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Safety evaluation of food enzyme trypsin from porcine pancreas

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Abstract

The food enzyme trypsin (EC 3.4.21.4) is extracted from porcine pancreas by Novozymes A/S. The food enzyme is intended to be used for hydrolysis of whey proteins employed as ingredients in infant formulae, follow-on formulae and in food for special medical purposes. 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 32 mg TOS/kg body weight (bw) per day for infants. The Panel considered that this value covers all population groups consuming these formulae. In the toxicological evaluation, clinical studies with pancreatic enzymes were considered. Hypersensitivity to the pharmaceuticals was identified as the major side effect. However, allergic reactions to porcine pancreatic enzymes in hydrolysed foods have not been reported. The Panel considered that a risk of allergic sensitisation to this food enzyme after consumption of products prepared by hydrolysis of milk could not be excluded in infants but considered the likelihood to be low. Based on the origin of the food enzyme from edible parts of animals, the data provided by the applicant and supported by the evaluation of clinical studies based on pancreatic enzymes and the estimated dietary exposure, the Panel concluded that the trypsin from porcine pancreas does not give rise to safety concern under the intended conditions of use.

Keywords: Food enzyme, trypsin, EC 3.4.21.4, porcine pancreas, pig

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1. Introduction

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

> 'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.
>
> 'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

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

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

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

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

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

1.1.1. Background as provided by the European Commission

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

Four applications have been introduced by the companies "Puratos NV sa.", "Novozymes A/S.", "Meito Sangyo Co., Ltd" and the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorisation for the food enzymes Inulinase from a genetically modified strain of *Aspergillus oryzae* (strain MUCL 44346), Trypsin from porcine pancreatic glands, Triacylglycerol lipase from *Candida cylindracea*, and Cellulase, Glucanase and Hemicellulase covering Xylanase and Mannanase from *Aspergillus niger* respectively.

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 four applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

[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, p. 15–24.

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 Inulinase from a genetically modified strain of *Aspergillus oryzae* (strain MUCL 44346), Trypsin from porcine pancreatic glands, Triacylglycerol lipase from *Candida cylindracea*, and Cellulase, Glucanase and Hemicellulase covering Xylanase and Mannanase from *Aspergillus niger* 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 food enzyme trypsin from porcine pancreatic glands.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for the authorisation of the food enzyme trypsin from porcine pancreatic glands.

Additional information was sought from the applicant during the assessment process in requests from EFSA sent on 9 March 2017 and on 15 February 2021 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 guidances of EFSA Scientific Committees.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the methodology described in the CEF Panel 'Statement on the exposure assessment of food enzymes' (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature	Trypsin
Synonyms	α-trypsin, ß-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.

The food enzyme is intended to be used for hydrolysis of whey proteins for use in infant formulae (IF), follow-on formulae (FOF) and food for special medical purposes (FSMP).[4]

3.1. Source of the food enzyme

The food enzyme is produced from the pancreas of pigs (*Sus scrofa domesticus*). The food enzyme is exclusively obtained from the pancreas of animals slaughtered and approved for human consumption, free of notifiable diseases (i.e. African swine fever (ASF), classical swine fever (CSF), food and mouth disease (FMD) and swine vesicular disease (SVD)). Verification is performed by veterinarians in charge of the registered establishments for the slaughtering, which are solely located in Europe. Pigs are not included in the list of the specific risk material defined by Commission

[4] Technical dossier/Additional data February 2021.

Regulation (EU) 2015/1162[5]. The porcine pancreas glands are collected following the requirements of the relevant EU hygiene regulations.

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

3.2. Production of the food enzyme

The food enzyme is manufactured in accordance with Regulations (EC) No 852/2004[6] and (EC) No 853/2004[7] with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current Good Manufacturing Practice.

Pancreases are [redacted]. In the initial extraction stage, glands are [redacted]. The pH of the suspension [redacted] ([redacted]). [redacted] food enzyme separated from the solids by centrifugation and concentrated [redacted]. [redacted] The concentrated food enzyme is submitted to a series of filtration steps, including ultrafiltration. At this stage, [redacted]. Finally, [redacted], collected by filtration and dried.[8] The applicant provided information on the identity of the substances used in the extraction 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 food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. A consistent protein pattern was observed across all batches. The gel showed a major protein band corresponding to an apparent molecular mass of about 24 kDa.[10] The sequence of trypsin is available in Uniprot.[11] The food enzyme was tested for chymotrypsin, phospholipase (lecitase), lipase, α-amylase and amyloglucosidase activities. An optional phospholipase removal step (see Section 3.2) was applied in the production of two of the tested batches. Chymotrypsin activity was detected in all batches and phospholipase activity in the batch produced without the optional phospholipase removal step.[12,13] No other enzymatic activities were reported.

The in-house determination of trypsin activity is based on hydrolysis of the substrate *N*-benzoyl-L-arginine ethyl ester (reaction conditions: pH 7.6, 25°C, 5 min). The enzymatic activity is determined by measuring the release of *N*-benzoyl-L-arginine spectrophotometrically at 253 nm. The enzyme activity is expressed in USP trypsin units (USP)/g. One USP is defined as the activity that induces a change in absorbance of 0.003 per minute under the conditions of the assay.[14]

The food enzyme has a temperature optimum between 60 and 70°C (pH 7.0), and a pH optimum around pH 9 (30°C). Thermostability was tested after pre-incubation of the food enzyme for 30 min at different temperatures. Under the conditions of the applied temperature stability assay (pH 7.0), trypsin activity decreased above 20°C, showing no residual activity above 70°C.[15]

[5] 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.
[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, p. 3–21.
[7] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin.
[8] Technical dossier/p. 41–45.
[9] Technical dossier/Annex 4.
[10] Technical dossier/p. 31–32.
[11] http://www.uniprot.org/uniprot/P00761
[12] Technical dossier/p. 38-39.
[13] LoDs: phospholipase = 15.0 LEU(L)/g; lipase = 0.02 KLU/g; α-amylase = 0.3 KNU(T)/g; amyloglucosidase = 0.825 AGU/g.
[14] Technical dossier/p. 36 and Annex 2.02.
[15] Technical dossier/p. 37–38 and Annex 6.

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme have been provided for three food enzyme batches used for commercialisation (Table 1).[16] The average total organic solids (TOS) content of the three food enzyme preparation batches was 11.0%. The average protease activity/TOS ratio of the three food enzyme batches for commercialisation is 3.0 KUSP/mg TOS.

Table 1: Composition of the food enzyme

Parameter	Unit	Batches		
		1	2	3[(a)]
Trypsin activity	KUSP/g batch[(b)]	339	357	305
Protein	% (w/w)	10.4	10.9	9.9
Ash	% (w/w)	0[(c)]	0[(c)]	0[(c)]
Water	% (w/w)	88.9	88.4	89.6
Total Organic Solids (TOS)[(d)]	% (w/w)	11.1	11.6	10.4
Trypsin activity/mg TOS	KUSP/mg TOS	3.1	3.1	2.9

(a): Produced without the optional [redacted] removal step.
(b): KUSP/g: Kilo USP Trypsin Units/g.
(c): < LOD (0.3%).
(d): TOS calculated as 100% − % water − % ash.

3.3.3. Purity

The lead content in the three commercial batches was below 0.5 mg/kg which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of arsenic, cadmium and mercury were below the limits of detection of the employed methodologies.[17,18]

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).[19]

The average total aerobic microbial count for three batches was 400 CFU/g.

The applicant ensures the absence of virus in the food enzyme by applying two viral inactivation steps (i.e. [redacted]).[20]

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 (ed.), 2011); however, it has not been reported to be commonly consumed in the European Union and data on the consumption by infants or other general population have not been identified by the Panel. Infants are among the end-users of the products manufactured with the protein hydrolysates obtained using this trypsin. 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 drugs 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 drugs, 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

[16] Technical dossier/p. 31 and Additional data February 2021.
[17] LoDs: Pb = 0.5 mg/kg; As = 0.3 mg/kg; Cd = 0.05 mg/kg; Hg = 0.05 mg/kg.
[18] Technical dossier/p. 33 and Additional data February 2021.
[19] Technical dossier/p. 33 and 35, and Additional data February 2021.
[20] Technical dossier/Additional data May 2017.

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,b) or repeated dose toxicity studies in neonatal animals (EFSA Scientific Committee, 2017) is not needed. The Panel examined the list of ingredients[9] used in the production process for obtaining the trypsin 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 drugs and with IF containing protein hydrolysates produced using pancreatic enzymes of porcine origin.

3.4.1. Preclinical studies in pancreatic enzymes used as drugs

The Panel identified some preclinical studies from the literature submitted for the marketing approval for the US Food and Drug Administration (FDA) performed *in vivo* in different animal models to test porcine pancreatic enzymes used as drugs (The Pharmacologists' review of NDA, 2008; Saruc et al., 2012). As the studies led to the approval as drugs and as clinical studies are available in humans, these preclinical 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 drugs and ii) IF containing protein hydrolysates produced using protease from porcine pancreas.

Drugs 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 units). The drug products 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 the pancreas 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 drug (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).[21] The most commonly reported undesired effects of drugs 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 pancreatic enzymes have been reported but the incidence is rare. No specific adverse effects were identified for infants.[21]

The Panel identified that the most concerning side effect documented by the consumption of the pancreatic enzymes used as drugs is the hypersensitivity to the product. However, the intact enzyme in this evaluation is inactivated by heat treatment.[22] The Panel considered that the likelihood of adverse effects of the intact enzyme to occur is low.

3.4.2.1. Clinical studies with infant formulae 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 was performed with the aim of investigating the safety and tolerability of porcine pancreatic enzymes. The studies analysed refer 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 were not selected to demonstrate the safety of the food enzyme, thus, their use in this evaluation is limited.

[21] http://www.fda.gov/ohrms/dockets/ac/08/briefing/2008- 4402b1-03-SOLVAY.pdf
[22] Technical dossier/p. 66.

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 trypsin extracted from porcine pancreas 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 matches were found.[23]

Pig is not a source included in the list of substances or products causing allergies or intolerances (EU Regulation 1169/2011).[24] 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 protein the allergenicity is ascribed.

Occupational respiratory allergies to enzyme dust of these pig 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 and Hartwig, 2017). These proteins from porcine pancreas were not reported to be food allergens.

Hydrolysis of milk is performed in order to reduce the allergenicity of milk proteins. Proteases produced with the aim of hydrolysing milk are made according to similar procedures as the pharmaceutical preparation. 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 formulae have been described in several surveys analysing the causes for anaphylactic reactions, and in particular those 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 food enzyme after consumption of formulae prepared by hydrolysis of milk in infants is 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 whey concentrate or whey isolate for use in IF, FOF and FSMP.[4] The recommended use level is up to 19,000 KUSP/kg protein dry matter, corresponding to 6,333 mg TOS/kg protein dry matter.[25]

The food enzyme hydrolyses the peptide bonds in whey proteins, specifically the four main milk whey proteins β-lactoglobulin, α-lactalbumin, bovine serum albumin and immunoglobulins during the manufacture of whey protein hydrolysate (WPH). After the enzymatic reaction, separation of the peptides by size is optional.[26]

The food enzyme–TOS almost entirely consists of protein (Table 1), therefore, all residual TOS present in the food enzyme is expected to be transferred into the WPH. Based on data provided on thermostability (see Section 3.3.1), it is expected that the food enzyme is inactivated during the hydrolysis of whey proteins.

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 FSMP, including pre-term

[23] Technical dossier pp. 56-58/Annex 5.
[24] 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.
[25] Technical dossier/pp. 49–52 and Additional data February 2021.
[26] Technical dossier/pp. 65–66.

formulae (PTF), formulae for cow milk protein allergy management (CMPA), formulae for absorption and digestive problems and other FSMP, 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 (formulae) 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 formulae 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 (EFSA Scientific Committee, 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 (formulae) feeding.

To ensure appropriate nutritional composition and food safety, specific compositional rules have been set by the European Commission for both IF and FOF[27] and FSMP.[28]

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 formulae prepared from protein hydrolysates of 1.9–2.8 g protein/100 kcal.

Concerning FSMP, 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 or clinical setting, etc.), therefore, no detailed compositional rules for such food products were established. FSMP 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 formulae equates to 1.96 g protein/100 mL formulae. 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,[29] information provided by Specialised Nutrition Europe (SNE) indicates a protein content ranging from 3.1 to 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 (FSMP) set out in legislation.

Based on the maximum use level of 6,333 mg TOS/g protein and the maximum protein content of 1.96 g protein/100 mL formulae, the exposure of infants from consumption of 260 mL formulae/kg bw per day calculates at 32.27 mg TOS/kg bw per day. The Panel considered that 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 formulae 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 food enzyme batch values	+/–

[27] 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.

[28] 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. OJ L 25, 2.2.2016.

[29] Available at https://www.efsa.europa.eu/en/consultations/call/call-input-data-exposure-assessment-food-enzymes-3rd-call

Sources of uncertainties	Direction of impact
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 formulae was used to calculate exposure	+
Use of conversion factor to extrapolate from powder to liquid formulae	+/–
Pre-term infant exposure representative for all concerned population groups	+
Exposure in infants is assumed to cover exposure in all concerned 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 for food enzyme–TOS is likely to have led to a considerable overestimation of the exposure.

4. Conclusion

Based on the origin of the food enzyme from edible parts of animals, the data provided by the applicant and the evaluation of clinical studies based on pancreatic enzymes and the estimated dietary exposure, the Panel concluded that the trypsin from porcine pancreas does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

1) Trypsin extracted from porcine pancreatic glands. February 2015. Submitted by Novozymes A/S.
2) Additional information. May 2017 and February 2021. Submitted by Novozymes A/S.
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

ASF	African swine fever (ASF)
bw	body weight
CMPA	formulae for cow milk protein allergy management
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
CSF	classical swine fever (CSF),
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
FMD	food and mouth disease
FOF	follow-on formulae
FSMP	food for special medical purposes
GLP	Good Laboratory Practice
GMO	genetically modified organism
IF	infant Formulae
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kilo Dalton
LoD	limit of detection
PTF	pre-term formulae
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SVD	swine vesicular disease
TOS	total organic solids
WHO	World Health Organization
WPH	whey protein hydrolysate