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SCIENTIFIC OPINION

Safety evaluation of the food enzyme 3-phytase from the genetically modified *Aspergillus niger* strain NPH

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Abstract

The food enzyme 3-phytase (*myo*-inositol-hexakisphosphate 3-phosphohydrolase EC 3.1.3.8) is produced with the genetically modified *Aspergillus niger* strain NPH by DSM Food Specialties. The genetic modifications do not give rise to safety concerns. The food enzyme was considered free from viable cells of the production organism and its DNA. It is intended to be used in three food manufacturing processes: processing of cereals and other grains for the production of (1) baked products and (2) distilled alcohol, and the processing of plant- and fungal-derived products for the production of (3) plant-based analogues of milk and milk products. Since no residual amounts of total organic solids (TOS) are carried over into distilled alcohol, dietary exposure was calculated only for the remaining two food manufacturing processes. It was estimated to be up to 0.553 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 833 mg TOS/kg bw per day, the highest dose tested, which, when compared with the estimated dietary exposure, resulted in a margin of exposure of at least 1506. 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 the risk of allergic reactions by dietary exposure cannot be excluded (except for distilled alcohol production), but the likelihood is 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

3-phytase, *Aspergillus niger*, EC 3.1.3.8, food enzyme, genetically modified microorganism, *myo*-inositol-hexakisphosphate 3-phosphohydrolase, phytate 6-phosphatase

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 microorganisms or products thereof including a product obtained by a fermentation process using microorganisms: (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 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[3] on food enzymes.

On January 2022, a new application has been introduced by the applicant "DSM Food Specialties B.V." for the authorisation of the food enzyme 3-Phytase from a genetically modified strain of *Aspergillus niger* (strain NPH).

1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments and the assessment of possible confidentiality requests of the following food enzyme: 3-Phytase from a genetically modified strain of *Aspergillus niger* (strain NPH), in accordance with Regulation (EC) No 1332/2008 establishing a common authorization procedure for food additives, food enzymes and food flavourings.[4]

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant submitted a dossier in support of the application for the authorisation of the food enzyme 3-phytase from the genetically modified *Aspergillus niger* strain NPH.

Following the request for additional data sent by EFSA on 11 November 2022, the applicant requested a clarification teleconference on 8 December 2022, after which the applicant provided additional data on 27 September 2023.

[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]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.

[4]OJ L 354, 31.12.2008, p. 1.

2.2 | Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009) and following the relevant 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[5] 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 26 May to 16 June 2023, for which no comments were received.

3 | ASSESSMENT

IUBMB nomenclature	3-Phytase
Systematic name	*myo*-inositol-hexakisphosphate 3-phosphohydrolase
Synonyms	Phytase
IUBMB no	3.1.3.8
CAS no	37288-11-2
EINECS no	609-386-0

3-Phytases catalyse the hydrolysis of phytic acid (myo-inositol hexakisphosphate) to 1D-myoinositol 1,2,4,5,6-pentakisphosphate and phosphate. The enzyme under assessment is intended to be used in three food manufacturing processes: processing of cereals and other grains for the production of 1) baked products and 2) distilled alcohol, and processing of plant- and fungal-derived products for the production of 3) plant-based analogues of milk and milk products.

3.1 | Source of the food enzyme

The 3-phytase is produced with the genetically modified filamentous fungus *A. niger* strain NPH (NPH54), which is deposited at the Westerdijk Fungal Biodiversity Institute culture collection (the Netherlands) with the deposit number CBS 101672.[6] The production strain was identified as *A. niger* by whole genome sequence (WGS) analysis, showing an average nucleotide identity (ANI) index > 99% with the reference strain *A. niger* CBS 513.88.[7]

3.1.1 | Characteristics of the parental and recipient microorganisms

The parental strain is *A. niger* NRRL 3122. The recipient strain *A. niger* DS 30620 was derived from the parental strain by classical mutagenesis and genetic modification (van Dijck et al., 2003). [redacted] The genetic modification steps made use of plasmids containing an ampicillin resistance gene.

3.1.2 | Characteristics of introduced sequences

The sequence encoding the 3-phytase is the *phyA* gene [redacted]

[5]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.

[6]Technical dossier/Risk Assessment/Source of the food enzyme/Annex 7.

[7]Technical dossier/Risk Assessment/Source of the food enzyme/Annex 4.

[redacted]

3.1.3 | Description of the genetic modification process

The aim of the genetic modification was to enable the production strain to synthesise 3-phytase [redacted]

The presence of multiple copies of the *phyA* gene in the production strain was confirmed by WGS analysis.[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 *A. niger* strain NPH differs from the recipient strain in its capacity to produce the 3-phytase [redacted]

The absence of vector backbone sequences, including the antimicrobial resistance gene used during the genetic modification, was confirmed by WGS analysis.[10]

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,[11] with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current Good Manufacturing Practice.[12]

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. 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.[13] 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.[14]

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 3-phytase is a single polypeptide chain of 467 amino acids.[15] The molecular mass of the mature protein, calculated from the amino acid sequence, is around 51 kDa. The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[16] A consistent protein pattern was observed across all batches. The gels showed a broad protein band

[8]Technical dossier/Risk Assessment/Source of the food enzyme/Annex 5.
[9]Technical dossier/Risk Assessment/Source of the food enzyme/Annex 4.
[10]Technical dossier/Risk Assessment/Source of the food enzyme/Annex 4.
[11]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.
[12]Technical dossier/Risk assessment/Manufacturing process of the food enzyme/Annex 13.
[13]Technical dossier/Risk assessment/Manufacturing process of the food enzyme/Annex 14.
[14]Technical dossier/Risk assessment/Manufacturing process of the food enzyme/Annex 15.
[15]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme.
[16]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/Annex 18.

migrating between 55 and 66 kDa, consistent with the expected mass of the enzyme. No other enzymatic activities were reported.[17]

The determination of 3-phytase activity is based on the amount of orthophosphate released by the hydrolysis of sodium phytate (reaction conditions: pH 5.5, 37°C). The enzyme activity is expressed in phytase units (FTU). One FTU is the amount of enzyme that liberates 1 µmol orthophosphate per min from sodium phytate.

The food enzyme has a temperature optimum around 45°C (pH 5.5) and a pH optimum around pH 5.5 (37°C). The thermostability was tested after a pre-incubation of the food enzyme for 10, 20 or 30 min at different temperatures (pH 5.5). The enzyme activity decreased above 30°C, showing no residual activity above 55°C after 10 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 two batches produced for the toxicological tests (Table 1).[19] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 27.8% and the mean enzyme activity/TOS ratio was 107.6 FTU/mg TOS.

TABLE 1 Composition of the food enzyme.

		Batches				
Parameters	**Unit**	**1**	**2**	**3**	**4[a]**	**5[b]**
Enzyme activity	FTU/g[c]	29,000	29,500	31,100	15,500	30,000
Protein	%	20.4	21.6	23.0	8.9	20.4
Ash	%	0.7	0.5	0.4	0.8	0.8
Water	%	71.8	72.1	71.2	80.7	72
Total organic solids (TOS)[d]	%	27.5	27.4	28.4	18.5	27.2
Activity/TOS ratio	FTU/mg TOS	105.5	107.7	109.5	83.8	110.3

[a]Batch used for the Ames test, *in vitro* chromosomal aberration test and repeated 90-day oral toxicity study in rats.

[b]Batch used for the *in vitro* micronucleus test.

[c]FTU: phytase units (see Section 3.3.1).

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

3.3.3 | Purity

The lead content in the three commercial batches was below 5 mg/kg[20,21] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).

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]

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 fumonisins (B1, B2 and B3) and ochratoxin A was examined in three food enzyme batches and all were below the limit of detection (LoD) of the applied methods.[24,25] Adverse effects caused by the possible presence of other secondary metabolites were addressed by the toxicological examination of the food enzyme–TOS.

The Panel considered that the information provided on the purity of the food enzyme was 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 analysed in triplicate. Three aliquots of 1 mL of product were each inoculated into 100 mL of non-selective medium and

[17]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme.

[18]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/Annex 19.

[19]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/Annexes: 2, 3.

[20]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/Annexes: 2, 3.

[21]LoD: Pb = 0.01 µg/mL.

[22]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/Annexes: 2, 3.

[23]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/Annexes: 2, 3.

[24]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/Annexes: 2, 3.

[25]LoDs: fumonisins (B1, B2 and B3) = 10 µg/kg each; ochratoxin A = 1 µg/kg.

incubated at 30°C for 6 days for resuscitation. From these, 10µL were spread on agar plates and incubated at 30°C for 6 days. No colonies were produced. A positive control was included.[26]

The absence of recombinant DNA in the food enzyme was demonstrated by polymerase chain reaction (PCR) analysis of three batches in triplicate. No DNA was detected with primers that would amplify a 972-bp fragment specific for the expression cassette, with a LoD of 10 ng spiked DNA/g food enzyme.[27]

3.4 | Toxicological data

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

The batches 4 and 5 (Table 1) used in the toxicological studies were considered similar to the batches used for commercialisation and, thus, 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, 1997a) and following Good Laboratory Practice (GLP).[28]

Four strains of Salmonella Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2uvrA were used with or without metabolic activation (S9-mix), applying the standard plate incorporation method. Two experiments were carried out in triplicate, using five concentrations of the food enzyme, ranging from 100 to 5000 µg dry matter/plate, corresponding to 96 to 4800 µg TOS/plate. No cytotoxicity was observed at any concentration of the food enzyme tested in any of the test strains. 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 3-phytase did not induce gene mutations under the test conditions applied in this study.

3.4.1.2 | *In vitro mammalian chromosomal aberration test*

The *in vitro* mammalian chromosomal aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP.[29]

Two separate experiments were performed with duplicate cultures of human peripheral whole blood lymphocytes treated with the food enzyme, either with or without metabolic activation (S9-mix). In a range-finding test, no cytotoxicity above 50% was seen at any concentration tested up to 5000 µg dry matter/mL.

In the first experiment, cells were exposed to the food enzyme and scored for chromosomal aberrations at concentrations of 1000, 3330 and 5000 µg dry matter/mL (corresponding to 960–4800 µg TOS/mL) in a short-term treatment (3 h exposure and 21 h recovery period), either with or without S9-mix. In the second experiment, the cells were exposed to the same concentrations and scored for chromosomal aberrations in two long-term treatments (24 h exposure or 48 h exposure without recovery) without S9-mix, and in a second short-term treatment (3 h exposure and 45 h recovery period) with S9-mix.

In the first experiment, the frequency of chromosome aberrations was statistically significantly increased compared to controls at the lowest concentration tested (1000 µg dry matter/mL) in the short-term treatment without S9-mix (5.5% compared to 0% in controls) in the absence of cytotoxicity. In the second experiment, the frequency of chromosomal aberrations was statistically significantly increased compared to controls at the mid-concentration tested of 3330 µg dry matter/mL in the long-term treatment (48 h without recovery) without S9-mix (8.5% compared to 1% in controls), associated with 40% cytotoxicity.

Both statistically significant findings, although not concentration-related, were outside the historical data for negative controls (0%–5% chromosome aberrations).

The Panel considered the results of the study as equivocal.

3.4.1.3 | *In vitro mammalian cell micronucleus test*

The *in vitro* mammalian cell micronucleus test was carried out according to the OECD Test Guideline 487 (OECD, 2016) and following GLP.[30]

[26]Technical dossier/Risk Assessment/Source of the food enzyme/Annex 10.

[27]Technical dossier/Additional data September 2023/Annex 6.

[28]Technical dossier/Risk Assessment/Toxicological data/Annex 20.

[29]Technical dossier/Risk Assessment/Toxicological data/Annex 21.

[30]Technical dossier/Additional data September 2023/Annex 23.

A preliminary test and two main experiments were performed with duplicate cultures of human peripheral whole blood lymphocytes. Based on the results from the preliminary test, the cell cultures were treated with the food enzyme with or without metabolic activation (S9-mix). In the first experiment, cells were exposed to the food enzyme and scored for the frequency of bi-nucleated cells with micronuclei (MNBN) at concentrations of 1000, 2000 and 5000 µg TOS/mL in a short-term treatment (4 hours 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 the same concentrations in a long-term treatment (44 h exposure without recovery period) without S9-mix.

In the short-term treatment, cytotoxicity of 33%, 27% and 32% (measured as cytostasis) was observed at concentrations of 1000, 2000 and 5000 µg TOS/mL respectively, with S9-mix.

In the long-term treatment, cytotoxicity of 19% (cytostasis) was observed at a concentration of 5000 µg TOS/mL.

The frequency of MNBN was not statistically significantly different to the negative controls at any concentrations tested.

The Panel concluded that the food enzyme 3-phytase did not induce an increase in the frequency of MNBNs under the test conditions applied in this study.

Conclusions on genotoxicity

The food enzyme 3-phytase was tested in a battery of *in vitro* genotoxicity studies. The test item in the presence or absence of S9 mix did not induce gene mutations in bacteria (four strains of *S.*Typhimurium, TA1535, TA1537, TA98 and TA100 and one strain of *E. coli*, WP2uvrA). Equivocal results were obtained in an *in vitro* chromosomal aberration assay which are overruled by the clearly negative results of an *in vitro* micronucleus test carried out under the same experimental conditions.

The Panel concluded that the food enzyme 3-phytase did not raise concern for genotoxicity.

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 the OECD Test Guideline 408 (OECD, 1998) with the following deviations: blood urea nitrogen was not determined and the microscopically examined brain regions were not specified.[31] The Panel considered that these deviations are minor and have no impact on the evaluation of the study.

Groups of 10 male and 10 female Wistar Crl:(WI) BR rats received by gavage the food enzyme in doses of 500, 1500 or 4500 mg/kg body weight (bw) per day, corresponding to 93, 278 or 833 mg TOS/kg bw per day. Controls received the vehicle (Milli U water).

One high-dose female was found dead on day 49. The Panel considered the death as incidental, because the animal died due to acute pyelonephritis, which was not related to the test item.

The body weight was statistically significantly increased on days 8, 15, 22, 29, 36, 43, 50, 57 and 64 of administration in low-dose males (+4%, +7%, +8%, +9%, +10%, +9%, +8%, +8%, +8%, respectively) and on days 29 and 36 in mid-dose males (+7%, +8%, respectively), but decreased on day 15 in mid-dose females (–5%). The Panel considered the changes as not toxicologically relevant, as they were recorded sporadically, there was no consistency between the changes in males and females, there was no dose–response relationship, the changes were small and they were without a statistically significant effect on the final body weight.

Haematological investigation revealed a statistically significant increase in the relative number of neutrophils in high-dose males (+47%) as well as a decrease in the white blood cell (WBC) count in mid-dose females (–23%) and in the partial thromboplastin time (PTT) in low-dose males (–6.5%). The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (all parameters), there was no dose–response relationship (WBC count and PPT), the change was small (PTT) and the change in neutrophils in males was not accompanied by a change in the WBC count.

Clinical chemistry investigation revealed a statistically significant increase in triglycerides in mid-dose males (+46%), in glucose in low- and mid-dose males (+16%, +14%, respectively) and in sodium in low-, mid- and high-dose females (+0.7%, +1.1%, +0.7%, respectively), a decrease in total bilirubin in mid-dose males (–20%), in chloride in low-, mid- and high-dose males (–3%, –3%, –3%, respectively) and in aspartate aminotransferase (ASAT) in mid-dose females (–44%). The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (all parameters), there was no dose–response relationship (all parameters) and the changes were small (sodium, chloride, ASAT).

Statistically significant increases were detected in the absolute liver weight in low-dose males (+15%) and in the relative (to body) spleen weight in high-dose females (+20%). A decrease in the relative (to body) weight of testes were seen in mid-dose males (–13%). The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (liver, spleen), there was no dose–response relationship (liver, testes) and there were no histopathological changes in the organs (liver, spleen, testes).

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

The Panel identified a no observed adverse effect level (NOAEL) of 833 mg TOS/kg bw per day, the highest dose tested.

[31]Technical dossier/Risk Assessment/Toxicological data/Annex 22.

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 enzyme produced with the genetically modified *A. niger* strain NPH 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.[32]

No information was available on oral and respiratory sensitisation or elicitation reactions of this 3-phytase.

Respiratory allergy following occupational inhalation of phytase have been reported (Baur et al., 2002; Budnik et al., 2017; Caballero et al., 2007; Kuske et al., 2020; O'Connor et al., 2001; van Heemst et al., 2009; Zober et al., 2002). However, several studies have shown that adults with occupational asthma to a food enzyme may be able to ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Armentia et al., 2009; Cullinan et al., 1997; Green & Beezhold, 2011; Poulsen, 2004). Information on adverse reactions upon ingestion of 3-phytase in individuals sensitised through the respiratory route has not been reported.

Yeast extract, 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 no potentially allergenic residues from this source are present in the food enzyme.

The Panel considered that the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded (except for distilled alcohol production), but the likelihood is low.

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme

The food enzyme is intended to be used in three food manufacturing 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.[33]

Food manufacturing process[a]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[b]
Processing of cereals and other grains		
Production of baked products	Flour	1.9–**46.5**
Production of distilled alcohol	Cereals	48.1–80.2
Processing of plant- and fungal-derived products		
Production of plant-based analogues of milk and milk products	Cereals, legumes and pulses, nuts, oil seeds	56–**186**

[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.

In baking processes, the food enzyme is added to flour during the preparation of the dough.[34] The 3-phytase hydrolyses phytate in cereals, increasing the bioavailability of minerals and digestibility of proteins.[35] The food enzyme–TOS remains in the baked foods.

In the distilled alcohol production, the food enzyme is added to the cereals before and during fermentation.[36] The hydrolysis by 3-phytase facilitates the release of phosphate as a nutrient in the fermentation step.[37] The food enzyme–TOS is not carried over with the distilled alcohols (EFSA CEP Panel, 2023).

[32]Technical dossier/Risk Assessment/Allergenicity/Annex 1 and Additional data September 2023.

[33]Technical dossier/Risk assessment/17. Use levels.

[34]Technical dossier/Risk assessment/12. Intended use in food.

[35]Technical dossier/Risk management/18. RM-Intended use in food.

[36]Technical dossier/ Risk assessment/12. Intended use in food.

[37]Technical dossier/Risk management/18. RM-Intended use in food.

In the production of plant-based dairy analogues, the food enzyme is added to a variety of plant materials (such as cereals, pulses, legumes, oil seeds and nuts, etc.).[38] The hydrolysis by 3-phytase reduces phytate concentrations, improving the digestibility of the final foods.[39] The food enzyme–TOS remains in the food products.

Based on data provided on thermostability (see Section 3.3.1) and the downstream processing steps applied, it is expected that the enzyme is inactivated or removed in the food manufacturing processes listed in Table 2.

3.5.2 | Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel, 2021), a dietary exposure was calculated only for food manufacturing processes where the food enzyme–TOS remains in the final foods: the production of baked products and the production of plant-based analogues of milk and milk products.

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). 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 1 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 41 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure was estimated to be 0.553 mg TOS/kg bw per day in infants at the 95th percentile.

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

	Estimated exposure (mg TOS/kg body weight per day)					
Population group	**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)	0.009–0.129 (11)	0.111–0.318 (15)	0.113–0.269 (19)	0.061–0.165 (21)	0.046–0.101 (22)	0.049–0.102 (22)
Min–max 95th (number of surveys)	0.051–0.553 (9)	0.280–0.503 (13)	0.226–0.505 (19)	0.137–0.349 (20)	0.101–0.215 (22)	0.097–0.174 (21)

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.

[38]Technical dossier/Risk assessment/12. Intended use in food/p. 1.

[39]Technical dossier/Risk management/18. RM-Intended use in food.

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	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Exclusion of one process from the exposure assessment: – production of distilled alcohol	–

Abbreviations: +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

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.

The exclusion of one food manufacturing process from the exposure assessment was based on > 99% of TOS removal. This is not expected to have an impact on the overall estimate derived.

3.6 | Margin of exposure

A comparison of the NOAEL (833 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.009–0.318 mg TOS/kg bw per day at the mean and from 0.051–0.553 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure (MoE) of at least 1506.

4 | CONCLUSIONS

Based on the data provided, the removal of TOS during the production of distilled alcohol and the derived margin of exposure for the two remaining food manufacturing processes, the Panel concluded that the food enzyme 3-phytase from the genetically modified *A. niger* strain NPH does not give rise to safety concerns under the intended conditions of use.

The CEP Panel considered the food enzyme free from viable cells of the production organism and recombinant DNA.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Application for the authorisation of 3-phytase from a genetically modified *Aspergillus niger* strain NPH as a new food enzyme. March 2022. Submitted by DSM Food Specialties.

Additional information. September 2023. Submitted by DSM Food Specialties.

ABBREVIATIONS

ANI	average nucleotide identity
bw	body weight
CAS	Chemical Abstracts Service
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
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
MNBN	bi-nucleated cells with micronuclei
MoE	margin of exposure
OECD	Organisation for Economic Co-operation and Development
PCR	polymerase chain reaction

TOS	total organic solids
WGS	whole genome sequence
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.

REQUESTOR

European Commission

QUESTION NUMBER

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LEGAL NOTICE

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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.

APPENDIX B

Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, 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, the 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, the 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, the 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, the 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, the 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).