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Supporting document

Risk and technical assessment – Application A1345

Dextransucrase from *Bacillus subtilis* (gene donor: *Streptococcus salivarius*) for use as a processing aid

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Executive summary

Danisco Australia Pty Ltd has applied to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of the enzyme dextransucrase (EC 2.4.1.5), from *Bacillus subtilis* containing the gene for dextransucrase from *Streptococcus salivarius* as a processing aid. The enzyme is proposed for use in the production of oligosaccharides and polysaccharides to reduce sucrose content and improve texture in foods containing sucrose.

The available evidence provides adequate assurance that the proposed use of dextransucrase from *B. subtilis* as a processing aid is technologically justified. Dextransucrase performs its technological function during food processing and, as such, meets the definition of a processing aid for the purposes of the Code. There are relevant identity and purity specifications for the enzyme in the Code, and the applicant provided evidence that their enzyme preparation meets these specifications.

No public health or safety concerns were identified with the use of the production organism, which is neither pathogenic nor toxigenic. Analysis of the modified production strain confirmed the presence and stability of the inserted DNA. No significant sequence homology between the enzyme and any known toxins or allergens was identified.

Glucose derived from wheat is used in the fermentation process to produce dextransucrase and therefore wheat may be present in the final enzyme preparation.

There was no evidence of genotoxicity *in vitro*. The no observed adverse effect level (NOAEL) in a 90-day oral gavage study in rats was 1000 mg total organic solids (TOS)/kg bw/day.

The theoretical maximum daily intake (TMDI) of this dextransucrase was calculated to be 3.58 mg TOS/kg bw/day. A comparison of the NOAEL and the TMDI results in a margin of exposure (MOE) of approximately 300. Based on the reviewed data it is concluded that in the absence of any identifiable hazard an Acceptable Daily Intake (ADI) ‘not specified’ is appropriate.

FSANZ concludes there are no safety concerns from the use of this dextransucrase from *B. subtilis* in the quantity and form required to perform its typical function in the production of oligosaccharides and polysaccharides to reduce sucrose content and improve texture in foods containing sucrose, which must be consistent with Good Manufacturing Practice.

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1. Introduction

Danisco Australia Pty Ltd[1] has submitted an application to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of dextransucrase (EC 2.4.1.5). This enzyme is produced by *Bacillus subtilis* expressing the gene for dextransucrase from *Streptococcus salivarius*.

The enzyme is proposed for use as a processing aid in the production of oligosaccharides and polysaccharides to reduce sucrose content and improve texture in foods containing sucrose. It will be utilised at the lowest effective level necessary to achieve the intended technological purpose, in accordance with Good Manufacturing Practice (GMP).[2]

1.1 Objectives of the assessment

The objectives of this risk and technical assessment were to:

- determine whether the proposed purpose is solely technological, and that the enzyme achieves its technological purpose as a processing aid in the quantity and form proposed to be used;
- evaluate potential public health and safety concerns that may arise from the use of this food enzyme by considering the:
    - safety and history of use of the host and donor organisms
    - characterisation of the genetic modification(s) to the production strain
    - safety of the enzyme.

2 Food technology assessment

2.1 Identity of the enzyme

The applicant provided information regarding the identity of the enzyme, which has been verified using the IUBMB[3] enzyme nomenclature reference database (McDonald et al. 2009). Details of the identity of the enzyme are provided below.

Accepted IUBMB name:	dextransucrase
Systematic name:	sucrose:(1→6)-α-D-glucan 6-α-D-glucosyltransferase
Other names/common names:	sucrose 6-glucosyltransferase; SGE; CEP; sucrose-1,6-α-glucan glucosyltransferase; sucrose:1,6-α-D-glucan 6-α-D-glucosyltransferase
IUBMB enzyme nomenclature:	EC 2.4.1.5
CAS number:	9032-14-8

[1] A subsidiary of International Flavors and Fragrances Inc

[2] GMP is defined in section 1.1.2—2 of the Code as follows: ***GMP*** *or* ***Good Manufacturing Practice****, with respect to the addition of substances used as food additives and substances used as processing aids to food, means the practice of:*
(a) limiting the amount of substance that is added to food to the lowest possible level necessary to accomplish its desired effect; and
(b) to the extent reasonably possible, reducing the amount of the substance or its derivatives that:
*(i) remains as a *component of the food as a result of its use in the manufacture, processing or packaging; and*
(ii) is not intended to accomplish any physical or other technical effect in the food itself;
(c) preparing and handling the substance in the same way as a food ingredient.

[3] International Union of Biochemistry and Molecular Biology.

Reaction: dextransucrase catalyses the below chemical reaction (see Figure 1)

sucrose + $[(1\rightarrow6)\text{-}\alpha\text{-D-glucosyl}]_n$ = D-fructose + $[(1\rightarrow6)\text{-}\alpha\text{-D-glucosyl}]_{n+1}$

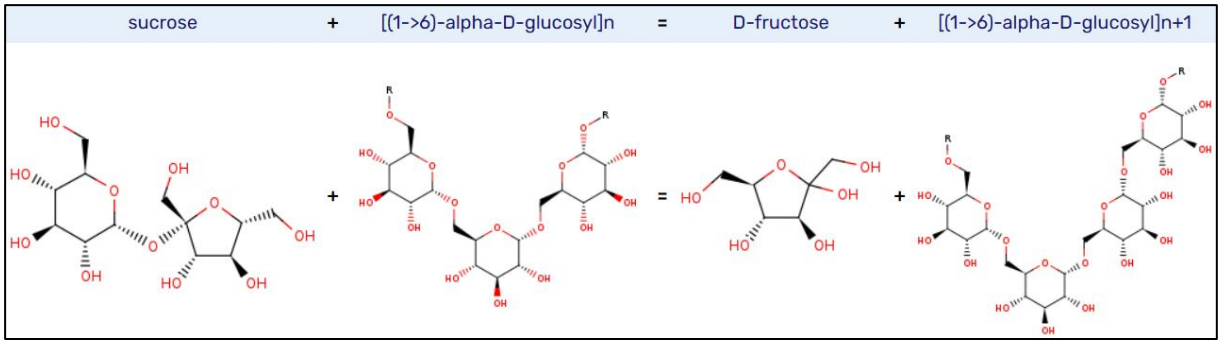

Figure 1: Reaction catalysed by dextransucrase (source: BRENDA)

2.2 Manufacturing process

2.2.1 Production of the enzyme

Enzymes from microorganisms are typically produced by controlled fermentation followed by removal of the production microorganism, purification, and concentration of the enzyme. Final standardisation with stabilisers, preservatives, carriers, diluents, and other approved food-grade additives and ingredients is carried out after the purification and concentration steps.

The formulated enzymes are referred to as enzyme preparations, which, depending upon the application in food, may be a liquid, semi-liquid or dried product. Enzyme preparations may contain either one major active enzyme that catalyses a specific reaction during food processing or two or more active enzymes that catalyse different reactions (FAO/WHO 2020).

Dextransucrase is produced by submerged fermentation of a *B. subtilis* strain carrying the gene for dextransucrase from *S. salivarius*. The batch or fed-batch fermentation process includes inoculating pure culture into fermentation media, followed by seed and main fermentation stages. Once fermentation is complete, the enzyme-containing media is separated from biomass and cell remnants, concentrated, and filtered. Both a polish and germ filtration step are utilised, to remove fine suspended particles and microbial particles, respectively. The resulting product is then formulated as a liquid enzyme preparation.

The typical composition of the enzyme preparation is shown in Table 1.

Table 1: Typical composition of dextransucrase preparation

Component	Approximate % w/w
Dextransucrase enzyme	1-5
Glycerine	46-67
Water	26-51
Calcium chloride	0-0.1

The applicant has stated the enzyme is manufactured with appropriate GMP controls and processes to ensure compliance with relevant specifications and that the final product does not contain hazardous impurities. The resultant product meets the general specifications for enzyme preparations used in food processing as established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC) (see also section 2.2.2 of this report).

Information on the raw materials used in the production and recovery of the enzyme preparation has been evaluated by FSANZ but deemed confidential commercial information (CCI) under section 114 of the *Food Standards Australia New Zealand Act 1991* (the FSANZ Act) and cannot be disclosed in this report.

2.2.2 Specifications for identity and purity

There are international general specifications for enzyme preparations used in the production of food, established by JECFA in its Compendium of Food Additive Specifications and in the FCC (13th edition), referenced in section S3—2 of Schedule 3 of the Code. Enzymes used as processing aids need to meet either of these specifications, or a relevant specification in section S3—3 of Schedule 3.

Schedule 3 of the Code includes specifications for arsenic and heavy metals (section S3—4) if they are not already detailed within specifications in sections S3—2 or S3—3.

The applicant provided certificates of analysis for three independent batches of the dextransucrase enzyme (section A.5 and Annex 5 of the application). Table 2 provides a comparison of the summary results of those analyses with the international specifications established by JECFA and the FCC, as well as those in the Code. Based on those results, the enzyme met all relevant specifications.

In addition, the specification for identity and purity of the enzyme product provided by the applicant indicates an absence of the production strain.

Table 2: Analysis of three representative standardised batches of enzyme compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes

Test parameters	*Test results*	*Specifications*		
		JECFA	*Food Chemicals Codex*	*The Code – section S3—4*
Lead (mg/kg)	0.04, 0.02, 0.04	≤5	≤5	≤2
Arsenic (mg/kg)	0.01, 0.01, <0.01	-	-	≤1
Cadmium (mg/kg)	<0.001	-	-	≤1

Test parameters	*Test results*	*Specifications*		
		JECFA	*Food Chemicals Codex*	*The Code – section S3—4*
Mercury (mg/kg)	<0.005	-	-	≤1
Coliforms (cfu/g)	<1	≤30	≤30	-
***Salmonella* (in 25 g)**	Negative	Absent	Negative	-
***Escherichia coli* (in 25 g)**	Negative	Absent	-	-
Antimicrobial activity	Negative	Absent	-	-

cfu = colony forming units

2.3 Technological purpose

The dextransucrase enzyme preparation is intended for use as a processing aid during the production of foods that contain sucrose. The applicant has provided the following as examples of sucrose-containing foods in which the enzyme could be used: dairy products, ice cream, processed fruits and vegetables, confectionary, cereal products, bread/bakery products, honey, and beverages. The applicant requested use of the enzyme at GMP levels, with typical use levels up to 3 mg of enzyme per gram of sucrose in the food.

The benefits of dextransucrase, as stated in the application, include:

- in-situ production of oligosaccharides (and polysaccharides)
- improved texture of food products
- reduction of sucrose content in food.

Dextransucrase facilitates the synthesis of glucose polymers (oligosaccharides and polysaccharides) by catalysing the hydrolysis of sucrose, a disaccharide composed of glucose and fructose, and the subsequent transfer of glucose onto a growing polymer chain (Schmid et al. 2019). In food processing, use of the enzyme reduces the sucrose content of the food because sucrose is hydrolysed during the enzymatic reaction (Nguyen et al. 2015).

The technological purpose stated by the applicant is consistent with the typical function of dextransucrase. This is supported by scientific literature, which verify the enzyme function (Monchois et al. 1999) and demonstrate the contribution of the enzyme to improved texture in various food products, including bread products with increased softness (Kajala et al. 2015) and dairy and plant-based milk products with enhanced viscosity (Tingirikari et al. 2014; Huang et al. 2025).

Dextransucrase performs its primary function during food manufacturing and does not perform a technological purpose in the food for sale, thus meeting the definition of a processing aid. The applicant provided data (section A.3 of the application) to highlight that the enzyme is inactivated by a UHT pasteurisation process or else activity is self-limited based on the availability of the substrate (sucrose). Information on the physical and chemical properties of the enzyme, as provided by the applicant, are summarised in Table 3.

Table 3: Dextransucrase enzyme preparation physical and chemical properties

Physical/chemical properties of commercial enzyme preparation	
Enzyme activity	85 GFTU[a]/g
Appearance	Liquid
Temperature optimum	Optimum activity within range 35–50°C
Thermostability	Enzyme activity starts to decrease when exposed to temperatures above 50°C with minimal activity at 55°C
pH range and optimum	Optimum pH within range 6.5–8.0

[a]GFTU = enzyme activity units, where it is understood that one GFTU is the amount of enzyme that hydrolyses 1 micromole of sucrose per minute under specified conditions (temperature, pH, substrate type). The enzyme activity of 85 GFTU/g is a representative minimum activity value and will differ based on the desired final product.

2.4 Allergen considerations

The applicant has provided an allergen statement for the enzyme preparation that is supplied to customers (Annex 6 of the application). Liquid glucose derived from wheat is used as a fermentation ingredient.

2.5 Food technology conclusion

The use of dextransucrase as a processing aid to produce glucose oligosaccharides and polysaccharides in the manufacture of sucrose-containing foods is consistent with the typical function of the enzyme. The reported advantages encompass enhanced food product texture as well as a reduction in sucrose content. The evidence presented to support its proposed use provides adequate assurance that the use of the enzyme, in the quantity and form proposed to be used (which must be consistent with GMP), is technologically justified.

Dextransucrase fulfils its primary function during the manufacture of food products and does not perform a technological purpose in the final food. It therefore functions as a processing aid for the purposes of the Code.

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

3 Safety assessment

The objective of this safety assessment is to evaluate any potential public health and safety concerns associated with the use of this dextransucrase enzyme as a processing aid.

Some information relevant to this section is CCI under section 114 of the FSANZ Act. This information has been evaluated by FSANZ but cannot be disclosed in this public report.

3.1 Source microorganism

B. subtilis is an aerobic, endospore forming, non-pathogenic, gram-positive bacterium widely distributed in soil, water sources, plants, air, animals, and food (Amuguni and Tzipori 2012, Earl et al. 2008, Su et al. 2020). The use of *B. subtilis* in food production can be traced back thousands of years to the fermentation of natto in Japan (Schallmey et al. 2004, Stulke et al. 2023). In recent decades *B. subtilis* has been used in a wide range of industries including food, feed, cosmetics, chemicals, and pharmaceuticals (Kaspar et al. 2019, Su et al. 2020).

FSANZ has previously assessed the safety of *B. subtilis* as the production organism for several enzyme processing aids. Schedule 18 of the Food Standards Code currently permits the following enzymes derived from *B. subtilis*: α-Acetolactate decarboxylase, α-amylase, β-amylase, β-galactosidase, aqualysin 1, asparaginase, endo-1,4-beta-xylanase, β-glucanase, hemicellulase multicomponent enzyme, maltogenic α-amylase, metalloproteinase, pullulanase, serine proteinase and subtilisin.

Internationally, *B. subtilis* has been assessed by multiple food regulatory agencies. The JECFA considers *B. subtilis* to be non-pathogenic and non-toxigenic, with a long history of safe use in industrial applications (Li 2024). Numerous enzyme products of *B. subtilis* have been granted the status of generally recognised as safe (GRAS) by the United States Food and Drug administration (U.S. FDA). Additionally, *B. subtilis* has been granted Qualified Presumption of Safety (QPS) status by the European Food Safety Authority (EFSA), with the qualification that there is an absence of toxigenic activity and acquired antimicrobial resistance genes (EFSA BIOHAZ Panel 2026).

The production strain in this application was generated by inserting a dextransucrase gene from *S. salivarius* into *B. subtilis* (See section 3.2 Characterisation of the genetic modification to the production strain for more information). CCI provided by the applicant confirms the production organism's identity as *B. subtilis.* Independent batch analysis for three samples of the final product, confirmed the absence of viable *B. subtilis* cells in all samples examined.

Additionally, the applicant applied the safe strain lineage concept of Pariza and Johnson (2001) to confirm the absence of toxigenic activity in the production organism. A safe strain lineage claim is supported by thorough characterisation of the host organism, well defined DNA inputs and modification techniques appropriate for food use. Using this concept, the information provided by the applicant showed that the risk of toxin production in the *B. subtilis* production strain is considered very low.

FSANZ has identified no microbiological safety concerns associated with the use of *B. subtilis* as a production organism for dextransucrase.

3.2 Characterisation of the genetic modification to the production organism

3.2.1 Description of the DNA to be introduced and the method of transformation

The gene encoding the dextransucrase enzyme was synthesised *in vitro* based on the native DNA sequence from *S. salivarius* available in public databases. Data provided by Danisco and analysed by FSANZ confirmed the identity of the dextransucrase enzyme.

A vector containing the dextransucrase expression cassette was integrated into the *B. subtilis* chromosome using standard molecular biology techniques. The dextransucrase gene was placed under the control of a promoter and terminator derived from *B. subtilis*. A native *B. subtilis* gene was used as a selectable marker to identify positive transformants.

3.2.2 Characterisation of the inserted DNA

Data provided by Danisco confirmed the presence of the inserted DNA in the genome of the production strain. No antibiotic-resistance markers are present in the final production strain.

3.2.3 Stability of the introduced DNA

The stability of the introduced DNA in the production strain was examined by genome sequencing. DNA extracted from cultures after prolonged fermentation and stock culture prior

to fermentation as a control were analysed. This data substantiates the stability of the dextransucrase gene in the genome of the production strain.

3.3 Safety of the enzyme

3.3.1 History of safe use

The enzyme that is the subject of this application has been approved for use in several countries. Letters of approval were provided on a confidential basis. Sales data were not available at the time of assessment but are not considered to be necessary because results of toxicology studies are provided.

3.3.2 Bioinformatic assessment of homology with known toxins

A BLAST search for homology of the enzyme against the complete UniProt[4] database (as of February 2026) was performed with a threshold E-value of 0.1. No matches reached the 35% sequence identity threshold considered significant (Negi et al. 2017). An additional BLAST search was performed against the UniProt animal toxin database. No matches were found.

3.3.3 Toxicology data

Animal studies

A 90-day repeat-dose oral gavage study of the dextransucrase was conducted in Sprague Dawley rats. The study is summarized in detail in Appendix 1. No treatment-related effects were observed, and the No Observed Adverse Effect Level (NOAEL) in both sexes was the highest dose tested, 1000 mg TOS/kg bw/day.

Genotoxicity assays

A bacterial reverse mutation assay (Ames test) and a chromosomal aberration assay were conducted and are summarized in Appendix 1. The results of both assays were negative.

3.3.4 Potential for allergenicity

The amino acid sequence of the dextransucrase was compared to known allergens in the Research and Resource Program (FARRP) AllergenOnline database[5] (version 23, January 2025) using the default settings. Search strategies included full sequence alignment, a sliding 80-amino acid search and an eight contiguous amino acid search. No matches were found using any of the search strategies.

Glucose used during fermentation of the production organism is derived from wheat.

3.3.5 Assessments by other regulatory agencies

No assessment reports by other national or supranational regulatory agencies are available for this dextransucrase.

4 Dietary exposure assessment

The objective of the dietary exposure assessment was to review the deterministic calculation

[4] UniProt database - https://www.uniprot.org

[5] Research and Resource Program (FARRP) AllergenOnline database - http://www.allergenonline.org

provided by the applicant as a 'worst case scenario' approach to estimating likely levels of dietary exposure, assuming that all the total organic solids (TOS) from the dextransucrase enzyme preparation remained in the food. The estimate of dietary exposure to the TOS can then be compared to an ADI or a NOAEL to estimate a margin of exposure (MOE) for risk characterisation purposes.

In their calculation, the applicant made the following assumptions:

- on average, Australians consume 66.4 grams of sugar daily (per capita consumption of free sugars based on sales of food and beverages)[6] (ABS 2023-24)
- 100% of daily sugar consumption is sucrose
- the highest intended use level in food ingredients and food products containing sucrose (3 mg active enzyme protein/g sucrose)
- 10% of food producers (market share) would use dextransucrase
- the average population body weight is 60 kg
- all the TOS from the enzyme preparation remains in the final food.

Based on these assumptions, the applicant calculated the theoretical maximum daily intake (TMDI) of the TOS from the enzyme preparation to be 0.64 mg TOS/kg body weight/day.

As assumptions made by the applicant differ from those that FSANZ would have made, FSANZ independently calculated the TMDI using the following inputs and assumptions that are conservative and reflective of a first tier in estimating dietary exposure:

- on average, in the most recent national nutrition survey, Australians reported consuming 43.4 grams free sugars daily (ABS 2025)
- 100% of food producers (market share) would use dextransucrase
- the average body weight of the Australian population aged 2 years and above is 70kg (ABS 2015).

All other inputs and assumptions used by FSANZ remained as per those used by the applicant. The TMDI of the TOS from the enzyme preparation based on FSANZ deterministic calculations is 3.58 mg TOS/kg bw/day.

Both the FSANZ and applicant's estimates of the dietary exposure to the TOS will be overestimates of the dietary exposure given the conservatisms in the calculations. This includes that it was assumed that all the TOS from the enzyme preparation remains in the final foods and beverages whereas the applicant has stated that it is likely to either be deactivated or removed during processing or would be present in insignificant quantities. In the rare case that inactive dextransucrase enzyme is present in the processed food and is ingested, it will not be absorbed intact. Instead, the enzyme is broken down by the digestive system into small peptides and amino acids, with the latter being absorbed and metabolised, which poses no human health risk.

5 Discussion

The use of dextransucrase as a processing aid to produce glucose oligosaccharides and polysaccharides in the manufacture of sucrose-containing foods is consistent with the typical function of the enzyme. The reported advantages encompass enhanced food product texture as well as a reduction in sucrose content. The evidence presented to support its proposed use provides adequate assurance that the use of the enzyme, in the quantity and form proposed to be used (which must be consistent with GMP), is technologically justified.

[6] Free sugars include all added sugars (sugars that have been added to foods during their processing or preparation) as well as the sugar that is naturally present in juice and honey.

Dextransucrase fulfils its primary function during the manufacture of food products and does not perform a technological purpose in the final food. It therefore functions as a processing aid for the purposes of the Code.

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

No public health or safety concerns were identified concerning the use of the production organism, which is neither pathogenic nor toxigenic. Analysis of the production strain confirmed the presence and stability of the inserted DNA. No significant sequence homology between the enzyme and any known toxins or allergens was identified.

Glucose derived from wheat is used in the fermentation process to produce dextransucrase and therefore wheat may be present in the final enzyme preparation.

There was no evidence of genotoxicity *in vitro*. A no observed adverse effect level (NOAEL) of 1000 mg TOS/kg bw/day was identified in a 90-day oral toxicity study in rats. The MOE was calculated by FSANZ by comparison of the NOAEL and the TOS and was calculated as approximately 300.

Based on the reviewed data it is concluded that in the absence of any identifiable hazard an Acceptable Daily Intake (ADI) ‘not specified’ is appropriate.

FSANZ concludes there are no safety concerns from the use of this dextransucrase from *B. subtilis* in the quantity and form required to perform its typical function in the production of oligosaccharides and polysaccharides to reduce sucrose content and improve texture in foods containing sucrose, which must be consistent with GMP.

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7 Appendix 1: Toxicology studies

Ninety-day oral toxicity study in Sprague Dawley rats. Adgyl Life Sciences Ltd, 2023. Regulatory status: GLP, in accordance with OECD Test Guideline 408 (June 2018).

The test article for this study was the applicant's dextransucrase from a single batch, with a purity of 75% and a TOS content of 12.12%. Deionised water was used as the vehicle and the negative control article. Rats, 10/sex/group, were housed in same-sex pairs under standard laboratory conditions and were 6 to 7 weeks old at study start, following 5 days of acclimation. Feed and water were provided *ad libitum*. Rats were dosed daily by oral gavage at 0, 250, 500 or 1000 mg TOS/kg bw/day at a constant dose volume per kg bw. Dose formulations were used within 48 hours, based on prior stability data. The highest dose of 1000 mg TOS/kg bw represented the undiluted test article as received. Concentrations of dose formulations were confirmed monthly during the in-life phase.

Observations and measurements recorded during the in-life phase included twice-daily morbidity/moribundity checks, daily cageside observations, weekly detailed observations, weekly bodyweights, and weekly feed consumption. Ophthalmological examinations were carried out prestudy and near the end of the in-life phase. A detailed functional observational battery was carried out in the final week of the in-life phase. Prior to scheduled necropsy, vaginal smears were taken from all females for determination of oestrous cycle, and blood and urine were collected from all rats. Blood was analysed for haematology, coagulation parameters, clinical chemistry, and measurement of thyroid function parameters. Urine was used for urinalysis. Terminal bodyweights were recorded before rats were killed and subject to detailed necropsy. Fresh organ weights were recorded for a standard list of organs, and a comprehensive list of organs and tissues were processed for histopathology.

Dose analyses confirmed the suitability of the dose formulations for use. All rats survived to scheduled termination and no treatment-related effects were observed on any parameters measured or calculated. The NOAEL was the highest dose administered, 1000 mg TOS/kg bw/day.

Table 1: Genotoxicity studies of Dextransucrase

Test	**Test system**	**Enzyme Concentrations**	**Results**
Bacterial reverse mutation assay (conducted in compliance with OECD TG 471 (2020))	*Salmonella enteriditis* var. Typhimurium TA98, TA100, TA1535, TA1537, *Escherichia coli* WP2*uvr*A	Preliminary test: 50, 100, 200, 400, 800, 1600, 3200, 5000 µg TOS/plate Definitive test: 50, 158, 500, 1581, 5000 µg TOS/plate	Negative ± S9
In vitro mammalian micronucleus assay (compliant with OECD TG 487 (2016))	Human peripheral blood lymphocytes	312.5, 1250 and 5000 µg/mL	Negative ± S9