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Safety evaluation of the food enzyme peroxidase from the genetically modified *Aspergillus niger* strain MOX

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Abstract

The food enzyme peroxidase (phenolic donor: hydrogen-peroxide oxidoreductase, EC 1.11.1.7) is produced with the genetically modified *Aspergillus niger* strain MOX by DSM Food Specialties B.V. 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. The food enzyme is intended to be used in whey processing. Dietary exposure to the food enzyme total organic solids (TOS) was estimated to be up to 0.635 mg 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 2,162 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure resulted in a margin of exposure of at least 3,405. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and no match 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 is 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, peroxidase, phenolic donor: hydrogen-peroxide oxidoreductase, EC 1.11.1.7, lactoperoxidase, *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 microorganisms or products thereof including a product obtained by a fermentation process using microorganisms: (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 Union 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 'Amano Enzyme Inc.', 'DSM Food Specialties BV.' and 'Novozymes A/S' for the authorisation of the food enzymes Glucoamylase from *Rhizopus oryzae* (strain AE-G), Beta-glucosidase from *Penicillium multicolour* (strain AE-GLY), Peroxidase from a genetically modified strain of *Aspergillus niger* (strain MOX), Beta-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-JA) and Triacylglycerol lipase from *Aspergillus niger* (strain AE-L) respectively.

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the 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, 11.03.2011, pp. 15–24.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Glucoamylase from *Rhizopus oryzae* (strain AE-G), Beta-glucosidase from *Penicillium multicolour* (strain AE-GLY), Peroxidase from a genetically modified strain of *Aspergillus niger* (strain MOX), Beta-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-JA) and Triacylglycerol lipase from *Aspergillus niger* (strain AE-L) 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 the food enzyme Peroxidase from a genetically modified strain of *Aspergillus niger* (strain MOX).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme peroxidase from a genetically modified *Aspergillus niger* (strain MOX).

Additional information was requested from the applicant during the assessment process on 27 July 2022 and 27 March 2023 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) 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	Peroxidase
Systematic name	Phenolic donor: hydrogen-peroxide oxidoreductase
Synonyms	Lactoperoxidase, guaiacol peroxidase, plant peroxidase
IUBMB No	EC 1.11.1.7
CAS No	9003-99-0
EINECS No	232-668-6

Peroxidases catalyse the reductive cleavage of hydrogen peroxide by two phenolic donors, resulting in two donor phenoxyl radicals and water. The resulting free radicals may react non-enzymatically with other compounds. The food enzyme under assessment is intended to be used in whey processing.

3.1. Source of the food enzyme

The peroxidase is produced with the genetically modified filamentous fungus *Aspergillus niger* strain MOX [redacted], which is deposited at the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS, the Netherlands) with deposit number [redacted].[4] The production strain was identified as *A. niger* by [redacted].[5]

[4] Technical dossier/Additional data January 2023/Annex 1.
[5] Technical dossier/Additional data January 2023/Annex 2.

3.1.1. Characteristics of the parental and recipient microorganisms

The recipient strain *A. niger* [redacted]

During the genetic modifications used to develop the recipient strain, [redacted].[6]

3.1.2. Characteristics of introduced sequences

The sequence encoding the peroxidase [redacted] was based on the amino acid sequence of the peroxidase from the basidiomycete *Mycetinis scorodonius* (formerly *Marasmius scorodonius*). [redacted][7] [redacted][8] [redacted]

3.1.3. Description of the genetic modification process

The purpose of the genetic modification was to enable the production strain to synthesise peroxidase from *M. scorodonius*.

[redacted][5]

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* MOX differs from the recipient strain in its capacity to synthesise peroxidase from *M. scorodonius*. The absence of the antimicrobial resistance genes used during the genetic modification was confirmed by WGS analysis.[5]

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

[6] Technical dossier/Annex II-3.
[7] Technical dossier/Annexes II-5 and II-7.
[8] Technical dossier/Annexes II-6 and II-8.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[9], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current good manufacturing practice.[10]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-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. The filtrate containing the enzyme is 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. 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.[11]

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 peroxidase is a single-polypeptide chain of 493 amino acids.[12] The molecular mass of the mature protein, calculated from the amino acid sequence, is 52.9 kDa.[13] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[14] A consistent protein pattern was observed across all batches. The gels showed a single major protein band between the marker proteins of 58 and 66 kDa in all batches, consistent with the expected mass of the enzyme. The protein profile also included bands of lesser staining intensity. No other enzyme activities were reported.[15]

The in-house determination of peroxidase activity is based on the oxidation of 2,20-azino-bis (3-ethylbenz-thiazole-6-sulfonic acid) (ABTS) in the presence of hydrogen peroxide (reaction conditions: pH 3.5, 37°C). The enzymatic activity is determined by measuring the release of oxidised ABTS spectrophotometrically at 405 nm. The enzyme activity is expressed in dairy bleaching unit (DBLU). One DBLU is defined as the amount of enzyme that oxidises 1 μmol ABTS per minute under the conditions of the assay.[16]

The food enzyme has a temperature optimum around 55°C (pH 3.5) and a pH optimum around pH 5.0 (37°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 and 60 min at different temperatures. Peroxidase activity decreased above 50°C for both incubation times, with no activity detected at 70°C.[17]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (batches 1–3) and two batches (4 and 5) prepared for the toxicological studies (Table 1).[18] The mean total organic solids (TOS) of the batches for commercialisation was 17.3% and the mean enzyme activity/TOS ratio was 93.8 DBLU/mg TOS.

[9] 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.
[10] Technical dossier/Annex I-5.
[11] Technical dossier/Annex I-7.
[12] Technical dossier/p. 44.
[13] Technical dossier/p. 45.
[14] Technical dossier/p. 42.
[15] Technical dossier/p. 46.
[16] Technical dossier/p. 45 and Annex I-2.
[17] Technical dossier/pp. 46–48.
[18] Technical dossier/p. 41 and Annexes I-1, I-3.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches				
		1	2	3	4[(b)]	5[(c)]
Peroxidase activity	DBLU/g[(a)]	13,450	17,550	16,600	11,935	65,280
Protein	%	7.6	10.4	11.4	ND	ND
Ash	%	0.4	0.5	0.6	1.02	5.53
Water	%	87.0	81.3	78.2	82.6	8.09
Total organic solids (TOS)[(d)]	%	12.6	18.2	21.2	16.4	86.4
Activity/TOS	DBLU/mg TOS	106.7	96.4	78.3	72.8	75.6

(a): DBLU: Dairy bleaching unit (see Section 3.3.1).
(b): Batch used for the genotoxicity studies.
(c): Batch used for the 90-day oral toxicity study in rats.
(d): TOS calculated as 100%–% water–% ash.

3.3.3. Purity

The lead content in the three commercial batches was below 5 mg/kg[19,20] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).

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).[19] No antimicrobial activity was detected in any of the tested batches.[19]

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 fumonisins and ochratoxin A was examined in three food enzyme batches and all were below the limit of detection (LoD) of the applied methods.[19,21] Adverse effects caused by the possible presence of other secondary metabolites were 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 and DNA of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated [redacted].[22]

The absence of recombinant DNA in the food enzyme was demonstrated [redacted].[23] At the request of the Panel, the applicant demonstrated that [redacted]

3.4. Toxicological data[24]

A battery of toxicological tests, including a bacterial reverse mutation test (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats, were provided. The batches 4 and 5 (Table 1) used in these studies have a lower activity/TOS value than the batches used for commercialisation, and thus are considered suitable as test items.

[19] Technical dossier/Annexes I-3, I-4.
[20] LoD: Pb = 1 mg/kg.
[21] LoDs: fumonisins (B1, B2, G1 and G2) = 10 μg/kg each; ochratoxin A = 0.1 μg/kg.
[22] Technical dossier/Additional data January 2023/Annex 3.
[23] Technical dossier/Additional data January 2023/Annex 4.
[24] Technical dossier/Additional data January 2023.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation test (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following good laboratory practice (GLP).[25]

Four strains of Salmonella Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA were used with or without metabolic activation (S9-mix), applying the standard plate incorporation method. The experiment was carried out in triplicate, using five different concentrations of the food enzyme ranging from 62 to 5,000 μg/plate, corresponding to 58–4,680 μg TOS/plate. No cytotoxicity was observed at any concentration of the test substance.

Growth stimulation, evident as a slightly more dense background lawn, was observed at 1,667 μg/plate and above in Salmonella Typhimurium TA98 strain in the presence of S9-mix and at 556 μg/plate and above in *E. coli* WP2uvrA with or without S9-mix.

Upon treatment with the food enzyme, there was no biologically relevant increase in the number of revertant colonies above the control values, in any strain tested, with or without S9-mix.

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

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[26] Two separate experiments were performed with duplicate cultures of human peripheral whole blood lymphocytes. The cell cultures were treated with the food enzyme either with or without metabolic activation (S9-mix).

In the first experiment, cells were exposed to the food enzyme and scored for chromosomal aberrations at concentrations of 625, 1,250 and 2,500 μg/mL (corresponding to 585, 1,170 and 2,340 μg TOS/mL) in a short-term treatment (4 h exposure and 20 h recovery period) either with or without S9-mix.

In the second experiment, cells were exposed to the food enzyme and scored for chromosomal aberrations at concentrations of 1,500, 3,000 and 5,000 μg/mL (corresponding to 1,404, 2,808 and 4,680 μg TOS/mL) in a short-term treatment (4 h exposure and 20 h recovery period) without S9-mix and in a long-term treatment (24 h exposure and 0 h recovery period) without S9-mix.

In the first experiment, the mitotic index of the highest concentration analysed (2,500 μg TOS/mL) was reduced to 66% and 61% of that of the concurrent controls in the presence and in the absence of S9-mix, respectively. In the second experiment, the mitotic index at the highest concentration analysed (5,000 μg TOS/mL) was reduced to 52% and 63% of that of the concurrent controls in the short-term treatment with S9-mix and in long-term treatment without S9-mix, respectively. The frequency of structural and numerical aberrations was not statistically significantly different to the negative controls at any concentration tested.

The Panel concluded that the food enzyme peroxidase did not induce an increase in the frequency of structural and numerical aberrations under the test conditions applied in this study.

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

The repeated dose 90-day oral toxicity study followed OECD Test Guideline 408 (OECD, 1998) and GLP.[27]

Groups of 10 male and 10 female Wistar rats received the food enzyme in the diet in doses of 0.7, 2 and 4%, i.e. 410, 1,100 and 2,400 mg test substance/kg body weight (bw) per day; corresponding to 385, 1,034 and 2,256 mg TOS/kg bw per day in males and 430, 1,200 and 2,300 mg test substance/kg bw per day, corresponding to 404, 1,128 and 2,162 mg TOS/kg bw per day in females.[28] Controls received the diet without food enzyme.

No mortality was observed.

The water consumption was increased in females in week 1, reaching statistical significance on day 4 of administration in low- and high-dose females (+11% and + 12%, respectively). The Panel

[25] Technical dossier/Additional data January 2023/Annex I-A.
[26] Technical dossier/Additional data January 2023/Annex I-B.
[27] Technical dossier/Additional data January 2023/Annex I-C.
[28] Technical dossier/Additional data January 2023.

considered the change as not toxicologically relevant as it was only recorded sporadically, it was only observed in one sex, and there was no dose–response relationship.

In the functional observations, a statistically significant decrease in the mean tail pinch response was observed in low-dose males (−38%). The Panel considered the change as not toxicologically relevant as it was only observed in one sex and there was no dose–response relationship.

Haematological investigations revealed a statistically significant decrease in absolute number of monocytes (−46%) and percentage of monocytes (−40%) in mid-dose males. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex, there was no dose–response relationship, there were no changes in other relevant parameters (other white blood cell parameters).

Clinical chemistry investigations revealed a statistically significant increase in fasting glucose in high-dose females (+18%), a decrease in potassium in high-dose females (−6%) and a decrease in aspartate aminotransferase (ASAT) in low- and high-dose males (−16% and −16%, respectively). The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (all parameters), there was no dose–response relationship (ASAT) and the changes were within the historical control values.[24]

The urinalysis revealed a statistically significant decrease in pH values in high-dose males (−9%), microscopic examination showed a decreased number of crystals in high-dose males (−60%) and an increase in the score for amorphous material in low-dose females. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (all parameters), there was no dose–response relationship (the score for amorphous material) and there were no histopathological changes in kidneys.

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

The Panel identified a no observed adverse effect level (NOAEL) of 2,256 mg TOS/kg bw per day in males and 2,162 mg TOS/kg bw per day in females, the highest doses tested.

3.4.3. Allergenicity

The allergenicity assessment considers only the food enzyme and not carriers or other excipients, which may be used in the final formulation.

The potential allergenicity of the peroxidase produced with the genetically modified *A. niger* strain MOX 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.[29]

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

Upon request, the applicant conducted a literature search of allergic reactions to fungal peroxidases and did not found any relevant reports. However, peroxidase from wheat is a major allergen causing bakers asthma (Sánchez-Monge et al., 1997). Several studies have shown that adults with occupational asthma to a food enzyme may be able to ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Poulsen, 2004; Armentia et al., 2009).

[redacted], a known source of allergens, is 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 no potentially allergenic residues from this source are 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.

[29] Technical dossier/Annex I-12.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in whey processing at the recommended use levels of 2.4–31.9 mg TOS/kg whey.[30]

Whey originating from cheese production (e.g. Cheddar) may contain the colouring agent annatto.[24] Before such whey can be used in other products, the colour should be removed. Peroxidase bleaches annatto by oxidation.[31] The food enzyme-TOS remains in the final whey products.

Based on data provided on thermostability (see Section 3.3.1), it is expected that the peroxidase is inactivated during pasteurisation.

3.5.2. Dietary exposure estimation

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 was estimated to be 0.635 mg TOS/kg bw per day in infants at the 95th percentile.

Table 3: Summary of the 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)	0.006–0.163 (11)	0.023–0.139 (15)	0.024–0.060 (19)	0.007–0.027 (21)	0.006–0.022 (22)	0.005–0.020 (22)
Min–max 95th (number of surveys)	0.035–0.635 (9)	0.053–0.547 (13)	0.051–0.138 (19)	0.021–0.080 (20)	0.019–0.067 (22)	0.013–0.044 (21)

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 4.

[30] Technical dossier/p. 67.
[31] Technical dossier/p. 66.

Table 4: 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	
Assuming that all the whey in foods are bleached whey	+
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	+/–

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

The conservative approach applied to estimate the dietary exposure to the 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.

3.6. Margin of exposure

A comparison of the NOAEL (2,162 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.005–0.163 mg TOS/kg bw per day at the mean and from 0.013 to 0.635 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure (MoE) of at least 3,405.

4. Conclusions

Based on the data provided and the derived margin of exposure, the Panel concluded that the food enzyme peroxidase produced with the genetically modified *Aspergillus niger* strain MOX does not give rise to safety concerns under the intended conditions of use.

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

5. Documentation as provided to EFSA

Application for authorisation of peroxidase from a genetically modified strain of *Aspergillus niger* (strain MOX). May 2015. Submitted by DSM Food Specialties B.V.

Additional data. January 2023. Submitted by DSM Food Specialties B.V.

Additional data. April 2023. Submitted by DSM Food Specialties B.V.

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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
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
MoE	margin of exposure
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
TOS	total organic solids
WGS	whole genome sequence
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.2023.8095#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
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).