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Safety evaluation of the food enzyme catalase from the genetically modified *Aspergillus niger* strain DP-Azw58

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Abstract

The food enzyme catalase (hydrogen-peroxide:hydrogen-peroxide oxidoreductase; EC 1.11.1.6) is produced with the genetically modified *Aspergillus niger* strain DP-Azw58 by Danisco US, Inc. The genetic modifications do not give rise to safety concerns. The food enzyme is considered free from viable cells of the production organism and its DNA. It is intended to be used in egg processing. Based on the maximum use levels, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 1 μg TOS/kg body weight (bw) per day in European populations. 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 1,288 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, results in a margin of exposure of at least 1.3×10^6. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and one 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 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: food enzyme, catalase, hydrogen-peroxide:hydrogen-peroxide oxidoreductase, EC 1.11.1.6, *Aspergillus niger*, genetically modified microorganism

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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 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 "Productos Nievi, SA" for the authorisation of the food enzyme rennet consisting of chymosin and pepsin from stomachs of young calves and sheep, "Avances Bioquímicos Alimentación, SL" for the authorisation of the food enzyme plant coagulant from the flowers of *Cynara cardunculus*, "Mitsubishi-Kagaku Foods Corporation" and "Kikkoman Biochemifa Company" for the authorisation of the food enzyme Tannase from *Aspergillus oryzae* (strains NBRC 110971 and 11-5, respectively) and from "Danisco US Inc." for the authorisation of the food enzymes Alpha-amylase from *Aspergillus niger* (DP-Azb60) and Catalase from a genetically modified strain of *Aspergillus niger* (DP-Azw58).

Following the requirements of Article 12.1 of Commission Regulation (EC) No 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] 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, p. 15–24.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment on the food enzymes rennet consisting of chymosin and pepsin from stomachs of young calves and sheep, plant coagulant from the flowers of *Cynara cardunculus*, Tannase from *Aspergillus oryzae* (strains NBRC 110971 and 11-5, respectively), Alpha-amylase from *Aspergillus niger* (DP-Azb60) and Catalase from a genetically modified strain of *Aspergillus niger* (DP-Azw58) 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 genetically modified *Aspergillus niger* (strain DP-Azw58).

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 a genetically modified *A. niger* (strain DP-Azw58).

Additional information was requested from the applicant during the assessment process on 25 June 2020 and was 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) 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 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 to the methodology described in the CEF Panel 'Statement on the exposure assessment of food enzymes' (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature: catalase
Systematic name : hydrogen-peroxide:hydrogen-peroxide oxidoreductase
Synonyms : catalase-peroxidase
IUBMB No : EC 1.11.1.6
CAS No : 9001-05-2

Catalases catalyse the decomposition of hydrogen peroxide, converting it to water and oxygen. The food enzyme is intended to be used in egg processing.

3.1. Source of the food enzyme

The catalase is produced with a genetically modified *A. niger* strain DP-Azw58 ([redacted])[4] which is deposited in the Westerdijk Fungal Biodiversity Institute (CBS) with the deposit number [redacted].[5] The production strain was identified by [redacted].[6]

3.1.1. Characteristics of the parental and recipient microorganisms

The parental microorganism is the filamentous fungus *A. niger* [redacted].

The recipient strain was developed from the parental strain [redacted]

[4] Technical dossier/Additional data April 2021/Annex AA.
[5] Technical dossier/Additional data April 2021/Annex AB.
[6] Technical dossier/Additional data April 2021/Annex AC.

[redacted][7]

3.1.2. Characteristics of the introduced sequences

[redacted][7]

3.1.3. Description of the genetic modification process

The purpose of the genetic modification was to enable the production strain to synthesise catalase [redacted][9]

3.1.4. Safety aspects of the genetic modification

The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain *A. niger* DP-Azw58 differs from the recipient strain [redacted]

No issues of concern arising from the genetic modifications were identified by the Panel.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[11], with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.

The production strain is grown as a pure culture using a typical industrial medium in a submerged batch or fed-batch fermentation system with conventional process controls in place. After completion of the fermentation and release of the intracellular enzyme by chemical lysis, 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.[12] 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.[13]

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.

[7] Technical dossier/2nd submission/Annex U.
[8] Technical dossier/Additional data April 2021/Annex 1.
[9] Technical dossier/2nd submission/Annex Y and Additional data April 2021/Annex AD.
[10] Technical dossier/2nd submission/Annex Y.
[11] 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.
[12] Technical dossier/2nd submission/Section 3.2.1.2.5.
[13] Technical dossier/Additional data April 2021/Annex AH.

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The catalase is a single polypeptide chain of [redacted] amino acids.[14] The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be [redacted] kDa.[8] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all batches. The gels showed a major protein band corresponding to an apparent molecular mass of about [redacted] kDa, consistent with the expected molecular mass of the enzyme. The protein profile also included bands of lower staining intensity.[15] No other enzymatic activities were reported.

The in-house determination of catalase activity is based on the decomposition of the substrate hydrogen peroxide (reaction conditions: pH 7.0, 25°C, 60 min). The enzymatic activity is determined by iodometric determination of residual hydrogen peroxide. The enzyme activity is expressed in catalase activity units (CAU)/g. One CAU is defined as that amount of catalase which will decompose 264 mg of hydrogen peroxide under the conditions of the assay.[16]

The food enzyme has a temperature optimum between 5°C and 15°C (pH 7.0) and a pH optimum between pH 5.5 and 6.5 (25°C). Thermostability was tested after a pre-incubation of the food enzyme at 95°C for different durations (pH 7.0). Catalase activity greatly decreased after 50 s, with no activity detected after 2 min.[17]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and for the batch produced for the toxicological tests (Table 1).[18] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation is 13.1% and the mean enzyme activity/TOS ratio is 109.1 CAU/mg TOS.

Table 1: Compositional data of the food enzyme

Parameters	Unit				
		1	2	3	4[(a)]
Catalase activity	CAU/g batch[(b)]	8,607	17,463	16,204	30,723
Protein	%	4.57	9.26	9.13	15.65
Ash	%	0.35	0.34	0.57	0.04
Water	%	92.17	83.69	83.67	79.84
Total organic solids (TOS)[(c)]	%	7.48	15.97	15.76	20.12
Activity/mg TOS	CAU/mg TOS	115.1	109.4	102.8	152.7

(a): Batch used for the toxicological studies.
(b): CAU: catalase activity units (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 which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of arsenic, cadmium and mercury in the batch used for toxicological studies were below the limits of detection of the employed methodologies.[19,20]

[14] Technical dossier/1st submission/Annex H.
[15] Technical dossier/2nd submission/p. 37.
[16] Technical dossier/1st submission/Annex D.
[17] Technical dossier/2nd submission/p. 40–42.
[18] Technical dossier/1st submission/Annex S, 2nd submission/p. 36 and Additional data April 2021/Annex AI.
[19] LODs: Pb = 0.02 mg/kg; As = 1 mg/kg; Cd = 0.2 mg/kg; Hg = 0.005 mg/kg.
[20] Technical dossier/1st submission/Annexes F and S and Additional data May 2021/Annex AM.

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). No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).[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, zearalenone and fumonisin was examined in all four food enzyme batches and was below the limit of detection (LOD) of the applied analytical methods.[22,23,24] Adverse effects caused by the potential presence of other secondary metabolites derived from the production strain are addressed by the toxicological examination of the food enzyme-TOS.

3.3.4. Viable cells and DNA of the production strain

The absence of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. [redacted] No growth was observed.[25]

The absence of recombinant DNA in the food enzyme was demonstrated by polymerase chain reaction (PCR) analysis of three batches in triplicate. [redacted][26]

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. Despite its slightly higher activity per unit TOS, batch 4 (Table 1) used in these studies is considered sufficiently representative of the batches used for commercialisation.

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).[27] 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 treat and plate assay. An initial toxicity-mutation assay was carried out using eight different concentrations of the food enzyme (from 1.5 to 5,000 μg total protein/plate, corresponding to 1.93 to 6,443 μg TOS/plate). Based on the results of the initial study, a mutagenicity study was carried out in triplicate using five different concentrations of the food enzyme (50, 150, 500, 1,500 and 5,000 μg/plate, corresponding to 64, 193, 640, 1,930 and 6,443 μg TOS/plate). A reduction of the number of revertants was observed in TA1535 strain in the main experiment in the presence of S9-mix. No cytotoxicity was observed in any other strain and concentration level of the test substance. 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 human peripheral blood lymphocytes according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[28]

[21] Technical dossier/1st submission/Annexes F and S.
[22] Technical dossier/Additional data April 2021/Annex AJ.
[23] LODs: Aflatoxin (total) = 5 μg/kg; Ochratoxin = 5 μg/kg; Sterigmatocystin = 100 μg/kg; T-2 toxin = 25 μg/kg; Zearalenone = 50 μg/kg ; Fumonisin=100 μg/kg.
[24] Technical dossier/1st submission/Annexes F and S, and Additional data April 2021/Annex AK.
[25] Technical dossier/Additional data April 2021/Annex AE.
[26] Technical dossier/Additional data April 2021/Annex AF.
[27] Technical dossier/Annex P.
[28] Technical dossier/Annex Q.

The dose-finding study was performed at concentrations ranging from 0.5 to 5,000 μg of food enzyme/mL (corresponding to 0.64 to 6,443 μg TOS/mL), and no inhibition of cell growth by 50% or more was observed. Based on these results, the cells were exposed to the food enzyme at 2,500, 3,500 and 5,000 μg food enzyme/mL (corresponding to 3,220, 4,510 and 6,443 μg TOS/mL, respectively) in a short-term treatment (4 h followed by 16 h recovery period) with and without metabolic activation (S9-mix), and a continuous treatment (20 h) in the absence of S9-mix. At the highest concentration tested, in the short-term treatment, a mitotic inhibition of 24% and 12% was observed in the presence and in the absence of S9-mix, respectively. At the highest concentration tested, in the continuous treatment without S9-mix, a 46% mitotic inhibition was observed. The frequency of structural and numerical chromosomal aberrations in treated cultures was comparable to that detected in negative controls and within the range of the laboratory historical solvent control data.

The Panel concluded that 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.[29] Groups of 10 male and 10 female Sprague–Dawley (Crl:CD(SD)) rats received by gavage the food enzyme in 250, 500 and 1,000 mg total protein/kg bw per day corresponding, respectively, to 322, 644.3 and 1,288 mg TOS/kg bw per day. Controls received the vehicle (deionised water).

No mortality was observed.

Statistically significant differences in body weight gain as compared to controls included a lower body weight gain on days 43–50 for high-dose males and a higher body weight on days 78–85 for low-dose males. Statistically significant differences to controls in feed intake were limited to an increase in low-dose males on days 15–22 and 85–91 and a decrease in mid-dose females on days 85–91. Feed efficiency was statistically significantly lower in high-dose males on days 1–8, in mid-dose males on days 29–36 and in low-dose males on days 78–85.

The Panel considered the differences in body weight gain, feed intake and feed efficiency not to be of toxicological relevance as they were generally small, sporadic and the body weights during the study and at termination, total body weight gains, total mean daily feed intake and total mean feed efficiency of treated males and females from the mid- and high-dose groups did not statistically significantly differ from controls.

A statistically significant increase in the motor activity as mean number of movements was observed in low- and high-dose males in week 1. This finding was considered by the Panel as incidental.

There was a statistically significant decrease in relative spleen weight in mid-dose males. The Panel considered this finding as incidental in absence of an apparent dose–response relationship and of histopathological changes in the organ.

No other statistically significant differences to controls were observed.

The Panel identified the no observed adverse effect level (NOAEL) of 1,288 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 catalase produced with the genetically modified *A. niger* strain DP-Azw58 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. [redacted].[30]

No information is available on sensitisation and elicitation reactions to the catalase under evaluation.

[redacted] Anaphylaxis, characterised by symptomatic hypotension with associated dyspnoea, urticaria and possibly gastrointestinal symptoms,

[29] Technical dossier/Annex R.
[30] Technical dossier/1st submission/Annex T.

has been observed after exposure to [redacted] as a drug, but is most common after parenteral drug administration and is rare with oral or cutaneous exposure ([redacted] Green and Beezhold, 2011). 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).[31]

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011)[32] are used as raw materials ([redacted]) 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. The food enzyme remains in the egg products.

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 occurring 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 egg processing at a recommended use level of 0.09 to 0.46 mg TOS/kg egg.[33] Egg includes fresh whole egg, egg white and egg yolk.[8]

A flowchart depicting the use of catalase in the egg processing was provided.[34] Whole egg, egg yolk or egg white is treated with hydrogen peroxide. After the pasteurisation step, catalase is added to remove the excess of hydrogen peroxide to obtain pasteurised egg products.

The food enzyme remains in the final pasteurised egg products. The egg products will be further used for production of different foods including bakery products and mayonnaise.

Based on the optimum catalase temperature data provided, 20% was reported as residual enzyme activity after 60 minutes at around 43°C. The catalase thermostability assay was performed at 95°C (see Section 3.3.1), therefore, it could not be used to estimate the degree of enzyme inactivation during egg cold pasteurisation (52°C).

3.5.2. Dietary exposure estimation

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 (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. 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 average 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 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),

[31] Technical dossier/Additional data April 2021/Annex AL.
[32] 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.
[33] Technical dossier/2nd submission/p. 59.
[34] Technical dossier/2nd submission/p. 58.

carried out in 23 European countries (Appendix B). The highest dietary exposure to the food enzyme–TOS was in young children, up to 1 μg TOS/kg bw per day in infants, toddlers and children.

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 (12)	0–0 (16)	0–0 (19)	0–0 (20)	0–0 (22)	0–0 (21)
Min–max 95th percentile (number of surveys)	0–0.001 (10)	0–0.001 (14)	0–0.001 (19)	0–0 (19)	0–0 (22)	0–0 (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	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS	+
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 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 occurrence and use levels of this specific food enzyme, is likely to have led to an overestimation of the exposure.

3.6. Margin of exposure

A comparison of the NOAEL (1,288 mg TOS/kg bw per day) from the 90-day study with the highest exposure estimates (0.001 mg TOS/kg bw per day at the 95th percentile) resulted in margin of exposure (MoE) of at least 1.3×10^6.

4. Conclusions

Based on the data provided and the derived MoE, the Panel concluded that the food enzyme catalase produced with the genetically modified *A. niger* strain DP-Azw58 does not give rise to safety concerns under the intended conditions of use.

The CEP Panel considers the food enzyme free from viable cells of the production organism and recombinant DNA.

Documentation provided to EFSA

1) Dossier "Application for authorisation of catalase from a genetically modified strain of *Aspergillus niger* (DP-Azw58) in accordance with Regulation (EC) No 1331/2008", March 2016. Submitted by Danisco US, Inc.
2) Additional information. April 2021. Submitted by Danisco US, Inc.

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Abbreviations

CAU	catalase activity units
CAS	Chemical Abstracts Service
CBS	Westerdijk Fungal Biodiversity Institute
CEF	EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
FAO	Food and Agriculture Organization of the United Nations
GMO	genetically modified organisms
IUBMB	International Union of Biochemistry and Molecular Biology
LOD	limit of detection
MoE	margin of exposure
NOAEL	no observed adverse effect level
PCR	polymerase chain reaction
SDS–PAGE	sodium dodecyl sulphate–polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	whole genome sequencing
WHO	World Health Organization

Appendix A – Dietary exposure estimates to the food enzyme–TOS in detail

Dietary exposure estimates to the food enzyme–TOS in detail

Information provided in this appendix is shown in an excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2021.6787).

The file contains two sheets, corresponding to two tables.

Table 1: Mean 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).