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Safety evaluation of the food enzyme phospholipase A1 from the genetically modified *Aspergillus oryzae* strain NZYM-LJ

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, Magdalena Andryszkiewicz, Giulio di Piazza, Natalia Kovalkovicova, Yi Liu and Andrew Chesson

Abstract

The food enzyme phospholipase A1 (phosphatidylcholine 1-acylhydrolase; EC 3.1.1.32) is produced with the genetically modified *Aspergillus oryzae* strain NZYM-LJ by Novozymes A/S. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and its DNA. The food enzyme is intended to be used in baking processes. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.09 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 957.3 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 at least 10,600. 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 concludes that this food enzyme does not give rise to safety concerns under the intended conditions of use.

Keywords: food enzyme, phospholipase A1, phosphatidylcholine 1-acylhydrolase, EC 3.1.1.32, *Aspergillus oryzae*, 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, Vittorio Silano (until 21 December 2020 †), Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis and Holger Zorn.

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Table of contents

1. Introduction

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

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

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

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

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

All food enzymes currently on the 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.

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 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.

An application has been introduced by the applicant "Novozymes A/S" for the authorisation of the food enzyme Phospholipase A1 from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-LJ).

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 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: Phospholipase A1 from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-LJ) in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and 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.

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

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of the food enzyme phospholipase A1 from a genetically modified *A. oryzae* (strain NZYM-LJ).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme phospholipase A1 from a genetically modified *A. oryzae* (strain NZYM-LJ). The dossier was updated on 16 June 2021.

Additional information was requested from the applicant during the assessment process on 13 December 2021 and received on 10 March 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) as well as in the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) and following the relevant existing guidance of EFSA Scientific Committees.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) 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	Phospholipase A1
Systematic name	Phosphatidylcholine 1-acylhydrolase
IUBMB No	EC 3.1.1.32
CAS No	9043-29-2
EINECS No	618–552-1

Phospholipases A1 catalyse the hydrolysis of the fatty acyl ester bond at the sn-1 position of the glycerol moiety, resulting in the formation of 2-acyl-1-lysophospholipids and free fatty acids. The food enzyme is intended to be used in baking processes.

3.1. Source of the food enzyme

The phospholipase A1 is produced with the genetically modified filamentous fungus *Aspergillus oryzae* strain NZYM-LJ, which is deposited at the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ, Germany), with deposit number [redacted].[4]

The production strain was identified as *A. oryzae* [redacted][5]

3.1.1. Characteristics of the parental and recipient microorganisms

The parental microorganism is *A. oryzae* strain [redacted]

The recipient strain was developed from the parental strain [redacted]

[4] Technical dossier/2nd submission/Annex A2.
[5] Technical dossier/2nd submission/Annex A1.

[redacted][6]

3.1.2. Characteristics of introduced sequences

The sequence encoding the phospholipase A1 [redacted][7]

3.1.3. Description of the genetic modification process

The purpose of the genetic modification was to enable the production strain to synthesise phospholipase A1 [redacted][8]

3.1.4. Safety aspects of the genetic modification

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

The production strain *A. oryzae* NZYM-LJ differs from the recipient strain in its capacity to produce the phospholipase A1 [redacted]

[6] Technical dossier/2nd submission/Annexes C1–C11.
[7] Technical dossier/2nd submission/Annex B1.
[8] Technical dossier/2nd submission/Annex D1.

[redacted][9]

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, [redacted] fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration. The filtrate containing the enzyme is 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 the fermentation and in the subsequent downstream processing of the food enzyme.[13]

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 phospholipase A1 is a single polypeptide chain of [redacted] amino acids. The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa.[14] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all batches. The gel showed a single major protein band migrating between the [redacted]- and [redacted]-kDa reference proteins, consistent with the expected mass of the enzyme.[15] The food enzyme was tested for α-amylase, protease and amyloglucosidase activities. Only amyloglucosidase activity was detected. No other enzymatic activities were reported.[16]

The in-house determination of phospholipase A1 activity is based on hydrolysis of L-α-phosphatidylcholine, (reaction conditions: pH 7.6, 30°C, 1 min). The released fatty acids lower the pH, which is monitored by adding the pH indicator bromothymol blue. The colour change is measured spectrophotometrically at 600 nm. The phospholipase A1 activity is quantified relative to an internal enzyme standard with units expressed as Phospholipase A1 Activity for baking applications/g (PLA(B)/g).[17]

The food enzyme has a temperature optimum around 30°C (pH 4.0) and a pH optimum around pH 4.0 (37°C).[18] Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 4.0). Enzyme activity decreased above 50°C, showing no residual activity above 80°C.[19]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and the batch produced for the toxicological tests (Table 1).[19] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation is 11.0% and the mean enzyme activity/TOS ratio is 2.7 PLA(B)/mg TOS.

[9] Technical dossier/2nd submission/Annex D2.
[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/2nd submission/p. 42 and Annex 5.
[12] Technical dossier/2nd submission/p. 44–51.
[13] Technical dossier/2nd submission/p. 45 and Annex 6.
[14] Technical dossier/2nd submission/p. 31 and Annex 1.
[15] Technical dossier/2nd submission/pp. 32–33.
[16] Technical dossier/2nd submission/p. 39 and Annex 3.02–3.04.
[17] Technical dossier/2nd submission/pp. 36–37 and Annex 3.01.
[18] Technical dossier/2nd submission/pp. 37–38 and Annex 9.
[19] Technical dossier/2nd submission/Annex 9.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Phospholipase A1 activity	PLA(B)/g batch[(b)]	234	233	405	138
Protein	%	6.8	8.3	8.2	6.0
Ash	%	0.3	0.4	0.5	2.4
Water	%	88.3	88.7	88.9	88.5
Total organic solids (TOS)[(c)]	%	11.4	10.9	10.6	9.1
Activity/mg TOS	PLA(B)/mg TOS	2.1	2.1	3.8	1.5

(a): Batch used for the toxicological studies.
(b): PLA(B)/g: Phospholipase A1 Activity/g (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 5 mg/kg which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[20] In addition, the levels of arsenic, cadmium and mercury were below the limits of detection (LoDs) of the employed methodologies.[21,22]

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).[23] No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).[22]

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al. 2018). The presence of aflatoxins, 2-nitropropionic acid, cyclopiazonic acid and of kojic acid was examined in the four food enzyme batches. The concentrations were below the respective LoDs of the applied methods. Adverse effects caused by the possible presence of other secondary metabolites are addressed by the toxicological examination of the food enzyme TOS.[22,24]

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 [redacted] No colonies were produced.[25]

The absence of recombinant DNA in the food enzyme was demonstrated [redacted][26]

3.4. Toxicological data

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

[20] Technical dossier/2nd submission/pp. 11, 34 and Annex 10.
[21] LoDs: Pb = 0.5 mg/kg; As = 0.3 mg/kg; Cd = 0.05 mg/kg; Hg = 0.05 mg/kg.
[22] Technical dossier/2nd submission/p. 34 and Annex 10.
[23] Technical dossier/2nd submission/p. 36 and Annex 10.
[24] LoDs: Aflatoxin B1 = 0.0003 mg/kg; Kojic acid* = 0.033/0.038/0.0030 mg/kg; 2-nitropropionic acid* = 0.29/0.25/0.42 mg/kg; cyclopiazonic acid = 0.0003 mg/kg (*LoD is matrix-dependent).
[25] Technical dossier/2nd submission/Annex E1.
[26] Technical dossier/2nd submission/Annex E2.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation test (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).[27] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA(pKM101) were used in the presence or absence of metabolic activation, applying the 'treat and plate assay'. Two separate experiments were carried out using six concentrations of the food enzyme (16, 50, 160, 500, 1,600 and 5,000 μg TOS/mL in the first experiment and 160, 300, 625, 1,250, 2,500 and 5,000 μg TOS/mL) in the second experiment. Toxic effects, evident as a slight thinning of the background bacterial lawn occurred in *S*. Typhimurium strains TA100, TA1535 and TA1537 at 5,000 μg TOS/mL in the first experiment, and in all *S*. Typhimurium strains at 2,500 μg/plate and above in the second experiment in both the absence and presence of S9-mix. Upon treatment with the food enzyme there was no increase in revertant colony numbers above the control values in any strain with or without S9-mix.

The Panel concluded that the food enzyme did not induce gene mutations under the test conditions employed 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 OECD Guideline 487 (OECD, 2016) and following GLP.[28] The experiment was performed in duplicate cultures of human peripheral whole blood lymphocytes. In a short treatment (3 + 21 h recovery time), the food enzyme was tested at 2,500, 3,500, 4,500 and 5,000 μg TOS/mL in the absence of S9-mix and at 1,000, 2,000, 3,000 and 3,500 μg TOS/mL in the presence of S9-mix. In the continuous 24-h treatment (24 + 24 h recovery time) in the absence of S9-mix, the cell cultures were treated at 500, 750, 1,000 and 1,500 μg TOS/mL. Cytotoxicity of 55% was observed at 1,500 μg/mL in the continuous treatment in the absence of S9-mix. Statistically significant increases of the frequency of binucleated cells with micronuclei (MNBN) were observed at a single intermediate concentration following 3 + 21-h treatments in the absence and presence of S9-mix (3,500 and 3,000 μg TOS/mL respectively) and at the highest concentration following 24-h continuous treatment in the absence of S9-mix (1,500 μg TOS/mL). However, these increases were marginally observed in 1 of the 2 replicate cultures exhibiting at 3,500 TOS/mL MNBN cell values slightly exceeding the historical control ranges (0.90% vs 0.0–0.7%) and were considered not biologically relevant. The frequency of MNBN was comparable to the negative controls at all the other concentrations tested. As only one intermediate concentration exceeded the current 95th percentile of the historical control range and no concentration-dependent increases in MNBN were observed, the Panel considered this finding of no biological relevance.

The Panel concluded that the food enzyme phospholipase A1 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.[29] Groups of 10 male and 10 female Crl:WI(Han) rats received by gavage amounts of the food enzyme, corresponding to 95.7, 315.9 or 957.3 mg TOS/kg body weight (bw) per day. Controls received the vehicle (reverse osmosis water).

No mortality was observed.

Functional observational battery tests revealed dose-dependent increases in total activity counts, total mobile counts and total rears in treated females, with statistical significance in high-dose females. The Panel considered these differences as not toxicologically relevant as all the values were within the normal variability of relevant historical control ranges in the laboratory.

Haematological investigation revealed statistically significant increased percentage of reticulocytes (+25%) and absolute reticulocytes (+24%) in low-dose females. The Panel considered these changes as not toxicologically relevant in the absence of a dose–response relationship, only observed in one sex and the values were within the range of the historical control ranges in the laboratory.

[27] Technical dossier/2nd submission/Annex 7.01.
[28] Technical dossier/2nd submission/Annex 7.02.
[29] Technical dossier/2nd submission/Annex 7.03.

Clinical chemistry investigation revealed statistically significantly decreased alanine aminotransferase (ALT) (−24%), calcium concentrations (−4%) and globulin concentrations (−19%) in mid-dose males. Creatine phosphokinase (CPK) was statistically significantly increased (+41%) in low-dose males and statistically significantly decreased (−41%) in mid-dose females. In high-dose females, lower value of total proteins (−8%) was reported. The Panel considered these changes as not toxicologically relevant as the changes were small (ALT, Ca, total protein), there was no dose–response relationship (all parameters except for total protein), the changes were only observed in one sex (all parameters), there was no consistency between the changes in males and females (CPK) and the values were within the range of the historical control ranges in the laboratory.

Statistically significant changes in organ weights included an increase in the liver to body weight ratio in mid- and high-dose females (+7%, +6%) and a decrease in the ovary to brain weight ratio in mid-dose females (−16%). The Panel considered these changes as not toxicologically relevant as the changes were small (relative liver weight), there was no dose–response relationship (relative liver weight and ovary to brain ratio), there were no macroscopic and histopathological changes and the values were within the normal variability of relevant historical control ranges in the laboratory.

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

The Panel identified a no observed adverse effect level (NOAEL) of 957.3 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 phospholipase A1 produced with the genetically modified *A. oryzae* strain NZYM-LJ 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 was Sch c 1, a glucoamylase produced by *Schizophyllum commune*, known as an occupational respiratory allergen.[30]

No information is available on oral and respiratory sensitisation or elicitation reactions of this phospholipase A1. In addition, no allergic reactions upon dietary exposure to any phospholipase A1 have been reported in the literature.

Glucoamylase from *S. commune* (Toyotome et al., 2014) 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*) may be able to ingest the corresponding allergen 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 food enzyme only a low number of case reports of allergic reactions upon oral exposure to α-amylase in individuals respiratory sensitised to α-amylase have been described in literature (Quirce et al., 1992; Losada et al., 1992; Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004).

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 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 of such reactions occurring is considered to be low.

[30] Technical dossier/2nd submission/pp. 63-66/Annex 8.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in baking processes at a recommended use level of 5–20 PLA(B)/kg flour. Based on 2.63 PLA(B)/mg TOS, this corresponds to 1.9–7.6 mg TOS/kg flour.[31]

In baking processes, the food enzyme is added to flour during the preparation of dough or batter. The phospholipase A1 hydrolyses flour lipids and releases lysophospholipids and free fatty acids, which have emulsifying properties. The food enzyme remains in the dough.

Based on data provided on thermostability (see Section 3.3.1), it is expected that the phospholipase A1 is inactivated during the baking processes.

3.5.2. Dietary exposure estimation

Chronic dietary exposure was estimated by combining the maximum recommended use level with the relevant FoodEx categories (EFSA CEP Panel, 2021) and individual consumption data. 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 average 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 2 provides an overview of the derived exposure estimates across all surveys. Detailed average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 41 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure at the 95th percentile to the food enzyme-TOS was estimated to be up to 0.09 mg TOS/kg bw per day in infants.

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

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.001–0.021 (11)	0.016–0.045 (15)	0.018–0.044 (19)	0.010–0.027 (21)	0.007–0.017 (22)	0.007–0.017 (22)
Min–max 95th percentile (number of surveys)	0.008–0.090 (9)	0.040–0.077 (13)	0.036–0.082 (19)	0.022–0.057 (20)	0.016–0.034 (22)	0.015–0.028 (21)

TOS: total organc 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 3.

[31] Technical dossier/p. 40.

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

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

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 overestimation of the exposure.

3.6. Margin of exposure

A comparison of the NOAEL (957.3 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.001–0-045 mg TOS/kg bw per day at the mean and from 0.008–0.090 mg TOS/kg bw per day at the 95th percentile, resulted in a margin of exposure (MoE) of at least 10,636.

4. Conclusions

Based on the data provided and the derived margin of exposure the Panel concludes that the food enzyme phospholipase A1 produced with the genetically modified *Aspergillus oryzae* strain NZYM-LJ 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 and recombinant DNA.

5. Documentation as provided to EFSA

Phospholipase A1 produced by a genetically modified strain of *Aspergillus oryzae* (strain NZYM-LJ). June 2021. Submitted by Novozymes A/S.

Additional information. March 2022. Submitted by Novozymes A/S.

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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

CFU	colony forming units
DRF	dose-range finding
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMM	genetically modified microorganism
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LoD	limit of detection
MoE	margin of exposure
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
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.2022.7381#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, 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, 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, Italy, Latvia, Netherlands, Portugal, 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).