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Scientific Opinion on the safety and suitability for use by infants of follow-on formulae with a protein content of at least 1.6 g/100 kcal

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Abstract

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on the safety and suitability for use by infants of follow-on formulae (FOF) based on cow's milk intact protein with a protein content of at least 1.6 g/100 kcal (rounded value) that meet otherwise the requirements of relevant EU legislation. If the formula under evaluation is considered to be safe and suitable for use by infants, the NDA Panel is also asked to advise on whether FOF based on goat's milk intact protein, soy protein isolates or protein hydrolysates are also safe and suitable for infants under the same conditions. The Panel concludes that the use of FOF with a protein content of at least 1.6 g/100 kcal from either intact cow's milk protein or intact goat's milk protein otherwise complying with the requirements of relevant EU legislation is safe and suitable for healthy infants living in Europe with an intake of complementary foods of a sufficient quality. This conclusion does not apply to infant formula (IF). The Panel also concludes that the safety and suitability of FOF with a protein content of at least 1.6 g/100 kcal manufactured from either protein hydrolysates or soy protein isolates cannot be established with the available data. The same conclusion applies to IF. The NDA Panel endorsed a draft of this scientific opinion on 14 December 2016 for public consultation. The draft document has been revised and updated according to the comments received, where appropriate.

Keywords: protein, infants, follow-on formula, safety, suitability, growth

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Summary

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on the safety and suitability for use by infants of follow-on formulae (FOF) based on cow's milk intact protein with a protein content of at least 1.6 g/100 kcal (rounded value) that meet otherwise the requirements of relevant European Union (EU) legislation. If the formula under evaluation is considered to be safe and suitable for use by infants, the NDA Panel is also asked to advise on whether FOF based on goat's milk intact protein, soy protein isolates or protein hydrolysates are also safe and suitable for infants under the same conditions.

For the scientific assessment, the NDA Panel considered: (a) the dietary protein requirements of infants in the second half of the first year of life, (b) the protein content of breast milk during the first year of lactation, (c) dietary protein intake of infants in Europe from breast milk, formula and complementary food (CF), (d) the overall contribution that a FOF with a protein content of 1.6 g/100 kcal could make towards protein requirements in the target population, assuming an intake of CF of a sufficient quality, following established feeding guidelines in Europe (e.g. from Member States), and (e) the application submitted by the food business operator, including two intervention studies in healthy term infants.

The Panel notes that:

a) population reference intakes (PRIs) of 8–9 g protein per day for girls and 9–10 g protein per day for boys aged 6 months, and a PRI of 10–11 g protein per day for girls and 11–12 g protein per day for boys aged 12 months have been established;
b) the mean content of true protein in breast milk by the end of the third month of lactation ranges between 1.3 and 1.6 g/100 kcal, tends to decrease thereafter to about 1.1–1.4 g/100 kcal by the end of the fourth month, corresponding to about 1.6 g/100 kcal of total protein The mean total protein is about 1.1 g/100 mL (1.6 g/100 kcal) at 6 months of lactation and tends to remain fairly stable thereafter;
c) the 5th and the 2.5th percentiles (P5th and P2.5th, respectively) of total protein intake in non-breastfed infants aged 6–12 months living in Europe was around or above the PRI for protein for that age group in all the studies and surveys available;
d) the P5th and P2.5th of total protein intake resulting from the consumption of FOF with a protein content of 1.6 g/100 kcal would remain at about or above the PRI for protein for infants aged 6–12 months who are not breastfed;
e) the two randomised, double-blind, controlled intervention studies provided by the applicant showed no differences in growth patterns between healthy term infants who consumed formulae with total protein contents of 1.61 g/100 kcal and 1.65 g/100 kcal from 3 months of age onwards and infants who consumed formulae with total protein contents of 2.15 g/100 kcal and 2.70 g/100 kcal, respectively. The control formula used in these studies contained 0.35 g/100 kcal (US study) and 0.90 g/100 kcal (Chile study) more protein than the current minimum requirement for protein content of a FOF (1.8 g/100 kcal).

The Panel also notes that the studies submitted were not specifically designed to meet the regulatory definitions for either infant formula (IF) or FOF laid down in Regulation (EU) No 609/2013[1], and that the information provided in relation to the type and amount of CF was not sufficient to calculate total energy and protein intake, nor the relative contribution of formulae and CF to total energy and protein intake. Therefore, the Panel considers that these studies do not provide, on their own, sufficient information to conclude on the safety and suitability of a FOF with a total protein content of 1.6 g protein/100 kcal.

The Panel notes, however, that:

a) the true protein content of human milk tends to decrease with feeding time to about 1.1–1.4 g/100 kcal by the end of the fourth month of lactation, corresponding to about 1.6 g/100 kcal of total protein, and remains fairly stable thereafter;

[1] 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. 35–56.

b) the P5th and P2.5th of protein intake from all sources (breast milk, formula and CF) in European infants between 6 and 12 months of age are at or above the PRI for protein for that age group;
c) the P5th and P2.5th of protein intake from all sources (formula and CF) in European infants between 6 and 12 months of age who are not breastfed would remain at or above the PRI for protein for that age group by assuming a protein content of 1.6 g/100 kcal in all FOF;
d) the two human intervention studies provided by the applicant did not show an adverse impact on growth resulting from the use of a formula containing about 1.6 g of protein/100 kcal as compared to control formulae containing 2.15 or 2.70 g of protein/100 kcal or the breastfed reference group.

Therefore, the Panel concludes that the use of FOF with a protein content of at least 1.6 g/100 kcal from intact cow's milk protein otherwise complying with the requirements of relevant EU legislation is safe and suitable for healthy infants living in Europe with an intake of complementary foods of a sufficient quality. This conclusion does not apply to IF.

On the basis of:

a) a previous evaluation by the Panel on the safety and suitability of goat's milk protein as a source of protein in IF and FOF (EFSA NDA Panel, 2012b), and
b) the Panel's conclusions regarding the safety and suitability of FOF with a protein content of at least 1.6 g/100 kcal from intact cow's milk protein otherwise complying with the requirements of relevant EU legislation,

the Panel concludes that the use of FOF with a protein content of at least 1.6 g/100 kcal from intact goat's milk protein otherwise complying with the requirements of relevant EU legislation is safe and suitable for healthy infants living in Europe with an intake of complementary foods of a sufficient quality. This conclusion does not apply to IF.

The Panel considers, however, that the safety and suitability of each FOF (and IF) manufactured from protein hydrolysates have to be established by clinical evaluation in the target population (EFSA NDA Panel, 2014). The Panel also considers that, given the higher minimum protein requirements established for FOF (and IF) manufactured from soy protein isolates (i.e. 2.25 g/100 kcal) and the lack of data available on the use of FOF from soy protein isolates in the target population, additional studies are required to establish the safety and suitability of FOF manufactured from soy protein isolates with a protein content of at least 1.6 g/100 kcal. Therefore, the Panel concludes that the safety and suitability of FOF with a protein content of at least 1.6 g/100 kcal manufactured from either protein hydrolysates or soy protein isolates cannot be established with the available data. The same conclusion applies to IF.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background

Commission Directive 2006/141/EC[2] lays down requirements for infant formulae and follow-on formulae placed on the market in the European Union (EU). Among others, it establishes that follow-on formula manufactured from cows' milk intact protein shall contain at least 1.8 g protein/100 kcal (Annex II, point 2.1).

Commission delegated Regulation (EU) 2016/127[3] revises the rules of Commission Directive 2006/141/EC and shall repeal and replace the Directive from 22 February 2020. Annex II, point 2.1 of the delegated Regulation maintains the minimum protein content of follow-on formula manufactured from cows' milk intact protein at 1.8 g/100 kcal.

The Commission has received a request from a food business operator for placing on the market a follow-on formula based on cow's milk intact protein with a protein content of at least 1.61 g/100 kcal, which is below the permitted levels of Directive 2006/141/EC and delegated Regulation (EU) 2016/127. In order to consider such request, the Commission needs to obtain the advice of the European Food Safety Authority (EFSA) and has asked the food business operator to send the scientific dossier to the Authority for assessment.

1.1.2. Terms of Reference

In accordance with Article 29 of Regulation (EC) No 178/2002[4], the European Commission requests EFSA to issue an opinion on the safety and suitability for use by infants of a follow-on formula (FOF) based on cow's milk intact protein with a protein content of at least 1.61 g/100 kcal.

If the formula under evaluation is considered to be safe and suitable for use by infants, EFSA is asked to advise whether a level of at least 1.61 g protein/100 kcal would be applicable to all FOFs. If this is not the case, the Authority is asked to advise on the specific criteria that need to be satisfied for the safety and suitability of such formulae to be demonstrated.

1.2. Interpretation of the Terms of Reference

The Panel interprets the terms of reference provided by the European Commission in the context of the background information given and the application submitted. The Panel understands that the European Commission seeks advice on:

a) whether a FOF based on cow's milk intact protein with a minimum protein content of 1.6 g/100 kcal (rounded value) is safe and suitable for infants provided that it meets otherwise the requirements[5] of relevant EU legislation,[6] and if so
b) whether FOF based on goat's milk intact protein, soy protein isolates or protein hydrolysates with a minimum protein content of 1.6 g/100 kcal (rounded value) are also safe and suitable for infants provided that they meet otherwise the requirements of relevant EU legislation.

[2] 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.
[3] 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.
[4] 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.
[5] Including the requirements with respect to the amino acid profile.
[6] Directive 2006/141/EC to be replaced by delegated Regulation (EU) 2016/127.

2. Data and methodologies

2.1. Data

EFSA was provided with a dossier related to a FOF based on cow's milk intact protein containing a minimum of 1.61 g protein/100 kcal but otherwise complying with the compositional criteria laid down in Directive 2006/141/EC. The dossier includes two intervention studies in infants, named 'US study' (Hayes and Northington, 2014; unpublished study report #1; published as Ziegler et al., 2015), and 'Chile study' (Yao, 2014; unpublished study report #2; published as Inostroza et al., 2014). The dossier was supplemented, upon request of EFSA, with additional information provided by the applicant on 28 June 2016 and on 29 September 2016. The intervention studies provided in the dossier were designed to assess the growth pattern of infants receiving a formula with standard protein content for the first three months of life and thereafter a formula with a protein content which is lower than currently authorised. These studies aimed to investigate whether lower protein content in formula to be fed from 3 to 12 months of age, in line with the decrease in the protein content of breast milk during that feeding period, would lead to growth rates closer to those of breastfed infants, as compared to infants fed a 'standard' formula.

The Panel will also take into account in the current assessment its previous opinions on Dietary Reference Values for protein (EFSA NDA Panel, 2012a), on nutrient requirements and dietary intake of infants and young children in Europe (EFSA NDA Panel, 2013), and on the essential composition of IF and FOF (EFSA NDA Panel, 2014), as well as data on the protein content of breast milk.

2.2. Methodologies

As outlined in the Panel's previous opinion on the essential composition of infant formula (IF) and FOF (EFSA NDA Panel, 2014), the minimum amounts of nutrients in formulae, including protein, should be based on generally accepted scientific evidence. While for IF compositional requirements may be based on the energy and nutrient requirements of infants and on the results of intervention studies in the target population in which the formula is the only source of energy and nutrients, evidence for proposing compositional requirements for foods which are not the sole source of energy and nutrients, such as FOF, is less strong, as other foods contribute to nutrient and energy intake in variable amounts. In its previous opinion, when proposing compositional requirements for FOF, the Panel assumed that complementary food (CF) would compensate for the higher energy and nutrient requirements of older infants and for the lower formula intake during that period. This is based on the assumption that infants in the target population have access to CF of a sufficient quality, following established feeding guidelines in Europe (e.g. from Member States).

For the present assessment of whether a FOF based on cow's milk intact protein with a protein content of at least 1.6 g/100 kcal (rounded value) is safe and suitable for infants provided that it meets otherwise the requirements[5] of relevant EU legislation,[6] the Panel will consider:

a) dietary protein requirements of infants in the second half of the first year of life;
b) protein content in breast milk during the first year of lactation;
c) dietary protein intake of infants in Europe from breast milk, formula and CF;
d) the overall contribution that a FOF with a protein content of 1.6 g/100 kcal could make towards protein requirements in the target population, assuming an intake of CF of a sufficient quality, following established feeding guidelines in Europe (e.g. from Member States);
e) the application submitted by the food business operator, including two intervention studies in healthy term infants.

The evaluation of the intervention studies provided by the food business operator will follow the general principles for the assessment of a modification of the composition of IFs or FOFs outside the established standards as laid down by the Scientific Committee on Food (SCF, 2003). In addition, the recommendations for the assessment of the safety and suitability of formulae for term infants of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) (Aggett et al., 2001), of the Committee on the Evaluation of the Addition of Ingredients New to Infant Formula of the Food and Nutrition Board of the United States (US) Institute of Medicine (IoM, 2004) and of the American Academy of Pediatrics (AAP, 1988), will be taken into account.

3. Assessment

3.1. Dietary protein requirements of infants in the second half of the first year of life

Dietary protein is an essential component of the diet, supplying the body with nitrogen (N) and amino acids as well as other non-protein metabolically active nitrogen-containing substances. The protein requirement of infants and young children comprises two components, the maintenance requirement and the growth requirement (EFSA NDA Panel, 2012a). In its previous opinion, the Panel established an average maintenance requirement of 0.66 g protein/kg body weight per day (105 mg N/kg body weight per day) for infants and young children aged from 6 to < 36 months, which was derived from nitrogen balance studies in adults. The average protein requirement for growth was estimated from average daily rates of protein deposition calculated from studies on whole-body potassium deposition, and adjusted by an efficiency of utilisation of dietary protein for growth of 58%. Together, these amounts constitute an average requirement (AR), to which 1.96 standard deviations were added to derive a population reference intake (PRI).

Using the 50th percentile of the reference body weights (kg) of European children (van Buuren et al., 2012), a PRI of 9 g protein per day for girls and 10 g protein per day for boys aged 6 months and a PRI of 11 g protein per day for girls and 12 g protein per day for boys aged 12 months were established (EFSA NDA Panel, 2012a). The use of the 50th percentile of the WHO Growth Standards (WHO Multicentre Growth Reference Study Group, 2006) as reference weights resulted in slightly lower PRIs for protein (in g/day) for the same age and sex groups, i.e. 8 g protein per day for girls and 9 g protein per day for boys aged 6 months and a PRI of 10 g protein per day for girls and 11 g protein per day for boys aged 12 months (EFSA NDA Panel, 2013).

3.2. Protein content of breast milk during the first year of lactation

Estimating the true protein content of breast milk is challenging because of the non-protein nitrogen fraction contained in it. Total nitrogen in human milk represents both protein, about 75%, and non-protein nitrogen, which is made up of urea (up to 50% of the non-protein nitrogen), amino acids and other nitrogen-containing compounds (SCF, 2003; FAO/WHO/UNU, 2007). The amount of nitrogen used by infants for protein synthesis is likely to include that from true protein, free amino acids and small peptides, and a proportion of urea nitrogen. Therefore, the amount of nitrogen in breast milk used for protein synthesis by infants is between the true protein content and the crude (total) protein calculated from total nitrogen.

In a meta-analysis of 21 studies reporting on energy and macronutrient composition of breast milk from mothers of healthy singleton infants born at term and who were exclusively breast fed at the time of breast milk sampling (Hester et al., 2012), crude (total) protein content expressed as mean (range) was 2.5 (1.4–6.5) g/100 mL (3.8 (2.2–10.0) g/100 kcal, n = 433) for colostrum (1–5 days); 1.7 (1.3–2.5) g/100 mL (2.6 (2.0–3.8) g/100 kcal, n = 308) for transitional milk (6–14 days); and 1.3 (0.8–2.1) g/100 mL (2.0 (1.2–3.2) g/100 kcal, n = 415) for mature human milk (> 14 days).

A meta-analysis of 41 published studies reports on the composition of preterm (26 studies, 843 mothers) and term (30 studies, 2,299 mothers) breast milk during the first 12 weeks of lactation (Gidrewicz and Fenton, 2014). Energy was estimated in 11 studies using bomb calorimetry, and in five studies by calculation using values for the energy contributions from fat, protein and carbohydrate. Protein was estimated based on total nitrogen in 23 studies and as a true protein estimate in 15 studies. Data on mean energy and protein content of breast milk from mothers of term infants by week of lactation is shown in **Table 1**.

Table 1: Mean energy and protein content of breast milk from mothers of term infants by week of lactation[(a)]

Time	Mean energy (SD) (kcal/100 mL)		Mean protein (SD) (g/100 mL)		Mean protein (SD) (g/100 kcal)	
	Bomb calorimetry	Calculated	True protein	Protein calculated from total nitrogen	True protein	Protein calculated from total nitrogen
4–7 days	66 (9)	68 (10)	1.6 (0.3)	2.0 (0.5)	2.4 (0.5)	2.9 (0.7)–3.0 (0.8)
2 week	66 (9)	–	1.3 (0.2)	1.8 (0.4)	2.0 (0.3)	2.7 (0.63)
3–4 week	66 (8)	70 (9)	1.1 (0.2)	1.5 (0.3)	1.6 (0.3)–1.7 (0.3)	2.1 (0.4)–2.3 (0.5)
5–6 week	63 (7)	–	1.0 (0.1)	1.1 (0.2)	1.6 (0.2)	1.7 (0.3)
7–9 week	63 (7)	69 (10)	0.9 (0.1)	1.3 (0.2)	1.3–1.4 (0.2)	2.1 (0.3)
10–12 week	63 (8)	68 (9)	1.0 (0.1)	1.2 (0.2)	1.5–1.6 (0.2)	1.8 (0.3)–1.9 (0.3)

(a): Adapted from Gidrewicz and Fenton (2014).

The true protein content of breast milk gradually decreased from the first week of lactation, being about 1.0 g/100 mL (corresponding to about 1.5–1.6 g/100 kcal) by the third month, which corresponds to 1.2 g/100 mL (1.8–1.9 g/100 kcal) of total protein. The amount of total protein (calculated from total nitrogen) in the second week of lactation was comparable to that reported for transitional breast milk (6–14 days) in the meta-analysis by Hester et al. (2012).

Table 2 shows the energy (calculated) and the interval from birth according to the true protein content (measured by infrared spectroscopy) of breast milk samples (n = 2,554) donated by 224 mothers of mostly term infants to a milk bank in Denmark (Michaelsen et al., 1990). The mean true protein content of all samples combined was 0.9 g/100 mL.

Table 2: Time interval from birth according to the true protein content of breast milk[(a)]

True protein (g/100 mL)	Mean interval from birth (weeks)	Mean (SD) energy (kcal/100 mL)	True protein (g/100 kcal)	No. of samples
≥ 1.3	3–4	74.7 (9.8)	≥ 1.7	70
1.1–1.29	6–7	71.4 (9.8)	1.5–1.8	193
0.9–1.09	11–12	67.4 (9.8)	1.3–1.6	572
0.7–0.89	15–16	64.6 (9.8)	1.1–1.4	800
< 0.7	19–20	64.2 (9.3)	< 1.1	108

(a): Adapted from Michaelsen et al. (1990).

The mean interval from birth gradually decreased with the increasing content of protein in breast milk. It was 3–4 weeks for samples containing ≥ 1.3 g/100 mL and 19–20 weeks for samples containing < 0.7 g/100 mL. The mean interval from birth was 11–12 weeks for samples containing 0.9–1.09 g/100 mL, which is consistent with the true protein content of breast milk by the third month of lactation (1.0 g/100 mL) reported by Gidrewicz and Fenton (2014).

A meta-analysis of 26 studies reporting on the protein content and protein composition of breast milk during the first year of life has also been published (Lönnerdal et al., 2017). In this meta-analysis, only studies reporting on true protein data (total nitrogen – non-protein nitrogen, with a 6.25 conversion factor) obtained using the Kjeldahl, Lowry, Biuret and bicinchoninic acid kits were included. The 26 articles provided 130 data points, 70% of which were for samples obtained during the first three weeks of lactation. The Panel notes that the number of breast milk samples analysed in studies reporting on the protein content of breast milk during the first year of lactation is low (e.g. Allen et al., 1991; Mitoulas et al., 2002).

Data on the true protein content of breast milk by month of lactation is reported as a median of 2.06 g/100 mL for colostrum (0–5 days after delivery), of 1.57 g/100 mL for mature milk (16–30 days) and of 1.1 g/100 mL for breast milk samples from 3 to 12 months of lactation on average. A linear regression analysis is provided in a figure considering all samples (mean and 95% CI of true protein content over the first year). The Panel notes that the mean true protein content of breast milk was about 1.4 g/100 mL at 3 months, 1.2 g/100 mL at 4 months, 1.1 g/100 mL at 6 months and about

1.0 g/100 mL at 8–12 months of lactation. The Panel also notes that the energy content of breast milk is not reported, and thus the true protein content per 100 kcal cannot be calculated.

In the context of the DARLING study, Nommsen et al. (1991) assessed the composition of breast milk in samples taken at 3, 6, 9 and 12 months of lactation in healthy mothers of term infants. The gross energy content and the total protein content of the breast milk samples are given in **Table** 3. Protein was analysed using a modified Lowry assay with bovine serum albumin as the standard, a method which tends to result in slightly elevated values for total protein (Nommsen et al., 1991).

Table 3: Gross energy and total protein content of breast milk during the first year of lactation[(a)]

Month of lactation	n	Mean (SD) gross energy (kcal/100 mL)	Mean (SD) total protein (g/100 mL)	Mean (SD) total protein (g/100 kcal)
3	58	69.7 (97)	1.2 (0.2)	1.7 (1.5)
6	45	70.7 (92)	1.1 (0.2)	1.6 (1.6)
9	28	70.9 (74)	1.2 (0.8)	1.6 (10.8)
12	21	70.6 (110)	1.2 (0.2)	1.7 (1.3)

(a): Adapted from Nommsen et al. (1991).

The mean total protein at three months of lactation (1.2 g/100 mL, 1.7 g/100 kcal) is comparable to the total protein content for that time period in the meta-analysis by Gidrewicz and Fenton (2014) (1.2 g/100 mL, 1.8 g/100 kcal). The mean total protein was 1.1 g/100 mL (1.6 g/100 kcal) at 6 months of lactation and remained fairly stable until the 12th month, which is consistent with the stable content of true protein in breast milk from the 6th to the 12th month of lactation reported in the meta-analysis by Lönnerdal et al. (2017).

The Panel notes that the mean content of true protein in breast milk by the end of the third month of lactation ranges between 1.3 and 1.6 g/100 kcal, tends to decrease thereafter to about 1.1–1.4 g/100 kcal by the end of the fourth month, corresponding to about 1.6–1.7 g/100 kcal of total protein. The mean total protein is about 1.1 g/100 mL (1.6 g/100 kcal) at 6 months of lactation and tends to remain fairly stable thereafter.

3.3. Dietary protein intake of infants in Europe

Data on mean energy and protein intake in infants living in Europe were gathered from published studies (**Table 4**). Details about the dietary data collection and on the assessment of breast milk intake are given in **Table 5**.

Data on mean energy and protein intake in infants living in Europe were also gathered form dietary surveys for which sufficient data were available in the EFSA Comprehensive European Food Consumption Database (**Table 6**).[7]

From the dietary surveys available in the EFSA Comprehensive European Food Consumption Database, mean energy and protein intake from formula and from CF in non-breastfed infants, and from CF only in (exclusively or partially) breastfed infants were also calculated (**Table 7**). Mean energy and protein intake by food group in non-breastfed infants are given in **Tables 8** (for infants 4 to < 6 months of age) and **9** (for infants aged ≥ 6 to 12 months).

[7] Details about the dietary surveys included in the EFSA Comprehensive Database are available at: https://dwh.efsa.europa.eu/bi/asp/Main.aspx?rwtrep=001

Table 4: Mean energy and protein intake in infants living in Europe from published studies

Age	Country	Study	Breastfeeding[a] (%)	N	Mean energy (SD)[b] (kcal/day)	Mean protein (SD)[b] (g/day)	P5th of protein intake[c]	P2.5th of protein intake[d]	Mean E% as protein[b]
4 month	UK	ALSPAC	All males	262	658 (123)	15.7 (3.4)	10.1	9.0	9.5
			All females	214	604 (118)	14.5 (3.3)	9.04	8.0	9.6
			100, no solids	53	626 (–)	12 (–)	–	–	7.6
			0, no solids	42	583 (–)	13 (–)	–	–	8.9
			100, solids	209	646 (–)	13 (–)	–	–	8.1
			0, solids	441	640 (–)	15 (–)	–	–	9.4
			Mixed, solids	107	667 (–)	14 (–)	–	–	8.4
6 month	France	National	0	58	668	20.0 (5.60)	10.7	–	11.9
	UK	Southampton	28	50	709 (652–818)[e]	19.7 (17.4–23.6)[e]	–	–	11.1[g]
	Germany	DONALD	48	302	645 (119)	16.3 (5.4)	7.4	5.7	10.1
	Belgium	CHOP	0	746	679 (659–702)[f]	19 (17–20)[f]	–	–	11.2
	Germany				634 (615–655)[f]	19 (18–20)[f]	–	–	12.0
	Italy				731 (710–753)[f]	23 (22–24)[f]	–	–	12.6
	Poland				725 (702–743)[f]	21 (20–22)[f]	–	–	11.6
	Spain				736 (719–754)[f]	18 (18–19)[f]	–	–	10.0
8 month	Belgium	CHOP	0	625	734 (707–761)[f]	24 (22–25)[f]	–	–	13.1
	Germany				694 (670–718)[f]	20 (19–22)[f]	–	–	11.5
	Italy				817 (796–838)[f]	28 (27–28)[f]		–	13.7
	Poland				812 (783–840)[f]	25 (24–26)[f]	–	–	12.3
	Spain				854 (831–878)[f]	30 (29–32)[f]	–	–	14.1
	Finland	STRIP	0	215	842 (148)	25.0 (6)	15.1	13.2	11.9
	UK	ALSPAC	0	618 m	840 (173)	29.0 (9.0)	14.1	11.4	13.8
				513 f	784 (156)	27.0 (8.0)	13.8	11.3	13.8

Age	Country	Study	Breastfeeding[a] (%)	N	Mean energy (SD)[b] (kcal/day)	Mean protein (SD)[b] (g/day)	P5th of protein intake[c]	P2.5th of protein intake[d]	Mean E% as protein[b]
9 month	Belgium	CHOP	0	617	770 (739–801)[f]	24 (22–25)[f]	–	–	13.5
	Germany				708 (684–731)[f]	20 (19–22)[f]	–	–	11.9
	Italy				850 (826–874)[f]	28 (27–28)[f]	–	–	14.1
	Poland				859 (850–892)[f]	25 (24–26)[f]	–	–	12.6
	Spain				872 (846–899)[f]	30 (29–32)[f]	–	–	14.7
	Iceland	1995–1996	37	80	760 (678–859)[e]	28.0 (21.7–35.3)[e]	–	–	14.4[g]
		2005	41	154	754 (629–859)[e]	22.7 (17.8–27.6)[e]	–	–	11.9[f]
	Netherlands	TNO	–	333	970 (175)	28.8 (6.2)	18.6	16.6	11.9
	Germany	DONALD	17	332	759 (122)	22.4 (5.3)	13.6	12.0	11.8
10–12 month	France	National	0	63	826 (160)	30.0 (11)	11.8	–	14.4

(a): Either exclusive or partial breastfeeding.
(b): Unless otherwise noted.
(c): Calculated from the mean and the SD assuming a normal distribution of intake.
(d): Calculated only for sample sizes n ≥ 180.
(e): Median (interquartile range).
(f): Mean (95% confidence interval).
(g): Median; – = not reported or not available; m = males; f = females.

Table 5: Methods for dietary assessment, estimation of breast milk intake and food composition databases used in published studies

Country	Study	Age (months)	Publications	Dietary assessment	Breast milk intake
France	National	6, 10–12	Fantino and Gourmet (2008)	3-day weighed-DR	
Germany	DONALD	3, 6	Hilbig and Kersting, (2006)	3-day weighed-DR	Measured by 'test-weighing' the infant before and after each breast milk meal
UK	ALSPAC	4	Noble and Emmett (2006)	24-h recall	Duration of each breastfeeding was used to estimate the volume of milk consumed; a feed lasting ≥ 10 min was assumed to be 125 mL, or a proportion of this if the feed was of shorter duration (i.e. 12.5 mL for 1 min)
UK	Southampton	6	Marriott et al. (2008)	4-day weighed-DR	Estimated using an algorithm based on length of suckling derived from published intake data
5 EU countries	CHOP	6–12	Damianidi et al. (2016)	3-day weighed-DR	–
Finland	STRIP	8	Lagström et al. (1997)	3-day DR	–
UK	ALSPAC	8	Noble and Emmett (2001)	3-day DR	–
Iceland	1995–1996	9	Thorisdottir et al. (2013)	2-day or 3-day weighed DR	Measured by 'test-weighing' the infant before and after each breast milk meal
	2005				
Netherlands	TNO	9	de Boer et al. (2006)	2-day DR	NR
Germany	DONALD	9	Schwartz et al. (2010)	3-day weighed-DR	Measured by 'test-weighing' the infant before and after each breast milk meal

DR = Dietary records; NR = not reported.

Table 6: Mean energy and protein intake in infants living in Europe from the EFSA Comprehensive European Food Consumption Database

Age	Breastfeeding[a]	Country	Survey	Dietary assessment	N	Mean energy intake (kcal/day)	Mean protein intake (g/day)	P5th of protein intake (g/day)	P2.5th of protein intake (g/day)	Mean E% as protein
4 to < 6 months	Yes	Bulgaria	NUTRICHILD	24-h recall, 3-day[(b),(e)]	64	679	11.2	6.9	–	7
		Denmark	IAT 2006_07	7-day DR[(c),(f)]	26	746	15.9	–	–	9
		UK	DNSIYC_2011	4-day DR[(d),(g)]	27	686	15.0	–	–	9
	No	Bulgaria	NUTRICHILD	24-h recall, 3-day	88	639	16.3	9.7	–	10
		Denmark	IAT 2006_07	7-day DR	12	745	18.2	–	–	10
		UK	DNSIYC_2011	4-day DR	49	639	15.9	–	–	10
≥ 6 to 12 months	Yes	Bulgaria	NUTRICHILD	24-h recall, 3-day[(b),(e)]	89	905	22.2	8.3	–	10
		Denmark	IAT 2006_07	7-day DR[(c),(f)]	315	832	23.0	10.1	8.9	11
		UK	DNSIYC_2011	4-day DR[(d),(g)]	264	804	24.5	13.0	12.0	12
	No	Bulgaria	NUTRICHILD	24-h recall, 3-day	343	859	27.4	14.1	11.9	13
		Denmark	IAT 2006_07	7-day DR	473	933	30.0	16.2	14.7	13
		UK	DNSIYC_2011	4-day DR	1,029	790	25.2	13.0	11.7	13

– = P5th and P2.5th of protein intake are only provided for study groups with a sample size ≥ 60 and 180 individuals, respectively. DR = dietary records.
(a): Either exclusive or partial breastfeeding.
(b): Method to estimate volume of breast milk intake per feeding occasion not reported, assumed to be 130 mL per feeding occasion.
(c): Volume of breast milk per feeding occasion calculated as follows (Dewey et al., 1984): 130 mL per breastfeeding if the infant was breastfed six times or more per day; 89 mL per breastfeeding if the infant was breastfed 3–5 times per day; 53 mL per breastfeeding if the infant was breastfed 1–2 times per day.
(d): Volume of breast milk per feeding occasion calculated based on the time for each feed, at 13.5 g/min with a maximum of 135 g per feed for infants aged 4–7 months and at 10 g/min with a maximum of 100 g per feed for infants aged 8–12 months.
(e): Breast milk assumed to contain 70.0 kcal/100 mL; 1.0 g of protein/100 mL and 1.4 g of protein/100 kcal.
(f): Breast milk assumed to contain 71.0 kcal/100 mL; 1.3 g of protein/100 mL and 1.8 g of protein/100 kcal.
(g): Breast milk assumed to contain 67.0 kcal/100 mL; 1.3 g of protein/100 mL and 1.9 g of protein/100 kcal.

Table 7: Mean energy and protein intake from formula and complementary food in infants from surveys in the EFSA Comprehensive European Food Consumption Database

Age	Breastfeeding[a]	Country	N[b]	Food group	Mean energy intake (kcal/day)	Mean protein intake (g/day)	Mean protein intake (g/100 kcal)[c]
4 to < 6 months	Yes	Bulgaria	64	CF	210	4.1	–
		Denmark	26	CF	255	6.0	–
		UK	27	CF	211	3.7	–
	No	Bulgaria	88 (62)	Formula[d]	298	6.5	2.18
				CF	341	9.8	–
		Denmark	12 (12)	Formula	495	12.0	2.42
				CF	250	6.2	–
		UK	49 (49)	Formula	493	10.9	2.21
				CF	146	6.0	–
≥ 6 to 12 months	Yes	Bulgaria	89	CF	554	17.3	–
		Denmark	315	CF	540	17.0	–
		UK	264	CF	405	16.6	–
	No	Bulgaria	343 (165)	Formula	91	2.2	2.42
				CF	749	24.8	–
		Denmark	473 (398)	Formula	225	6.1	2.71
				CF	708	23.9	–
		UK	1,029 (986)	Formula	349	7.4	2.12
				CF	441	17.6	–

CF = Complementary food.
(a): Either exclusive or partial breastfeeding.
(b): For non-breastfed infants, the number of infants consuming formula is indicated in parenthesis.
(c): Calculated from mean energy and protein intake from formula.
(d): Any (infant and follow-on) formula.

Table 8: Mean energy and protein intake by food group in non-breastfed infants aged 4–6 months

Food group	Bulgaria (n = 88)		Denmark (n = 12)		UK (n = 49)	
	Mean energy (kcal/day)	Mean protein (g/day)	Mean energy (kcal/day)	Mean protein (g/day)	Mean energy (kcal/day)	Mean protein (g/day)
Animal and vegetable fats and oils	8.6	0.0	27.4	0.0	1.0	0.0
Composite food (including frozen products)	0.2	0.0	0.0	0.0	0.4	0.0
Eggs and egg products	3.1	0.2	0.0	0.0	0.4	0.0
Fish and other seafood (including amphibians, reptiles, snails and insects)	0.0	0.0	0.4	0.1	0.7	0.1
Food for infants and small children	129.5	2.4	105.5	3.0	95.1	3.0
Fruit and fruit products	7.9	0.1	14.7	0.1	11.5	0.1
Fruit and vegetable juices	15.6	0.1	0.0	0.0	0.7	0.0
Grains and grain-based products	26.6	0.6	47.5	1.1	4.7	0.1
Herbs, spices and condiments	0.0	0.0	0.0	0.0	0.3	0.0
Infant formula and follow-up formula	298.0	6.5	495.2	12.0	493.4	10.9
Legumes, nuts and oilseeds	0.4	0.0	3.7	0.3	1.9	0.2
Meat and meat products (including edible offal)	7.3	0.6	1.0	0.1	2.3	0.4
Milk and dairy products	98.1	5.5	11.1	0.7	13.5	0.8
Snacks, desserts, and other foods	2.1	0.1	0.0	0.0	3.9	0.1
Starchy roots and tubers	9.0	0.2	27.8	0.6	5.0	0.1
Sugar and confectionary	31.6	0.0	4.7	0.0	0.7	0.0
Vegetables and vegetable products (including fungi)	1.2	0.0	5.9	0.3	3.7	0.2

Table 9: Mean energy and protein intake by food group in non-breastfed infants aged 6–12 months

Food group	Bulgaria (n = 343)		Denmark (n = 473)		UK (n = 1029)	
	Mean energy (kcal/day)	Mean protein (g/day)	Mean energy (kcal/day)	Mean protein (g/day)	Mean energy (kcal/day)	Mean protein (g/day)
Animal and vegetable fats and oils	70.4	0.0	87.0	0.0	15.8	0.0
Composite food (including frozen products)	1.3	0.1	0.0	0.0	19.2	0.9
Eggs and egg products	13.0	0.9	2.5	0.2	2.9	0.2
Fish and other seafood (including amphibians, reptiles, snails and insects)	1.3	0.2	7.9	1.0	7.2	0.8
Food for infants and small children	99.7	2.4	68.9	1.9	121.4	4.0
Fruit and fruit products	35.6	0.3	62.4	0.7	33.2	0.4
Fruit and vegetable juices	24.8	0.2	4.7	0.0	1.7	0.0
Grains and grain-based products	183.1	4.3	186.3	5.3	78.7	2.4
Herbs, spices and condiments	0.9	0.1	2.0	0.0	3.9	0.1
Infant formula and follow-up formula	90.8	2.2	225.1	6.1	349.0	7.4
Legumes, nuts and oilseeds	6.5	0.4	5.9	0.4	5.5	0.4
Meat and meat products (including edible offal)	48.3	5.2	49.4	3.7	21.9	2.9
Milk and dairy products	166.5	9.2	154.5	8.7	79.3	4.4
Snacks, desserts, and other foods	7.7	0.1	12.0	0.4	12.1	0.2
Starchy roots and tubers	24.0	0.6	34.8	0.8	20.9	0.4
Sugar and confectionary	50.6	0.0	17.1	0.0	5.9	0.1
Vegetables and vegetable products (including fungi)	14.3	0.6	12.5	0.6	10.4	0.5

Mean protein intakes from all sources were above the PRI for protein in all surveys from the EFSA Comprehensive Database for both breastfed and formula-fed infants aged 6–12 months (**Table 6**). Mean protein intake from all sources were also reported in published studies which accurately estimated breast milk intake (by weighing the infant before and after each breast milk meal) and/or which used more accurate methods for dietary assessment (3- or 4-day weighted dietary records). At 4 months of age, the lowest mean protein intake (12 g/day) was reported for infants exclusively breastfed in the ALSPAC cohort. Mean protein intakes were slightly higher (13 g/day) in breastfed infants who had already received some solid food. The lowest mean protein intake (16.3 g/day) for infants aged 6 months or older was reported in a German cohort (DONALD study), in which the proportion of breastfed infants was the highest (48%) among all the studies available (**Table 4**). Mean protein intakes from all sources were above the PRI for protein for infants aged 6–12 months in all the studies.

In the dietary surveys for which data on (exclusively or partially) breastfed infants and formula-fed infants were available separately (**Table 6**), mean protein intakes were systematically higher in formula-fed infants than in breastfed infants, as previously reported by others (Heinig et al., 1993). Breast milk was assumed to contain from 1.4 to 1.9 g of protein/100 kcal, depending on the survey. The lower mean protein intake reported for Bulgarian infants could be explained in part by the assumed lower protein content in breast milk (1.4 g/100 kcal). The protein content of formula ranged from 2.1 to 2.7 g of protein/100 kcal, depending on the survey and age category (**Table 7**). This is higher than the minimum protein content allowed by EU legislation (Directive 2006/141/EC and Commission delegated Regulation (EU) 2016/127) for (infant and follow-on) formula manufactured from intact cow's or goat's milk proteins (1.8 g/100 kcal).

In breastfed infants, mean protein intake from CF ranged between 3.7 and 6.0 g/day in infants aged 4 to < 6 months, and was already well beyond the PRI for protein in infants aged 6–12 months (about 17 g/day). In formula-fed infants aged 4–6 months, mean protein intake from formula ranged from 6.5 to 12.0 g/day, while mean protein intakes from CF were about 6 g/day. In Bulgaria, where mean protein intake from formula was the lowest (6.5 g/day), mean protein intake from CF was much higher (9.8 g/day), mostly coming from cow's milk and dairy products other than formula (**Table 8**). This is due to a replacement of IF with cow's milk (rather than with FOF) at the time of the introduction of CF. In formula-fed infants aged 6–12 months, mean protein intakes from CF were at or above the PRI in all countries. The contribution of formula to total protein intake varied widely, being lower in countries (Bulgaria and Denmark) with the highest protein intake from cow's milk and dairy products and from meat and meat products (**Table 9**).

Whenever the data available allowed doing so, the 5th and the 2.5th percentiles (P5th and P2.5th, respectively) of protein intake were calculated by assuming a normal distribution of protein intake data **(Table 4)** or extracted from individual data **(Table 6**). Otherwise, IQRs or 95% CI were considered (**Table 4**). The Panel notes that the P5th and P2.5th of total protein intake in non-breastfed infants aged 6–12 months was around or above the PRI for protein for that age group in all the studies (**Table 4**) and surveys **(Table 6)** available.

3.4. Contribution that a FOF with a protein content of at least 1.6 g/100 kcal could make towards protein requirements in the target population

Consumption of a FOF with a protein content of about 1.6 g/100 kcal would provide about 9 g of protein per day in the first months of complementary feeding (assuming an intake of about 500 mL/day) and about 4.5 g of protein per day (assuming an intake of about 250 mL/day) by the end of the first year of life. This is about 1 g and 0.5 g of protein less than the estimated intake from a formula containing a minimum of 1.8 g/100 kcal, as currently authorised. The Panel notes, however, that the protein content of (infant and follow-on) formula in the European surveys available (from 2.1 to 2.7 g of protein/100 kcal, **Table 7**) was higher than the minimum authorised.

Using individual data from the three surveys which were available in the EFSA Comprehensive Food Consumption Database, total protein intake in non-breastfed infants aged 6–12 months was recalculated by assuming that: (a) all FOF consumed by the infants contained 1.6 g of protein/100 kcal; (b) the energy content of the individual FOFs did not change; (c) protein intake from other sources (IF, CF) did not change. The mean, P5th and P2.5th of total protein intake under these conditions are shown in **Table 10**.

Table 10: Protein intake in European non-breastfed infants aged 6–12 months, assuming a protein content of FOF of 1.6 g/100 kcal

Country	N	Mean energy intake (kcal/day)	Mean protein intake (g/day)	P5th of protein intake (g/day)	P2.5th of protein intake (g/days)
Bulgaria	343	859	27.2	13.7	11.9
Denmark	473	933	29.4	15.8	13.8
UK	1,029	790	24.4	12.6	11.2

As expected, total protein intakes resulting from the consumption of FOF with a protein content of 1.6 g/100 kcal would be lower than those reported in the original surveys **(Table 6)**. The Panel notes, however, that the P5th and P2.5th of total protein intake would remain at about or above the PRI for protein for infants aged 6–12 months.

3.5. Application submitted by the food business operator

The applicant provided two human intervention studies aiming to investigate whether protein content in formula to be fed from 3 to 12 months of age that is closer to the protein content of breast milk during that feeding period would lead to growth rates more in line with those of breastfed infants, as compared to infants fed a 'standard' formula.

3.5.1. Composition of the formulae used in the two human intervention studies

The formulae investigated in the US and Chile studies contain a minimum of 1.61 g protein/100 kcal (1.61 and 1.65 g/100 kcal, respectively), calculated from total nitrogen analysis by the Kjeldahl method and using a nitrogen conversion factor of 6.25. The protein source is based on intact proteins derived from skimmed milk and a proprietary preparation of demineralised whey. The demineralised whey is obtained from modified caseinoglycomacropeptide (CGMP)-reduced sweet whey produced using a patented process (Patent No PCT/EP1998/003176). The whey preparation used in the formula has a CGMP content which is reduced by at least 85%. The whey protein-to-casein ratio of the final product is 60:40. The applicant indicated that the protein sources have been used in other FOF currently marketed by the applicant, and that the use of CGMP-reduced sweet whey has allowed for a lower protein content of the FOF, while still meeting the requirements of Directive 2006/141/EC with respect to the amino acid pattern. The energy content and the amount of carbohydrates, fat, vitamins and minerals also comply with the compositional requirements laid down in Directive 2006/141/EC.

The macronutrient composition of the intervention and control formulae used in the US and the Chile studies are outlined in **Table 11**.

Table 11: Macronutrient composition of study formulae in comparison to the compositional requirements for FOF manufactured from cow's or goat's milk proteins as laid down in Directive 2006/141/EC

	Unit	Directive 2006/141/EC	US study		Chile study	
			Intervention	Control	Intervention	Control
Energy	kcal/100 mL	60–70	67.2	64.6	62.8	65.6
Protein	g/100 kcal	1.8–3.5	1.61	2.15	1.65	2.70
Fat	g/100 kcal	4.0–6.0	5.46	5.21	5.30	5.03
Carbohydrates	g/100 kcal	9.0–14.0	11.10	11.13	11.41	10.98
Cyst(e)ine	mg/100 kcal	38	28	38	28	46
Histidine	mg/100 kcal	40	40	49	39	64
Isoleucine	mg/100 kcal	90	95	125	100	166
Leucine	mg/100 kcal	166	166	222	180	298
Lysine	mg/100 kcal	113	132	185	142	234
Methionine	mg/100 kcal	23	33	46	42	69
Phenylalanine	mg/100 kcal	83	103	88	108	179

	Unit	Directive 2006/141/EC	US study		Chile study	
			Intervention	Control	Intervention	Control
Threonine	mg/100 kcal	77	94	141	84	137
Tryptophan	mg/100 kcal	32	31	31	34	57
Tyrosine	mg/100 kcal	76	52	68	69	113
Valine	mg/100 kcal	88	94	137	102	168
Methionine + Cyst(e)ine	mg/100 kcal	61[(a)]	61	84	70	115
ratio			1.2	1.2	1.5	1.5
Tyrosine + Phenylalanine	mg/100 kcal	159[(b)]	155	156	177	292
ratio			0.5	0.8	0.6	0.6

(a): The concentrations of cyst(e)ine and methionine may be added together if the methionine:cyst(e)ine-ratio is not > 3.
(b): The concentrations of tyrosine and phenylalanine may be added together if the tyrosine:phenylalanine-ratio is not > 2.

The applicant states that the tyrosine and phenylalanine content (calculated as sum) and tryptophan content in the intervention and control formulae of the US study were slightly lower than required by Directive 2006/141/EC, and that the histidine content in the intervention formula of the Chile study was slightly lower than required by Directive 2006/141/EC, but that the final marketed product will comply with the specifications laid down in the Directive.

In the Chile study, the intervention formula also contained 2×10^7 colony forming units (CFU) *Bifidobacterium lactis* (CNCM I-3446) and 2×10^7 CFU *Lactobacillus rhamnosus* (CGMCC 1.3724) per gram of powder formula, while the control formula did not contain these bacteria.

The intervention formulae in both studies had a whey protein-to-casein ratio of 60:40.

3.5.2. Human intervention studies

The two randomised, double-blind, controlled intervention studies were conducted in Chile (Inostroza et al., 2014) and in the US (Ziegler et al., 2015). These studies assessed growth rates in healthy term infants who consumed (low-protein, intervention) formulae with protein contents of 1.61 g/100 kcal (n = 97) (US study) and 1.65 g/100 kcal (n = 89) (Chile study) from 3 months of age onwards, against those of infants who consumed (control) formulae with protein contents of 2.15 g/100 kcal (n = 97) and 2.70 g/100 kcal (n = 87), respectively, and against those of a breastfed reference group (n = 76 and n = 112, respectively). In the Chile study, only infants from overweight and obese mothers were recruited.

In both studies, the primary outcome was weight gain between 3 and 6 months of age. Secondary outcomes included, among others, weight gain at time points beyond 6 months of age, weight changes, changes in length and head circumference, and changes in serum albumin and blood urea nitrogen (BUN). Adverse events were registered.

Statistical analyses were conducted in completers and per protocol (PP) in both studies. In the US study, 10 infants in the intervention group, 10 infants in the control group and 7 infants in the breastfed reference group discontinued the study. The numbers in the Chile study were 23, 11 and 11, respectively. Reasons for withdrawal were provided.

Despite the original protocols foreseeing exclusive formula or breastfeeding up to the age of 6 months and the introduction of CF thereafter (control formula was allowed from 6 to 12 months to the breastfed reference groups, if desired), small amounts of CF were provided to some infants from around 4 months of age onwards. In the US study, a total of nine infants consumed > 4 teaspoons of CF per day before the age of 6 months and were excluded from the PP analysis. In the Chile study, CF in amounts > 4 teaspoons per day were introduced before 6 months of age in 66 infants (28 in the intervention, 24 in the control and 14 in the breastfed reference group), who were not excluded from the statistical analysis. In the breastfed reference group, one formula feeding per day was allowed from 3 months of age onwards in the Chile study. Supplemental formula feeding at parent's discretion in the US study (control formula) and discontinuation of breastfeeding at mother's discretion in the Chile study (commercially available formula with a protein content of 2.4 g/100 kcal and energy content of 67 kcal/100 mL) were allowed after 6 months.

The information provided in these studies did not allow the calculation of energy and protein intake from CF at any time point, and thus of the total energy and protein intake at time points in which infants consumed CF (4–12 months in the Chile study and 6–12 months in the US study).

Mean daily energy and protein intake from formula at different time points in the US and Chile studies are given in Table 12.

Table 12: Mean daily energy and protein intake from formula at different time points in the US and Chile studies

	n	Mean (SD) volume intake (mL/day)	Mean (SD) energy intake (kcal/day)	Mean (SD) protein intake (g/day)
US Study				
4 months				
Low-protein formula	83	905 (216)	608 (145)	9.8 (2.3)
Control formula	85	894 (180)	578 (116)	12.4 (2.5)
6 months				
Low-protein formula	83	917 (232)	616 (156)	9.9 (2.5)
Control formula	84	902 (184)	583 (119)	12.5 (2.6)
8 months				
Low-protein formula	80	850 (208)	571 (140)	9.2 (2.3)
Control formula	82	857 (179)	554 (116)	11.9 (2.5)
12 months				
Low-protein formula	76	719 (239)	483 (161)	7.8 (2.6)
Control formula	78	725 (241)	468 (156)	10.1 (3.3)
Chile study				
4 months				
Low-protein formula	75	820 (268)	515 (168)	8.5 (2.8)
Control formula	80	868 (228)	569 (150)	15.4 (4.0)
6 months				
Low-protein formula	62	980 (248)	615 (156)	10.2 (2.6)
Control formula	74	957 (172)	628 (113)	17.0 (3.0)
9 months				
Low-protein formula	55	896 (256)	563 (161)	9.3 (2.7)
Control formula	64	869 (242)	570 (159)	15.4 (4.3)
12 months				
Low-protein formula	47	854 (324)	536 (203)	8.8 (3.4)
Control formula	63	747 (217)	490 (142)	13.2 (3.8)

The mean volume of formula consumed by infants did not differ significantly between the low protein and the control groups at any time point in any of the studies. In this context, mean energy intake from formula was comparable between the two formula groups while mean protein intake was systematically higher in the control formula vs the low-protein formula groups in both studies, as per study design. The Panel notes that the mean volume intake of formula in the low-protein formula and control formula groups in both studies at 9 and 12 months of age was high.

The results of anthropometric measurements in the low-protein formula, control formula and breastfed reference groups in both studies are given in Appendices A–D. In both studies, weight gain (in g/day) was somewhat lower in the infants consuming the low protein formula than in infants consuming the control formula for the time period 3–6 and 6–12 months of age, but this difference only reached statistical significance for weight gain between 3 and 6 months in the Chile study. Other anthropometric measures (i.e. weight, length and head circumference at different time points in absolute values and as change from baseline) generally followed this pattern in both studies, although statistically significant differences between groups were not detected at any time point.

In the US study, both formula groups showed statistically significantly higher weight gain and higher weight and length in absolute values at different time points as compared to the breastfed reference group. In the Chile study, the low-protein formula group and the breastfed reference group

did not differ significantly in weight gain, weight and length. The concentrations of serum albumin and BUN remained within the normal range in all groups during the intervention in both studies. Reported adverse events were similar in the intervention and control groups.

The Panel notes that, in both studies, no differences in growth patterns were observed between infants in the control vs the low-protein formulae, including the time period of 3–6 months of age when the formula was fed almost exclusively, except for a lower weight gain in the low-protein formula group (time period 3–6 months) in the Chile study. In the US study, growth was higher in the low-protein groups as compared to the breastfed reference group, whereas in the Chile study, infants in the low-protein formula group had a similar growth pattern to breastfed infants. Mean weight-for-age z-scores were, at all ages, at or above the median of WHO Growth Standards. The control formula used in these studies contained 0.35 g/100 kcal (US study) and 0.90 g/100 kcal (Chile study) more protein than the current minimum requirement for protein content of a FOF (1.8 g/100 kcal). In both studies, at all-time points, the difference in mean protein intake from formula between the control formula group and the low-protein formula group was about 3 g/day or greater.

The Panel also notes that the studies submitted were not specifically designed to meet the regulatory definitions for either IF or FOF laid down in Regulation (EU) No 609/2013[1], and that the information provided in relation to the type and amount of CF was not sufficient to calculate total energy and protein intake, nor the relative contribution of formulae and CF to total energy and protein intake. Therefore, the Panel considers that these studies do not provide, on their own, sufficient information to conclude on the safety and suitability of a FOF with a protein content of 1.6 g protein/100 kcal.

3.6. Comparison between the human intervention studies provided and European dietary surveys with respect to mean energy and protein intake from formula and complementary food in the target population

In the European surveys which allowed calculation of mean energy and protein intake from both (infant and follow-on) formula and CF in formula-fed infants, the protein content of formula was between 2.1 and 2.7 g/100 kcal (Table 7). The lower end is close to the protein content of the control formula used in the US study (2.15 g/100 kcal) and the upper end is close to the protein content of the control formula used in the Chile study (2.70 g/100 kcal). Mean energy and protein intakes from formula were, however, lower in infants aged 4 to < 6 months in the European surveys than in infants at 4 months of age in the two formula groups (low protein and control) in both intervention studies (US and Chile studies). In infants aged 6–12 months, mean energy and protein intakes from formula in the European surveys were about half (or lower) than in the US and Chile studies. This suggests that the contribution of formula (vs CF) to total protein intake in the target population (infants at the time of the introduction of complementary feeding and up to 12 months of age) may be lower in Europe than in the intervention studies provided. Therefore, the impact on total protein intake of lowering the protein content of a follow-on formula to about 1.6 g/100 kcal would also be lower in Europe. However, direct comparisons regarding total energy and protein intake and energy and protein intake from CF between the European surveys and the intervention studies provided cannot be made.

4. Conclusions

4.1. On the safety and suitability for use by infants of FOF with a protein content of at least 1.6 g/100 kcal from intact cow's milk protein otherwise complying with the requirements of relevant EU legislation

The Panel considers that the two intervention studies provided by the applicant do not provide, on their own, sufficient information to conclude on the safety and suitability of FOF with a total protein content of 1.6 g protein/100 kcal.

The Panel notes, however, that:

a) the true protein content of human milk tends to decrease with feeding time to about 1.1–1.4 g/100 kcal by the end of the fourth month of lactation, corresponding to about

1.6–1.7 g/100 kcal of total protein. The mean total protein is about 1.1 g/100 mL (1.6 g/100 kcal) at 6 months of lactation and tends to remain fairly stable thereafter;

b) the P5th and P2.5th of protein intake from all sources (breast milk, formula and CF) in European infants between 6 and 12 months of age are at or above the PRI for protein for that age group;
c) the P5th and P2.5th of protein intake from all sources (formula and CF) in European infants between 6 and 12 months of age who are not breastfed would remain at or above the PRI for protein for that age group by assuming a protein content of 1.6 g/100 kcal in all FOF; and
d) the two human intervention studies provided by the applicant did not show an adverse impact on growth resulting from the use of a formula containing about 1.6 g of protein/100 kcal as compared to control formulae containing 2.15 or 2.70 g of protein/100 kcal or the breastfed reference group.

Therefore, the Panel concludes that the use of FOF with a protein content of at least 1.6 g/100 kcal from intact cow's milk protein otherwise complying with the requirements of relevant EU legislation is safe and suitable for healthy infants living in Europe with an intake of complementary foods of a sufficient quality. This conclusion does not apply to IF.

4.2. On the safety and suitability for use by infants of FOF with a protein content of at least 1.6 g/100 kcal from intact goat's milk protein, soy protein isolates or protein hydrolysates otherwise complying with the requirements of relevant EU legislation

On the basis of:

a) a previous evaluation by the Panel on the safety and suitability of goat's milk protein as a source of protein in IF and FOF (EFSA NDA Panel, 2012b), and
b) the Panel's conclusions regarding the safety and suitability of FOF with a protein content of at least 1.6 g/100 kcal from intact cow's milk protein otherwise complying with the requirements of relevant EU legislation (Section 4.1),

the Panel concludes that the use of FOF with a protein content of at least 1.6 g/100 kcal from intact goat's milk protein otherwise complying with the requirements of relevant EU legislation is safe and suitable for healthy infants living in Europe with an intake of complementary foods of a sufficient quality. This conclusion does not apply to IF.

The Panel considers, however, that the safety and suitability of each FOF (and IF) manufactured from protein hydrolysates have to be established by clinical evaluation in the target population (EFSA NDA Panel, 2014). The Panel also considers that, given the higher minimum protein requirements established for FOF (and IF) manufactured from soy protein isolates (i.e. 2.25 g/100 kcal) and the lack of data available on the use of FOF from soy protein isolates in the target population, additional studies are required to establish the safety and suitability of FOF manufactured from soy protein isolates with a protein content of at least 1.6 g/100 kcal. Therefore, the Panel concludes that the safety and suitability of FOF with a protein content of at least 1.6 g/100 kcal manufactured from either protein hydrolysates or soy protein isolates cannot be established with the available data. The same conclusion applies to IF.

Documentation provided to EFSA

Application for the placing on the market of a follow-on formula with a new minimum protein content of 1.61 g of protein/100 kcal. April 2016. Nestlé Nutrition.

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Abbreviations

AAP	American Academy of Pediatrics
AR	average requirement
BUN	blood urea nitrogen
CF	complementary food
CFU	colony forming units
CGMP	caseinoglycomacropeptide
DR	dietary records
E%	percentage of total energy intake
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
FAO	Food and Agriculture Organization of the United Nations
FOF	follow-on formula
IF	infant formula
IoM	Institute of Medicine
IQR	Inter-quartile range
NDA	Dietetic Products, Nutrition and Allergies
P2.5th	percentile 2.5
P5th	percentile 5
PP	per protocol
PRI	population reference intake
SCF	Scientific Committee on Food
WHO	World Health Organization

Appendix A – Absolute weight, weight gain and weight change in the 'US study'

	n	Intervention Mean ± SD	n	Control Mean ± SD	n	Breastfed Mean ± SD	Intervention vs control Mean difference (95% CI)[a]	Intervention vs breastfed Mean difference (95% CI)[a]	Control vs breastfed Mean difference (95% CI)[a]
Weight gain (g/day)							**Weight gain (g/day)**		
3–6 months									
Completers	92	20.09 ± 4.62	91	20.65 ± 5.54	109	17.43 ± 4.89	−0.67 (−2.11 to 0.77)	–	–
PP	–	–	–	–		–	−0.69 (−2.20 to 0.81)	–	–
6–12 months									
Completers	86	11.54 ± 3.04	87	12.31 ± 3.08	103	10.39 ± 3.13	−0.80 (−1.73 to 0.13)	1.04 (0.12 to 1.95)	1.84 (0.93 to 2.75)
PP	78	11.45 ± 3.00	81	12.22 ± 3.05	97	10.28 ± 3.03	−0.85 (−1.80 to 0.10)	0.96 (0.03 to 1.89)	1.81 (0.90 to 2.73)
Weight (kg)							**Weight (g)**		
3 months									
Completers	97	5.96 ± 0.70	97	5.91 ± 0.68	112	5.78 ± 0.70	–		
PP	85	5.99 ± 0.68	85	5.89 ± 0.69	105	5.79 ± 0.68	–		
6 months									
Completers	93	7.62 ± 0.84	91	7.62 ± 0.89	109	7.24 ± 0.87	−71.06 (−193.4 to 51.25)	167.86 (47.71 to 288.01)[1]	238.92 (118.28 to 359.57)[2]
PP	84	7.68 ± 0.84	85	7.62 ± 0.90	103	7.27 ± 0.89	−71.08 (−199.9 to 57.76)	175.72 (49.63 to 301.81)	246.80 (121.83 to 371.77)
12 months									
Completers	87	9.86 ± 1.12	87	9.97 ± 1.21	104	9.20 ± 1.10	−231.7 (−473.2 to 9.86)	385.61 (148.70 to 622.51)[1]	617.28 (380.58 to 853.98)[2]
PP	79	9.89 ± 1.12	81	9.95 ± 1.21	98	9.20 ± 1.12	−239.7 (−484.6 to 5.11)	378.73 (139.63 to 617.83)	618.48 (382.08 to 854.88)
Weight change (kg)							**Weight change (kg)**		
6–12 months									
Completers	86	2.20 ± 0.58	87	2.35 ± 0.59	103	1.96 ± 0.59	−0.16 (−0.34 to 0.02)	0.21 (0.03 to 0.38)[3]	0.37 (0.19 to 0.54)[2]
PP	78	2.18 ± 0.57	81	2.33 ± 0.58	97	1.94 ± 0.56	−0.17 (−0.35 to 0.01)	0.19 (0.02 to 0.37)	0.36 (0.19 to 0.53)[2]

(a): Adjusted for baseline and gender.
(1): $p \leq 0.01$
(2): $p \leq 0.0001$
(3): $p < 0.05$.

Appendix B – Length and head circumference in the 'US study'

	n	Intervention	n	Control	n	Breastfed	Intervention vs control	Intervention vs breastfed	Control vs breastfed
		Mean ± SD		Mean ± SD		Mean ± SD	Mean difference (95% CI)[a]	Mean difference (95% CI)[a]	Mean difference (95% CI)[a]
Length (cm)							**Length (mm)**		
3 months									
Completers	97	59.54 ± 2.24	97	59.44 ± 2.06	112	59.62 ± 2.04	–		
PP	85	59.62 ± 2.20	85	59.35 ± 2.03	105	59.61 ± 2.03	–		
6 months									
Completers	94	65.61 ± 2.30	91	65.75 ± 2.34	110	65.11 ± 2.32	−1.43 (−4.51 to 1.65)	6.03 (3.02 to 9.04)[1]	7.46 (4.41 to 10.51)[2]
PP	85	65.72 ± 2.35	85	65.63 ± 2.30	104	65.16 ± 2.35	−1.48 (−4.63 to 1.67)	5.79 (2.73 to 8.86)[1]	7.27 (4.19 to 10.35)[2]
12 months									
Completers	87	74.52 ± 2.53	87	74.71 ± 2.77	105	73.34 ± 2.69	−2.37 (−7.24 to 2.50)	12.04 (7.28 to 16.80)[2]	14.41 (9.63 to 19.19)[2]
PP	79	74.58 ± 2.55	81	74.55 ± 2.74	99	73.35 ± 2.74	−2.95 (−8.08 to 2.18)	11.67 (6.68 to 16.65)[2]	14.62 (9.64 to 19.61)[2]
Head circumference (cm)							**Head circumference (mm)**		
3 months									
Completers	97	40.27 ± 1.24	96	40.34 ± 1.27	112	40.27 ± 1.23	–		
PP	85	40.26 ± 1.16	84	40.41 ± 1.15	105	40.25 ± 1.26	–		
6 months									
Completers	94	42.99 ± 1.30	91	43.19 ± 1.29	110	42.92 ± 1.26	−0.57 (−2.24 to 1.10)	0.89 (−0.74 to 2.52)	1.46 (−0.19 to 3.11)
PP	85	43.00 ± 1.23	85	43.18 ± 1.25	104	42.88 ± 1.26	−0.09 (−1.67 to 1.49)	1.11 (−0.43 to 2.65)	1.20 (−0.35 to 2.74)
12 months									
Completers	86	46.18 ± 1.39	87	46.47 ± 1.49	104	46.04 ± 1.39	−1.36 (−3.82 to 1.11)	1.76 (−0.64 to 4.17)	3.12 (0.70 to 5.54)[3]
PP	79	46.23 ± 1.32	81	46.43 ± 1.44	98	45.98 ± 1.37	−0.43 (−2.73 to 1.86)	2.34 (0.10 to 4.57)[3]	2.77 (0.54 to 5.00)[3]

(a): Adjusted for baseline and gender.
(1): $p < 0.001$.
(2): $p < 0.0001$.
(3): $p < 0.05$.

Appendix C – Absolute weight, weight gain and weight change in the 'Chile study'

	n	Intervention	n	Control	n	Breastfed	Intervention vs control	Intervention vs breastfed	Control vs breastfed
		Mean ± SD		Mean ± SD		Mean ± SD	Mean difference (95% CI)[a]	Mean difference (95% CI)[a]	Mean difference (95% CI)[a]
Weight gain (g/day)							**Weight gain (g/day)**		
3–6 months									
Completers	66	18.97 ± 4.19	76	20.74 ± 5.01	65	20.07 ± 5.79	−2.26 (−3.88 to −0.64)[1]	−0.72 (−2.46 to 1.01)	1.54 (−0.13 to 3.21)
PP	55	19.17 ± 4.16	68	21.02 ± 4.88	57	20.35 ± 5.83	–	–	–
6–12 months									
Completers	54	10.97 ± 3.05	66	12.13 ± 3.03	61	10.18 ± 3.85	−0.88 (−2.10 to 0.35)	0.77 (−0.50 to 2.05)	1.65 (0.45 to 2.85)[1]
PP	47	11.05 ± 3.03	60	12.09 ± 3.15	54	9.99 ± 3.94	−0.76 (−2.09 to 0.58)	1.16 (−0.24 to 2.57)	1.92 (0.63 to 3.21)[1]
Weight (kg)							**Weight (g)**		
6 months									
Completers	66	8.03 ± 0.67	76	8.17 ± 0.95	65	8.50 ± 1.12	−142.91 (−377.33 to 91.52)	3.75 (−245.49 to 252.99)	146.65 (−93.14 to 386.45)
PP	55	8.09 ± 0.68	68	8.21 ± 0.97	57	8.52 ± 1.11	−161.08 (−502.24 to 180.08)	71.78 (−286.92 to 430.48)	232.85 (102.29 to 568.00)
12 months									
Completers	54	10.08 ± 0.86	66	10.36 ± 1.10	61	10.42 ± 1.27	−315.70 (−566.93 to −64.46)[3]	132.25 (−129.81 to 394.32)	447.95 (199.13 to 696.77)[2]
PP	47	10.14 ± 0.87	60	10.40 ± 1.13	54	10.43 ± 1.28	−284.55 (−655.77 to 86.68)	273.83 (−112.04 to 659.70)	558.38 (211.97 to 904.79)[1]
Weight change (kg)							**Weight change (kg)**		
6–12 months									
Completers	54	2.01 ± 0.55	66	2.20 ± 0.55	61	1.89 ± 0.71	−0.14 (−0.37 to 0.08)	0.11 (−0.12 to 0.35)	0.26 (0.04 to 0.48)[3]
PP	47	2.03 ± 0.54	60	2.20 ± 0.57	54	1.86 ± 0.73	−0.12 (−0.37 to 0.12)	0.19 (−0.07 to 0.44)	0.31 (0.07 to 0.54)[3]

(a): Adjusted for baseline, gender, prepregnancy BMI of the mother (as continuous variable), antibiotic use, introduction of complementary food prior to 6 months of age (yes/no) and ethnicity;
(1): $p \leq 0.01$.
(2): $p \leq 0.001$.
(3): $p < 0.05$.

Appendix D – Length and head circumference in the 'Chile study'

	n	Intervention	n	Control	n	Breastfed	Intervention vs control	Intervention vs breastfed	Control vs breastfed
		Mean ± SD		Mean ± SD		Mean ± SD	Mean difference (95% CI)[a]	Mean difference (95% CI)[a]	Mean difference (95% CI)[a]
Length (cm)							**Length (mm)**		
3 months									
Completers	89	59.80 ± 2.01	87	60.34 ± 2.07	76	61.20 ± 1.95	−6.21 (−12.03 to −0.39)[1]	−14.60 (−20.71 to −8.49)[2]	−8.39 (−14.47 to −2.30)[3]
PP	65	59.84 ± 1.95	73	60.30 ± 2.01	65	61.08 ± 1.93	−6.46 (−12.94 to 0.02)	−14.05 (−20.83 to −7.27)[2]	−7.59 (−14.07 to −1.11)[1]
6 months									
Completers	66	66.04 ± 1.78	76	66.54 ± 2.25	65	67.09 ± 2.25	0.08 (−5.36 to 5.53)	1.86 (−3.97 to 7.68)	1.77 (−3.74 to 7.29)
PP	55	66.20 ± 1.82	68	66.56 ± 2.14	57	67.04 ± 2.18	4.12 (−3.75 to 11.99)	8.64 (0.21 to 17.06)[1]	4.51 (−3.26 to 12.28)
12 months									
Completers	54	74.23 ± 1.99	66	74.77 ± 2.34	61	75.37 ± 2.60	−0.75 (−6.58 to 5.08)	1.58 (−4.54 to 7.70)	2.33 (−3.40 to 8.05)
PP	47	74.44 ± 1.97	60	74.81 ± 2.33	54	75.36 ± 2.64	3.19 (−5.33 to 11.71)	8.33 (−0.70 to 17.35)	5.13 (−2.89 to 13.16)
Head circumference (cm)							**Head circumference (mm)**		
3 months									
Completers	89	40.22 ± 1.06	87	40.50 ± 1.11	76	40.66 ± 1.19	−3.19 (−6.29 to −0.10)[1]	−4.71 (−7.96 to −1.47)[3]	−1.52 (−4.75 to 1.72)
PP	65	40.29 ± 0.97	73	40.46 ± 1.07	65	40.74 ± 1.15	−2.52 (−5.92 to 0.89)	−4.97 (−8.53 to −1.40)[3]	−2.45 (−5.86 to 0.96)
6 months									
Completers	66	43.10 ± 1.17	76	43.27 ± 1.24	65	43.43 ± 1.36	0.73 (−1.24 to 2.69)	0.79 (−1.28 to 2.86)	0.06 (−1.92 to 2.03)
PP	55	43.22 ± 1.05	68	43.26 ± 1.17	57	43.51 ± 1.31	1.12 (−1.72 to 3.96)	1.76 (−1.25 to 4.77)	0.64 (−2.14 to 3.42)
12 months									
Completers	54	46.02 ± 1.32	66	46.34 ± 1.09	61	46.40 ± 1.36	−0.76 (−2.87 to 1.34)	0.90 (−1.28 to 3.08)	1.67 (−0.38 to 3.71)
PP	47	46.11 ± 1.16	60	46.35 ± 0.97	54	46.50 ± 1.32	−2.19 (−5.32 to 0.93)	0.80 (−2.46 to 4.07)	3.00 (0.11 to 5.88)[1]

(a): Adjusted for baseline, gender, prepregnancy BMI of the mother (as continuous variable), antibiotic use, introduction of complementary food prior to 6 months of age (yes/no) and ethnicity.
(1): $p < 0.05$.
(2): $p \leq 0.001$.
(3): $p \leq 0.01$.