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Safety evaluation of the food enzyme 4-α-glucanotransferase from *Aeribacillus pallidus* (strain AE-SAS)

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Abstract

The food enzyme 4-α-glucanotransferase (1,4-α-D-glucan:1,4-α-D-glucan 4-α-D-glycosyltransferase, EC 2.4.1.25) is produced with a non-genetically modified *Aeribacillus pallidus* (previously identified as *Geobacillus pallidus*) strain from Amano Enzyme Inc. The food enzyme is intended to be used in baking processes and in starch processing for the production of modified dextrins. For baking processes, based on the maximum use levels recommended and individual data from the EFSA Comprehensive European Food Database, dietary exposure to the food enzyme–Total Organic Solids (TOS) was estimated to be up to 0.050 mg TOS/kg body weight (bw) per day. Exposure assessment for the modified dextrins was not considered necessary. Genotoxicity tests did not raise a safety concern. Systemic toxicity was assessed by a repeated dose 90-day oral toxicity study in rats. From this study, the Panel identified a no observed adverse effect level (NOAEL) of at least 900 mg TOS/kg bw per day, the highest dose tested. When the NOAEL value is compared to the estimated dietary exposure to the food enzyme used in baking, this results in a Margin of Exposure (MOE) of at least 18,000. The Panel considers that any additional exposure to the food enzyme from the use of modified dextrins will be covered by the above MOE. A search was made for similarity of the amino acid sequence of the food enzyme with those of known allergens. One match was found with a known respiratory allergen, an α-amylase. The Panel considered that an allergic reaction upon oral ingestion of 4-α-glucanotransferase produced by *A. pallidus* AE-SAS in individuals respiratory sensitised to α-amylase cannot be excluded, but the likelihood is considered to be low. Overall, the Panel concluded that, under the intended conditions of use and based on the data provided, this food enzyme does not give rise to safety concerns.

Keywords: food enzyme, 4-α-glucanotransferase, 1,4-α-D-glucan:1,4-α-D-glucan 4-α-D-glycosyltransferase, EC 2.4.1.25, *Aeribacillus pallidus*

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1. Introduction

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

> 'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.
>
> 'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

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

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;
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 approval via an EU Community list.

The 'Guidance on submission of a dossier on food enzymes for safety 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 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.

Five applications have been introduced by the companies "Danisco US Inc." for the authorisation of the food enzyme Mucorpepsin from *Rhizomucor rniehei*, "Novozymes A/S" for the authorisation of the food enzymes Acetolactate decarboxylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-JB) and Glucose isomerase from *Streptomyces murinus* (strain NZYM-GA), and Amano Enzyme Inc." for the authorisation of the food enzymes 4-alpha-glucanotransferase from *Geobacillus pallidus* (strain AE-SAS) and Tannase from *Aspergillus niger* (strain AE-TAN).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the five applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter 11 of that Regulation.

[1] Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, 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, pp. 1–6.

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

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Mucorpepsin from *Rhizomucor miehei*, Acetolactate decarboxylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-JB), Glucose isomerase from *Streptomyces murinus* (strain NZYM-GA), 4-alpha-glucanotransferase from *Geobacillus pallidus* (strain AE-SAS) and tannase from *Aspergillus niger* (strain AE-TAN) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme 4-α-glucanotransferase from *Geobacillus pallidus* (strain AE-SAS) (now identified as *Aeribacillus pallidus*).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme 4-α-glucanotransferase from *G. pallidus* (now identified as *A. pallidus*) (strain AE-SAS).

Additional information was requested to the applicant during the assessment process on 20 December 2017 and was consequently provided (see 'Documentation provided to EFSA').

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009) and following the relevant existing guidances of EFSA Scientific Committees.

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

3. Assessment

IUBMB nomenclature:	4-α-glucanotransferase
Systematic name:	1,4-α-D-glucan:1,4-α-D-glucan 4-α-D-glycosyltransferase
Synonyms:	amylomaltase; dextrin glycosyltransferase; dextrin transglycosylase; maltodextrin glycosyltransferase
IUBMB No:	EC 2.4.1.25
CAS No:	9032-09-1

The 4-α-glucanotransferase catalyses the hydrolysis of 1,4-α-glycosidic linkages of starch or starch hydrolysates, and successively transfers a segment of (1→4)-α-D-glucans to a new position in an acceptor molecule, which may be glucose or a (1→4)-α-D-glucan. Addition to C3 or C6 of glucose residues can result in a more highly branched structure. The food enzyme is intended to be used in baking processes and starch processing for the production of modified dextrins.

3.1. Source of the food enzyme

The 4-α-glucanotransferase is produced with a strain of the non-genetically modified bacterium *A. pallidus* AE-SAS (previously *G. pallidus* AE-SAS).

The original isolate from which the production strain derives was found in soil and was deposited in the Japanese National Institute of Technology and Evaluation, Patent Microorganisms Depositary under the name *Geobacillus pallidus* with the accession number NITE BP-770.[4] The production strain was derived from the original isolate through conventional mutagenesis. A DNA–DNA hybridisation test made with the type species of *A. pallidus* (Miñana-Galbis et al., 2010) showed a homology greater than 70% and confirmed the identity of the production strain as *A. pallidus*.

[4] Technical dossier/Additional information October 2018/Annex 4.

Strains described as *A. pallidus* or *G. pallidus,* are not generally considered pathogenic. However, as they would previously have been considered to be members of the genus *Bacillus* and as some bacilli are known to produce cytotoxic agents, a cytotoxicity assay was made. Vero cells were exposed to the culture supernatant from *A. pallidus* AE-SAS with lactate dehydrogenase activity as a measure of cell damage. Appropriate positive and negative controls were included. No evidence of cytotoxicity with *A. pallidus* AE-SAS was found.[5]

The production strain was tested for its susceptibility to a battery of antibiotics based on those recommended for the testing of *Bacillus* spp. or for 'other Gram-positive strains'. The minimum inhibitory concentration (MIC) values obtained were in all cases below the cut-off values given in the latest guidance on the testing for antibiotic susceptibility (EFSA FEEDAP Panel, 2018).[6]

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[7], with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with current Good Manufacturing Practice (GMP).

The production strain is grown as a pure culture using a typical industrial medium in a contained, submerged, 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. As a final step, the concentrated food enzyme solution undergoes microfiltration to remove any bacterial cells. 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.[8,9]

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 4-α-glucanotransferase is a single polypeptide of 767 amino acids, including a signal sequence of 34 amino acids. The protein pattern of the food enzyme was examined by size-exclusion chromatography (three batches). Each showed one major protein peak and a number of other minor peaks.[10] However, their identity and molecular mass were not given. The apparent molecular mass based on the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) pattern is about 83 kDa.[11] No enzyme side activities were reported.[12]

The in-house determination of 4-α-glucanotransferase activity is based on the hydrolysis of the substrate maltotetraose (reaction conditions: pH 6.5, 40°C, incubation time 60 min). The enzyme activity is determined by measuring the release of glucose using a colorimetric method. One unit of activity is defined as the quantity of enzyme that liberates 1 μmol of glucose per minute under the conditions of the assay.[13]

The food enzyme has a temperature optimum of 50°C (pH 6.5) and a pH optimum between pH 6.5 and 8.0 (40°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at pH 6.5 and different temperatures. Activity decreases rapidly above 65°C, with about 20% activity remaining at 75°C.[14]

[5] Technical dossier/Additional information October 2018/Annex 6.
[6] Technical dossier/Additional information October 2018/Annex 8.
[7] 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.
[8] Technical dossier/Annexes 5 and 6.
[9] Technical dossier/Additional information October 2018.
[10] Technical dossier/p. 21.
[11] Technical dossier/p. 22.
[12] Technical dossier/p. 23–24.
[13] Technical dossier/Annex 2.
[14] Technical dossier/p. 24–25.

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for four food enzyme batches, three food enzyme batches intended for commercialisation and one batch produced for the toxicological tests (Table 1).[15] The average Total Organic Solids (TOS) of the three commercial batches was 8.2% (w/w) (range 7.9–8.3%). The average enzyme activity/TOS ratio of the commercial food enzyme batches is 103 U/mg TOS (Table 1).

Table 1: Compositional data of the food enzyme

Parameter	Units	Batches			
		1	2	3	4[(a)]
4-α-Glucanotransferase activity	U/g batch[(b)]	11,000	7,000	7,300	11,200
Protein	%	6.7	7.1	6.2	NA[(c)]
Ash	%	0.3	0.4	0.2	0.7
Water	%	91.4	91.3	91.9	90.3
Total Organic Solids (TOS)[(d)]	%	8.3	8.3	7.9	9.0
4-α-glucanotransferase-activity/mg TOS	U/mg TOS	133	84	92	125

(a): Batch used for the toxicological studies.[16]
(b): U/g batch: 4-α-glucanotransferase units/g.
(c): Not analysed.
(d): 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 0.01 mg/kg,[17,18] which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).

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 should not exceed 30 colony forming units (CFU) per gram.[18] No antimicrobial activity was detected in any of these batches.[18]

3.3.4. Viable cells of the production strain

No experimental data was provided on the number of viable cells of the production strain in the food enzyme. However, the Panel notes that the final stage of manufacture involves a microfiltration step using membranes with a pore size sufficient to retain bacterial cells.

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. Batch 4 (Table 1) used for all toxicological testing is considered sufficiently similar to the batches used for commercialisation to be a valid test item.

Although the toxicological tests were made in accordance with the relevant Japanese guidelines, these guidelines are based on and mimic in all important respects those produced by the OECD.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

The Ames test was performed according to the test guidelines (Notification No. 1604, 1999) of the Japanese Ministry of Health and Welfare and complying with the OECD guideline 471 (OECD, 1997)

[15] Technical dossier/p. 21 and Annex 3.
[16] Technical dossier/Additional information October 2018/Annex 1.
[17] LOD: lead = 0.01 mg/kg.
[18] Technical dossier/Annex 3.

and following Good Laboratory Practice (Ordinance No. 21, Ordinance No. 114, 1997 and 2008).[19] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2*uvrA* were used in the presence or absence of metabolic activation, applying the pre-incubation method. Experiments were carried out using five different concentrations (6.25%, 12.5%, 25%, 50% and 100% of the food enzyme, corresponding to 562.5, 1,125, 2,250, 4,500 and 9,000 μg TOS/plate, respectively). No toxicity was observed at any concentration level of the test substance. Upon treatment with the food enzyme there was no significant increase in revertant colony numbers above control values in any strain with or without S9-mix.

The Panel concluded that the food enzyme 4-α-glucanotransferase did not induce gene mutations in the bacterial reverse mutation assay under the conditions of the study.

3.4.1.2. *In vitro* chromosomal aberration test

The *in vitro* chromosome aberration test was carried out according to the test guidelines (Notification No. 1604, 1999) of the Japanese Ministry of Health and Welfare, and following Good Laboratory Practice (GLP) (Ordinance No. 21, Ordinance No. 114, 1997 and 2008).[20] The food enzyme was tested for its ability to induce chromosomal aberrations in Chinese hamster lung fibroblast (CHL/IU) cells at concentrations up to 100% of the food enzyme (corresponding to 9,000 μg TOS/mL final culture concentration). Two experiments were performed. In the short treatment (6 + 18 h recovery) in the presence and in the absence of the S9-mix and in the long treatment (24 + 0 h) in the absence of the S9-mix, the concentrations scored for chromosome aberration were 100%, 50% and 25% of the food enzyme preparation 10,000 μg food enzyme/mL, corresponding to 9,000, 4,500 and 2,225 μg TOS/mL respectively. No cytotoxicity was observed at any concentration tested. The food enzyme did not induce a statistically significant increase in structural or numerical chromosomal aberrations in CHL/IU cells in either of the two independently repeated experiments.

The Panel concluded that the food enzyme 4-α-glucanotransferase 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 was performed according to the test guidelines (Notification No. 29, Notification No. 655, 1996 and 1999) and following GLP (Ordinance No. 21, Ordinance No. 114, Ordinance No. 0613007, 1997 and 2008) of the Japanese Ministry of Health and Welfare.[21] Groups of 12 male and 12 female Sprague–Dawley (Crl:CD(SD)SPF) rats received by gavage 10 mL/kg body weight (bw) per day of the undiluted food enzyme or 50% and 25% dilutions of the food enzyme (doses established following a 14-day dose range finding study). These corresponded to 225, 450 and 900 mg TOS/kg bw per day. Controls received the vehicle (water).

No mortalities occurred during the study. However, abnormally high values in the white blood cells and lymphocyte counts were noted in one high dose male. Necropsy at the end of the study revealed enlargement of the spleen, liver and pancreatic lymph nodes and histopathology confirmed a diagnosis of malignant lymphoma.

Weekly feed intake was statistically significantly higher in mid-dose males from day 52 to the termination with exception on days 66 and 87. For females, a statistically significantly higher feed intake was recorded in low-dose females on days 17 and 21 and in mid-dose females on day 17. As the differences in feed intake were not dose related and there were no test article-related differences in body weights between the control and treated groups, the recorded differences were considered of no toxicological relevance.

A small but statistically significant decrease in red blood cells was recorded in low-dose males, but not in other male dose groups or in females. This observation was considered as incidental. Other than the findings for the single animal with malignant lymphoma, no other significant changes were seen in the haematological parameters measured.

Statistically significant increases in absolute and relative weights of the ovary in high-dose females were seen but these changes were small and not accompanied by any histological differences or by effects on other reproductive sites (uterus, oviduct etc.). A statistically significant increase in absolute weight of the spleen in mid-dose males was recorded but values for the high-dose group appeared compromised by the results for the single animal diagnosed with lymphoma which led to a high

[19] Technical dossier/Annex 7.
[20] Technical dossier/Annex 8.
[21] Technical dossier/Annex 9.1, 9.2 and 9.3.

standard deviation. As relative spleen weights in males did not differ and no effects on absolute and relative spleen weight were shown in females, these results were considered by the Panel as of no toxicological concern. Statistically significant increases in absolute and relative weights of the salivary gland in mid-dose males only were also noted which were not dose dependent and were considered as incidental.

No other statistically significant changes were detected.

The Panel identified that the no observed adverse effect level (NOAEL) was 900 mg TOS/kg bw 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 4-α-glucanotransferase produced with the *A. pallidus* AE-SAS was assessed by comparing its amino acid sequence with those of known allergens according to the scientific opinion on the assessment of allergenicity of genetically modified 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. The matching allergen was Asp o 21, an α-amylase produced by *Aspergillus oryzae*.[22]

No information is available on oral and respiratory sensitisation or elicitation reactions of this 4-α-glucanotransferase.

α-Amylase from *A. oryzae* (Brisman and Belin, 1991; Quirce et al., 1992, 2002; Sander et al., 1998; Brisman, 2002) is known 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*) can ingest the corresponding respiratory allergens without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Poulsen, 2004; Armentia et al., 2009). Taking into account the wide use of α-amylase as a food enzyme, only a low number of case reports has been described in literature that focused on allergic reactions upon oral exposure to α-amylase in individuals respiratory sensitised 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 4-α-glucanotransferase, produced with the *A. pallidus* (strain AE-SAS), 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 considers 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 two food processes. Intended uses and the recommended use levels are summarised in Table 2.

Table 2: Intended use and recommended use levels of the food enzyme as provided by the applicant

Food manufacturing process[(a)]	Raw material	Recommended dosage of the food enzyme
Baking processes	Flour	0.4–4.2 mg TOS/kg flour
Starch processing for the production of modified dextrins	Starch	0.8–8.4 mg TOS/kg starch

TOS: Total Organic Solids.

(a): The description provided by the applicant has been harmonised by EFSA according to the 'EC working document describing the food processes in which food enzymes are intended to be used' - not yet published at the adoption of this opinion.

[22] Technical dossier/Annex 10.

In baking processes, the food enzyme is used to facilitate handling of the dough, improving the baking process and its consistency. The food enzyme is added during the preparation of the dough at the beginning of this process. The food enzyme remains in the dough. Based on data provided on thermostability (see Section 3.3.1), it is anticipated that the 4-α-glucanotransferase is inactivated during baking processes.

In starch processing for production of modified dextrin, the food enzyme is used to increase the degree of branching of gelatinised starch and starch hydrolysate, thus reduces retrogradation. The Panel notes that soluble dextrins are typically subject to extensive purification steps likely to result in the removal of the food enzyme–TOS.

3.5.2. Dietary exposure estimation

For the 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 (Table 2) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period and normalised for bodyweight. 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 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 3 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 3: Summary of estimated dietary exposure to food enzyme–TOS in six population groups following use in baking

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)	0.001–0.012 (10)	0.009–0.025 (14)	0.010–0.024 (19)	0.006–0.015 (18)	0.004–0.010 (19)	0.004–0.009 (18)
Min–max 95th percentile (number of surveys)	0.005–0.050 (8)	0.022–0.043 (12)	0.020–0.046 (19)	0.012–0.031 (17)	0.009–0.019 (19)	0.008–0.015 (18)

TOS: Total Organic Solids.

The exposure estimates provided in Table 3 do not include exposure to the food enzyme when used in starch processing for the production of modified dextrins, because of the difficulty in identifying the foods to which modified dextrins are added. However, the Panel notes that soluble dextrins are typically subject to extensive purification steps likely to result in the removal of the food enzyme–TOS.

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), 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	
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	+/–
Exposure to dextrins from starch modified by the food enzyme was not estimated	–

TOS: Total Organic Solids.
+: uncertainty with potential to cause overestimation of exposure; –: uncertainty with potential to cause underestimation of exposure.

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

3.6. Margin of exposure

A comparison of the NOAEL (900 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.001–0.025 mg TOS/kg bw per day at the mean and from 0.005 to 0.050 mg TOS/kg bw per day at the 95th percentile from baking processes only, resulted in a margin of exposure (MOE) of at least 18,000.

The Panel considers the MOE sufficient to accommodate any potential additional exposure to the food enzyme used for the production of modified dextrins from starch.

4. Conclusions

Based on the data provided and the derived MOE calculated for the baking processes, the Panel concluded that the food enzyme 4-α-glucanotransferase from *A. pallidus* (strain AE-SAS) does not give rise to safety concerns under the intended conditions of use.

Documentation provided to EFSA

1) Technical dossier '4-alpha-glucanotransferase from *Geobacillus pallidus* (strain AE-SAS)'. February 2015. Submitted by Amano Enzyme Inc.
2) Additional information. October 2018. Submitted by Amano Enzyme Inc.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids

CFU	colony forming units
CHL/IU	Chinese hamster lung fibroblast
EC	Enzyme Commission
FAO	Food and Agricultural Organization
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
MIC	minimum inhibitory concentration
MOE	Margin of Exposure
OECD	Organisation for Economic Cooperation and Development
SDS–PAGE	sodium dodecyl sulfate–poly acrylamide 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 (https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.5628).

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 the FoodEx categories to the dietary exposure to the food enzyme–TOS

Appendix B – Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than one day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, 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).