any laxative effects from D-allulose from consuming a single food/ food category in a single eating occasion or over a single day. Estimates of dietary intake of D-allulose were also extracted to support the microbiological risk assessment.

The dietary intake assessment for D-allulose was undertaken using FSANZ's dietary modelling computer program Harvest[11] and deterministic methodologies using Microsoft Excel. A summary of the general FSANZ approach to conducting the dietary intake assessment for this application is on the FSANZ website. A detailed discussion of the FSANZ methodology and approach to conducting dietary intake assessments is set out in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ, 2009).

[11] Harvest is FSANZ's custom-built dietary modelling program that replaced the previous program, DIAMOND, which does the same calculations just using a different software program.

3.5.2.2 Food consumption data used and population groups assessed

The food consumption data used for the dietary intake assessments were:

- 2011-12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS), one 24-hour food recall survey of 12,153 Australians aged 2 years and above, with a second 24-hour recall undertaken for 64% of respondents (ABS, 2015).
- 2002 New Zealand National Children's Nutrition Survey (2002 NZ CNS), one 24-hour food recall covering 3,275 New Zealand school children aged 5-14 years, with 25% of respondents also completing a second 24-hour recall (Ministry of Health 2005).
- 2008–09 New Zealand Adult Nutrition Survey (2008 NZ ANS): a 24-hour recall of 4,721 New Zealanders aged 15 years and above, with a second 24-hour recall undertaken for 25% of respondents. (Ministry of Health 2011a; Ministry of Health 2011b).

The design of these nutrition surveys and the key attributes, including survey limitations, are set out on the FSANZ website.

In this assessment, dietary intakes were estimated for 'consumers only' (e.g. consumers of foods containing D-allulose). Nutrition survey respondents who had no consumption of these foods were not included in the results presented. All results were weighted to make them representative of the respective populations.

For the chronic dietary intake assessment, one day of food consumption data from both of the New Zealand surveys were used, whereas the average of two days of data from the 2011-12 NNPAS was used for Australia. Two day average intakes better reflect longer term estimates of dietary intake and therefore are a better estimate of the chronic dietary intake.

For the microbiological risk assessment, data from day one only of all nutrition surveys were used.

For the assessment of evaluating the potential for laxative effects, the 97.5th percentile (P97.5) consumption of each food category was extracted from the nutrition survey data via Harvest and multiplied by the proposed maximum use level deterministically to determine the dietary intake. This was done using the pooled data from day 1 and 2 from the 2011-12 NNPAS (not averaged; for respondents with two days of data; if a respondent had two days of data they were considered as two separate people in the dataset) and from day 1 only for the New Zealand surveys. All results were weighted to make them representative of the respective populations. Using high consumption amounts at the 97.5th percentile for short term or acute dietary exposure assessments is international best practice and is appropriate to assesses risk based on high consumers within a population (FAO/WHO, 2020).

The toxicological assessment did not identify any population sub-groups or at-risk groups for which there were specific safety considerations or where separate chronic dietary intake estimates were needed. Therefore, the whole survey population from each of the nutrition surveys were used for the chronic dietary intake assessment. For the laxative effect hazard assessment, data were not available for children, therefore the relevant endpoints for adults were used for the short term dietary intake assessment which was undertaken for the whole survey populations. For the microbiological assessment, data were required to be separated for children (up to 14 years), adults (15 to 64 years) and older adults (65 years and above).

3.5.2.3 Scenarios assessed

Two scenarios were assessed to estimate chronic dietary intakes of D-allulose:

> '*Added D-allulose*' included the intake of D-allulose from foods that the applicant proposed to include the added D-allulose (Table 6).
>
> '*Naturally occurring D-allulose*' included the intake of D-allulose from the foods that contain naturally occurring D-allulose (Table A1.1/ Appendix-1).

Both scenarios included foods consumed 'as is' (e.g. tabletop sweeteners), and when used in recipes (e.g. cakes) where FSANZ's recipe database in the Harvest Food Additive model was applied. A hydration factor was applied when the reported food consumption amount in the nutrition survey was in the dried form (e.g. jelly crystals) to ensure the consumption amount used in the assessment was in the form of 'as consumed'.

For the short-term dietary intake assessment, and the data extracted for the microbiological risk assessment, an '*Added D-allulose*' scenario was used.

3.5.2.4 Concentration data used

The concentration data used in the dietary intake assessment for the *Added D-allulose* scenario were sourced from the application where they were presented as 'maximum use level % (w/w)'. A summary of the concentrations used in estimating the dietary intake is shown in Table 5. The concentration data that were published by Oshima *et al.* (2006) and included in the application were used for the dietary intake assessment for the *Naturally occurring D-allulose* scenario (Table A1.1/ Appendix-1). Where uncertainties in the concentration data existed, FSANZ used conservative assumptions to determine the highest concentration levels in order to ensure that the estimated dietary intake was not underestimated.

Table 5 Concentrations used in dietary intake assessment for the added D-allulose scenarios

Food category (as proposed by the applicant)	**Maximum use level % (w/w) proposed by the applicant**	**Proposed maximum use level used by FSANZ**	
		% (w/w)	**g/ kg**
Bakery products (bread rolls, cakes, cake-type rolls, pastries, doughnuts, biscuits (including cookies, shortbread, butter milk and whole wheat biscuits, crackers)); reduced energy	10	10	100
Beverages (water based, non-alcoholic); low- and reduced energy, low- and reduced sugar (including sweetened teas, instant coffees but not including cereal/nut/legume-based milk analogues)	3.5	3.5	35
Breakfast cereals and cereal based bars; regular	2	5[#]	50[#]
Breakfast cereals and cereal bars; reduced energy; reduced sugar	5	5	50
Chewing gum	50	50	500
Icings and frostings	5	5	50
Frozen dairy desserts (ice cream, soft serve, sorbet); low- and reduced- energy and low- and reduced sugar	5	5	50
Yogurt and frozen yogurt; low- and reduced energy; low- and reduced sugar	5	5	50
Dressings for salads	5	5	50
Gelatins, pudding and fillings; low- and reduced energy, low- and reduced sugar	10	10	100
Hard candies/confectionery; low- and reduced energy	50	50	500
Soft candies/confectionery; low- and reduced energy (not including chocolate)	25	50[#]	500[#]
Jams and jellies	10	10	100
Sugar products	10	n/a[†]	n/a[†]
Sugar substitutes	100	100	1000
Sweet sauces and syrups; low- and reduced-energy, low- and reduced sugar	10	10	100
Fat-based cream (used in modified fat/energy cookies, cakes, pastries, and pie)	5	5	50

[#]Higher maximum use level was applied to represent the worst case scenario.
[†]not applicable; this was excluded to minimize overestimations as it is a general food category that includes most of the other listed food categories.

3.5.2.5 Assumptions and limitations of the dietary intake assessment

The dietary intake assessment was designed to calculate the most realistic estimate of dietary intake of D-allulose as possible. However, where significant uncertainties in the data and information exist, conservative assumptions were generally used to ensure that the estimated dietary intake is not an underestimation.

Assumptions made in the dietary intake assessment included:

- Where there was no subcategory for low- and reduced-energy/sugar and/or where significant uncertainties in the available data exist, the entire food category was included in the assessment (e.g. biscuits & crackers).
- The same proposed maximum use level was appropriate for both 'regular' and 'reduced energy and reduced sugar' categories of the 'breakfast cereals and cereal based bars' (Table 5).
- The same proposed maximum use level was appropriate for both 'hard and soft candies/confectionery' (Table 5).
- Where different 'maximum use levels' were proposed for a food ingredient and its final product, the highest 'maximum use level' was used for the assessment (e.g. fat-based cream in cookies).
- As proposed by the applicant and where possible, cereal/nut/legume-based milk analogues, and chocolates were excluded from the assessment.
- Only foods that contained a known concentration of the naturally occurring D-allulose were included in the naturally occurring assessment (Table A1.1/ Appendix-1). Concentration of the naturally occurring D-allulose in the other foods was assumed to be zero for the purpose of the dietary intake assessment as D-allulose is a rare sugar and present in small quantities in nature (Ahmed *et al*. 2022).
- For the short-term intake assessment, consumption of only one food category at a time was considered. Therefore, the short-term assessment doesn't consider if two or more of the foods proposed to contain added D-allulose were consumed in the same meal/eating occasion.
- The eating occasion data available and assessed from the 2011-12 NNPAS are also considered applicable for the New Zealand.

In addition to the specific assumptions made in relation to this dietary intake assessment, there are a number of limitations associated with the nutrition surveys from which the food consumption data are used for the assessment. A discussion of these limitations is included in Section 6 of the *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

3.5.2.6 Estimated dietary intakes of D-allulose

Chronic dietary intakes

Results of the chronic dietary intake assessment for the added D-allulose and the naturally occurring D-allulose are summarised in Table 6 and Table A1.2/ Appendix-1 respectively. The results are presented as the mean and the 90th percentile (P90) intakes in g/day and g/kg bw (body weight)/day. Mean and P90 intakes are derived from the distribution of D-allulose intakes from each individual consumer within the survey population. Where results are expressed on a body weight basis, each individual's body weight was used. All the results are shown for three consumer populations separately as 2 years and above for Australia, and 5-14 years and 15 years and above for New Zealand.

For Australian consumers, the mean chronic dietary intake of added D-allulose based on proposed maximum use levels from the application is 10.2 g/day and 0.17 g/kg bw/day while the P90 is 21.3 g/day and 0.36 g/kg bw/day. For all the population groups assessed, the highest estimated mean chronic dietary intake of added D-allulose was 12.1 g/day and 0.34 g/kg bw/day for New Zealand children aged 5-14 years. The highest estimated P90 chronic dietary intake of added D-allulose was 27.3 g/day for New Zealand consumers aged 15 years and above, and 0.73 g/kg bw/day for New Zealand consumers aged 5-14 years.

Table 6 Estimated chronic dietary intakes for consumers of D-allulose based on added sources at maximum use levels proposed by the applicant

Country	Age group	Proportion of consumers to respondents (%)	Estimated dietary intake of the added D-allulose			
			g/ day		g/kg bw/day	
			Mean	P90	Mean	P90
Australia*	2 years and above	93.3	10.2	21.3	0.17	0.36
New Zealand#	5-14 years	93.2	12.1	26.0	0.34	0.73
	15 years and above	83.6	11.7	27.3	0.15	0.35

*Based on food consumption data from Day 1 and 2. Average of intakes across the two days presented.
#Based on food consumption data from Day 1 only.

The dietary intakes of naturally occurring D-allulose were found to be negligible in comparison to the *'added D-allulose'* scenario for all three populations. For instance, the P90 dietary intake of the naturally occurring D-allulose for all the populations assessed is ≤ 0.4 g/day and ≤ 0.01 g/kg bw/day (Table A1.2/ Appendix-1). The dietary intake results of the naturally occurring D-allulose scenario are therefore not further discussed.

Assessment of potential laxative effects

The short-term dietary intake of added D-allulose from each food category was estimated based on consumption data for high consumers (P97.5) as high intakes are associated with gastrointestinal symptoms including a laxative effect if the intake exceeds 0.4 g/kg bw in a single eating occasion or 0.9 g/ kg bw during a 24-hour period (see section 3.2.5). For this assessment, it was assumed that the consumption over a 24-hour period was equivalent to a single eating occasion (in other words, a bolus dose) in order to provide a worst-case scenario. Estimates of short-term dietary intake are provided in Table 7 with the consumption data on which the estimates are based shown in Table A2.1.

There were five food categories for which consumption resulted in estimated dietary intakes of D-allulose above 0.4 g/kg bw for all three population groups assessed (Table 7). They were mainly non-brewed soft drinks ('carbonated, cola, intense sweetened' and 'carbonated, not cola type, sugar sweetened'), desserts ('non-dairy, jelly') and bakery products ('pies and pastries, sweet' and 'doughnuts, sweet'). The same five food categories produced intakes above 0.9 mg/kg bw/day for some of the population groups assessed, particularly for New Zealanders aged 5-14 years. In addition to these five categories, there were five other food categories (e.g. some other beverages, 'puddings and dumplings' and 'muffins') where there was an exceedance of the 0.9 mg/kg bw/day for one of the population groups assessed.

It was noted that the proportion of consumers who had daily intakes of D-allulose above 0.4 g/kg bw (as shown by shading in Table 7) was less than 10% for all the food categories for the Australian population and New Zealand adults aged 15 years and above. The proportion of consumers exceeding 0.4 g/kg bw was above 10% (≤19%) for three food

categories (1: 'Non-brewed soft drink, carbonated, not cola type, sugar sweetened', 2: 'Desserts, dairy, ice cream and ice confection' and 3: 'Bread and related products, wheat base, unspecified type') for the New Zealand population aged 5-14 years.

Table 7 Estimated high daily intake (P97.5) of added D-allulose*

Harvest food classification code	Harvest food classification name	Australia 2 years and above		New Zealand 5-14 years		New Zealand 15 years and above	
		% consumers	Dietary intake (g/kg bw/day)	% consumers	Dietary intake (g/kg bw/day)	% consumers	Dietary intake (g/kg bw/day)
1.5.3	Dried yoghurt powder	NC	NC	NC	NC	NR	NR
4.3.4.2	Low joule chutneys, jams & spreads	2.6	0.08	NR	NR	NR	NR
5.2.1	Bubble gum & chewing gum	<1	0.14	3.6	0.65	NR	NR
5.2.4.1	Sugar confectionery, confectionery, intense sweetened	NR	NR	NR	NR	NR	NR
5.4	Icings & frostings	10.1	0.06	11.1	0.13	7.5	0.03
6.3.2	Breakfast biscuits & flakes	17.7	0.12	30.8	0.15	18.2	0.07
6.3.4	Breakfast cereals, unspecified form	<1	0.19	NR	NR	NR	NR
7.1.4	Fancy breads	1.5	0.44	4.1	0.70	2.6	0.31
7.1.6	Bread and related products, wheat base, unspecified type	14.7	0.37	**19.0	0.51	17.9	0.27
7.2	Biscuits, crackers, cakes, pastries & scones	<1	0.34	1.3	0.41	3.4	0.37
7.2.1	Biscuits & crackers	34.2	0.27	56.6	0.33	34.7	0.13
7.2.2.1	Cakes	6.6	0.70	9.5	0.72	9.6	0.27
7.2.2.2.1	Muffins, sweet	1.9	0.76	5.5	0.93	4.6	0.33
7.2.3	Pastries	12.6	0.34	18.4	0.35	14.1	0.23
11.4	Tabletop sweeteners	5.5	0.06	NR	NR	3.4	0.06
14.1.3.1.1.1.2	Non-brewed soft drink, carbonated, cola, intense sweetened	6.7	0.72	2.0	0.96	3.9	0.59
14.1.3.1.1.1.2.1	Non-brewed soft drink, carbonated, cola, intense sweetened, decaffeinated	<1	0.67	NC	NC	NR	NR
14.1.3.1.1.2.1	Non-brewed soft drink, carbonated, not cola type, intense sweetened	<1	0.62	NR	NR	1.2	0.33
14.1.3.1.1.2.2	Non-brewed soft drink, carbonated, not cola type, sugar sweetened	9.3	0.68	**16.8	0.93	9.7	0.6
14.1.3.1.1.2.3	Non-brewed soft drink, carbonated, not cola type, unspecified sweetener	NC	NC	NR	NR	1.0	1.68

Harvest food classification code	Harvest food classification name	Australia 2 years and above		New Zealand 5-14 years		New Zealand 15 years and above	
		% consumers	Dietary intake (g/kg bw/day)	% consumers	Dietary intake (g/kg bw/day)	% consumers	Dietary intake (g/kg bw/day)
14.1.3.1.1.3.1	Non-brewed soft drink, carbonated, energy, intense sweetened	NR	NR	NC	NC	NR	NR
14.1.3.1.3.1.1	Non-brewed soft drinks, cordial powders, intense sweetened	NC	NC	NR	NR	NR	NR
14.1.3.1.3.2.1	Non-brewed soft drinks, cordials, not powder, intense sweetened	<1	1.03	NR	NR	NR	NR
14.1.3.1.4	Non-brewed soft drinks, iced tea, sold as ready to drink	NC	NC	NR	NR	NR	NR
14.1.5.1.2.2	Coffee beverage, decaffeinated, instant powder/granules	2.8	0.30	NR	NR	1.6	2.01
14.1.5.1.3.2	Coffee beverage, unspecified caffeine content, instant powder/granules	NC	NC	NR	NR	NR	NR
20.1.3.1	Beverages, non-alcoholic, tea-based	2.2	0.58	NC	NC	NR	NR
20.2.1.3.1	Desserts, dairy, ice cream and ice confection	3.6	0.26	**13.6	0.48	7.1	0.22
20.2.1.3.5	Desserts, dairy, yoghurt	<1	0.51	NR	NR	NR	NR
20.2.1.4.1	Desserts, non-dairy, jelly	1.2	0.92	2.6	1.28	1.2	0.76
20.2.1.4.1.2.1	Desserts, non-dairy, jelly, dry mix, intense sweetened	NR	NR	NC	NC	NC	NC
20.2.1.4.1.2.2	Desserts, non-dairy, jelly, dry mix, sugar sweetened	NR	NR	NC	NC	NR	NR
20.2.1.4.3	Desserts, non-dairy, puddings and dumplings	<1	0.42	NR	NR	1.1	1.1
20.2.1.4.7.1	Cream, imitation	NR	NR	NC	NC	NC	NC
20.2.2.3	Cereal products, bars	6.2	0.11	18.9	0.15	5.2	0.09
20.2.2.5	Breakfast cereals, ready to eat	11.7	0.16	4.3	0.39	12.9	0.14
20.2.2.6	Breakfast cereals, porridge	<1	0.58	NR	NR	1.0	0.23
20.2.3.1.4	Bakery products, sweet, pies and pastries	2.4	0.44	1.9	2.95	2.7	0.49
20.2.3.1.7	Bakery products, sweet, doughnuts	<1	0.57	1.9	1.02	<1	0.48
20.2.7.2	Salad dressings	5.1	0.04	3.9	0.07	12.9	0.04

*Based on food consumption data for Australia from the pooled dataset of Day 1 and Day 2 for 7735 respondents and Day 1 only for New Zealand. All intakes were calculated for consumers only. Intake values ≥ 0.4 g/kg bw are shaded.
** Where >10% and ≤19% of consumers had daily intakes above 0.4 g/kg bw.
NC-not applicable as no consumption has been reported in the survey.
NR not reported due to too few consumers to ensure a reliable 97.5 percentile consumption value.

Validation of the data and method used for the short-term intake assessment

Estimated short term dietary intakes based on consumption data derived from a 24-hour period were compared to an adverse effect endpoint for laxative effects based on a bolus dose (akin to a single eating occasion) of 0.4 g/kg bw. Therefore an evaluation was undertaken to determine how many times consumers ate a food within one category in a 24-hour period to determine if this direct comparison is appropriate. In order to analyse the number of eating occasions of each food category per day, day 1 and 2 eating occasion data available for the 2011-12 NNPAS were investigated for food categories that had intakes of D-allulose above the 0.4 g/kg bw limit (grey shaded in Table 7). It revealed that more than 90% of the consumers had consumed most of the food categories only once per day. This means that comparing D-allulose intakes derived using consumption data derived from a 24-hour period with a single bolus dose endpoint in this assessment is appropriate and undertaking short term intake estimations based on eating occasion data would therefore not be likely to change the conclusions made from the assessment. There were three food categories 1) 'Non-brewed soft drink, carbonated, cola, intense sweetened' 2) 'Non-brewed soft drinks, cordials, not powder, intense sweetened' and 3) 'Beverages, non-alcoholic, tea-based' (e.g. iced tea) that had been consumed more than once per day by about 30% of consumers. However, the estimated highest single intake of added D-allulose (i.e. from the single eating occasions as opposed to the sum of the eating occasions over 24 hours) from these three food categories was lower than the 0.4 g/kg bw for around 90% of those consumers.

Maximum use concentration to not exceed the level that can cause laxative effects

As a result of the consumption of some foods potentially causing a laxative effect, an additional analysis was undertaken to determine the maximum amount of D-allulose (%w/w) that could be added to each food category before causing a laxative effect. This was based on the daily intake limit of 0.4 g/kg bw and high consumption (P97.5) of each food category for the three population groups assessed. This was done for each food category in its own right, assuming there would be no consumption of other foods containing D-allulose in the same eating occasion or meal. Where possible, similar food/ food groups were combined together to derive a high (P97.5) consumption value in order to determine a single maximum possible concentration for the entire category (e.g. breakfast cereals included 'breakfast cereals and cereal based bars; regular/ reduced sugar', 'breakfast biscuits & flakes', 'breakfast cereals, ready to eat' and 'breakfast cereals unspecified forms'; this was also done for all biscuits, cakes and pastries, and all water based flavoured drinks). When there was insufficient consumption data to determine a reliable food consumption amount to use in the calculation, a higher level/ major food category was used from which that particular food/ food category belongs to in order to derive a representative consumption amount (e.g. consumption data from 'sugar confectionary' was extracted to represent consumption for 'sugar confectionary, intense sweetened'; this was also done for sweet sauces and syrups). This was so that a maximum possible concentration could be derived for all food categories where D-allulose was proposed to be used.

The resulting highest concentration before causing a laxative effect was reviewed across all food categories assessed for all population groups assessed. It was found that there was no consistent threshold or concentration across all food categories that caused a laxative effect and it was dependent on the foods. For some foods, a threshold was not able to be determined mostly due to their low consumption and small number of consumers (e.g. dried yoghurt powder and imitation cream). This was not an issue given the assessment included 'yoghurt' as a ready to eat food, and a back calculation for imitation cream based on the laxative effect level and proposed maximum use level resulted in a daily consumption amount that would be unrealistic to achieve. Each of the population groups assessed had a maximum concentration estimated that was slightly different as a result of different consumption amounts and body weights. Therefore, one rounded value was determined for the food category, usually based on worst case scenario which was mostly the New Zealand children given their lower body weight in comparison to other population groups. The results are presented in Table 8 with a comparison to the proposed maximum use level provided in the application. For foods where the amount of D-allulose that could be added was higher than the proposed maximum use level in the application, these are not included in the table.

It should be noted that this assessment is based on the consumption of each food category individually, and does not include the possibility of two or more foods being eaten in the same eating occasion or meal. Whilst this is a possibility given the types of foods assessed, it would be unlikely that both or all of the foods eaten in combination would be consumed at the high (P97.5th) consumption level, therefore reducing the potential for a laxative effect in that situation. The data were not evaluated in this way to determine the likelihood or degree of this occurring and if resulting intakes in this scenario would exceed 0.4 g/kg bw.

Table 8 Comparison between the proposed maximum use level and the concentration that could cause a laxative effect for foods/ food categories

Food/ food category proposed by the applicant*	Proposed maximum use % (w/w) by the applicant	% (w/w) D-allulose to cause a laxative effect*
Bubble gum/chewing gum	50	30
Confectionery	50	10
Breads	10	5
Biscuits, cakes and pastries	10	5
Water based flavoured drinks (including iced teas)	3.5	1.5
Coffee based beverages	3.5	1
Dairy based desserts	5	4
Non-dairy based desserts (e.g. jelly)	10	3
Breakfast cereals, hot porridge type	5	3.5

* This table only includes foods/ food categories for which the concentration that could cause a laxative effect is lower than the proposed maximum use level.

Estimated dietary intakes for the microbiological safety assessment

Dietary intakes of added D-allulose were extracted based on a single day of consumption data for the microbiological risk assessment. Based on normal reported food consumption patterns, where consumers may have eaten one or more foods containing added D-allulose, estimates of dietary intake are shown in Table 9. Mean and P90 intakes are derived from the distribution of D-allulose intakes from each individual consumer within the survey population. Where results are expressed on a body weight basis, each individual's body weight was used. All the results are shown for each population sub group separately as children up to 14 years, adults 15-64 years and older adults aged 65 years and above for both Australia and New Zealand.

On a grams per day basis, mean intakes are slightly lower for older adults aged 65 years and above compared to children up to 14 years and adults 15-64 years. However, on a body weight basis, intakes of added D-allulose were highest for children up to 14 years at both the mean and 90th percentile intakes; intakes for children were around 2.5 times higher on a body weight basis compared to older adults 65 years and above.

Table 9 Estimated dietary intakes for consumers of D-allulose based on added sources at maximum use levels proposed by the applicant from day one only

Country	Age group (years)	Proportion of consumers to respondents (%)	Estimated dietary intake of the added D-allulose			
			g/day		g/kg bw/day	
			Mean	P90	Mean	P90
Australia	2-14	87.6	10.7	22.4	0.37	0.69
	15-64	80.9	13.0	29.3	0.17	0.39
	65 and above	84.7	9.6	21.5	0.13	0.29
New Zealand	5-14	93.2	12.1	26.0	0.34	0.73
	15 -64	82.9	12.4	28.6	0.16	0.36
	65 and above	86.9	8.8	21.1	0.12	0.27

3.5.2.7 Major contributing foods/food categories

Foods contributing to dietary intakes of D-allulose are only reported for the chronic scenario and one day of food consumption data from three surveys were used. The food categories that contributed >5% to the estimated dietary intake of the added D-allulose are summarised in Table A2. 2/ Appendix-2. 'Beverages (water based, non-alcoholic)' (e.g. 'non-brewed soft drink, carbonated, cola/not cola type, intense/sugar sweetened') and 'bakery products' (e.g. bread and related products, biscuits & crackers, cakes and pastries) are the major contributing food categories that are common for all three population groups assessed. 'Frozen dairy desserts' (e.g. desserts, dairy, ice cream and ice confection) and 'breakfast cereals and cereal based bars' (e.g. breakfast biscuits & flakes) are also major contributors for New Zealand consumers aged 5-14 years.

3.5.2.8 Discussion and conclusion of the dietary intake assessment for D-allulose

For the chronic assessment, the mean dietary intakes of the added D-allulose, on a g/day basis, are similar for all three population groups assessed. This is mainly due to the common consumption patterns that were identified through major contributing food categories to the estimated dietary intake for both Australian and New Zealand populations. However, the P90 dietary intake of the added D-allulose, on a g/day basis, for Australian population group is considerably lower because the results are a two-day average and will take into consideration that some respondents may not have consumed the food on both days of the nutrition survey. It is obvious that for New Zealand consumers aged 5-14 years, both the mean and the P90 dietary estimates of the added D-allulose, on a g/ kg bw basis, are higher due to lower body weights for that age group in comparison to the other two population groups assessed. As the dietary intakes of the naturally occurring D-allulose are shown to be negligible, a total dietary intake from both added and naturally occurring D-allulose was not calculated.

In the short-term assessment, although there were less than 10% of consumers of most food categories that had intakes above the level that causes a laxative effect (0.4 g/kg bw), there is the potential for a laxative effect to occur based on normal food consumption patterns. There was a higher proportion of high consumers (>10%) of children in the 5-14 years New Zealand population who had estimated intakes of the added D-allulose above 0.4 g/kg bw for three food categories (1: 'Non-brewed soft drink, carbonated, not cola type, sugar sweetened', 2: 'Desserts, dairy, ice cream and ice confection' and 3: 'Bread and related products, wheat base, unspecified type'). This is mainly due to their lower body weight as compared to the mean body weight of the other two populations assessed. In an additional analysis it was found that lower concentrations of D-allulose for some food categories could result in intakes that do not exceed the level that causes laxative effects if eaten without consuming other foods containing D-allulose in the same eating occasion. These levels were lower than the maximum use levels provided in the application for some food categories.

3.5.3 Dietary exposure assessment for D-psicose-3-epimerase

Due to the uniqueness of the proposed manufacturing process including the purification steps (as outlined in section 2.2.2 and 3.4.4), the presence of D-psicose-3-epimerase in the final D-allulose products is expected to be negligible. The analytical results of the commercial D-allulose preparations have confirmed that the protein content was below the limit of quantification (LOQ) of 0.625mg/g (limit of detection (LOD): 0.1 mg-N/g), indicating that residual protein could not be detected in the samples (see section 3.4.4.). Therefore, the likelihood of consumer exposure to the D- psicose 3-epimerase enzyme is considered to be negligible through the consumption of D-allulose manufactured using this unique method. This is consistent with the conclusion made by EFSA (2021) for their safety evaluation of the D-psicose-3-epimerase from the genetically modified *Corynebacterium glutamicum* strain FIS002 in the production of D-allulose using a similar manufacturing process. JECFA (FAO/WHO, 2022) has also assessed the dietary exposure to the D-psicose-3-epimerase enzyme from *A. globiformis* M30 expressed in *E. coli* K-12 W3110 that is used to produce D-allulose. Although it was assumed for the purpose of the JECFA dietary exposure assessment that all the enzyme remained in the final food, the Committee expected that the enzyme would not be present in D-allulose. Therefore, no dietary exposure to the enzyme would be expected from the final foods and therefore, FSANZ has not undertaken a dietary exposure assessment for D- psicose-3-epimerase.

3.6 Nutrition Assessment

3.6.1 Introduction

The application requests permission for the use of D-allulose as a novel food, which is intended to be used as a low-energy substitute for sugar ingredients and therefore requires the determination of an energy factor for labelling purposes. The application requests an energy factor for D-allulose of 1.0 kilojoule per gram (kJ/g) to be added to Schedule 11—2(3) of the Food Standards Code.

The objectives of the nutrition assessment are to determine:

1) The components of the equation for metabolisable energy of D-allulose.

2) Whether D-allulose could interfere with the absorption of any other nutrients, at the proposed levels.

3.6.2 Summary of Evidence for Calculating Metabolisable Energy

To support calculation of metabolisable energy, the application included published papers describing in vitro, animal, and human studies (Whistler et al. 1974; Matsuo et al. 2002; Matsuo et al. 2003; Iida et al. 2010; Hishike et al. 2013; Tsukamoto et al. 2014; Kishida et al. 2019), and an unpublished report describing a human study (Williamson et al. 2014).

FSANZ did not identify any additional relevant studies. If data from included studies were present only in graphs the required data were extracted using the online program WebplotDigitizer Version 4.4.1.

3.6.2.1 Human Studies

Iida et al. (2010) describe three human trials and one *in vitro* fermentation study (summarised in Section 4.2.3) to determine if D-allulose that is absorbed in the small intestine is metabolised, and whether any non-absorbed proportion is fermented by intestinal bacteria. Healthy male and female Japanese subjects were recruited, with exclusion criteria including diabetes or any systemic disease. Subjects fasted after an alcohol-free evening meal the night before testing for study 1, and excluded dietary fibre and lactic acid before studies 2 and 3.

The first crossover study included 6 participants (age 33.3 ± 3.7 (mean ± SD) years; weight 57.7 ± 10.2 kg) who consumed water (negative control), starch hydrolysate (positive control) or D-allulose (0.35 g/kg bodyweight, equivalent to a dose of ~20 g) as a 100 mL aqueous solution. Samples were administered at intervals of at least one week. Subjects urinated immediately prior to administration of test substances. Respiratory exchange (volume of carbon dioxide produced and volume of oxygen used) was measured shortly after dosing for 180 min, urine was collected at the end of measurement and urine nitrogen estimated. Carbohydrate energy expenditure (CEE) was calculated using the Weir method (Weir 1949) $(71.80 * VCO_2) – (50.8 * VO_2) – (44.4 * UN)$. [12] The area under the curve for CEE was significantly higher for starch hydrolysate compared to D-allulose (2.33 ± 0.95 vs 0.02 ± 0.62 kJ/min per kg bw; $p < 0.01$). The authors concluded that D-allulose is not metabolised into energy when consumed by humans at a typical dose because no increase in CEE following

[12] VCO_2: carbon dioxide produced (L/min); VO_2: oxygen consumed (L/min); UN: nitrogen excreted in urine (g/min).

D-allulose ingestion was observed.

The second randomised crossover study of 14 participants (age 34.1 ± 3.1 (mean ± SD) years; weight 59.5 ± 11.9 kg) measured D-allulose excretion in urine and fermentability in the large intestine. D-allulose or fructo-oligosaccharide (FOS; positive control) was consumed at 0.33, 0.17 and 0.08 g/kg, equivalent to doses of 20, 10 and 5 g, respectively. Non-ingestion was used as a negative control. Study arms were undertaken at one-week intervals. Participants consumed an alcohol-free evening meal formulated to exclude dietary fibre or lactic acid bacteria before 9 pm the night before testing. Expired air was collected immediately before consumption of test samples, and after consumption, at one-hour intervals for 10 hours, and breath hydrogen was measured using a gas chromatograph breath analyser. Urine D-allulose concentration was measured at 0-12 hours, 12-24 hours and 24-48 hours using high performance liquid chromatography (HPLC).

Cumulative urine excretion over 48 hours, expressed as percentage of dose administered was 66.2 ± 12.6% (mean ± SD) for 20 g D-allulose; 78.6 ± 10.6% for 10 g D-allulose and 78.8 ± 11.7% for 5 g D-allulose.
Breath hydrogen excretion was significantly higher in the 20 g (0.33 g/kg, based on 57 kg average bw) dose FOS group compared to the 20 g D-allulose group, (AUC[13]: 506.9 ± 437.5 (mean ± SD) vs 56.0 ± 91.0 ppm*hr; $p < 0.01$) and in the 10 g (0.17 g/kg, based on 57 kg average bw) FOS group (291.0 ± 227.5 vs 26.7 ± 33.7 ppm*hr; $p < 0.01$). The AUC for FOS breath hydrogen was plotted against energy consumed and used to derive D-allulose energy values of 0.89 kJ/g, 0.86 kJ/g and 1.61 kJ/g for doses of 20 g, 10 g and 5 g respectively, however details of the calculations were not provided.

The third study investigated the fermentability of D-allulose in the large intestine. Eight subjects (age 36.1 ± 8.0 (mean ± SD) years; weight 61.8 ± 11.6 kg) consumed 5 g D-allulose (0.08 g/kg based on 57 kg average bw) three times a day for 8 weeks. Expired air was collected hourly for eight hours before treatment and on the first and last day of treatment. There was no statistically significant difference in breath hydrogen excretion before and after 15 g D-allulose ingestion for 8 weeks.

The authors concluded that it is unlikely for an individual to consume 20 g D-allulose at one meal and therefore absorption of approximately 80% is likely (i.e. based on the cumulative urinary excretion of intact D-allulose following the 10 g and 5 g doses), with 20% passing to the large intestine.

Williamson et al. 2014 (unpublished) describe a single-dose, open-label non-randomised radiotracer study using D-[^{14}C(U[14])]-allulose that involved 8 healthy adult males (no age or other subject details provided). Subjects received a single oral dose of solution that contained 15 g allulose (no further details provided) that was measured using accelerator mass spectrometry. Samples were collected at the following time points after dosing: expired air: 15, 30, 60, 90 min and hourly from 2-6 hours; blood: 15, 30, 60, 90 min and hourly from 2-6 hours,12, 24, 48, 72, 96,120 hr; urine: 0-12, 12-24, 24-48, 48-72, 72-96, 96-120,120-144, 144-168 hr; faeces: 0-24, 24-48, 48-72, 72-96, 96-120 hr.

The mean percentage of dose in expired air over a 6 hour period was calculated to be 0.03%, with most readings below the limit of quantification.

[13] AUC: Area under the concentration versus time curve.
[14] U refers to uniform labelling of all carbon atoms.

The mean percentage recovery of radioactivity in urine and faeces during the 168 hour collection period was 81.5 ± 13.9 (SD)% and 3.12 ± 1.23% respectively. Only 48% of radioactivity was recovered from the urine of one participant, which the authors attributed to incomplete sample collection.

Radiotracer was observed in plasma 15 min after administration and peaked at one hour with most cleared by 24 hours. The authors reported that 80.3% of the radioactivity was recovered as D-allulose in 7 of the 8 participants in the 24 hour composite plasma sample. Variability was not reported.

The authors conclude that based on the comparison to a radiolabelled study of the non-metabolisable sugar erythritol, the calorific value of D-allulose is close to zero.

3.6.2.2 Animal Studies

Whistler et al. (1974) describe a metabolism study in which D-[^{14}C-U]-allulose (272 mCi/mole) was administered intravenously (n=4) or orally by stomach tube (n not provided) to rats ((150-200 g; strain or sex not reported) that had fasted for 24 hours. Urine and exhaled air were collected for 6 hours in the intravenous study, and for 7 and 72 hours respectively in the oral study.

In the intravenous study 97 – 98% of radioactivity was excreted in the urine as D-allulose and 0.6% was exhaled as carbon dioxide after six hours. It was concluded that intravenously administered D-allulose is rapidly removed by the kidneys and only metabolised to a small degree.

When administered orally by stomach tube, 4% of radioactivity was recovered in carbon dioxide and 35.4% in urine at 7 hours. At 72 hours, 15.1%, 37.3% and 12.5% of radioactivity was recovered in carbon dioxide, urine and faeces respectively. The carcass was reported to contain 38.7% of radioactivity. The authors also note that 12.3% of radioactivity was excreted in carbon dioxide at 24 hours.

Matsuo et al. (2002) describe a study in male Wistar rats age 3 weeks (no SD provided) that were fed CD-2 commercial rodent diet and water *ad libitum* for 3 days, and a basal diet for 4 days. Animals (n = 70) were randomly divided into 14 groups. All rats in one group were killed on day 0 (day 0 control) for body composition analysis. The remaining rats were fed a basal diet with 0, 0.5, 1, 1.5 or 2.0 g sweetener (sucrose, D-fructose or D-allulose) for 20 days. Faeces were collected during the last 7 days to determine nitrogen excretion. On the final day, rats were fasted overnight and killed. Change in body energy content over the 20 day period was calculated by multiplying the average energy content of the animals (Donato and Hegsted 1985) killed at the beginning of the experiment with the starting weight of each animal, which was subtracted by the value at 20 days. Body weight gain and body energy gain increased with increasing dietary concentrations of sucrose and D-fructose, but not with D-allulose**.** Caecal contents, faeces and nitrogen excretion increased with an increase in D-allulose dose (Table 10), but were not affected by increasing sucrose or D-fructose dose. Daily faecal excretion was significantly higher than control diet for the 1.0, 1.5 and 2.0 g daily D-allulose supplement groups (786 ± 27, 851 ± 49, 831 ± 30 vs 658 ± 9 mg/day (mean ± SE; $p < 0.05$), corresponding to an increase in weight of 12.8%, 12.9% and 8.7% of the D-allulose dose respectively. The authors suggest the increase in caecal and faecal content indicate that D-allulose is a fermentative saccharide similar to a soluble fibre.

Table 10 Caecal contents, faeces and nitrogen excretion in rats following D-allulose supplementation for 20 days (mean ± SE in groups of 5 rats).

Daily dose (g)	Total dose (g)	Caecal contents (g)	Faeces (mg/day)	Nitrogen (mg/day)
0	0	12.8 ± 0.8[a]	658 ± 9[a]	22.9 ± 0.9[a]
0.5	10	10.1 ± 1.2[a]	713 ± 20[a]	26.4 ± 1.2[b]
1.0	20	11.3 ± 0.9[a]	786 ± 27[b]	39.2 ± 1.5[c]
1.5	30	16.4 ± 1.0[b]	851 ± 49[c]	42.1 ± 2.0[c]
2.0	40	16.0 ± 1.0[b]	831 ± 30[c]	49.1 ± 1.9[d]

Values with a different superscript in each column are significantly different ($p < 0.05$).

Matsuo et al. (2003) investigated the absorption, excretion and fermentation of D-allulose in male Wistar rats when given orally as part of controlled diets in 3 studies. In the first study, following a twelve hour fast, 8 rats (mean weight 138 ± 4 g (assumed to be SEM)) were fed a diet containing D-allulose at a concentration that was reported to be equivalent to 5 g/kg body weight, although no further details were provided. Urine and faeces were then collected at intervals of 24 hours for 72 hours and excretion of D-allulose was assayed by HPLC. Over the 0-24 hour collection period 11-15% and 8-13% of D-allulose doses were recovered from urine and faeces respectively, but were not detected in the samples collected 24-48 and 48-72 hours after administration. The authors suggest that the remaining D-allulose is metabolised or remains in the rat body.

In the second experiment 18 rats (mean weight 140 ± 4 g (assumed to be SEM)) were fed a standard diet until 6 weeks old; fasted for 12 hours after which time they received 5 g/kg body weight D-allulose orally, however no further details were provided. Six rats were killed at 1 hour, 3 hours and 7 hours after oral administration and blood was collected. D-allulose in serum, and stomach, small intestine and caecum contents were determined. Serum D-allulose concentration decreased progressively after administration. D-allulose content in the stomach was higher at 1 hour (26-37% of dosage) than at 3 hours (0.4 – 0.6%), and by 7 hours it was not detected. Similarly 6-10%, 2-3% and 1-3% of the dosage was found in the small intestine at 1, 3 and 7 hours respectively and 18% and 10-19% of the dosage was found in the caecum at 3 and 7 hours.

The third experiment used 26 rats (mean weight 76 ± 2 g (SEM)) randomised into 4 groups and fed a standard diet until 4 weeks old, followed by an *ad libitum* synthetic high carbohydrate diet including 0, 10, 20 or 30% D-allulose, 5% corn oil and 65, 55, 45, or 35% corn starch for 34 days. Following a 12 hour fast animals were killed. The caecum was removed, and caecal weight, surface area and caecal content weight measured.

Caecal short chain fatty acid (SCFA: acetic, propionic and butyric acid) concentrations increased with increasing amounts of D-allulose in the diet. Acetic and butyric acid concentrations for the 0%, 10%, 20% and 30% D-allulose treatments were significantly different from each other (acetic acid: 5.27 ± 0.75, 7.43 ± 0.56, 11.47 ± 1.22, 22.29 ± 3.48 mmol (mean ± SEM); butyric acid: 1.06 ± 0.23, 2.96 ± 0.53, 5.42 ± 0.58, 9.0 ± 1.37 mmol; $p < 0.05$). Propionic acid concentrations were significantly higher for the 20% and 30% doses ($p < 0.05$) compared to control (1.27 ± 0.12, 1.68 ± 0.36, 2.08 ± 0.33, 2.51 ± 0.22 mmol for the 0%, 10%, 20%, and 30% doses). SCFA concentrations were positively correlated with caecal weight and surface area. The authors conclude that D-allulose is partly absorbed in the digestive tract and is excreted into urine and faeces and is fermentable due to the production of SCFAs in the caecum.

Tsukamoto et al. (2014) investigated the pharmacokinetics of D-allulose after oral and intravenous administration of radiolabelled [1-^{14}C]-D-allulose in rats. Male Wistar rats (n=30; 280-320 g) were fasted for 24 hours before a catheter was inserted into the femoral vein. Anesthesia (pentobarbital sodium 1%) was continued until the animals were sacrificed. D-allulose (0.6 mL, providing a dose of 100 mg/kg bw) was administered by oral gavage. Rats were sacrificed at 10, 30, 60, and 120 minutes after administration. Total bladder urine was measured and gastrointestinal organ contents (stomach, small intestine, large intestine, cecum) were collected and homogenised for all samples.

Following oral administration, blood concentrations (mean ± SD) of isotope-labelled D-allulose were 11.3 ± 6.4, 41.8 ± 16.2, 48.5 ± 15.6 and 39.2 ± 9.5 µg/g at 10, 30, 60 and 120 min, respectively. Radioactivity of D-allulose in urine was 0%; 3193.6 ± 2870.4 µg: 10%; 5832.0 ± 1682.5 µg: 19%; and 11125.2 ± 2403.0 µg: 37% at 10, 30, 60, and 120 min respectively.

Following intravenous administration, blood concentrations of isotope-labelled D-allulose were 132.0 ± 22.1, 83.0 ± 26.5, 77.2 ± 23.5 and 40.1 ± 31.5 µg/g (± SD) at 10, 30, 60 and 120 min, respectively. Radioactivity of D-allulose in urine was 4773.6 ± 1376.6 (SD), 7303.9 ± 3662.3, 13,230.7 ± 3292.9 and 15,231.7 ± 4649.0 µg/unit at 10, 30, 60, and 120 min respectively, which the authors estimated to be equivalent to 15%, 24%, 44% and 50% of the administered dose. The authors noted that orally administered D-allulose was partly absorbed from the gastrointestinal tract into the blood and excreted into urine.

Seven days after a single dose of 100 mg/kg bodyweight, radioactivity in the blood, gastrointestinal contents and urine was almost zero, with the authors suggesting that more than 99% of the orally administered dose was excreted.

In summary, based on radiolabelling studies, approximately 37% of the administered D-allulose was found in urine 120 min after a single oral dose of 100 mg/kg bodyweight.

Kishida et al. (2019) observed increased plasma D-allulose concentrations in GLUT5-induced rats that consumed D-fructose compared to rats that consumed glucose prior to oral administration of D-allulose (C_{max} 3.47 ± 0.23 vs 1.11 ± 0.05 (mean ± SE) mmol/L; T_{max} 52.5 ± 7.5 vs 75.0 ± 8.7 min for fructose and glucose-fed rats respectively). A competitive study found that plasma D-allulose levels were delayed when D-allulose was gavaged with D-fructose compared to D-allulose alone, indicating competition between D-allulose and D-fructose. Experiments using radiolabelled sugars including D-[^{14}C]-fructose and D-[^{14}C]-glucose indicated that D-allulose shares transporters with both D-fructose and D-glucose with D-allulose causing a decrease of 54.8% ± 5.8% of D-fructose uptake but also a decrease of 51.2% ± 9% of D-glucose uptake. The authors conclude that D-allulose may be transported by SGLT1 as well as GLUT5.

3.6.2.3 In vitro studies

Iida et al. (2010) investigated D-allulose fermentability by intestinal bacteria using 35 typical intestinal bacteria strains (6 *Bacteroides*, 7 *Bifidobacterium*, 5 *Clostridium*, 5 *Lactobacillus* and 12 other strains, sources not described). Bacteria were grown in culture media alone or media containing either 0.5% D-glucose or 0.5% D-allulose. A low level of D-allulose fermentability was observed in 4 strains only – *Bacteroides thetaiotaomicron* GAI# 5628*, Bacteroides uniformis* GAI# 5466^{T}*, Bifidobacterium dentium* CIFL# N0121^{T} and *Ruminococcus productus* JCM 1471^{T}. The authors concluded that the quantity of D-allulose that arrives in the large intestine is not readily fermented by intestinal bacteria.

As discussed in Section 3.2.1.1, an in-vitro study by Hishiike et al. (2013) investigated the transport of D-allulose across cell membranes in a Caco-2 cell monolayer model. Caco-2 cells are a human intestinal adenocarcinoma cell line that exhibit enterocyte-like properties and are used to study the absorption of nutrients. The permeation of D-allulose across the monolayer was not affected by the addition of phlorizin, an inhibitor of the sodium-glucose transport protein SGLT1, but was accelerated by treatment with forskolin, which induces the fructose transporter GLUT5 gene. The authors conclude that the transport route of D-allulose is the same as D-fructose, and D-allulose has similar permeability to fructose.

3.6.2.4 Assessment by other regulatory agencies

The United States Food and Drug Administration (FDA) assessed the scientific evidence on the cariogenic potential, metabolism, glycaemic response and caloric value of D-allulose (FDA 2019). FDA concluded that, based on Iida et al. (2010), summarised above, the energy value generated from the fermentation of allulose in the large intestine as compared to that of FOS, a non-digestible carbohydrate, provided an estimated caloric value that is no more than 0.4 kcal/g (equivalent to 1.7 kJ/g). However, as noted above (Section 4.2.1), details of the energy calculations were not provided by Iida et al. (2010).

A guidance document for industry was published, regarding the declaration of D-allulose on nutrition and supplement labels and the caloric content of D-allulose (FDA 2020). FDA stated that it will exercise enforcement discretion for the exclusion of D-allulose from the amount of total sugars and added sugars declared on the label and use a general factor of 0.4 kcal/g for D-allulose when determining energy on the nutrition and supplement facts label.

3.6.3 Calculation of the components of equation for metabolisable energy

Section 3.2.5 B.2 of the FSANZ Application Handbook (FSANZ 2019) states that the proposed energy factor for a food ingredient must be calculated using the equation:

ME = GE - FE - UE - GaE - SE where

ME means metabolisable energy
GE means gross energy (as measured by bomb calorimetry)
FE means energy lost in faeces.
UE means energy lost in urine.
GaE means energy lost in gases produced by fermentation in the large intestine.
SE means energy content of waste products lost from surface areas.

3.6.3.1 Gross energy (GE)

The gross energy of a food or food ingredient is the total chemical energy measured from complete combustion to carbon dioxide and water in a bomb calorimeter (Wierdsma et al. 2014). The applicant requested the value of 15.2 kJ/g for GE, based on the FSANZ assessment for D-tagatose, an epimer of D-fructose (FSANZ 2004). FSANZ considers that the value of 15.2 kJ/g was an editorial error and the correct gross energy value for D-allulose based on bomb calorimetry is 15.7 kJ/g (Levin et al. 1995). D-allulose is also an epimer of D-fructose and therefore the same GE for fructose is suitable for D-allulose.

The gross energy (GE) for D-allulose, based on bomb calorimetry of fructose, is 15.7 kJ/g

3.6.3.2 Urinary energy (UE)

Urinary energy is the percentage of ingested food that is excreted unchanged in urine. HPLC can be used to identify and quantify different molecules in a mixture and therefore can be used to quantify unchanged D-allulose in urine. FSANZ considers that in general, HPLC is a suitable method to quantify urinary energy. Isotopic tracer methods are also considered to be an acceptable source of evidence to substantiate the energy factor calculation (FSANZ 2019), noting that they cannot distinguish between metabolised and non-metabolised products unless combined with other techniques.

One human study (Iida et al. 2010) calculated the 48 hour excretion rates for D-allulose of 66.2% for 20 g dose for a person of average weight (based on 0.33 g/kg body weight) and 78.6% and 78.8% for a 10 or 5 g (0.17 and 0.8 g/kg body weight) dose respectively, using HPLC. The authors consider that 20 g dose is unlikely to be consumed in one sitting, which FSANZ agrees with, based on estimated dietary intake levels (Section 3.5.2). An unpublished human radiotracer study reported that 81% of orally administered radiolabelled D-allulose was collected in urine in the 168 hour collection period (Williamson et al. 2014).

FSANZ notes that urinary excretion rates differ between rats (37%: Tsukamoto et al. 2014) and humans (66-79%: Iida et al. 2010; 81%: Williamson 2014).
Based on the available evidence from human studies, FSANZ considers that 80% of D-allulose is excreted unchanged in urine.

The Urinary energy (UE) for ingested D-allulose is 80% of gross energy.

3.6.3.3 Faecal energy (FE)

Food ingredients that are not absorbed pass through the gastrointestinal tract and may be fermented by bacteria in the large intestine. The energy lost from D-allulose in faeces (FE) must be calculated. In the case of ingredients that are fermented or partly fermented the following equation can be used (FSANZ 2019):

FE = uFE + mFE + oFE

uFE: unchanged faecal energy; the proportion of the food ingredient that is excreted unchanged in the faeces

mFE: microbial faecal energy; microbial mass in faeces that is produced from the proportion of the food ingredient that reaches the large intestine and is fermented. FSANZ has set a default value of 30% for mFE for ingredients fermented or partly fermented in the large intestine.[15]

oFE: other faecal energy; other produced substances excreted into the faeces from the proportion of the food ingredient that escapes absorption, such as short chain fatty acids or other metabolites. FSANZ has set a default value of 0% for oFE for ingredients fermented or partly fermented in the large intestine.

[15] If default values are not used for mFE, oFE, GaE of fermented food ingredients, or for SE, then the value for that respective component of the energy factor equation must be substantiated

Several studies considered the fermentability of D-allulose in the large intestine, including breath hydrogen excretion tests that estimate the degree of fermentation. Iida et al. (2010) investigated the D-allulose fermentability by 35 typical intestinal bacterial strains and noted no fermentability in 31 strains and a low level of fermentability in four strains. No significant difference in breath hydrogen excretion was observed. These data indicate that at least some fermentation of D-allulose occurs in the large intestine, but do not directly quantify the extent of fermentation.

Two studies report faecal excretion of D-allulose in rats. Whistler (1974) reported that 12.5% of radioactivity from radiolabelled D-allulose was recovered in rat faeces 72 hours after oral administration. Matsuo et al. (2003) reported that 8-13% of the administered D-allulose dose was recovered from rat faeces 24 hours after oral administration of 5 g/kg body weight, with nothing recovered at 48 or 72 hours, as measured by HPLC. In a second experiment, following dosing with 5 g/kg body weight, 18% and 10-19% of D-allulose was recovered from the caecum of rats 3 and 7 hours after administration respectively, with no detection at 1 hour.

A study by Williamson (2014) reported that 3% of the radioactivity was recovered in human faeces in the 168-hour collection period following dosing.

The available evidence from rat studies indicates that the faecal energy (FE) of D-allulose could range from 8% to 19% of total energy, while the Williamson (2014) unpublished human study reports a value of 3%.

No data were available to calculate the unchanged faecal energy (uFE) in order to use the equation for fermented or partly fermented ingredients. If uFE was set to zero, and default values were used for mFE (30%) and oFE (0%), the final value for FE would be 4.71 kJ/g (0 + (30% * 15.7 kJ/g) + 0). FSANZ notes that the calculations using default values are likely to be an overestimate as UE was calculated to be 80% and therefore the combined energy from urine and faeces would exceed 100%, resulting in the energy factor for D-allulose of zero.

Based on the available human data, FSANZ considers that the faecal energy for D-allulose is 3% of gross energy.

The Faecal energy (FE) for ingested D-allulose is 3% of gross energy.

3.6.3.4 Gaseous energy (GaE)

Gaseous energy is the energy lost in gases produced by fermentation in the large intestine. FSANZ has set a default value of 5% for GaE for ingredients fermented or partly fermented in the large intestine (FSANZ 2019).

In a study by Iida et al. (2010) breath hydrogen concentration was estimated in subjects that consumed 5, 10, and 20 g doses of D-allulose (based on average weight of 70 kg), with a dose-response observed. However, gaseous energy from D-allulose fermentation was not quantified. Therefore, the default value of 5% GaE is used to calculate the energy factor.

The Gaseous energy (GaE) for ingested D-allulose is 5% of gross energy.

3.6.3.5 Surface area energy (SE)

Surface area energy is the energy content of waste products lost from surface areas. FSANZ has set a default value of 0% for SE for ingredients fermented or partly fermented in the large intestine.

The applicant did not provide any data relating to the calculation of surface area energy of D-allulose and FSANZ did not identify any additional studies that measured SE. Therefore the default value of 0% will be applied.

The Surface area energy (SE) for ingested D-allulose is 0% of gross energy.

Based on the above estimates the components of the equation for metabolisable energy of D-allulose are:

Gross Energy	=	15.7 kJ/g
Urinary Energy	=	80% of gross energy
Fecal Energy	=	3% of gross energy
Gaseous Energy	=	5% of gross energy
Surface area Energy	=	0% of gross energy

3.6.4 Effect of D-allulose on absorption of other nutrients

FSANZ considered whether D-allulose could interfere with the absorption of other nutrients, at the proposed level. The applicant did not provide any evidence to indicate that D-allulose affected the absorption of other nutrients. FSANZ undertook a literature search and did not identify any relevant studies to indicate that D-allulose inhibits the absorption of other nutrients.

3.6.5 Key Findings of the Nutrition Assessment

FSANZ considered the available evidence for calculation of the components of the equation for metabolisable energy of D-allulose, as required by the Application Handbook. It is concluded that:

Gross energy (GE) = 15.7 kJ/g
Urinary energy (UE) = 80% of gross energy
Fecal energy (FE) = 3% of gross energy
Gaseous energy (GaE) = 5% of gross energy
Surface area energy (SE) = 0% of gross energy

No evidence was identified to indicate that D-allulose consumption would affect the absorption of other nutrients.

3.7 Microbiology assessment

3.7.1 Objectives of the microbiology assessment

The microbiology assessment evaluated information on the safety of the intake of D-allulose. The application also requests approval of the D-psicose-3-epimerase enzyme produced by the organisms *M. foliorum* to be used as a processing aid.

3.7.2 Microbiology assessment of human toxicokinetic and toxicology studies

Section 3.2 covers the toxicokinetic and toxicology studies from a toxicological perspective. This section evaluates chronic (≥8 weeks) human trials from a microbiological perspective.

Four chronic intake studies were identified: Iida et al. (2008), Hayashi et al. (2010), Han et al. (2018a) and Tanaka et al. (2020). A fifth study, Han et al. (2018b) was a dosing study which investigated the maximum single dose for occasional ingestions and maximum daily intake for regular ingestion (Appendix 3). No microbiological concerns were identified in the study. A summary of the chronic intakes studies is presented in Table 11.

Table 11 Summary of daily intake amounts and frequency for human trials.

Reference	Study details	Intake	Duration
Iida et al. (2008)	study 4	5g x 3 times/day	8 weeks
Hayashi et al. (2010)		5g x 3 times/day	12 weeks
Han et al. (2018a)	low dose	4g x 2 times/day	12 weeks
	high dose	7g x 2 times/day	12 weeks
Tanaka et al (2020)	low dose	5g/day	48 weeks
	high dose	15g/day	48 weeks

Estimated dietary intakes for consumers of D-allulose based on added sources at maximum use levels proposed by the applicant from day one only are presented in Table 9. The total daily intake ranged between 5g/day (Tanaka et al., 2000) up to 15g/day (Iida et al., 2008; Hayashi et al., 2010 and Tanaka et al., 2020). The estimated mean daily intake for 15-64 years in Australia and New Zealand are 13.0 g/day and 12.4 g/day respectively. The 15-64 year age group dietary intakes (3.5.2.6 Estimated dietary intakes for the microbiological safety assessment) was requested as it is a more representative age group than 2 years and above for Australia or 15 years and above for New Zealand (see Table 6). The corresponding P90 intakes are 29.3 g/day and 28.6 g/day for Australia and New Zealand, respectively are up to double those in the human trials. The estimated intakes were calculated of consumers that may have eaten one or more foods containing D-allulose. The use of multiple smaller intakes of D-allulose is likely to be a more realistic eating pattern than a single large quantity consumed in the single dose which are used for investigation into postprandial plasma glucose studies (Section 3.2). The use of two days of consumption data would likely reduce the estimated intakes.

Study information on adverse events (where available) or reasons for study dropouts in the human trials did not raise concerns regarding the microbiological safety for chronic consumption of D-allulose (Appendix 3).

A feature of the human trials was inclusion and exclusion criteria for potential study participants (Appendix 3). Inclusion criteria with undefined terms like “normal” (Iida et al., 2010; Han et al., 2018b) or “healthy” (Hayashi et al., 2010) were used. Two studies including fasting blood glucose levels to excluded diabetics and border-line diabetics (Hayashi et al.,

2010) or to include border-line diabetics and exclude diabetics (Tanaka et al., 2020). The list of exclusion criteria in the studies was often extensive with Tanaka et al. (2020) having a list of 16 exclusion criteria. Commonly cited exclusion criteria included potential participants who had diabetes, hepatic and renal function disorders, pregnancy or were lactating. As a result of these exclusions, no assessment can be made regarding the microbiological safety for the intake of D-allulose in these subpopulations.

3.7.3 Metabolism of D-allulose by microorganisms

Bilt et al (2017) analysed a range of *Klebsiella* species for their ability to ferment D-allulose Strains used in this study included environmental clinical and hospital sourced isolates. For the klebsiella pneumonia strains 15 of the 20 were found to be able to respire the sugar. Iida et al (2007) tested faecal bacteria did not find the single *Klebsiella* pneumonia strain capable of growing on D-allulose.

3.7.4 Identification of D-allulose metabolism genes

The potential for *K. pneumoniae* to utilise D-allulose has been indicated in a few studies. Martin et al. (2018) undertook a case-control study comparing infected and asymptomatic colonised patients to identify bacterial genes associated with *K. pneumoniae* infection. 38 patients met the case definitions for extraintestinal infection, either bacteraemia or pneumonia. Each infected patient (n=38) was matched to two asymptomatically colonized controls (n=76) based on age range (within 10 years), gender, and sample collection date (within 3 weeks). The gene frequency differences between bacterial isolates were analysed using a novel comparative genomics technique, termed pathogenicity associated locus sequencing (PAL-Seq), and then assessed as to whether candidate virulence genes were independent predictors of infection, whether they improved prediction of infection when incorporated into a clinical model, and whether they had a distinct phenotype in a rodent model of pneumonia. Genes associated with a D-allulose utilization locus were identified to be associated with infection, though not incorporated in the final model. The identified genes KP1_RS12850 and KP1_RS12840 differed significantly by 84.2% compared to 56.6% and 81.5% and 56.6% between infected and asymptomatically colonised patients respectively. D-allulose was experimentally identified as the relevant substrate because deletion of the permease gene in this locus impaired growth on D-allulose.

3.7.5 *in silico* identification of D-allulose metabolism genes in microorganisms

The BfR (German Federal Institute for Risk Assessment) undertook bioinformatic sequence analysis to determine the distribution and significance of the gene cluster coding D-allulose metabolization enzymes in *Klebsiella* spp. Isolates identified by Martin et al. (2018) (BfR 2020). A small number of bacterial species were shown to contain the gene cluster and a complete D-allulose usage system was most frequently detected in *Klebsiella* spp., with the highest proportion in *K. pneumoniae* (5025 *K. pneumoniae* isolates and 199 isolates from six other *Klebsiella* spp.). Sporadically conserved sequences from all genes were identified in less than four isolates for *Proteus* spp., *Enterobacter* spp., *Serratia* spp., *Cedecea* spp., *Gibbsiella quercinecans*, and *Escherichia coli*. Additionally, the D-allulose utilisation gene cluster was determined to be firmly anchored in the *Klebsiella* chromosome, and horizontal gene transfer to other species is unlikely.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used to identify microbial genomes which included the D-allulose-6-phosphate 3-epimerase gene.

A variety range of pathogenic *E. coli* types were identified including uropathogenic (O6:K15:H31 and O127:H6), enterohaemorrhagic (O103:H2), enterotoxigenic (O78:H11:K80), adherent-invasive (O83:H1) and extra intestinal pathogenic (O18:K1). Commensal and laboratory research *E. coli* strains were also identified. *K. pneumoniae* strains which carry the D-allulose metabolism gene were recovered from multiple specimens including urine, perirectal swabs and sputum.

D-allulose-6-phosphate 3-epimerase gene sequences from the human KEGG strains were analysed using the NCBI blast database which found a high degree of similarity to other isolates from the same species. The D-allulose-6-phosphate 3-epimerase gene appears to be widely spread in both *E. coli* and *K. pneumoniae*.

3.7.6 Clinical evidence

The majority of *K. pneumoniae* infections globally are opportunistic hospital acquired infections generally displaying as pneumonia, urinary tract and wound infections (Gorrie et al. 2017; Wyres et al. 2020). A key risk factor in these infections is intestinal carriage and the most at risk are the young, old, and immunocompromised. An Australian study confirmed *K. pneumoniae* colonisation is a significant risk factor for infection in hospital intensive care units, and ~50% of *K. pneumoniae* infections result from patients' own microbiota (Gorrie et al. 2017). It is speculated that infection is due to overgrowth and lack of immunological control of commensal *K. pneumoniae* strains (Wyres et al. 2020). *K. pneumoniae* can also act as a pathogen in the community causing severe community-acquired infections in healthy people but with different risk factors to hospital acquired infections.

Martin et al. (2018) noted that the absorption of D-allulose into the small intestine and excretion via the urine provide two potential sites where colonising *K. pneumoniae* could encounter D-allulose. The BfR also noted (BfR 2020) that the few studies where D-allulose was administered to healthy humans and/or persons with metabolic disorders do not provide data on the distribution of D-allulose in the parts of the body where *K. pneumoniae* spp. is known to colonise - including the gastrointestinal tract, the urogenital tract (with the exception of excretion of D-allulose in urine), and the respiratory tract (Iida et al., 2008; Iida et al., 2010; Kimura et al., 2017; Braunstein et al., 2018; Han et al., 2018a; Han et al., 2018b; Hayashi et al., 2010; Norhona et al., 2018). The BfR hypothesised that it was possible that the presence of D-allulose could enhance intestinal *K. pneumoniae* colonisation and be a risk factor for progression to disease. However, the source and function of allulose during pneumonia is unclear and this has not been confirmed (BfR 2020).

3.7.7 D-allulose concentrations in body compartments

No studies identified. None of the toxicokinetic or toxicology studies assessed measured the D-allulose concentration in urine.

3.7.8 Discussion concerning the microbiological safety of D-allulose

The review of human D-allulose intake studies identified no specific concerns for either short or long term intake of the sugar in healthy adults. Study exclusion criteria consistently removed potential participants with had diabetes, hepatic and renal function disorders, pregnancy or were lactating. The exclusion criteria do highlight potential vulnerable subpopulations. This finding is particularly relevant to the microbiological safety assessment as the very high percentage of sugar absorbed in the small intestine through the kidney with rapid excretion to the urine is relevant to potential urinary tract infections.

Evidence from the toxicological metabolism studies did not support gut microflora as a major contributor to the degradation of D-allulose with the majority that entered the large intestine being excreted in the faeces.

The D-allulose metabolism gene was used as a proxy for the identification of microorganisms that may be capable of D-allulose metabolism. The use of the KEGG database identified a variety range of pathogenic *E*. *coli* types including uropathogenic, enterohaemorrhagic and extra intestinal pathogenic strains as well as a many of *K*. *pneumoniae* strains which carry the D-allulose metabolism gene. Gene sequences from the KEGG strains were analysed using the NCBI blast database which found a high degree of similarity to other isolates from the same species. This analysis extended on the BfR (2020) genomic analysis and confirmed that the D-allulose metabolism gene is distributed within the *E*. *coli* and *K*. *pneumoniae* species.

The *in silico* analysis provided genotypic information but phenotypic confirmation is required to demonstrate microbiological species that carry the D-allulose metabolism gene are capable of growing on the D-allulose as a sole carbon source. Bilt et al. (2017) demonstrated *K*. *pneumonia* isolates were able to metabolise D-allulose.

A weakness in the evidence for this microbiological safety assessment is the lack of D-allulose concentration information in body compartments. None of the toxicokinetic or toxicology studies reported the D-allulose concentration in urine. The development of physiologically based kinetic models to predicted the D-allulose concentrations in different body compartments could help design experimental studies to better understand the relationship between intakes, internal concentrations and the potential for pathogen growth and disease.

3.7.9 Other microbiological considerations

The production strain of *M*. *foliorum* SYG27B-MF was originally isolated from ginseng. The identity was determined using a genomic sequence of the 16S rRNA gene (Kim et al., 2018).

The specifications for this application include several microbiological parameters for D-allulose crystalline powder and syrups. These are total plate count: ≤1000 CFU/g; coliforms: not detected; *Salmonella*: not detected; and *Staphylococcus aureus*: not detected. The same parameters apply for both the powder and syrup forms. These microbiological parameters for D-allulose do not pose a risk to consumer.

4 Discussion

4.1 Assessment of D-allulose

Samyang have provided assurance of their ability to produce D-allulose suitable for use in foods as a sugar replacer and which is consistent with specifications set out in scientific literature. Their manufacturing plant operates in accordance with standard GMP, under International Standards Organisation (ISO) 9001:2000 and Hazard Analysis and Critical Control Point (HACCP) certification. The D-allulose crystalline powder and syrups are stable under typical storage conditions and when contained in a food matrix typical of the proposed end use.

Most (80%) of an oral dose of D-allulose is rapidly absorbed from the small intestine, but rapidly excreted in the urine. There is some metabolism of D-allulose by microbiota in the large intestine, but it appears that most D-allulose that reaches the large intestine is excreted unchanged in the faeces.

D-allulose is of very low acute toxicity, with an acute oral LD50 in the rat of approximately 16 g/kg bw. A No Observed Adverse Effect Level (NOAEL) of 5000 mg/kg bw/day, the highest dose tested, was identified in a 90-day rat study. Results of genotoxicity assays were negative, and D-allulose was not associated with carcinogenicity in an 18-month chronic study in rats. D-allulose was not associated with adverse reproductive or developmental effects in rats.

Laxative effects, attributed to the osmotic effect of D-allulose that is not absorbed from the gastrointestinal tract, have been observed in laboratory animals and in humans. The lowest single dose of D-allulose associated with gastrointestinal effects was 0.4 g/kg bw (28 g in a 70 kg adult) and repeated intake should not exceed a total intake of 0.9 g/kg bw/day (Han et al 2018b).

Estimated mean and high chronic dietary intakes of D-allulose ranged between 150 and 730 mg/kg bw/day across the population groups and scenarios assessed based on maximum use levels noted in the application. Estimated dietary intakes from naturally occurring sources of D-allulose were very low compared to intakes from added sources.

There were number of single food categories (Table 7) from which the daily intake of D-allulose exceeded 0.4 g/kg bw for high consumers (P97.5). There were also some food categories for which consumption amounts over 24-hours resulted in D-allulose intakes above 0.9 g/kg bw/day. For the majority of food categories where an exceedance was estimated, there were less than 10% of consumers exceeding, however for three foods the proportion exceeding the laxative effect threshold was up to nearly double that. For high consumers of these foods, a lower concentration of added D-allulose would be needed to result in intakes of D-allulose under the level that causes laxative effects if eaten as one D-allulose containing food per eating occasion. Chronic dietary exposures would be lower than those estimated (Table 6) if proposed use levels were lower than those originally requested in the application.

FSANZ considered the available evidence for calculation of the components of the equation for metabolisable energy of D-allulose, as required by the Application Handbook. It is concluded that:

Gross energy (GE) = 15.7 kJ/g
Urinary energy (UE) = 80% of gross energy
Fecal energy (FE) = 3% of gross energy
Gaseous energy (GaE) = 5% of gross energy
Surface area energy (SE) = 0% of gross energy.

No evidence was identified to indicate that D-allulose consumption would affect the absorption of other nutrients.

No public health or safety concerns were identified in the microbiological safety assessment of D-allulose and healthy adults. D-allulose intakes for

Chronic consumption trials (≥8 weeks duration) were biased towards healthy adults. Exclusion criteria for these trials highlighted potential vulnerable subpopulations such as diabetics. The high percentage of absorption of untransformed D-allulose in the small intestine through the kidneys to urine does flag potential issues with urinary tract infections. from bacteria such as *Klebsiella pneumoniae* which are able to metabolise D-allulose. Genomic analysis also found that a variety of *E. coli* sub-types including uropathogenic strains have the gene for metabolising D-allulose. The microbiological safety of subpopulations, such as diabetics, consuming D-allulose has not been established.

4.2 Assessment of D-psicose 3-epimerase

The evidence presented to support the proposed use provides adequate assurance that the use of enzyme D-psicose 3-epimerase, in the form and requested amount (i.e. at a level consistent with GMP) is technologically justified and has been demonstrated to be effective in achieving its stated purpose. The enzyme meets relevant identity and purity specifications in the Code. D-psicose 3-epimerase is appropriately categorised as a processing aid as defined in the Code.

D-psicose 3-epimerase has a five-year history of safe use for the production of D-allulose, and the applicant has provided evidence that there is negligible likelihood of consumer exposure to the production organism, the intact enzyme, or residues from the enzyme. No significant homology was found with any known toxins or allergens. Toxicological studies of D-psicose 3-epimerase are not considered to be necessary.

No public health and safety risks were identified to be associated with the use of *M. foliorum* in the production of D-psicose 3-epimerase.

5 Conclusions

It is concluded that there is no toxicological risk to public health and safety from consumption of D-allulose in food based on the proposed maximum use levels in the application, or from the use of D-psicose 3-epimerase in the production of D-allulose. An Acceptable Daily Intake (ADI) “not specified” is appropriate for both D-allulose and D-psicose 3-epimerase.
Based on previous assessments of rare sugars and polyols that may induce osmotic laxation, a recommendation against consuming a high single dose of D-allulose may be appropriate. The lowest dosage of D-allulose associated with gastrointestinal symptoms is 0.4 g/kg bw (400 mg/kg bw; 28 g for a 70 kg adult) as a single dose. High consumption of some food categories was estimated to result in dietary intakes of D-allulose above this level. Lower maximum use levels of D-allulose for some food categories could result in intakes that do not exceed the level that causes laxative effects if eaten without consuming other foods containing D-allulose in the same eating occasion.

No public health or safety concerns were identified in the microbiological safety assessment of D-allulose and healthy adults. D-allulose intakes for chronic human feeding trials (≥8 weeks duration) were similar to the estimated dietary intakes for single day of consumption. Intakes for older adults (65 years and above) were less than the 15-64 years group. Exclusion criteria for the human feeding trials highlighted that no microbiological safety data is available for vulnerable sub-populations such as diabetics. The high percentage of absorption of untransformed D-allulose in the small intestine through the kidneys to urine does flag potential issues with urinary tract infections. Uro-pathogenic bacteria such as *Klebsiella pneumoniae* are able to metabolise D-allulose. The microbiological safety of some subpopulations, such as diabetics, exposed to D-allulose has not been established.

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Appendix 1. Concentration data used and results of the dietary intake assessment of naturally occurring D-allulose

Table A1.1 Naturally occurring concentrations of D-allulose used in the chronic dietary intake assessment

Food	Concentration (mg/100 g food) reported in the application/literature[†]	Concentration (mg/100 g food) used by FSANZ for dietary intake calculation[#]
Bakery products		
Sponge cake	11.0	11.0
Corn-snack	47.0	47.0
Rice cracker	27.3	27.3
Cookie	26.7	76.5*
Brown sugar drop	76.5	76.5
Fried dough cake	95.6	95.6
Chocolate-chip cookie	6.4	76.5*
Cereal	2.2	2.2
Dishes		
Fish broiled with soy	39.1	39.1
Simmered dishes of dried radish strips	8.1	8.1
Fermented soybeans	7.8	7.8
Seasonings		
Caramel sauce	83.0	83.0
Brown sugar	71.1	71.1
Meat sauce	15.8	15.8
Demiglace	16.3	130.6*
Maple syrup	57.9	57.9
Ketchup	39.8	130.6*
Worcester sauce	130.6	130.6
Beverages		
Coke	38.3	38.3
Coffee	0.5	0.5
Fruit juice	21.5	21.5
Tomato juice	2.4	2.4
Fruits		
Dried fig	29.6	38.7*
Dried kiwi fruit	9.4	38.7*
Raisin	38.7	38.7
Canned peaches	1.5	8.4*
Can of mandarin oranges	8.4	8.4
Canned cherries	2.0	8.4*

[#]All the values (mg/100 g food) were converted to g of D-allulose in kg of food (g/kg) before calculating dietary intake.

[*]Higher concentration level was used when similar foods were proposed to contain different concentrations to represent the worst case scenario.

[†] Oshima et al. (2006).

Table A1.2 Estimated chronic dietary intake of D-allulose from naturally occurring sources

Country	Age group	% Proportion of consumers to respondents	Estimated dietary intake of the naturally occurring D-allulose†			
			g/day		g/kg bw/day	
			Mean	P90	Mean	P90
Australia*	2 years and above	100	0.2	0.3	0.003	0.006
New Zealand#	5-14 years	99.9	0.2	0.4	0.005	0.011
	15 years and above	99.7	0.2	0.4	0.002	0.005

*Based on food consumption data from Day 1 and 2. Average of intakes across the two days presented.
#Based on food consumption data from Day 1 only.
†All population intakes were calculated for consumers only.

Appendix 2. Additional results related to dietary intake assessment of added D-allulose

Table A2.1 Estimated high consumption (P97.5) of foods that are proposed to contain added D-allulose, for use in the short-term dietary intake assessment*

Harvest food classification code	Harvest food classification name	Australia 2 years and above		New Zealand 5-14 years		New Zealand 15 years and above	
		% consumers	Consumption (g/kg bw/day)	% consumers	Consumption (g/kg bw/day)	% consumers	Consumption (g/kg bw/day)
1.5.3	Dried yoghurt powder	NC	NC	NC	NC	NR	NR
4.3.4.2	Low joule chutneys, jams & spreads	2.6	0.8	NR	NR	NR	NR
5.2.1	Bubble gum & chewing gum	<1	0.3	3.6	1.3	NR	NR
5.2	Sugar confectionery**	11.0	1.7	37.6	4.8	17.7	2.2
5.2.4.1	Sugar confectionery, confectionery, intense sweetened	NR	NR	NR	NR	NR	NR
5.4	Icings & frostings	10.1	1.3	11.1	2.5	7.5	0.6
6.3.2, 6.3.4, 20.2.2.5	Breakfast cereals, ready to eat**	29.0	2.9	3.7	34.6	29.8	2.2
6.3.2	Breakfast biscuits & flakes	17.7	2.4	30.8	3.0	18.2	1.5
6.3.4	Breakfast cereals, unspecified form	<1	3.8	NR	NR	NR	NR
7.1.4	Fancy breads	1.5	4.4	4.1	7.0	2.6	3.1
7.1.6	Bread and related products, wheat base, unspecified type	14.7	3.7	19.0	5.1	17.9	2.7
7.2, 7.2.1, 7.2.2.1, 7.2.2.2.1, 7.2.3, 20.2.3.1.4, 20.2.3.1.7	Biscuits, cakes and pastries**	48.7	4.8	73.0	7.2	54.6	3.6
7.2	Biscuits, crackers, cakes, pastries & scones	<1	3.4	1.3	4.1	3.4	3.7
7.2.1	Biscuits & crackers	34.2	2.7	56.6	3.3	34.7	1.3
7.2.2.1	Cakes	6.6	7.0	9.5	7.2	9.6	2.7
7.2.2.2.1	Muffins, sweet	1.9	7.6	5.5	9.3	4.6	3.3
7.2.3	Pastries	12.6	3.4	18.4	3.5	14.1	2.3
11.4	Tabletop sweeteners	5.5	0.1	NR	NR	3.4	0.1

Harvest food classification code	Harvest food classification name	Australia 2 years and above		New Zealand 5-14 years		New Zealand 15 years and above	
		% consumers	Consumption (g/kg bw/day)	% consumers	Consumption (g/kg bw/day)	% consumers	Consumption (g/kg bw/day)
14.1.3.1.1.1.2, 14.1.3.1.1.1.2.1, 14.1.3.1.1.2.1, 14.1.3.1.1.2.2, 14.1.3.1.1.2.3, 14.1.3.1.1.3.1, 14.1.3.1.3.2.1	Water based flavoured drinks**	17.7	20.7	20.4	27.4	15.9	17.7
14.1.3.1.1.1.2	Non-brewed soft drink, carbonated, cola, intense sweetened	6.7	20.7	2.0	27.4	3.9	16.9
14.1.3.1.1.1.2.1	Non-brewed soft drink, carbonated, cola, intense sweetened, decaffeinated	<1	19.2	NC	NC	NR	NR
14.1.3.1.1.2.1	Non-brewed soft drink, carbonated, not cola type, intense sweetened	<1	17.7	NR	NR	1.2	9.6
14.1.3.1.1.2.2	Non-brewed soft drink, carbonated, not cola type, sugar sweetened	9.3	19.5	16.8	26.5	9.7	17.2
14.1.3.1.1.2.3	Non-brewed soft drink, carbonated, not cola type, unspecified sweetener	NC	NC	NR	NR	1.0	48.1
14.1.3.1.1.3.1	Non-brewed soft drink, carbonated, energy, intense sweetened	NR	NR	NC	NC	NR	NR
14.1.3.1.3.1.1	Non-brewed soft drinks, cordial powders, intense sweetened	NC	NC	NR	NR	NR	NR
14.1.3.1.3.2.1	Non-brewed soft drinks, cordials, not powder, intense sweetened	<1	29.5	NR	NR	NR	NR
14.1.3.1.4, 20.1.3.1	Tea beverages (incl Chai)**	67.5	21.5	22.3	24.2	82.0	28.8
14.1.3.1.4	Non-brewed soft drinks, iced tea, sold as ready to drink	NC	NC	NR	NR	NR	NR
14.1.5.1.2.2, 14.1.5.1.3.2	Coffee beverages**	2.8	8.6	NR	NR	1.9	57.3
14.1.5.1.2.2	Coffee beverage, decaffeinated, instant powder/granules	2.8	8.6	NR	NR	1.6	57.3
14.1.5.1.3.2	Coffee beverage, unspecified caffeine content, instant powder/granules	NC	NC	NR	NR	NR	NR
20.1.3.1	Beverages, non-alcoholic, tea-based	2.2	16.5	NC	NC	NR	NR
20.2.1.3.1	Desserts, dairy, ice cream and ice confection	3.6	5.1	13.6	9.5	7.1	4.5

Harvest food classification code	Harvest food classification name	Australia 2 years and above		New Zealand 5-14 years		New Zealand 15 years and above	
		% consumers	Consumption (g/kg bw/day)	% consumers	Consumption (g/kg bw/day)	% consumers	Consumption (g/kg bw/day)
20.2.1.3.5	Desserts, dairy, yoghurt	<1	10.1	NR	NR	NR	NR
20.2.1.4.1	Desserts, non-dairy, jelly	1.2	9.2	2.6	12.8	1.2	7.6
20.2.1.4.1.2.1	Desserts, non-dairy, jelly, dry mix, intense sweetened	NR	NR	NC	NC	NC	NC
20.2.1.4.1.2.2	Desserts, non-dairy, jelly, dry mix, sugar sweetened	NR	NR	NC	NC	NR	NR
20.2.1.4.3	Desserts, non-dairy, puddings and dumplings	<1	4.2	NR	NR	1.1	11.0
20.2.1.4.7.1	Cream, imitation	NR	NR	NC	NC	NC	NC
20.2.2.3	Cereal products, bars	6.2	2.1	18.9	3.0	5.2	1.8
20.2.2.5	Breakfast cereals, ready to eat	11.7	3.2	4.3	7.7	12.9	2.7
20.2.2.6	Breakfast cereals, porridge	<1	11.6	NR	NR	1.0	4.6
20.2.3.1.4	Bakery products, sweet, pies and pastries	2.4	4.4	1.9	29.5	2.7	4.9
20.2.3.1.7	Bakery products, sweet, doughnuts	<1	5.7	1.9	10.2	<1	4.8
20.2.6.1	Sauces and syrups, sweet**	3.1	1.9	6.1	2.5	7.1	1.3
20.2.7.2	Salad dressings	5.1	0.8	3.9	1.5	12.9	0.8

* Based on food consumption data for Australia from the pooled dataset of Day 1 and Day 2 for 7735 respondents and Day 1 only for New Zealand. All intakes were calculated for consumers only.

NC- no consumption has been reported in the survey.

NR - not reported due to too few consumers to ensure a reliable 97.5 percentile consumption value.

** Categories where consumption was derived for either a combination of similar food categories, or a higher level/major food group, for use in the determination of the highest use level that would result in the laxative effect level not being exceeded.

Table A2.2 A summary of major contributing (>5%) food categories to chronic dietary intake of added D-allulose

Country	Age group	Food category (as proposed by the applicant)	Harvest classification		% Contribution to estimated dietary intake [#]
			Harvest classification Code	Harvest classification Name	
Australia	2 years and above	Beverages (water based, non-alcoholic); low- and reduced energy, low- and reduced sugar	14.1.3.1.1.1.2	Non-brewed soft drink, carbonated, cola, intense sweetened	14
			14.1.3.1.1.2.2	Non-brewed soft drink, carbonated, not cola type, sugar sweetened	16
		Bakery products, reduced energy	7.1.6	Bread and related products, wheat base, unspecified type	10
			7.2.1	Biscuits & crackers	12
			7.2.2.1	Cakes	7
			7.2.3	Pastries	7
New Zealand	5-14 years	Beverages (water based, non-alcoholic); low- and reduced energy, low- and reduced sugar	14.1.3.1.1.2.2	Non-brewed soft drink, carbonated, not cola type, sugar sweetened	19
		Bakery products, reduced energy	7.1.6	Bread and related products, wheat base, unspecified type	7
			7.2.1	Biscuits & crackers	17
			7.2.2.1	Cakes	5
			7.2.2.2.1	Muffins, sweet	6
			7.2.3	Pastries	8
		Frozen dairy desserts, low- and reduced- energy and low- and reduced sugar	20.2.1.3.1	Desserts, dairy, ice cream and ice confection	8
		Breakfast cereals and cereal based bars; regular	6.3.2	Breakfast biscuits & flakes	6
	15 years and above	Beverages (water based, non-alcoholic); low- and reduced energy, low- and reduced sugar	14.1.3.1.1.1.2	Non-brewed soft drink, carbonated, cola, intense sweetened	7
			14.1.3.1.1.2.2	Non-brewed soft drink, carbonated, not cola type, sugar sweetened	15
		Bakery products, reduced energy	7.1.6	Bread and related products, wheat base, unspecified type	9
			7.2.1	Biscuits & crackers	10
			7.2.2.1	Cakes	6
			7.2.2.2.1	Muffins, sweet	5
			7.2.3	Pastries	9

[#]Based on g/day food consumption data from Day 1 only for all the surveys.

Appendix 3. Summary of long-term human trials for D-allulose

Table A3.1 Long-term (≥8 weeks) human trials for D-allulose consumption

Subjects	Study summary	Inclusion criteria	Exclusion criteria	Results and reported study limitations	Reference
21 healthy male and female volunteers in four studies	Eight subjects Study 4: 5g of D-allulose x 3 times/d for 8 weeks	Healthy	(1) Treatment for diabetes (2) Notable systemic disease (3) Hepatic or renal function disorders (4) Proven ineligible by a physician Study 3: subjects susceptible to diarrhoea excluded	Limitations: none reported No evidence of microbiological concerns.	Iida et al. (2010)
Age range 20-40 years BMI ≥ 23 kg/m^2	Placebo control low D-allulose (4g x 2 times/day) and high D-allulose (7g x 2 times/day) groups 6 x 24 hour food recalls 12 week study duration	Younger adults Resided in Daegu city region	(1) Hypertensive taking diuretics (2) Taking oral hypoglycemic agents or insulin injection (3) Serious cardiac, renal, hepatic, thyroid, or cerebrovascular disease (4) Serious cystic or gastrointestinal disease, gout, or porphyria (5) Psychiatric problems such as depressive disorder, schizophrenia, alcoholic, and drug intoxication (6) Taking functional food products that may affect the results of this study; (7) A history of surgery within the past 6 months (8) Cancer diagnosis and treatment (9) Asthma or other allergies (10) Pregnant or in lactation period	A total of 10 of 144 subjects dropped out due to personal reasons, 13 subjects were excluded due to low compliance Limitations: Small sample size Relatively young ages of participants No evidence of microbiological concerns.	Han et al. (2018a)

Subjects	Study summary	Inclusion criteria	Exclusion criteria	Results and reported study limitations	Reference
Maximum dose: 15 males and 15 females Age range 21 to 30 years Regular ingestion: 10 male and 9 female. Average age 23.84 years	Gradual increase to 1.0 g/kg bw 11 weeks	Healthy, normal range BMI based on Asian criteria, non-adapted to D-allulose consumption	(1) Hypertensive taking diuretic (2) Patient taking oral hypoglycemic agent or insulin injection (3) Serious cardiac, renal, hepatic, thyroid or cerebrovascular disease (4) Serious cystic or gastrointestinal disease, gout or porphyria (5) Psychiatric problems such as depressive disorder, schizophrenia, alcoholism, drug intoxication (6) Using functional food products that may affect the results of this study (7) A history of surgery within 6 months (8) Cancer diagnosis and treatment (9) Asthma or other allergy (10) A history of surgery within the past 6 months (11) Drinking frequently (12) Anticipated difficulties following the taking experimental material schedule (13) Pregnant or currently lactating	Symptoms include diarrhoea, nausea, abdominal distention and abdominal pain 29 subjects completed the maximum dose trial. One subject had incomplete reporting of symptoms. 18 subjects reported for the regular ingestion trial. One subject had incomplete reporting of symptoms. Limitations: (1) small sample size; (2) recruitment of young subjects with normal range BMI – symptoms may not be representative for individuals who are old, obese or with certain metabolic and GI diseases (specific diseases not identified); (3) duration of wash-out period and GI adaption Intake for study was a large quantity of D-allulose after fasting compared to typical consumption of smaller amounts during the day in foods such as pastries or confectionary. No evidence of microbiological concerns.	Han et al. (2018b)
18 normal subjects in treatment group	3 x 5 g D-allulose per day Total duration 18 weeks: 2 week observation pre-treatment, 12 week treatment and 4 week observation post-treatment	Normal adult men and women Fasting blood glucose level of <110 mg/dL	Not reported	One subject dropped-out. A single male in the treatment group had moderate symptoms after four weeks from the start of treatment: diarrhoea, borborygmus and increased defecation frequency. The relationship between the symptoms and treatment were unclear. Amongst all participants abdominal wind (62.5%, 5/8) and increased frequency of defecation (50%, 4/8) were the most common symptoms in the treatment group. Abdominal wind was the most commonly reported symptom in the Control group (55.6 %, 5/9). Limitations: none reported No evidence of microbiological concerns.	Hayashi et al. (2010)

Subjects	Study summary	Inclusion criteria	Exclusion criteria	Results and reported study limitations	Reference
90 adults including border-line diabetics Adult men and women aged 20 to 65 years Average age for each of the three groups was ca. 50 years	High-dose (15g D-allulose/day) Low dose (5 g D-allulose/day) Placebo Daily test beverage 52 weeks with 48 weeks of consumption with a 4 week observation period after consumption	Fasting blood glucose concentration of 100-125 mg/dL	(1) undergoing medical treatment by drugs (2) daily intake of dietary supplements related to blood glucose or lipids (3) pregnancy, breastfeeding, or expected pregnancy (4) subjects who had blood collected (> 200 mL; total blood or directed donation) within 4 weeks prior to starting the examination (5) men who had blood collected (> 400 mL) within 12 weeks prior to starting the examination (6) women who had blood collected (> 400 mL) within 16 weeks prior to starting the examination (7) men who had more than 1200 mL of blood collected within 12 months prior to starting the examination when the planned blood sampling amount of this study was added (8) women who had more than 800 mL of blood collected within 12 months prior to starting the examination when planned blood sampling amount of this study was added (9) concurrent participation in other clinical studies or within four weeks of finishing another study (10) heavy alcohol use of more than 60 g/day or heavy smoker beyond 21 cigarettes/day (11) individuals with extremely random dietary habits, rotating shift workers, midnight workers (12) serious damage to the liver, the kidney, the heart (13) subjects with a history of circulatory diseases (14) diabetic patients (15) allergy to the test substance (16) anyone judged as ineligible to participate in this study by the principal physician.	Trial dropouts: Placebo: 1 (osteoporosis) Low-dose D-allulose: 2 (anaphylactic shock and diabetes mellitus) High-dose D-allulose: 2 (atrophic gastritis and rheumatoid arthritis) All drop-outs were deemed to be unrelated to the D-allulose consumption Adverse events: Placebo: 42 Low dose D-allulose: 34 High dose D-allulose: 38 No details of adverse events were provided. All adverse events were deemed to be unrelated to the D-allulose consumption Limitations: none reported No evidence of microbiological concerns based on trial dropout reasons.	Tanaka et al. (2020)