

ADOPTED: 3 April 2019

doi: 10.2903/j.efsa.2019.5680

Safety evaluation of the food enzyme alpha-amylase from non-genetically modified *Aspergillus niger* strain (strain DP-Azb60)

EFSA Panel on Food Contact Materials, Enzymes, Processing Aids (CEP),
Vittorio Silano, José Manuel Barat Baviera, Claudia Bolognesi, Beat Johannes Brüscheiler,
Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi,
Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren,
Laurence Vernis, Holger Zorn, Klaus-Dieter Jany*, Boet Glandorf, André Penninks*,
Davor Želježic*, Magdalena Andryszkiewicz, Davide Arcella, Yi Liu, Annamaria Rossi,
Karl-Heinz Engel* and Andrew Chesson

Abstract

The food enzyme alpha-amylase (4- α -D-glucan glucanohydrolase; EC 3.2.1.1) is produced with a non-genetically modified *Aspergillus niger* (strain DP-Azb60) by Danisco US Inc. The food enzyme is free from viable cells of the production organism. The α -amylase is intended to be used in baking processes. Based on the maximum use levels, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.503 mg TOS/kg body weight (bw) per day. Genotoxicity tests with the food enzyme did not indicate a genotoxic 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 (NOAEL) at the highest dose of 1,000 mg TOS/kg bw per day that, compared with the estimated dietary exposure, results in a sufficiently high margin of exposure (of at least 1,988). Similarity of the amino acid sequence to those of known allergens was searched and one match was found to Asp o 21, an alpha-amylase from *Aspergillus oryzae*. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is considered 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.

© 2019 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

Keywords: food enzyme, α -amylase, 4-alpha-D-glucan glucanohydrolase, amylase, glycogenase, EC 3.2.1.1, *Aspergillus niger*

Requestor: European Commission

Question number: EFSA-Q-2016-00273

Correspondence: fip@efsa.europa.eu

* Member of the former Working Group on 'Enzymes' of the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) /Food Contact Materials, Enzymes and Processing Aids (CEP).

Panel members: José Manuel Barat Baviera, Claudia Bolognesi, Beat Johannes Brüscheweiler, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Alicja Mortensen, Gilles Rivière, Vittorio Silano, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn.

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.

Suggested citation: EFSA Panel on Food Contact Materials, Enzymes, Processing Aids (CEP), Silano V, Barat Baviera JM, Bolognesi C, Brüscheweiler BJ, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Jany K-D, Glandorf B, Penninks A, Želježić D, Andryszkiewicz M, Arcella D, Liu Y, Rossi A, Engel K-H and Chesson A. Scientific Opinion on the safety evaluation of the food enzyme alpha-amylase from non-genetically modified *Aspergillus niger* strain (strain DP-Azb60). EFSA Journal 2019;17(5):5680, 14 pp. <https://doi.org/10.2903/j.efsa.2019.5680>

ISSN: 1831-4732

© 2019 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

This is an open access article under the terms of the [Creative Commons Attribution-NoDerivs](https://creativecommons.org/licenses/by/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.



The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.



Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the requestor.....	4
1.1.1. Background as provided by the European Commission.....	4
1.1.2. Terms of Reference.....	4
1.2. Interpretation of the Terms of Reference.....	5
2. Data and methodologies.....	5
2.1. Data.....	5
2.2. Methodologies.....	5
3. Assessment.....	5
3.1. Source of the food enzyme.....	5
3.2. Production of the food enzyme.....	5
3.3. Characteristics of the food enzyme.....	6
3.3.1. Properties of the food enzyme.....	6
3.3.2. Chemical parameters.....	6
3.3.3. Purity.....	7
3.3.4. Viable cells of the production strain.....	7
3.4. Toxicological data.....	7
3.4.1. Genotoxicity.....	7
3.4.1.1. Bacterial reverse mutation test.....	7
3.4.1.2. <i>In vitro</i> mammalian chromosomal aberration test.....	8
3.4.2. Repeated dose 90-day oral toxicity study in rodents.....	8
3.4.3. Allergenicity.....	9
3.5. Dietary exposure.....	9
3.5.1. Intended use of the food enzyme.....	9
3.5.2. Dietary exposure estimation.....	9
3.5.3. Uncertainty analysis.....	10
3.6. Margin of exposure.....	10
4. Conclusions.....	11
Documentation provided to EFSA.....	11
References.....	11
Abbreviations.....	12
Appendix A – Dietary exposure estimates to the food enzyme–TOS in details.....	13
Appendix B – Population groups considered for the exposure assessment.....	14

1. Introduction

Article 3 of the Regulation (EC) No 1332/2008¹ provides definitions 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 entered 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 1331/2008² established European Union 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:

- i) it does not pose a safety concern to the health of the consumer at the level of use proposed;
- ii) there is a reasonable technological need; and
- iii) 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 an approval via a Union list.

The 'Guidance on submission of a dossier on a food enzyme for evaluation' (EFSA CEF Panel, 2009) 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 'Productos Nievi, SA' for the authorisation of the food enzyme rennet consisting of chymosin and pepsin from stomachs of young calves and sheep, 'Avances Bioquímicos Alimentación, SL' for the authorisation of the food enzyme plant coagulant from the flowers of *Cynara cardunculus*, 'Mitsubishi-Kagaku Foods Corporation' and 'Kikkoman Biochemifa Company' for the authorisation of the food enzyme Tannase from *Aspergillus oryzae* (strain NBRC 110971 and 11-5, respectively) and from 'Danisco US Inc.' for the authorisation of the food enzymes Alpha-amylase from *Aspergillus niger* (DP-Azb60) and Catalase from a genetically modified strain of *A. niger* (DP-Azw58).

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

1.1.2. Terms of Reference

The European Commission requests the EFSA to carry out the safety assessments on the food enzymes rennet consisting of chymosin and pepsin from stomachs of young calves and sheep, enzyme plant coagulant from the flowers of *Cynara cardunculus*, Tannase from *A. oryzae* (strain NBRC 110971

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 1493/199, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, p. 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, p. 1–6.

and 11-5), Alpha-amylase from *A. niger* (DP-Azb60) and Catalase from a genetically modified strain of *A. niger* (DP-Azw58) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of the food enzyme α -amylase from *A. niger* (strain DP-Azb60).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier supporting the application for authorisation of the food enzyme α -amylase from a non-genetically modified *A. niger* strain DP-Azb60.

Additional information was sought from the applicant during the assessment process in requests from EFSA sent on 15 November 2017 and on 10 December 2018 and was consequently provided (see 'Documentation provided to EFSA').

Following the request for additional information data sent by EFSA on 15 November 2017, EFSA requested a clarification teleconference, which was held on 15 May 2018.

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,b) and following the relevant existing guidances of EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier for safety evaluation of a food enzyme' (EFSA CEF Panel, 2009) has been followed for the evaluation of this application with the exception of the exposure assessment, which was carried out in accordance with the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature:	Alpha-amylase
Systematic name:	4- α -D-glucan glucanohydrolase
Synonyms:	Endo-amylase, 1,4- α -D-glucan glucanohydrolase
IUBMB No:	EC 3.2.1.1
CAS No:	9000-90-2
EINECS No:	232-565-6.

The enzyme α -amylase catalyses the hydrolysis of α -1,4-glycosidic linkages in starch (amylose and amylopectin), glycogen and related polysaccharides and oligosaccharides, resulting in the generation of soluble dextrans and other oligosaccharides. It is intended to be used in baking processes.

3.1. Source of the food enzyme³

The α -amylase is produced with a non-genetically modified strain of *A. niger* (DP-Azb60), which is deposited at the [REDACTED] with deposit number [REDACTED].⁴

The production strain *A. niger* has been taxonomically identified using whole genome sequence analysis.⁵

3.2. Production of the food enzyme

The food enzyme is manufactured according to Food Hygiene Regulation (EC) No 852/2004⁶, with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with current Good Manufacturing Practice (GMP).⁷

³ Technical dossier/2nd submission/p. 42-47.

⁴ Technical dossier/Additional data April 2018/Annex L.

⁵ Technical dossier/Additional data April 2018.

⁶ 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, 321 pp.

⁷ Technical dossier/2nd submission/p. 47-55 and Technical dossier/1st submission/Annex L/Annex K.

The production strain is grown as a pure culture using a typical industrial medium in a batch or 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 leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained while most of the low molecular weight 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.⁸

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 α -amylase is a single polypeptide chain of [REDACTED] amino acids.⁹ The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. A consistent protein pattern was observed across all batches. One of the three major bands observed in the gel corresponds to an apparent molecular mass of about [REDACTED] kDa which is consistent with the amino acid sequence of the α -amylase.¹⁰ No other enzymatic side activities were reported.¹¹

The in-house determination of α -amylase activity is based on the hydrolysis of starch (reaction conditions: pH 3.8, 60°C, 10 min) and is measured by the rate at which the iodine-staining capacity decreases. The α -amylase activity is quantified relative to an internal enzyme standard and expressed in α -amylase Units/g.¹²

The food enzyme has been characterised with regard to its temperature and pH profiles. It has a temperature maximum around 70°C (pH 4.6) and a pH maximum around pH 3.6 (70°C).¹³ Thermostability was tested after a pre-incubation of the food enzyme for 20 min at different temperatures. Under the conditions (pH 4.6) of the applied temperature stability assay, the α -amylase showed almost no residual activity above 80°C after 5 min incubation.¹⁴

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme have been provided for four food enzyme batches, three batches used for commercialisation and one batch used for the toxicological tests (Table 1).¹⁵ The average total organic solids (TOS) content of the three commercial enzyme batches was 24.99% (range 15.06–30.55%). The average enzyme activity/TOS ratio of the three batches for commercialisation is 7.1 Units/mg TOS.

Table 1: Compositional data provided for the food enzyme/food enzyme preparation

Parameter	Unit	Batch			
		1	2	3	4 ^(a)
α -amylase activity	Units/g ^(b)	1,024	2,136	2,189	1,078
Protein	%	8.93	17.22	17.21	9.10
Ash	%	0.29	0.50	0.25	0.36
Water	%	84.65	70.14	69.20	83.20
Total organic solids (TOS) ^(c)	%	15.06	29.37	30.55	16.44
α -amylase Units/mg TOS	Units/mg TOS	6.80	7.30	7.20	6.56

(a): Batch used for the toxicological studies; also includes 0.51% of preservatives.

(b): Units/g batch: α -amylase activity (see Section 3.3.1).

(c): TOS calculated as 100% – % water – % ash.

⁸ Technical dossier/1st submission/Annex M; Additional information April 2018/Annex E.

⁹ Technical dossier/1st submission/Annex S.

¹⁰ Technical dossier/2nd submission/p. 36 and Additional information April 2018/Annex D.

¹¹ Technical dossier/2nd submission/p. 37 and Annex J.1/J.2.

¹² Technical dossier/1st submission/Annex E.

¹³ Technical dossier/2nd submission/p. 40 and

¹⁴ Technical dossier/2nd submission/p. 41 and Technical dossier/1st submission/Annex J.3.

¹⁵ Technical dossier/2nd submission/p. 35 and Additional information June 2018.

3.3.3. Purity

The lead content in the three commercial batches was below 5 mg/kg¹⁶ and in the batch used for toxicological studies was below 0.05 mg/kg¹⁷ which complies with the specification for lead (\leq 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of arsenic, cadmium and mercury in the batch used for toxicological testing were below the limits of detection of the employed methods.¹⁸

The food enzyme complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *Escherichia coli* and *Salmonella* species are absent in 25 g of sample and total coliforms are not more than 30 colony forming units (CFU) per gram.¹⁹ No antimicrobial activity was detected in any of these batches (FAO/WHO 2006).²⁰

The presence of mycotoxins was reported as negative in the certificate of analysis of the two food enzyme preparation batches.²¹ In addition, the presence of mycotoxins (T-2 toxin, zearalenone, ochratoxin, sterigmatocystin, fumonisin, vomitoxin) was examined in the batch used for toxicological testing and they were below the limits of detection²² of the applied analytical methods.²³

3.3.4. Viable cells of the production strain

The production strain was recorded as absent in the certificate of analysis of the nine different batches for commercialisation.²⁴

3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats has been provided. The batch 4 (Table 1) used in these studies has similar protein pattern as the batches used for commercialization, but has lower chemical purity, and thus is considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

The Ames test was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline of Chemicals No. 471, Bacterial Reverse Mutation Test (OECD, 1997) and following Good Laboratory Practice (GLP) in four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2uvrA in the presence or absence of metabolic activation by S9-mix. The 'treat and plate' method was applied, the only exception was the positive control plates for WP2uvrA in the presence of S9 (2AA) where the plate incorporation method was used.²⁵ Based on the results obtained in the dose range finding test performed at 33.3, 66.7, 100, 333, 667, 1,000, 3,333 and 5,000 μ g/plate of total protein dosed (corresponding to 5.82, 11.66, 17.49, 58.23, 116.65, 174.90, 582.30 and 874.41 μ g TOS/plate, respectively), five concentrations were selected for the main test ranging from 333 to 5,000 μ g/plate (corresponding to 58.23 and 874.41 μ g TOS/plate, respectively) in the absence and in presence of S9-mix. No precipitation and growth inhibition were observed in any strain at any dose level tested. No statistically significant increases in the number of revertant colonies were observed in any tester strain, in the absence or presence of metabolic activation. Therefore, the Panel concluded that the food enzyme has no mutagenic activity under the conditions employed in this study.

¹⁶ LOD: Pb = 5 mg/kg; Additional information June 2018/Annex C, D, E.

¹⁷ LOD: Pb = 0.05 mg/kg; Additional information June 2018/Annex N.

¹⁸ LODs: As = 0.1 mg/kg, Hg = 0.005 mg/kg, Cd = 0.01 mg/kg; Additional information June 2018/Annex N.

¹⁹ Additional information June 2018/Annex C, D, E.

²⁰ Additional information June 2018/Annex C, D and E.

²¹ Additional information June 2018/Annex J.

²² LODs: T-2 toxin = 25 mg/kg; zearalenone = 25 mg/kg; ochratoxin = 0.2 mg/kg; sterigmatocystin = 10 mg/kg; fumonisin = 50 mg/kg; vomitoxin = 200 mg/kg (Additional information June 2018/Annex N).

²³ Technical dossier/1st submission/Annex H.

²⁴ Additional information February 2019/Annex P.

²⁵ Technical dossier/2nd submission/Annex O; Additional information April 2018.

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* chromosome aberration test was carried out according to the OECD Test Guideline 473 (OECD, 2014) and following GLP in human peripheral blood lymphocytes.²⁶ A dose-range finding test was performed at concentrations ranging from 1 to 5,000 μg of total protein/mL, applying a short-term treatment (4 h followed by 18 h recovery) both in the presence and absence of S9-mix, and a continuous treatment (24 h) in the absence of S9-mix. No substantial toxicity was observed at any concentration in any test condition.

Based on these results, the analysis of chromosome aberrations in the main experiment was performed at 1,000, 2,500 and 5,000 $\mu\text{g}/\text{mL}$ (corresponding to 174.88, 437.20 and 874.41 μg TOS/mL, respectively) for all test conditions. Cytotoxicity at the highest dose, detected as reduction in the mitotic index in relation to the vehicle, was not higher than 30%. In all the tested conditions, the frequency of structural and numerical chromosomal aberrations in treated cultures was comparable to the values detected in negative controls and within the range of the laboratory historical solvent control data.

The Panel concluded that the food enzyme α -amylase did not induce chromosome aberrations under the test conditions employed for this study.

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

A repeated dose 90-day oral toxicity study in rodents was performed according to OECD Test Guideline 408 (OECD, 1998), and following GLP.²⁷ Groups of 10 male and 10 female CrI:CD(SD) rats were given by gavage the food enzyme corresponding to 263, 500 and 1,000 mg TOS/kg body weight (bw) per day. The control group received water, which served as a vehicle.

One male in each of the treated groups was either found dead or euthanised due to a gavage error.

Females from all-dose groups had slightly lower body weights throughout the study. This effect appeared in a dose-dependent manner; the difference to controls attained statistical significance at the high dose on days 85 and 90. Final body weights in the low-, mid- and high-dose groups were 3.2, 5.2 and 8.8% lower than controls. Similarly, lower overall body weight gains (days 1–90) were seen in a dose-dependent manner (6.0, 11.4, 17.7%) with a statistical significance to controls in the high-dose group only.

Statistically significantly lower food intake and food efficiency were sporadically recorded during weekly intervals in females from all-dose groups as compared to controls. The overall feed intakes (days 1–90) at low-, mid- and high dose were 3.8, 5.1 and 5.8% lower than in controls. The difference to controls attained statistical significance in the mid- and high dose.

The overall food efficiency (days 1–90) was 2.0, 6.3 and 12.7% lower than controls in the low-, mid- and high-dose females, respectively. The difference to the control group was statistically significant only at the high dose.

The lower body weight, body weight gain, food intake and food efficiency in treated females were considered by the Panel as not adverse as the differences in body weight and feed intake were below 10%. In addition, the clinical condition of animals was not affected and there were other treatment-related effects on other parameters measured. The Panel further noted that body weight and food intake parameters of treated males were comparable to controls.

Among haematological parameters, statistically significant differences to controls included increased red blood cell and platelet counts and decreased mean corpuscular haemoglobin value in mid-dose males, and increased mean corpuscular haemoglobin concentration in mid- and high-dose females.

Among clinical chemistry parameters, the only statistically significant difference to controls was a decreased activity of sorbitol dehydrogenase in the high-dose males.

Urinalysis revealed a statistically significant increase in urine volume and a statistically significant decreased specific gravity in high-dose males.

The differences of haematology, clinical chemistry and urinalysis parameters from controls were minor, lacked dose–response relationship, were confined to one sex and there were no correlative changes in other related parameters. Therefore, these findings were considered by the Panel not to be of toxicological significance.

The absolute ovary weight and the ovary weight relative to brain weight were statistically significantly decreased at the high dose as compared to controls. The differences were minor and in

²⁶ Technical dossier/2nd submission/Annex P.

²⁷ Technical dossier/2nd submission/Annex Q.

the absence of microscopic changes in the organ, they were considered by the Panel as not treatment related.

No other adverse effects attributable to the treatment were observed.

Overall, the Panel identified a no-observed-adverse-effect level (NOAEL) of 1,000 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

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

The potential allergenicity of the α -amylase produced with the non-genetically modified *A. niger* strain DP-Azb60 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, 2017). Using higher than 35% identity in a window of 80 amino acids as the criterion, one match was found with TAKA-amylase-A, also called Asp o 21 an alpha-amylase from *A. oryzae*.¹⁰

No information is available on oral and respiratory sensitisation or elicitation reactions of this α -amylase from *A. niger* strain DP-Azb60. α -amylase from *A. oryzae* (Brisman and Belin, 1991; Quirce et al., 1992, 2002; Sander et al., 1998; Brisman, 2002) is described as an occupational respiratory allergen associated with baker's asthma. However, several studies have shown that adults with occupational asthma to a food enzyme (as described for α -amylase from *A. oryzae*) may be able to ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). Taking into account the wide use of α -amylase, only a low number of case reports has been described in literature focussed on allergic reactions upon oral exposure to α -amylase in individuals sensitised by inhalation to α -amylase (Losada et al., 1992; Quirce et al., 1992; Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004). Therefore, it can be concluded that an allergic reaction upon oral ingestion of this α -amylase, produced with *A. niger* strain DP-Azb60, in individuals sensitised by inhalation to α -amylase cannot be ruled out, but the likelihood of such reaction to occur is considered to be low.

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

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in baking processes at the recommended use level up to 42.3 mg TOS/kg flour.²⁸

In baking processes, α -amylase is used to improve the properties of the final food, such as to increase volume of final bread, to improve crumb structure, colour and taste.

The food enzyme remains in the dough. Based on data provided on thermostability (see Section 3.3.1), it is anticipated that the α -amylase is inactivated during baking processes.

3.5.2. Dietary exposure estimation

For baking processes, chronic exposure was calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016). The assessment involved selection of relevant food categories from the EFSA Comprehensive European Food Consumption Database and application of process and technical conversion factors (Annex B in EFSA CEF Panel, 2016).

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant (see Section 3.5.1) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from individual FoodEx categories was subsequently summed up, averaged over the total survey period 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

²⁸ Technical dossier/2nd submission/p. 59.

the total population and per age class. Surveys with only one day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 2 provides an overview of the derived exposure estimates across all surveys. Detailed average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 35 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B).

Table 2: Summary of estimated dietary exposure to food enzyme–TOS in six population groups

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	Infants	Toddlers	Children	Adolescents	Adults	The elderly
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.008–0.118 (10)	0.089–0.253 (14)	0.102–0.244 (19)	0.055–0.156 (18)	0.042–0.097 (19)	0.041–0.086 (18)
Min–max 95th percentile (number of surveys)	0.046–0.503 (8)	0.223–0.431 (12)	0.199–0.459 (19)	0.124–0.317 (17)	0.091–0.190 (19)	0.082–0.150 (18)

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 3.

Table 3: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/-
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/-
Model assumptions and factors	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS	+
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions in disaggregation FoodEx categories	+/-
Use of technical factors in the exposure model	+/-

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

The conservative approach applied to the exposure estimate to food enzyme–TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to a considerable overestimation of the exposure.

3.6. Margin of exposure

A comparison of the NOAEL (1,000 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.008–0.253 mg TOS/kg bw per day at the mean and from 0.046–0.503 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MOE) of at least 1,988.

4. Conclusions

Based on the data provided, and the derived MOE for baking processes, the Panel concluded that the food enzyme α -amylase produced with the non-genetically modified *A. niger* strain DP-Azb60 does not give rise to safety concerns under the intended conditions of use.

Documentation provided to EFSA

- 1) Dossier 'Alpha-amylase produced by a non-genetically modified strain of *Aspergillus niger* strain DP-Azb60'. March 2015. Submitted by Danisco US Inc.
- 2) Additional information, April 2018. Submitted by Danisco US Inc.
- 3) Additional information, June 2018. Submitted by Danisco US Inc.
- 4) Additional information, February 2019. Submitted by Danisco US Inc.

References

- Armentia A, Dias-Perales A, Castrodeza J, Dueñas-Laita A, Palacin A and Fernández S, 2009. Why can patients with baker's asthma tolerate wheat flour ingestion? Is wheat pollen allergy relevant? *Allergologia et Immunopathologia*, 37, 203–204.
- Baur X and Czuppon AB, 1995. Allergic reaction after eating alpha-amylase (Asp o 2)-containing bread. A case report. *Allergy*, 50, 85–87.
- Brisman J, 2002. Baker's asthma. *Occupational and Environmental Medicine*, 59, 498–502; quiz 502, 426.
- Brisman J and Belin L, 1991. Clinical and immunological responses to occupational exposure to alpha-amylase in the baking industry. *British Journal of Industrial Medicine*, 48, 604–608.
- Cullinan P, Cook A, Jones M, Cannon J, Fitzgerald B and Newman Taylor AJ, 1997. Clinical responses to ingested fungal α -amylase and hemicellulase in persons sensitized to *Aspergillus fumigatus*? *Allergy*, 52, 346–349.
- EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. *EFSA Journal* 2006;5(1):438, 54 pp. <https://doi.org/10.2903/j.efsa.2007.438>
- EFSA (European Food Safety Authority), 2009a. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. *EFSA Journal* 2009;7(5):1051, 22 pp. <https://doi.org/10.2903/j.efsa.2009.1051>
- EFSA (European Food Safety Authority), 2009b. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. *EFSA Journal* 2009;7(5):1051, 22 pp. <https://doi.org/10.2903/j.efsa.2009.1051>
- EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in exposure assessment. *EFSA Journal* 2011;9(3):2097, 34 pp. <https://doi.org/10.2903/j.efsa.2011.2097>
- EFSA CEF Panel (EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids), 2009. Guidance of the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on the submission of a dossier on food enzymes. *EFSA Journal* 2009;7(8):1305, 26 pp. <https://doi.org/10.2903/j.efsa.2009.1305>
- EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2016. Exposure assessment of food enzymes. *EFSA Journal* 2016;14(11):4581, 9 pp. <https://doi.org/10.2903/j.efsa.2016.4581> and Annex B – Process-specific technical data used in exposure assessment of food enzymes (accessible at https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903%2Fj.efsa.2016.4581&file=efs24581-sup-0001-Annex_B.pdf)
- EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Guerche P, Jones H, Manachini B, Messean A, Nielsen EE, Nogue F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Eigenmann P, Epstein M, Hoffmann-Sommergruber K, Koning F, Lovik M, Mills C, Moreno FJ, van Loveren H, Selb R and Fernandez Dumont A, 2017. Guidance on allergenicity assessment of genetically modified plants. *EFSA Journal* 2017;15(5):4862, 49 pp. <https://doi.org/10.2903/j.efsa.2017.4862>
- FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th meeting. FAO JECFA Monographs, 3, 63–67. Available online: <http://www.fao.org/3/a-a0675e.pdf>
- Kanny G and Moneret-Vautrin DA, 1995. Alpha-amylase contained in bread can induce food allergy. *Journal of Allergy and Clinical Immunology*, 95, 132–133.
- Losada E, Hinojosa M, Quirce S, Sánchez-Cano M and Moneo I, 1992. Occupational asthma caused by alpha-amylase inhalation: clinical and immunologic findings and bronchial response patterns. *Journal of Allergy and Clinical Immunology*, 89(1 Pt 1), 118–125.

- Moreno-Ancillo A, Domínguez-Noche C, Gil-Adrados AC and Cosmes PM, 2004. Bread eating induced oral angioedema due to alpha-amylase allergy. *Journal of Investigational Allergology and Clinical Immunology*, 14, 346–347.
- OECD (Organisation for Economic Co-Operation and Development), 1997. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 471: Bacterial reverse mutation test. 21 July 1997. 11 pp. Available online: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en;jsessionid=9zfgzu35paaq.x-oecd-live-01
- OECD (Organisation for Economic Co-Operation and Development), 1998. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 408: Repeated dose 90-day oral toxicity study in rodents. 21 September 1998. 10 pp. Available online: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en
- OECD (Organisation for Economic Co-Operation and Development), 2014. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 473: *In vitro* mammalian chromosomal aberration test. 26 September 2014. 10 pp. Available online: <https://doi.org/10.1787/9789264224223-en>
- Poulsen LK, 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel food. *Molecular Nutrition and Food Research*, 48, 413–423.
- Quirce S, Cuevas M, Díez-Gómez M, Fernández-Rivas M, Hinojosa M, González R and Losada E, 1992. Respiratory allergy to Aspergillus-derived enzymes in bakers' asthma. *Journal of Allergy and Clinical Immunology*, 90(6 Pt 1), 970–978.
- Quirce S, Fernandez-Nieto M, Bartolome B, Bombin C, Cuevas M, Sastre J, 2002. Glucoamylase: another fungal enzyme associated with baker's asthma. *Annals of Allergy, Asthma & Immunology*, 89, 197–202.
- Sander I, Raulf-Heimsoth M, Siethoff C, Lohaus C, Meyer HE and Baur X, 1998. Allergy to Aspergillus-derived enzymes in the baking industry: identification of beta-xylosidase from *Aspergillus niger* as a new allergen (Asp n 14). *Journal of Allergy and Clinical Immunology*, 102, 256–264.

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
CFU	Colony Forming Units
EC	Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kilo Dalton
MOE	Margin of Exposure
NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Cooperation and Development
SDS-PAGE	Sodium Dodecyl Sulfate-PolyAcrylamide Gel Electrophoresis
TOS	Total Organic Solids
WHO	World Health Organization

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

Information provided in this appendix is shown in an excel file (downloadable <https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.5680>).

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, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, United Kingdom
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, United Kingdom
Children ^(a)	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom
The elderly ^(a)	From 65 years of age and older	Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011).