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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 HIPP-Werk Georg Hipp OHG (dossier submitted by meyer.science GmbH).**

Torsten Bohn, Jacqueline Castenmiller, Stefaan de Henauw, Karen-Ildico Hirsch-Ernst, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J. Mcardle, Androniki Naska, Carmen Pelaez, et al.

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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 HIPP-Werk Georg Hipp OHG (dossier submitted by meyer.science GmbH)

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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 infant and follow-on formula by HIPP-Werk Georg Hipp OHG. The dossier that was submitted to the European Commission aimed at requesting an amendment of Regulation (EU) 2016/127 with respect to the protein sources that may be used in 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 1.9 g/100 kcal and consumed as the sole source of nutrition by infants for 3 months led to growth equivalent to a formula manufactured from intact cow's milk protein with the same protein content. 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 1.9 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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**Requestor:** European Commission following an application by meyer. science

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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<sup>1</sup> 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<sup>2</sup> 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<sup>3</sup> and revises them, based on the opinion of the European Food Safety Authority (EFSA) of 2014<sup>4</sup>. 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 to 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.

<sup>1</sup> 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.

<sup>2</sup> OJ L 25, 2.2.2016, p. 1.

<sup>3</sup> 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.

<sup>4</sup> 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<sup>5</sup>, 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, 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 whey protein concentrate and used in infant formula<sup>6</sup> and follow-on formula<sup>7</sup> is based on the data supplied in the dossier submitted to EFSA (EFSA-Q-2019-00304) 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.<sup>8</sup> 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.<sup>9</sup>

<sup>5</sup> 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.

<sup>6</sup> 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.

<sup>7</sup> 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.

<sup>8</sup> 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>

<sup>9</sup> <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.<sup>7</sup> Previous EFSA work<sup>10</sup> and the regulatory framework<sup>11</sup> were also taken into account.

As the infant 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<sup>7</sup>) 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 (WPC) powder derived from cow's milk with a minimum protein content of [REDACTED]<sup>12</sup> of the dry matter, minimum 4% fat and minimum 1.5% lactose. It was described that the whey comes as the [REDACTED]. The incoming whey is subjected to analytical controls [REDACTED]. The whey is then [REDACTED] to form the WPC. The raw material specifications of the WPC were provided by the food business operator. Individual intact proteins in the source material have been identified by the food business operator, based on a publication on the composition of whey proteins (Walstra, 1999).

#### Protein processing

The protein hydrolysate is produced under a hazard analysis and critical control points (HACCP) management system, under good manufacturing practice (GMP) standards, and according to additional Food Safety System Certification (FSSC) 22000 requirements. A certificate of FSSC 22000 certification and reports from official controls of food safety standards are provided.

In order to produce the hydrolysate, the source material (WPC) is hydrated and heated to [REDACTED]. The protein content and the pH are then adjusted to [REDACTED], respectively. The total duration of the enzymatic hydrolysis is [REDACTED] (at [REDACTED]) during which time the pH is kept constant.

The food enzymes used have been described in the dossier by the food business operator. The individual food enzymes employed in the process are currently under safety assessment by the EFSA CEP Panel. First, the serine endopeptidase, [REDACTED] is added to achieve a concentration (activity of enzyme per kg substrate protein) of [REDACTED] where the activity unit ([REDACTED]) is proprietary to the food enzyme supplier. After [REDACTED], the second enzyme, a metalloprotease, [REDACTED] is added to achieve a concentration (activity of enzyme per kg substrate protein) of [REDACTED] where again the activity unit ([REDACTED]) is proprietary to the food enzyme supplier. The two enzymes are used at the weight ratio of [REDACTED] (total enzyme protein, i.e. the sum of the two enzymes) per 1,000 kg total substrate protein.

The food enzymes are inactivated in a heat treatment step of the protein hydrolysate at [REDACTED] that lasts [REDACTED].

<sup>10</sup> 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>

<sup>11</sup> [https://ec.europa.eu/food/safety/labelling\\_nutrition/special\\_groups\\_food/children\\_en](https://ec.europa.eu/food/safety/labelling_nutrition/special_groups_food/children_en)

<sup>12</sup> Nx6.38.

[REDACTED]

The Panel notes that no analytical data were provided with respect to the residual enzymatic activity in the protein hydrolysate. However, considering the duration and temperature applied, [REDACTED] the Panel considers that there will be no/negligible residual enzyme activity in the final product.

### Degree of hydrolysis and molecular weight distribution of peptides, 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) method, 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 the DH. The Panel notes that the data presented by the food business operator are not equivalent to the DH and are only considered to approximate the DH. The average of this ratio, expressed as a percentage, based on six independently produced batches was [REDACTED] with a standard deviation (SD) of [REDACTED] (range: [REDACTED]). The target value as indicated by the food business operator for this ratio is [REDACTED]. A certificate of analysis for the six batches was provided.

According to the food business operator, the maximum level of residual proteins in the protein hydrolysate is [REDACTED] protein equivalent/g protein. The amount of residual protein in the protein hydrolysate was quantified by using enzyme-linked immunosorbent assay (ELISA) of the six independently produced batches of the protein hydrolysate, showing that this limit value was not exceeded. A peptide fingerprint of the most abundant peptides, with six or more amino acids and a minimum number of peptide spectrum matches of five, in the protein hydrolysate was provided in the dossier. Six independently produced batches were analysed. Peptides were identified by mass spectrometry with the method described in the dossier. The target level of free amino acids in the protein hydrolysate is [REDACTED] w/w of the powder. Data on batch-to-batch variability have been presented in the dossier for the six independently produced batches of the protein hydrolysate. All batches were well within the limit value, with the highest content at [REDACTED] w/w. The amino acid analyses were based on the publications by Schuster (1988) and Henderson et al. (2000).

Target values for the molecular weight distribution of peptides and data on the batch-to-batch variability of six batches were presented. The food business operator explained [REDACTED] was provided instead of certificates of analyses.

The target values as minimum and maximum percentages for each molecular weight range are presented below. The range of measured values for the six batches are given in brackets.

< 175 Da:	[REDACTED]
< 375 Da:	[REDACTED]
375–750 Da:	[REDACTED]
750–1,250 Da:	[REDACTED]
1,250–2,500 Da:	[REDACTED]
> 2,500 Da:	[REDACTED]

The molecular weight distribution of peptides was measured using size exclusion chromatography (SEC) with UV detection at [REDACTED]. Information on the column ([REDACTED]) used was provided by the food business operator, as was a validation report, providing details on the accuracy, the precision and the robustness of the method.

The Panel considers 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 the fraction of the hydrolysed protein.



### 3.2. Uncertainties related to the characterisation of the protein hydrolysate

The Panel notes that no analytical data were provided with respect to the residual enzymatic activity in the protein hydrolysate. The consideration of the Panel that there will be no/negligible residual enzyme activity in the final product, considering the duration and temperature used, [REDACTED]

[REDACTED] is based on expert judgement rather than on data provided by the food business operator.

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

The infant formula manufactured from the protein hydrolysate and that was used in the clinical studies provided, complies with the compositional criteria of Regulation (EU) 2016/127.

The protein content of this formula is 0.45 g/100 kJ (1.9 g/100 kcal) and is based on a calculation of total nitrogen  $\times$  6.25. [REDACTED] are added as free amino acids to the formula. The amino acid profile meets the one laid down in Annex IIIA of Regulation (EU) 2016/127. Data on the batch-to-batch variability of the amino acid content of the formula and the associated certificates of analyses were provided by the food business operator.

The infant formula, produced in powder form, is manufactured following GMP and the HACCP standards. The production site is certified according to British Retail Consortium (BRC) food safety standards. The associated certificates have been provided.

Data on concentrations of furosine and carboxymethyllysine (CML) in four batches of infant formula manufactured from the specific protein hydrolysate as well as in nine batches of infant formula manufactured from intact cow's milk protein have been provided by the food business operator together with the certificates of analyses. Concentrations of furosine and [REDACTED] of CML [REDACTED]

[REDACTED] in line with what has been reported in the literature for these types of products (i.e. furosine: 130–1,226 mg/100 g protein; CML: 4.2–32.2 mg/100 g protein) (Fenaille et al., 2006; Delatour et al., 2009; Sabater et al., 2018; Prosser et al., 2019). The analysis of CML was carried out using high-performance liquid chromatography (HPLC) coupled to mass spectrometry, [REDACTED]

[REDACTED] according to the validated method described by Schwarzenbolz et al. (2016). Furosine analysis was performed according to the validated method described by Henle et al. (1991) using ion exchange chromatography-UV [REDACTED]

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

For the substantiation of the nutritional safety and suitability of the infant and follow-on formula, the food business operator provided an unpublished full study report (Jochum et al., 2021, unpublished) of one pertinent randomised controlled trial (RCT) in 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 protein, HF) or an infant formula manufactured from intact cow's milk protein (control formula, CF), both with a protein content of 0.45 g/100 kJ (1.9 g/100 kcal). The CF was a commercially available infant formula that complied with Commission Delegated Regulation (EU) 2016/127.

This non-inferiority study was conducted in 21 centres in four European countries (Bulgaria, Czech Republic, Germany and Hungary). Included were healthy term infants up to 25 days of age from singleton pregnancies with a birth weight between the  $\geq$  3rd and  $\leq$  97th percentile for gestational age and without a family history of physician-diagnosed and treated atopic disease. The food business operator states that care was taken to ensure that the decision of the mother to breast-feed or not was not influenced by the investigators.

Eligible infants were randomised at the enrolment visit (V0) between the age of 0 and 25 days using a central randomisation system. Randomisation was stratified by sex and country and followed a

dynamic allocation procedure. An exclusively breast-fed, non-randomised reference group was also included. Study products, similar in appearance, were packaged in identical containers, which were dispensed in boxes of four containers each. Each box was labelled with a randomly assigned unique code. The blinding was maintained throughout the study. The unblinding for data analysis occurred after the lock of the database.

The primary outcome was weight gain from baseline (V1,  $30 \pm 7$  days of age) to V4 ( $120 \pm 7$  days of age). Secondary outcomes were weight gain at intermediate time points (V2  $60 \pm 3$  days of age, and V3  $90 \pm 3$  days of age), length gain, head circumference (HC) gain, body mass index (BMI) increase as well as weight, length, HC and BMI of infants as absolute values and as variable-for-age and weight-for-length z-scores (based on World Health Organization (WHO) child growth standards using the R 'anthro' package<sup>13</sup>). Other secondary outcomes were formula intake, energy and macronutrient intake, stool frequency, consistency, amount and colour, sleeping behaviour and gastrointestinal tolerance. Adverse events (AEs) and concomitant medications were also recorded.

Anthropometric measurements were taken according to standard operating procedures by trained personnel. Infants were weighed naked while lying on a calibrated scale. Length was measured to the nearest 0.1 cm using a length mat. HC was measured to the nearest 0.1 cm using an insertion tape. All measurements were performed in duplicate and recorded twice. If the difference between the two values was  $> 10$  g for weight and  $> 10$  mm for length and HC, the duplicate measurements were repeated. Measurements were performed at the study sites in 1,420 cases and in five cases (3 at V0, 1 at V1 and 1 at V4) at the caregivers' homes. Measurements at intermediate time points (V2 and V3) could, according to the protocol, in exceptional cases, also be performed by the parents under supervision of an investigator remotely connected via videocall, using calibrated equipment. This option was used on two occasions, for one infant at V2 and another one at V3.

Data on formula intake were collected using 3-day diaries filled in by caregivers before each study visit. This diary also contained questions on the consumption, if any, of energy or non-energy-containing liquids, complementary foods and formula other than the study formulae. Stool frequency and characteristics were assessed using the [REDACTED] and the sleeping behaviour using a modification of the [REDACTED] and the gastrointestinal tolerance using the [REDACTED].

Statistical analyses were preplanned and the statistical analysis plan was submitted by the food business operator. A linear mixed model (SAS PROC MIXED) was used to analyse mean daily weight gain between V1 (baseline) and V4 using infant formula group and sex as fixed factors and centre as random factor. Weight at V1 was used as a covariate. As sensitivity analyses, an unadjusted analysis was performed as well as an analysis that adjusted, in addition to the before mentioned factors/variables, for birth weight, gestational age, age and BMI of the mother at delivery and educational level of the mother. Mixed models were also used in the analyses of continuous secondary outcomes. A mixed Poisson regression model was used for ordinal variables (i.e. number, amount, consistency and colour of stool) and a mixed logistic ordinal model for data expressed as proportion (related to the outcome of gastrointestinal tolerance).

Sample size calculations were performed assuming an average weight gain per day of 29 g in both formula groups (i.e. 0 g difference) with an SD of 7 g. Using a one-sided t-test procedure, a non-inferiority margin of  $-3$  g/day, a type I error of 2.5% and a type II error of 20%, 87 infants were to be enrolled per formula group. Assuming a drop-out rate of 40%, it was calculated that 145 infants per formula group were needed.

Finally, 160 infants were randomised to the group consuming the formula manufactured from hydrolysed protein (HF group) and 158 to the formula manufactured from intact cow's milk protein (CF group). Additionally, 41 infants were included in the exclusively breast-fed reference group. Most formula-fed infants were recruited in Bulgaria ( $n = 298$ ), two were recruited in the Czech Republic, one in Germany and 17 in Hungary, while all exclusively breast-fed infants were from Bulgaria.

One infant in each formula group stopped the study before V1 (baseline) because of AEs. In addition, another infant was withdrawn during the study in the CF group because of an AE and an additional infant was lost to follow-up in this group.

The intention-to-treat (ITT) population was made up of all infants who were randomised (i.e. 160 in the HF group and 158 in the CF group). The full analysis set (FAS) consisted of all infants who participated in V1 and who had no major violation of the inclusion or non-inclusion criteria. In the FAS,

<sup>13</sup> <https://cran.r-project.org/web/packages/anthro/index.html>

the two infants who stopped the study before the baseline visit was excluded as well as one infant in the HF group who was already 26 days old when enrolled. This resulted in 158 infants in the HF group and 157 in the CF group in the FAS. In addition, from the per-protocol (PP) population, infants were excluded if they did not complete the study (two in the CF group), if they had consumed foods and/or drinks other than the study products (six infants in each group, if they were consuming non-energy-containing liquids (except one who had still received some breast milk at V1), if they had a study visit outside the window (one in the HF group) or not at the study site at V1 or V4 (one in the CF group; the infant with the second home visit performed in this study discontinued the study), if they did not meet the eligibility criteria (two in the HF group) and if they received corticosteroids (one in the HF group). Thus, the PP population was composed of 149 infants in the HF group and 148 infants in the CF group. In the exclusively breast-fed reference group, all infants completed the study without protocol violations.

The mean daily weight gain of infants between V1 and V4 in the PP population (primary analysis) was 28.95 (95% CI 27.21–30.68) g/day in the HF group and 28.85 (27.10–30.61) g/day in the CF group. The mean difference in weight gain was 0.09 g/day with the one-sided lower 97.5% CI being at 0.86 g/day when adjusted for sex and baseline weight (V1), thus confirming the non-inferiority assumption. This was also the case in the FAS and in the unadjusted and fully adjusted analyses both in the PP population and the FAS. There were no statistically significant differences in length gain

and HC gain (PP population) between formula groups. Growth in terms of z-scores was also not statistically significantly different between formula groups.

The Panel notes that a number of infants had weight-for-length z-scores of  $\leq -3$  at, at least one measurement time point, the number being similar between formula groups. Following the review of the individual data of these infants, the Panel considers that these low z-scores were likely a result of imprecise length measurements. In this context, the Panel notes that the deviations of individual anthropometric measures from the WHO standards were discussed by a blinded data quality committee and decisions regarding the handling of these values were made according to prespecified criteria and documented.

The weight gain observed in the formula-fed infants was similar to the weight gain in the exclusively breast-fed infants (29.2 (SD 4.1) g/day). However, the absolute weight of exclusively breast-fed infants was around 100 g below the weight of formula-fed infants both at V1 and V4. Length gain in exclusively breast-fed infants was higher than in formula-fed infants ( ). Length-for-age z-scores were higher in exclusively breast-fed infants than in formula-fed infants and weight-for-length z-scores lower.

Formula intake was comparable between the HF and the CF groups at V1 and V4 (PP population: ). There was a statistically significant difference in formula intake at V3, but this amounted to an average of around

There was no statistically significant difference between formula groups in stool frequency, consistency and amount. The HF group had a higher frequency of green stools compared with the CF group.

Gastrointestinal tolerance of the infant formula was not different between groups throughout the study, based on the total scores. Also, when individual scores of the were compared, no significant differences between formula groups were observed.

**Sleeping behaviour** was also not statistically significantly different between the HF and the CF groups.

AEs occurred in 3.8% (n = 6) of infants in the HF group and 2.5% (n = 4) of infants in the CF group in the FAS. Of these, one event in the CF group was considered definitely related to the study product (crying, fussing and rash), while all other events were considered to be unrelated to the formula consumed. In addition, one infant in each group had stopped the study before V1 (baseline) because of an adverse event (fussing and crying, and fussing and regurgitation). These infants were not included in the FAS and therefore not considered in the description above. The Panel notes that the incidence of AEs was similar in both groups and mostly unrelated to the study formulae.

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 0.45 g/100 kJ (1.9 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 the same protein content.

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 0.45 g/100 kJ (1.9 g/100 kcal) protein<sup>14</sup> 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.

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 0.45 g/100 kJ (1.9 g/100 kcal) protein<sup>14</sup> 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 the 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 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 0.45 g/100 kJ (1.9 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 assessment of the nutritional safety and suitability of a specific protein hydrolysate used in infant formula. July 2019. Submitted by meyer.science GmbH.

### Steps taken by EFSA

- 1) The technical dossier was received by EFSA on 17/04/2019.
- 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 16/04/2019.
- 3) The scientific evaluation procedure started on 18/07/2019.
- 4) On 30/09/2019, 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 11/10/2019 and was restarted on 11/12/2019.
- 5) On 14/01/2020, 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 04/02/2020 and was restarted on 21/12/2021.
- 6) During its meeting on 27/01/2022, 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 HIPP-Werk Georg Hipp OHG (dossier submitted by meyer.science GmbH).

<sup>14</sup> Calculated as Nx6.25.

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## Abbreviations

AE	adverse event
AN	amino nitrogen
AU	activity unit
BMI	body mass index
BRC	British Retail Consortium
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	carboxymethyllysine
Da	Dalton

DH	degree of hydrolysis
ELISA	enzyme-linked immunosorbent assay
FAS	full analysis set
FSSC	Food Safety System Certification
GMO	genetically modified organism
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
HC	head circumference
HF	formula manufactured from hydrolysed protein
HPLC	high performance liquid chromatography
ITT	intention-to-treat
NDA	Panel on Nutrition, Novel Foods and Food Allergens
OPA	o-phthaldialdehyde
PP	per protocol
RCT	randomised controlled trial
SD	standard deviation
SEC	Size Exclusion Chromatography
UV	ultra-violet
WHO	World Health Organization
WPC	whey protein concentrate
w/w	weight for weight