SCIENTIFIC OPINION

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Safety evaluation of the food enzyme dextranase from *Collariella gracilis* strain ATCC-16153

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Abstract

The food enzyme dextranase (6-α-D-glucan 6-glucanohydrolase, EC 3.2.1.11) is produced with the non-genetically modified *Collariella gracilis* strain ATCC-16153 by Mitsubishi-Kagaku Foods Corporation. The food enzyme is free from viable cells of the production organism. The food enzyme is intended to be used in sugar production and processing. As residual amounts of total organic solids (TOS) are removed during the production of refined sugars, dietary exposure was calculated only for unrefined sugar products. Based on the maximum use levels, dietary exposure to the food enzyme TOS was estimated to be up to 15 μg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not raise a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level (NOAEL) of 110 mg TOS/kg bw per day, the highest dose tested, which, when compared with the estimated dietary exposure, results in a sufficiently high margin of exposure (MoE) of at least 7,300. Similarity of the amino acid sequence of the food enzyme to those of known allergens was searched and no match was found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood is considered to be low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

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Keywords: food enzyme, dextranase, 6-α-D-glucan 6-glucanohydrolase, EC 3.2.1.11, dextran hydrolase, *Collariella gracilis*

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] provides definition for 'food enzyme' and 'food enzyme preparation'.

> 'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.
>
> 'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

- it does not pose a safety concern to the health of the consumer at the level of use proposed;
- there is a reasonable technological need;
- its use does not mislead the consumer.

All food enzymes currently on the European Union market and intended to remain on that market, as well as all new food enzymes, shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and approval via an EU Community list.

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) lays down the administrative, technical and toxicological data required.

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background as provided by the European Commission

Only food enzymes included in the European Union (EU) Community list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7(2) of Regulation (EC) No 1332/2008 on food enzymes.

Six applications have been introduced by the companies "Decernis, LLC", "Keller and Heckman LLP", the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) and "Novozyme A/S" for the authorisation of the food enzymes Cyclomaltodextrin glucanotransferase from *Geobacillus stearothermophilus*, Dextranase from *Chaetomium gracile*, Subtilisin from *Bacillus licheniformis*, Mucorpepsin from *Rhizomucor miehei*, Animal rennet consisting of chymosin and pepsin from the abomasum of *Bos primigenius* (cattle), *Bubalus bubalis* (buffalo), *Capra aegagrus hircus* (goat) and *Ovis aries* (sheep), and Lipase from a genetically modified strain of *Aspergillus niger* (strain NZYM-DB) respectively.

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the six applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

[1] Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, pp. 7–15.

[2] Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, pp. 1–6.

[3] Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.3.2011, pp. 15–24.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Cyclomaltodextrin glucanotransferase from *Geobacillus stearothermophilus*, Dextranase from *Chaetomium gracile*, Subtilisin from *Bacillus licheniformis*, Mucorpepsin from *Rhizomucor miehei*, Animal rennet consisting of chymosin and pepsin from the abomasum of *Bos primigenius* (cattle), *Bubalus bubalis* (buffalo), *Capra aegagrus hircus* (goat) and *Ovis aries* (sheep), and Lipase from a genetically modified strain of *Aspergillus niger* (strain NZYM-DB) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme Dextranase from *Collariella gracilis* (initially indicated as *Chaetomium gracile*) strain ATCC-16153.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme Dextranase from *Collariella gracilis* (strain ATCC-16153).

Additional information was requested from the applicant during the assessment process on 14 March 2019 and was consequently provided (see 'Documentation provided to EFSA').

Following the request for additional data sent by EFSA on 14 March 2019, the applicant requested a clarification teleconference which was held on 2 May 2019.

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009b) as well as in the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) and following the relevant existing guidance's of EFSA Scientific Committees.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance with the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature	Dextranase
Systematic name	6-α-D-glucan 6-glucanohydrolase
Synonyms	dextran hydrolase, dextranase DL 2, endodextranase
IUBMB no	EC 3.2.1.11
CAS no	9025-70-1
EINECS no	232-803-9

Dextranase catalyses the hydrolysis of 1,6 α-D glucosidic linkages in dextran. It is intended to be used in sugar production and processing.

3.1. Source of the food enzyme

The dextranase is produced with the filamentous fungus *Collariella gracilis* strain ATCC-16153 (formerly *Chaetomium gracile*) which is deposited at the Biological Resource Center of the National Institute of Technology and Evaluation (NBRC, Japan), with deposit number [redacted].[4]

[4] Technical dossier/Additional information June 2020/Annex 2.

The production strain was obtained from the reference strain of *C. gracilis* ATCC-16153. The strain identity was confirmed by sequence analysis of the Internal Transcribed Spacer (ITS) region showing 100% homology with ATCC-16153.[5]

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[6], with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with current Good Manufacturing Practice (GMP).[7]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which the enzyme protein is retained while most of the low molecular weight material passes the filtration membrane and is discarded. The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[8]

The Panel considered that sufficient information has been provided on the manufacturing process and the quality assurance system implemented by the applicant to exclude issues of concern.

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The dextranase is a single polypeptide chain of [redacted] amino acids.[9] The molecular mass of the protein, derived from the amino acid sequence, was calculated to be [redacted] kDa. The food enzyme preparation was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A consistent protein pattern was observed across all batches. The gels showed the target protein band corresponding to an apparent molecular mass of about [redacted] kDa.[10] The food enzyme preparation was tested for amylase, invertase, cellulase, glucanase, pectinase, peptidase and lipase activities. Only low levels of amylase, cellulase and peptidase activity were detected.[11] No other enzymatic activities were reported.

The in-house determination of dextranase activity is based on hydrolysis of the substrate dextran (reaction conditions: pH 5.0, 40°C, 10 min). The enzymatic activity is determined by measuring the amount of reducing sugar liberated from dextran. One unit (U) of enzyme activity is defined as the amount of enzyme, which releases reducing sugars equivalent to 1 μmol glucose in 1 min under the conditions of the assay.[12]

The food enzyme has a temperature optimum between 50°C and 65°C and a pH optimum between pH 4 and 7. The enzyme preparation is stable below 70°C in the presence of high sugar concentrations.[13]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch produced for the toxicological tests (Table 1).[14] The average total organic solids (TOS) of the three food enzyme batches for commercialisation is 0.87%. The average enzyme activity/TOS ratio of the three food enzyme batches for commercialisation is 621 U/mg TOS.

[5] Technical dossier/Additional information June 2020/Annex 1.
[6] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.
[7] Technical dossier/1st submission/Annex 17 and Annex 18.
[8] Technical dossier/1st submission/p. 28–30 and Additional information June 2020/Annex 3.
[9] Technical dossier/1st submission/Annex 37.
[10] Technical dossier/Additional information June 2020/Annex 4.
[11] Technical dossier/1st submission/p. 19 and Annex 8.
[12] Technical dossier/1st submission/Annex 7.
[13] Technical dossier/1st submission/p. 18.
[14] Technical dossier/Additional information June 2020.

Table 1: Compositional data of the food enzyme preparation

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Dextranase activity	U/g batch[(b)]	5,520	5,301.5	5,278.5	9,070
Protein	%	0.5	0.5	0.5	0.6
Ash	%	0.2	0.2	0.2	0.1
Water	%	42.9	42.9	43	98.8
[redacted] **(excipient)**	%	56	56	56	0
Total organic solids (TOS)[(c)]	%	0.9	0.9	0.8	1.1
Activity/mg TOS	U/mg TOS	613.3	589.1	659.8	824.5

(a): Batch used for the toxicological studies.
(b): UNIT: Dextranase units (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash – % excipient.

3.3.3. Purity

The lead content in the three commercial batches and in the batch used for toxicological studies was not more than 5 mg/kg[14] which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).

The food enzyme preparation complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). No antimicrobial activity was detected in any of these batches[15] (FAO/WHO, 2006).

Strains of *Collariella*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites. The presence of mycotoxins (aflatoxins B1, B2, G1, G2; sterigmatocystine; zearalenone; ochratoxin and chaetochromin A) was examined in five food enzyme preparation batches, different from those reported in Table 1 and were below the limits of detection (LoD) of the applied analytical methods.[16,17] The potential presence of other secondary metabolites is addressed by the toxicological examination of the food enzyme–TOS.

The Panel considered that the information provided on the purity of the food enzyme is sufficient.

3.3.4. Viable cells of the production strain

The absence of the viable cells of the production strain in the food enzyme was demonstrated in three independent commercial batches. [redacted] No colonies were detected.[18]

3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats has been provided. The batch 4 (Table 1) used in these studies has similar protein pattern as the batches used for commercialisation and is considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good

[15] Technical dossier/1st submission/p. 27 and Technical dossier/1st submission/Annex 2/Additional information June 2020.
[16] LoD: aflatoxins: B1, B2, G1 and G2 = 0.001 mg/kg; sterigmatocystine = 0.02 mg/kg; zearalenone = 0.005 mg/kg; ochratoxin = 0.001. mg/kg; chaetochromin A = 0.02 mg/kg.
[17] Technical dossier/1st submission/Annex 2 and Annex 3.
[18] Technical dossier/Additional information June 2020/Annex 8.

Laboratory Practice (GLP).[19] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA were used in the presence or absence of metabolic activation (S9-mix), applying the preincubation method. A dose-range finding experiment was carried out using seven concentrations of the enzyme ranging from 1.1 to 1,100 μg TOS/plate. No growth inhibition was observed. Two separate main experiments were carried out in triplicate using five different concentrations of the food enzyme (68.8; 137.5; 275; 550; 1,100 μg TOS/mL). A slight toxic effect, evident as a reduction in the number of revertants, occurred in TA1537 in the absence of S9-mix at 137.5 μg/plate only in the first experiment. Upon treatment with the food enzyme, there was no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

The Panel concluded that the food enzyme did not induce gene mutations under the test conditions employed in this study.

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out in a fibroblasts cell line from lungs of Chinese hamster (CHL/IU) according to OECD Test Guideline 473 (OECD, 2016) and following GLP.[20]

The dose-finding study was performed at concentrations ranging from 34.4 to 1,100 μg TOS/mL. Inhibition of cell growth by 50% or more was observed at 275 μg TOS/mL in a short-term treatment (6 h followed by 18 h recovery period) with metabolic activation (S9-mix) and at 550 μg TOS/mL in a short-term treatment and a continuous treatment (24 h) without metabolic activation. Based on these results, the cells were exposed to the food enzyme at 75, 150 and 300 μg TOS/mL, with and without metabolic activation, in both short and a continuous treatment (24 h). Cytotoxic effects were observed at the highest concentration tested (at 300 μg TOS/mL, 47%, 48% and 43% relative increase in cell count for short-term treatment in the presence of S9-mix, in the absence of S9-mix and for the continuous treatment, respectively). The frequency of structural and numerical chromosomal aberrations in treated cultures was comparable to the values detected in negative controls and within the range of the laboratory historical solvent control data.

The Panel concluded that the food enzyme did not induce chromosome aberrations under the test conditions employed for this study.

3.4.2. Repeated dose 90-day oral toxicity study in rodents

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[21] Groups of 10 male and 10 female Sprague-Dawley (Crl:CD(SD)) rats received by gavage the food enzyme in three doses corresponding to 27.5, 55 and 110 mg TOS/kg bw per day. Controls received the vehicle (water).

No mortality was observed.

A statistically significant increase in feed intake was observed in mid-dose males on days 65 and 79. The spontaneous motor activity of low-dose males showed a statistically significant increase in rearing count after administration. These observations were isolated, and the Panel considered them as not treatment-related.

Haematological investigation revealed a small but statistically significant lower prothrombin time (PT) in low- and mid-dose females, a statistically significant lower white blood cell count in mid- and high-dose females and a statistically significant lower lymphocytes count in mid-dose females.

Clinical chemistry investigation revealed that low-dose females had a statistically significant decrease in alanine phosphatase and a statistically significant increase in total cholesterol. Total protein concentration was statistically significantly increased in mid-dose females and albumin was statistically significantly increased in low- and mid-dose females. The percentage of γ-globulin was statistically significantly decreased in all treated females.

All the changes in haematology and blood chemistry parameters were considered by the Panel as not treatment-related because the differences were without an apparent dose dependency and restricted to one sex.

Urinalysis showed a statistically significant increase in specific gravity of the urine in low-dose males and a statistically significant increase in potassium in mid-dose females. The Panel considered these

[19] Technical dossier/Additional information June 2020/Annex 5.
[20] Technical dossier/Additional information June 2020/Annex 6.
[21] Technical dossier/ Additional information June 2020/Annex 7.

findings not to be treatment-related as they were observed without dose dependency and were restricted to one sex.

There was a statistically significant increase in the absolute and relative weight of the thyroid gland in high-dose males and a statistically significant increase in relative weight of the pituitary gland in mid-dose females. The Panel considered these changes as incidental because there was either no apparent dose–response relationship, the changes were not accompanied by histopathological findings and the values were within the normal variability of relevant historical control ranges in the laboratory.

No other statistically significant differences to controls were observed.

The Panel identified the no observed adverse effect level (NOAEL) of 110 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

The allergenicity assessment considers only the food enzyme and not any carrier or other excipient which may be used in the final formulation.

The potential allergenicity of the dextranase produced with the genetically modified *C. gracilis* strain ATCC-16153 was assessed by comparing its amino acid sequence with those of known allergens according to the scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.

No information is available on oral and respiratory sensitisation or elicitation reactions of this dextranase.

A literature search on the Google Scholar and PubMed databases using the search terms "dextranase allergen" or "dextranase allergy" did not produce any result.[22]

According to the information provided, [redacted] that may cause allergy, is used as raw material in the media fed to the microorganisms. However, these proteins will be digested during the fermentation process and consumed by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial biomass and fermentation solids will be removed. Therefore, potentially allergenic residues of these foods employed as protein sources are not expected to be present.

The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood of such reactions to occur is considered to be low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in refined sugar production at a recommended use level of 0.018–0.09 mg TOS/kg raw juice after the diffusion step and 1.8–10.8 mg TOS/kg raw molasses before the purification step.[23] Therefore, the highest maximum use level of 10.8 mg TOS/kg raw molasses was used in the dietary exposure estimation.

In sugar production, dextranase is added to the raw juice beet or crushed sugar cane coming from the crushers, in order to hydrolyse dextrans, which may be present due to microbial spoilage. Dextranase can also be added to the raw molasses before the purification step, or to the cane syrup during the re-melt stage. Degradation of dextrans helps to reduce the viscosity of the raw juice and improve the sucrose yield.[24]

During the refined sugar production process, protein components are removed at several steps, such as decantation, centrifugation, discoloration and crystallisation. Information provided by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) and the European Association of Sugar Manufacturers (CEFS) indicate that 99% of the enzyme–TOS is eliminated during the beet or cane sugar production process (Documentation provided to EFSA No 3). When using sucrose content as the proxy to estimate the residual food enzyme–TOS, it is estimated that 90–94% (beet juice intermediary), 98.2–99.9% (commercial beet sugar) and 98.7–99.8% (commercial cane

[22] Technical dossier/1st submission/Annex 34 and Annex 35.
[23] Additional information June 2020/Annex 9.
[24] Technical dossier/1st submission/pp. 54–57.

sugar) of enzymes added to the raw material are removed during refined sugar production (Documentation provided to EFSA No 4).

The Panel considered the available information sufficient to conclude that residual amounts of TOS are removed during the production of the refined sugars (white sucrose). However, this consideration cannot be applied to unrefined sugar products (e.g. beet molasses, cane syrups). Although the applicant did not detect the dextranase activity, the viable cells of *C. gracile* and the Chaetochromin A[25] , this information was not considered sufficient to prove the absence of residual TOS.

Molasses (also referred to as black treacle) is a by-product of the refined sugar production in the form of an uncrystallised syrup. In the EU, molasses is mainly used as animal feed and in biofuels production. However, due to its nutritional value and flavour characteristics, the beet molasses has also emerged as alternative to sweeten foods, e.g. breakfast cereals, sauce and bread. Certain products, e.g. speculaas and lebkuchen, have traditionally been produced with molasses.

3.5.2. Dietary exposure estimation

As residual amounts of TOS are removed during the production of refined sugars (see Section 3.5.1), foods containing refined sugar as an ingredient were excluded from the estimation.

Concerning unrefined 'brown sugar', CEFS clarified that 'brown sugar', which is currently not legally defined at EU level, covers a broad range of different sugars with brown colour, and which do not all correspond to raw cane sugar. According to CEFS' members, brown sugars containing cane molasses or caramelised sugar syrup are considered to be niche products in the EU and only make up a small fraction. Such sugars were therefore excluded from the exposure assessment, since the Comprehensive Database does not clearly differentiate these products from refined white sugar.

Similarly, the Comprehensive Database does generally not provide information on the raw material used (i.e. cane, beet) to produce the unrefined sugar products (beet and cane molasses/syrups), and consumption of such products could not be separated into the different categories. While it is acknowledged that use of such products may not be equal, in the absence of being able to distinguish products coming from the two different sources, dietary exposure assessment of these products was combined, and was reflected in the undifferentiated inclusion of both the beet molasses and the unrefined cane syrup in the open call.[26]

Only foods containing molasses and cane syrups were considered. Exposure estimates were calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016). The calculation involved the selection of relevant food groups and the application of process-specific technical conversion factors (Appendix A). These input data were subject to a stakeholder consultation through open calls[26] and adjusted in accordance with feedback received.

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant (see Section 3.5.1) with the relevant FoodEx categories (Appendix A), based on individual consumption data. Exposure from individual FoodEx categories was subsequently summed up, averaged over the total survey period and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only one day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 2 provides an overview of the derived exposure estimates across all surveys. Detailed average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix B – Tables 1 and 2. For the present assessment, food consumption data were available from 40 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 23 European countries (Appendix C).

[25] Technical dossier/Table 15 and Appendix 19, LoQ = 60 mg/kg.
[26] https://www.efsa.europa.eu/en/consultations/call/call-input-data-exposure-assessment-food-enzymes-6th-call

Table 2: Summary of estimated dietary exposure to food enzyme–TOS in six population groups

Population group	Estimated exposure (μg TOS/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.00–0.11 (12)	0.01–0.64 (16)	0.04–3.78 (19)	0.02–2.04 (20)	0.01–0.76 (22)	0.00–0.33 (21)
Min–max 95th percentile (number of surveys)	0.00–0.59 (10)	0.05–2.49 (14)	0.22–15.03 (19)	0.06–10.34 (19)	0.02–4.68 (22)	0.02–0.85 (21)

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the 'EFSA Opinion related to uncertainties in dietary exposure assessment' (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 3.

Table 3: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/underreporting/misreporting/ no portion size standard	+/–
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Minor FoodEx categories found to only sporadically contain molasses were excluded from the exposure assessment	–
'Brown sugar' produced through use of cane molasses or caramelised sugar syrup was excluded, due to it being a niche product on the European market	–
Assumption that dextrans, which may be present due to microbial spoilage, are always present thus necessitating treatment with dextranase	+
The transfer of food enzyme–TOS into cane and beet molasses/syrups was assumed to be 100%	+
No distinction was made between beet molasses and cane syrups used as ingredients in foods	+/–
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

+: uncertainty with potential to cause overestimation of exposure; –: uncertainty with potential to cause underestimation of exposure.

The conservative approach applied to the exposure estimate to food enzyme–TOS, in particular, assumptions made on the use levels of this specific food enzyme, is likely to have led to a considerable overestimation of the exposure.

The exclusion of minor FoodEx categories and unrefined 'brown sugar' from the exposure assessment is not expected to have an impact on the overall estimate derived.

3.6. Margin of exposure

A comparison of the NOAEL (110 mg TOS/kg bw per day) from the 90-day toxicity study in rats with the derived exposure estimates of 0.0–3.78 μg TOS/kg bw per day at the mean and from 0.0–15.03 μg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MoE) of at least 7,319.

4. Conclusions

Based on the data provided, removal of TOS during the production of refined sugars and the derived MoE for foods containing unrefined sugar products, the Panel concluded that the food enzyme dextranase produced with the non-genetically modified *C. gracilis* strain ATCC-16153 does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

1) Dextranase. October 2015. Submitted by Keller and Heckman LLP on behalf of Mitsubishi-Kagaku Foods Corporation.
2) Additional information. June 2020. Submitted by Keller and Heckman LLP on behalf of Mitsubishi-Kagaku Foods Corporation.
3) Information on the transfer of enzymes into foods for refined sugar production and processing. October 2017. Provided by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP).
4) Information on the transfer of enzymes into foods for refined sugar production and processing. October 2020. Provided by the European Association of Sugar Manufacturers - Comité Européen des Fabricants de Sucre (CEFS).

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
MoE	margin of exposure
OECD	Organisation for Economic Cooperation and Development
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WHO	World Health Organization

Appendix A – FoodEx categories used to derive intake estimates of FE–TOS and the respective conversion factors

FoodEx hierarchical code	FoodEx matrix description	FoodEx hierarchical level	f1 (Convertingraw unrefined sugar product to unrefined sugar product)	f2 (Average fraction of unrefined sugar product in respective FoodEx category)	f3 (Percentage of FoodEx category containing unrefined sugar product)
A.01.04.001.004	Wheat bread, brown	4	1	0.01	0.1
A.01.04.001.005	Wheat bread, brown, gluten free	4	1	0.01	0.1
A.01.04.001.006	Wheat bread, brown, with oil seeds	4	1	0.01	0.1
A.01.04.001.007	Wheat bread, with bran	4	1	0.01	0.1
A.01.04.001.008	Wheat bread, with oil seeds	4	1	0.01	0.1
A.01.04.001.009	Wheat germ bread	4	1	0.01	0.1
A.01.04.001.013	Wheat rolls, brown	4	1	0.01	0.1
A.01.04.001.014	Wheat rolls, brown and oil seeds	4	1	0.01	0.1
A.01.04.001.015	Wheat rolls, with oil seeds	4	1	0.01	0.1
A.01.04.001.017	Wheat toast bread, brown	4	1	0.01	0.1
A.01.04.002	Rye bread and rolls	3	1	0.01	0.1
A.01.04.003	Mixed wheat/rye bread and rolls	3	1	0.01	0.1
A.01.04.004	Multigrain bread and rolls	3	1	0.01	0.1
A.01.04.006.004	Muesli bread	4	1	0.01	0.1
A.01.04.006.005	Oat bread	4	1	0.01	0.1
A.01.06.001	Cereal flakes	3	1	0.04	0.03
A.01.06.002	Muesli	3	1	0.001	0.03
A.01.06.003	Cereal bars	3	1	0.001	0.01
A.01.07.001.020	Fruit cake	4	1	0.01	0.25
A.01.07.001.024	Gingerbread	4	1	0.1	1
A.01.07.001.044	Lebkuchen	4	1	0.1	1
A.01.07.002.008	Speculaas	4	1	0.1	1
A.10.04.001	Candies, with sugar	3	1	0.001	0.01
A.10.04.011	Liquorice candies	3	1	0.001	0.13
A.10.04.012	Gum drops	3	1	0.001	0.01
A.10.04.013	Jelly candies	3	1	0.001	0.01
A.10.06.001	Molasses	4	1	1	1
A.10.06.005	Sugar beet syrup	4	1	1	1
A.10.06.006	Treacle	4	1	1	1
A.16.05.001	Mustard, sweet	3	1	0.035	1
A.16.05.007	Barbecue sauce	3	1	0.05	1
A.16.05.015	Mixed condiment	3	1	0.05	0.25
A.16.08.002	Brown sauce	3	1	0.05	1
A.19.02.002	Rice and meat meal	3	1	0.01	0.1
A.19.02.003	Rice, meat and vegetables meal	3	1	0.01	0.1
A.19.03.002	Potatoes and meat meal	3	1	0.01	0.1
A.19.03.003	Potatoes, meat and vegetables meal	3	1	0.01	0.1

FoodEx hierarchical code	FoodEx matrix description	FoodEx hierarchical level	f1 (Convertingraw unrefined sugar product to unrefined sugar product)	f2 (Average fraction of unrefined sugar product in respective FoodEx category)	f3 (Percentage of FoodEx category containing unrefined sugar product)
A.19.04.001	Beans and meat meal	3	1	0.01	0.1
A.19.04.003	Beans, meat and vegetables meal	3	1	0.01	0.1
A.19.05.004	Meat stew	3	1	0.01	0.1
A.20.02.001	Ice cream, milk-based	3	1	0.01	1

Appendix B – Dietary exposure estimates to the food enzyme–TOS in details

Information provided in this appendix is shown in an Excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2020.6309#support-information-section).

The file contains two sheets, corresponding to two tables.

Table 1: Average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey

Table 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey

Appendix C – Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than one day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, United Kingdom
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain, United Kingdom
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain, Sweden, United Kingdom
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden, United Kingdom
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden, United Kingdom

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011).