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# Nutritional safety and suitability of a specific protein hydrolysate derived from a whey protein concentrate and used in an infant formula and follow-on formula manufactured from hydrolysed protein by FrieslandCampina Nederland 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 a whey protein concentrate and used in an infant and follow-on formula manufactured by FrieslandCampina Nederland 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. 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 2.4 g/100 kcal and consumed as the sole source of nutrition by infants for 3 months led to a growth equivalent to a formula manufactured from intact cow's milk protein with a protein content of 2.1 g/100 kcal. Data on gastrointestinal tolerance of the formula did not raise any concerns. No experimental data have been provided on the nutritional safety and suitability of this protein source in follow-on formula. Given that it is consumed with complementary foods and the protein source is 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 2.4 g/100 kcal protein and complies with the compositional criteria of Regulation (EU) 2016/127 and the amino acid pattern in its Annex IIIA.



**Keywords:** protein hydrolysate, characterisation, infant formula, follow-on formula, nutritional safety, suitability, clinical trial

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# 1. Introduction

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

### 1.1.1. Background

Commission Directive 2006/141/EC[1] 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) 2016/127[2] 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 Council[3] 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] 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.

[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. https://doi.org/10.2903/j.efsa.2014.3760

### 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 EFSA 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 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 submission has been made. The conclusions 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 formula[6] and follow-on formula[7] is based on the data supplied in the dossier submitted to EFSA (EFSA-Q-2020-00025) 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 formula 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 is 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] https://www.efsa.europa.eu/sites/default/files/applications/apdeskapplworkflownutriinfant.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 hydrolysates. Previous EFSA work[10] and the regulatory framework 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 guidance[8]) as it is not expected that protein 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 (WPC) [redacted]. Certificates of analysis of five batches of the WPC were provided by the food business operator. Protein contents of the WPC ranged between [redacted][11]. Individual intact proteins in the source material have been identified by their molecular weight by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and the information has been provided in the submitted dossier.

***Protein processing***

The protein hydrolysate is produced under Good Manufacturing Practice (GMP), and ISO 9001:2015 (Quality Management System) and ISO 22000:2005 (Food Safety Management System), as indicated in two certificates provided in the submitted dossier.

In order to produce the hydrolysate, the source material (i.e. WPC) is hydrated and heated to [redacted]. The temperature and pH are kept at [redacted] and [redacted] for the hydrolysis, respectively.

The protein hydrolysate is produced in a [redacted] The food enzymes and their sources have been identified. The individual food enzymes employed in the process are currently under safety assessment by the EFSA CEP Panel. [redacted] is added to the WPC in an amount (weight of enzyme/weight of substrate protein) of [redacted]. The activity of enzyme/weight of substrate, expressed as [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] the hydrolysis [redacted]. The enzymes are inactivated [redacted]. After the hydrolysis, the protein hydrolysate is concentrated [redacted] and spray dried.

The food business operator specifies that, based on the information on thermostability provided by the food enzyme suppliers, no residual enzymatic activity is expected to be detectable beyond temperatures of [redacted] for the first food enzyme and beyond [redacted] for the second food enzyme. Thermostability curves were provided for both enzymes and confirm the statement of the food business operator.

[redacted]

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

The average degree of hydrolysis (DH) is [redacted] with a standard deviation (SD) of [redacted].

The calculation of DH is based on the formula DH[%] = $h/h_{tot} \times 100\%$, where h, that represents the number of cleaved peptide bonds, is derived by measuring the increase in free amino groups. Free

[10] https://ec.europa.eu/food/safety/labelling_nutrition/special_groups_food/children_en
[11] Calculated as [redacted]

amino nitrogen (AN) and total nitrogen (TN) after hydrolysis have been measured with [redacted] (indicated to have been validated by the [redacted] and by formol titration (USP 23-NF18) for free AN; no validation reports provided). Analytical measures were obtained for five independent batches, for which certificates of analysis were provided for free AN and TN after hydrolysis. Free AN and TN before hydrolysis has been calculated based on published amino acid sequence data. The values for free AN/TN before hydrolysis were subtracted from free AN/TN after hydrolysis to derive h. The total number of peptide bonds in the source material (i.e. WPC), $h_{tot}$, was derived from a published database and divided by calculated TN before hydrolysis.

The molecular weight distribution of peptides, based on the same five independent batches as above, for which certificates of analysis were provided, are on average % (SD):

1–500 Da: [redacted]
500–1,000 Da: [redacted]
1,000–2,000 Da: [redacted]
2,000–5,000 Da: [redacted]
5,000–10,000 Da: [redacted]
> 10,000 Da: [redacted]

The molecular weight distribution of peptides was measured by ultra performance liquid chromatography-size exclusion chromatography with ultraviolet (UV) detection at 214 nm (UPLC-SEC, acidic method pH = 2; internal method based on Smyth and FitzGerald (1997)). This method was indicated to have been validated and briefly described by the food business operator. Name of the column used (ACQUITY UPLC Protein BEH SEC column) and system calibration, including details on the calibrators, was provided by the food business operator upon request.

Data on the amount of residual proteins (defined as the fraction > 10,000 Da) and on the amount of peptides (defined as the fraction < 10,000 Da) in the protein hydrolysate were provided. These data were derived from analysis of the molecular weight distribution of peptides as described above and based on the total protein content calculated from TN, analysed as described above. Data on the content of free amino acids in the protein hydrolysate were also provided. Free amino acids were measured according to Schuster (1988) by reversed-phase high-performance liquid chromatography (RP-HPLC), and tryptophan by an Association of Official Analytical Collaboration (AOAC) official method of analysis, in a laboratory that was presented as being accredited for amino acid analyses. Values were obtained for five independent batches (including four of the previously mentioned batches) and certificates of analysis have been provided.

Regarding Maillard reaction products, the concentrations of blocked and total lysine (i.e. reactive and blocked), furosine and carboxymethyl-lysine (CML) in five independent batches of the protein hydrolysate have been provided (including one not previously investigated batch and four others investigated for some of the parameters mentioned above). Certificates of analysis have been provided. Analytical methods applied for furosine and total lysine were based on [redacted], respectively, and were briefly described. The method used to analyse CML was also briefly described by the food business operator. Validation reports for the methods used to analyse furosine and CML have been provided. Blocked lysine was calculated from values of furosine and total lysine.

Contrary to the confidential specifications provided, 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. 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 formula for which the dossier has been submitted is sufficiently characterised with respect to the fraction of the hydrolysed protein.

## 3.2. Characterisation of the formula manufactured from the protein hydrolysate used in the clinical study provided

The infant formula, manufactured from the protein hydrolysate assessed in Section 3.1, and that is used in the unpublished clinical study provided, complies with the compositional criteria laid down in Commission Delegated Regulation (EU) 2016/127[2].

The infant formula is produced under ISO 22000:2005, ISO/TS 22002–1:2009 and additional Food Safety System Certification (FSSC) 22000 requirements, according to a FSSC certificate provided in the dossier.

This infant formula has a protein content of 0.57 g/100 kJ (2.4 g/100 kcal) and an amino acid profile complying with Annex IIIA of Commission Delegated Regulation (EU) 2016/127[2]. During the manufacturing process of this powdered formula, free amino acids (L-phenylalanine and L-tyrosine) are added to the formula and additional heat treatments are applied, including homogenisation, pasteurisation and spray drying.

Furosine and CML have been analysed, according to the methods described in Section 3.1, in three batches of the infant formula. The concentrations fell within the range of furosine and CML concentrations found in seven different commercially available infant formulae with intact protein, as analysed by the food business operator. Certificates of analyses have been provided.

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

## 3.3. Nutritional safety and suitability of the infant formula

### 3.3.1. Human intervention studies

The food business operator performed a literature search in PubMed on 16 January 2016 (no time limit specified) for human intervention studies on measures of growth of infants who exclusively consumed formula manufactured from whey protein hydrolysates. Through this search, the food business operator identified six potentially pertinent studies, reported in seven papers (Giovannini et al., 1994; Exl et al., 2000; Schmelzle et al., 2003; Florendo et al., 2009; Rzehak et al., 2009; Rzehak et al., 2011; Borschel et al., 2014). These studies were conducted with formulae other than the infant formula for which the dossier has been submitted. The Panel considers that no conclusions can be drawn from them for the scientific assessment of the nutritional safety and suitability of the protein hydrolysate for which the dossier has been submitted.

The food business operator also presented an unpublished full study report (Manios and Kantaras, 2023, unpublished) of one pertinent randomised controlled trial (RCT) on 345 healthy term infants. The infants were exclusively fed for 3 months with either the infant formula for which the dossier has been submitted (formula manufactured from hydrolysed whey protein, HF) or an infant formula manufactured from intact cow's milk protein (control formula, CF). The HF had a protein content of 0.57 g/100 kJ (2.4 g/100 kcal) and the CF of 0.50 g/100 kJ (2.1 g/100 kcal). The CF was a commercially available infant formula and the composition complied with Commission Delegated Regulation (EU) 2016/127.

The equivalence study was conducted in Greece (Attica, Thessaly and Thessaloniki). Healthy term infants up to 28 days of age with a birthweight between 2,500 and 4,200 g were included in the study. The infants had to be exclusively formula-fed for at least 5 days prior to inclusion in the study. Exclusively formula-fed infants with caregivers willing to participate in the study were identified by private paediatricians. Their eligibility was verified by a junior paediatrician during a home visit during which also the baseline anthropometric measurements were taken. Eligible infants were then randomised in blocks of eight, stratified by gender and using central randomisation, to consume one of four coded formulae. Two of the codes pertained to the HF and two the CF. In case of inclusion of twins, the first twin was randomised and the second allocated to the same group. Formulae were provided in blank tins with one of the four codes printed on them. The blinding for study personnel was maintained throughout the study. The unblinding for data analysis occurred after the lock of the database.

The primary outcome was average weight gain per day between baseline and 17 weeks of age. The primary analysis was identified as the one done on the per protocol (PP) population. Secondary outcomes were length gain, head circumference (HC) gain, absolute weight, length and HC, body mass index (BMI) and variable (weight, length, HC and BMI)-for-age and weight-for-length z-scores (based on World Health Organization (WHO) child growth standards using WHO Anthro for personal computers, version 3.2.2, 2011). Other outcomes were formula intake, gastrointestinal tolerance, stool frequency, stool consistency, amount and colour. Adverse events and serious adverse events were also recorded.

Anthropometric measurements were taken by junior paediatricians at home visits according to standard operating procedures. Home visits took place at baseline, 8 weeks of age (± 1 day),

13 weeks of age (± 1 day) and 17 weeks of age (± 1 day). Infants were weighed without clothes with a clean nappy while lying on a scale that was calibrated each month and checked with a reference weight before each measurement. Measurements were taken around the same time of the day at each visit. Length was measured to the nearest 0.1 cm using an infantometer and HC was measured to the nearest 0.1 cm using a non-elastic tape. All measurements were performed in duplicate. If the difference was more than 20 g, 0.7 cm or 0.5 cm, respectively, a third measurement was taken. The mean of two or the median of three was used in the analysis.

Gastrointestinal tolerance was assessed using the Infant Gastrointestinal Symptoms Questionnaire (IGSQ) (Riley et al., 2015) and a crying diary, and stool characteristics using the Amsterdam Infant Stool Scale (Bekkali et al., 2009). Formula intake was recorded in diaries for 7 days before each study visit and from day 3 to 7 and day 10 to 14 after the baseline visit. Parents were also asked to record any vomiting or regurgitation and had to hand in the empty formula tins.

Statistical analyses were carried out using SAS PROC MIXED.

In the primary analysis, study formula and gender were included as fixed interaction terms with time (formula × time, formula × time × time, gender × time, and gender × time × time), and gender, birth weight and maternal gestational diabetes were used as covariates. A Huynh-Feldt covariance matrix was used.

Unadjusted analyses for the primary outcome using the two one-sided-t-test (TOST) procedure, as well as summary statistics for other anthropometric outcomes were also presented.

Sample size was calculated assuming a mean difference in weight gain of 0.9 g/day, an SD of 6 g/day and using an equivalence margin of ±3 g/day. When using a significance level of 5% and a power of 80%, it was calculated that 103 infants would need to be enrolled per formula group. Assuming a 25% drop-out rate, 138 infants needed to be enrolled per group (in total 276 infants).

After enrolment of 121 infants a pre-planned interim analysis was conducted by an independent statistician comparing the intervention and control groups in the PP population. This led to an increase in sample size to a total of 345 infants.

Baseline characteristics of parents were similar in both groups except for maternal gestational diabetes and paternal smoking that were both higher in the group consuming the HF. Baseline characteristics of infants were similar in the full analysis set (FAS), i.e. all randomised participants who were fed at least once with the study product. It consisted of 173 and 172 infants in the HF and CF groups at baseline, respectively, and 138 and 150 infants at 17 weeks of age. No imputations of missing data were made. In the PP population (122 in the HF and 142 in the CF group), statistically significant differences at baseline were observed for HC-for-age z-scores and BMI-for-age z-scores, which were in the magnitude of around 0.1 z-scores with higher values observed in the HF group. The Panel considers that this magnitude is not of biological relevance.

In the PP population (primary analysis), weight gain per day was similar in both groups and amounted to a mean (SD) of around 30.9 (6.2) g/day. The adjusted analyses of the data showed an adjusted mean difference of −0.08 g/day (90% confidence interval (CI) −1.25–1.10). The unadjusted analysis showed similar results, with the mean difference being 0.09 g/day (90% CI −1.36–1.18). The results of the analyses in the FAS population were similar. The Panel notes that the 90% CIs of both the PP and the FAS 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.

There were also no statistically significant differences in length and HC gain between groups, i.e. 0.002 cm (90% CI −0.001–0.006) cm and −0.002 cm (90% CI −0.004 to 0), respectively (adjusted analyses, PP population).

Results from the adjusted analysis on absolute weight, length, HC and BMI, and respective variables as z-scores also showed no biologically relevant differences in growth parameters between groups.

Average formula intake in the group of all infants who consumed the allocated formula was similar in both groups and amounted to on average (SD) 687 (153) mL/day and 710 (161) mL/day in the HF and CF groups, respectively, at day 8 from baseline and to 901 (179) mL/day and 906 (180) mL/day, respectively, at the end of the study.

Results for gastrointestinal tolerance and stool characteristics were not presented, as results of the analyses were not yet available at the time of submission of the anthropometric data to EFSA. Individual data on adverse events that occurred during the study were provided. Gastrointestinal complaints occurred in 15 out of 173 infants in the HF and 13 out of 172 infants in the CF group, three of which were classified as serious adverse events (one in the HF and two in the CF group). They were considered by the investigators as definitely related to the study formula in six infants in the HF group (vomiting: one infant; posseting: one infant; strong odour on the clothes, skin, urine and faeces of the

infant and gas production: one infant; diarrhoea > 1 day: one infant; diarrhoea > 1 day and vomiting: one infant colics and constipation: one infant) and one in the CF group (diarrhoea > 1 day). None of them was classified as serious adverse event. Gastrointestinal complaints possibly related to the study formula (as classified by the investigators) occurred in five infants in the HF group (posseting/vomiting after feeding: three infants (in one infant also decreased appetite); vomiting and gas production: one infant; colics and bloated abdomen: one infant and classified as serious adverse event) and in two infants in the CF group (esophagitis, milk protein allergy: one infant; posseting: one infant, none considered as serious adverse event).

Adverse events (not restricted to gastrointestinal complaints) were the reason for withdrawal of 12 infants in the HF group and 9 infants in the CF group. Four infants in each group stopped the intervention because the infants disliked the milk. The Panel notes that the number of infants with gastrointestinal complaints was similar between groups. Although the number of infants with gastrointestinal complaints that were considered by the investigators to be possibly or definitely related to the study formula was higher in the HF group there was no consistent pattern in those symptoms that would allow to relate those symptoms with sufficient certainty to the intervention formula. The Panel considers that the data submitted with respect to gastrointestinal complaints recorded as adverse events do not give rise to concern. This judgement is based on the observation that overall gastrointestinal complaints were similar in both groups and occurred in a limited number of infants (< 10%), that no consistent pattern in the occurrence of individual symptoms could be identified and that the described complaints are commonly occurring in infants of that age, which introduces some uncertainty regarding whether the complaints were related to the study products.

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 0.57 g/100 kJ (2.4 g/100 kcal) and consumed as the sole source of nutrition for 3 months leads to growth that is equivalent to an infant formula manufactured from intact cow's milk protein with a protein content of 0.50 g/100 kJ (2.1 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 0.57 g/100 kJ (2.4 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 0.57 g/100 kJ (2.4 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.

### 3.4. Uncertainties related to the nutritional safety and suitability of the infant formula

The Panel notes that results on the gastrointestinal tolerance and stool characteristics were not provided. The considerations of the Panel that there is no concern with respect to the tolerance of the study formula are based on adverse event reporting and expert judgement. The considerations made by the Panel in this relation are given in Section 3.3.

## 4. Conclusions

The Panel concludes that:

- the protein hydrolysate for which the dossier has been submitted and that is to be used in the manufacture of infant and follow-on formula 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 terms of reference, 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 0.57 g/100 kJ (2.4 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

Dossier for the authorisation of infant formula Frisolac HA manufactured from protein hydrolysate in accordance to Commission Delegated Regulation (EU) 2016/127. April 2020. Submitted by FrieslandCampina Nederland B.V.

## 6. Steps taken by EFSA

1) The technical dossier was received by EFSA on 08/01/2020.
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 23/01/2020.
3) The scientific evaluation procedure started on 29/04/2020.
4) On 11/05/2020, the Working Group on Protein Hydrolysates 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 20/05/2020 and was restarted on 26/06/2020.
5) On 14/07/2020, the Working Group on Protein Hydrolysates 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 28/08/2020.
6) On 05/10/2020, 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 15/10/2020 and was restarted on 20/02/2023.
7) On 24/03/2023, 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 31/03/2023 and was restarted on 19/04/2023.
8) During its meeting on 23/05/2023, the NDA Panel, having evaluated the data, adopted an opinion on the 'Nutritional safety and suitability of a specific protein hydrolysate derived from a whey protein concentrate and used in an infant formula and follow-on formula manufactured from hydrolysed protein by FrieslandCampina Nederland B.V.'.

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## Abbreviations

| | |
|---|---|
| AN | amino nitrogen |
| AOAC | Association of Official Analytical Collaboration |
| BMI | body mass index |
| CEF | Panel on Food Contact materials, Enzymes, Flavourings and Processing Aids |
| CEP | Panel on Food Contact Materials, Enzymes and Processing Aids |
| CF | control formula |
| CI | confidence interval |
| CML | carboxymethyl-lysine |
| DH | degree of hydrolysis |
| [redacted] | [redacted] |
| FAS | full analysis set |
| FSSC | Food Safety System Certification |
| GMP | Good Manufacturing Practice |
| h | number of cleaved peptide bonds |
| HC | head circumference |
| HF | formula manufactured from hydrolysed protein |
| IDF | International Dairy Federation |
| IGSQ | Infant Gastrointestinal Symptoms Questionnaire |
| ISO | International Organization of Standardization |
| NDA | Panel on Nutrition, Novel Foods and Food Allergens |
| PP | per-protocol |
| [redacted] | [redacted] |
| RCT | randomised controlled trial |
| RP-HPLC | reversed phase high performance liquid chromatography |
| SD | standard deviation |
| SDS–PAGE | sodium dodecyl sulfate–polyacrylamide gel electrophoresis |
| TOST | two one-sided-t-test |
| TN | total nitrogen |

| | |
|---|---|
| UPLC-SEC | ultra performance liquid chromatography-size exclusion chromatography |
| USP | United States Pharmacopoeia |
| UV | ultraviolet |
| WHO | World Health Organization |
| WPC | whey protein concentrate |