SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 23 March 2022

doi: 10.2903/j.efsa.2022.7279

# Safety evaluation of the food enzyme dextranase from the *Collariella gracilis* strain AE-DX

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Claude Lambré, José Manuel Barat Baviera, Claudia Bolognesi, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn, Magdalena Andryszkiewicz, Yi Liu, Sandra Rainieri and Andrew Chesson

## 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 AE-DX by Amano Enzyme Inc. The food enzyme is considered free from viable cells of the production organism. The food enzyme is intended to be used in refined sugar production from sugar beet or sugar cane. Since residual amounts of total organic solids (TOS) are removed by crystallisation during the production of refined white sucrose, dietary exposure was not considered necessary for refined sugars. However, beet molasses and cane syrups, by-products from sugar production, could enter the food chain. Based on the maximum use levels recommended, dietary exposure was estimated to be up to 0.39 mg TOS/kg body weight (bw) per day via the consumption of unrefined sugars. Genotoxicity tests did not indicate 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 lowest observed adverse effect level (LOAEL) of 940.5 mg TOS/kg bw per day, the lowest dose tested, which when compared with the estimated dietary exposure, results in a margin of exposure of more than 800. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and no matches were 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 for this to occur 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.



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

**Requestor:** European Commission

**Question number:** EFSA-Q-2015-00685

**Correspondence:** fip@efsa.europa.eu

**Panel members:** José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis and Holger Zorn.

**Legal notice:** The full opinion will be published in accordance with Article 12 of Regulation (EC) No 1331/2008 once the decision on confidentiality will be received from the European Commission.

**Declarations of interest:** The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

**Acknowledgments:** The Panel wishes to thank Helen Regina for the support provided to this scientific output.

**Suggested citation:** EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Andryszkiewicz M, Liu Y, Rainieri S and Chesson A, 2022. Scientific Opinion on the safety evaluation of the food enzyme dextranase from the *Collariella gracilis* strain AE-DX. EFSA Journal 2022;20(5):7279, 15 pp. https://doi.org/10.2903/j.efsa.2022.7279

**ISSN:** 1831-4732



The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

# Table of contents

# 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.

Five applications have been introduced by the companies "Danisco US Inc." for the authorisation of the food enzymes Alpha-amylase from a genetically modified strain of *Trichoderma reesei* (DP-Nzb48) and Termolysin from *Geobacillus caldoproteolyticus* (DP-Fzj32), and "Armano Enzyme Inc." for the authorisation of the food enzymes AMP deaminase from *Streptomyces murinus* (strain AE-DNTS), Beta-galactosidase from *Aspergillus oryzae* (strain AE-LA) and Dextranase from *Chaetomium erraticum* (strain AE-DX).

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

### 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 Alpha-amylase from a genetically modified strain of *Trichoderma*

[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.

*reesei* (DP-Nzb48), Thermolysin from *Gebacillus caldoproteolyticus* (DP-Fzj32), AMP deaminase from *Streptomyces murinus* (strain AE-DNTS). Beta-galactosidase from *Aspergillus oryzae* (strain AE-LA) and Dextranase from *Chaetomium erraticum* (strain AE-DX) 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 *Chaetomium erraticum* (strain AE-DX).

Recent data identified the production microorganism as *Collariella gracilis* (Section 3.1). The dextranase is produced with the filamentous fungus Collariella gracilis strain AE-DX (formerly Chaetomium erraticum). Therefore, this name will be used in this opinion instead of *Chaetomium erraticum*.

## 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 *Chaetomium erraticum* (strain AE-DX).

Additional information was sought from the applicant during the assessment process in a request from EFSA sent on 22 March 2019 and on 02 March 2020 was consequently provided (see 'Documentation provided to EFSA').

Following the reception of additional data by EFSA on 31 July 2019, EFSA requested a clarification teleconference on 17 February 2020, after which the applicant provided additional data on 29 June 2020.

### 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) and following the relevant guidance documents of the EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) as well as the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) have been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a).

## 3. Assessment

| | |
|---|---|
| IUBMB nomenclature | Dextranase |
| Systematic name | 6-α-D-glucan 6-glucanohydrolase |
| Synonyms | dextran hydrolase; entodextranase; dextranase; endo-dextranase |
| IUBMB No | EC 3.2.1.11 |
| CAS No | 9025-70-1 |
| EINECS No | 232-803-9 |

Dextranases catalyse the hydrolysis of α-1,6 and α-1,4 glucosidic linkages in dextrans. The food enzyme is intended to be used in refined sugar production from sugar beet or sugar cane.

### 3.1. Source of the food enzyme

The dextranase is produced with the filamentous fungus *Collariella gracilis* strain AE-DX (formerly *Chaetomium erraticum*), [redacted] as strain number [redacted] and is currently deposited in the Culture Collection of the Biological Resource Center, National Institute of Technology and Evaluation, Japan (NBRC) with deposit number [redacted]. The production strain was identified as *C. gracilis* [redacted][4]

[4] Technical dossier/Additional information January 2022/Annex 1.

## 3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[5], with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.[6]

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 enzyme protein is retained while most of the low molecular mass material passes the filtration membrane and is discarded.[7] 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. The molecular mass calculated on the basis of the amino acid sequence was [redacted] kDa. The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all batches. The gel showed a major protein band consistent with the expected mass of the enzyme and a number of other bands of lower staining intensity.[9]

No other enzymatic activities were reported.

The in-house determination of dextranase activity is based on hydrolysis of dextran and is expressed in UNIT/g. Potassium ferricyanide is used to develop a blue colouration which is back titrated with sodium thiosulfate until the colour disappears. One UNIT is defined as the quantity of dextranase that releases reducing sugars equivalent to 1 μmol of sodium thiosulfate in 1 min under the conditions of the assay.[10]

The food enzyme has a temperature optimum around 60°C and a pH optimum of around 6.0 (37°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures. Under the conditions of the applied temperature stability assay, dextranase activity decreased above 60°C and showed no residual activity above 70°C.[11]

### 3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three food enzyme batches used for commercialisation and for two batches used for toxicological studies (Table 1). The mean total organic solids (TOS) of the three batches used for commercialisation is 32.6% and the mean enzyme activity/TOS ratio is 195.7 Units/mg TOS.

[5] 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.
[6] Technical dossier/1st submission/p. 34 and Annex 4.
[7] Technical dossier/1st submission/p. 34-41 and Annex 5.
[8] Technical dossier/1st submission/Annex 6.
[9] Technical dossier/Additional information July 2019.
[10] Technical dossier/1st submission/Annex 2.
[11] Technical dossier/1st submission/p. 30.

**Table 1:** Composition of the food enzyme batches

| Parameter | Unit | Batches | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4[(a)] | 5[(b)] |
| Dextranase activity | U/g batch[(c)] | 62,200 | 63,700 | 64,800 | 65,500 | 72,300 |
| Protein | % | 1.2 | 1.4 | 1.2 | NA[(c)] | NA[(d)] |
| Ash | % | 5.0 | 5.2 | 4.0 | 6.0 | 5.8 |
| Water | % | 63.2 | 59.5 | 65.3 | 62.3 | 61.2 |
| Total Organic Solids (TOS)[(e)] | % | 31.8 | 35.3 | 30.7 | 31.7 | 33.0 |
| Activity/mg TOS | U /mg TOS | 195.6 | 180.5 | 211.1 | 206.6 | 219.1 |

(a): Batch used for chromosomal aberration test.
(b): Batch used for reverse mutation assay and 90-day systemic toxicity study.
(c): U/g batch: dextranase units/g (see Section 3.3.1).
(d): NA: not analysed.
(e): TOS calculated as 100% – % water – % ash.

### 3.3.3. Purity

The lead content in the three commercial batches was below 0.01 mg/kg[12,13] and in the batches used for toxicological testing below 3 mg/kg[14,15] which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, the level of arsenic[16] in the batches used for toxicological testing was below the limit of detection (LOD) of the employed method.[14]

The food enzyme complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*)[17] as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).[13]

Strains of *Collariella*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites. The presence of aflatoxins: B1, B2, G1, G2, HT-2 toxin, T-2 toxin, zearalenone, ochratoxin A, sterigmatocystin and fumosin FB1 and FB2 was examined in the three commercial batches and all were below the LOD of the applied analytical methods.[13,18] The applicant was unable to produce data on the presence of chaetochromins. However, the possible presence of these and other toxic 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 viable cells of the production strain in the food enzyme was demonstrated [redacted] No colonies were formed.[19]

## 3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation test (Ames test), an *in vitro* mammalian chromosomal aberration test, and a repeated dose 90-day oral toxicity study in rats has been provided. Batch 4 (Table 1) was used in *in vitro* mammalian chromosomal aberration test and batch 5 in the Ames test and repeated dose 90-day oral toxicity study. The batches employed had a similar composition as those used for commercialisation and are considered suitable as test items.

[12] LOD: Pb = 0.01 mg/kg.
[13] Technical dossier/1st submission/Annex 3.
[14] Technical dossier/1st submission/p. 53.
[15] LOD: Pb = 3 mg/kg.
[16] LOD: As = 2 mg/kg.
[17] Technical dossier/1st submission/p. 53 and Annex 3.
[18] LOD: aflatoxins: B1, B2, G1, G2 = 0.2 μg/kg; ochratoxin A = 0.5 μg/kg; HT-2, T-2 toxins, zearalenone and sterigmatocystin = 10 μg/kg; fumosin FB1 and FB2 = 5.0 μg/kg.
[19] Technical dossier/Additional data January 2022/Annex 3.

### 3.4.1. Genotoxicity

A bacterial reverse mutation assay (Ames test) was made according to the Japanese test guidelines (notification 1604, 1999 and 0920, 2012) which accords with the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following GLP according to the Japanese system of the Japanese Ministry of Health and Welfare (ordinance N 21, 1997; N 114, 2008 and notification N 0613007, 2008) in four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2 *uvrA*.[20]

Five concentrations of the test substance (from 313 to 5,000 μg dry matter/plate corresponding to 266, 532, 1,064, 2,128 and 4,710 μg TOS/plate) were evaluated with and without activation (S9-mix) using the pre-incubation method.

No cytotoxicity, determined as cell growth inhibition, was observed at any concentrations of the test substance evaluated in any of the strains used. The test substance did not induce any increase in the number of revertants, either with or without metabolic activation in comparison to the vehicle controls.

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

#### 3.4.1.1. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out according to the Guideline for Genotoxicity Test on Drugs of the Pharmaceutical and Medical Safety Bureau of the Japanese Ministry of Health and Welfare (Notification Iyakushin N 1604, November, 1999), in compliance with OECD Test Guideline 473 (OECD, 1997b), according to the Japanese Ministry of Health and Welfare Ordinance N. 21 'Good Laboratory Practice Standard Ordinance for Nonclinical Laboratory Studies on Safety of Drugs' (2008) and following GLP.[21]

The food enzyme was tested for its ability to induce chromosomal aberrations in Chinese Hamster Lung cells (CHL/IU) with and without metabolic activation (S9-mix). A preliminary range finding test was carried out at concentrations up to 5,000 μg food enzyme/mL (corresponding to 1,585 μg TOS/mL) and no inhibition of cell growth by 50% or higher was observed. On the basis of these results, two main experiments were performed in duplicate cultures. In the first experiment, CHL/IU cells were exposed to the food enzyme in a short-treatment experiment (6 h + 20 h) in presence and in absence of metabolic activation (S9-mix). In the second experiment, the cells were exposed continuously to the food enzyme in absence of S9-mix during 26 h (long-term treatment). In both experiments, the concentrations scored were 160, 310, 630, 1,300, 2,500, and 5,000 μg food enzyme/mL, corresponding to 50.7, 98.3, 199.7, 412.1, 792.5 and 1,585 μg TOS/mL, respectively.

No significant cytotoxic effects, evaluated as cell growth reduction, were detected in any of the treatments. The frequency of structural and numerical aberrations was not statistically significantly different to the negative controls at all concentrations tested.

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

A repeated dose 90-day oral toxicity study was performed in accordance with the test guidelines of the Japanese Ministry of Health and Welfare) (Notification N 24 (1989), N 29 (1996), N 655 (1999) and N 0219 (2010), and the GLP (Ordinance N 21 (1997), N 114 (2008) and Notification N 0219 (2010)). The Applicant states that the guidelines followed can be considered equivalent to the OECD Guideline 408 (OECD, 1998) as they have been harmonised according to ICH (Europe, Japan, USA).

Groups of 12 male and 12 female Sprague–Dawley (Crl:CD(SD)) rats received by gavage the test substance in doses corresponding to 940.5, 1,881 and 3,762 mg TOS/kg body weight (bw) per day. Controls received the vehicle (water).

No mortality was observed.

Haematological parameter evaluation revealed statistically significantly higher counts in white blood cells (+29%), lymphocytes (+33%) and eosinophils (+56%) in high-dose females. Although the increases in white blood cells and lymphocyte counts appeared dose-related, these results were not accompanied by any inflammatory changes in the organs and the values were within the range of

[20] Technical dossier/1st submission/Annex 7.
[21] Technical dossier/1st submission/Annex 8.

historical control data from the laboratory. Therefore, these findings were considered to be of no toxicological relevance.

Clinical chemistry investigation revealed a statistically significant higher triglyceride level in high-dose males (+100%), a statistically significantly higher concentration of phospholipids (+16%), statistically significant lower concentrations of potassium (−9%) and chloride (−3%), and aspartate transaminase (−45%) and lactate dehydrogenase activities (−62%) in high-dose females. These findings were considered to be physiological fluctuations as they were within the range of historical control data.

Urinalysis showed that high-dose females produced a significant greater volume of urine (+49%) than the controls. However, this finding was not considered toxicologically significant, since no abnormalities in the histopathological examination or in blood chemistry parameters related to kidney damage were detected, although a slight but statistically significant increase in the absolute kidney weight was recorded.

Statistically significantly higher concentrations of Na were detected in the urine of males and females of the mid- (+33% and +46%, respectively) and high-dose groups (+124% and +169%, respectively); statistically significant greater concentrations of K were detected in high-dose females (+30%). The increase in electrolyte concentration was due to the presence of Na and K in the test substances as confirmed by the applicant with electrolyte detection in the test substance. Additionally, a significant reduction of K and Cl was detected in the urines of mid-dose males (−30% and −23%, respectively) and statistically significantly lower values in osmolality were detected in the urine of low-dose females (−32%). Those changes were not dose-related. Therefore, they were not considered toxicologically relevant.

A statistically significant increase in absolute weights of liver was observed in the high-dose males and females (+25% and +17%, respectively). Additionally, liver relative weight increased significantly in males in all dose groups (+7%, +12% and +18%) and in high-dose females (+9%). For kidney, the absolute and relative weights were statistically significantly increased in high-dose males (+15% and +8%, respectively) and the absolute kidney weight was statistically significantly increased in high-dose females (+18%). High-dose females also showed a statistically significant lower relative brain weight (−7%) with respect to the controls. Significant lower relative weight of adrenals in high-dose males were observed (−18%). A significant increase of the pituitary gland absolute weight in high-dose males was also observed (+12%). These changes were considered to be of no toxicological relevance as no adverse histological changes were observed in these organs.

Histology results showed a minimal hypertrophy of centrilobular hepatocytes in 3 of 12 high-dose males which was considered by the Panel as not adverse.

At necropsy, thickening of the limiting ridge in the stomach was observed in 2 mid-dose males and in 10 males and 5 females of the high-dose group. Hyperplasia of the stomach limiting ridge was observed in 7 males and 2 females of the low-dose group, 12 males and 9 females of the mid-dose group and in 12 males and 12 females of the high-dose group. Furthermore, histopathological examination showed hyperplasia of mucosal cells in glandular stomach in 11 and 12 males and in 4 and 12 females of the mid- and high-dose group, respectively. These changes were considered by the Panel as test compound related as the incidence increased with increment of the doses. Although the findings in the limiting ridge of the rat may not be relevant for humans as there is no similar structure, the Panel considered the lowest dose that produced indication of irritation as a lowest observed adverse effect level (LOAEL) rather than a no observed adverse effect level (NOAEL) to provide greater protection.

The Panel identified a LOAEL of 940.5 mg TOS/kg bw per day.

### 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 by *C. gracilis* strain AE-DX 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.[9]

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

The Panel is unaware of reported allergic reactions to other dextranases.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[22]) are used as raw materials ([redacted])[23] in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these foods employed as protein sources are not expected to be present.

Quantifying the risk for allergenicity is not possible in view of the individual susceptibility to food allergens. Allergenicity can be ruled out only if the proteins are fully removed. In the production of refined sugars, experimental data showed a significant removal (> 99%) of protein. However, traces of protein could be present in refined white sugar.

The Panel considers 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 up to 8 mg TOS/kg sugar beet or sugar cane.[24]

In sugar production, dextranase is added to the raw beet juice or crushed sugar cane coming from the crushers to hydrolyse dextrans, which may be present due to microbial spoilage. Dextranase can also be added to the raw molasses before 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.[25] The food enzyme-TOS is removed from the refined sucrose by crystallisation (EFSA CEP Panel, 2021b) .

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 biofuel 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, bread. Certain products, e.g. speculaas and lebkuchen, have traditionally been produced with molasses.

Data from the European Association of Sugar Manufacturer (CEFS) on the raw beet juice suggest that the beet molasses for human consumption are at least 90% pure. The applicant did not detect any protein bands by separating the beet molasses samples on SDS–PAGE and visualising by silver staining.[26] However, no data was provided to establish the purity of cane syrups (EFSA CEP Panel, 2021b). Altogether, the Panel considered this information insufficient to prove absence of residual TOS in unrefined sugars, in particular, the cane syrups.

### 3.5.2. Dietary exposure estimation

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

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 of sugar consumption. Since the EFSA Comprehensive European Food Consumption Database does not clearly differentiate these products from refined white sugar, such sugars were also excluded from the estimation.

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant with the individual consumption data from the EFSA Comprehensive European Food Consumption Database. The estimation involved selection of relevant food categories and application

[22] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

[23] Technical dossier/ Annex 6.

[24] Technical dossier/1st submission/p. 45 and Additional Information July 2019.

[25] Technical dossier/1st submission/p. 44 and Additional Information July 2019.

[26] Additional data June 2020.

of technical conversion factors (EFSA CEP Panel, 2021b). 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 A – 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 B). The highest dietary exposure to the food enzyme-TOS was estimated to be 0.39 mg TOS/kg bw per day in children of 3–9 years old.

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

| Population group | Estimated exposure (mg 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) | 0–0.003 (12) | 0–0.016 (16) | 0.001–0.098 (19) | 0–0.053 (20) | 0–0.020 (22) | 0–0.009 (21) |
| Min–max 95th percentile (number of surveys) | 0–0.015 (10) | 0.001–0.064 (14) | 0.006–0.390 (19) | 0.002–0.268 (19) | 0.001–0.121 (22) | 0.001–0.022 (21) |

TOS: total organic solids.

### 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 | – |
| 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 | +/– |

| Sources of uncertainties | Direction of impact |
|---|---|
| Use of recipe fractions in disaggregation FoodEx categories | +/– |
| Use of technical factors in the exposure model | +/– |

TOS: total organic solids.
+: 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 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 LOAEL (940.5 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0–0.098 mg TOS/kg bw per day at the mean and from 0–0.390 mg TOS/kg bw per day at the 95th percentile and applying an additional uncertainty factor of 3 to extrapolate from the LOAEL, resulted in a margin of exposure (MOE) of at least 804.

## 4. Conclusions

Based on the data provided, the 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 AD-EX does not give rise to safety concerns under the intended conditions of use.

## 5. Documentation as provided to EFSA

1) Technical dossier "Dextranase from *Chaetomium erraticum* AE-DX". February 2015. Submitted by Amano Enzyme Inc.
2) Additional information. July 2019, March 2020, June 2020 and January 2022. Submitted by Amano Enzyme Inc.
3) Information on the transfer of enzymes into foods for refined sugar production and processing. October 2017. Provided by AMFEP.
4) Information on the transfer of enzymes into foods for refined sugar production and processing. June 2020. Provided by CEFS.

## References

EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2006;5(1):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority), 2009a. Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. EFSA Journal 2009;7(8):1305, 26 pp. https://doi.org/10.2903/j.efsa.2009.1305

EFSA (European Food Safety Authority), 2009b. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. EFSA Journal 2009;7 (5):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Silano V, Barat Baviera JM, Bolognesi C, Brüschweiler BJ, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Glandorf B, Herman L, Aguilera J and Chesson A, 2019. Statement on the characterisation of microorganisms used for the production of food enzymes. EFSA Journal 2019;17(6):5741, 13 pp. https://doi.org/10.2903/j.efsa.2019.5741

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Glandorf B, Herman L, Aguilera J, Andryszkiewicz M, Gomes A, Kovalkovicova N, Liu Y, Rainieri S and Chesson A, 2021a. Scientific Guidance for the submission of dossiers on Food Enzymes. EFSA Journal 2021;19(10):6851, 37 pp. https://doi.org/10.2903/j.efsa.2021.6851

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, van Loveren H, Vernis L, Zorn H, Liu Y and Chesson A, 2021b. Statement on the process-specific technical data used in exposure assessment of food enzymes. EFSA Journal 2021;19(12):7010, 38 pp. https://doi.org/10.2903/j.efsa.2021.7010

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. https://doi.org/10.2903/j.efsa.2010.1700

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th meeting. FAO JECFA Monographs, 3, 63–67. Available online: https://www.fao.org/3/a-a0675e.pdf

OECD (Organisation for Economic Co-Operation and Development), 1997a. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 471: Bacterial reverse mutation test. 21 July 1997. 11 pp. Available online: https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1639400502&id=id&accname=guest&checksum=D2E89C16911BE1BEB13BBC206B402098

OECD (Organisation for Economic Co-Operation and Development), 1997b. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 473: In vitro mammalian chromosomal aberration test. 21 July 1997. 10 pp. Available online: https://www.oecd-ilibrary.org/environment/test-no-473-in-vitro-mammalian-chromosome-aberration-test_9789264071261-en

OECD (Organisation for Economic Co-Operation and Development), 1998. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 408: Repeated dose 90-day oral toxicity study in rodents. 21 September 1998. 10 pp. Available online: https://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en

## 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 |
| CFU | colony forming units |
| EINECS | European Inventory of Existing Commercial Chemical Substances |
| FAO | Food and Agricultural Organization of the United Nations |
| GLP | Good Laboratory Practice |
| GMM | genetically modified microorganism |
| GMO | genetically modified organism |
| IUBMB | International Union of Biochemistry and Molecular Biology |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| LOAEL | lowest observed adverse effect level |
| LoD | limit of detection |
| NOAEL | no observed adverse effect level |
| MoE | margin of exposure |
| OECD | Organisation for Economic Cooperation and Development |
| PCR | polymerase chain reaction |
| SDS–PAGE | sodium dodecyl sulfate–polyacrylamide gel electrophoresis |
| TOS | total organic solids |
| WHO | World Health Organization |

# Appendix A – 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.2022.7279 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 B – 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** | 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, 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).