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# Safety evaluation of the food enzyme catalase from the non-genetically modified *Aspergillus niger* strain CTS 2093

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, Jaime Aguilera, Magdalena Andryszkiewicz, Yi Liu, Giulio di Piazza, Sandra Rainieri and Andrew Chesson

## Abstract

The food enzyme catalase (hydrogen-peroxide:hydrogen-peroxide oxidoreductase; EC 1.11.1.6) is produced with the non-genetically modified *Aspergillus niger* strain CTS 2093 by Shin Nihon Chemical Co., Ltd. It is considered free from viable cells of the production organism. The food enzyme is intended to be used in eight food manufacturing processes: baking processes, cereal-based processes, coffee processing, egg processing, vegetable processing for juice production, processing of tea, herbal and fruit infusions, herring roe processing and milk processing for cheese production. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 3.61 mg TOS/kg body weight (bw) per day in European populations. In addition, it is used in the production of acacia gum with the highest dietary exposure at the 95th percentile of 0.018 mg TOS/kg bw per day in infants, when acacia gum is used as a food additive. 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 no observed adverse effect level of 56 mg TOS/kg bw per day, the mid-dose tested, which, when compared with the estimated dietary exposure, resulted in a margin of exposure of 16. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and one match with a respiratory allergen was found. The Panel considered that, under the intended conditions of use, the risk of allergic reactions by dietary exposure cannot be excluded, but the likelihood for this to occur is low. Based on the data provided, the Panel considered the margin of exposure as insufficient to exclude safety concerns under the intended conditions of use.



**Keywords:** food enzyme, catalase, hydrogen-peroxide:hydrogen-peroxide oxidoreductase, EC 1.11.1.6, caperase, *Aspergillus niger*

**Requestor:** European Commission

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**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.

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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.

Five applications have been introduced by the companies "Intertek Scientific & Regulatory Consultancy" for the authorisation of the food enzymes Catalase from *Aspergillus niger* (strain CTS 2093), Glucose oxidase from *Penicillium chrysogenum* (strain PGO 19–162), Tannase from *Aspergillus oryzae* (strain TAN 206) and Glucoamylase from *Rhyzopus oryzae* (strain CU634-1775), and "RDA Scientific Consultants GmbH" for the authorisation of the food enzyme Phospholipase D from *Streptomyces netropsis* (DSZM No. 40093).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011 implementing Regulation (EC) No 1331/2008[3], 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 Catalase from *Aspergillus niger* (strain CTS 2093), Glucose oxidase

[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.

from *Penicillium chrysogenum* (strain PGO 19–162), Tannase from *Aspergillus oryzae* (strain TAN 206), Glucoamylase from *Rhyzopus oryzae* (strain CU634-1775) and Phospholipase D from *Streptomyces netropsis* (DSZM No. 40093) 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 catalase from *Aspergillus niger* strain CTS 2093.

## 2. Data and methodologies

### 2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme catalase from *Aspergillus niger* strain CTS 2093.

Additional information was requested from the applicant during the assessment process on 13 December 2021 and 12 April 2022 and consequently provided (see 'Documentation provided to EFSA').

### 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 '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 with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a).

## 3. Assessment

| | |
|---|---|
| IUBMB nomenclature | Catalase |
| Systematic name | Hydrogen-peroxide:hydrogen-peroxide oxidoreductase |
| Synonyms | Caperase |
| IUBMB No | EC 1.11.1.6 |
| CAS No | 9001-05-2 |
| EINECS No | 232-577-1 |

Catalases catalyse the decomposition of hydrogen peroxide, converting it to water and oxygen. The enzyme under assessment is intended to be used in eight food processes: baking processes, cereal-based processes, coffee processing, egg processing, vegetable processing for juice production, processing of tea, herbal and fruit infusions, herring roe processing and milk processing for cheese production. In addition, it is also used in production of acacia gum, an authorised food additive (E 414) in the EU.

### 3.1. Source of the food enzyme

The catalase is produced with the non-genetically modified filamentous fungus *Aspergillus niger* strain CTS 2093, which is deposited at the CABI Bioscience Genetic Resource Collection (UK), with the deposit number 388406.[4] This strain has been obtained from a parental strain originally isolated from food, following several rounds of classical mutagenesis, selecting for higher enzyme production and a reduced ability to produce selected toxic metabolites.[5]

The production strain was identified as *A. niger* by [redacted].[6]

[4] Technical dossier/Annex I.2.
[5] Technical dossier/Annex I.3.
[6] Technical dossier/Additional data March 2022/Attachment A.

## 3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[7], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[8]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, [redacted] 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 filtrate 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.[9] 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.[10]

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 catalase is a single polypeptide chain of 730 amino acids.[11] The molecular mass of the mature protein, calculated from the amino acid sequence, is 80.4 kDa. The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches. The gels showed a single major protein band migrating slightly below the 97.4-kDa reference protein, consistent with the expected molecular mass of the enzyme.[12] No other enzymatic activities were reported.[13]

The in-house determination of catalase activity is based on decomposition of hydrogen peroxide (reaction conditions: pH 7.0, 30°C, 25 min). The enzymatic activity is determined by the iodometric determination of residual hydrogen peroxide. The enzyme activity is expressed in Units/g or Units/mL (U/g or U/mL). One unit is defined as the amount of enzyme which degrades one μmol of hydrogen peroxide per minute under the conditions of the assay.[14]

The food enzyme has a temperature optimum around 50°C (pH 7.0) and a pH optimum around pH 7.0 (30°C). Thermostability was tested after a pre-incubation of the food enzyme for 15 min at different temperatures (pH 7.0). Enzyme activity decreased above 50°C, showing no residual activity above 75°C.[15]

### 3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (1–3) and one batch (4) produced for the toxicological tests (Table 1).[16] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 5.9% and the mean enzyme activity/TOS ratio 4,141 U/mg TOS.

[7] 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.
[8] Technical dossier/Additional data March 2022/Attachment B.
[9] Technical dossier/pp. 32–35.
[10] Technical dossier/p. 33 and Annex III.
[11] Technical dossier/p. 17 and Annex VII_1.
[12] Technical dossier/pp. 17–18.
[13] Technical dossier/p. 25.
[14] Technical dossier/p. 21 and Annex II_1.
[15] Technical dossier/p. 21–24.
[16] Technical dossier/pp. 36–37, 50–51 and Annexes: IV and V.

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

| Parameters | Unit | Batches | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4[(a)] |
| **Catalase activity** | U/g batch[(b)] | 202,000 | 256,000 | 263,000 | 249,000 |
| **Protein** | % | 3.2 | 2.4 | 3.1 | 2.7 |
| **Ash** | % | 1.4 | 1.1 | 1.4 | 1.4 |
| **Water** | % | 92.0 | 93.9 | 92.4 | 93.0 |
| **Total organic solids (TOS)[(c)]** | % | 6.6 | 5.0 | 6.2 | 5.6 |
| **Activity/TOS** | U/mg TOS | 3,061 | 5,120 | 4,242 | 4,446 |

(a): Batch used for the toxicological studies.
(b): U: Unit/g (see Section 3.3.1).
(c): TOS calculated as 100% − % water − % ash.

### 3.3.3. Purity

The lead content in the three commercial batches and in the batch used for toxicological studies was below 5 mg/kg,[17,18] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). Arsenic was also determined and found to be <3 mg/kg.[19]

The food enzyme complies with the microbiological criteria for total coliforms, *Escherichia coli* and *Salmonella*, as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[20] No antimicrobial activity was detected in any of the tested batches.[21]

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of aflatoxins, ochratoxin A, sterigmatocystin, T-2 toxin and zearalenone was examined in three food enzyme batches and all were below the limit of detection (LoD) of the applied methods.[22,23] Adverse effects caused by the possible 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 viable cells of the production strain in the food enzyme was demonstrated in three independent batches plated on a non-selective agar and incubated for 6 days. No colonies were produced. A positive control was included.[24]

## 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. The batch 4 (Table 1) used in these studies has a similar composition and activity/TOS value 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, 1997a) and following Good Laboratory Practice (GLP).[25]

[17] Technical dossier/pp. 20, 36 and Annex IV_1.
[18] Limit of quantification (LOQ): Pb = 0.05 mg/kg. As = 3 mg/kg.
[19] Technical dossier/ pp. 20, 36, Annex IV_1 and Additional data March 2022.
[20] Technical dossier/ pp. 20, 36 and Annex IV_1.
[21] Technical dossier/ pp. 20, 36 and Annex IV_2.
[22] Technical dossier/pp. 20, 31, 37 and Annex IV_3.
[23] LoDs: aflatoxins (B1, B2, G1, G2) and ochratoxin A = 0.5 μg/kg each; sterigmatocystin = 20 μg/kg; T2-toxin and zearalenone = 50 μg/kg each.
[24] Technical dossier/Additional data March 2022.
[25] Technical dossier/Annex VI/1. Ames test.

Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2uvrA were used in the presence or absence of metabolic activation (S9-mix), applying the preincubation method. Two experiments were performed in triplicate. A dose-range finding study was carried out at a concentration range between 1,021 and 24,900 U/plate (corresponding to 23 and 5,600 μg TOS/plate) and a main test with five concentrations of the food enzyme (from 1,560 to 24,900 U/plate, corresponding to 350, 700, 1,400, 2,800 and 5,600 μg TOS/mL).

No cytotoxicity was observed at any concentration level of the test substance in either the dose finding study or the main test. 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 on cultured Chinese hamster lung cells (CHL/IU) according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[26]

The dose-finding study was performed at concentrations ranging from 778 to 24,900 U/mL (corresponding to 170 and 5,600 μg TOS/mL) and from 195 to 24,900 U/mL (corresponding to 43 and 5,600 μg TOS/mL) in a short-term treatment (6 h followed by 18 h recovery period) with and without metabolic activation (S9-mix), and in a continuous treatment (24 h) in the absence of S9-mix.

In the continuous treatment, cell growth inhibition over 50% was observed at the highest concentration tested (5,600 μg TOS/mL). No cell growth inhibition was observed in the short-term treatment with or without S9-mix. Based on these results, chromosomal aberrations were scored on cells exposed to the food enzyme at 6,230, 12,500 and 24,900 U/mL (corresponding to 1,400, 2,800 and 5,600 μg TOS/mL) and at 3,110, 6,230 and 12,500 U/mL (corresponding to 700, 1,400 and 2,800 μg TOS/mL) for the short-term treatment with and without S9-mix, respectively. For the continuous treatment, chromosomal aberrations were scored at 1,526, 3,310 and 6,230 (corresponding to 340, 1,400 and 2,800 μg TOS/mL).

Cytotoxic effects were observed at the highest concentration in the short-term treatment with S9-mix (76.8% relative cell growth) and at 3,110 and 6,230 U/mL in the continuous treatment (63.2% and 64.3% relative cell growth, 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 food enzyme did not induce chromosomal 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 followed the guidelines of Japanese Ministry of Health and Welfare (1996)[27] and GLP.[28] The study is in accordance with OECD Test Guideline 408 (OECD, 1998) with the following deviations: detailed clinical observations and functional observations were not performed, urea was not investigated, epididymides were not weighed and in the microscopic examination the regions of the brain examined were not specified. The Panel considered that these deviations are minor and do not impact the evaluation of the study.

Groups of 10 male and 10 female Sprague–Dawley (Crl:CD(SD)) rats received by gavage the food enzyme in doses corresponding to 5.6, 56 and 560 mg TOS/kg body weight (bw) per day. Controls received the vehicle (water for injection).

No mortality was observed.

The feed consumption was statistically significantly increased in low-dose males (+12%) from day 78 to day 85. The Panel considered this change as not toxicologically relevant as it was only recorded for 1 week, it was only observed in one sex, there was no dose–response relationship, there was no statistically significant change in the final feed consumption and there were no statistically significant changes in the final body weight and body weight gain.

The haematological investigation revealed a statistically significant decrease in haematocrit (HCT, −4.6%), haemoglobin (HGB, −4.5%) and red blood cell count (RBC, −4.3%) in high-dose males, a

[26] Technical dossier/Annex VI/2. Chromosomal aberration test.
[27] Guideline for designation of Food Additives and for Revision of standards for use of food additives, Notification 29, Environmental Health Bureau, Ministry of Health and Welfare, Japan, March 22, 1996.
[28] Technical dossier/Annex VI/3. 90-day Study.

decrease in mean corpuscular volume (MCV, −4%) in low-dose males and a decrease in HGB (−3.3%) and in mean corpuscular haemoglobin concentration (MCHC, −1.7%) in high-dose females. The Panel considered these changes as not toxicologically relevant as they were only observed in one sex (all parameters except for HGB), there was no dose–response relationship (HTC, HGB in males, RBC, MCV) and the changes were small (all parameters).

The clinical chemistry investigation revealed a statistically significant increase in the sodium concentration (+0.7%) in mid-dose females. The Panel considered the change as not toxicologically relevant as it was only observed in one sex, the change was small and there was no dose–response relationship.

The urinalysis revealed statistically significant increases in sodium concentration and in the total sodium excretion in low-dose males (+48% and +62%, respectively), in high dose males (+76% and +94%, respectively) and in high-dose females (+63% and +68%, respectively), in the total potassium excretion in low-dose males (+25%) and in low-dose females (+29%) and in chloride concentration and the total chloride excretion in mid-dose males (+43% and +53%, respectively). The Panel considered these changes as not toxicologically relevant as there was no dose–response relationship (potassium, chloride, sodium in males), there were no changes in serum electrolytes and in other relevant clinical chemistry parameters (such as creatinine, blood urea nitrogen) and there were no histopathological changes in the kidneys. Furthermore, the Panel agreed with the applicant that the increased urinary sodium concentration could be due to the urinary excretion of ash sodium present at about 1.4% in the test substance.

Statistically significant changes in organ weights included an increase in absolute liver weight in low-dose males (+14%), a decrease in relative testis weight (−11%) in high-dose males and a decrease in absolute and relative ovary weights (−20% and −23%, respectively) in high-dose females. The Panel considered changes in liver weight as not toxicologically relevant, as the change was small, there was no dose–response relationship and it was observed only in one sex. Concerning the changes in gonad weights seen in both sexes, the Panel noted that, while there were no histopathological findings, the magnitude of change observed in high-dose females constitutes an uncertainty regarding the adversity of these findings.

No other statistically significant or biologically relevant differences to controls were reported.

The Panel identified a no observed adverse effect level (NOAEL) of 56 mg TOS/kg bw per day, the mid-dose tested, based on the reduction of absolute and relative ovary weights and relative testis weight observed in the high-dose groups.

### 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 catalase produced with the genetically modified *A. niger* strain CTS 2093 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, one match was found. The matching allergen was Pen c 30, a catalase from *Penicillium citrinum*.[29]

The matching allergen from *P. citrinum* is a respiratory allergen. In addition, catalase from the fungus *Metarhizium anisopliae* has been found to react with IgE in sera from asthmatic patients (Ward et al., 2009). However, several studies have shown that adults sensitised to an enzyme through the respiratory tract can commonly ingest the corresponding respiratory allergens without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Brisman, 2002; Poulsen, 2004; Armentia et al., 2009).

No information was available on oral and respiratory sensitisation or elicitation reactions of this catalase.

[redacted] and [redacted], substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[30]), are used as raw materials. In addition, [redacted], a known

[29] Technical dossier/pg. 52-52/Annex VII_1.

[30] 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.

source of allergens, is also present 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 materials employed as protein sources are not expected to be present in the food enzyme.

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

## 3.5. Dietary exposure

### 3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in eight food manufacturing processes at the recommended use levels summarised in Table 2.

**Table 2:** Intended uses and recommended use levels of the food enzyme preparation[(d)]

| Food manufacturing process[(a)] | Raw material (RM) | Maximum recommended use level (mg TOS/kg RM)[(b)] |
|---|---|---|
| Baking processes | Flour | **4.8** |
| Cereal-based processes | Flour | **4.8** |
| Coffee processing | Coffee beans | **96** |
| Egg processing | Egg | **4.8** |
| Vegetable processing for vegetable juice production[(c),(e)] | Vegetables | **4.8** |
| Processing of tea, herbal and fruit infusions | Tea leafs | **96** |
| Herring roe processing[(f)] | Herring roe | **2.0** |
| Milk processing for cheese production | Milk | **0.00024** |

TOS: total organic solids.
(a): The name has been harmonised by EFSA according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.
(b): Numbers in bold were used for calculations.
(c): The Panel noted that this food enzyme will not be used in the production of fruit juices (Additional data July 2022/Answer 3 and Attachment A).
(d): Additional data March 2022 and July 2022.
(e): Additional data July 2022/Answer 3.
(f): Additional data July 2022/Answer 2.

In the first six food manufacturing processes shown in Table 2, the food enzyme is used in combination with glucose oxidase. The action of glucose oxidase generates hydrogen peroxide, which is subsequently removed by the catalase activity. In the last two food manufacturing processes shown in Table 2, the foods are first treated with hydrogen peroxide to remove potential microbial contaminations. Catalase is then added to remove excessive hydrogen peroxide. The food enzyme–TOS remains in the final foods.

Based on data provided on thermostability (see Section 3.3.1), the catalase may remain active in vegetable juices and cheese depending on the pasteurisation conditions, but is expected to be inactivated in the other food processes.

In addition to the uses listed in Table 2, the applicant also intends to use this food enzyme in the production of acacia gum at the recommended use level of 6.1 mg TOS/kg crude acacia gum.[31] In the production of acacia gum, crude acacia gum is dissolved in water and treated with hydrogen peroxide and subsequently with catalase.[32] The food enzyme–TOS remains in the acacia gum.

Acacia gum (E 414) is an authorised food additive in the EU according to Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives.[33] In the framework of Regulation (EC) No 1333/2008

[31] Additional data July 2022/Answer 3.
[32] Additional data July 2022/Attachment B/Figure E14.
[33] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on Food additives. OJ L 354, 16.12.2008, pp. 1–33.

and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) re-evaluated acacia gum used as food additive in 2017. The re-evaluation was supported by a public call for occurrence data (usage level and/or concentration data) on acacia gum (E 414). In response to this public call, updated information on the actual use levels of acacia gum (E 414) in foods was made available to EFSA by industry.

### 3.5.2. Dietary exposure estimation

Two sets of estimation were calculated: one for the dietary exposure of the food enzyme–TOS via the uses listed in the Table 2, the other for the use of acacia gum as a food additive.

For the uses listed in Table 2, chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021a). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2021b). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) 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 1 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 3 provides an overview of the derived exposure estimates across all surveys. Detailed mean 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 41 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure at the 95th percentile was estimated to be 3.61 mg TOS/kg bw per day in toddlers.

**Table 3:** 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)** | 0.005–0.750 (12) | 0.017–0.867 (15) | 0.021–0.941 (19) | 0.015–0.538 (21) | 0.014–0.573 (22) | 0.013–0.782 (23) |
| **Min–max 95th percentile (number of surveys)** | 0.018–2.924 (11) | 0.033–3.610 (14) | 0.039–3.208 (19) | 0.027–1.523 (20) | 0.031–2.045 (22) | 0.027–1.734 (22) |

TOS: total organic solids.

Since an exposure assessment to acacia gum (E 414) was carried out by the EFSA ANS Panel as part of the re-evaluation program and published in 2017, the so-derived exposure estimates were used in this opinion and combined with the food enzyme use levels in the assessment of exposure to food enzymes used in the production of acacia gum. For the assessment of acacia gum (E 414), food consumption data were available from 33 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 19 European countries (Appendix B). Chronic exposure estimates were obtained. Two different exposure assessment scenarios were considered by the ANS Panel, i.e. a maximum level exposure assessment and a refined exposure assessment scenario (EFSA ANS Panel, 2017).

For the purposes of assessing exposure to the food enzyme–TOS under assessment, the CEP Panel decided to use the most conservative acacia gum exposure estimates, i.e. data derived using the maximum level exposure scenario. The so-derived exposure estimates to acacia gum were combined with the use level for the food enzyme provided by the applicant (i.e. 6.1 mg TOS/kg crude acacia gum). Table 4 provides an overview of the derived exposure estimates across all surveys. The highest dietary exposure at the 95th percentile was estimated to be 0.018 mg TOS/kg bw per day in infants.

**Table 4:** Summary of dietary exposure to acacia gum (E 414) from their use as food additives in the maximum level exposure assessment scenario and subsequent exposure to the food enzyme, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

| Population group | 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 |
| **Regulatory maximum level exposure scenario (mg acacia gum (E 414)/kg bw per day** | | | | | | |
| **Min–max mean** | 242.1–880.9 | 309.8–1,398.0 | 314.5–1,056.0 | 196.2–671.0 | 87.8–350.9 | 69.2–278.4 |
| **Min–max 95th percentile** | 705.5–2,952.2 | 1,108.08–2,767.2 | 824.4–1,994.0 | 458.1–1,433.1 | 221.6–811.4 | 150.0–657.1 |
| **Estimated exposure (mg TOS/kg bw per day)** | | | | | | |
| **Min–max mean** | 0.001–0.005 | 0.002–0.009 | 0.002–0.006 | 0.001–0.004 | 0.001–0.002 | 0–0.002 |
| **Min–max 95th percentile** | 0.004–0.018 | 0.007–0.017 | 0.005–0.012 | 0.003–0.009 | 0.001–0.005 | 0.001–0.004 |

bw: body weight; TOS: total organic solids.

The data sources of these two sets of exposure estimation are different and should not be summed to derive the highest intake. Therefore, the results are kept separately. Since the estimates shown in Table 3 exceeds those shown in Table 4 by two orders of magnitude, to avoid excessive overestimation, the Panel chose only the estimates shown in Table 3 to derive the margin of exposure.

### 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 5.

**Table 5:** 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 | + |
| Selection of broad FoodEx categories for the exposure assessment | + |
| Use of recipe fractions to disaggregate FoodEx categories | +/– |
| Use of technical factors in the exposure model | +/– |
| Overall intake via all sources of dietary exposure | |
| Only the highest intake at the P95 percentile shown in Table 3 is used to derive the lowest margin of exposure | – |

TOS: total organic solids.
+: Uncertainty with potential to cause overestimation of exposure.
–: Uncertainty with potential to cause underestimation of exposure.

For figures reported in Tables 3 and 4, the conservative approach applied to the exposure estimate to food enzyme–TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to overestimation of the exposure.

The choice of using only figures reported in Table 3 to derive the margin of exposure (MoE) may lead to underestimation of the overall intake. However, estimates in Table 3 exceeds greatly those in Table 4, thus, the underestimation, if it occurs, would be minimal.

### 3.6. Margin of exposure

A comparison of the NOAEL (56 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.005–0.941 mg TOS/kg bw per day at the mean and from 0.018 to 3.61 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure of 16.

## 4. Conclusions

Based on the data provided, the Panel considered the margin of exposure as insufficient to exclude safety concerns for the food enzyme catalase produced with *A. niger* strain CTS 2093 under the intended conditions of use.

## 5. Documentation as provided to EFSA

Application for the Authorisation of Catalase from Aspergillus niger Strain CTS 2093. March 2015. Submitted by Shin Nihon Chemical Co., Ltd.

Additional data. March 2022. Submitted by Shin Nihon Chemical Co., Ltd.

Additional data. July 2022. Submitted by Shin Nihon Chemical Co., Ltd.

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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 |
| HCT | haematocrit |
| HGB | haemoglobin |
| IUBMB | International Union of Biochemistry and Molecular Biology |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| kDa | kiloDalton |
| LoD | limit of detection |
| LOQ | limit of quantification |
| MCHC | mean corpuscular haemoglobin concentration |
| MCV | mean corpuscular volume |
| MoE | margin of exposure |
| OECD | Organisation for Economic Cooperation and Development |
| RBC | red blood cell |
| 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.2023.7843#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 1 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, Spain |
| Toddlers | From 12 months up to and including 35 months of age | Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain |
| Children | From 36 months up to and including 9 years of age | Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Spain, Sweden |
| Adolescents | From 10 years up to and including 17 years of age | Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden |
| 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 |
| 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 |

(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).