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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 Neova Technologies Inc. The serine protease complex is intended to be used for hydrolysis of whey proteins employed as ingredients of infant formulae and follow-on formulae. Based on maximum use levels and the maximum permitted protein content in infant formulae, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be 18 mg TOS/kg body weight (bw) per day for infants. 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, 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 cannot be excluded in infants, but considers the likelihood 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 1332/2008<sup>1</sup> 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<sup>2</sup> 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.

Five applications have been introduced by the companies "Meiji Seika Pharma Co., Ltd." for the authorisation of the food enzyme Cellulase from *Talaromyces cellulolyticus*/*Talaromyces pinophilus* (strain *Acremonium cellulolyticus*); "Danisco US Inc." for the authorisation of the food enzymes Aspergillopepsin I from a genetically modified strain of *Trichoderma reesei* (strain DP-Nzq40) and Triacylglycerol lipase from genetically modified strain of *Hansenula polymorpha* (strain DP-Jzk33); "Neova Technologies Inc." for the authorisation of the food enzyme Trypsin and Chymotrypsin from porcine pancreatic glands, and "Novozymes A/S" for the authorisation of the food enzyme Peptidase from a strain of *Aspergillus oryzae* (strain NZYM-EX).

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

<sup>1</sup> 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.

<sup>2</sup> 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.

<sup>3</sup> Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.3.2011, pp. 15–24.

### 1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Cellulase from *Talaromyces cellulolyticus*/*Talaromyces pinophilus* (strain *Acremonium cellulolyticus*); Aspergillopepsin I from a genetically modified strain of *Trichoderma reesei* (strain DP-Nzq40), Triacylglycerol lipase from a genetically modified strain of *Hansenula polymorpha* (strain DP-Jzk33); Trypsin and Chymotrypsin from porcine pancreatic glands and Peptidase from a strain of *Aspergillus oryzae* (strain NZYM-EX) 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 serine protease complex containing trypsin and chymotrypsin obtained 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 enzyme serine protease complex containing trypsin and chymotrypsin from porcine pancreas.

Additional information was requested from the applicant during the assessment process on 19 August 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 two declared activities:

IUBMB nomenclature	Trypsin
Synonyms	$\alpha$ -trypsin, $\beta$ -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, $\alpha$ -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.

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).

### 3.1. Source of the food enzyme

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

#### 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.<sup>4</sup> Pigs are not included in the list of the specific risk material defined by Commission Regulation (EU) 2015/1162<sup>5</sup>. The porcine pancreas glands are collected following the requirements of the relevant EU hygiene regulations.<sup>6</sup>

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).<sup>7</sup>

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 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.<sup>8,9,10</sup>

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

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, calculated from the amino acid sequence, is 24.4 kDa.<sup>13</sup> 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).<sup>14</sup> The food enzyme was analysed via high-performance liquid chromatography and no other enzymatic activities were reported.<sup>15</sup>

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

<sup>4</sup> Technical dossier/Section 3.2/p. 23 and Annex 3-2-1-60.

<sup>5</sup> 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.

<sup>6</sup> Regulations (EC) No 853/2004 and (EC) No 854/2004.

<sup>7</sup> Technical dossier/Section 3.2/pp. 24–25.

<sup>8</sup> Technical dossier/Annex 3-2-1-23 and Additional data November 2020/p. 4, Annexes 2 and 3.

<sup>9</sup> Health Canada. Food and Drug Act. <http://laws-lois.justice.gc.ca>. (Last amended June 19, 2013).

<sup>10</sup> Health Canada. Food and Drug Regulations. <http://laws-lois.justice.gc.ca>. (Last amended May 16, 2014).

<sup>11</sup> Technical dossier/Section 3.2/pp. 26–36.

<sup>12</sup> Technical dossier/Section 3.2/p. 33 and Annexes 3-2-1-8, 3-2-1-24 to 3-2-1-35.

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

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

<sup>15</sup> Technical dossier/Section 3.2/p. 4 and Annex 3-2-1-4; Additional data November 2020/pp. 5–9 and Annex 4.

nm. The enzyme activity is expressed in USP trypsin units (USPT)/mg. One USPT is defined as the activity causing a change of absorbance of 0.003 per minute under the conditions of the assay.<sup>16</sup>

The in-house determination of chymotrypsin activity is based on hydrolysis of the substrate *N*-acetyl-L-tyrosine ethyl ester (ATEE) (reaction conditions: pH 7.0, 25°C, 5 min). The enzymatic activity is determined by measuring the decrease in absorbance at 237 nm. The enzyme activity is expressed in USP chymotrypsin units (USPC)/mg. One USPC is defined as the activity causing a change of absorbance of 0.0075 per minute under the conditions of the assay.<sup>17</sup>

The trypsin in the serine protease complex has a temperature optimum between 45°C and 70°C (pH 7.6), while chymotrypsin has a temperature optimum between 30°C and 60°C (pH 7.0) and both activities show a pH optimum around pH 8 (25°C).<sup>18</sup> Thermostability was tested after a pre-incubation of the food enzyme for 1 min at 80°C. Under the conditions (pH 7.0) of the applied temperature stability assay, no residual trypsin activity was detected after 1 minute, and no residual chymotrypsin activity was detected immediately after adjustment of the temperature to 80°C.<sup>19</sup>

### 3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme preparation were provided for three batches used for commercialisation (Table 1).<sup>20</sup> The average total organic solids (TOS) of the three food enzyme preparation batches for commercialisation is 60.4% and the average enzyme activity/TOS ratio is 4,555 USPT/mg TOS for trypsin and 530 USPC/mg TOS for chymotrypsin.

**Table 1:** Composition of the food enzyme preparation

Parameters	Unit	Batches		
		1	2	3
<b>Trypsin activity</b>	USPT/mg batch <sup>(a)</sup>	2,798	2,681	2,765
<b>Chymotrypsin activity</b>	USPC/mg batch <sup>(b)</sup>	307	301	350
<b>Protein</b>	%	60.2	59.2	57.3
<b>Ash</b>	%	0.2	0.1	0.1
<b>Water</b>	%	2.8	3.6	3.6
<b>Excipient</b>	%	33.95	37.08	37.54
<b>Total organic solids (TOS)<sup>(c)</sup></b>	%	63.1	59.2	58.8
<b>Trypsin activity/mg TOS</b>	USPT/mg TOS	4,434	4,529	4,702
<b>Chymotrypsin activity/mg TOS</b>	USPC/mg TOS	487	508	595

(a): USPT/mg: Trypsin USP Units/mg (see Section 3.3.1).

(b): USPC/mg: Chymotrypsin USP Units/mg (see Section 3.3.1).

(c): TOS calculated as 100% – % water – % ash – % excipient.

### 3.3.3. Purity

The lead content in six commercial batches was below 5 mg/kg which complies with the specification for lead ( $\leq 5$  mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).<sup>21,22</sup>

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 enzymes used in food processing (FAO/WHO, 2006). Total aerobic microbial counts for six analysed batches were between 20 and 355 CFU/g.<sup>23</sup>

Three batches of the food enzyme preparation were tested for presence of the following viruses: hepatitis E, norovirus genotype I and norovirus genotype II. Three representative batches were also

<sup>16</sup> Technical dossier/Section 3.2/p. 13 and Annex 3-2-1-5.

<sup>17</sup> Technical dossier/Section 3.2/p. 16 and Annex 3-2-1-6.

<sup>18</sup> Technical dossier/Section 3.2/pp. 18–20; Additional information EFSA-Q-2015-00372.

<sup>19</sup> Technical dossier/Section 3.2/pp. 38–39; Additional data November 2020/p. 10.

<sup>20</sup> Technical dossier/pp. 8 and 11; Additional information EFSA-Q-2015-00372.

<sup>21</sup> LoD: Pb = 5 mg/kg.

<sup>22</sup> Technical dossier/Section 3.2/p. 8, Table 6 and Annexes 3-2-1-9 to 3-2-1-14; Additional information EFSA-Q-2015-00372.

<sup>23</sup> Technical dossier/Section 3.2/p. 8, Table 6 and Annexes 3-2-1-9 to 3-2-1-14; Additional information EFSA-Q-2015-00372.

tested for the presence of porcine parvovirus and porcine circovirus. All tested samples were negative.<sup>24</sup>

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; Toldrà, 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 are the end-users of the formulae manufactured with the protein hydrolysates obtained using this protease complex. 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; Gubergriets 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 pig 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 protease complex 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 preclinical 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 pharmaceutical preparation and since 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 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, triacylglycerollipase (25,000 PhEur units), amylase (18,000 PhEur units) and proteases (1,000 PhEur 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 the pancreas enzymes, including proteases.

<sup>24</sup> Technical dossier/Additional data November 2020/pp. 11–15 and Annexes 5 and 6.



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).<sup>25</sup> The most commonly reported undesired effects of pharmaceuticals 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 infants.<sup>25</sup>

The Panel identified as the most concerning side effect documented by the consumption of the pancreatic enzymes used as a pharmaceutical the hypersensitivity to the product. The Panel considers that such an effect of the intact enzyme to occur cannot be excluded.

#### 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 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

The allergenicity assessment considers only the food enzyme and not any carrier or other excipient which may be used in the final formulation.

Pig is not a source included in the list of substances or products causing allergies or intolerances (EU Regulation 1169/2011).<sup>26</sup> 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 pig pancreas enzymes have been described in workers upon industrial exposure and in medical laboratory technicians (Colten et al., 1975; van Kampen et al., 2017; Kempf et al., 1999). 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 proteases produced with the aim of hydrolysing the 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

<sup>25</sup> [https://www.accessdata.fda.gov/drugsatfda\\_docs/nda/2009/020725s000SumR.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/020725s000SumR.pdf)

<sup>26</sup> 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.

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 protease complex is intended to be used for hydrolysis of cow milk whey proteins to be used as an ingredient of IF and FOF at a recommended use level between 0.4% and 0.6% relative to the protein content of the starting material (whey protein concentrate, demineralised whey concentrate), which is equivalent to 2,416–3,624 mg of TOS/kg whey protein (WP).<sup>27</sup>

The protease complex hydrolyses the peptide bonds in WP, specifically the four main milk whey proteins  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, bovine serum albumin and immunoglobulins during the manufacture of hydrolysed whey protein (WPH).<sup>28</sup>

Based on data provided on thermostability (see Section 3.3.1), it is expected that the remaining protease complex is inactivated. An ultra-high temperature treatment step is included during the WPH manufacture to inactivate the food enzyme, which was supported by the absence of residual enzymatic activities in the finished WPH.<sup>29</sup>

Healthy infants are the end-users of the formulae manufactured with the protein hydrolysates obtained using this protease complex. The potential target food categories include IF and FOF.<sup>30</sup>

#### 3.5.2. Dietary exposure estimation

Chronic exposure to the food enzyme–TOS was calculated for full-term infants on enteral (formula) feed, 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 preterm 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 FOF.<sup>31</sup>

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.

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,<sup>32</sup> information provided by Specialised Nutrition Europe (SNE) indicates a protein content ranging from 3.1 to 5 g protein in 100 g of products containing entirely or partially hydrolysed protein, which is in line with the requirements set out in legislation.

Based on the maximum use level of 3.62 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 18.45 mg TOS/kg bw per day.

<sup>27</sup> Technical dossier/Section 3.2/p. 44; Additional data November 2020/pp. 18–19 and Annex 10.

<sup>28</sup> Technical dossier/Section 3.2/pp. 40–41.

<sup>29</sup> Technical dossier/Section 3.2/p. 37–39; Additional data November 2020/pp. 16–17 and Annex 9; LoQ = 0.01 units/mg.

<sup>30</sup> Technical dossier/Section 3.2/p. 42.

<sup>31</sup> 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.

<sup>32</sup> Available at <https://www.efsa.europa.eu/en/consultations/call/call-input-data-exposure-assessment-food-enzymes-3rd-call>

### 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
<b>Model input data</b>	
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 food enzyme batch values	+/-
<b>Model assumptions and factors</b>	
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	+
Use of conversion factor to extrapolate from powder to liquid formula	+/-

TOS: total organic solid.

+: 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 a considerable 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 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.

## 5. Documentation as provided to EFSA

- 1) Dossier "Authorization of a Serine protease complex consisting of trypsin and chymotrypsin from porcine pancreas". February 2015. Submitted by Neova Technologies Inc. in.
- 2) Additional information. November 2020. Submitted by Bioseutica B.V. on behalf of Neova Technologies Inc. in.
- 3) Summary report on technical data and dietary exposure. June 2016. 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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- 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>
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## Abbreviations

ATEE	<i>N</i> -acetyl-L-tyrosine ethyl ester
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
EC	Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FOF	Follow-on-Formulae
IF	Infant Formulae
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LOD	limit of detection
LOQ	limit of quantification
NDA	new drug application
PhEur	European Pharmacopoeia
SNE	Specialised Nutrition Europe
TOS	total organic solids
USPC	USP chymotrypsin unit
USPT	USP trypsin unit
WHO	World Health Organization
WP	whey protein
WPH	hydrolysed whey protein