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SCIENTIFIC OPINION

Safety evaluation of the food enzyme 3-phytase from the non-genetically modified *Aspergillus niger* strain PHY93-08

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Abstract

The food enzyme 3-phytase (myo-inositol-hexakisphosphate 3-phosphohydrolase EC 3.1.3.8) is produced with the non-genetically modified *Aspergillus niger* strain PHY93-08 by Shin Nihon Chemical Co., Ltd. The food enzyme is free from viable cells of the production organism. It is intended to be used in nine food manufacturing processes. Since residual amounts of food enzyme–total organic solids (TOS) are removed in two of the food manufacturing processes, dietary exposure was calculated only for the remaining seven processes. It was estimated to be up to 0.763 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not raise safety concerns. 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 2560 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 3355. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and no matches were found. The Panel considered that the risk of allergic reactions upon dietary exposure cannot be excluded, 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, myo-inositol-hexakisphosphate 3-phosphohydrolase, phytase

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 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.

1.1 | Background and Terms of Reference as provided by the requestor

1.1.1 | Background as provided by the European Commission

Only food enzymes included in the European Union (EU) Community list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008 on food enzymes.

Five applications have been introduced by the applicant "Intertek Scientific & Regulatory Consultancy" for the authorization of the food enzymes 3-Phytase from *Aspergillus niger* (strain PHY93-08), Alpha-amylase from *Aspergillus niger* (strain AS 29–286), Invertase and Exo-beta-glucosidase from *Aspergillus niger* (strain IN 319), Alpha-galactosidase from *Aspergillus niger* (strain AGS614), and Lactase from *Aspergillus oryzae* (strain GL 470).

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

1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the following food enzymes 3-Phytase from *Aspergillus niger* (strain PHY93-08), Alpha-amylase from *Aspergillus niger* (strain AS 29–286), Invertase and Exo-beta-glucosidase from *Aspergillus niger* (strain IN 319), Alpha-galactosidase from *Aspergillus niger* (strain AGS614), and Lactase from *Aspergillus oryzae* (strain GL 470) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

[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 food enzyme 3-Phytase from *Aspergillus niger* (strain PHY93-08).

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme 3-phytase from the non-genetically modified *Aspergillus niger* strain PHY93-08.

Additional information was requested from the applicant during the assessment process on 20 January 2021 and received on 20 July 2021 (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, 2009a) 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, 2009b) 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. Additional information was requested in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021) and the guidance on the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

3 | ASSESSMENT

IUBMB nomenclature	3-phytase
Systematic name	Myo-inositol-hexakisphosphate 3-phosphohydrolase
Synonyms	Phytase; Phytate 6-phosphatase
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 food enzyme under assessment is intended to be used in nine food manufacturing processes: processing of cereals and other grains for the production of (1) baked products, (2) brewed products, (3) starch and gluten fractions, (4) distilled alcohol and (5) non-wine vinegars; processing of plant- and fungal-derived products for the production of (6) coffee extracts, (7) soy sauce, (8) protein hydrolysates from plants and fungi and (9) plant extracts.

3.1 | Source of the food enzyme

The 3-phytase is produced with the non-genetically modified filamentous fungus *Aspergillus niger* strain PHY93-08, which is deposited at the CABI Bioscience culture collection (UK), with the deposit number SD139.[4]

The production strain was identified as *A. niger* by [redacted][5] The production strain was isolated from a food source and selected for high level of phytase activity and absence of mycotoxin production.

[4]Technical dossier/Annex I-2.

[5]Technical dossier/Additional data July 2021/Annex A.

3.2 | Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[6] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[7]

The production strain is grown as a pure culture using a typical industrial medium in a solid-state fermentation system with conventional process controls in place. After completion of the fermentation, water is added and the solid biomass is then removed from the resulting suspension 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.[8] 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.[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 3-phytase is a single polypeptide chain of [redacted] amino acids.[10] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa. The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches. The gel showed a major protein band migrating between the marker proteins of [redacted] kDa due to glycosylation.[11] No other enzymatic side activities were reported.[12]

The in-house determination of 3-phytase activity is based on the hydrolysis of phytate (reaction conditions: [redacted]) and is determined by measuring the release of phosphoric acid [redacted]. The phytase activity is expressed in U/g or U/mL. One unit is defined as the quantity of enzyme which releases 1 µmol of phosphoric acid from phytate per minute under the conditions of the assay.[13]

The food enzyme has a temperature optimum around 50°C (pH 5.5) and a pH optimum around pH 5.8 (37°C). Thermostability was tested after a pre-incubation of the food enzyme for 15 min at different temperatures (pH 5.5). The 3-phytase activity decreased above 50°C, showing no residual activity after pre-incubation at 60°C.[14]

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).[15] The average total organic solids (TOS) of the three food enzyme batches for commercialisation was 25.8% and the average enzyme activity/TOS ratio was 14.4 U/mg TOS.

TABLE 1 Composition of the food enzyme.

		Batches				
Parameters	**Unit**	**1**	**2**	**3**	**4**[a]	**5**[b]
3-Phytase activity	U/g[c]	3710	3670	3800	4020	4590
Protein	%	16.7	17.5	17.1	15.7	NA
Ash	%	2.1	2.2	2.2	3.3	0.3
Water	%	72.3	72.1	71.6	71.0	78.2
Total organic solids (TOS)[d]	%	25.6	25.7	26.2	25.7	21.5
Activity/TOS ratio	U/mg TOS	14.5	14.3	14.5	15.6	21.3

[a]Batch used for most of the toxicological studies.

[b]Batch used for in vivo mammalian alkaline comet assay in rodents.

[c]U/g: (see Section 3.3.1).

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

[6]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.

[7]Technical dossier/pp. 38.

[8]Technical dossier/pp. 32–38.

[9]Technical dossier/pp. 33/Annex III.

[10]Technical dossier/pp. 17/Annex VII.

[11]Technical dossier/pp. 17.

[12]Technical dossier/pp. 25, 41.

[13]Technical dossier/Annex II-1.

[14]Technical dossier/pp. 21–24.

[15]Technical dossier/pp. 52/Annex IV/Annex V and Additional data July 2021/Annex B.

3.3.3 | Purity

The lead content in the three commercial batches and in the batches used for toxicological studies was below 5 mg/kg[16] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, the concentrations of arsenic were either below the limits of detection (LoDs) of the employed methods, or when detected, found at concentrations which did not give rise to safety concerns.[17,18]

The food enzyme complies with the microbiological criteria for total coliforms, *Escherichia coli* and *Salmonella* as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).[19] No antimicrobial activity was detected in any of the tested batches.[20]

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 the mycotoxins aflatoxins B1, B2, G1 and G2, fumonisins B1 and B2, ochratoxin A, sterigmatocystin, T-2 toxin and zearalenone was examined in three food enzyme batches and all were below the LoD of the applied method.[21,22] Adverse effects caused by 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 was sufficient.

3.3.4 | Viable cells of the production strain

The absence of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. One gram of product was inoculated on non-selective agar plates and incubated at 30°C for 6 days. No colonies of the production strain were produced. A positive control was included.[23]

3.4 | Toxicological data

A battery of toxicological tests, including a bacterial gene mutation assay (Ames test), an in vitro mammalian chromosomal aberration test, an in vivo micronucleus assay, an in vivo mammalian alkaline comet assay and a repeated dose 90-day oral toxicity study in rats, has been provided. The batches 4 and 5 (Table 1) were considered suitable as test items.

3.4.1 | Genotoxicity

3.4.1.1 | *In vitro studies*

3.4.1.1.1 | Bacterial reverse mutation test

A bacterial reverse mutation assay (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).[24]

Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2*uvrA* were used in the presence or absence of metabolic activation (S9-mix), applying the pre-incubation, and treat and wash methods.

The range-finding study was performed in triplicate at concentrations ranging from 35.09 to 25,600 µg TOS/mL, applying the pre-incubation method. The growth of background bacteria in each strain was accelerated, resulting in the formation of colonies larger than those in the negative control. An increase in the number of revertants above the control values was observed in *S*. Typhimurium TA98, TA100 and TA1535 both in the presence and absence of S9-mix and in *S*. Typhimurium TA1537 in the absence of S9-mix.

Based on the results of the range finding study showing the absence of toxicity over the concentration range tested, the first main study was performed in triplicate at concentrations of 800, 1600, 3200, 6400, 12,800 and 25,600 µg TOS/mL in *E. coli* WP2*uvrA* in the presence or absence of S9-mix and *S*. Typhimurium TA1537 in the presence of S9-mix, applying the pre-incubation method. A two-fold increase in the number of revertants was observed in strain TA1535.

[16]Technical dossier/pp. 52/Annex IV-1 and Additional data July 2021/Annex B.

[17]LoDs: Pb = 0.05 mg/kg; As = 0.1 mg/kg.

[18]Technical dossier/pp. 52/Annex IV-1 and Additional data July 2021/Annex B.

[19]Technical dossier/pp. 52/Annex IV-1 and Additional data July 2021/Annex B.

[20]Technical dossier/pp. 52/Annex IV-2 and Additional data July 2021/Annex B.

[21]LoD: aflatoxins B1, B2, G1 and G2 = 0.5 µg/kg each; fumonisins B1 and B2 = 0.5 µg/kg each; ochratoxin A = 0.5 µg/kg; sterigmatocystin = 100 µg/kg; T-2 toxin = 0.1 mg/kg; zearalenone = 100 µg/kg.

[22]Technical dossier/Annex IV-3 and Additional data July 2021/Annex B.

[23]Technical dossier/Additional data July 2021.

[24]Technical dossier/Annex VI-1.

The second main and confirmatory studies were performed in triplicate at concentrations of 800, 1600, 3200, 6400, 12,800 and 25,600 µg TOS/mL in *S.* Typhimurium TA98, TA100, and TA1535 both in the presence and absence of S9-mix and in *S.* Typhimurium TA1537 in the absence of S9-mix, applying the treat and wash 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.

The Panel concluded that the food enzyme 3-phytase did not induce gene mutations under the test conditions employed in this study.

3.4.1.1.2 | *In vitro mammalian chromosomal aberration test*

The in vitro mammalian chromosomal aberration test was carried out in Chinese hamster lung fibroblast (CHL/IU) cells according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[25]

The growth inhibition test was performed at concentrations ranging from 800 to 25,600 µg TOS/mL and the 50% cell growth inhibition concentration (IC_{50}) was calculated to be 251 and 232 U/mL (corresponding to 15,984 and 14,774 µg TOS/mL) in the short-term treatment in the absence and in the presence of S9-mix, respectively, and 218 U/mL (corresponding to 13,883 µg TOS/mL) in the long-term treatment. Based on these results, the cells were exposed to the food enzyme at 8788, 12,545 and 17,895 µg TOS/mL in the short-term treatment (6 hours followed by 18 hours recovery period) with and without metabolic activation (S9-mix), and at 6145, 8788, 12,545 and 17,895 µg TOS/mL in the long-term treatment (24 h) in the absence of S9-mix.

Cytotoxicity was observed at the highest concentration of 17,895 µg TOS/mL after all treatments (51% for short-term treatment with and without S9-mix and 61% for long-term treatment). The frequency of structural chromosomal aberrations in treated cultures was increased in a concentration-related manner, with statistically significant difference at the concentration of 17,895 µg TOS/mL in the short-term and long-term treatments without S9-mix and at the concentration of 12,545 µg TOS/mL in the short-term treatment with S9-mix. The frequency of numerical chromosomal aberrations in treated cultures was comparable to the values detected in negative controls and within the range of the laboratory historical solvent control data.

The Panel concluded that food enzyme 3-phytase induced structural chromosomal aberrations under the test conditions employed for this study.

3.4.1.2 | *In vivo studies*

3.4.1.2.1 | *In vivo mammalian erythrocyte micronucleus test*

The in vivo mammalian erythrocyte micronucleus test in rats was carried out according to the OECD Test Guideline 474 (OECD, 1997c) and following GLP.[26]

Five male Crl:CD(SD)[SPF] rats per group were orally administered the food enzyme dissolved in distilled water at doses of 640, 1280 and 2560 mg TOS/kg body weight (bw) per day (Batch 4) for 2 consecutive days. Rats were sacrificed 24 h after dosing. A negative control group received distilled water and a positive control group received 2 mg/kg bw of mitomycin C intravenously.

No mortalities, body weight changes and clinical signs of toxicity were reported after treatment with the test item. For each animal, 2000 immature erythrocytes (IE) were scored for the presence of micronucleated immature erythrocytes (MNIE).

No statistically significant increases in the frequency of MNIE and no substantial decrease in the proportion of IE to total number of analysed erythrocytes were observed in animals treated with the food enzyme, compared with vehicle control values.

The Panel concluded that the food enzyme did not induce MNIE in bone marrow when tested up to 2560 mg TOS/kg bw per day under the experimental conditions employed; however, this study was considered inconclusive because no data on bone marrow exposure were provided.

3.4.1.2.2 | *In vivo mammalian alkaline comet assay in rodents*

An in vivo mammalian alkaline Comet assay in liver and duodenum of Crl:CD(SD) [SPF] rats was carried out according to the OECD Test Guideline 489 (OECD, 2016) and following GLP.[27] The range of doses applied in the main study were selected based on the results of a single oral dose toxicity study and of a 2-week repeated oral dose toxicity study in rats, in which no mortality and no treatment-related clinical signs after dosing at 2310 mg TOS/kg bw per day were observed. Since there were no differences in toxicity between sexes, only males were used in the main study.

[25]Technical dossier/Annex VI-2.

[26]Technical dossier/Annex VI-3.

[27]Technical dossier/Additional data, 22 July 2021/Attachment D.

Five male rats were dosed once daily by oral gavage up to the maximum administrable dose: 578, 1160 and 2310 mg TOS/kg bw per day for two consecutive days at 21-h interval. At 3 h after the final administration, the liver and duodenum samples were collected and slides were prepared.

No mortality, body weight changes, treatment-related clinical signs or macroscopic lesions in the liver and duodenum were observed in any animal group.

Measurements of tail intensity (% DNA in tail) were obtained from 150 cells/animal. No statistically significant increase in mean tail intensity values for animals treated with the food enzyme were observed in liver or duodenum of any treated group compared to the concurrent vehicle control group. The positive control (EMS, 200 mg/kg per day) induced a statistically significant increase in the mean tail intensity in liver and duodenum, however, within the range of the historical positive control database.

No increase in the mean frequencies of hedgehogs was observed in any of the treatment groups as compared with the negative control group.

The food enzyme did not induce DNA damage in liver or duodenum of rats, administered via oral gavage, as analysed by the Comet assay. The Panel considered that the negative results of this study allow the concern for clastogenicity to be ruled out.

Conclusions on genotoxicity:

The food enzyme was tested in a battery of in vitro and in vivo genotoxicity studies. The test item in the presence or absence of S9-mix, did not induce gene mutations in bacteria. The results of an in vitro study carried out in Chinese hamster lung fibroblasts showed the induction of structural chromosomal aberrations in presence and absence of metabolic activation. Negative results were reported in an in vivo Comet assay performed in liver and duodenum of rats treated at the maximum feasible doses. The Panel concluded on the basis of the in vitro and in vivo studies that there is no concern for genotoxicity of the food enzyme 3-phytase.

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.[28] Groups of 10 male and 10 female Sprague–Dawley (Crl:CD(SD)) [SPF] rats received by gavage the food enzyme in 25.6, 256 and 2560 mg TOS/kg bw per day. Controls received the vehicle (water for injection).

No mortality was observed.

Haematological investigation revealed statistically significant increases in red blood cell count, haemoglobin concentration and haematocrit levels (+4% for all parameters) in high-dose females. The Panel considered the changes as not toxicologically relevant as they were small, they were only observed in one sex and the changes were within the historical control values.

Clinical chemistry investigation revealed a statistically significant increase in blood glucose (+17%) in high-dose males and a statistically significant decrease in total protein concentration (−4%) in low-dose males. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (both parameters), the change was small (total protein) and the changes were within the historical control values.

Statistically significant changes in organ weights detected were a decrease in relative liver weight (−5%) in mid-dose males and a statistically significant increase in absolute ovary weight (+22%) in mid-dose females. The Panel considered the changes as not toxicologically relevant as there was no-dose response relationship, the change was small and only observed in one sex (liver), there were no histopathological changes in the liver and ovaries, and in other relevant organs/tissues (for ovary weight in uterus and in vagina).

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

The Panel identified the no observed adverse effect level (NOAEL) of 2560 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 3-phytase produced with *Aspergillus niger* strain PHY93-08 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.[29]

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

[28]Technical dossier/Annex VI-5; Technical dossier/Additional data, 22 July 2021/Attachment C.

[29]Technical dossier/pp. 53–56/Annex VII.

Respiratory allergy following occupational inhalation of phytase have been reported (Baur et al., 2002; Caballero et al., 2007; Doekes et al., 1999; O'Connor et al., 2001; van Heemst et al., 2009). In addition, *Aspergillus niger* is a source of inhalant allergens (Vermani et al., 2015). However, several studies have shown that individuals respiratorily sensitised 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; Poulsen, 2004). Information on adverse reactions upon ingestion of 3-phytase in individuals sensitised through the respiratory route has not been reported.

[redacted], a substance that may cause allergies (listed in the Regulation (EU) No 1169/2011[30]) is used as a raw material. 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, 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 nine food manufacturing processes[31] at the recommended use levels summarised in Table 2.

TABLE 2 Intended uses and use levels of the food enzyme as provided by the applicant.[32]

Food manufacturing process[a]	Raw material (RM)	Maximum recommended use level (mg TOS/kg RM)[b,c]
Processing of cereals and other grains		
• Production of baked products	Flour	**41.5**
• Production of brewed products	Cereals	**138.4**
• Production of starch and gluten fractions	Cereals	138.4
• Production of distilled alcohol	Cereals	138.4
• Production of non-wine vinegar	Cereals	**138.4**
Processing of plant- and fungal-derived products		
• Production of coffee extracts	Coffee beans	**226.8**
• Production of soy sauce	Soybean, wheat	138.4
• Production of protein hydrolysates from plants and fungi	Soybean	**276.7**
• Production of plant extracts	Plant material	**138.4**

Abbreviation: TOS, total organic solids.

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

[b]Based on the conversion of 3729 U/g of enzyme = 14.4 Unit/mg TOS.[33]

[c]The numbers in bold represent the maximum recommended use levels, which were used for calculation.

In the production of baked products, 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. The food enzyme–TOS remains in the baked foods.

In the production of brewed products, the food enzyme is added to cereals during the mashing or the saccharification steps.[35] The hydrolysis of phytate releases 1D-myoinositol 1,2,4,5,6-pentakisphosphate and phosphate to improve alcoholic fermentation by yeast. The food enzyme–TOS remains in the brewed products.

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

[31]Additional data July 2021/Attachment E.

[32]Additional data July 2021/Table 4.

[33]Additional data July 2021/Annex E/p. 5.

[34]Additional data July 2021/Attachment E/p. 6.

[35]Additional data July 2021/Attachment E/pp. 7, 8, 19.

In the production of starch and gluten fractions, the 3-phytase is added during the homogenisation and agglomeration step. It is used to reduce the steeping time and to aid the separation of different fractions.[36] The food enzyme–TOS is removed by repeated washing (EFSA CEP Panel, 2023).

In the production of distilled alcohol, the food enzyme is added to the cereals before and during fermentation.[37] The food enzyme–TOS is not carried over with the distilled alcohols (EFSA CEP Panel, 2023).

In the production of coffee extracts, the food enzyme is added to coffee beans after the water extraction to improve the filtration process.[38] The food enzyme–TOS remains in the coffee products.

In the production of non-wine vinegar, the food enzyme is added to cereals or to fruits during the saccharification or the fermentation steps. The hydrolysis by 3–phytase reduces the formation of haze and lees.[39] The food enzyme–TOS remains in the vinegars.

In the production of soy sauce, the food enzyme is added after the fermentation step,[40] while in the production of soy paste, it is added during the fermentation.[41] The hydrolysis by 3–phytase prevents the formation of haze and lees in soy sauce.[42] The food enzyme–TOS remains in soy products.

In the production of protein hydrolysates from plants and fungi, the 3-phytase is added to soy milk to separate β-conglycinin[43] and after the suspension step to enhance the proteolytic activity of proteases.[44] The food enzyme–TOS remains in the final protein hydrolysates, which is an ingredient in a variety of final foods.

In the production of plant extracts, the food enzyme is added to ground plant materials to enhance the activity of proteases.[45] The food enzyme–TOS remains in the plant extracts.

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

3.5.2 | Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel, 2021), dietary exposure was calculated only for the seven food manufacturing processes where the food enzyme–TOS remain in the final foods.

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 one day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 3 provides an overview of the derived exposure estimates across all surveys. Detailed mean and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 48 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 26 European countries (Appendix B). The highest dietary exposure was estimated to be 0.763 mg TOS/kg bw per day in adults 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)	0–0.097 (12)	0.008–0.257 (15)	0.010–0.214 (19)	0.010–0.132 (21)	0.086–0.252 (22)	0.068–0.278 (23)
Min–max 95th percentile (number of surveys)	0–0.276 (11)	0.026–0.399 (14)	0.026–0.428 (19)	0.041–0.261 (20)	0.213–0.763 (22)	0.150–0.538 (22)

Abbreviation: TOS, total organic solids.

[36] Additional data July 2021/Attachment E/p. 9.
[37] Additional data July 2021/Attachment E/p. 10.
[38] Additional data July 2021/Attachment E/p. 11.
[39] Additional data July 2021/Attachment E/p. 12.
[40] Additional data July 2021/Attachment E/p. 14.
[41] Additional data July 2021/Attachment E/p. 15.
[42] Additional data July 2021/Attachment E/p. 14.
[43] Additional data July 2021/Attachment E/p. 18.
[44] Additional data July 2021/Attachment E/p. 17.
[45] Additional data July 2021/Attachment E/p. 16.

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	
Selection of broad FoodEx categories for the exposure assessment	+
Exposure to food enzyme–TOS always calculated based on the recommended maximum use level	+
Use of recipe fractions to disaggregate FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Exclusion of two processes from the exposure estimation: – Production of starch and gluten fractions – Production of distilled alcohol	–

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

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 overestimation of the exposure.

The exclusion of two food manufacturing processes from the exposure estimation was based on > 99% of TOS removal. This is not expected to impact on the overall estimate derived.

3.6 | Margin of exposure

A comparison of the NOAEL (2560 mg TOS/kg bw per day) identified from the 90-day rat study with the derived exposure estimates of 0–0.278 mg TOS/kg bw per day at the mean and from 0 to 0.763 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure of at least 3355.

4 | CONCLUSIONS

Based on the data provided, the removal of TOS during two food manufacturing processes and the derived margin of exposure for the remaining seven food manufacturing processes, the Panel concluded that the food enzyme 3-phytase produced with the non-genetically modified *A. niger* strain PHY93-08 does not give rise to safety concerns under the intended conditions of use.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Application for the Authorisation of 3-Phytase from *Aspergillus niger* Strain PHY93-08 as a Food Enzyme in the European Union Pursuant to Regulation (EC) No 1332/2008 of the European Parliament and Council of 16 December 2008. March 2015. Submitted by Shin Nihon Chemical Co., Ltd.

Additional information. July 2021. Submitted by Shin Nihon Chemical Co., Ltd.

ABBREVIATIONS

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

IE	immature erythrocytes
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
MNIE	micronucleated immature erythrocytes
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
TOS	total organic solids
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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NOTE

The full opinion will be published in accordance with Article 12 of Regulation (EC) No 1331/2008 once the decision on confidentiality will be received from the European Commission.

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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 one day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia*, Serbia*, Slovenia, Spain
Children	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia*, Serbia*, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina*, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina*, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden
The elderly[a]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden

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

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