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Safety evaluation of the food enzyme glucose oxidase from the genetically modified *Trichoderma reesei* strain AR-352

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Claude Lambré, José Manuel Barat Baviera, Claudia Bolognesi, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn, Boet Glandorf, Jaime Aguilera, Magdalena Andryszkiewicz, Elsa Nielsen, Karin Nørby, Yi Liu, Rita Ferreira De Sousa and Andrew Chesson

Abstract

The food enzyme glucose oxidase (β-D-glucose:oxygen 1-oxidoreductase; EC 1.1.3.4) is produced with the genetically modified *Trichoderma reesei* strain AR-352 by AB Enzymes GmbH. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism, but the absence of its recombinant DNA could not be established. The food enzyme is intended to be used in four food manufacturing processes, namely baking processes, cereal-based processes, grain treatment for the production of starch and gluten fractions, and egg processing. Since residual amounts of total organic solids (TOS) are removed by repeated washing during the production of starch and gluten, dietary exposure was calculated only for the remaining three processes. Dietary exposure to the food enzyme–TOS was estimated to be up to 0.13 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not indicate a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 1,000 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, results in a margin of exposure of more than 7,800. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and one match was found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood for this to occur is considered to be low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns, under the intended conditions of use.

Keywords: food enzyme, EC 1.1.3.4, glucose oxidase, β-D-glucose:oxygen 1-oxidoreductase, glucose oxyhydrase, *Trichoderma reesei*, genetically modified microorganism

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Correspondence: fip@efsa.europa.eu

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

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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 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, 2009a) 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[1] on food enzymes.

An application has been introduced by the applicant "AB Enzymes GmbH" for the authorisation of the food enzyme Glucose oxidase from a genetically modified strain of *Trichoderma reesei* (strain AR-352).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011[3] implementing Regulation (EC) No 1331/2008[2], the Commission has verified that the application falls within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002, the European Commission requests the European Food Safety Authority to carry out the safety assessment on the following food enzyme: Glucose oxidase from a genetically modified strain of *Trichoderma reesei* (strain AR-352), in accordance with Article 17.3 of Regulation (EC) No 1331/2008[2] establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

[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.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme glycose oxidase from a genetically modified *T. reesei* (strain AR-352).

The dossier was updated on 26 August 2021.

Additional information was requested from the applicant during the assessment process on 24 November 2021 and received on 24 February 2022 (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, 2009b) and following the relevant guidance documents of the EFSA Scientific Committee.

The 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) as well as the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) have been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a).

3. Assessment

IUBMB nomenclature	Glucose oxidase
Systematic name	β-D-glucose:oxygen 1-oxidoreductase
Synonyms	glucose oxyhydrase; β-D-glucose oxidase, D-glucose oxidase
IUBMB No	EC 1.1.3.4
CAS No	9,001-37-0
EINECS No	232–601-0

Glucose oxidase catalyses the oxidation of glucose to D-glucono-1,5-lactone (glucono δ-lactone), thereby reducing molecular oxygen to hydrogen peroxide. It is intended to be used in four food manufacturing processes, namely baking processes, cereal-based processes, grain treatment for the production of starch and gluten fractions, and egg processing.[5]

3.1. Source of the food enzyme

The glucose oxidase is produced with the genetically modified filamentous fungus *Trichoderma reesei* strain AR-352 [redacted], which is deposited at the Westerdijk Fungal Biodiversity Institute culture collection (CBS, the Netherlands), with deposit number [redacted][3] The production strain was identified as *T. reesei* [redacted][4]

3.1.1. Characteristics of the parental and recipient microorganisms

[redacted][5]

The recipient strain, [redacted]

[3] Technical dossier/Volume II/Annex 5.
[4] Technical dossier/Additional information February 2022/Annex 1.
[5] Technical dossier/Volume II/Annex 1.

[redacted][6]

3.1.2. Characteristics of introduced sequences

[redacted][7][redacted][8]

3.1.3. Description of the genetic modification process

The purpose of genetic modification was to enable the production strain to produce glucose oxidase from [redacted][9]

3.1.4. Safety aspects of the genetic modification

The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain *T. reesei* AR-352 differs from the recipient strain [redacted] No issues of concern arising from the genetic modifications were identified by the Panel.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[10], with food safety procedures based on hazard analysis and critical control points, and in accordance with current Good Manufacturing Practice.[11]

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, 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 mass material passes the filtration membrane and is discarded.[12] The applicant provided information on the identity of the substances used to control fermentation and in the subsequent downstream processing of the food enzyme.[13]

[6] Technical dossier/Volume II/Annexes 2 and 3.
[7] Technical dossier/Volume II/Annex 7.
[8] Technical dossier/Volume II/Annex 10.
[9] Technical dossier/Volume II/Annex 6.
[10] 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.
[11] Technical dossier/Volume I/p. 17/Annex 8.
[12] Technical dossier/Volume I/p. 17–25/Annex 10.
[13] Technical dossier/Volume I/p. 18, 20–23/Annex 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 glucose oxidase is a single polypeptide chain of [redacted] amino acids.[14] The molecular mass of the mature protein, calculated from the amino acid sequence, was [redacted] kDa.[14] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. 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 [redacted] kDa, consistent with the expected mass of the enzyme.[15] The food enzyme was tested for the presence of other enzyme activities. Endo-1,4-β-glucanase, xylanase and protease activities were detected.[16] No other enzymatic activities were reported.

The in-house determination of glucose oxidase activity is based on the oxidation of glucose to D-glucono-1,5-lactone (reaction conditions: [redacted]) with the production of hydrogen peroxide. Enzyme activity is determined by measuring the hydrogen peroxide produced with a peroxidase assay and detected spectrophotometrically [redacted]. The enzyme activity is expressed in Glucose Oxidase (GOX) units/g. One GOX is defined as the amount of enzyme that oxidises 1 μmol glucose per minute.[17]

The food enzyme has a temperature optimum around 25°C (pH 7.0) and a pH optimum between pH 4.0 and 6.0 (25°C). Thermostability was tested after a pre-incubation of the food enzyme for 2–60 min at different temperatures (pH 7.0). Glucose oxidase activity decreased above 55°C, showing no residual activity at 85°C after 2 min of pre-incubation.[18]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch produced for the toxicological tests (Table 1).[19] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation is 19.7% and the mean enzyme activity/TOS ratio is 71.2 GOX/mg TOS.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Glucose oxidase activity	GOX/mg[(b)]	13.1	14.6	14.1	39.9
Protein	%	11.7	15.9	15.8	68.3
Ash	%	0.5	0.4	0.6	3.5
Water	%	82.8	77.9	78.6	5.1
Total organic solids (TOS)[(c)]	%	16.7	21.7	20.8	91.4
Activity/mg TOS	GOX/mg TOS	78.4	67.3	67.8	43.8

(a): Batch used for the toxicological studies.
(b): GOX: Glucose Oxidase unit (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The lead content in the three commercial batches and in the batch used for toxicological studies was below 0.05 mg/kg[20] which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, the

[14] Technical dossier/Volume I/p. 9/Annex 2.
[15] Technical dossier/Volume I/Annex 1.
[16] Technical dossier/Volume I/p. 3, 14/Annexes: 3 and 4.
[17] Technical dossier/Volume I/p. 12/Annexes: 4 and 5.
[18] Technical dossier/Volume I/p. 12–14/Annex 6.
[19] Technical dossier/Volume I/p. 11, 39/Annexes: 3, 13, 15 and 16.
[20] Technical dossier/Volume I/p. 11–12/Annexes: 3 and 4.

levels of arsenic, cadmium and mercury were below the limits of quantification (LoQs) of the employed methods.[20,21]

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).[20] No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).[20]

Strains of *Trichoderma*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of T-2 and HT-2 toxins was examined in the three food enzyme batches and both toxins were below the LoQ of the applied methods.[20,22] Adverse effects due to the possible presence of other secondary metabolites are addressed by the toxicological examination of the food enzyme TOS.

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

3.3.4. Viable cells and DNA of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches of the enzyme liquid concentrate, each analysed in quadruplicate. Five millilitres of product was inoculated on non-selective agar plates and incubated at 30°C for 5 days. No colonies were produced. A positive control was included.[23]

The analysis provided to test the possible presence of DNA from the production strain in the food enzyme was insufficient to demonstrate the absence of recombinant DNA, because a limit of detection of 10 ng/g of sample could not be established for all three batches.[24]

3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* micronucleus 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 commercialisation, and has lower chemical purity, thus, is considered suitable as a test item. The test sample for the bacterial reverse mutation test was inactivated in order to avoid possible interferences by the formation of hydrogen peroxide.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP).[25] In a pre-experiment, two strains of *Salmonella* Typhimurium (TA98 and TA100) were tested with eight concentrations of the food enzyme (3.16 to 5,000 μg TOS/plate) in the presence or absence of metabolic activation (S9-mix) applying the plate incorporation method and with triplicate plating. No cytotoxicity or precipitation were seen at any concentration tested.

Two main experiments were performed with five strains of *S*. Typhimurium (TA98, TA100, TA 102, TA1535 and TA1537) which were tested with six concentrations of the food enzyme (31.6, 100, 316, 1,000, 2,500 and 5,000 μg TOS/plate) ± S9-mix, applying the pre-incubation method. Upon treatment with the food enzyme, there was no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix, except for TA1537 with S9-mix in the first main test, where a mutation factor of up to 3.2 was observed. In the second main experiment, this result was not confirmed and no significant increase in revertant colonies were seen compared to the controls at any test condition.

The Panel concluded that the food enzyme glucose oxidase did not induce gene mutations under the test conditions employed in this study.

[21] LoQs: Pb, Cd and Hg = 0.05 mg/kg; As = 0.5 mg/kg.
[22] LoQs: T2 and HT-2 toxin = 10 μg/kg each.
[23] Technical dossier/Volume I/Annex 4 and Additional information February 2022/Annex 2.
[24] Technical dossier/Additional information February 2022/Annex 3.
[25] Technical dossier for glucose oxidase p. 37/ Annex 13/Additional information February 2022.

3.4.1.2. *In vitro* micronucleus assay

The *in vitro* micronucleus test was carried out according to OECD Draft Guideline 487 (OECD, 2010) and following GLP.[26] A cytotoxicity test and two main separate experiments were performed in duplicate cultures of human peripheral whole blood lymphocytes. Cells were exposed to the test substance in a short-term-treatment (4 + 40 h recovery time) with and without S9-mix. Additionally, a continuous 44-h treatment without S9-mix was included (44 + 0 h recovery time). In the cytotoxicity test, the food enzyme was tested at 12 concentrations (2.0 to 5,000 μg TOS/mL). High cytotoxicity was observed at 31.3 μg TOS/mL in the absence of S9-mix and at 500 μg TOS/mL in the presence of S9-mix. Based on this result, concentrations tested in the short-term treatments were 5, 10 and 15 μg TOS/mL (without S9-mix) and 50, 100, 200 and 275 μg TOS/mL (with S9-mix). In the continuous treatment, concentrations of 0.1, 0.25, 0.5, 1.0 and 2.5 μg TOS/mL (without S9-mix) were applied. The frequency of bi-nucleated cells with micronuclei (MNBN) was statistically significant in the short-term treatments at the mid concentration (10 μg/mL) without S9-mix and in the highest concentration (275 μg TOS/mL) with S9-mix in the presence of 60% cytotoxicity. In the continuous treatment, no significant increase above the control was observed. The Panel noted that all these values were within the 95% of the historical control range and therefore were not considered to be biologically relevant.

The Panel concluded that the food enzyme glucose oxidase did not induce an increase in the frequency of MNBNs in cultured human peripheral blood lymphocytes, under the test conditions employed 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 in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[27] Groups of 10 male and 10 female Wistar Crl:WI(Han) rats received by gavage the food enzyme in doses of 100, 300 or 1,000 mg TOS/kg body weight (bw) per day. Controls received the vehicle (water).

One low-dose female and one high-dose female were found dead on days 27 and 82, respectively, due to misdosing.

The clinical chemistry investigation revealed a statistically significant increase in total bilirubin in mid-dose females (+25%). The Panel considered this change as not toxicologically relevant since it was only observed in one sex and there was no dose–response relationship.

There was a statistically significant decrease in the absolute thymus weight in treated females in all dose groups (−16%, −16%, −19%) and in the relative thymus weight in mid- and high-dose females (−18%, −18%). The Panel considered these changes as not toxicologically relevant as there was no dose–response relationship and additionally there were no histopathological changes in the thymus.

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

The Panel identified the 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 glucose oxidase produced with the genetically modified *T. reesei* strain AR-352 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, one match was found. The matching allergen [redacted][28]

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

According to the information provided, [redacted] a known source of allergens, is present in the media fed to the microorganisms. However, during the fermentation process, this product will be

[26] Technical dossier for glucose oxidase p. 37/Annex 14/Additional information February 2022.
[27] Technical dossier for glucose oxidase p. 37/Annex 15.
[28] Technical dossier/Volume I/p. 40–42/Annex 2.

degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these materials employed as protein sources are not expected to be present in the food enzyme.

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 for this 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 four food processes at the recommended use levels summarised in Table 2.

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

Food manufacturing process(a)	Raw material (RM)	Maximum recommended use level (mg TOS/kg RM)(b)
Baking processes	Flour	**10**
Cereal-based processes	Flour	**10**
Grain treatment for the production of starch and gluten fractions	Cereals	18
Egg processing	Whole liquid egg or egg white	**10**

TOS: total organic solids.
(a): The description has been harmonised according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.
(b): The numbers in bold were used for calculation.
(c): Technical dossier/p. 30.

In baking processes and cereal-based processes, glucose oxidase is added to flour during the making of dough or batter.[29] The oxidation of glucose releases hydrogen peroxide, which interacts with the gluten protein. The modification of the gluten protein structure improves the dough consistency, crumb structure or crust surface of the final products. The food enzyme remains in the final foods.

In grain treatment for the production of starch and gluten fractions, the food enzyme is added to cereals during homogenisation.[30] The treatment with glucose oxidase induces the formation of protein–protein bonds, which may strengthen the protein network. The food enzyme is removed in the final starch and gluten fractions by repeated washing and purification steps applied during grain treatment (EFSA CEP Panel, 2021).

In egg processing, glucose oxidase is added to the whole egg or egg white.[31] This de-sugaring step prevents the browning in the drying process. The food enzyme remains in the final egg products.

3.5.2. Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel, 2021a), a dietary exposure was calculated only for food manufacturing processes where the food enzyme–TOS remains in the final foods, namely baking processes, cereal-based processes and egg processing.

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level provided by the applicant with the individual data from the EFSA Comprehensive European Food Consumption Database. The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2021b). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period

[29] Technical dossier/p. 31–32.
[30] Additional data February 2022.
[31] Technical dossier/p. 34.

(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 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 41 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure to the food enzyme–TOS was estimated to be 0.127 mg TOS/kg bw per day at the 95th percentile in children.

Table 3: 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)	0.006–0.035 (11)	0.034–0.076 (15)	0.037–0.068 (19)	0.019–0.042 (21)	0.013–0.027 (22)	0.012–0.026 (22)
Min–max 95th percentile (number of surveys)	0.030–0.119 (9)	0.070–0.119 (13)	0.063–0.127 (19)	0.035–0.081 (20)	0.026–0.052 (22)	0.023–0.044 (21)

TOS: total organic solids.

3.5.3. Uncertainty analysis

In accordance with the 'guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment' (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 4.

Table 4: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/underreporting/ misreporting/no portion size standard	+/–
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
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	+/–
Exclusion of other processes from the exposure assessment – Grain treatment for the production of starch and gluten fractions	–

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

The exclusion of one food manufacturing process (grain treatment for the production of starch and gluten fractions) from the exposure assessment was based on > 99% of TOS removal during these processes and is not expected to have an impact on the overall estimate derived.

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 0.006–0.076 mg TOS/kg bw per day at the mean and from 0.023–0.127 mg TOS/kg bw per day at the 95th percentile, resulted in a margin of exposure (MoE) of at least 7,874.

4. Conclusions

Based on the data provided, the removal of TOS during grain treatment and the derived margin of exposure for baking, cereal-based process and egg processing, the Panel concluded that the food enzyme glucose oxidase produced with the genetically modified *T. reesei* strain AR-352 does not give rise to safety concerns under the intended conditions of use.

The CEP Panel considers the food enzyme free from viable cells of the production organism but could not conclude on the absence of recombinant DNA.

5. Documentation as provided to EFSA

Application for Authorisation of a Glucose oxidase from a genetically modified strain of *Trichoderma reesei* in accordance with Regulation (EC) No 1331/2008. March 2021. Submitted by AB Enzymes GmbH.

Additional information. August 2021. Submitted by AB Enzymes GmbH.

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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
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMO	genetically modified organism
GMC	glucose-methanol-choline
GOX	glucose oxidase
IUBMB	International Union of Biochemistry and Molecular Biology
LoQ	limit of quantification
MoE	margin of exposure
MNBN	micronucleated bi-nucleated cells
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
RM	raw material
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.2022.7372#support-information-section).

The file contains two sheets, corresponding to two tables.

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

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

Appendix B – Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than 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
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, 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, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, 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, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden

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