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SCIENTIFIC OPINION

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Safety evaluation of the food enzyme asparaginase from the genetically modified *Aspergillus niger* strain ASP

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Abstract

The food enzyme asparaginase (L-asparagine amidohydrolase; EC 3.5.1.1) is produced with the genetically modified *Aspergillus niger* strain ASP by DSM Food Specialties B.V. The genetic modifications do not give rise to safety concerns. The food enzyme was considered free from viable cells of the production organism and its DNA. The food enzyme is intended to be used in the prevention of acrylamide formation in foods and in the processing of yeast and yeast products. Dietary exposure to the food enzyme-total organic solids (TOS) was estimated to be up to 0.792 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 at the highest dose tested of 1038 mg TOS/kg bw per day, which when compared with the estimated dietary exposure, resulted in a margin of exposure of at least 1311. 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 the risk of allergic reactions upon 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

Asparaginase, *Aspergillus niger*, EC 3.5.1.1, EFSA-Q-2013-00895, EFSA-Q-2021-00176, food enzyme, genetically modified microorganism, L-Asparagine amidohydrolase

CONTENTS

1 | INTRODUCTION

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

'Food enzyme' means a product obtained from plants, animals or 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.

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

1.1.1 | Background as provided by the European Commission in 2013

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.

Three applications have been introduced by the companies DSM Food Specialties B.V, Novozymes A/S and Kerry Ingredients & Flavours for the authorisation of the food enzymes asparaginase from a genetically modified strain of *Aspergillus niger* (strain DS 53180), glucoamylase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BE) and a peroxidase obtained from soy bean hulls, 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 three 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 in 2013

The European Commission requests the European Food Safety Authority to carry out the safety assessments of the food enzymes asparaginase from a genetically modified strain of *Aspergillus niger* strain DS 53180), glucoamylase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BE) and a peroxidase obtained from soy bean hulls in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.1.3 | Background as provided by the European Commission in 2021

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.

[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.

Asparaginase from a genetically modified strain of *Aspergillus niger* (strain ASP)[4] is a food enzyme included in the Register of food enzymes to be considered for inclusion in the European Union (EU) Community list and thus subject to risk assessment by the European Food Safety Authority (EFSA). In the initial dossier with reference EFSA-Q-2013-00895, the applicant request for the authorisation of the above food enzyme is baking process and other cereal-based processes, potato processing and the production of processed flavourings from yeast extract in accordance with Regulation (EC) No 1331/2008.

On 26 February 2021, a new application has been introduced by the applicant "DSM Food Specialties B.V." for an extension of the conditions of use for the above food enzyme in coffee processing, fruit and vegetable processing and flavouring production.

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011 implementing Regulation (EC) No 1331/2008, the Commission has verified that the application falls within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

Taking into account that the above food enzyme is subject to a risk assessment by EFSA, in accordance with Regulation (EC) No 1331/2008, it is appropriate to address the safety of the proposed extension of the condition of use within the scientific opinion evaluating the safety of that food enzyme.

1.1.4 | Terms of Reference in 2021

In accordance with Article 29 of Regulation (EC) No 178/2002, the European Commission requests the European Food Safety Authority to carry out the safety assessment of an extension of the conditions of use for the following food enzyme: asparaginase from a genetically modified strain of *Aspergillus niger* (strain ASP) in accordance with Regulation (EC) No 1331/2008, establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

1.2 | Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's requests in 2013 and 2021 to carry out the safety assessment of the food enzyme asparaginase from a genetically modified *Aspergillus niger* strain ASP (DS 53180).

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme Asparaginase from *Aspergillus niger* strain ASP. The dossier was updated on 26 February 2021 with an application for extension of use of the food enzyme.

Additional information requested from the applicant during the assessment process on 08 July 2014, 19 November 2014, 24 March 2015, 16 November 2021, December 2022 and January 2024 was received on 29 October 2014, 15 December 2014, 18 May 2015, 14 September 2022, 7 September 2023 and 12 April 2024, respectively (see 'Documentation provided to EFSA').

Spontaneous additional information was submitted by the applicant in May 2020 (see 'Documentation provided to EFSA').

Following the reception of additional data by EFSA on 14 September 2022, EFSA requested a clarification teleconference on 13 October 2022.

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, 2009a) 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, 2009b) 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. Additional information was requested in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021) and the guidance on the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

[4]The EC provided clarification to the Terms of Reference regarding the name of the production strain on November 2013, specifically, strain name DS 53180 was replaced by strain name ASP.

3 | ASSESSMENT

IUBMB nomenclature	Asparaginase
Systematic name	L-asparagine amidohydrolase
Synonyms	asparaginase II; L-asparaginase; α-asparaginase
IUBMB no	EC 3.5.1.1
CAS no	9015-68-3
EINECS no	232–765-3

Asparaginases catalyse the hydrolysis of L-asparagine, releasing L-aspartic acid and ammonia. The enzyme under application is intended to be used in two food manufacturing processes as described in the EFSA guidance (EFSA CEP Panel, 2023): (1) the prevention of acrylamide formation in foods and (2) the processing of yeast and yeast extracts.

3.1 | Source of the food enzyme

The asparaginase is produced with the genetically modified filamentous fungus *Aspergillus niger* strain ASP ([redacted]), which is deposited in the Westerdijk Fungal Biodiversity Institute culture collection (the Netherlands) with the deposition number [redacted].[5]

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

3.1.1 | Characteristics of the parental and recipient microorganisms

The recipient strain *A. niger* [redacted].[7]

During the genetic modifications used to develop the recipient strain, the [redacted] was inserted and later deleted.

3.1.2 | Characteristics of introduced sequences

The sequence encoding the asparaginase ([redacted] gene) is from *A. niger*. [redacted].[8],[9]

3.1.3 | Description of the genetic modification process

The purpose of the genetic modification was to enable the production strain to overproduce asparaginase. For this purpose, [redacted]

[5]Technical dossier/Spontaneous data submission May 2020/Additional data October 2014/Annex II-16.
[6]Technical dossier/Annex II-2/Additional data September 2022 Annex 1 and additional information April 2024/Annexes 1 and 2.
[7]Technical dossier/Additional information October 2014/Annex II-3.
[8]Technical dossier/Annex II-5 and II-7.
[9]Technical dossier/Annex II-6 and II-8.

[redacted][10]

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* ASP differs from the recipient strain in its ability to overproduce asparaginase. The integration the [redacted] gene was shown by Southern blot analysis.[11] The absence of vector backbone sequences, including the [redacted] gene, was confirmed by Southern blot analysis.[12]

No issues of concern arising from the genetic modification 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,[13] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current good manufacturing practice.[14]

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 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.[15] 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.[16]

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 asparaginase is a single polypeptide chain of 361 amino acids.[17] The molecular mass of the mature protein, calculated from the amino acid sequence, is around 40 kDa.[18] 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 major protein band corresponding to an apparent molecular mass of about 50 kDa, indicating the molecular mass of the enzyme after glycosylation.[19] No other enzymatic activities were reported.[20]

The in-house determination of asparaginase activity is based on the hydrolysis of L-asparagine (reaction conditions: pH 5.0, 37°C, 30 min) and determined by measuring the release of ammonia with phenol nitroprusside detected spectrophotometrically at 600 nm. The asparaginase activity is expressed in asparaginase units/g (ASPU/g). One ASPU is defined as the amount of enzyme required to liberate 1 µmol of ammonia from L-asparagine per minute under the conditions of the assay.[21]

The food enzyme has a temperature optimum around 55°C (pH 5.0) and a pH optimum around pH 4.5 (37°C). Thermostability was tested after a pre-incubation of the food enzyme for different time periods and temperatures (pH 5.0). No residual activity was found above 64°C after 10 min pre-incubation.[22]

[10]Technical dossier/Annexes II-9 and II-10.
[11]Technical dossier/Additional data October 2014/Annex II-12.
[12]Technical dossier/Additional data October 2014/Additional data Part II.
[13]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.
[14]Technical dossier/p. 62/Annex I-5.
[15]Technical dossier/p. 62-69/Annex I-6.
[16]Technical dossier/Annex I-7; Additional data May 2015.
[17]Technical dossier/p. 51–52.
[18]Technical dossier/p. 52.
[19]Technical dossier/p. 50, 84; Additional data October 2014 Part I.
[20]Technical dossier/p. 55; Additional data October 2014 Part I.
[21]Technical dossier/p. 52–53/Annex I-2.
[22]Technical dossier/p. 53–54; Additional data October 2014 Part I.

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches intended for commercialisation and one batch produced for the toxicological tests (Table 1).[23] The mean total organic solids (TOS) of the three food enzyme batches intended for commercialisation was 22.3% and the mean enzyme activity/TOS ratio was 38.1 ASPU/mg TOS.

TABLE 1 Composition of the food enzyme.

		Batches			
Parameters	**Unit**	**1**	**2**	**3**	**4[a]**
Asparaginase activity	ASPU/g[b]	8680	8400	8370	34,552
Protein	%	14.1	14.6	13.1	57.8
Ash	%	0.67	0.68	0.61	2.0
Water	%	77.2	75.7	78.2	8.3
Total organic solids (TOS)[c]	%	22.1	23.6	21.2	89.7
Activity/TOS ratio	ASPU/mg TOS	39.3	35.6	39.5	38.5

[a]Batch used for the toxicological studies.
[b]ASPU/g: Asparaginase Units/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 2 mg/kg[24] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[25]

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).[26] No antimicrobial activity was detected in any of the tested batches.[27]

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, fumonisins, ochratoxin A, HT-2 toxin, T-2 toxin and zearalenone was examined in three food enzyme batches and each was below the limit of detection (LoD) of the applied method.[28,29] 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 was 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 in three independent batches analysed in triplicate. One gram of product was added to [redacted]. No colonies were produced. A positive control was included.[30]

The absence of recombinant DNA in the food enzyme was demonstrated by [redacted] analysis of three batches of the food enzyme in triplicate. No DNA was detected with [redacted].[31]

[23]Technical dossier/p. 50, 83/Annexes: I-1, I-3, I-18, I-19, I-20.
[24]LoD: Pb = 0.006 mg/L sample solution.
[25]Technical dossier/p. 51, 83/Annexes: I-3, I-4.
[26]Technical dossier/p. 51, 83/Annexes: I-3, I-4.
[27]Technical dossier/p. 51, 83/Annexes: I-3, I-4.
[28]Technical dossier/p. 51, 83/Annexes: I-3, I-4; Additional data October 2014 Part I.
[29]LoDs: aflatoxins and ochratoxin A = 0.1 μg/kg each; fumonisins, HT-2 toxin and T-2 toxin = 10 μg/kg each; zearalenone = 3 μg/kg.
[30]Technical dossier/Additional information September 2022/Annex 2.
[31]Technical dossier/Additional information September 2022/Annex 3 and additional information September 2023/Annex 1.

3.4 | Toxicological data

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, was provided. Batch 4 (Table 1) used in these studies has activity/TOS value as the batches used for commercialisation and was considered suitable as a test item.

3.4.1 | Genotoxicity

3.4.1.1 | *Bacterial reverse mutation test*

A bacterial reverse mutation test (Ames test) was made according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following good laboratory practice (GLP).[32]

Four strains of Salmonella Typhimurium (TA1535, TA100, TA1537 and TA98) and *Escherichia coli* WP2uvrA were used in the presence or absence of metabolic activation (S9-mix), applying the 'plate incorporation assay'. One experiment in triplicate was performed using five concentrations of the food enzyme from 62 to 5000 µg/plate, corresponding to 56, 166, 499, 1495 and 4484 µg TOS/plate.

No cytotoxicity was observed at any concentration 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 asparaginase did not induce gene mutations under the test conditions employed in this study.

3.4.1.2 | *In vitro mammalian chromosomal aberration test*

An in vitro mammalian chromosomal aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP in human peripheral blood lymphocytes with and without metabolic activation (S9-mix).[33]

Two separate chromosomal aberration tests were conducted in duplicate cultures. In the first experiment, the cultures were exposed at concentrations of 2000, 3000 and 5000 µg of food enzyme/mL (corresponding to 1794, 2690 and 4484 µg TOS/mL), applying a 4 h treatment followed by 20-h recovery period in the presence and absence of S9-mix. In the second experiment, 3000, 4000 and 5000 µg of food enzyme/mL (corresponding to 2690, 3587 and 4484 µg TOS/mL, respectively) were tested in a short-term treatment with the S9-mix and in a continuous 24-h treatment in the absence of S9-mix.

Slight cytotoxicity was observed after the short-term treatment with and without metabolic activation. The test substance was clearly cytotoxic at the highest concentration tested after the continuous treatment (mitotic index was reduced to 46% of that of the concurrent controls). The enzyme preparation did not induce a significant increase in structural or numerical chromosome aberrations in cultured human blood lymphocytes, in the two independently repeated experiments.

The Panel concluded that the food enzyme asparaginase 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 was performed in accordance with the OECD Test Guideline 408 (OECD, 1998) and following GLP.[34] Groups of 20 male and 20 female Wistar rats (Crl:WI(Wu)) received 0.2%, 0.6% or 1.8% of the food enzyme in the diet in doses corresponding to 117, 351 and 1038 mg TOS/kg body weight (bw) per day for males, and 135, 405 and 1194 mg TOS/kg bw per day for females. Controls received the same diet with no enzyme added.

No mortality was observed.

Haematological investigations showed a statistically significant increase in the absolute differential count (+46%) and the relative differential count (+38%) of monocytes in high-dose males on day 8 of the study, but not on day 44 or at termination, a decrease in the absolute basophile count in low-, mid- and high-dose males (−38%, −31% and −46%, respectively) and in the percentage of basophils in low- and high-dose males (−33% and −33%, respectively) at termination. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (all parameters), the changes were small (all parameters), they were only recorded sporadically (monocytes) and there were no changes in other relevant parameters (total leucocyte count).

Clinical chemistry examinations showed a statistically significant increase in blood urea concentration in low- and mid-dose females on day 44 (+11% and +29%, respectively). The Panel considered the change as not toxicologically relevant as it was not observed at termination, it was only observed in one sex and there was no dose–response relationship.

[32]Technical dossier EFSA-Q-2013-00895/Annex I-18.

[33]Technical dossier EFSA-Q-2013-00895/Annex I-19.

[34]Technical dossier EFSA-Q-2013-00895/Annex I-20.

Urinalysis revealed a statistically significant increase in triple phosphate crystals in the sediment in high-dose males. The Panel considered the change as not toxicologically relevant as it was only observed in one sex and there were no changes in other urinalysis parameters.

Statistically significant changes in organ weights detected were decreases in the relative weights of testes (–9%) and epididymides (–7%) in the low-dose group. The Panel considered the changes as not toxicologically relevant as there was no dose–response relationship (both organs), the changes were small (both organs) and there were no histopathological changes in the testes and epididymides.

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

The Panel identified a no observed adverse effect level (NOAEL) of 1038 mg TOS/kg bw per day, the highest dose tested in males.

3.4.3 | Allergenicity

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

The potential allergenicity of the asparaginase produced with the *Aspergillus niger* strain ASP 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.[35]

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

Asparaginases are used in the treatment of different types of cancer and may cause sensitisation and anaphylactic responses (Bryant, 2001; Marini et al., 2019). However, sequence homology analysis did not reveal matches of the enzyme that is the subject of this application with the asparaginases used in the clinic. Moreover, there are no reports of allergic reaction to asparaginases when consumed via food.

Aspergillus species, including *A. niger*, the production microorganism, are a source of respiratory allergens (Kauffman et al., 1984; Shen & Han, 1998; Vermani et al., 2015). However, several studies have shown that individual adults respiratorily sensitised with occupational asthma to a food enzyme may be able to ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Armentia et al., 2009; Cullinan et al., 1997; Poulsen, 2004).

[redacted], a known source of allergens, is present in the medium fed to the microorganisms. However, during the fermentation process, this product will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. Taking into account the fermentation process and downstream processing, including the removal of the fungal biomass, the Panel considered that potentially allergenic residues from this source are not expected to be present in the food enzyme.

The Panel considered that a 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 two food manufacturing processes at the recommended use levels summarised in Table 2.

TABLE 2 Intended uses and recommended use levels of the food enzyme as provided by the applicant.[36,37,38]

Food manufacturing process[a]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[b]
Prevention of acrylamide formation in foods		
– Baked products	Flour	10–45
– Cereal-based products (e.g. crackers, tortilla chips)	Cereals	10–45
– Cereal-based products for infants	Grain, flour	10–40
– French fries	Potato flour	20–80

(Continues)

[35]Technical dossier/p. 84–86 and Annex I-21.
[36]Technical dossier EFSA-Q-2021-00176/p. 16.
[37]Additional data September 2022/Part I.
[38]Outcome of the clarification teleconference October 2022.

TABLE 2 (Continued)

Food manufacturing process[a]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[b]
– Potato-based snacks (e.g. sliced crisps)	Potatoes	20–80
– Coffee products	Coffee beans	65–**130**
– Fruits and vegetable concentrates	Fruits and vegetables	10–24
– Fruits and vegetable products (e.g. prune puree)	Fruits and vegetables	20–48
Processing of yeast and yeast products	Yeast[39]	120–**160**

[a]The name has been harmonised by EFSA according to the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

[b]Numbers in bold were used for calculation.

For the prevention of acrylamide formation from asparagine, the food enzyme can be added to a variety of raw materials before high temperature treatment (e.g. baking, frying, roasting). For baked/fried/roasted foods, the food enzyme is added to starch-rich food commodities (e.g. flour, potato, coffee, prunes) at various stages. For bread and extruded snacks, it is added to flour or potato flakes during dough making.[40] Green coffee beans are treated before roasting.[41] Potato products are dipped into an enzyme solution before baking.[42] Fruit and vegetable products are treated with the food enzyme after the cutting phase.[43] The asparaginase hydrolyses the free L-asparagine to release L-aspartic acid and ammonia. The food enzyme-TOS remain in the final processed foods.

In yeast processing, the food enzyme is added to yeast[44] to release glutamic acid from glutamine that enhances the flavour of the yeast extracts. The resulting yeast extracts may be further processed with sugars and other raw materials to obtain flavouring preparations that are used as ingredients in a wide range of foods.[45,46] The food enzyme-TOS remains in the yeast extracts.

Based on data provided on thermostability (see Section 3.3.1) and the downstream processing step applied in the food processes, it is expected that this asparaginase will be inactivated during most of the food manufacturing processes but may retain some activity in processes involving less severe conditions.

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, 2021). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2023). 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 48 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 26 European countries (Appendix B). The highest dietary exposure was estimated to be 0.792 mg TOS/kg bw per day in infants at the 95th percentile.

[39]Additional data September 2022/Part I/Answer 5.
[40]Technical dossier 2013–00895/p. 102–103.
[41]Technical dossier 2021–00176/p. 13.
[42]Technical dossier 2013–00895/p. 103.
[43]Technical dossier 2021–00176/p. 12.
[44]Additional information September 2022/part I/Answer 5.
[45]Technical dossier 2013–00895/p. 74.
[46]Additional information September 2022/part I/Answer 5.

TABLE 3 Summary of the estimated dietary exposure to food enzyme–TOS in six population groups.

	Estimated exposure (mg TOS/kg body weight 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
Min–max mean (number of surveys)	0.05–0.312 (12)	0.079–0.250 (15)	0.04–0.188 (19)	0.015–0.12 (21)	0.039–0.087 (22)	0.035–0.075 (23)
Min–max 95th percentile (number of surveys)	0.189–0.792 (11)	0.185–0.715 (14)	0.096–0.362 (19)	0.042–0.229 (20)	0.076–0.168 (22)	0.066–0.127 (22)

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.

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	
Selection of broad FoodEx categories for the exposure assessment	+
Exposure to food enzyme–TOS always calculated based on the recommended maximum use level	+
Although different use levels were provided for the 'prevention of acrylamide formation in foods', the highest value of the recommended maximum use level was used in the calculation	+
The calculation included also cocoa beans as possible raw material, in addition to those reported in Table 2	+
For yeast processing, although the food enzyme is not used to treat yeast cell wall, the food categories chosen for calculation cover also those containing mannoproteins resulting from the treatment of yeast cell wall	+
Use of recipe fractions to disaggregate FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Assumption that 100% of TOS remains in the final foods	+

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

The conservative approach applied to estimate the 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 an overestimation of the exposure.

3.6 | Margin of exposure

A comparison of the NOAEL (1,038 mg TOS/kg bw per day) identified from the 90-day rat study with the derived exposure estimates of 0.015–0.312 mg TOS/kg bw per day at the mean and from 0.042–0.792 mg TOS/kg bw per day at the 95th percentile, resulted in a margin of exposure of at least 1311.

4 | CONCLUSIONS

Based on the data provided and the derived margin of exposure, the Panel concluded that the food enzyme asparaginase produced with the genetically modified *Aspergillus niger* strain ASP 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 asparaginase from a genetically modified strain of *Aspergillus niger*. October 2013. Submitted by DSM Food Specialties.

Application for extension of use of asparaginase from genetically modified strain *Aspergillus niger*. February 2021. Submitted by DSM Food Specialties.

Additional information. October 2014, December 2014, May 2015, September 2022, September 2023, April 2024. Submitted by DSM Food Specialties.

Spontaneous information. May 2020. Submitted by DSM Food Specialties.

Summary report on genetically modified microorganism part report. January 2014. Delivered by National Food Institute, Technical University of Denmark (Lyngby, Denmark).

Summary report on genotoxicity and subchronic toxicity study report. January 2014. Delivered by FoBiG (Freiburg, Germany).

ABBREVIATIONS

bw	body weight
CAS	Chemical Abstracts Service
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
kDa	kiloDalton
LoD	limit of detection
OECD	Organisation for Economic Cooperation and Development
TOS	total organic solids
WHO	World Health Organization

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBERS

EFSA-Q-2013-00895 and EFSA-Q-2021-00176

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NOTE

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

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Dietary exposure estimates to the food enzyme–TOS in details

Appendix A can be found in the online version of this output (in the 'Supporting 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, Republic of North Macedonia*, Serbia*, 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, Republic of North Macedonia*, Serbia*, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina*, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina*, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, 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, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden

*Consumption data from these pre-accession countries are not reported in Table 3 of this opinion; however, they are included in Appendix B for testing purpose.

[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).