Safety evaluation of a food enzyme containing trypsin and chymotrypsin from porcine pancreas.

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

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Abstract

The food enzyme is a serine protease complex containing trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) obtained from porcine pancreas by Paninkret Chem.-Pharm. Werk GmbH. The food enzyme is currently only used in protein processing to hydrolyse milk proteins. Milk protein hydrolysates and peptides are mainly used in formulae intended to have reduced allergenicity. Based on the recommended use level and the high consumption of formulae in very young babies, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be 180 mg TOS/kg body weight (bw) per day for infants and toddlers. Toxicological evaluation was based on the available clinical studies with pancreatic enzymes. Hypersensitivity to the product was identified as the major side effect. However, the intact enzymes are inactivated during preparation of food products; therefore, the Panel considered that the likelihood of adverse effects of the intact enzyme to occur is low. The Panel considered that a risk of allergic sensitisation to these protein hydrolysates after consumption cannot be excluded, but the likelihood of occurrence was considered to be low. Based on the origin of the food enzyme from edible parts of animals, the data provided and the evaluation of clinical studies with pancreatic enzymes and the estimated dietary exposure, the Panel concluded that the food enzyme does not give rise to safety concerns when used in the production of infant formulae based on milk protein hydrolysates.

Keywords: Trypsin, chymotrypsin, EC 3.4.21.4, EC 3.4.21.1, pig pancreas

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

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

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

1.1.1. Background as provided by the European Commission

Only food enzymes included in the 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.

Three applications have been introduced by the company "Paninkret Chem. Pharm. Werk GmbH" and "AB Enzymes GmbH" for the authorisation of the food enzymes trypsin and chymotrypsin from pig pancreas, pectin lyase from a genetically modified strain of *Trichoderma reesei* (strain RF 6199) and endo 1,4-beta xylanase from a genetically modified strain of *Aspergillus acidus* (strain RF 7398).

Following the requirements of Article 12.1 of Commission Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the three 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 trypsin and chymotrypsin from pig pancreas, pectin lyase from a genetically modified strain of *Trichoderma reesei* (strain RF 6199) and endo 1,4-beta xylanase from a

[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.

genetically modified strain of *Aspergillus acidus* (strain RF 7398) 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 enzymes trypsin and chymotrypsin from porcine pancreas.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzymes trypsin and chymotrypsin obtained from porcine pancreas.

Additional information was requested from the applicant during the assessment process on 19 May 2015; 21 January 2016; 4 July 2016; 10 March 2017 and 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

The food enzyme is a serine protease complex containing two 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.

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 a lower extent), releasing polypeptides.

The food enzyme is currently only intended to be used in protein processing to hydrolyse milk proteins in the production of formulae based on milk protein hydrolysates.

3.1. Source of the food enzyme

The food enzyme is extracted 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, and free of notifiable diseases[4] (i.e. African swine fever (ASF), classical swine fever

[4] Technical Dossier/English translation/page 8.

(CSF), food and mouth disease (FMD) and swine vesicular disease (SVD)). Verification is performed by veterinarians. The source material is obtained only from EU approved abattoirs located in Europe. Pigs are not included in the list of the specific risk material defined by Commission Regulation (EU) 2015/1162[5] and pancreas is an edible offal as defined in Regulation (EC) No 853/2004[6].

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 complex is manufactured in accordance with Regulations (EC) No 852/2004[7] and (EC) No 853/2004, with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[8]

The food enzyme is extracted from [redacted] pancreas of pigs using [redacted] filtration leaving [redacted] containing the food enzyme. The filtrate is then submitted to a series of filtration and concentration steps, including filter press and sterile filtration. The food enzyme is then standardised [redacted] 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

Trypsin is a single polypeptide chain of [redacted] amino acids. The molecular mass, derived from the amino acid sequence, is [redacted] kDa.[10] Chymotrypsin is composed of three polypeptide chains with a molecular mass around [redacted] kDa.[11] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all tested batches. Minor lipase and amylase activities were also reported by the applicant.[12,13]

The in-house determination of protease activity is based on the hydrolysis of haemoglobin, resulting in the release of peptides that are not precipitated by addition of trichloroacetic acid (reaction conditions: pH 6.8, 37°C, 10 min). After hydrolysis of haemoglobin, the reaction is stopped by addition of trichloroacetic acid, and the amount of released peptides is determined spectrophotometrically at 280 nm. The enzyme activity is expressed in Units of Haemoglobin (UHb)/g. One UHb is defined as the amount of enzyme which releases an amount of soluble fragments from haemoglobin equivalent to one μmol tyrosine per minute under the conditions of the assay.[14]

The food enzyme has a temperature optimum around 40°C (at pH 8 and 10) or around 50°C (at pH 4 and 6), and a pH optimum around 8.0 (at 30–50°C) or around 6.0 (at 60°C). Thermostability was tested after a pre-incubation for 5 min at different temperatures. Under the conditions of the applied temperature stability assay (pH 6.8), no protease activity remained at 90°C.[15]

3.3.2. Chemical parameters

Data on the chemical parameters have been provided for three batches of the food enzyme preparation (Table 1). The average total organic solids (TOS) of the three batches was 57.6% and the average protease activity/TOS ratio was 2.4 UHb/mg TOS.

[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] Regulations (EC) No 853/2004 and (EC) No 854/2004.
[7] 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.
[8] Technical dossier/Additional data April 2016.
[9] Technical dossier/Additional data July 2015.
[10] http://www.uniprot.org/uniprot/P00761
[11] http://www.uniprot.org/uniprot/G1ARD6
[12] Technical dossier/p. 7, and Additional data July 2014 and June 2016.
[13] LoQ: Phospholipase = 62.5 U/L.
[14] Technical dossier/p. 6-7 and Annex 3.
[15] Technical dossier/English translation of missing Information July 2014.

Table 1: Composition of the food enzyme preparation

Parameter	Unit	Batches		
		1	2	3
Protease activity	UHb/g batch[(a)]	1,404	1,297	1,451
Protein	%	59.9	61.9	59.4
Ash	%	4.4	4.6	4.2
Water	%	5.3	6.1	6.2
[redacted] (excipient)	%	31.4	30.5	34.6
Total organic solids (TOS)[(b)]	%	58.9	58.8	55.0
Protease Activity/mg TOS	UHb/mg TOS	2.4	2.2	2.6

(a): UHb/g: Units of Haemoglobin /g (see Section 3.3.1).
(b): TOS calculated as 100% – % water – % ash – % [redacted]

3.3.3. Purity

The lead content in three commercial batches was below 0.38 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 were below the lowest specification level set for food additives (1 mg/kg[16], EC Regulation 231/2012[17]).

The food enzyme preparation 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).

Ten batches of the food enzyme preparation were tested for presence of hepatitis E virus, and the results were negative.[18] No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).[19]

The Panel considered that the information provided on the purity of the food enzyme preparation was 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 EU and data on the consumption by infants or the 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 food enzyme. Therefore, the Panel decided that, for this enzyme, a toxicological evaluation was 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 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 used in the production process for obtaining trypsin and chymotrypsin from porcine pancreas. None of the ingredients presented genotoxic hazard. For this

[16] Technical dossier/Session 1.2.
[17] Regulation (EC) No 231/2012 of the European Parliament and of the Council of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83/1, 22.3.2012.
[18] Technical dossier/Additional data May 2017.
[19] Technical dossier/p. 5.

reason, the Panel decided that for this food enzyme, produced with the process described and with the ingredients employed, genotoxicity was of no concern and experimental data were 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 infant formulae 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 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) infant formulae 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 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 drug (Smyth, 1996).

Post-marketing data of pancrealipase have been available since 2009 and included in the summary of product characteristics of the drug CREON® (pancrelipase delayed-release capsules).[20] 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.[20]

The Panel identified as the most concerning side effect documented by the consumption of the pancreatic enzymes used as drugs the hypersensitivity to the product. However, the intact enzymes in this evaluation are heat-inactivated during the production of milk protein hydrolysates.[21] The Panel considered that the likelihood of adverse effects of the intact enzyme to occur is low.

3.4.3. Clinical studies with infant formulae containing protein hydrolysates

Several clinical studies on infant formulae 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 infant formulae produced with protein hydrolysates obtained with porcine pancreatic protease; however, no information on the exact composition of the formulas is indicated in the studies. The available studies on infant formulae 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 in 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.

[20] http://www.fda.gov/ohrms/dockets/ac/08/briefing/2008- 4402b1-03-SOLVAY.pdf
[21] Technical dossier/2nd submission July 2014/p. 6.

3.4.4. Allergenicity

Pig is not a source included in the list of substances or products causing allergies or intolerances (EU Reg. 1169/2011).[22] However, in studies performed on enzymes of porcine origin employed as drugs, 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 drug 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 pancreas 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 pig pancreas were not reported 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 hydrolysis of milk is made according to similar procedures as the drug. 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 towards 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 the 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, 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 currently only used for the hydrolysis of milk proteins at a recommended use level of 1.2 kg food enzyme/1,000 kg milk[23], which corresponds to 691 mg TOS/kg milk. The applicant specified that cow's milk is the raw material treated by this food enzyme.[23]

Milk protein hydrolysates and peptides are mainly used in formulae intended to have reduced allergenicity. Therefore, this assessment covers only the use of this food enzyme in the production of formulae based on milk protein hydrolysates. Other food applications of protein hydrolysates are not considered in this assessment.

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 milk protein hydrolysates and peptides. Based on data provided on thermostability (see Section 3.3.1), it is expected that the food enzyme is inactivated during the hydrolysis. After hydrolysing milk with this food enzyme at 37°C for 30 min, the food enzyme is inactivated by heating the reaction products to 90°C for 5 min.[21]

3.5.2. Dietary exposure estimation

Infants and adults allergic or intolerant to milk proteins are the end-users of the formulae manufactured with the milk protein hydrolysates obtained using this food enzyme. However, consumption data for infant formulae and follow-on formulae that are based on milk protein hydrolysates are still scarce at present in the European Food Consumption Database. Having considered that the amount of formulae consumed by allergic babies should be similar to that of healthy babies, the Panel decided to use

[22] 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.

[23] Technical dossier/Additional data February 2021.

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

Combining the use level recommended by the applicant with this consumption value resulted in a chronic exposure to the food enzyme–TOS of 180 mg TOS/kg bw per day.

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	+/–
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 use level	+/–
Use of conversion factor to extrapolate from powder to liquid formulae	+/–
Exposure in infants of 14-27 days old is assumed to cover exposure in young children up to three years old.	+

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 an overestimation of the exposure.

4. Conclusion

Based on the origin of the food enzyme from edible parts of animals, the data provided and the evaluation of clinical studies with pancreatic enzymes and the estimated dietary exposure, the Panel concluded that the food enzyme containing trypsin and chymotrypsin from porcine pancreas does not give rise to safety concerns when used in the production of infant formulae based on milk protein hydrolysates.

5. Comment

The applicant indicated that in addition to the current use of this food enzyme in the production of formulae based on milk protein hydrolysates, further applications are under consideration. Such food applications will require additional assessments.

6. Documentation as provided to EFSA (if appropriate)

1) 3PE Technical Dossier. August 2013, updated November 2013 and July 2014. Submitted by Paninkret chem.-pharm. Werk GmbH.
2) Additional information. July 2015, April 2016, June 2016, December 2016, May 2017 and March 2021. Submitted by Paninkret chem.-pharm. Werk GmbH.
3) Summary report on technical data and dietary exposure. April 2015. Delivered by Hylobates Consulting and BiCT (Rome and Lodi, Italy).

References

Borschel MW and Baggs GE, 2015. A new hydrolyzed formula is well tolerated in infants with suspected food protein allergy or intolerance. Open Nutrition Journal, 9, 1–4.

Borschel MW, Baggs GE and Barrett-Reis B, 2014. Growth of healthy term infants fed ready-to-feed and powdered forms of an extensively hydrolyzed casein-based infant formula: a randomized, blinded, controlled trial. Clinical Pediatrics (Phila), 53, 585–592.

Brady MS, Rickard K, Yu PL and Eigen H, 1991. Effectiveness and safety of small vs. large doses of enteric coated pancreatic enzymes in reducing steatorrhea in children with cystic fibrosis: a prospective randomized study. Pediatric Pulmonology, 10, 79–85.

Colten HR, Polakoff PL, Weinstein SF and Strieder D, 1975. Immediate hypersensitivity to hog trypsin resulting from industrial exposure. The New England Journal of Medicine, 292, 1050–1053.

De Silva IL, Mehr SS, Tey D and Tang MLK, 2008. Pediatric anaphylaxis: a 5 years retrospective review. European Journal of Allergy and Clinical Immunology, 63, 1071–1076. https://doi.org/10.1111/j.1398-9995.2008.01719.x

EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2006;5(1):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority), 2009a. Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. EFSA Journal 2009;7(8):1305, 26 pp. https://doi.org/10.2903/j.efsa.2009.1305

EFSA (European Food Safety Authority), 2009b. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. EFSA Journal 2009;7 (5):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in exposure assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2016. Exposure assessment of food enzymes. EFSA Journal 2016;14(11):4581, 9 pp. https://doi.org/10.2903/j.efsa.2016.4581 and Annex B – Process-specific technical data used in exposure assessment of food enzymes (accessible at https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903%2Fj.efsa.2016.4581&file=efs24581-sup-0001-Annex_B.pdf)

EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), 2020. Scientific Opinion on the re-evaluation of lecithins (E 322) as a food additive in foods for infants below 16 weeks of age and follow up of its re-evaluation as food additive for uses in foods for all population groups. EFSA Journal 2020;18(11):6266, 37 pp. https://doi.org/10.2903/j.efsa.2020.6266

EFSA Scientific Committee, 2017. Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age. EFSA Journal 2017;15(5):4849, 58 pp. https://doi.org/10.2903/j.efsa.2017.4849

FAO/WHO (Food and Agriculture Organization of the United States/World Health Organization), 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th meeting. FAO JECFA Monographs 3, 63–67. Available online: http://www.fao.org/3/a-a0675e.pdf

Graff GR, Maguiness K, McNamara J, Morton R, Boyd D, Beckmann K and Bennett D, 2010. Efficacy and tolerability of a new formulation of pancrelipase delayed-release capsules in children aged 7 to 11 years with endocrine pancreatic insufficiency and cystic fibrosis: a multicentre, randomized, double-blind, placebo-controlled, two-period crossover, superiority study. Clinical Therapy, 32, 89–103.

Gubergrits N, Malecka-Panas E, Lehman GA, Vasileva G, Shen Y, Sander-Struckmeier S, Caras S and Whitcomb DC, 2011. A 6-month, open-label clinical trial of pancrelipase delayed-release capsules (Creon) in patients with exocrine pancreatic insufficiency due to chronic pancreatitis or pancreatic surgery. Alimentary Pharmacology and Therapeutics, 33, 1152–1161.

Jakobsson I, Lothe L, Ley D and Borschel MW, 2000. Effectiveness of casein hydrolysate feedings in infants with colic. Acta Paediatrica, 89, 18–21.

van Kampen V and Hartwig A, 2017. Trypsin and Chymotrypsin [MAK Value Documentation, 2016].

Kashirskaya NY, Kapranov NI, Sander-Struckmeier S and Kovalev V, 2015. Safety and efficacy of Creon® micro in children with exocrine pancreatic insufficiency due to cystic fibrosis. Journal of Cystic Fibrosis, 14, 275–281.

Kempf W, Oman H and Wüthrich B, 1999. Allergy to proteases in medical laboratory technicians: a new occupational disease? Journal of Allergy and Clinical Immunology, 104, 700–701.

Littlewood JM, Connett GJ, Sander-Struckmeier S and Henniges F and Creon 40,000 Study Group, 2011. A 2-year post-authorization safety study of high-strength pancreatic enzyme replacement therapy (pancreatin 40,000) in cystic fibrosis. Expert Opinion on Drug Safety, 10, 197–203.

Marti DL, Johnson RJ and Mathews Jr KH, 2011. Where's the (Not) Meat? By products From Beef and Pork Production / LDP-M-209-01. A Report from the Economic Research, Service/USDA.

Mehl A, Wahn U and Niggermann B, 2005. Anaphylactic reactions in children – a questionnaire-based survey in Germany. European Journal of Allergy and Clinical Immunology, 60, 1440–1445. https://doi.org/10.1111/j.1398-9995.2005.00909.x

Pharmacologist's review of NDA (20, 725), 2008. Available online: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/020725s000PharmR.pdf (Accessed: May 2020).

Samady W, Trainor J, Smith B and Gupta R, 2018. Food-induced anaphylaxis in infants and children. Annals of Allergy, Asthma & Immunology, 121, 360–365.

Sampson HA, Bernhisel-Braodbent J, Yang E and Scanlon SM, 1991. Safety of casein hydrolysate formula in children with cow milk allergy. Journal of Pediatrics, 118, 520–525.

Sander-Struckmeier S, Beckmann K, Janssen-van Solingen G and Pollack P, 2013. Retrospective analysis to investigate the effect of concomitant use of gastric acid-suppressing drugs on the efficacy and safety of pancrelipase/pancreatin (CREON®) in patients with pancreatic exocrine insufficiency. Pancreas, 42, 983–989.

Saruc M, Nozawa F, Yalniz M, Itami A and Pour PM, 2012. Effects of porcine pancreatic enzymes on the pancreas of hamsters. Part 1: basic studies. Journal of the Pancreas (Online), 13, 476–481.

Smyth RL, 1996. Fibrosing colonopathy and cystic fibrosis. Archives of Disease in Childhood, 74, 464–468.

Somaraju URR and Solis-Moya A, 2020. Pancreatic enzyme replacement therapy for people with cystic fibrosis. Cochrane Database of Systematic Reviews, Issue 8, Art.No. CD008227. https://doi.org/10.1002/14651858.cd008227.pub4

Toldrá F, 2011. Handbook of Analysis of Edible Animal By-Products(ed.). CRC Press, Boca Raton, FL. Print ISBN: 978-1-4398-0360-8. eBook ISBN: 978-1-4398-0361-5.

Whitcomb DC, Lehman GA, Vasileva G, Malecka-Panas E, Gubergrits N, Shen Y, Sander-Struckmeier S and Caras S, 2010. Pancrelipase delayed-release capsules (CREON) for exocrine pancreatic insufficiency due to chronic pancreatitis or pancreatic surgery: a double-blind randomized trial. American Journal of Gastroenterology, 105, 2276–2286.

Worm M, Eckermann O, Dölle S, Aberer W, Beyer K, Hawranek T, Hompes S, Koehli A, Mahler V, Nemat K, Niggemann B, Pföhler C, Rabe U, Reissing A, Rietschel E, Scherer K, Treudler R and Ruëff F, 2014. Triggers and treatment of anaphylaxis: an analysis of 4,000 cases from Germany, Austria and Switzerland. Deutsches Ärzteblatt International, 111, 367–375. https://doi.org/10.3238/arztebl.2014.0367

Abbreviations

ASF	African swine fever
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
CSF	classical swine fever
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
FMD	food and mouth disease
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
SVD	swine vesicular disease
kDa	kiloDalton
LoD	limit of detection
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
WHO	World Health Organization