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Safety evaluation of a food enzyme containing trypsin, chymotrypsin, elastase and carboxypeptidase from porcine pancreas

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Abstract

The food enzyme is a protease complex, containing trypsin (EC 3.4.21.4), chymotrypsin (EC 3.4.21.1), pancreatic elastase (EC 3.4.21.36) and carboxypeptidase B (EC 3.4.17.2), obtained from porcine pancreas by Neova Technologies Inc. The food enzyme is intended to be used for hydrolysis of whey proteins employed as ingredients of infant formulae, follow-on formulae and in food for special medical purposes (tube feeding). Based on maximum use levels and the maximum permitted protein content in infant formula, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be 36 mg TOS/kg body weight (bw) per day for infants. The Panel estimates that this value covers all population groups. In the toxicological evaluation, clinical studies with pharmaceutical preparations containing pancreatic enzymes were considered. Hypersensitivity to the pharmaceuticals was identified as the major side effect. However, the intact enzymes are removed during preparation of food products; therefore, the Panel considers that the likelihood of adverse effects of the intact enzyme to occur is small. Low molecular weight peptides derived from the enzyme are still likely to be present in the protein hydrolysate. The Panel considered that a risk of allergic sensitisation to these peptides after consumption of products prepared by hydrolysis of milk, cannot be excluded in infants, but the likelihood to occur is considered to be low. Based on the origin of the food enzyme from edible parts of animals, the data provided by the applicant, supported by the evaluation of clinical studies with pharmaceutical preparations based on pancreatic enzymes, the Panel concluded that the porcine pancreatic enzymes do not give rise to safety concern under the intended conditions of use.

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008¹ 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 1331/2008² 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:

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

All food enzymes currently on the European Union market and intended to remain on that market, as well as all new food enzymes, shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and approval via an EU Community list.

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) lays down the administrative, technical and toxicological data required.

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

1.1.1. Background as provided by the European Commission

Only food enzymes included in the European Union (EU) Community list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008³ on food enzymes.

Five applications have been introduced by the companies "Novozymes A/S", "Puratos NV sa", "Neova Technologies Inc." and "Amano Enzyme Inc." for the authorisation of the food enzymes Asparaginase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-OA), Xylanase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-CE), Xylanase from a genetically modified strain of *Bacillus subtilis* LMG S-24584, Protease complex consisting of trypsin, chymotrypsin, elastase and carboxypeptidase from pig pancreas, and Cellulase from *Trichoderma viride* (strain AE-CT).

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, p. 7–15.

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011⁴ implementing Regulation (EC) No 1331/2008⁵, 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 assessment on the food enzymes Asparaginase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-OA), Xylanase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-CE), Xylanase from a genetically modified strain of *Bacillus subtilis* LMG S-24584, Protease complex consisting of trypsin, chymotrypsin, elastase and carboxypeptidase from pig pancreas, and Cellulase from *Trichoderma viride* (strain AE-CT) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

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 a food enzyme containing trypsin, chymotrypsin, elastase and carboxypeptidase obtained from porcine pancreas.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for the authorisation of a food enzyme containing trypsin, chymotrypsin, elastase and carboxypeptidase from porcine pancreas.

Additional information was sought from the applicant during the assessment process in a request from EFSA sent on 21 January 2016, on 3 October 2016 and on 11 June 2020, and was consequently provided (see 'Documentation provided to EFSA').

2.2. Methodologies

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

The food enzyme under application contains four declared activities:

IUBMB nomenclature	Trypsin
Synonyms	α -trypsin, β -trypsin
IUBMB No.	EC 3.4.21.4
CAS No.	9002-07-7
EINECS No.	232-650-8

Trypsin is a serine endopeptidase that catalyses the hydrolysis of peptide bonds on the carboxyl-terminal (C-terminal) side of the amino acids lysine and arginine, releasing polypeptides.

⁴ 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, p. 15–24.

⁵ 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, p. 1–6.

IUBMB nomenclature	Chymotrypsin
Synonyms	Chymotrypsin A and B, α -chymar ophth
IUBMB No.	EC 3.4.21.1
CAS No.	9004-07-3
EINECS No.	232-671-2

Chymotrypsin, also a serine endopeptidase, catalyses the hydrolysis of peptide bonds on the C-terminal side of the amino acids tryptophan, tyrosine, phenylalanine and leucine (to lower extent), releasing polypeptides.

IUBMB nomenclature	Pancreatic elastase
Synonyms	Elastase, pancreatopeptidase E, pancreatic elastase I, serine elastase
IUBMB No.	EC 3.4.21.36
CAS No.	9004-06-2
EINECS No.	232-670-7

Elastase is a serine endopeptidase that catalyses the hydrolysis of peptide bonds on the C-terminal side of the amino acids valine, alanine and glycine, releasing polypeptides.

IUBMB nomenclature	Carboxypeptidase B
Synonyms	Protaminase; pancreatic carboxypeptidase B
IUBMB No.	EC 3.4.17.2
CAS No.	9025-24-5
EINECS No.	232-788-9

Carboxypeptidase B is an exopeptidase with a preferential release on the C-terminal side of the amino acids lysine, arginine and ornithine.

The food enzyme is intended to be used in protein processing for the production of whey protein hydrolysates to be used as ingredients in infant formulae (IF) and follow-on formulae (FOF), and in food for special medical purposes (FSMP) (tube feeding).

3.1. Source of the food enzyme

The food enzyme is produced from the pancreas of pigs (*Sus scrofa domesticus*).

3.1.1. Information on the animal source material

The food enzyme is exclusively obtained from the pancreas of animals slaughtered and approved for human consumption, free of diseases (i.e. African swine fever, classical swine fever, foot and mouth disease, and swine vesicular disease). Verification is performed by veterinarians in charge of the registered establishments for the slaughtering.⁶ Pigs are not included in the list of the specific risk material defined by Commission Regulation (EU) 2015/1162⁷. The porcine pancreas glands are collected following the requirements of the relevant EU hygiene regulations.⁸

Possible parasites (i.e. *Trichinella spiralis*, *Taenia solium* and *Toxoplasma gondii*) and bacterial contamination (e.g. *Yersinia enterocolitica*, *Listeria monocytogenes*, *Salmonella* spp.) are removed within the technological process (by freezing and filtering, respectively).⁹

No issues of concern arising from the safety of the source material were identified by the Panel.

⁶ Technical dossier/p. 38/ Annex 3-2-1-94.

⁷ Commission Regulation (EU) No 2015/1162 of 15 July 2015 amending Annex V to Regulation (EC) No 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies.

⁸ Regulations (EC) No 853/2004 and (EC) No 854/2004.

⁹ Technical dossier/p. 38–39.

3.2. Production of the food enzyme

The food enzyme is manufactured according to Canadian Food and Drug Act and Regulations which lays down the general principles and requirements of food law in the country of manufacture, implementing the Codex general principles of food hygiene and in accordance with current Good Manufacturing Practice.¹⁰

The food enzyme is extracted from minced pancreas of pigs using [REDACTED]. After [REDACTED] of the proteases ([REDACTED]), the tissue material is removed by filtration leaving the [REDACTED] solution containing the food enzyme. The filtrate is then submitted to a series of filtration and concentration steps, including ultrafiltration in which the enzyme protein is retained while most of the low molecular weight material passes the filtration membrane and is discarded. The food enzyme is then dried and stabilised.¹¹ The applicant provided information on the identity of the substances used in the extraction and in the subsequent downstream processing of the food enzyme.¹²

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

Trypsin is a single polypeptide chain of 231 amino acids. The molecular mass, derived from the amino acid sequence, is 24.4 kDa.¹³ Chymotrypsin is composed of three polypeptide chains. There are three isoenzymes present in the food enzyme: chymotrypsin A and B (231 amino acids, 24.1 kDa each), and chymotrypsin C (268 amino acids, 28.9 kDa).¹⁴ Elastase is a single polypeptide chain of 266 amino acids. The molecular mass, derived from the amino acid sequence, is 28.8 kDa.¹⁵ The sequences of the main carboxypeptidases, A1 and B, are available in Uniprot.^{16,17,18} The food enzyme was tested for amylase, phospholipase and lipase activities, which were detected in all three commercial batches shown in Table 1 at the mean activity of 141,000 amylase units (AU)/g, 167 phospholipase units (PU)/g and 533 lipase units (LU)/g, respectively.¹⁹ No other enzymatic side activities were reported.

The in-house determination of protease activity of the food enzyme is based on the hydrolysis of a casein solution releasing aromatic amino acids (reaction conditions: pH 8, 40°C, 60 min). The reaction is stopped by adding trichloroacetic acid which precipitates intact casein remaining in the solution. The precipitate is removed by filtration and the enzymatic activity is then determined spectrophotometrically at 280 nm in the filtrate. The protease activity is quantified relative to an USP Pancreatin Protease standard and expressed in USP Protease Units (USPP)/g.²⁰

The in-house determination of trypsin activity is based on the hydrolysis of the substrate *N*-benzoyl-L-arginine ethyl ester hydrochloride, releasing *N*-benzoyl-L-arginine (reaction conditions: pH 7.6, 25°C). The enzymatic activity is determined by quantifying the formed product spectrophotometrically at 253 nm. The enzyme activity is expressed in USP trypsin unit (USPT)/g. One USPT is defined as the activity causing a change of absorbance of 0.003 per minute under the conditions of the assay.²¹

The chymotrypsin, elastase and carboxypeptidase B activities are quantified based on the same principle as for trypsin, but using different substrates and reaction conditions: *N*-acetyl-L-tyrosine ethyl ester (reaction conditions: pH 7.0, 25°C), succinyl-Ala-Ala-Ala-*p*-nitroanilide (Suc Ala₃NA) (reaction conditions: pH 8.0, 25°C) and hippuryl-L-arginine (reaction conditions: pH 7.65, 25°C), respectively. The hydrolysis of the substrates is followed spectrophotometrically (237, 410 and 254 nm, respectively).

¹⁰ Technical dossier/Annex 3-2-1-31.

¹¹ Technical dossier/Section 3.2/p. 40–46.

¹² Technical dossier/p. 47.

¹³ <http://www.uniprot.org/uniprot/P00761>

¹⁴ <http://www.uniprot.org/uniprot/G1ARD6>

¹⁵ <http://www.uniprot.org/uniprot/P00772>

¹⁶ <http://www.uniprot.org/uniprot/P09954>

¹⁷ <http://www.uniprot.org/uniprot/P09955>

¹⁸ Technical dossier/ Section 3.2/p. 3–4 and Additional data February 2016.

¹⁹ Technical dossier/Section 3.2/p. 8.

²⁰ Technical dossier/Section 3.2/p. 13–14 and Annex 3-2-1-13.

²¹ Technical dossier/ Section 3.2/Annex 3-2-1-14.

The enzymatic activities are expressed as USP chymotrypsin units (USPC)/g, elastase units (EIU)/g and carboxypeptidase units (CU)/g. One USPC is defined as the activity causing a change of absorbance of 0.0075 per minute under the conditions of the assay. One EIU is defined as the activity that hydrolyses 1 μmol of Suc Ala₃NA under the conditions of the assay. One CU is defined as the activity that hydrolyses 1 μmol of hippuryl-L-arginine under the conditions of the assay.²²

The overall protease activity against casein has a temperature optimum around 50°C (pH 8) and a pH optimum at pH 10, which corresponds to the highest pH tested (50–60°C).²³ Thermostability was tested after a pre-incubation of the food enzyme at 80°C. Under the conditions (pH 7.0) of the applied temperature stability assay, the food enzyme showed 0.6% residual activity after 0.5 and 1.5 min, and 0.5% residual activity after 2.5 min.²⁴

3.3.2. Chemical parameters

Data on the chemical parameters have been provided for three solid food enzyme preparation batches used for commercialisation (Table 1).²⁵ The average total organic solids (TOS) content of the three food enzyme preparation batches was 44.2%. The average protease activity/TOS ratio was 694 USP/mg TOS.

Table 1: Composition of the food enzyme preparation

Parameter	Unit	Batches		
		1	2	3
Protease complex activity	USPP/g batch ^(a)	287,000	334,000	298,000
Trypsin	USPT/g batch ^(b)	338,000	367,000	415,000
Chymotrypsin	USPC/g batch ^(c)	101,000	97,000	119,000
Elastase	EIU/g batch ^(d)	100	100	100
Carboxypeptidase	CU/g batch ^(e)	1,700	1,600	2,100
Protein	%	41.5	47.1	42.5
Ash	%	2.8	3.1	2.6
Water	%	4.0	4.0	4.0
█ (excipient)	%	51.1	43.6	52.2
Total organic solids (TOS) ^(f)	%	42.1	49.3	41.2
Protease activity/mg TOS	USPP/mg TOS	682	677	723
Trypsin activity/mg TOS	USPT/mg TOS	803	744	1,007
Chymotrypsin activity/mg TOS	USPC/mg TOS	240	197	289
Elastase activity/mg TOS	EIU/mg TOS	0.2	0.2	0.2
Carboxypeptidase activity/mg TOS	CU/mg TOS	4	3	5

(a): USPP/g: Protease USP Units/g (see Section 3.3).

(b): USPT/g: Trypsin USP Units/g (see Section 3.3).

(c): USPC/g: Chymotrypsin USP Units/g (see Section 3.3).

(d): EIU/g: Elastase Units/g (see Section 3.3).

(e): CU/g: Carboxypeptidase Units/g (see Section 3.3).

(f): TOS calculated as 100% – % water – % ash – % diluents.

3.3.3. Purity

The lead content in the three commercial batches was below 5 mg/kg which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).²⁶

The food enzyme preparation complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications and considerations for

²² Technical dossier/ Section 3.2/Annex 3-2-1-15, 3-2-1-16 and 3-2-1-17.

²³ Technical dossier/Section 3,2/p. 32–33.

²⁴ Technical dossier/Section 3.2/p. 53.

²⁵ Technical dossier/p. 7 and Additional data February 2016 and Additional data with the CoA.

²⁶ Technical dossier/Additional data February 2016.

enzymes used in food processing (FAO/WHO, 2006). Total aerobic microbial counts for six analysed batches were between 10 and 28 CFU/g.²⁷

Three batches of the food enzyme preparation were tested for presence of the following viruses: hepatitis E, norovirus genotype I and norovirus genotype II. A representative batch was also tested for the presence of porcine parvovirus and porcine circovirus. All tested samples were negative.²⁸

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

3.4. Toxicological data

Porcine pancreas is edible offal as defined in Regulation (EC) No 853/2004 and it is described as a meat by-product (Marti et al., 2011; Toldrá, 2011); however, it has not been reported to be commonly consumed in the EU and data on the consumption by infants or other general population have not been identified by the Panel. Healthy infants as well as infants and adults requiring tube feeding are the end-users of the formulae and of tube feeding products manufactured with the protein hydrolysates obtained using this food enzyme. Therefore, the Panel decided that, for this enzyme, a toxicological evaluation is necessary.

Human data on the safety of pancreatic enzymes are available from their therapeutic use. Pancreatic enzymes of porcine origin have been used for decades in pharmaceuticals used to treat patients with pancreatic insufficiency, including infants, with the diagnosis of cystic fibrosis (Brady et al., 1991; Graff et al., 2010; Whitcomb et al., 2010; Gubergrits et al., 2011; Littlewood et al., 2011; Sander-Struckmeier et al., 2013; Kashirskaya et al., 2015; Somaraju and Solis-Moya, 2020). It should be noted, however, that pancreatin, which is the active ingredient in pharmaceuticals is composed of not only proteases but contains lipase and amylase.

Clinical trials with infants receiving formulae containing protein hydrolysates produced with pancreatic enzymes were also available. These studies, however, were not designed to evaluate the safety of pancreatic enzymes.

As human data are considered to provide a direct evidence for risk assessment, the Panel decided to use available clinical studies for the toxicological assessment of this food enzyme. With this approach, the performance of 90-day studies in rodents (EFSA, 2009a), or repeated dose toxicity studies in neonatal animals (EFSA Scientific Committee, 2017), is not needed. The Panel examined the list of ingredients used in the production process for obtaining the protease complex from porcine pancreas. None of the ingredients presented genotoxic hazard. For this reason, the Panel decided that for this enzyme, produced with the process described and with the ingredients employed, genotoxicity is of no concern and experimental data are not necessary.

Considering all the above, the toxicological assessment of this food enzyme has been performed using the information provided by clinical studies with pharmaceutical preparations and with IF containing protein hydrolysates produced using pancreatic enzymes of porcine origin.

3.4.1. Preclinical studies in pancreatic enzymes used as pharmaceutical preparations

The Panel identified some pre-clinical studies from the literature submitted for the marketing approval for Food and Drug Administration (FDA) performed *in vivo* in different animal models to test porcine pancreatic enzymes used as pharmaceutical preparations (Pharmacologist's review of NDA, 2008; Saruc et al., 2012). As the studies led to the approval as a drug and since clinical studies are available in humans, these pre-clinical studies were not considered in this assessment.

3.4.2. Clinical studies

Possible adverse effects of pancreases on humans were estimated by assessing clinical studies performed on: i) pancreatic enzymes of porcine origin used as pharmaceuticals and ii) IF containing protein hydrolysates produced using protease from porcine pancreas.

Pharmaceuticals produced from porcine pancreas are indicated in patients with pancreatic insufficiency, including infants, with the diagnosis of cystic fibrosis. They contain pancreatin, a preparation of the three pancreatic enzymes combined, e.g. per unit of a 300 mg dosage form, triacylglycerol lipase (25,000 PhEur units); amylase (18,000 PhEur units) and proteases (1,000 PhEur

²⁷ Technical dossier/p. 10, Table 4 and Annexes 3.2.1.54-3.2.1.59.

²⁸ Technical dossier/Additional information June 2017/Annex 4.

units). The pharmaceutical preparations have been commercially available for several decades. Therefore, clinical studies on pancreatin containing drugs are a source of information on the tolerability and safety of pancreatic enzymes, including proteases.

The most serious reported adverse effect of pharmaceutical porcine pancreatic enzymes is fibrosing colonopathy. This rare phenomenon is associated with very high-dose and prolonged use of the pharmaceutical preparations (Smyth, 1996).

Post-marketing data of pancrelipase have been available since 2009 and included in the summary of product characteristics of the drug CREON[®] (pancrelipase delayed-release capsules).²⁹ The most commonly reported undesired effects of pharmaceutical preparations produced from porcine pancreas are gastrointestinal disorders that are generally of mild or moderate severity. Pruritus, urticaria and rash, blurred vision, myalgia, muscle spasm, and asymptomatic elevations of liver enzymes have been reported but the incidences are rare. No specific adverse effects were identified for infants²⁹.

The Panel identified as the most concerning side effect documented by the consumption of the pancreatic enzymes used as pharmaceuticals the hypersensitivity to the product. However, the intact enzymes in this evaluation are removed during preparation of food products, including IF and tube feeding. For these reasons, the Panel considers that the likelihood of adverse effects of the intact enzyme to occur is low.

3.4.2.1. Clinical studies with IF containing protein hydrolysates

Several clinical studies on IF containing protein hydrolysates produced with porcine pancreatic enzymes were identified and evaluated by the Panel. However, none of the studies were performed with the aim of investigating the safety and tolerability of porcine pancreatic enzymes. The studies analysed referred to IF produced with protein hydrolysates obtained with porcine pancreatic protease; however, no information on the exact composition of the formulae is indicated in the studies. The available studies on IF containing the enzyme (Sampson et al., 1991; Jakobsson et al., 2000; Borschel et al., 2014; Borschel and Baggs, 2015) did not report significant adverse effects on infants. However, these studies were not carried out on the food enzyme itself and the endpoints evaluated are not selected to demonstrate the safety of the food enzyme, therefore, their use in this evaluation is limited.

3.4.3. Allergenicity

Pig is not a source included in the list of substances or products causing allergies or intolerances (EU Regulation 1169/2011).³⁰ However, in studies performed on enzymes of porcine origin employed as pharmaceutical preparations, adverse allergic incidences have been reported. Such effects can be related directly to the enzymes, as the enzymes are the basic ingredient of the drugs. Nevertheless, since the enzymes that make the pharmaceutical preparation comprise a mixture of pancreatic enzymes including lipase, amylase and protease, it is not clear in these cases to which enzyme protein the allergenicity is directed.

Occupational respiratory allergies to enzyme dust of these porcine pancreatic enzymes have been described in workers upon industrial exposure and in medical laboratory technicians (Colten et al., 1975; Kempf et al., 1999; van Kampen et al., 2017). Whereas these proteins from pig pancreas are oral allergens, as evidenced by the pharmaceutical use, they are not known to be food allergens.

Hydrolysis of milk is performed in order to reduce the allergenicity of milk proteins. The protease produced with the aim of hydrolysing the milk is made according with similar procedures as the pharmaceutical preparation. However, the intact enzyme is removed from the hydrolysate during manufacture, but low molecular weight peptides derived from the enzyme are likely to be present. Foods in which the enzyme has been applied have been on the market with only rare reports of adverse allergic reactions in infants (EFSA FAF Panel, 2020). The specificity of these adverse reactions has not been established. Although the immune system of infants is not fully developed occasional cases of anaphylactic reactions on food have been reported (Mehl et al., 2005).

No reports on anaphylactic reactions resulting from the exposure to hydrolysed formula have been described in several surveys analysing the causes for anaphylactic reactions, and in particular those

²⁹ https://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/020725s000SumR.pdf

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.

due to food (De Silva et al., 2008; Worm et al., 2014; Samady et al., 2018). The total number of subjects included in these three surveys was more than 1,400. The Panel concluded that a risk of allergic sensitisation to the peptides after consumption of formulae prepared by hydrolysis of milk in infants, if it exists, must be low. However, allergic reactions may not readily be evident at such a young age, but it is possible that exposure to the allergens at this young age may result in sensitisation that becomes evident later in life.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used for hydrolysis of cow milk³¹ whey proteins to be used as an ingredient of IF, FOF and in FSMP (tube feeding), at a recommended use level between 0.8% and 1.6% relative to the protein content of the starting material (whey protein concentrate, demineralised whey concentrate),³² which is equivalent to 3.53–7.06 mg TOS/g protein.^[33]

The food enzyme hydrolyses the peptide bonds in whey protein (WP), specifically the four main milk WP β -lactoglobulin, α -lactalbumin, bovine serum albumin and immunoglobulins during the manufacture of hydrolysed whey protein (WPH). The protein hydrolysate consisting of small peptides is harvested via ultrafiltration (UF) [redacted] ([redacted]),³³ which retain the food enzyme protein (molecular weight ranging 24–48 kDa). However, low molecular weight peptides derived from the enzyme are likely to be transferred into the WPH. The food enzyme–TOS almost entirely consists of protein (Table 1); therefore, any residual TOS present in WPH is likely to comprise only these low molecular weight peptides.

Analytical data were provided by the applicant showing absence of residual enzymatic activity in the finished product.³⁴

Healthy infants, as well as infants and adults requiring tube feeding are the end-users of the formulae and of tube feeding products manufactured with the protein hydrolysates obtained using this food enzyme. The potential target food categories include IF, FOF and FSMPs, including pre-term formulae (PTF), formulae for cow milk protein allergy management (CMPA), formulae for absorption and digestive problems, and other FSMPs such as tube feeds for infants, toddlers, children and adults.

3.5.2. Dietary exposure estimation

Chronic exposure to the food enzyme–TOS was calculated for pre-term and full-term infants on enteral (formula) feeding and was carried out in accordance with the recommendations of the EFSA Scientific Committee (2017) on the risk assessment of substances present in food intended for infants below 16 weeks of age.

The Scientific Committee derived a formula consumption value of 260 mL/kg body weight (bw) per day, derived from 95th percentile consumption during the period of 14–27 days of life (2017). This time reflects the highest relative consumption on a body weight basis and also covers the potential high consumption rates of pre-term infants on enteral (formula) feeding.

To ensure appropriate nutritional composition and food safety, specific compositional rules have been set by the European Commission for both IF and FOF³⁵ and FSMPs.³⁶

In the case of IF and FOF, the legislation (Regulation (EU) 2016/127) prescribes a min-max energy content of 60–70 kcal/100 ml ready to consume product and a min-max protein content for formula prepared from protein hydrolysates of 1.86–2.8 g protein/100 kcal.

Concerning FSMPs, the legislation (Regulation (EU) 2016/128) notes that the composition may differ substantially depending on the intended use and target population (e.g. age, disease, disorder or medical condition standard or nutrient adapted, clinical setting, etc.), and therefore, no detailed

³¹ Technical dossier/Additional data September 2020/Annex 7.

³² Additional data September 2020.

³³ Technical dossier/p. 51; Table 1 and Figure 28.

³⁴ Technical dossier/Annex 3-2-1-46 & Additional data September 2020/Annex 8, LoQ = 0.17 u/mg.

³⁵ Commission Delegated Regulation (EU) 2016/127 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for infant formula and follow-on formula and as regards requirements on information relating to infant and young child feeding. OJ L 025, 2.2.2016, p. 1.

³⁶ Commission Delegated Regulation (EU) 2016/128 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for food for special medical purposes (Official Journal of the European Union, L 25, 2 February 2016).

compositional rules for such food products were established. FSMPs developed to satisfy the nutritional requirements of infants, however, should be based on that of IF and FOF, allowing for derogations when this is necessary for the intended use of the product.

Based on maximum energy and maximum protein content provided for in the legislation, the maximum protein content per 100 mL prepared formula equates to 1.96 g protein/100 mL formula. The recommended consumption value by the EFSA Scientific Committee of 260 mL/kg bw therefore may contain up to 5.1 g of protein. Following the 3rd EFSA call for input data for the exposure assessment of food enzymes, namely a call for data on protein components in IF and FOF,³⁷ information provided by Specialised Nutrition Europe (SNE) indicates a protein content ranging from 3.1-5 g protein in 100 g of products (including SFMPs) containing entirely or partially hydrolysed protein, which is in line with the requirements (IF/FOF) and recommendations (FSMPs) set out in legislation.

Based on the maximum use level of 7.06 mg TOS/g protein and the maximum permitted protein content of 1.96 g protein/100 mL formula, the exposure of infants from consumption of 260 mL formula/kg bw per day calculates at 35.99 mg TOS/kg bw per day. The Panel considers this exposure estimate also covers other population groups including adults, since exposure in infants reflects the highest relative consumption on a body weight basis (EFSA Scientific Committee, 2017).

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 2.

Table 2: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: 95th percentile formula consumption for the period of 14–27 days of life was used to calculate exposure	+/-
Use level (mg TOS/g protein) was derived based on average FE batch values	+/-
Model assumptions and factors	
100% transfer of the food enzyme–TOS into the final foodstuff	+
Exposure to food enzyme–TOS was calculated based on the recommended maximum use level	+
Maximum permitted protein content in formula was used to calculate exposure	+
Exposure in infants is assumed to cover exposure in all population groups	+
Use of conversion factor to extrapolate from powder to liquid formula	+/-
Pre-term infant exposure representative for all population groups	+

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 is likely to have led to an overestimation of the exposure.

4. Conclusions

Based on origin of the food enzyme from edible parts of animals, the data provided by the applicant and supported by the evaluation of clinical studies with pharmaceutical preparations based on pancreatic enzymes, the Panel concluded that the porcine pancreatic enzymes do not give rise to safety concerns under the intended conditions of use.

³⁷ Available online: <https://www.efsa.europa.eu/en/consultations/call/call-input-data-exposure-assessment-food-enzymes-3rd-call>

5. Documentation as provided to EFSA

- 1) Dossier "Food enzyme Protease complex consisting of trypsin, chymotrypsin, elastase and carboxypeptidase from pancreas of pigs" Submitted by Neova Technologies Inc. in December 2014.
- 2) Additional information was received from Neova Technologies Inc. in February 2016, June 2017 and from Bioseutica on behalf of Neova Technologies Inc. in September 2020.
- 3) Summary report on technical data and dietary exposure. November 2015. Delivered by Hylobates Consulting and BiCT (Rome and Lodi, Italy).
- 4) Response to EFSA information request on study evaluation of infants fed on extensively hydrolysed infant formula. 16 January 2020. Specialised Nutrition Europe (SNE).
- 5) "Transfer of food enzymes into protein hydrolysates that are used in infant formulae and follow-on formulae". March 2019. Provided by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP).

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Abbreviations

AU	amylase units
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
CMPA	cow milk protein allergy management
CU	carboxypeptidase unit
EC	Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
EIU	elastase unit
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FOF	follow-on formulae
FSMP	Food for Special Medical Purposes
IF	infant formula

GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kilo Dalton
LoQ	limit of quantification
LU	lipase units
NDA	new drug application
PhEur	European Pharmacopoeia
PTF	pre-term formulae
PU	phospholipase units
SNE	Specialised Nutrition Europe
TOS	total organic solids
UF	ultrafiltration
USPC	USP chymotrypsin unit
USPP	USP protease unit
USPT	USP trypsin unit
WHO	World Health Organization
WP	whey protein
WPH	hydrolysed whey protein