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Nutritional safety and suitability of a specific protein hydrolysate derived from whey protein concentrate and used in an infant and follow-on formula manufactured from hydrolysed protein by Danone Trading ELN B.V.

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Abstract

The European Commission asked EFSA to deliver an opinion on the nutritional safety and suitability of a specific protein hydrolysate. It is derived from whey protein concentrate and used in an infant and follow-on formula by Danone Trading ELN B.V, which submitted a dossier to the European Commission to request an amendment of Regulation (EU) 2016/127 with respect to the protein sources that may be used in the manufacture of infant and/or follow-on formula. This opinion does not cover the assessment of the safety of the food enzymes used in the manufacture of the protein hydrolysate. The protein hydrolysate under evaluation is sufficiently characterised with respect to the fraction of the hydrolysed protein. In the pertinent intervention study provided, an infant formula manufactured from the protein hydrolysate with a protein content of 2.3 g/100 kcal and consumed as the sole source of nutrition by infants for 3.5 months led to growth equivalent to a formula manufactured from intact cow's milk protein (2 g protein/100 kcal). No experimental data have been provided on the nutritional safety and suitability of this protein source in follow-on formula. However, given that it is consumed with complementary foods and the protein source is considered nutritionally safe and suitable in an infant formula that is the sole source of nutrition of infants, the Panel considers that the protein hydrolysate is also a nutritionally safe and suitable protein source for use in follow-on formula. The Panel concludes that the protein hydrolysate under evaluation is a nutritionally safe and suitable protein source for use in infant and follow-on formula, as long as the formula in which it is used contains a minimum of 2.3 g/100 kcal protein and complies with the compositional criteria of Commission Delegated Regulation (EU) 2016/127 and the amino acid pattern in its Annex IIIA.

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Table of Contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the requestor.....	4
1.1.1. Background.....	4
1.1.2. Terms of Reference.....	5
1.2. Interpretation of the Terms of Reference.....	5
2. Data and methodologies.....	5
2.1. Data.....	5
2.2. Methodologies.....	6
3. Assessment.....	6
3.1. Characterisation of the protein hydrolysate.....	6
3.2. Uncertainties related to the characterisation of the protein hydrolysate.....	7
3.3. Characterisation of the formula manufactured from the protein hydrolysate used in the clinical studies provided.....	8
3.4. Nutritional safety and suitability of the infant and follow-on formula.....	8
4. Conclusions.....	10
5. Documentation as provided to EFSA (if appropriate).....	10
Steps taken by EFSA.....	10
References.....	11
Abbreviations.....	11

1. Introduction

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

1.1.1. Background

Commission Directive 2006/141/EC¹ lays down harmonised rules applicable in the entire EU to infant formulae and follow-on formulae. The Directive allows the use of protein hydrolysates as source of protein in infant formulae and follow-on formulae under certain conditions (Articles 5–7; Annex I, point 2.2; Annex II, point 2.2 and Annex VI).

Commission delegated Regulation (EU) 2016/127² transfers the existing rules of Directive 2006/141/EC under the new framework of Regulation (EU) No 609/2013 of the European Parliament and of the Council³ and revises them, based on the opinion of the European Food Safety Authority (EFSA) of 2014.⁴ In that opinion, EFSA noted that *'the safety and suitability of each specific formula containing protein hydrolysates has to be established by clinical studies. Information on protein sources and the technological processes applied should also be provided. In this context, the Panel notes that one particular formula containing partially hydrolysed whey protein has been evaluated for its safety and suitability by the Panel (...) and has been authorised for use by Directive 2006/141/EC'*. EFSA also noted that *'the criteria given in Directive 2006/141/EC alone are not sufficient to predict the potential of a formula to reduce the risk of developing allergy to milk proteins. Clinical studies are necessary to demonstrate if and to what extent a particular formula reduces the risk of developing short- and long-term clinical manifestations of allergy in at-risk infants who are not exclusively breast fed'*.

Taking into account EFSA's opinion, the delegated Regulation establishes that infant formula and follow-on formula manufactured from protein hydrolysates should only be allowed to be placed on the market if their composition corresponds to the one positively assessed by EFSA so far and prohibits the use of health claims describing the role of infant formula in reducing the risk of developing allergy to milk proteins. The requirements of Commission delegated Regulation (EU) 2016/127 shall apply to infant formula and follow-on formula manufactured from protein hydrolysates from 22 February 2021.

Pursuant to Recital 21 of the Regulation, these requirements may be amended in the future in order to allow the placing on the market of formulae manufactured from protein hydrolysates with a composition different from the one already positively assessed, following a case-by-case evaluation of their safety and suitability by EFSA. In addition, if, after the assessment by EFSA, it is demonstrated that a specific formula manufactured from protein hydrolysates reduces the risk of developing allergy to milk proteins, further consideration will be given to how to adequately inform parents and caregivers about that property of the product.

The requirements of Commission delegated Regulation (EU) 2016/127 shall apply to infant formula and follow-on formula manufactured from protein hydrolysates from 22 February 2021. It can be expected that, before that date, dossiers on formulae manufactured from protein hydrolysates will be presented by food business operators for assessment by EFSA with a view to request possible modifications of the conditions applicable to these products in the delegated Regulation.

In this context, it is considered necessary to ask EFSA to provide scientific advice to the Commission on dossiers on formulae manufactured from protein hydrolysates submitted by food business operators for assessment by EFSA in the future.

EFSA will be informed by the Commission by letter when the applicant has been asked by the Commission to transmit the dossier to EFSA for scientific assessment.

¹ Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC, OJ L 401, 30.12.2006, p. 1.

² OJ L 25, 2.2.2016, p. 1.

³ Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009, OJ L 181, 29.6.2013, p. 35.

⁴ EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2014. Scientific Opinion on the essential composition of infant and follow-on formulae. EFSA Journal 2014;12(7):3760.

1.1.2. Terms of Reference

In accordance with Article 29 of Regulation (EC) No 178/2002,⁵ the European Commission requests the European Food Safety Authority to issue scientific opinions on infant and follow-on formula manufactured from protein hydrolysates in particular, depending on the nature of the application, on:

- 1) the safety and suitability for use by infants of a specific formula manufactured from protein hydrolysates;
If the formula under evaluation is considered to be safe and suitable for use by infants, the European Food Safety Authority is also asked to advise on the minimum specific criteria on protein source, protein processing and protein quality of the formula that need to be satisfied for the safety and suitability of such formulae to be demonstrated.
- 2) the product's efficacy in reducing the risk of developing allergy to milk proteins;
- 3) the product's efficacy in reducing the risk of developing allergy/allergic manifestations to allergens in general.

1.2. Interpretation of the Terms of Reference

The interpretation by the Panel on Nutrition, Novel Foods and Food Allergens (NDA) is that the safety of food enzymes or their combination that are used in the manufacture of the protein hydrolysate, is not to be assessed in this opinion. The assessment of the safety of the individual food enzymes is performed by the EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) according to the guidance and statements of the CEF/CEP Panel (EFSA CEF Panel, 2009, 2016; EFSA CEP Panel, 2019). This assessment is ongoing at the time of the adoption of the present opinion.

Therefore, the conclusions of the Panel are related to the nutritional safety and suitability of the specific protein hydrolysate used to manufacture the infant and follow-on formula for which the dossier has been submitted. They are not related to the safety of the protein hydrolysate in general, including the safety of the individual enzymes or their combination. Neither are they related to the safety of the final formula. This is justified as the composition of the formula with respect to substances other than the protein fraction should comply with the compositional requirements laid down in Commission Delegated Regulation (EU) 2016/127 in order to ensure the nutritional safety and suitability for use by infants. The conclusions of the Panel also do not refer to the efficacy of the formula in reducing the risk of developing allergic manifestations.

2. Data and methodologies

2.1. Data

The assessment of the nutritional safety and suitability of the specific protein hydrolysate derived from a whey protein concentrate and used in infant formula⁶ and follow-on formula⁷ is based on the data supplied in the dossier submitted to EFSA (EFSA-Q-2019-00652) and the additional information provided by the food business operator upon request.

A common and structured format for the presentation of dossiers related to infant and follow-on formulae manufactured from protein hydrolysates is described in the EFSA scientific and technical guidance for the preparation and presentation of an application for authorisation of an infant and/or follow-on formula manufactured from protein hydrolysates.⁸ As outlined in this guidance, it is the duty of the food business operator who submitted the dossier to provide all available scientific data which are pertinent to the dossier. The procedure followed by EFSA for handling dossiers on formulae manufactured from protein hydrolysates, the various steps in the procedure and estimated timelines are described online.⁹

⁵ Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, OJ L 31, 1.2.2002, p. 1.

⁶ Infant formula means food intended for use by infants during the first months of life and satisfying by itself the nutritional requirements of such infants until the introduction of appropriate complementary feeding.

⁷ Follow-on formula means food intended for use by infants when appropriate complementary feeding is introduced and which constitutes the principal liquid element in a progressively diversified diet of such infants.

⁸ EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2017. Scientific and technical guidance for the preparation and presentation of an application for authorisation of an infant and/or follow-on formula manufactured from protein hydrolysates. EFSA Journal 2017;15(5):4779, 24 pp. <https://doi.org/10.2903/j.efsa.2017.4779>.

⁹ <http://www.efsa.europa.eu/sites/default/files/applications/apdeskaplworkflownutriinfant.pdf>

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance for the preparation and presentation of an application for authorisation of an infant and/or follow-on formula manufactured from protein hydrolysates⁷. Previous EFSA work¹⁰ and the regulatory framework¹¹ were also taken into account.

As the formula in which the protein hydrolysate under evaluation is used, is marketed only in powder form, stability data were not evaluated for the formula (even though requested in the scientific and technical guidance⁷) as it is not expected that hydrolysis continues in powdered formulae.

3. Assessment

3.1. Characterisation of the protein hydrolysate

Protein source:

The protein hydrolysate under evaluation is produced from whey protein concentrate from cow's milk that is [REDACTED]

[REDACTED] The raw material specifications of these ingredients were provided by the food business operator. Information on individual intact proteins in the source material has been provided (name and average molecular weight), based on a publication on the composition of whey proteins (Walstra, 1999).

Protein processing:

The protein hydrolysate is produced under ISO 22000:2005, ISO/TS 22002-1:2009 and additional Food Safety System Certification (FSSC) 22000 requirements, according to a certificate provided in the dossier, following Good Hygiene Practices (GHP), Good Manufacturing Practices (GMP) and the Hazard Analysis and Critical Control Point (HACCP) system. A HACCP flow chart was provided.

In order to produce the hydrolysate, the source material is hydrated and heated to [REDACTED]

[REDACTED] The total duration of the hydrolysis is [REDACTED] minutes (at [REDACTED]) during which the pH is kept at [REDACTED]

The food enzymes used have been identified by the food business operator. The individual food enzymes employed in the process are currently under safety assessment by the EFSA CEP Panel. The serine endopeptidase, [REDACTED]

[REDACTED] is added to the source material in an amount (weight of enzyme/weight of substrate protein) of [REDACTED]. The activity of enzyme/weight of substrate, expressed as [REDACTED]

At the same time, a protease/peptidase complex [REDACTED]

[REDACTED] is added in an amount (weight of enzyme/weight of substrate protein) of [REDACTED]. The activity of enzyme/weight of substrate, expressed as [REDACTED]

[REDACTED] The raw material specifications of the food enzymes were provided in the submitted dossier.

The food enzymes are inactivated in a heat treatment step at [REDACTED] °C during the production process of the formula. This heat treatment step is applied for a duration of [REDACTED]

Residual enzymatic activity was measured in 10 batches of the infant formula or follow-on formula (5 and 5, respectively) in an external laboratory and was below the limit of quantification [REDACTED] of the original enzymatic activity) in all batches. The method applied and its calibration were described by the food business operator. Taking into account that the limit of quantification was [REDACTED] of the original enzymatic activity, the Panel notes that the sensitivity of the method was not satisfactory. However,

¹⁰ EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2014. Scientific Opinion on the essential composition of infant and follow-on formulae. EFSA Journal 2014;12(7):3760, 106 pp. <https://doi.org/10.2903/j.efsa.2014.3760>.

¹¹ https://ec.europa.eu/food/safety/labelling_nutrition/special_groups_food/children_en

considering the time and temperature applied, the Panel considers that the food enzymes are deactivated.

Degree of hydrolysis, molecular weight distribution, content of free amino acids and residual proteins

The degree of hydrolysis (DH) was approximated by the food business operator by calculating the ratio between free amino nitrogen (AN) in the protein hydrolysate, analysed by the *o*-phthaldialdehyde (OPA) assay, and the total number of peptide bonds in the source material, h_{tot} derived from published data on the amino acid composition of the source material. The food business operator presents this ratio as DH. The Panel notes that the data presented by the food business operator are not equivalent to DH and are only considered to approximate DH. The average of this ratio, expressed as a percentage, based on 10 independently produced batches was [REDACTED] with a standard deviation (SD) of [REDACTED].

Data on the content (%) of peptides and residual proteins in the hydrolysate have not been provided. The Panel notes that the amount of residual protein could be approximated by the > 10,000 Da fraction and the amount of peptides by the < 10,000 Da fraction of the molecular weight distribution of peptides (as described below). The food business operator states that, as a release criterion for the content of residual proteins, a maximum concentration of [REDACTED] in the hydrolysate and formula, is used (more than 1,100 analytical data points were represented in a graph for the period 2014–2018, and 308 analytical values from 2017 to 2019 were provided). The release criterion was met in [REDACTED] presented and in [REDACTED] given. Values exceeding the release criterion trigger an investigation of the possible cause. [REDACTED] is measured [REDACTED] (method described and presented as validated). The food business operator explained that this criterion was chosen as an indicator of residual intact protein, [REDACTED].

Target values for the molecular weight distribution of peptides and data on the batch-to-batch variability on 82 batches were presented. The target values as minimum and maximum percentage for each molecular weight range are presented below. However, these target values were not met in the analyses [REDACTED] for which data were presented. The highest percentages of non-conformities occurred in the fractions of 1,000–3,000 Da, 5,000–10,000 Da and > 10,000 Da.

1–500 Da: [REDACTED]
 500–1,000 Da: [REDACTED]
 1,000–3,000 Da: [REDACTED]
 3,000–5,000 Da: [REDACTED]
 5,000–10,000 Da: [REDACTED]
 >10,000 Da: [REDACTED]

The molecular weight distribution of peptides was measured by the analytical laboratory of the food business operator (certified ISO 9001:2025 for 'Quality management systems') by gel permeation chromatography–size exclusion chromatography (GPC–SEC) with UV detection at [REDACTED]. The method was indicated to have been validated. Information on the column [REDACTED] used was provided as well as information on the calibration of the system with regard to molecular weight, including details on the calibrators used and data indicating the reproducibility of the method.

The Panel notes that the non-confidential specifications provided by the food business operator upon request by EFSA with respect to the temperature and pH applied during hydrolysis, the temperature used to inactivate the food enzymes, the DH, and the molecular weight distribution of peptides were broad and could not be used in the characterisation of the protein hydrolysate (contrary to the confidential specifications provided). Therefore, they are not reported in the Opinion.

The Panel considers that the protein hydrolysate that has been used in the manufacture of the infant and follow-on formulae for which the dossier has been submitted is sufficiently characterised with respect to the fraction of the hydrolysed protein.

3.2. Uncertainties related to the characterisation of the protein hydrolysate

The Panel notes that the detection limit of the method applied for measuring the residual enzymatic activity in the final protein hydrolysate was insufficient to conclude with certainty that the food

enzymes are effectively inactivated. The consideration of the Panel that the time and temperature used are sufficient to inactivate the food enzymes is based on expert judgment rather than on data provided by the applicant.

3.3. Characterisation of the formula manufactured from the protein hydrolysate used in the clinical studies provided

The infant formula manufactured from the protein hydrolysate that is used in the clinical studies provided¹³ complies with the compositional criteria of Regulation (EU) 2016/127,² except for its contents of alpha-linolenic acid (ALA) and docosahexaenoic acid (DHA) which are in line with Directive 2006/141/EC.¹ The protein content of this formula is 9.6 g/100 kJ (2.3 g/100 kcal). No free amino acids are added to the formula. The amino acid profile meets the one laid down in Annex IIIA of Regulation (EU) 2016/127.

The infant and follow on formulae, produced in powder form, are produced under ISO 22000:2005, ISO/TS 22002-1:2009 and additional FSSC 22000 requirements, according to a FSSC certificate provided in the dossier for each production site, following GHP, GMP and the HACCP system (HACCP flow chart provided).

Data on the concentrations (mg/100 g protein) of furosine, available lysine, carboxymethyl-lysine (CML) and carboxyethyl-lysine (CEL) in the final formulae (three batches of infant formula and one batch of follow-on formula; two samples per batch analysed in two replicates) were provided. They were analysed by liquid chromatography coupled to tandem mass spectrometry, according to a method described in a signed report of the external laboratory that performed the analysis. This report also provided information on the analytical performance, the linearity range, the recovery and the accuracy of the method, including information on the validation of the method. Three publications on the method were also provided. The food business operator indicated that concentrations of these Maillard reaction products in the infant or follow-on formula manufactured from the hydrolysed protein (measured at two different steps of the product process or at the end of two different shelf-lives) were in the same range as those observed in infant or follow-on formula produced from intact cow's milk protein (one batch for each formula, at the end of the same two tested shelf-lives).

The Panel considers that the infant formula that is used in the pertinent human intervention study is sufficiently characterised.

3.4. Nutritional safety and suitability of the infant and follow-on formula

The food business operator performed a search in MEDLINE, Embase, CAB Abstracts, BIOSIS Previews and Current Contents including literature from 1 January 2015 to 9 July 2019 (search strings provided in the dossier). In this literature search, the food business operator identified five publications on one clinical trial (PATCH study: 'Prevention of Allergy Through Cow's Milk Hydrolysate') (Tang et al., 2014; Wopereis et al., 2014, 2016, 2018; Boyle et al., 2016) which were submitted as supportive evidence. The unpublished study report (No authors listed, 2012, unpublished study report) of this study was also provided. In addition, two unpublished study reports were provided by the food business operator, one as directly pertinent evidence (TENUTO study) (No authors listed, 2019, unpublished study report) and one as supportive evidence (GIRAFFE study: 'growth of infants who are formula-fed exclusively') (No authors listed, 2016, unpublished study report).

The Panel notes that, in the PATCH study (No authors listed, 2012, unpublished study report), the intervention and the control formulae were not the only source of nutrition, as partially breast-fed infants were also included in the study. The Panel considers that no conclusions can be drawn from this study for the evaluation of the nutritional safety and suitability of a protein hydrolysate to be used in a formula declared to be suitable as the sole source of nutrition in infants.

The Panel also notes that, in the GIRAFFE study (No authors listed, 2016, unpublished study report), the formula manufactured from hydrolysed protein was compared with another formula manufactured from hydrolysed protein that has not yet been assessed for its nutritional safety and suitability under Regulation (EU) 2016/127. Therefore, the Panel considers that no conclusions can be drawn from this study for the evaluation of the nutritional safety and suitability of either formula.

¹³ The follow-on formula is not investigated in the studies provided (Section 3.3).

Pertinent human intervention study

In a randomised controlled clinical trial (TENUTO study) (No authors listed, 2019, unpublished study report) performed in five countries (15 centres), healthy term exclusively formula-fed infants with a maximum age of 14 days were randomised, stratified by gender and centre and using a web-based randomisation system. They either consumed the formula manufactured from hydrolysed protein described in Section 3.1 (protein 9.6 g/100 kJ (2.3 g/100 kcal); intervention group) or an intact cow's milk protein formula (protein 8.4 g/100 kJ (2.0 g/100 kcal); control group) up to the age of 4 months.

The primary outcome of the study was weight gain from age \leq 14 days to day 119. Secondary outcomes were length, head circumference (HC), anthropometric measures expressed as z-scores, frequency of adverse events and symptoms of digestive intolerance.

Power calculations were performed assuming a SD of the difference of 6 g/day and a difference in weight gain of 0.5 g/day between groups. In order to reach 80% power, the authors calculated that 78 infants per group were needed. Assuming a 30% drop-out rate, 112 infants per group were envisaged to be recruited. Following a pre-planned interim analysis aimed at re-estimating the sample size, the sample size was increased to 134. For the interim analysis, groups were unblinded for the statistician and an independent Expert Committee, but not for the staff involved in the research.

Infants were weighed by the investigators naked while lying on a calibrated electronic scale that was accurate to 10 g. Length was measured to the nearest 0.1 cm using a length board. Prior to each visit, formula intake was noted by caregivers in a diary for 7 days. The number and the consistency of stools, regurgitation and vomiting were also recorded. Diary entries were double-checked by investigators with caregivers. Infants were considered as compliant if they only consumed the study formula and no other formula or complementary foods.

Anthropometric data were analysed by a mixed model with a random intercept, a random slope and a random quadratic term for each subject. Fixed effects were group, time, time^{squared}, sex, centre and birth weight and interaction terms for group x time, group x time^{squared}, sex x time and sex x time^{squared}. A prespecified equivalence margin for weight gain of 3 g/day was used. Other outcomes were analysed either by using an equivalence approach (i.e. length and HC gain) or by using a superiority approach (i.e. attained weight/length/HC, absolute weight/length/HC change, weight/length/HC-for age z-scores).

A total of 268 subjects (134/group) were randomised. Baseline characteristics and parental characteristics of subjects were comparable between groups, as was formula intake throughout the study. In the intervention group, 28 subjects and, in the control group, 23 subjects dropped out or were withdrawn from the analysis. Reasons for termination are reported and the number of infants who terminated the study early for varying reasons (e.g. adverse events, withdrawal, protocol violation) was comparable between groups. For the per protocol (PP) analysis, another six subjects in the intervention and four subjects in the control group were withdrawn from the analysis.

In the PP population ($n = 100$ in the intervention and $n = 107$ in the control group), the mean difference in weight gain/day between groups from baseline to 4 months was -1.2 g/day. The 90% confidence intervals (CIs) (used for judging equivalence) was -2.42 to 0.02 g/day. In the intention-to-treat (ITT) analysis (all subjects randomised), the mean difference was -1.1 (90% CI -2.3 to 0.1) g/day. The 90% CIs of both the ITT and the PP population fell within the prespecified equivalence margin and allowed to demonstrate the equivalence of the intervention to the control formula with respect to weight gain. Results presented for other anthropometric outcomes that were investigated were consistent with those observed for weight gain.

Adverse events were reported spontaneously by caregivers or were taken into account when observed by investigators. With respect to adverse events possibly, probably or definitely related to the study products, there was a statistically significant difference observed between the intervention and the control group. In the intervention group, 13.5% ($n = 18$) showed adverse events of any kind that could be related to the study product, while this was the case for 6% ($n = 8$; $p = 0.041$) in the control group. Gastrointestinal symptoms as described by the caregivers and classified by investigators (e.g. abdominal discomfort, abdominal pain, constipation, diarrhoea, regurgitation, vomiting) were the most frequent related adverse events that were reported. However, the Panel notes that no consistent pattern with respect to the frequency of occurrence of gastro-intestinal symptoms was observed, which makes it difficult to attribute the individual symptoms to the study formulae. There were also no appreciable differences reported with respect to stool frequency and stool consistency.

The Panel considers that this study shows that an infant formula manufactured from the protein hydrolysate described in Section 3.1 with a protein content of 9.6 g/100 kJ (2.3 g/100 kcal) and consumed

as the sole source of nutrition for 3.5 months leads to growth that is equivalent to an infant formula manufactured from intact cow's milk protein with a protein content of 8.4 g/100 kJ (2.0 g/100 kcal).

The Panel concludes that the protein hydrolysate under evaluation is a nutritionally safe and suitable protein source for use in infant formula, as long as the infant formula in which it is used contains a minimum of 9.6 g/100 kJ (2.3 g/100 kcal) protein and complies with the compositional criteria of Commission Delegated Regulation (EU) 2016/127 and the amino acid pattern in Annex IIIA of the Regulation.

No experimental data have been provided on the nutritional safety and suitability of this protein source in follow-on formula. However, given the fact that follow-on formula is consumed in conjunction with complementary foods and the protein source is considered nutritionally safe and suitable in an infant formula that is the sole source of nutrition of infants, the Panel considers that the protein hydrolysate under evaluation is also a nutritionally safe and suitable protein source for use in follow-on formula, as long as the follow-on formula in which it is used contains a minimum of 9.6 g/100 kJ (2.3 g/100 kcal) protein and complies with the compositional criteria of Commission Delegated Regulation (EU) 2016/127 and the amino acid pattern in Annex IIIA of the Regulation.

4. Conclusions

The Panel concludes that:

- the protein hydrolysate that has been used in the manufacture of the infant and follow-on formula for which the dossier has been submitted is sufficiently characterised with respect to its fraction of hydrolysed protein;
- the minimum specific criteria for characterisation of the protein hydrolysate with respect to the protein source, protein processing and protein quality, as requested in the ToR, are those given in Section 3.1;
- the protein hydrolysate for which the dossier has been submitted is a nutritionally safe and suitable protein source for use in infant and follow-on formula, as long as the formula in which it is used contains a minimum of 9.6 g/100 kJ (2.3 g/100 kcal) protein and complies with the other compositional criteria of Commission Delegated Regulation (EU) 2016/127 and the amino acid pattern in Annex IIIA of the Regulation.

5. Documentation as provided to EFSA (if appropriate)

Dossier for the authorisation of infant and follow on formulae manufactured from protein hydrolysates. February 2020. Submitted by Danone Trading ELN B.V.

Steps taken by EFSA

- 1) The technical dossier was received by EFSA on 18/10/2019.
- 2) A letter from the European Commission with the request for a scientific opinion on the safety and suitability for use by infants of an infant and follow-on formula manufactured from protein hydrolysate was received by EFSA on 07/11/2019.
- 3) The scientific evaluation procedure started on 27/02/2020.
- 4) On 06/03/2020, the Working Group on protein hydrolysate-based formula of the NDA Panel agreed on a list of questions for the food business operator to provide additional information to accompany the dossier. The scientific evaluation was suspended on 13/03/2020 and was restarted on 13/05/2020.
- 5) On 25/06/2020, the Working Group on protein hydrolysate-based formula of the NDA Panel agreed on a list of questions for the food business operator to provide additional information to accompany the dossier. The scientific evaluation was suspended on 28/07/2020 and was restarted on 04/09/2020.
- 6) During its meeting on 22/10/2020, the NDA Panel, having evaluated the data, adopted an opinion on the nutritional safety and suitability of a specific protein hydrolysate derived from whey protein concentrate and used in an infant and follow-on formula manufactured from hydrolysed protein by Danone Trading ELN B.V.

References

- Boyle RJ, Tang ML, Chiang WC, Chua MC, Ismail I, Nauta A, Hourihane JOB, Smith P, Gold M, Ziegler J, Peake J, Quinn P, Rao R, Brown N, Rijniere A, Garssen J and Warner JO and the Patch study investigators, 2016. Prebiotic-supplemented partially hydrolysed cow's milk formula for the prevention of eczema in high-risk infants: a randomized controlled trial. *Allergy*, 71, 701–710. <https://doi.org/10.1111/all.12848>
- EFSA CEF Panel (EFSA Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2009. Guidance of the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on the Submission of a Dossier on Food Enzymes for Safety Evaluation by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids. *EFSA Journal* 2009;7(8):1305, 26 pp. <https://doi.org/10.2903/j.efsa.2009.1305>
- EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2016. Panel statement on the exposure assessment of food enzymes. *EFSA Journal* 2016;14(11):4581, 9 pp. <https://doi.org/10.2903/j.efsa.2016.4581>
- EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), 2019. Statement on the characterisation of microorganisms used for the production of food enzymes. *EFSA Journal* 2019;17(6):5741, 13 pp. <https://doi.org/10.2903/j.efsa.2019.5741>
- No authors listed, 2012. unpublished study report. The effect of early nutrition in high-risk infants on allergy prevention during the first 12 months of life. 262 pp.
- No authors listed, 2016. unpublished study report. Study to investigate the nutritional efficacy and suitability of hypoallergenic infant formulae with lowered protein content in healthy full-term infants. 222 pp.
- No authors listed, 2019. unpublished study report. A randomised, controlled, double-blind, parallel group, multi-country study to investigate the effects of an infant formula containing partially hydrolysed proteins on growth, safety, and tolerance in healthy term infants. 125 pp.
- Tang M, Nauta A, Rijniere A, Knippels L, Hourihane J, Chiang W, Boyle R, Garssen J and Warner J, 2014. Hypoantigenic and immune modulatory properties of a partially hydrolyzed cow's milk formula supplemented with prebiotic oligosaccharides. *Allergy: European. Journal of Allergy and Clinical Immunology*, 69.
- Walstra P, 1999. Principles of milk properties and processes. CRC Press, Boca Raton, FL, USA.
- Wopereis H, Sim K, Shaw A, Martin R, Oozeer R, Warner J, Kroll J and Knol J, 2014. The developmental gut microbiota is modulated towards a pattern closer to breastfed infants by a partially hydrolyzed cow's milk formula supplemented with specific prebiotic oligosaccharides. *Allergy: European. Journal of Allergy and Clinical Immunology*, 69.
- Wopereis H, Sim K, Shaw A, Oozeer R, Warner J, Kroll J and Knol J, 2016. Decreased microbial conversion of lactic acid into butyrate in infants developing eczema. *Allergy: European. Journal of Allergy and Clinical Immunology*, 71, 168–169.
- Wopereis H, Sim K, Shaw A, Warner JO, Knol J and Kroll JS, 2018. Intestinal microbiota in infants at high risk for allergy: Effects of prebiotics and role in eczema development. *Journal of Allergy and Clinical Immunology*, 141, 1334–1342 e1335. <https://doi.org/10.1016/j.jaci.2017.05.054>

Abbreviations

ALA	alpha-linolenic acid
AN	amino nitrogen
■	■
CEL	carboxyethyl-lysine
CEP	Panel on Food Contact Materials, Enzymes and Processing Aids
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CI	confidence interval
CM	carboxymethyl-lysine
DH	degree of hydrolysis
DHA	docosahexaenoic acid
■	■
FSSC	Food Safety System Certification
GHP	Good Hygiene Practices
GIRAFFE	growth of infants who are formula-fed exclusively
GMO	genetically modified organism
GMP	Good Manufacturing Practices
GPC-SEC	gel permeation chromatography-size exclusion chromatography
HACCP	Hazard Analysis and Critical Control Point
HC	head circumference
ISO	International Organization for Standardization

ITT	intention-to-treat
██████	████████████████████
NDA	Panel on Nutrition, Novel Foods and Food Allergens
OPA	<i>o</i> -phthaldialdehyde
PATCH	Prevention of Allergy Through Cow's Milk Hydrolysate'
PP	per protocol
RCT	randomised controlled trial
SD	standard deviation
TN	total nitrogen
ToR	Terms of Reference
TS	technical specification
UV	ultraviolet
w/w%	percentage on a weight-per-weight basis