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SCIENTIFIC OPINION

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

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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 AGN by DSM Food Specialties B.V. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and its DNA. It is intended to be used to prevent acrylamide formation in food processing. The dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 1.434 mg TOS/kg body weight (bw) per day in European populations. The toxicity studies were carried out with an asparaginase from *A. niger* (strain ASP). The Panel considered this food enzyme as a suitable substitute for the asparaginase to be used in the toxicological studies, because the genetic differences between the production strains are not expected to result in a different toxigenic potential, and the raw materials and manufacturing processes of both food enzymes are comparable. 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 1038 mg TOS/kg bw per day, which, when compared with the estimated dietary exposure, resulted in a margin of exposure of at least 724. 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 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

asparaginase, *Aspergillus niger*, EC 3.5.1.1, food enzyme, genetically modified microorganism, L-asparagine amidohydrolase, α-asparaginase

CONTENTS

1 | INTRODUCTION

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

'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

- it does not pose a safety concern to the health of the consumer at the level of use proposed;
- there is a reasonable technological need;
- its use does not mislead the consumer.

All food enzymes currently on the European Union market and intended to remain on that market, as well as all new food enzymes, shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and approval via an EU Community list.

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.

Two applications have been introduced by the company "DSM Food Specialties B.V." for the authorisation of the food enzymes Asparaginase from a genetically modified strain of *Aspergillus niger* (strain AGN) and Polygalacturonase from a genetically modified strain of *Aspergillus niger* (strain EPG).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011 implementing Regulation (EC) No 1331/2008,[3] the Commission has verified that the two applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Asparaginase from a genetically modified strain of *Aspergillus niger* (strain AGN) and Polygalacturonase from a genetically modified strain of *Aspergillus niger* (strain EPG) 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 asparaginase from a genetically modified strain of *A. niger* (strain AGN).

[1]Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, pp. 7–15.

[2]Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, pp. 1–6.

[3]Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.3.2011, pp. 15–24.

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 a genetically modified strain of *A. niger* (strain AGN).

Additional information was requested from the applicant during the assessment process on 19 February 2015 and 23 January 2023 and received on 20 April 2015 and 7 July 2023 (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, 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).

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 assessment is intended to be used to prevent acrylamide formation during food processing.

3.1 | Source of the food enzyme

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

3.1.1 | Characteristics of the parental and recipient microorganisms

[redacted][6]

[4]Technical dossier/Additional data July 2023/Annex 3.

[5]Technical dossier/Additional data July 2023/Annex 4.

[6]Technical dossier/Annex II-3.

3.1.2 | Characteristics of introduced sequences

The sequence encoding the asparaginase [redacted] was obtained [redacted] from *A. niger*. [redacted][7] [redacted].

[redacted][8] [redacted][9] [redacted].

3.1.3 | Description of the genetic modification process

The purpose of the genetic modification was to enable the production strain to overproduce asparaginase. [redacted][10] [redacted][11] [redacted].

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* AGN differs from the recipient strain [redacted] by its capacity to overproduce asparaginase [redacted][12] [redacted].[13]

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,[14] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice (GMP).[15]

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.[16] 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.[17]

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/Annex II-10.
[8]Technical dossier/Annex II-5 and II-7.
[9]Technical dossier/Annex II-6 and II-8.
[10]Technical dossier/Annex II-9.
[11]Technical dossier/Annex II-11.
[12]Technical dossier/Additional data July 2023/Annex 4.
[13]Technical dossier/Additional data received in 109226/Annex 3.
[14]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.
[15]Technical dossier/Annex I-5.
[16]Technical dossier/pp. 62–67 and Annex I-6.
[17]Technical dossier/Annex I-7 and Additional data July 23/Additional information to EFSA on AGN part I.

3.3 | Characteristics of the food enzyme

3.3.1 | Properties of the food enzyme

The enzyme is a single polypeptide chain of 378 amino acids. The molecular mass of the mature protein, calculated from the amino acid sequence, is around 40 kDa. The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches. The gels showed a major protein band with an apparent molecular mass of about 50 kDa, consistent with the expected mass of the glycosylated enzyme.[18] The protein profile also included other bands of different staining intensity. No other enzymatic activities were reported.

The in-house determination of asparaginase activity is based on the hydrolysis of L-asparagine (reaction conditions: pH 7.0, 37°C, 30 min), determined by measuring the release of ammonia by a colorimetric assay at 600 nm. The activity of asparaginase is expressed in neutral asparaginase units (NASPU). One NASPU is defined as the amount of enzyme required to release one µmol of ammonia from L-asparagine per minute under the conditions of the assay.[19]

The food enzyme has a temperature optimum around 50°C (pH 6.0) and a pH optimum around 6.0 at 37°C. Thermostability was tested after a pre-incubation of the food enzyme at different temperatures for different time periods (pH 7.0). The asparaginase activity decreased above 55°C, showing no residual activity above 65°C after 15 min. incubation.[20]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation.[21] The mean total organic solids (TOS) of the three food enzyme batches was 13.4% and the mean enzyme activity/TOS ratio was 82.0 NASPU/mg TOS (Table 1).

TABLE 1 Composition of the food enzyme.

		Batches		
Parameters	**Unit**	**1**	**2**	**3**
Enzyme activity	NASPU/g[a]	9810	11,000	11,900
Protein	%	6.2	6.6	7.5
Ash	%	0.65	0.45	0.75
Water	%	87.4	87.1	83.7
Total organic solids (TOS)[b]	%	12.0	12.5	15.6
Activity/TOS ratio	NASPU/mg TOS	81.8	88.0	76.3

[a]NASPU: Neutral asparaginase units (see Section 3.3.1).
[b]TOS calculated as 100% – % water – % ash.

3.3.3 | Purity

The lead content in the three commercial batches was below 5 mg/kg,[22,23] 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). No antimicrobial activity was detected in any of the tested batches.[24]

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 ochratoxin A and fumonisins B1, B2 and B3 was examined in the three food enzyme batches and all were below the limit of detection (LoD) of the applied methods.[25,26] 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.

[18]Technical dossier/Additional data July 23/Additional information to EFSA on AGN part I.
[19]Technical dossier/Annex I-2.
[20]Technical dossier/Additional data July 23/Additional information to EFSA on AGN part I.
[21]Technical dossier/pp. 50 and 82.
[22]Technical dossier/Annex I-3.
[23]LoD: 0.004–2 mg/kg.
[24]Technical dossier/Annex I-4.
[25]Technical dossier/Annex I-4; Additional data July 23/Annex 1.
[26]LoDs: ochratoxin A = 0.1 µg/kg; fumonisins (B1, B2, B3) = 10 µg/kg each.

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 [redacted].[27]

The absence of recombinant DNA in the food enzyme concentrate was demonstrated [redacted].[28]

3.4 | Toxicological data

3.4.1 | Choice of test item

No toxicological studies were provided for the asparaginase food enzyme produced with the *A. niger* strain AGN. Instead, the applicant argued that the assessment of this asparaginase could be based on toxicological data from another food enzyme – an asparaginase produced with the *A. niger* strain ASP, previously submitted to EFSA (Question No EFSA-Q-2013-00895) following the EFSA guidance (EFSA, 2009b).

The production strains of both asparaginases were developed from the same recipient strain [redacted] using the same genetic modification system [redacted], with different genes of interest. The genetic modification in *A. niger* ASP only differs from that of *A. niger* AGN in the gene of interest [redacted]. No rounds of mutagenesis have been applied in the development of the production strains from the recipient and all the genetic modifications have been described throughout. No partial inserts of the AGN expression cassette were introduced into the genome, as confirmed by WGS analysis.[29] Therefore, the genetic differences between *A. niger* AGN and *A. niger* ASP are not expected to result in a different toxigenic potential.

The batch of asparaginase from *A. niger* ASP, used for toxicological studies, was produced according to a standard procedure similar to the one described in Section 3.1.5 of this opinion. According to the applicant, the raw materials used[30] and the steps involved in the manufacturing of both asparaginase food enzymes are comparable in both processes,[31] and the temperature and pH conditions used during fermentation are similar. The batch used for toxicological analysis had a TOS content of 89.7%.[32]

In view of the above, the Panel considered the asparaginase produced with the *A. niger* strain ASP as an acceptable substitute for the food enzyme under application.

A battery of toxicological tests made with the substitute food enzyme has been provided, 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.

3.4.2 | Genotoxicity

3.4.2.1 | *Bacterial reverse mutation test*

A bacterial reverse mutation assay (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).[33]

Four strains of *Salmonella* Typhimurium (TA1535, TA100, TA1537, 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 (62, 185, 556, 1667 and 5000 µg/plate, corresponding to 56, 166, 499, 1495 and 4484 µg TOS/plate). No cytotoxicity was observed at any concentration level of the test substance. A slightly denser background lawn was observed with all strains at the highest concentrations tested.

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.

[27]Technical dossier/Additional data July 2023/Annex 5.
[28]Technical dossier/Additional data July 2023/Annex 6.
[29]Technical dossier/Additional data July 2023/Annex 4.
[30]Technical dossier/Additional data July 2023/Annex 8.
[31]Technical dossier/Additional data July 2023/Annex 7.
[32]Technical dossier/Additional data July 2023/Annex 9.
[33]Technical dossier/Annex I-8.

3.4.2.2 | *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).[34]

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 hours treatment in the absence of S9-mix.

A slight cytotoxicity was observed after the pulse treatment with and without metabolic activation. The test substance was clearly cytotoxic at the highest concentration analysed after a 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 chromosomal aberrations in cultured human blood lymphocytes, in either of the two independently repeated experiments.

The Panel concluded that the food enzyme did not induce chromosome aberrations in cultured human blood lymphocytes under the test conditions employed for this study.

3.4.3 | 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.[35] Groups of 20 male and 20 female Wistar WU rats (Crl:WI(Wu)) received 0.2%, 0.6% or 1.8% food enzyme in the feed. These dietary concentrations corresponded to 117, 351 or 1038 mg TOS/kg bw per day for males and to 135, 405 or 1194 mg TOS/kg bw per day for females, based on the registered feed intake. Controls received the feed with no food enzyme added.

No mortality was observed.

Haematological examination revealed a statistically significant increase in the absolute differential count (+46%) and in the relative differential count (+38%) of monocytes (Mono) in the high-dose males in week 2, a decrease in the absolute differential count of basophils (Baso) in the low-, mid- and high-dose males (–38%, –31% and –46%, respectively) and in the relative differential count of Baso in low-dose and high-dose males (–33% and –33%, respectively) at the end of the treatment. The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (Mono, Baso), they were only recorded sporadically (Mono), there was no dose–response relationship (Baso), the changes were small (Mono, Baso) and there were no changes in other relevant parameters (i.e. in the total white blood cell count).

Clinical chemistry investigations revealed a statistically significant decrease in activity of sorbitol dehydrogenase (SDH) in high-dose males (–22%) and in mid- and high-dose females (–19% and –22%, respectively) in week 2, a decrease in activity of alanine aminotransferase (ALT) in low-dose males (–13%) in week 2 and an increase in urea concentration in low- and mid-dose females in week 7 (+11% and +29%, respectively). The Panel considered the changes as not toxicologically relevant, as they were only recorded sporadically (all parameters), they were only observed in one sex (ALT, urea), there was no dose–response relationship (ALT, urea) and the change was small (ALT).

Urinalysis revealed a statistically significant increase in triple phosphate crystals in urinary sediment from high-dose males in week 13. The Panel considered the change as not toxicologically relevant, as it was only observed in one sex and there were no changes in other relevant parameters (e.g. in weight and morphology of the kidneys).

Statistically significant changes in organ weights included a decrease in relative weights of testes (–9%) and epididymides (–7%) in low-dose males. The Panel considered the changes as not toxicologically relevant, as there was no dose–response relationship (both parameters) and the changes were small (both parameters).

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

No adverse effects were observed at the highest dietary concentrations tested, equal to 1038 mg TOS/kg bw per day for males and to 1194 mg TOS/kg bw per day for females.

The Panel identified a no observed adverse effect level (NOAEL) of 1038 mg TOS/kg bw per day.

3.4.4 | 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 *A. niger* strain AGN 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'

[34]Technical dossier/ Annex I-9.

[35]Technical dossier/Annex I-11.

(EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.[36]

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

Asparaginase administered intramuscularly or intravenously is used in the treatment of different types of cancer, where it may cause sensitization (Bryant, 2001; Marini et al., 2019). However, no allergic reactions after oral exposure have been reported.

[redacted], a known source of allergens, is present in the media fed to the microorganisms. However, during the fermentation process, [redacted] 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 the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is low.

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme

The food enzyme is intended to be used to prevent acrylamide formation in various 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.[c]

Food manufacturing process[a]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[b]
Prevention of acrylamide formation in foods		
– Baked products	Flour	1–70
– Cereal-based products (e.g. crackers, tortilla chips)	Cereals	1–70
– French fries	Potato flour	9–**104**
– Potato-based snacks (e.g. sliced crisps)	Potatoes	9–104

Abbreviation: TOS, total organic solids.

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

[b]The number in bold represent the maximum recommended use level which was used for calculation.

[c]Technical dossier/p. 74 and Additional data July 2023.

The food enzyme can be added to a variety of raw materials before high temperature treatment (e.g. baking, frying, roasting) to prevent the formation of acrylamide. For baked/fried/toasted foods, the food enzyme is added to a variety of starch-rich food commodities (e.g. flour, potato) at various stages. For bread and extruded snacks, it is added to flour or potato flakes during dough making. Potato products are blanched with or dipped into an enzyme solution before frying.[37] This enzymatic treatment prevents the formation of acrylamide from asparagine under high temperature processing conditions. The food enzyme–TOS remains in the final processed foods.

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.

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, 2021b). 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

[36]Technical dossier/pp. 83–85 and Annex I-12.

[37]Technical dossier/pp. 98–101.

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 1.434 mg TOS/kg bw per day in infants at the 95th percentile.

TABLE 3 Summary of the estimated dietary exposure to the 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)	0.043–0.627 (12)	0.069–0.291 (15)	0.033–0.150 (19)	0.012–0.095 (21)	0.031–0.069 (22)	0.028–0.061 (23)
Min–max 95th percentile (number of surveys)	0.163–1.434 (11)	0.163–0.937 (14)	0.079–0.299 (19)	0.033–0.182 (20)	0.061–0.134 (22)	0.054–0.102 (22)

Abbreviation: 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 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	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Although different use levels were provided, the highest value of the recommended maximum use level was used in the calculation	+
In addition to the types of raw materials shown in Table 2, the calculation included also coffee beans and plums as possible raw materials	+

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

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 an overestimation of the exposure.

3.6 | Margin of exposure

The comparison of the NOAEL (1038 mg TOS/kg bw per day) from the 90-day study in rats with the derived exposure estimates of 0.012–0.627 mg TOS/kg bw per day at the mean and from 0.033 to 1.434 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure of at least 724.

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 *A. niger* strain AGN does not give rise to safety concerns under the intended conditions of use.

The CEP Panel considered the food enzyme to be 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 (strain AGN). June 2014. Submitted by DSM Food Specialties B.V.

Additional information. April 2015. Submitted by DSM Food Specialties B.V.

Additional information. July 2023. Submitted by DSM Food Specialties B.V.

Summary report on technical data and dietary exposure related to asparaginase from a genetically modified strain of *Aspergillus niger* (strain AGN) by DSM. April 2015. Delivered by contractor Hylobates Consulting (Rome, Italy) and BiCT(Villanova del Sillaro, Italy).

ABBREVIATIONS

ALT	alanine aminotransferase
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
GMP	Good Manufacturing Practice
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
NASPU	neutral asparaginase units
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
RM	raw material
SDH	sorbitol dehydrogenase
TOS	total organic solids
WGS	whole genome sequence
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.

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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

Information provided in this appendix is shown in an excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2024.8617#support-information-section).

The file contains two sheets, corresponding to two tables.

Table 1: Average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey.

Table 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey.

APPENDIX B

Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia[b], Serbia[b], 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[b], Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina[b], Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro[b], Netherlands, Portugal, Romania, Serbia[b], Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina[b], Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro[b], Netherlands, Portugal, Romania, Serbia[b], 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[b], Netherlands, Portugal, Romania, Serbia[b], 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).

[b]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.