Safety evaluation of the 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 Ningbo Linzyme Biosciences Co., Ltd. It is intended to be used for the hydrolysis of whey proteins for use in infant formulae and follow-on formulae. 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 16.8 mg TOS/kg body weight (bw) per day for infants. 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 proteins could not be excluded in infants, but it considered the likelihood to be low. Based on the origin of the food enzyme from an edible tissue of pigs, the data provided by the applicant, the information from 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.

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Keywords: Food enzyme, Trypsin, (EC 3.4.21.4), porcine pancreas, pig

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Question number: EFSA-Q-2020-00667
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Note: The full opinion will be published in accordance with Article 12 of Regulation (EC) No 1331/2008 once the decision on confidentiality will be received from the European Commission.

Declarations of interest: The declarations of interest of all scientific experts active in EFSA’s work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Suggested citation: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivièreme G, Steffensen I-L, Tiustos C, Van Loveren H, Vernis L, Zorn H, Andryszkiewicz M, Liu Y, Rainieri S and Chesson A, 2022. Scientific Opinion on the safety evaluation of the food enzyme trypsin from porcine pancreas. EFSA Journal 2022;20(1):7008, 12 pp. https://doi.org/10.2903/j.efsa.2022.7008

ISSN: 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

† Deceased.
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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008 provides definition for ‘food enzyme’ and ‘food enzyme preparation’.

‘Food enzyme’ means a product obtained from plants, animals or microorganisms or products thereof including a product obtained by a fermentation process using microorganisms: (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 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 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 EU 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.

An application has been introduced by the applicant “Ningbo Linzyme Biosciences Co., Ltd.” for the authorisation of the food enzyme Trypsin from Porcine Pancreas.

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011 implementing Regulation (EC) No 1331/2008, the Commission has verified that the application falls within the scope of the food enzyme Regulation and contains 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 following food enzyme: Trypsin from Porcine Pancreas in accordance with Article 29 of Regulation (EC) No 178/2002, and Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

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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, p. 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, p. 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.

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2. Data and methodologies

2.1. Data

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

Additional information was requested from the applicant during the assessment process on 21 June 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 guidance documents of EFSA Scientific Committee.

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, 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.

The food enzyme is intended to be used for the hydrolysis of whey proteins for use in infant formulae (IF) and follow-on formulae (FOF).

3.1. Source of the food enzyme

The food enzyme is extracted from the pancreas of pigs (Sus scrofa domesticus) and is obtained exclusively from the pancreas of animals slaughtered and approved for human consumption free of notifiable diseases (i.e. African Swine Fever, Classical Swine Fever, Food and Mouth Disease and Swine Vesicular Disease). Full details of the verification process performed by veterinarians in charge of the registered establishments for the slaughtering were provided. Porcine tissue is not included in the list of the specific risk material defined by Commission Regulation (EU) 2015/1162. 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 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.

Pancreases are enzyme extraction. The pH during which the extraction takes place. The solution, is separated from the solids by

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4 Technical dossier p. 18–21 and Annex VIII.
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 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. 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. 18/Annex I.
3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

Trypsin is a single polypeptide chain of 223 amino acids. The molecular mass, derived from the amino acid sequence, is 23.8 kDa. 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 two major bands. One of around kDa, corresponding to the single peptide β-trypsin and the other migrating between and kDa, corresponding to the two cleaved peptides of α-trypsin. Chymotrypsin and activities were detected in all batches. No other enzymatic activities were reported.

The in-house determination of trypsin activity is based on the hydrolysis of N-Benzoyl-L-arginine ethyl ester (reaction conditions: ). The enzymatic activity is determined by measuring the release of N-Benzoyl-L-arginine spectrophotometrically at 253 nm. The enzyme activity is expressed in United States Pharmacopeia Trypsin units (USP)/mg. One USP is defined as the activity that induces a change of 0.003 absorbance units per minute under the conditions of the assay.

The food enzyme has a temperature optimum at around 37°C and a pH optimum between pH 7.0 and 9.0 (Buck et al., 1962). Thermostability was tested after a pre-incubation of the food enzyme at 75°C for different durations. Under the conditions of the assay (pH 7.0), trypsin activity decreased by more than 90% after 3 min of incubation, showing no residual activity after 5 min.

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1). The mean total organic solids (TOS) was 33% and the mean enzyme activity/TOS ratio was about 4,000 USP/mg TOS.

Table 1: Composition of the food enzyme preparation

| Parameters                  | Unit                  | Batches          |
|-----------------------------|-----------------------|------------------|
|                             |                       | 1    | 2    | 3    |
| Trypsin activity            | USP/mg batch(a)       | 1,306 | 1,335 | 1,312 |
| Protein                     | %                     | 32.0  | 32.0  | 30.0  |
| Ash                         | %                     | 0.2   | 0.1   | 0.1   |
| Water                       | %                     | 0.4   | 0.6   | 0.5   |
| (excipient)                 | %                     | 66.0  | 66.0  | 67.0  |
| Total organic solids (TOS)(b) | %                     | 33.4  | 33.3  | 32.4  |
| Trypsin activity/mg TOS     | USP/mg TOS            | 3,910 | 4,009 | 4,049 |

(a): USP/mg: United States Pharmacopeia Trypsin units/mg (see Section 3.3.1).
(b): TOS calculated as 100% – % water – % ash – % excipient.

9 Technical dossier p. 22/Annexes: XII and XIII.
10 Technical dossier p. 7–8.
11 Technical dossier p. 8–9/Annex II.
12 Technical dossier p. 9–10; 12/Annex III.
13 Technical dossier p. 15–17/Annex III.
14 Technical dossier p. 17/Annex V.
15 Technical dossier p. 12 and Additional information July 2021.
3.3.3. Purity

The lead content in the three commercial batches was below 5 mg/kg, which complies with the specifications for lead 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 limits of detection (batch 1) and quantitation (batches 2 and 3), respectively, of the employed methods.\(^{16,17}\)

Three batches of the food enzyme preparation were analysed for the presence of \textit{Enterobacteriaceae} (< 10 CFU/g) and \textit{Salmonella} (not detected in 25 g).\(^{18}\)

The steps of the manufacturing process (i.e. ...) are considered sufficient to exclude the presence of viruses and other microorganisms from the raw material in the food enzyme.\(^{19}\) One batch of the food enzyme preparation was tested for the presence of porcine circovirus type 2 (PCV2), and the results were negative.\(^{20}\)

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; Toldrà (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 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; Gubergritis et al., 2011; Littlewood et al., 2011; Sander-Struckmeier et al., 2013; Kashirskaya et al., 2015; Somaraju and Solis-Moya, 2020).

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 direct evidence for risk assessment, the Panel decided to use those clinical studies for the toxicological assessment of this food enzyme. Using this approach, 90-day studies in rodents (EFSA, 2009a,b) or repeated dose toxicity studies in neonatal animals (EFSA Scientific Committee, 2017) are not considered necessary. The Panel examined the list of ingredients used in the production process for obtaining the trypsin under assessment. 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 to 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.

\(^{16}\) LoDs: Pb = 0.02 mg/kg; As = 0.003 mg/kg for batch 1 and LoQs Pb = 0.05 mg/kg; As = 0.04 mg/kg LoQ for batches 2 and 3.

\(^{17}\) Technical dossier p. 9–10, 13/Annex IV and additional information July 2021.

\(^{18}\) Technical dossier p. 9–10, 13 and Additional information July 2021.

\(^{19}\) Technical dossier p. 10–11 and Annex XIV.

\(^{20}\) Technical dossier p. 13.
3.4.2. Clinical studies

Possible adverse effects of pancreases in humans upon oral ingestion were investigated 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). 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.

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. None of the studies was performed with the aim of investigating the safety and tolerability of porcine pancreatic enzymes. The studies refer to IF produced with protein hydrolysates obtained with porcine pancreatic protease, but the exact composition of the formulae is not 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.

3.4.3. Allergenicity

The allergenicity assessment considers only the food enzyme and not carriers or other excipients that 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). However, in studies performed on enzymes of porcine origin employed as pharmaceutical preparations, adverse allergic incidences have been reported. Such effects can be related directly to the enzymes, as the enzymes are the basic ingredient of the drugs. Nevertheless, since the enzymes that make the pharmaceutical preparation comprise a mixture of pancreatic enzymes including lipase, amylase and protease, it is not clear in these cases to which enzyme protein the allergenicity is directed.

Occupational respiratory allergies to enzyme dust of these porcine pancreatic enzymes have been described in workers upon industrial exposure and in medical laboratory technicians (Colten et al., 1975; Kempf et al., 1999; van Kampen and Hartwig, 2017). These proteins from porcine pancreas were not reported to be food allergens.

21 https://www.fda.gov/ohrms/dockets/ac/08/briefing/2008-4402b1-03-SOLVAY.pdf
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.
The Panel noted that an allergic reaction upon oral ingestion of this trypsin, produced by porcine pancreas, in individuals respiratory sensitised to trypsin cannot be ruled out, but the likelihood of such a reaction to occur is considered to be low.

Hydrolysis of milk proteins is performed in order to reduce their allergenicity. The protease produced with the aim of hydrolysis of milk proteins is made according to similar procedures as the drug. 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 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 proteins in infants, if it exists, is low. 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 protein for use in IF and FOF. The recommended use level was given as enzyme/substrate ratio and corresponded to a range between 1.32 and 3.30 mg TOS/g whey protein.

A flow chart was provided by the applicant, showing the manufacturing of whey protein hydrolysates to be included in children formulae. The food enzyme trypsin is added to whey protein in two consecutive hydrolysis steps.

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.

#### 3.5.2. Dietary exposure estimation

Infants are the end users of the products manufactured with the protein hydrolysates obtained using this food enzyme. To ensure appropriate nutritional composition and food safety, specific compositional rules have been set by the European Commission for both IF and FOF. Based on maximum energy and maximum protein content provided for IF and FOF in Regulation (EU) 2016/1272, the maximum protein content per 100 mL prepared formula equates to 1.96 g protein/100 mL formula.

Chronic exposure to the food enzyme-TOS was calculated 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, which derived 260 mL/kg body weight (bw) per day from the 95th percentile consumption during the period of the first 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.

Using the maximum use level of 3.30 mg TOS/g protein, the exposure of the food enzyme-TOS from consumption of 260 mL formulae/kg bw per day was calculated at 16.8 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.

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23 Technical dossier p. 22 and Additional information July 2021.
24 Technical dossier p. 24.
25 Technical dossier/Annex VI.
26 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 25, 2.2.2016, p. 1.
The conservative approach applied to the exposure estimate for food enzyme–TOS is likely to have led to an overestimation of the exposure.

3.6. Margin of exposure

Since no toxicological test was considered necessary by the Panel, the margin of exposure was not calculated.

4. Conclusions

Based on the origin of the food enzyme from an edible part of pigs, the data provided by the applicant, 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) Food Enzyme Trypsin from porcine pancreas. February 2021. Submitted by Ningbo Linzyme Biosciences Co., Ltd.
2) Additional information. July 2021. Submitted by Ningbo Linzyme Biosciences Co., Ltd.
3) Response to EFSA information request on study evaluation of infants fed on extensively hydrolysed infant formula. 16 January 2020. Specialised Nutrition Europe (SNE).
4) 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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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 the first 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 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 | + |

+: Uncertainty with potential to cause overestimation of exposure.
-: Uncertainty with potential to cause underestimation of exposure.
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Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| 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 |
| EINECS       | European Inventory of Existing Commercial Chemical Substances |
| FAO          | Food and Agricultural Organization of the United Nations |
| FDA          | Food and Drug Administration |
| 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 |
| SDS-PAGE     | sodium dodecyl sulfate-polyacrylamide gel electrophoresis |
| TOS          | total organic solids |
| WHO          | World Health Organization |