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SCIENTIFIC OPINION

Safety evaluation of the food enzyme protein–glutamine γ-glutamyltransferase from the non-genetically modified *Streptomyces mobaraensis* strain M2020197

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Abstract

The food enzyme protein–glutamine γ-glutamyltransferase (protein–glutamine: amine γ-glutamyltransferase; EC 2.3.2.13) is produced with the non-genetically modified *Streptomyces mobaraensis* strain M2020197 by Taixing Dongsheng Bio-Tech Co. Ltd. The identity of the production strain and the absence of viable cells could not be established. The food enzyme is intended to be used in eight food manufacturing processes: processing of cereals and other grains for the production of (1) baked products, (2) cereal-based products other than baked; processing of dairy products for the production of (3) fermented dairy products, (4) cheese, (5) dairy desserts; processing of plant- and fungal-derived products for the production of (6) meat analogues, (7) plant-based analogues of milk and milk products; processing of meat and fish products for the production of (8) modified meat and fish products. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 3.498 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not indicate a safety concern. The systemic toxicity was assessed by a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 91 mg TOS/kg bw per day. The calculated margin of exposure for each age group was 36 (infants), 26 (toddlers), 50 (children), 99 (adolescents), 115 (adults) and 133 (the elderly). 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 a risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is low. The safety of the food enzyme could not be established given the derived margins of exposure. Therefore, the Panel concluded that the food enzyme could not be considered safe under the intended conditions of use.

KEYWORDS

EC 2.3.2.13, food enzyme, genetically modified microorganism, protein–glutamine γ-glutamyltransferase, protein–glutamine: amine γ-glutamyltransferase, *Streptomyces mobaraensis*, transglutaminase

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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:

- 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 EU 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.[2]

On 2 July 2021, a new application was introduced by the applicant "Taixing Dongsheng Bio-Tech Co., Ltd." for the authorization of the food enzyme Transglutaminase from a non-genetically modified strain of *Streptomyces mobaraensis* M2020197.

1.1.2 | Terms of Reference

The European Commission requests EFSA to assess the safety and possible confidentiality requests of the food enzyme Transglutaminase from a non-genetically modified strain of *Streptomyces mobaraensis* M2020197, in accordance with the Regulation (EC) No 1331/2008, establishing a common authorization procedure for food additives, food enzymes and food flavourings.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant submitted a dossier in support of the application for authorisation of the food enzyme transglutaminase from a non-genetically modified strain of *Streptomyces mobaraensis* M2020197. The dossier was updated on 29 June 2021.

Additional information was requested from the applicant during the assessment process on 19 December 2022 and received on 27 January 2023 (see 'Documentation provided to EFSA').

[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.

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2.2 | Methodologies

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

The 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021) and the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023) have been followed for the evaluation of the application.

2.3 | Public consultation

According to Article 32c (2) of Regulation (EC) No 178/2002[3] and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 3 August to 24 August 2023.[4] No comments were received.

3 | ASSESSMENT

IUBMB nomenclature	Protein–glutamine γ-glutamyltransferase
Systematic name	Protein–glutamine: amine γ-glutamyltransferase
Synonyms	Transglutaminase; Factor XIIIa; fibrinoligase
IUBMB No	EC 2.3.2.13
CAS No	80,146–85-6
EINECS No	616–952-0

Protein–glutamine γ-glutamyltransferases catalyse the amide-transferase reaction between the γ-glutamyl group of glutamine residues and the ε-amino group of lysine residues in proteins, resulting in intra- and intermolecular cross-linkings of proteins. In the absence of amino substrates, transglutaminases catalyse the deamidation of glutamyl residues involving water as an acyl acceptor.

The enzyme under assessment is intended to be used in eight food manufacturing processes: processing of cereals and other grains for the production of (1) baked products, (2) cereal-based products other than baked; processing of dairy products for the production of (3) fermented dairy products, (4) cheese, (5) dairy desserts; processing of plant- and fungal-derived products for the production of (6) meat analogues and (7) plant-based analogues of milk and milk products; processing of meat and fish products for the production of (8) modified meat and fish products.

3.1 | Source of the food enzyme

The protein–glutamine γ-glutamyltransferase is produced with the non-genetically modified bacterium *Streptomyces mobaraensis* strain M2020197, which is deposited at the China Center for Type Culture Collection (CCTCC) with deposit number [redacted].[5] *S. mobaraensis* [redacted] was obtained from the wild-type strain *S. mobaraensis* DSM 40587 by selection for high levels of transglutaminase activity.

In order to identify the production strain, its whole genome sequence (WGS) was compared to the WGS of the parental strain (*S. mobaraensis* DSM 40587), but not to that of the type strain (*S. mobaraensis* DSM 40847) that is publicly available. As the parental strain has not been unequivocally identified, no conclusion can be made on the identity of the production strain.

The WGS of the production strain was searched for sequences involved in toxigenicity or virulence and no match of concern was found.[6] Insufficient information was provided to allow the appraisal of the methodology used and the outcome of the analysis on AMR genes in the genome.[7]

As the identity of the production strain could not be established and the absence of genes of concern was not confirmed, the Panel was unable to evaluate the safety of the production strain.

[3]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.
[4]Available at: https://connect.efsa.europa.eu/RM/s/publicconsultation2/a0l0900000D94B0/pc0608
[5]Technical dossier/Source of the Food Enzyme/Annex C.
[6]Technical dossier/Source of the Food Enzyme/pp. 4–5.
[7]Technical dossier/Source of the Food Enzyme/pp. 6–12.

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3.2 | Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[8] with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current Good Manufacturing Practice.[9]

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 and centrifugation. The filtrate is then further purified and concentrated, including an ultrafiltration step. It is then acidified, centrifuged several times and precipitated with [redacted]. The enzyme paste obtained is finally [redacted].[10] The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[11]

The Panel considered that sufficient information has been provided on the manufacturing process and the quality assurance system implemented by the applicant to exclude issues of concern.

3.3 | Characteristics of the food enzyme

3.3.1 | Properties of the food enzyme

The protein–glutamine γ-glutamyltransferase is a single polypeptide chain of 331 amino acids.[12] The molecular mass of the mature protein, calculated from the amino acid sequence, is 37.8 kDa.[13] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.[14] A consistent protein pattern was observed across all batches. The gel showed a single major protein band corresponding to an apparent molecular mass of about 35 kDa, consistent with the expected mass of the enzyme. No other enzymatic activities were reported.[15]

The in-house determination of protein–glutamine γ-glutamyltransferase activity [redacted].[16]

The food enzyme has a temperature optimum between [redacted] and [redacted] (pH [redacted]) and a pH optimum between pH [redacted] and [redacted] ([redacted]°C). The thermostability was tested after a pre-incubation of the food enzyme up to 16 min at different temperatures. The enzyme activity decreased above 55°C, showing no residual activity above 70°C.[17]

3.3.2 | Chemical parameters

Complete data on the chemical parameters of the food enzyme were provided for three batches (Table 1).[18] The mean total organic solids (TOS) of the three food enzyme batches was 91.1% and the mean enzyme activity/TOS ratio was 13.8 U/mg TOS.

[8]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.
[9]Technical dossier/Manufacturing process/p. 1, p. 6/Annex F; Additional data January 2023/Manufacturing process.
[10]Technical dossier/Manufacturing process/pp. 1-3/Annex F.
[11]Technical dossier/Manufacturing process/p. 1/Annex F.
[12]Technical dossier/Chemical composition, properties, purity/pp. 1–2.
[13]Technical dossier/Chemical composition, properties, purity/p. 1.
[14]Additional data January 2023/Chemical composition, properties, purity.
[15]Technical dossier/Chemical composition, properties, purity/p. 6.
[16]Technical dossier/Chemical composition, properties, purity/p. 3/Annex A.
[17]Technical dossier/Chemical composition, properties, purity/pp. 3–6.
[18]Technical dossier/Manufacturing process/p. 5/Chemical composition, properties, purity/Annex D/Methods of analysis.

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TABLE 1 Composition of the food enzyme.

Parameters	Unit	Batches		
		1	2[a]	3[b]
Protein–glutamine γ-glutamyltransferase activity	U/g[c]	13,851	13,742	10,184
Protein	%	85.3	85.0	85.2
Ash	%	5.8	5.7	6.0
Water	%	3.0	3.2	3.2
Total organic solids (TOS)[d]	%	91.2	91.1	90.9
Activity/TOS ratio	U/mg TOS	15.2	15.1	11.2

[a]Batch used for the Ames test.

[b]Batch used for the *in vitro* micronucleus assay and the repeated dose 90-day oral toxicity study in rats.

[c]UNIT: U (see Section 3.3.1).

[d]TOS calculated as 100% – % water – % ash.

3.3.3 | Purity

The lead content in the three batches was below 5 mg/kg,[19] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). For arsenic, the average concentration determined in the three batches was 0.2 mg/kg.[20,21] The Panel considered this concentration as not of concern.

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).[22] No antimicrobial activity was detected in any of the tested batches.[23]

The presence of ochratoxin A, aflatoxin (B1, B2, G1 and G2), zearalenone, sterigmatocystin and T-2 toxin was examined in the three food enzyme batches and were below the limits of quantification (LoQs) of the applied analytical methods.[24,25]

The Panel considered that the information provided on the purity of the food enzyme was sufficient.

3.3.4 | Viable cells of the production strain

The absence of viable cells of the production strain in the food enzyme was not demonstrated due to shortcomings in the detection procedure, such as the method used to determine the identity of detected colonies and a lack of appropriate positive controls.[26]

3.4 | Toxicological data

A battery of toxicological tests, including a bacterial reverse mutation test (Ames test), an *in vitro* micronucleus test and a repeated dose 90-day oral toxicity study in rats, has been provided.

The batches 2 and 3 (Table 1) used in these studies were those used for commercialisation and were considered suitable as test items.

3.4.1 | Genotoxicity

3.4.1.1 | *Bacterial reverse mutation test*

A bacterial reverse mutation test (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP).[27]

[19]Technical dossier/Manufacturing process/p. 4/Chemical composition, properties, purity/Annex D/Methods of analysis/GB_T-5009.74-2003-limit-test-heavy-metals-in-food-additives_eng; Additional data January 2023/Applicant's comments/Methods of analysis.

[20]Technical dossier/Manufacturing process/p. 4/Chemical composition, properties, purity/Annex D/Methods of analysis GB_T-5009.74-2003-limit-test-heavy-metals-in-food-additives_eng.

[21]LoQ: Pb = 0.05 mg/kg; As – LoD/LoQ – not provided.

[22]Technical dossier/Manufacturing process/p. 4/Chemical composition, properties, purity/Annex D/Methods of analysis.

[23]Technical dossier/Manufacturing process/p. 4/Chemical composition, properties, purity/Annex D/Methods of analysis/Antimicrobial Activity Analysis Method.

[24]Technical dossier/Manufacturing process/p. 6/Chemical composition, properties, purity/Annex D/Methods of analysis.

[25]LoQs: ochratoxin A = 1 μg/kg; aflatoxin B1 and G1 = 0.1 μg/kg; aflatoxin B2 and G2 = 0.03 μg/kg, zearalenone = 20 μg/kg; sterigmatocystin and T-2 toxin = 10 μg/kg.

[26]Technical dossier/Source of the Food Enzyme/Annex E.

[27]Technical dossier/Risk assessment/Toxicological data/Genotoxicity/Annex G.

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Five strains of *Salmonella* Typhimurium (TA98, TA100, TA102, TA1535 and TA1537) were used with or without metabolic activation (S9-mix), applying the standard plate incorporation method (experiment I) and the pre-incubation method (experiment II). The experiments were carried out in triplicate, using six different concentrations of the food enzyme ranging from 31.6 to 5000 µg/plate, corresponding to 29, 91, 288, 911, 2277 and 4555 µg TOS/plate.

Precipitation was observed d in both experiments at 2277 µg TOS/plate and above with and without S9-mix. No cytotoxicity was observed at any concentration tested in any tester strain. Upon treatment with the food enzyme, there was no biologically relevant increase in the number of revertant colonies above the control values in any strain tested, with or without S9-mix.

The Panel concluded that the food enzyme protein–glutamine γ-glutamyltransferase did not induce gene mutations under the test conditions applied in this study.

3.4.1.2 | In vitro *mammalian cell micronucleus test*

The *in vitro* mammalian cell micronucleus test was carried out according to the OECD Test Guideline 487 (OECD, 2014) and following GLP.[28] Two experiments were performed with duplicate cultures of human peripheral whole blood lymphocytes. The cell cultures were treated with the food enzyme with or without metabolic activation (S9-mix).

In a pre-experiment, no cytotoxicity above 50% was seen at any concentration tested up to 5000 µg/mL with and without S9-mix. On the basis of these results, in the first experiment, cells were exposed to the food enzyme and scored for the frequency of binucleated cells with micronuclei (MNBN) at concentrations of 3000, 4000 and 5000 µg/mL (corresponding to 2727, 3636 and 4545 µg TOS/mL) with a short-term treatment (4 h exposure and 40 h recovery period), either with or without S9-mix. In the second experiment, cells were exposed to the food enzyme and scored for MNBN at concentrations of 2000, 3000 and 5000 µg/mL (corresponding to 1818, 2727 and 4545 µg TOS/mL) with a long-term treatment (44 h exposure without recovery period) without S9-mix.

No cytotoxicity or precipitation was seen, neither with the short-term treatment with or without S9-mix, nor with the long-term treatment. The frequency of MNBN was not statistically significantly different to the negative controls at any concentrations tested.

The Panel concluded that the food enzyme protein–glutamine γ-glutamyltransferase did not induce an increase in the frequency of MNBNs under the test conditions applied in this study.

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

The repeated dose 90-day oral toxicity study was performed following GLP[29] and in accordance with the OECD Test Guideline 408 (OECD, 1998). Groups of 10 male and 10 female Wistar Crl: WI(Han) (SPF) rats received the food enzyme by gavage in doses of 100, 300 or 1000 mg/kg body weight (bw) per day, corresponding to 91, 273 and 909 mg TOS/kg bw per day. Controls received the vehicle (water for injection, sterile water).

One mid-dose female and one control male were found dead on Days 25 and 60, respectively. Furthermore, three animals were euthanized due to a disturbed clinical condition: a high-dose male on Day 14, a mid-dose female on Day 20 and a high-dose female on Day 40. Clinical signs (abnormal breathing and/or cyanosis) in the one mid-dose female and the high-dose rats suggested a gavage accident. At microscopic examination, the cause of death could not be established in any of the animals euthanised prematurely. Microscopic stress-related findings (thymic atrophic/involution, adrenocortical hypertrophy, vacuolation of adrenal cortex, decreased secretion in seminal vesicles and vaginal mucification) were variably observed in all animals examined. Moreover, in high-dose animals, changes in the glandular stomach, indicative of a local irritant effect were observed with minimal severity. The Panel considered that the local irritant effect of the test-item in the airways, related to gavage technique, could have been the cause of the clinical signs leading to euthanasia in one mid-dose female and two high-dose rats.

The body weight was statistically significantly decreased at the end of the first week of administration in high-dose males (−6%). The Panel considered the change as not toxicologically relevant, as it was only recorded on one occasion and in one sex, the change was small and without a statistically significant effect on the final body weight and the final body weight gain.

The total and average feed consumption (Days 1–90) was slightly decreased in all treated groups (males: −2%, −6% and −6%, females: −4%, −7% and −3% in the low-, mid- and high-dose groups, respectively). The Panel considered the changes as not toxicologically relevant, as they were without a statistically significant effect on the final body weight and the final body weight gain.

In the functional observations, a statistically significant increase in the score for an animal sleeping in the cage in mid- and high-dose females (0.5 and 0.5, vs. 0.0 in the controls) and a decrease in a score for an animal moving in the cage in mid-and high-dose females (−50% at both doses) in Week 6, and a decrease in the spontaneous motor activity in mid- and high-dose males in Week 11 (−13% and −15%, respectively) and in high-dose males in Week 12 (−25%) were recorded. The Panel considered these changes as not toxicologically relevant, as they were only recorded sporadically (all parameters), they were only observed in one sex and there was no dose–response relationship (parameters in Week 6).

[28]Technical dossier/Risk assessment/Toxicological data/Genotoxicity/Annex G.

[29]Technical dossier/Risk assessment/Toxicological data/Subchronic toxicity/Annex G.

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Haematological investigations revealed a statistically significant decrease in the percentage of lymphocytes in high-dose males (–16%). The Panel considered the change as not toxicologically relevant, as it was only observed in one sex, the change was small and there were no changes in other relevant parameters (e.g. in total number of white blood cells).

Clinical chemistry investigations revealed a statistically significant decrease in blood urea in low- and high-dose males (–13% and 18%, respectively). The Panel considered the change as not toxicologically relevant, as it was only observed in one sex, there was no dose–response relationship and there were no histopathological correlates in the kidneys.

Microscopic examination of the animals surviving to the end of the treatment revealed histopathological changes in the glandular stomach in the mid- and high-dose groups with minimal to moderate severity. These were mucosal necrosis (males: 1/10 and 5/9 vs. 1/9 in the control group; females: 2/8 and 2/9 vs. 0/10 in the control group), inflammation (males: 3/10 and 6/9 vs. 0/9 in the control group; females: 2/8 and 3/9 vs. 0/10 in the control group), regeneration of mucosa (males: 5/10 and 3/9 vs. 0/10 in the control group, females: 2/9 in the high-dose group vs. 0/10 in the control group), and mucus neck cell hypertrophy at the high dose only (males: 4/9 vs. 0/9 in the control group; females 2/9 vs. 0/10 in the control group). The Panel considered the changes in the glandular stomach as test-item-related. They could be a sequelae of local contact with an irritant test item, as indicated by mucus neck cell hypertrophy, although other mechanisms could not be excluded.

In the lungs, inflammation in the high-dose group (males 3/9 vs. 1/10 in the controls; females 3/9 vs. 0/10) and alveolar macrophages (males 6/9 vs. 3/9 in the controls; females 3/9 vs. 0/10) were observed. The Panel considered the changes as related to the gavage technique, i.e. to incidental influx or regurgitation of the dosing solution.

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

The Panel identified a no observed adverse effect level (NOAEL) of 91 mg TOS/kg bw per day, based on the changes observed in the glandular stomach at the mid- and high-doses.

3.4.3 | Allergenicity

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

The potential allergenicity of the protein–glutamine γ-glutamyltransferase produced with the *S. mobaraensis* strain M2020197 was assessed by comparing its amino acid sequence with those of known allergens according to the 'Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms' (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.[30]

No information was available on oral and respiratory sensitisation or elicitation reactions of this protein–glutamine γ-glutamyltransferase.

A case report (Sander et al., 2020) has shown that transglutaminase can cause occupational asthma. However, several studies have shown that adults with occupational asthma may be able to ingest respiratory allergens without acquiring clinical symptoms of food allergy (Armentia et al., 2009; Brisman, 2002; Poulsen, 2004). Transglutaminase has recently been implicated in the pathogenesis of celiac disease due to its functional similarity to endogenous tissue transglutaminase, which is an autoantigen of coeliac disease (Lerner & Matthias, 2019; Torsten & Aaron, 2018). The potential immunogenic effect of these transglutaminase complexes could be brought about by cross-linking of transglutaminase to proteins that may be structurally similar to gluten, thus triggering immunological reactions in individuals with celiac disease (BfR, 2011). However, the food products to which the enzyme is added are typically thermally treated, resulting in the inactivation of the enzyme, thus rendering it unable to form these cross-linked products.

[redacted], a product that may cause allergies (listed in the Regulation (EU) No 1169/2011[31]) is used as a raw material. In addition, [redacted], a known source of allergens, is also present in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the yeast biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that no potentially allergenic residues are present in the food enzyme.

The Panel considered that a risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is low.

[30]Technical dossier/Allergenicity/pp. 1-3/Annex H.

[31]Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

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3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme

The food enzyme is intended to be used in eight food manufacturing processes at the recommended use levels summarised in Table 2.[32]

TABLE 2 Intended uses and recommended use levels of the food enzyme as provided by the applicant.

Food manufacturing process[a]	Raw material (RM)	Maximum use level (mg TOS/kg RM)[b]	
Processing of cereals and other grains			
• Production of baked products	Flour	24.8	Bread products
		54.2	Fine bakery products
		57.8	Raw doughs and pre-mixes
		57.8	Pizza and pizza-like dishes
• Production of cereal-based products other than baked	Flour	**57.8**	Breakfast cereals
		17.34	Pasta and similar
Processing of dairy products			
• Production of fermented dairy products	Milk	**4.34**	
• Production of cheese	Milk	**97.56**	
• Production of dairy desserts[c]	Milk	**17.34**	
Processing of plant- and fungal-derived products			
• Production of meat analogues[c]	Vegetable protein	**21.69**	
• Production of plant-based analogues of milk and milk products	Vegetable protein	**43.35**	Cheese analogues
	Vegetable protein	8.67	Yoghurt analogues
	Soybeans	19.87	Tofu
Processing of meat and fish products			
• Production of modified meat and fish products	Meat and fish	**65.04**	

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

[b]The numbers in bold were used for calculation.

[c]These food manufacturing processes were not included in the (EFSA CEP Panel, 2023). Appendix C was created to assist their calculation.

In all of the involved food manufacturing processes, the transglutaminase catalyses the cross-linking between glutamine and lysine residues, modifying the physical properties (e.g. breaking strength and moisture retention) of the foods and impacts their sensory attributes such as mouthfeel.[33]

In the production of baked products and cereal-based products other than baked, the food enzyme is added to flour during the mixing step to make dough.[34] The food enzyme–TOS remains in the final foods.

In the production of fermented dairy products, the food enzyme is added to milk usually during the fermentation step.[35] The food enzyme–TOS remains in the final foods.

In the production of cheese, the food enzyme is usually added to milk together with the starter culture during coagulation.[36] The food enzyme–TOS remains in the final foods.

In the production of dairy desserts, the food enzyme is added to milk usually after pasteurisation and during the cooling step.[37] The food enzyme–TOS remains in the final foods.

[32]Technical dossier/Intended uses and use levels/Table 4.7.1.4–1.

[33]Technical Dossier/Intended Use(s) in Food and Use Level(s) (Proposed Normal and Maximum Use Levels), p. 1.

[34]Additional information January 2023/Annex J 221223.

[35]Technical Dossier/Annex J/pp. 8–9.

[36]Technical Dossier/Annex J/p. 1.

[37]Technical Dossier/Annex J/p. 2.

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In the production of meat analogues, the food enzyme is added to vegetable proteins during the mixing, cutting and emulsifying steps.[38] The food enzyme–TOS remains in the final foods.

In the production of plant-based analogues of milk and milk products, the food enzyme is usually added to plant materials during the fermentation step.[39] The food enzyme–TOS remains in the final foods.

In the production of modified meat and fish products, the food enzyme is added to meat or fish during the mixing, the cutting and the emulsifying steps[40] in all the described processes except in the production of mechanically separated meat (MSM), in which it is added during the transfer of the MSM to the dosing machine for packaging.[41] The food enzyme–TOS remains in the final foods.

The applicant measured the transglutaminase activity in a selection of foods produced without thermal treatment and found that the residual activity was negligible.[42] Together with the data on thermostability (see Section 3.3.1), the Panel considered that the food enzyme is inactivated in most of the processed foods (e.g. ripened cheese, sausages, canned meat products). However, the enzyme may remain active in some foods (e.g. freshly prepared cheese, plant-based analogues of meat or dairy products), depending on the specific food manufacturing process conditions.

3.5.2 | Dietary exposure estimation

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2023) and input data provided in Appendix C. 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 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 mean and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 48 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 26 European countries (Appendix B). The highest dietary exposure was estimated to be 3.498 mg TOS/kg bw per day in toddlers at the 95th percentile.

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

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.220–0.710 (12)	0.641–1.402 (15)	0.476–0.906 (19)	0.225–0.488 (21)	0.190–0.344 (22)	0.163–0.334 (23)
Min–max 95th percentile (number of surveys)	0.790–2.532 (11)	1.129–3.498 (14)	0.839–1.805 (19)	0.435–0.915 (20)	0.382–0.789 (22)	0.358–0.684 (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.

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

[38]Technical Dossier/Annex J/p. 6.

[39]Technical Dossier/Annex J/pp. 3–5, p. 7, pp. 10–11.

[40]Technical Dossier/Annex J/pp. 2–21.

[41]Technical Dossier/Annex J/p 0.22.

[42]Technical Dossier/Reaction and fate in foods to which the food enzyme is added/Annex I.

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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	
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Exposure from the production of plant-based analogues of milk and milk products was calculated using the TOS indicated for cheese analogues	+
Exposure from the production of baked products, including bread, was calculated using the TOS indicated for pizza and pizza-like dishes	+
Exposure from cereal-based processes, including pasta, was calculated using the TOS indicated for breakfast cereals	+
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.

Abbreviation: TOS, total organic solids.

3.6 | Margin of exposure

The comparison of the NOAEL (91 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.163–1.402 mg TOS/kg bw per day at the mean and from 0.358–3.498 mg TOS/kg bw per day at the 95th percentile resulted in margins of exposure (MOE) for infants, toddlers, children, adolescents, adults and the elderly of at least 36, 26, 50, 99, 115 and 133, respectively.

4 | CONCLUSIONS

The safety of the food enzyme could not be established given the low margins of exposure in all age groups. Furthermore, the safety of the production strain and the absence of viable cells of the production strain were not demonstrated. Therefore, the Panel concluded that the food enzyme protein–glutamine γ-glutamyltransferase produced with the non-genetically modified *Streptomyces mobaraensis* strain M2020197 could not be considered safe under the intended conditions of use.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Application for authorisation of transglutaminase from *Streptomyces mobaraensis* strain M2020197. June 2021. Submitted by Taixing Dongsheng Bio-Tech Co., Ltd.

Additional information. January 2023. Submitted by Taixing Dongsheng Bio-Tech Co., Ltd.

ABBREVIATIONS

AMR	Antimicrobial resistance gene
bw	body weight
CAS	Chemical Abstracts Service
CCTCC	China Center for Type Culture Collection
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
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
MOE	margin of exposure
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development

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TOS	total organic solids
WGS	whole genome sequencing
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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SUPPORTING INFORMATION

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

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APPENDIX A

Dietary exposure estimates to the food enzyme–TOS in details

Appendix A can be found in the online version of this output (in the 'Supporting information' section). The file contains two sheets, corresponding to two tables.

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

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

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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, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia*, Serbia*, Slovenia, Spain
Children	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia*, Serbia*, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina*, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina*, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden
The elderly[a]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden

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

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

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APPENDIX C

FoodEx1 categories and technical conversion factors considered for food manufacturing processes not included in the EFSA guidance

TABLE C.1 Input table used to estimate the exposure to the 'production of meat analogues'.

FoodEx_code	FoodEx_name	FoodEx hierarchical level	Tf1	Tf2	Tf3
A.06.09	Sausages	2	1	0.50	0.11
A.06.12	Meat imitates (unspecified)	4	1	0.20	1.00
A.06.12.001	Textured soy protein	4	1	0.20	1.00
A.06.12.002	Quorn (mycoprotein)	4	1	0.50	1.00
A.19.05	Meat-based meals (unspecified)	4	1	0.43	0.30
A.19.05.001	Meat burger	4	1	0.40	0.50
A.19.05.002	Meat balls	4	1	0.57	0.44
A.19.05.003	Goulash	4	1	0.40	0.18
A.19.05.004	Meat stew	4	1	0.43	0.10

TABLE C.2 Input table used to estimate the exposure to the 'production of dairy desserts'.

FoodEx_code	FoodEx_name	FoodEX hierarchical level	Tf1	Tf2	Tf3
A.20.02.001	Ice cream, milk-based	3	8.00	0.1	1

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