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A REQUEST TO AMEND THE MINIMUM PROTEIN REQUIREMENT IN FOLLOW-ON FORMULA IN THE AUSTRALIA NEW ZEALAND FOOD STANDARDS CODE

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Request to Amend the Minimum Protein Requirement in Follow-on Formula in the Australia New Zealand Food Standards Code

ABBREVIATIONS

AI	adequate intake
AUSNUT	Australian Food, Supplement and Nutrient
BMI	body mass index
CCNFSDU	Codex Committee on Nutrition and Foods for Special Dietary Uses
CI	confidence intervals
EAR	Estimated Average Requirement
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
EWG	electronic working group
FSANZ	Food Standards Australia New Zealand
HPF	higher-protein formula
InFANT	Infant Feeding Activity and Nutrition Trial
IOM	Institute of Medicine
IQR	interquartile range
ITT	intention-to-treat
LPF	lower-protein formula
NDA Panel	Panel on Dietetic Products, Nutrition and Allergies
NHANES	National Health and Nutrition Examination Survey
NHMRC	National Health and Medical Research Council
NNS	National Nutrition Survey
NUTTAB95	NUTrient data TABle for use in Australia
OECD	Organisation for Economic Co-operation and Development
PP	per protocol
PRI	Population Reference Intake
RDA	recommended dietary allowance
SD	standard deviation
The Code	Australia New Zealand Food Standards Code
U.S.	United States
WHO	World Health Organization

Request to Amend the Minimum Protein Requirement in Follow-on Formula in the Australia New Zealand Food Standards Code

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1.0 EXECUTIVE SUMMARY

Dietary protein is an essential component of the diet, supplying the body with nitrogen and amino acids (EFSA, 2017), which are needed for the synthesis of nucleic acids, hormones and vitamins (IOM, 2005). Proteins are the major structural components of all cells in the body (IOM, 2005) and are essential in growth and development (Dupont, 2003), including the development of the brain and bones (Bonjour *et al.*, 2001). Proteins also function as enzymes and transport carriers (IOM, 2005).

The purpose of this application is to request an amendment to the Code, specifically **Standard 2.9.1** (Infant formula products) (FSANZ, 2017)[1], **Division 3** (Infant formula and follow-on formula), **2.9.1–9** (Infant formula and follow-on formula – composition), **(2) (b)** which states "Follow-on formula must have a protein content of no less than 0.45 g/100 kJ and no more than 1.3 g/100 kJ". We are requesting to vary the minimum protein requirement in follow-on formula from "no less than 0.45 g/100 kJ" (equivalent to 1.88 g protein/100 kcal) to **"no less than <u>0.38 g/100 kJ</u>"** (equivalent to 1.6 g/100 kcal)[2].

As Codex is revising a standard, and EU has already amended a standard, that permits a protein minimum of 1.6 g/100 kcal or 0.38g/100kJ (under set conditions) for follow-on formula, aligning Australian and New Zealand standards with these standards will help facilitate harmonisation opportunities and promote international trade (this is assuming other nutritional parameters do not present hurdles for harmonisation). This harmonisation will also reduce the number of future technical barriers to trade. Based on human milk composition data from Mitoulas *et al.* (2002), the proposed minimum protein quantity recommended in this application for follow-on formula for use by infants aged 6 to 12 months of 1.6 g total protein/100 kcal (0.38g/100kJ) more closely aligns with, yet still exceeds by approximately 25%, the levels of protein occurring in the breast milk of Australian mothers during the 6th and 12th months of lactation (an average of 1.26 g total protein/100 kcal, equivalent to 0.30g/100kJ).

This request to decrease the minimum level of protein, an already-permitted nutrient in follow-on formula for infants 6 to 12 months of age, is to achieve growth rates, measured by infants' length, weight, and head circumference, that are more comparable to breastfed infants. This is done in accordance with the Australia and New Zealand's Food Regulation Ministerial Council's Food Regulation Standing Committee's position (2011) which states "the composition of breast milk should be used as a primary reference for determining the composition of infant formula and follow-on formula" and "the composition of follow-on formula must be safe, suitable for the intended use and **must strive to achieve as closely as possible the normal growth and development […] of healthy full-term breastfed infants at the appropriate age**" (Australian and New Zealand Food Regulation Ministerial Council, 2011). In line with these guidelines, clinical and epidemiological evidence obtained via systematic review is presented to highlight the safety and potential efficacy associated with this reduction of protein. Dietary intake data of the relevant population group is also reviewed.

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[1] Available at: https://www.legislation.gov.au/Details/F2017C00332/Download

[2] For the conversion from g/100 kJ to g/100 kcal, a conversion factor of 4.18 was used. As such, 0.45 g/100 kJ = 1.881 g/100 kcal (rounded to 1.88 g/100 kcal in this application) and 0.38 g/100 kJ=1.5884 g/100 kcal, rounded to 1.6 g/100 kcal in this application.

2.0 APPLICANT DETAILS

(a) **Organisation's name:** Nestlé Australia Ltd and Nestlé New Zealand Limited

(b) **Name of contact person:**

(c) **Address:** Nestlé Australia Ltd, Building D, 1 Homebush Bay Drive, Rhodes, NSW, 2138, AUSTRALIA

(d) **Telephone number:**

(e) **Email address:**

(f) **Nature of applicant's business:** Nestlé is a manufacturer and importer of a wide variety of foods for the Australian and New Zealand markets and is globally one of the largest manufacturers of infant formula products and other foods. Nestlé currently imports and markets infant formula products which are regulated in Section 2.9.1 of the Australia New Zealand Food Standards Code ('the Code')

(g) **Details of other individuals, companies or organisations associated with the application:** Not applicable

3.0 PURPOSE OF THE APPLICATION

The purpose of this application is to request an amendment to the Code, specifically **Standard 2.9.1** (Infant formula products) (FSANZ, 2017)[3], **Division 3** (Infant formula and follow-on formula), **2.9.1–9** (Infant formula and follow-on formula – composition), **(2) (b)** which states:

> "Follow-on formula must have a protein content of no less than 0.45 g/100 kJ and no more than 1.3 g/100 KJ".

We are requesting to vary the minimum protein requirement in follow-on formula from "no less than 0.45 g/100 kJ" (equivalent to 1.88 g protein/100 kcal) to **"no less than 0.38 g/100 kJ"** (equivalent to 1.6 g/100 kcal)[4].

To achieve this purpose, this application addresses the requirements stated in Sections 3.3.3 and 3.6.2 of the Food Standards Australia New Zealand (FSANZ) *Application Handbook*, that relate to "Substances used for a nutritive purpose" and "Infant formula products", respectively (FSANZ, 2016a). The former is relevant because we are requesting, in this application, a *reduction* in the minimum requirement for a *nutritive substance* (protein), a macronutrient. The latter is relevant because this request is specifically being made for *follow-on formula (for infants 6 to 12 months).*

[3] Available at: https://www.legislation.gov.au/Details/F2017C00332/Download
[4] For the conversion from g/100 kJ to g/100 kcal, a conversion factor of 4.18 was used. As such, 0.45 g/100 kJ = 1.881 g/100 kcal (rounded to 1.88 g/100 kcal in this application) and 0.38 g/100 kJ=1.5884 g/100 kcal, rounded to 1.6 g/100 kcal in this application.

4.0 JUSTIFICATION FOR THE APPLICATION

Breast milk is universally accepted as the optimal and preferred source of nutrition for infants – the World Health Organization (WHO) acknowledges that "breastfeeding is an unequalled way of providing ideal food for the healthy growth and development of infants" (WHO, 2017). While the multifactorial benefits of breastfeeding are undisputed, for infants who are not breastfed or are partially breastfed, the Australian Government's *National Health and Medical Research Council* (NHMRC) recommends commercial infant formulas as "the only suitable and safe alternative" to breast milk until 12 months of age (NHMRC, 2012a).

In accordance with the Australia and New Zealand's Food Regulation Ministerial Council's Food Regulation Standing Committee's position on the "Regulation of Infant Formula Products" (Australian and New Zealand Food Regulation Ministerial Council, 2011), "the composition of breast milk should be used as a primary reference for determining the composition of infant formula and follow-on formula" and "the composition of follow-on formula must be safe, suitable for the intended use and **must strive to achieve as closely as possible the normal growth and development […] of healthy full-term breastfed infants at the appropriate age**" (Australian and New Zealand Food Regulation Ministerial Council, 2011). In line with these guidelines, the food regulatory measure being proposed herein to decrease the minimum protein requirement in follow-on formula for infants 6 to 12 months of age is intended to achieve a closer match between the "total protein" levels in follow-on formula with the "total protein" levels in breast milk from the 6th to 12th month of lactation, and also to more closely match the growth outcomes of infants fed follow-on formula with the growth of breastfed infants.

With respect to whether similar applications have been made in other countries, in 2015, Nestlé submitted an application to the European Commission (EC) in the European Union (EU) to market a follow-on formula with a protein content of 1.6 g protein/100 kcal (0.38g/100kJ) – a level below the required minimum of 1.8 g protein/ 100 kcal (0.43g/100kJ) for follow-on formula manufactured from cows' milk protein, as stated in Point 2.1 of Annex II ("Essential Composition of Follow-on Formulae When Reconstituted as Instructed by the Manufacturer") of Directive 2006/141/EC (EC, 2006). In response to this application, the EC requested that the European Food Safety Authority (EFSA) deliver a scientific opinion on the safety of follow-on formula based on cows' milk intact protein with a protein content of at least 1.6 g/100 kcal (0.38g/100kJ). The EFSA's Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) concluded in their Scientific Opinion that follow-on formulae (comprised of cows' and goats' milk protein) with a protein content of at least 1.6 g/100 kcal (0.38g/100kJ) were safe and suitable, together with an intake of complementary foods of a sufficient quality (EFSA, 2017). The EFSA's Scientific Opinion (EFSA, 2017) is, indeed, a pivotal line of evidence that supports the safety of the proposed compositional change in follow-on formula described in this application. Since then, Annex II to Delegated Regulation (EU) 2016/127 has been amended to permit 1.6g/100 kcal (0.38g/100kJ) minimum protein in follow-on formula.

There are other lines of evidence that provide justification for the request to decrease the minimum protein requirement in follow-on formula (for infants 6 to 12 months of age) as described below.

- The protein needs of infants decrease during the 1st year of life (Dewey *et al.,* 1996). Concurrently, the protein content of breast milk decreases during the 1st year of lactation (Michaelsen *et al.,* 1990; Allen *et al.,* 1991; Nommsen *et al.,* 1991; Mitoulas *et al.,* 2002[5]; Saarela *et al.,* 2005; Hester *et al.,* 2012; Gidrewicz and Fenton, 2014; Lönnerdal *et al.,* 2017), with appreciable decreases (≥25%) occurring by the 6th month of lactation (Hytten, 1954; Prentice *et al.,* 1981; Butte *et al.,* 1984a; Allen *et al.,* 1991). Lönnerdal *et al.* (2017) visually depicted these

[5] Mitoulas *et al.* (2002) measured the composition of breast milk throughout the 1st year of lactation in healthy mothers living in Australia.

concurrent changes by superimposing estimated protein requirements in infants over their 1st year, as calculated by Dewey *et al.* (1996), with the true protein content in breast milk during the 1st year of lactation, as estimated from a systematic review and meta-analysis of 26 original articles published between 1973 and 2011 (Lönnerdal *et al.,* 2017), 2 of which were conducted in Australia (Arnold *et al.,* 1987 and Mitoulas *et al.,* 2002); see Section 5.2.1.3.1. Indeed, based on breast milk composition data generated from mothers living in Australia, it is noteworthy that at the 6th, 9th, and 12th months of lactation, the "total protein" levels of breast milk averaged 0.80±0.04 g/100 mL (=1.28 g/100 kcal; 0.31g/100kJ), 0.83±0.05 g/100 mL (=1.24 g/100 kcal; 0.30g/100kJ), and 0.83±0.06 g/100 mL (=1.25 g/100 kcal; 0.30g/100kJ), respectively, in contrast to levels of 1.05±0.04 g/100 mL (=1.62 g/100 kcal; 0.39g/100kJ) in the 1st month of lactation[6] (Mitoulas *et al.,* 2002). Notably, based on changes in protein measured as g/100 mL or g/100 kcal in Mitoulas *et al.* (2002), from the 1st to the 6th months, reductions in protein levels averaged 23.5 and 20.6%, respectively. Importantly, Mitoulas *et al.* (2002) reported that the total protein content of breast milk at the 6th and 9th months of lactation was statistically significantly lower than at the 1st, 2nd, and 4th months of lactation ($p<0.05$)[7].

- Based on data from Mitoulas *et al.* (2002), mean levels of "total protein" in breast milk from Australian mothers from the 6th to the 12th months of lactation was approximately 1.3 g protein/100 kcal (0.31g/100kJ). Furthermore, the total protein levels occurring in the breast milk of Australian mothers from the 6th to the 12th month of lactation are lower than the minimum required amount in follow-on formula as per the Australia New Zealand Food Standards Code (0.45 g protein/100 kJ, which is equivalent to 1.88 g protein/100 kcal). The proposed minimum protein quantity recommended in this application for follow-on formula for use by infants aged 6 to 12 months of 1.6 g total protein/100 kcal (0.38g/100kJ) more closely aligns with, and exceeds, levels of total protein occurring in the breast milk of Australian mothers during the 6th and 12th months of lactation (approximately 1.3 g total protein/100 kcal or 0.31g/100kJ).

- Compared to breastfed infants, formula-fed infants have greater body weight gains in infancy (Dewey, 1998; Kramer *et al.,* 2004; Victora *et al.,* 1998 cited in Koletzko *et al.,* 2009) and evidence exists to indicate that rapid weight gain in infancy is associated with an increased risk of overweight in children (Péneau *et al.*, 2011; Weng *et al.,* 2012).

- Different intakes of metabolizable substrates, specifically protein (Koletzko *et al.,* 2005), between formula-fed and breastfed infants may explain the greater weight gain in formula-fed infants. Indeed, protein intake per kilogram of body weight is estimated to be 55 to 80% higher in formula-fed than breastfed infants (Alexy *et al.,* 1999 cited in Koletzko *et al.,* 2009). This observation is supported by the study by Conn *et al.* (2009), wherein the food and nutrient intakes of 9-month old infants (n=341; 180 boys and 161 girls; median weight of 9.2 kg; median

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[6] Protein levels in g/100 kcal were derived using data from Table 2 in Mitoulas *et al.* (2002), wherein the protein level (g/L) and energy content (KJ/mL) of breast milk were reported for the 1st, 2nd, 4th, 6th, 9th, and 12th months of lactation. In the 6th month of lactation, the mean protein (g/L) and energy (KJ/mL) contents ± standard error of 16 breast milk samples from 8 mothers were 8.03±0.38 g/L and 2.62±0.09 KJ/mL, respectively. In the 9th month of lactation, the mean protein and energy contents of 12 breast milk samples from 6 mothers were 8.34±0.45 g/L and 2.81±0.09 KJ/mL, respectively. In the 12th month of lactation, the mean protein and energy contents of 10 breast milk samples from 5 mothers were 8.34±0.57 g/L and 2.79±0.14 KJ/mL, respectively. In contrast, in the 1st month of lactation, the mean protein and energy contents of 18 breast milk samples from 9 mothers were 10.5±0.4 g/L and 2.72±0.06 KJ/mL, respectively.

[7] The protein content of breast milk at 12 months, although not significantly different from its level in breast milk at 9 months (as indicated by very comparable mean protein values between 9 and 12 months) was also not significantly different from levels of protein in breast milk at the 1st, 2nd, and 4th months of lactation (even though the mean protein level at 12 months was lower than the mean levels of protein during the first 4 months of lactation). This is likely because of the higher standard error of the mean at 12 months as compared to the standard error of the mean in the first 4 months of lactation, likely a consequence of the smaller sample size at 12 months. See Table 5.2.1.3.1-2.

length of 71.2 cm) living in Australia were measured from 1999 to 2001. Based on dietary intake data (that reflected consumption patterns 'over the past month') ascertained through structured open-ended questions and with a food frequency format, the mean±standard deviation (SD) protein intake reported for 'breastfed infants' (n=121)[8] and 'not breastfed infants' (n=220) was 23±7 g protein/day and 30±8 g protein/day, respectively, a difference which reached statistical significance (p<0.001). Based on these nutrient intakes generated for 9-month old infants living in Australia, it appears that breastfed infants have about a 30% lower daily protein intake as compared to infants who are not breastfed. In the Melbourne (Australia) Infant Feeding Activity and Nutrition Trial (InFANT), dietary intake data were collected for children at ages 9 and 18 months, and 3.5 and 5 years, using three 24-hour dietary recalls (Lioret *at al.,* 2013; Campbell *et al.,* 2017). Based on analyses of these data, it was observed that at 9 months of age, both the earlier introduction of solids and the primary milk source being formula/dairy or mixed (as opposed to exclusively breast milk), were associated with the consumption of significantly more protein per 1, 000 kcal (Campbell *et al.,* 2017).

- Epidemiological evidence exists that links high protein intakes in infancy to overweight (including obesity) in childhood (Rolland-Cachera *et al.,* 1995; Gunnarsdottir and Thorsdottir, 2003; Günther *et al.,* 2007; Öhlund *et al.,* 2010), contributing to the 'early protein hypothesis' – *i.e.,* that high protein intakes in excess of metabolic requirements early in life enhance weight gain in infancy and increase obesity risk later in life (Koletzko *et al.,* 2005). In the study by Inostroza *et al.* (2014), wherein infants were fed a lower protein formula (LPF) (1.65 g/100 kcal) or a higher protein formula (HPF) (2.7 g/100 kcal) from 3 to 12 months (with complementary foods introduced in an unrestricted fashion from 6 to 12 months), at 6 and 12 months of age, the percentage of infants whose weight was >90th percentile of the WHO standards was significantly lower among infants fed the LPF *versus* the HPF (10.6% *versus* 22.4%, respectively, at 6 months[9] and 18.5% *versus* 31.8%, respectively, at 12 months[10]). The lower protein intake achieved by breastfed *versus* formula-fed infants may be among the reasons why breastfed infants are at lower risk of overweight/obesity later in life (Arenz *et al.,* 2004; Harder *et al.,* 2005; Owen *et al.,* 2005; Hester *et al.,* 2012; Weng *et al.,* 2012).

- Dietary surveys informing on the protein intakes of 9-month old infants were conducted in Australia (Melbourne or Adelaide) in 1999 to 2001 (Conn *et al.,* 2009) and 2008 to 2009 (Lioret *et al.,* 2013). From these 2 studies, the mean (±SD) protein intakes of 9-month old infants were 29.0±10.9 g protein/day for girls and boys (Lioret *et al.,* 2013; n=177; breastfed or not breastfed + complementary foods); 26±8 g protein/day for girls (Conn *et al.,* 2009; n=161; breastfed or not breastfed + complementary foods); and, 29±8 g protein/day for boys (Conn *et al.,* 2009; n=180; breastfed or not breastfed + complementary foods). The <u>mean</u> daily protein intakes (~26 to 29 g/day) and also the <u>median</u> daily protein intakes (25 to 29 g/day) across both these studies indicate that 9-month old infants living in Australia (Melbourne or Adelaide) are far exceeding (by about 2-fold) the Australian Government NHMRC's adequate intake (AI) for dietary protein (14 g/day) for older infants (7 to 12 months of age) and the Institute of Medicine's (IOM) recommended dietary allowance (RDA) for dietary protein for older infants (7 to 12 months of age). Protein intakes in excess of metabolic requirements have public health implications, as discussed above. Indeed, dietary intake analyses conducted by Campbell *et al.* (2017), who

[8] Regarding the contribution of breast milk to daily nutrient intakes, Conn *et al.* (2009) stated the following: "The quantity of breast milk consumed was estimated from information on the frequency of feeding only, as descriptions of the duration of feeds were often too variable or vague to be useful in this regard. The volume of breast milk per feed was calculated from the data of Dewey *et al.* (1984). Where breast-feeding occurred 6 or more times daily, the assigned volume was 130 mL per feed, with 4 or 5 feeds per day assigned 101 mL per feed and up to 3 feeds per day assigned 55 mL per feed. The nutritional content of breast milk was obtained from published values (Department of Health and Social Security, 1977)".
[9] Odds ratio = 5.3, 95% confidence intervals (CI) =1.2 to 23.5.
[10] Odds ratio = 3.6; 95% CI =1.1 to 11.2.

report on the same study cohort as described by Lioret *et al.* (2013), provide evidence that protein intakes at 9 and 18 months of age can predict intakes at 5 years – *i.e.,* the residualised protein intakes at 9 months were significantly associated with intakes at 18 months (p=0.007) and 5 years (p=0.006). Lowering the currently excessive protein intakes in older infants (6 to 12 months of age) who are not breastfed could result in potential health benefits in the local Australian-New Zealand context, namely a reduced risk of excessive protein intakes during and after infancy and a reduced risk of overweight/obesity later in life.

- In 2 randomised, double-blind, controlled intervention studies conducted in Chile (Inostroza *et al.,* 2014) and in the United States (U.S.) (Ziegler *et al.,* 2015), the effects, on infant growth (weight, length, head circumference), of a LPF (1.61 or 1.65 g/100 kcal in Ziegler *et al.,* 2015 and Inostroza *et al.,* 2014, respectively, equivalent to 0.39g/100kJ) were compared to a HPF (2.15 g/100 kcal or 2.70 g/100 kcal in Ziegler *et al.,* 2015 and Inostroza *et al.,* 2014 , respectively, equivalent to 0.51 and 0.65g/100kJ respectively) and also to a breastfed reference group. The formulas were administered from 3 to 12 months of age[11]. Collectively, across both studies (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015), 141 infants received a LPF and 166 infants were breastfed[12]. At 6 and 12 months, as compared to breastfed infants, there were no adverse effects in infants receiving the LPF on infant weight, length, and head circumference. As summarised in Appendices A to D in EFSA (2017), across both studies for 'completers' and the 'per protocol population', <u>at 6 and 12 months</u>, the LPF led to <u>**mean increases**</u> in weight (kg), length (cm), and head circumference (cm) compared to the breastfed reference group; <u>**mean increases**</u> in weight gain (g/day) and weight change (kg) were also observed with the LPF compared to the breastfed reference group during this time period (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015)[13]. Indeed, in both studies for 'completers' and the 'per protocol population', <u>from 6 to 12 months</u>, weight gain (g/day) and weight change (kg) in the LPF group *versus* the breastfed reference group were more similar as compared to the HPF group *versus* the breastfed reference group (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015). These studies are discussed further in Section 5.2.1.3.2 of this application.

- In establishing the AI for protein for infants 7 to 12 months of age, the NHMRC considered the mean protein intake from complementary foods for breastfed infants aged 7 to 12 months to be 7.1 g protein/day [U.S. National Health and Nutrition Examination Survey (NHANES) III data – IOM, 2005; NHMRC, 2014)], with 6.6 g protein/day delivered by human milk. In the absence of human milk contributing to protein intakes, it is of interest to assess whether infants consuming the LPFs in 2 pivotal studies (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015) averaged protein intakes of at least 6.6 g/day. In Ziegler *et al.* (2015), actual average intakes of the LPF were 917 mL/day at 6 months of age, 850 mL/day at 8 months of age, 810 mL/day at 10 months of age, and 719 mL/day at 12 months of age. Given the composition of the LPF was 1.08 g protein per 100 mL, this corresponds to a daily protein intake from the principal milk source of 9.9 g/day at 6 months, 9.18 g/day at 8 months, 8.75 g/day at 10 months, and 7.77 g/day at 12 months. The intake data from the LPF group in Inostroza *et al*. (2014) were similar, with 980 mL/day (10.2 g protein/day) consumed at 6 months of age, 896 mL/day (9.3 g protein/day) consumed at 9 months of age, and 854 mL/day (8.8 g protein/day) consumed at 12 months of age (EFSA, 2017)[14]. Thus, in Ziegler *et al.* (2015) and Inostroza *et al. (*2014), from 6 to 12 months of age, the

[11] In both studies (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015), complementary foods were unrestricted from 6 to 12 months of age.

[12] These values represent the number of infants analyzed across both studies, in combination, at 12 months.

[13] The difference in weight gain achieved with the intake of a LPF *versus* infants in the breastfed reference group from 6 to 12 months was 1.04 g/day (95% CI=0.12 to 1.95) for 'completers' in Ziegler *et al.* (2015) and 0.77 g/day (95% CI=-0.50 to 2.05) for 'completers' in Inostroza *et al.* (2014).

[14] It should be noted that the formula intakes reported in the supplementary tables in Inostroza et al. (2014) are not correct; however, they are correctly reported in Table 12 in EFSA (2017).

average daily protein intakes of infants that was consumed from their principal milk source (LPF) ranged from 7.77 to 10.2 g protein/day, levels which are above 6.6 g protein/day – the amount of protein typically consumed by breastfed infants 7 to 12 months of age.

- In 2012, the Australian Government's NHMRC published a literature review titled *Literature Review: Infant Feeding Guidelines* (NHMRC, 2012b) wherein the NHMRC acknowledged that formula-fed infants grow at a different rate than breastfed infants and the former are heavier at 12 months of age and have a slightly increased risk of later obesity (WHO European Region 2007 cited in NHMRC, 2012b). The NHMRC highlighted the findings of Koletzko *et al*. (2009)[15], also stating that "a lower protein intake in infancy might diminish the later risk of overweight and obesity". Additionally, due to the potential for excess protein to increase risk of obesity later in life, in the NHMRC's *Eat for Health: Infant Feeding Guidelines – Information for health workers* (NHMRC, 2012a), it is stated that "it is preferable to use a formula with a lower protein level".

From the rationale outlined above, no public health or safety issues are foreseen with the proposed compositional change in follow-on formula. There is also a potential benefit in achieving growth and development outcomes that are closer to those of breastfed infants.

The Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) is currently drafting a revised *Standard for Follow-Up Formula* (CODEX STAN 156-1987 – Codex Alimentarius, 2017b)[16], which will specify a minimum protein content of 1.8 g/100 kcal (for cows' and goats' milk protein), with an associated footnote 6 permitting that '*A lower minimum protein level between 1.6 and 1.8 g/100kcal (0.38 and 0.43 g/100kJ) in follow-up formula based on non-hydrolysed milk protein can be accepted. Such follow-up formula and follow-up formula based on hydrolysed protein should be evaluated for their safety and suitability and assessed by a competent national and/or regional authority based on clinical evidence*' (CXS 156-1987 – for adoption at Step 5).

4.1 Regulatory Impact Information

4.1.1 Costs and Benefits of the Application

<u>(a) Consumers</u>

The proposed reduction in the minimum level of protein in follow-on formula for infants 6 to 12 months of age is consistent with efforts to manufacture products for infants that better match the nutrient composition of human milk, as set forth by the principles in the Australia and New Zealand Food Regulation Ministerial Council's Policy Guideline on the *Regulation of Infant Formula Products* (Australian and New Zealand Food Regulation Ministerial Council, 2011). These efforts are intended to help ensure that infants living in Australia and New Zealand consume an adequate, but not excessive, intake of protein to support normal growth and development; excess protein intakes could put undue

[15] In Koletzko *et al.* (2009), 1138 healthy, formula-fed infants were randomly assigned to receive either cow milk-based infant and follow-on formula with lower (**1.77** and 2.2 g protein/100 kcal, respectively) or higher (2.9 and 4.4 g protein/100 kcal, respectively) protein contents for the 1st year; a breastfed reference group was also included (n=619). Weight, length, weight-for-length and BMI were determined at 3, 6, 12, and 24 months of age. This study was <u>excluded</u> by the applicant and was not considered a "pertinent study" to support the proposed composition change in the protein quantity of follow-on formula since the LPF in Koletzko *et al.* (2009) did not align with our compositional requirement of 1.61±0.05 g protein/100 kcal.

[16] CODEX STAN 156-1987 states that follow-up formula (suitable for infants aged 6 months on and for young children) contain "Not less than **3.0 g per 100 available calories** (or 0.7 g per 100 available kilojoules) of protein of nutritional quality equivalent to that of casein or a greater quantity of other protein in inverse proportion to its nutritional quality. The quality of the protein shall not be less than 85% of that of casein. The total quantity of protein shall not be more than 5.5 g per 100 available calories (or 1.3 g per 100 available kilojoules)".

stress on immature organs as they work to excrete it. The Australian Infant Feeding Guidelines from the NHMRC (2012a) state "It is preferable to use a formula with a lower protein level" due to the evidence on excessive protein leading to later overweight and obesity.

Epidemiological evidence exists that links high protein intakes in infancy to overweight (including obesity) in childhood (Rolland-Cachera *et al.,* 1995; Gunnarsdottir and Thorsdottir, 2003; Günther *et al.,* 2007; Ohlund *et al.,* 2010), contributing to the 'early protein hypothesis' – *i.e.,* that high protein intakes in excess of metabolic requirements early in life enhance weight gain in infancy and increase obesity risk later in life (Koletzo *et al.,* 2005). Thus, the provision of adequate, but not excessive, amounts of protein in the 1st year of life can lead to health benefits in so far as reducing the risk of overweight and obesity in later life (Koletzko *et al.,* 2009; Inostroza *et al.,* 2014; Weber *et al.,* 2014; Ziegler *et al.,* 2015). When summarising the programming effects of early nutrition, Koletzko *et al.* (2017) stated that *"*In conclusion, it is very encouraging that the **conclusive evidence** for programming effects of infant protein supply provided by the European Union funded collaborative research has been rather rapidly adopted into policies, regulatory standards, and infant feeding practices, and hence **contributes effectively to the primary prevention of obesity and its associated disorders across populations**".

The specific economic impact of reducing the protein content in infant formula products has recently been studied (Marsh *et al.,* 2016). Health economic modelling in this study suggested this intervention had the ability to "translate into considerable health and economic benefits in the long term". The modelling showed a highly significant 3.9% reduction in direct health costs, mostly driven by reductions in obesity-related morbidity. This modelling was completed in the context of a Mexican healthcare system; as such, it is difficult to directly translate the cost and health savings to the Australian context. The modelling did, however, suggest a "10.5% reduction in the likelihood of developing obesity".

It has been well documented (Australian Institute of Health and Welfare, 2017) that the obesity rates in Australia are among the highest in the world; thus, any possible reduction in these rates would have enormous health and economic benefits locally. Both Australia and Mexico are in the top 5 countries for high obesity rates currently [Organisation for Economic Co-operation and Development (OECD) Obesity Report – OECD, 2017)]. The Australian Institute of Health and Welfare (2017) reported that 5% of the total burden of disease in Australia is attributable to overweight and obesity, and that it is a "major public health issue". It is clear therefore that the reduction in excessive protein (to levels closer to those found in human milk) has the potential to provide economic benefits to the healthcare system in an Australian context and has no potential to increase costs or negatively affect health in the Australian context.

Apart from the above health-related benefits, introducing a protein minimum of 1.6 g/100 kcal (0.38 g/100 kJ) into regulations for Australia and New Zealand, may likely facilitate harmonisation and trade, given Codex is in the process of revising their standards, and EU has already amended their regulations, to permit this amount under set conditions. Harmonisation of international requirements will assist with continuation of supply for the consumer and facilitate greater access to innovation in the interests of the formula-fed infant, when breastfeeding is not possible.

(b) Industry

See separate Confidential information.

(c) Government

No significant financial impact is anticipated, where the Application is paid.

4.1.2 Impact on International Trade

The proposed change is unlikely to negatively impact trade; rather, reduction of a protein minimum in follow-on formula will broaden the existing range of infant products available.

As Codex is revising a standard, and EU has already amended a standard, that permits a protein minimum of 1.6 g/100 kcal or 0.38g/100kJ (under set conditions) for follow-on formula, aligning Australian and New Zealand standards with these standards will help facilitate harmonisation opportunities and promote international trade (this is assuming other nutritional parameters do not present hurdles for harmonisation). This harmonisation will also reduce the number of future technical barriers to trade.

5.0 INFORMATION TO SUPPORT THE APPLICATION

Section 18 ("Objectives of the Authority in developing or reviewing food regulatory measures and variations of food regulatory measures") of the FSANZ Act (FSANZ, 2016b) states that in developing or reviewing food regulatory measures and variations of food regulatory measures, regard should be given to a risk analysis using (i) the best available scientific evidence and (ii) the promotion of consistency between domestic and international food standards. To ensure this application discusses the "best available evidence", a transparent, comprehensive, and relevant electronic literature search was conducted, as described below. In light of FSANZ's interest in promoting consistency between domestic and international food standards, as further described in Section 5.2.4, pertinent evidence in assessing our request to amend the Code is EFSA's (an internationally-recognised scientific authority) *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* or 0.38g/100kJ (EFSA, 2017) and the CCNFSDU revision (for adoption at Step 5) to the *Standard for Follow-Up Formula* (Codex Alimentarius, 2017a - CX/NFSDU 17/39/4 Rev.1). Both of these authoritative and credible bodies, under set conditions, support a decrease in the minimum protein requirement in follow-on formula used by infants 6 to 12 months of age, to 1.6 g/100 kcal (0.38g/100kJ).

Description of Literature Search Methods and Results

The applicant understands that a request to FSANZ to amend the Code must include human studies as supporting evidence for nutritional safety. For applications for follow-on formula (intended for older infants from 6 to 12 months of age), the applicant understands that FSANZ requires that human studies monitor and report growth measures for a minimum period of 2 months within the relevant age range (*i.e.,* 6 to 12 months). These important considerations were accounted for in the literature search methods described herein.

The applicant commissioned a comprehensive, systematic, and transparent search of the scientific literature to identify the totality of relevant scientific evidence on the nutritional effects of a follow-on formula with a protein to energy ratio that is aligned with that proposed in this application – *i.e.,* 1.6 g protein/100 kcal (0.38g/100kJ). The intent of the literature search was to retrieve studies conducted in *healthy term infants* up to 12 months of age wherein the effects, on growth, of a follow-on formula containing a 'lower protein' quantity (*i.e.,* 1.6±0.05 g[17] protein/100 kcal; 0.38g/100kJ) was compared with a follow-on formula containing a 'higher protein' quantity. Since the current FSANZ standard requires that follow-on formula have a protein content of no less than 0.45 g/100 kJ, which is equivalent to 1.88 g protein/100 kcal, the 'higher protein formula' had to have a protein content of ≥1.8 g protein/100 kcal. With these points in mind, the keywords used to execute the literature search are

[17] ± 0.05 g protein was considered by the applicant to be an acceptable deviation from the proposed minimum protein requirement of 1.6 g/100 kcal.

outlined below in Table 5-1. Importantly, the search required that keywords in each keyword category appear in either the title or abstract of publications. A search of the scientific literature was conducted using the electronic search tool ProQuest Dialog™. Four literature databases were searched on 1 December 2017, as described in Table 5-2 below. No limitations were placed on the publication language or publication date. Furthermore, the criteria used to establish the relevance of the retrieved articles are summarised in Table 5-3.

Table 5-1 Keywords Used to Execute the Literature Search

Keyword Category	Keywords
Matrix	Formula*[a]
Protein exposure	high* protein (NEAR/3[b]) or (high-protein) or (increased-protein) or protein increased (NEAR/3) **or** low* protein (NEAR/3) or (low-protein) or reduced-protein or protein reduced (NEAR/3)
Study population	Infant* or baby or babies
Health outcomes	*Weight or length or growth

[a] An asterisk (*) provides flexibility in the word ending (*e.g.,* formula* would retrieve "formula" or "formulas", or "formulae") or the beginning of the word (*e.g.,* *weight would retrieve "overweight" or "bodyweight" or "weight").
[b] "NEAR/3" requires that the words (*e.g.,* high protein) appear within 3 words of each other with either term appearing first within the record – *e.g.,* "high in protein".

Table 5-2 Summary of the Electronic Databases searched by ProQuest Dialog™

Electronic Database	Date Range	Update Frequency
BIOSIS Previews®	1926 to present	Weekly
CAB ABSTRACTS	1910 to present	Weekly
Embase®	1947 to present	Daily
MEDLINE®	1946 to present	Daily

Table 5-3 Criteria Used to Establish Literature Relevance

Inclusion Criteria
• A full-length article published in a peer-reviewed journal • A randomised and controlled intervention trial[a] conducted in formula-fed healthy term infants, regardless of maternal weight • The studies included an intervention group fed a LPF containing 1.6±0.05 g protein/100 kcal + an intervention group fed a HPF containing ≥1.8 g protein/100 kcal ± a breastfed reference group • The formula must, at the very least, have been fed to infants 6 to 12 months of age for ≥2 months • Health outcomes measured related to infant growth (*e.g.,* length, weight) or measures of nutritional status (*e.g.,* levels/intakes of vitamins, minerals or other relevant parameters) • Systematic reviews/meta-analyses that included infant intervention studies with the above-described study features • Unpublished study reports on infant intervention studies with the above-described study features
Exclusion Criteria
• A full-length article published in a non-peer-reviewed source (*e.g.,* website, magazine, *etc.*) • Published in abstract form only (full study report not available) or as a short communication (*e.g.,* letter to the editor, commentary, *etc.*) • Animal or *in vitro* study • Human observational study • Non-systematic research synthesis study (*e.g.*, narrative review) • Non-randomised or non-controlled intervention study conducted in formula-fed infants • Infants were not healthy term infants (*i.e.,* low birth weight or pre-term infants) • Formula fed for <2 months between 6 to 12 months of age • Formula fed to infants from 0 to 6 months of age and not beyond • Interventions did not include a LPF (1.6±0.05 g protein/100 kcal) and a HPF (≥1.8 g/100 kcal) • Non-relevant outcomes were measured • The study was a duplicate record in the literature search

Table 5-3 Criteria Used to Establish Literature Relevance

• Systematic reviews/meta-analyses on studies investigating lower *versus* higher protein formulas but not specifically on studies investigating effects of a LPF (1.6±0.05 g/100 kcal) and a HPF (≥1.8 g/100 kcal)

HPF = higher-protein formula; LPF = lower-protein formula.
[a] FSANZ states that human infant studies **must** include a control group (*i.e.,* an infant formula-fed group that is not exposed to the proposed compositional change), an exposure group (*i.e.,* a formula-fed group that is exposed to the proposed compositional change, plus a breastfed reference group. If a breastfed reference group was not included, a rationale for its omission is required.

The relevance of the retrieved articles was determined at 3 stages using the titles[18], abstracts[19], and then the full-length versions[20] of the publications. At each stage, the inclusion/exclusion criteria described above in Table 5-3 were applied to determine literature relevance.

As illustrated in Figure 5-1 below, the literature search resulted in the identification of 755 titles. Abstracts were retrieved for 144 of the records. The applicant was also aware of 1 additional abstract for which a full-length version of the publication was *not available*[21]. Of the 145 abstracts, 45 were considered to be potentially relevant and their full-length versions were reviewed. Three additional articles [Picone *et al.* (1989) and Fomon *et al.* (1995, 1999)] were identified *via* hand-searching [*i.e.,* upon review of the reference list of Ziegler *et al.* (2015)]; as such, a total of 48 full-length versions of publications were reviewed for relevance.

Of the 48 full-length articles reviewed, 46 met 1 or more of the exclusion criteria and 2 met all of the inclusion criteria (and none of the exclusion criteria) [Inostroza *et al*. (2014); Ziegler *et al*. (2015)]. The references of the excluded and included full-length articles are provided in Tables 5-4 and 5-5, respectively. All reviewed articles were in English.

[18] Titles of articles were reviewed, and abstracts of titles determined to be potentially relevant were retrieved. Reasons for the exclusion of titles were logged.
[19] Abstracts of articles were reviewed, and the full-length versions of abstracts determined to be potentially relevant were retrieved. Reasons for the exclusion of abstracts were logged.
[20] The full-length versions of articles were reviewed, and those determined not to meet all of the inclusion criteria or to meet any of the exclusion criteria were excluded. Reasons for the exclusion of full-length publications were logged.
[21] This abstract describes the growth of children at 3 and 5 years of age. Importantly, their growth during infancy was investigated in 1 of the 2 full-length publications included in this application as a pertinent safety and efficacy study on the effects of a lower-protein formula (Ziegler *et al.,* 2015). Because a full-length publication of this abstract is not available, this abstract was not discussed in this application.

Figure 5-1 Literature Search Summary

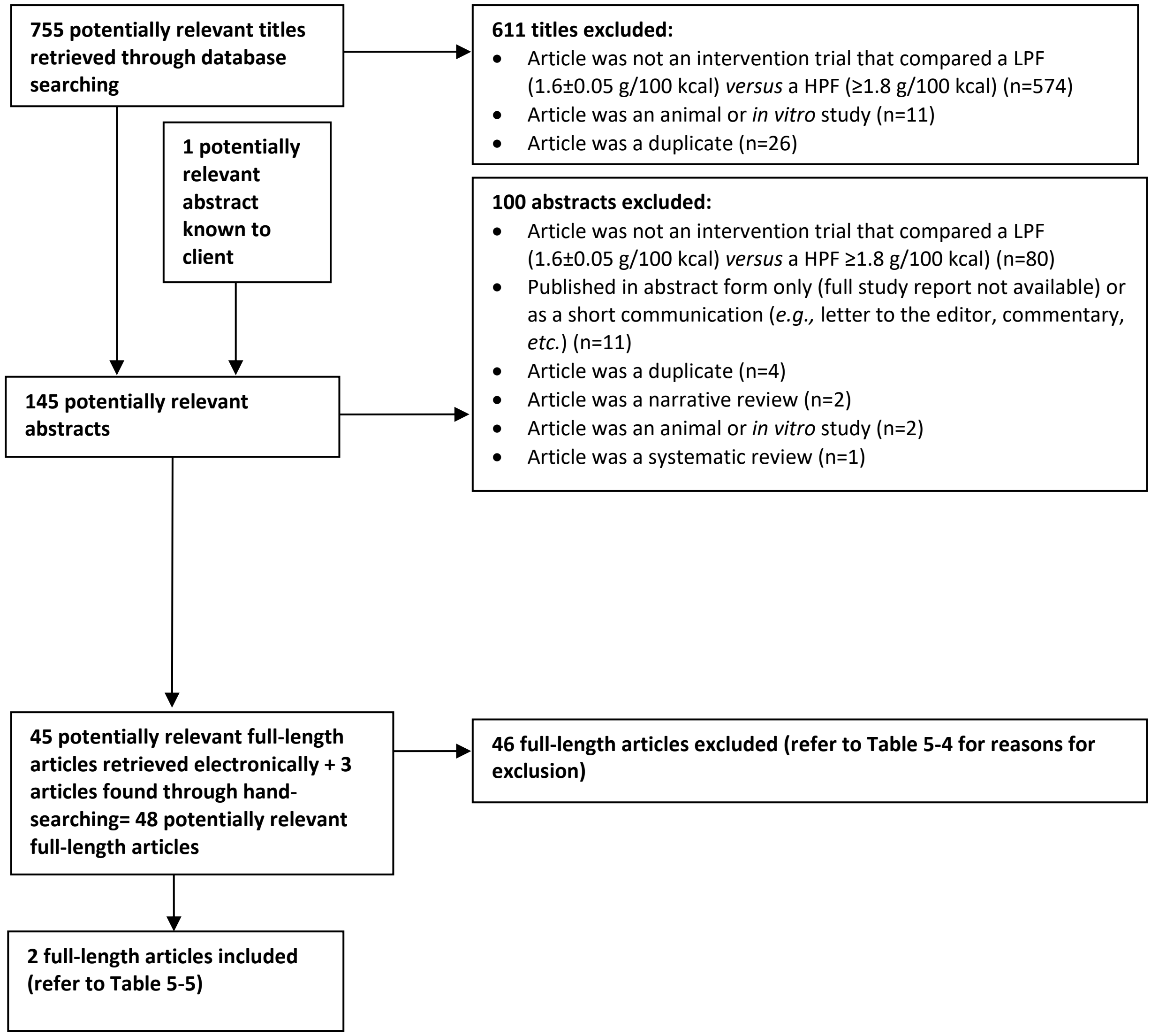

Table 5-4 Publications Excluded at the Full-Length Review Stage (n=46)

	Reference	Reason for Exclusion
1.	Akeson PM, Axelsson IE, Räihä NC (1998). Growth and nutrient intake in three- to twelve-month-old infants fed human milk or formulas with varying protein concentrations. J Pediatr Gastroenterol Nutr 26(1):1-8.	Article was not an intervention trial that compared a LPF (1.6±0.05 g/100 kcal) *versus* a HPF ≥1.8 g/100 kcal) (n=24)
2.	Alexander DD, Yan J, Bylsma LC, Northington RS, Grathwohl D, Steenhout P, et al. (2016). Growth of infants consuming whey-predominant term infant formulas with a protein content of 1.8 g/100 kcal: a multicenter pooled analysis of individual participant data. Am J Clin Nutr 104(4):1083-1092. DOI:10.3945/ajcn.116.130633.	
3.	Axelsson I, Borulf S, Righard L, Räihä N (1987). Protein and energy intake during weaning: I. Effects on growth. Acta Paediatr Scand 76(2):321-327. DOI:10.1111/j.1651-2227.1987.tb10468.x.	
4.	Axelsson IE, Jakobsson I, Räihä NC (1988). Formula with reduced protein content: effects on growth and protein metabolism during weaning. Pediatr Res 24(3):297-301. DOI:10.1203/00006450-198809000-00004.	
5.	Axelsson IEM, Ivarsson SA, Räihä NCR (1989). Protein intake in early infancy effects on plasma amino acid concentrations insulin metabolism and growth. Pediatr Res 26(6):614-617. DOI:10.1203/00006450-198912000-00020.	
6.	Carver JD, Wu PY, Hall RT, Ziegler EE, Sosa R, Jacobs J, et al. (2001). Growth of preterm infants fed nutrient-enriched or term formula after hospital discharge. Pediatrics 107(4):683-689.	
7.	Costa-Orvay JA, Figueras-Aloy J, Romera G, Closa-Monasterolo R, Carbonell-Estrany X (2011). The effects of varying protein and energy intakes on the growth and body composition of very low birth weight infants. Nutr J 10:140 [8pp]. DOI:10.1186/1475-2891-10-140.	
8.	Decsi T, Volker V, Szasz M, Ezer E, Mehes K (1992). Comparative study of breast-feeding and formula feeding in term infants. Orv Hetil 133(33):2087-2091 [Hungarian, English abstract].	
9.	Escribano J, Luque V, Ferre N, Mendez-Riera G, Koletzko B, Grote V, et al. (2012). Effect of protein intake and weight gain velocity on body fat mass at 6 months of age: *The EU Childhood Obesity Programme*. Int J Obesity 36(4):548-553. DOI:10.1038/ijo.2011.276.	
10.	Fomon SJ. Ziegler EE, Nelson SE, Frantz JA (1995). What is the safe protein-energy ratio for infant formulas? Am J Clin Nutr 62(2):358-363.	
11.	Fomon SJ, Ziegler EE, Nelson SE, Rogers RR,Frantz JA (1999). Infant formula with protein-energy ratio of 1.7 g/100 kcal is adequate but may not be safe. J Pediatr Gastroenterol Nutr 28(5):495-501.	
12.	Grote V, Kries RV, Closa-Monasterolo R, Scaglioni S, Gruszfeld D, Sengier A, et al. (2010b). Protein intake and growth in the first 24 months of life. J Pediatr Gastroenterol Nutr 51(Suppl. 3):S117-S118. DOI:10.1097/MPG.0b013e3181f96064.	
13.	Gruszfeld D, Weber M, Gradowska K, Socha P, Grote V, Xhonneux A, et al. (2016). Association of early protein intake and pre-peritoneal fat at five years of age: Follow-up of a randomized clinical trial. Nutr Metab Cardiovasc Dis 26(9):824-832. DOI:10.1016/j.numecd.2016.04.005.	
14.	Kashyap S, Forsyth M, Zucker C, Ramakrishnan R, Dell RB, Heird WC (1986). Effects of varying protein and energy intakes on growth and metabolic response in low birth weight infants. J Pediatr 108(6):955-963. DOI:10.1016/S0022-3476(86)80940-4.	
15.	Kirchberg FF, Harder U, Weber M, Grote V, Demmelmair H, Peissner W, et al. (2015). Dietary protein intake affects amino acid and acylcarnitine metabolism in infants aged 6 months. J Clin Endocrinol Metab 100(1):149-158. DOI:10.1210/jc.2014-3157.	
16.	Koo WWK, Hockman EM (2006). Posthospital discharge feeding for preterm infants: effects of standard compared with enriched milk formula on growth, bone mass, and body composition. Am J Clin Nutr 84(6):1357-1364.	
17.	Lönnerdal B, Chen CL (1990). Effects of formula protein level and ratio on infant growth, plasma amino acids and serum trace elements. I. Cow's milk formula. Acta Paediatr Scand 79(3):257-265. DOI:10.1111/j.1651-2227.1990.tb11454.x.	
18.	Räihä N C R, Heinonen K, Rassin DK, Gaull GE (1976). Milk protein quantity and quality in low birth weight infants: Part 1. Metabolic responses and effects on growth. Pediatrics 57(5):659-674.	
19.	Räihä N, Minoli I, Moro G (1986). Milk protein intake in the term infant. I. Metabolic responses and effects on growth. Acta Paediatr Scand 75(6):881-886. DOI:10.1111/j.1651-2227.1989.tb11204.x.	
20.	Roggero P, Giannì ML, Amato O, Liotto N, Morlacchi L, Orsi A, et al. (2012). Growth and fat-free mass gain in preterm infants after discharge: a randomized controlled trial. Pediatrics 130(5):e1215-e1221.	

Table 5-4 Publications Excluded at the Full-Length Review Stage (n=46)

	Reference	Reason for Exclusion
21.	Schulze KF, Stefanski M, Masterson J, Spinnazola R, Ramakrishnan R, Dell RB, et al. (1987). Energy expenditure, energy balance, and composition of weight gain in low birth weight infants fed diets of different protein and energy content. J Pediatr 110(5):753-759. DOI:10.1016/S0022-3476(87)80019-7.	
22.	Socha P, Grote V, Gruszfeld D, Janas R, Demmelmair H, Closa-Monasterolo R, et al. (2011). Milk protein intake, the metabolic-endocrine response, and growth in infancy: data from a randomized clinical trial (The European Childhood Obesity Trial Study Group). Am J Clin Nutr 94(6, Suppl.):1776S-1784S. DOI:10.3945/ajcn.110.000596.	
23.	Svenningsen NW, Lindroth M, Lindquist B (1982). Growth in relation to protein intake of low birth infants. Early Hum Dev 6(1):47-58. DOI:10.1016/0378-3782(82)90056-1.	
24.	Weber M, Grote V, Closa-Monasterolo R, Escribano J, Langhendries JP, Dain E, et al. (2014). Lower protein content in infant formula reduces BMI and obesity risk at school age: follow-up of a randomized trial (The European Childhood Obesity Trial Study Group). Am J Clin Nutr 99(5):1041-1051. DOI:10.3945/ajcn.113.064071.	
25.	Chierici R, Sawatzki G, Thurl S, Tovar K, Vigi V (1992). Experimental milk formulae with reduced protein content and desialylated milk proteins: influence on the faecal flora and the growth of term newborn infants. Acta Paediatr 86(6):557-563. DOI:10.1111/j.1651-2227.1997.tb08934.x.	Intervention was not administered to infants 6 to 12 months of age (n=3)
26.	Lien EL, Davis AM, Euler AR (2004). Growth and safety in term infants fed reduced-protein formula with added bovine alpha-lactalbumin. J Pediatr Gastroenterol Nutr 38(2):170-176.	
27.	Picone TA, Benson JD, Moro G, Minoli I, Fulconis F, Rassin DK, et al. (1989). Growth, serum biochemistries, and amino acids of term infants fed formulas with amino acid and protein concentrations similar to human milk. J Pediatr Gastroenterol Nutr 9(3):351-360.[a]	
28.	Boehm G, Lorenz I, Bergmann L, Müller DM, Beyreiss K (1987). Metabolic consequences of high protein intake in premature infants appropriate for gestational age. Biomed Biochim Acta 46(1):89-95.	Study population was not healthy, full-term infants (n=2)
29.	Wauben I, Westerterp K, Gerver WJ, Blanco C (1995). Effect of varying protein intake on energy balance, protein balance and estimated weight gain composition in premature infants. Eur J Clin Nutr 49(1):11-16.	
30.	Escribano J, Closa R, Luque V, Zaragoza M, Ferre N, Grote V (2010). The effects of increased protein intake on kidney size and function in healthy infants: A randomized clinical trial. Pediatr Nephrol 25(3):576. DOI:10.1007/s00467-009-1423-3.	Published in abstract form only (n=6)
31.	Grote V, Schiess S, Koletzko B (2010a). Solid introduction and growth in the first two years of life in formula-fed children. J Pediatr Gastroenterol Nutr 50(Suppl. 2):E24 [abstract PA-N-065]. DOI:10.1097/01.mpg.0000383075.98243.67.	
32.	Olling CCJ (1983). The effect of the amount of protein on the protein utilization, growth and renal load in feeding young infants. Kiel Milchwirtsch Forschungsber 35(3):445-449.	
33.	Patro-Gołąb B, Zalewski B, Kouwenhoven SMP, Karaś J, Koletzko B, van Goudoever JB, et al. (2016a). The effect of different protein concentration in infant formula on growth, body composition, and later risk of obesity: a systematic review. J Pediatr Gastroenterol Nutr 62(Suppl. 1):674. DOI:10.1097/01.mpg.0000484500.48517.e7.	
34.	Olling CC, Magendans-Post AP, Post AG, Wauters EA (1985). Protein requirements of young infants. The effect of protein content in food on growth, protein intake and protein utilization. Tijdschr Kindergeneeskd 53(1):11-20 [Dutch, English abstract].	
35.	Rezza E, Colombo U, Bucci G (1971). Early postnatal weight gain of low weight newborns: relationships with various diets and with intrauterine growth. Helv Paediatr Acta 28(3):340-352.	
36.	Premji S, Fenton T, Sauve R (2006a). Does amount of protein in formula matter for low-birthweight infants? A Cochrane systematic review. JPEN J Parenter Enteral Nutr 30(6):507-514. DOI:10.1177/0148607106030006507.	Duplicate (n=1)
37.	Haschke F, Grathwohl D, Detzel P, Steenhout P, Wagemans N, Erdmann P (2016). Postnatal high protein intake can contribute to accelerated weight gain of infants and increased obesity risk. In: Fewtrell MS, Haschke F, Prescott SL, editors. *Preventive Aspects of Early Nutrition*. 85th Nestlé Nutrition Institute Workshop, Nov. 16-19, 2014, London, UK. (Nestlé Nutrition Institute Workshop Series, vol. 85). Vevey, Switzerland: Nestec, Ltd. / Basel, Switzerland: S. Karger AG, pp. 101-109. DOI:10.1159/000439492.	Narrative review (n=3)

Table 5-4 Publications Excluded at the Full-Length Review Stage (n=46)

	Reference	Reason for Exclusion
38.	Michaelsen KF, Larnkjær A, Mølgaard C (2012). Amount and quality of dietary proteins during the first two years of life in relation to NCD risk in adulthood. Nutr Metab Cardiovasc Dis 22(10):781-786. DOI:10.1016/j.numecd.2012.03.014.	
39.	Tijhuis MJ, Doets EL, Noordegraaf-Schouten MV (2014). *Extensive Literature Search and Review as Preparatory Work for the Evaluation of the Essential Composition of Infant and Follow-on Formulae and Growing-up Milk.* (EFSA Supporting Publication 2014-EN-551). European Food Safety Authority (EFSA). Available at: https://www.efsa.europa.eu/en/supporting/pub/en-551.	
40.	Koletzko B, Broekaert I, Demmelmair H, Franke J, Hannibal I, Oberle D, et al. (2005). Protein intake in the first year of life: a risk factor for later obesity? The E.U. childhood obesity project. In: Koletzko B, Dodds P, Akerblom H, Ashwell M, editors. *Early Nutrition and its Later Consequences: New Opportunities. Perinatal Programming of Adult Health — EC Supported Research*. (Advances in Experimental Medicine and Biology). New York (NY): Springer Science + Business Media, Inc, pp. 69-79.	Non-peer reviewed source (n=1)
41.	Patro-Gołąb B, Zalewski B, Kouwenhoven SMP, Karaś J, Koletzko B, van Goudoever JB, et al. (2016b). Protein concentration in milk formula, growth, and later risk of obesity: a systematic review. J Nutr 146(3):551-564. DOI:10.3945/jn.115.223651.	Systematic review (n=5)
42.	Abrams SA, Hawthorne KM, Pammi M (2015). A systematic review of controlled trials of lower-protein or energy-containing infant formulas for use by healthy full-term infants. Adv Nutr 6(2):178-188. DOI:10.3945/an.114.006379.	
43.	Tonkin EL, Collins CT, Miller J (2014). Protein intake and growth in preterm infants: a systematic review. Glob Pediatr Health [published online – Oct. 15, 2014]. DOI:10.1177/2333794X14554698.	
44.	Fenton TR, Premji SS, Al-Wassia H, Sauve RS (2014). Higher versus lower protein intake in formula-fed low birth weight infants. Cochrane Database Syst Rev (4):CD003959. DOI:10.1002/14651858.CD003959.pub3.	
45.	Premji SS, Fenton TR, Sauve RS (2006b). Higher versus lower protein intake in formula-fed low birth weight infants. Cochrane Database Syst Rev (1):CD003959. DOI:10.1002/14651858.CD003959.pub2.	
46.	Martin F-PJ, Moco S, Montoliu I, Collino S, Da Silva L, Rezzi S, et al. (2014). Impact of breast-feeding and high- and low-protein formula on the metabolism and growth of infants from overweight and obese mothers. Pediatr Res 75(4):535-543. DOI:10.1038/pr.2013.250.[a]	Kin publication (n=1)[b]

[a] Article was identified *via* hand-searching – *i.e.,* upon review of the reference list of Ziegler *et al.* (2015).
[b] Article is a kin publication of Inostroza *et al.* (2014) in which the same study population was studied. Martin *et al.* (2014) highlights changes in metabolic outcomes, such as urinary metabolites and excretion patterns, and amino acid and short-chain fatty acid metabolism in stool. Since Inostroza *et al.* (2014) highlights changes in nutritional parameters related to growth, such as length and weight, it was considered to be the more pertinent publication; as such, Martin *et al.* (2014) was excluded from evaluation and Inostroza *et al.* (2014) was included.

Table 5-5 Relevant Publications Meeting Study Eligibility Criteria (n=2)

	Reference	PDF
1.	Ziegler EE, Fields DA, Chernausek SD, Steenhout P, Grathwohl D, Jeter JM, et al. (2015). Adequacy of infant formula with protein content of 1.6 g/100 kcal for infants between 3 and 12 months. J Pediatr Gastroenterol Nutr 61(5):596-603. DOI:10.1097/MPG.0000000000000881.	
2.	Inostroza J, Haschke F, Steenhout P, Grathwohl D, Nelson SE, Ziegler EE (2014). Low-protein formula slows weight gain in infants of overweight mothers. J Pediatr Gastroenterol Nutr 59(1):70-77. DOI:10.1097/MPG.0000000000000349.	

5.1 Application to Vary the Standards for Substances Added to Food – Substances Used for a Nutritive Purpose

5.1.1 Information on the Use of the Nutritive Substance

5.1.1.1 Information on the purpose of the use of a nutritive substance in food

Dietary protein is an essential component of the diet, supplying the body with nitrogen and amino acids (EFSA, 2017), which are needed for the synthesis of nucleic acids, hormones and vitamins (IOM, 2005). Proteins are the major structural components of all cells in the body (IOM, 2005) and are essential in growth and development (Dupont, 2003), including the development of the brain and bones (Bonjour *et al*., 2001). Proteins also function as enzymes and transport carriers (IOM, 2005).

The principal proteins in human milk are whey proteins (*e.g.,* α-lactalbumin, lactoferrin, immunoglobulins, and lysozyme) and casein proteins (*e.g.,* β-casein and κ-casein) (Lönnerdal *et al.,* 2017). Human milk also contains milk fat globule membrane proteins; however, they only contribute a small percentage to the true protein content of human milk (Patton and Huston*,* 1986).

Proteins play a particularly important function in infancy (*i.e.,* birth to 12 months), when growth and brain development are at their peak. In fact, during the 1st year of life, ~87% of protein intake over and above that used for maintenance is utilised for tissue synthesis (Dewey *et al.,* 1996 cited in Dupont, 2003). It is estimated that protein synthesis in infants averages 6.9 g/kg/day (Young *et al*., 1975; IOM, 2005). Many proteins in human milk have demonstrated roles beyond nutrition, providing enzymatic activity, enhancing nutrient absorption, stimulating growth, modulating the immune system and defending against pathogens by inhibiting bacterial adhesion (Lönnerdal *et al.,* 2017).

The types of protein used in infant and follow-on formulas (*i.e.,* whey and casein) and their ratio are intended to mimic the whey to casein ratio observed in human milk for most of the lactation period (Nagasawa *et al.,* 1972; Nagra, 1989; Kunz and Lönnerdal, 1992; Montagne *et al.,* 2000; Lönnerdal and Kelleher, 2009). Similarly, the amino acid profile of infant formulas is intended to mimic their profile in human milk with the intent that proteins provided in infant and follow-on formulas achieve similar functions (*i.e.,* infant growth and development) to naturally-occurring proteins in human milk.

The request made in this application to decrease the minimum level of protein (Section 3.0), an already-permitted nutritive substance in follow-on formula for infants 6 to 12 months of age, is for achieving growth rates, measured by infants' length, weight, and head circumference, that are more comparable to breastfed infants.

In 2 randomised, double-blind, controlled intervention studies conducted in Chile (Inostroza *et al.,* 2014) and in the U.S. (Ziegler *et al.,* 2015), the effects, on infant growth, of a LPF (1.61 or 1.65 g/100 kcal in Ziegler *et al.,* 2015 and Inostroza *et al.,* 2014, respectively; 0.39g/100kJ) were compared to a HPF (2.15 g/100 kcal or 2.70 g/100 kcal in Ziegler *et al.,* 2015 and Inostroza *et al.,* 2014, respectively, 0.51 or 0.65g/100kJ respectively) and also to a breastfed reference group. The formulas were administered from 3 to 12 months of age[22]. In both studies, from 6 to 12 months for 'completers' and the 'per protocol population', weight gain (g/day) and weight change (kg) in the lower-protein group *versus* the breastfed reference group were more similar as compared to the higher-protein group *versus* the breastfed reference group (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015). Importantly, across both studies for 'completers' and the 'per protocol population', compared to breastfed infants, the LPF did not adversely affect the weight, length and head circumference of infants at 6 and 12 months [*i.e.,* the LPF

[22] In both studies (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015), complementary foods were unrestricted from 6 to 12 months of age.

led to mean increases in weight (kg), length (cm) and head circumference (cm) compared to breastfed infants]; see Appendices A to D in EFSA (2017). These studies support both the safety and efficacy of protein at the proposed level of 1.6 g total protein/100 kcal.

5.1.1.2 General data requirements for supporting evidence

The commercial product for which approval is being sought is a follow-on formula delivering a minimum of **1.6 g (0.38g/100kJ) of total protein from milk or other edible food constituents of animal origin/100 kcal of formula[23].**

The follow-on formula will otherwise meet the requirements stated in Standard 2.9.1 (Infant formula products) (FSANZ, 2017), as they relate to energy content, fat content, potential renal solute load, and levels of L-amino acids[24], vitamins, minerals, and electrolytes, and other permitted optional ingredients (*e.g.,* lactic acid-producing microorganisms, inulin-type fructans, and galacto-oligosaccharides).

The content of protein (1.61 or 1.65 g total protein/100 kcal; 0.39g/100kJ) administered to infants receiving the LPFs in the relevant efficacy/safety/tolerability studies (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015) described in Section 5.2 is comparable to the level of protein proposed in this application for inclusion into follow-on formulas (1.6 g total protein/100 kcal; 0.38g/100kJ).

5.1.2 Technical Information on the Use of the Nutritive Substance

This application is not being lodged to extend the use of a nutritive substance; as such, the application requirements stated under B.1 to B.7 in Section 3.3.3 (Substances Used for a Nutritive Purpose) of FSANZ's *Application Handbook* are considered "not applicable".

5.1.3 Information Related to the Safety of the Nutritive Substance

This application is not being lodged for a new nutritive substance nor for extending the use of a currently-permitted nutritive substance; as such, the application requirements stated under C.1 to C.3 in Section 3.3.3 (Substances Used for a Nutritive Purpose) of FSANZ's *Application Handbook* are considered "not applicable".

Protein is an essential nutrient for infants as it is required to meet their physiological needs for growth and development. Protein is currently approved for addition to infant formula products including follow-on formula; therefore, safety with regard to addition of protein is not addressed here. In this Application, a decrease in the minimum requirement for protein in follow-on formula for infants 6 to 12 months of age is requested; as such, we do not consider the toxicokinetics and metabolism of the nutritive substance to be relevant. For this Application, we consider adequate growth and development to be measures of safety, which have been discussed in preceding sections.

Two infant intervention studies (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015) provide clinical evidence that the proposed compositional change is safe and well tolerated. In these 2 pertinent studies, the effects on anthropometric parameters (length, weight, head circumference) of a LPF containing 1.61 or 1.65 g total protein/100 kcal (0.39g/100kJ), that aligns with the proposed compositional change of a minimum 1.6 g total protein/100 kcal (0.38g/100kJ), was compared to a HPF (2.15 or 2.7 g total protein/100 kcal; 0.51 – 0.65g/100kJ) and a reference group comprised of breastfed infants. The formulas were fed to

[23] In Standard 2.9.1, Section 2.9.1-3 (Definitions), an "infant formula product" is defined as "a product based on milk or other edible food constituents of animal or plant origin which is nutritionally adequate to serve by itself either as the sole or principal liquid source of nourishment for infants, depending on the age of the infant" (Australian Government, 2017a).
[24] By adding appropriate amounts of free amino acids and micronutrients during the manufacturing process, manufacturers will ensure that the amino acid and micronutrient profiles of the proposed follow-on formula will comply with Standard 2.9.1.

infants from 3 to 12 months of age; thus, anthropometric changes were monitored for a minimum period of 2 months within the relevant age range of infants for the use of a follow-on formula (6 to 12 months), as required by FSANZ. The key characteristics and results of both these studies are further described in Section 5.2.1.3.2.

5.1.4 Information on Dietary Intake of the Nutritive Substance

5.1.4.1 A detailed list of the food groups or foods in which the use of a nutritive substance is proposed, or changes to currently permitted foods in which a nutritive substance is used

The nutritive substance (protein) is proposed for use at a lower level (minimum 1.6 g protein/100 kcal *versus* 1.88 g protein/100 kcal; equivalent to 0.38g/100kJ versus 0.45g/100kJ) in **follow-on formula used by infants 6 to 12 months of age**. It should be noted that the targeted levels (average quantity) will be higher in the commercial product. The follow-on formula will otherwise meet the requirements stated in Standard 2.9.1 (Infant formula products) (FSANZ, 2017), as they relate to the essential composition of energy content, fat content, potential renal solute load, and levels of L-amino acids, vitamins, minerals, and electrolytes[25] and other permitted optional ingredients (*e.g.,* lactic acid-producing microorganisms, inulin-type fructans, and galacto-oligosaccharides).

5.1.4.2 The maximum proposed level of the use of the nutritive substance for each food group or food, or the proposed changes to the currently permitted use levels

The nutritive substance (protein) is proposed for use at a lower minimum level of **1.6 g total protein/ 100 kcal** (0.38 g/100 kJ) *versus* 1.88 g total protein/100 kcal (0.45 g/100 kJ, as required in Standard 2.9.1) in follow-on formula used by infants 6 to 12 months of age.

This Application does not propose to amend the current maximum level for protein in follow-on formula, which is currently stated as 1.3 g/100 kJ (~5.4 g/100 kcal) in Standard 2.9.1. However, the Applicant anticipates this could be part of a future proposal by FSANZ.

5.1.4.3 For foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs), information on the likely level of consumption

No national Australian or New Zealand nutrition survey data currently exist for children <2 years of age to model the likely consumption of protein (from all dietary sources) of infants 6 to 12 months of age, should follow-on formula be re-formulated to meet a lower minimum protein requirement (*i.e.,* 1.6 g total protein/100 kcal or 0.38g/100kJ). Since European dietary intake data exist for this age group, the EFSA was able to conduct protein intake modelling in this regard (EFSA, 2017).

As summarised in EFSA's Scientific Opinion (2017), using individual data from 3 surveys conducted in Bulgaria (24-hour dietary recall over 3 days), Denmark (7-day dietary record), and the United Kingdom (4-day dietary record), which were available in the EFSA Comprehensive Food Consumption Database, the 5th and 2.5th percentiles of total protein intake in non-breastfed infants 6 to 12 months of age were calculated assuming:

1. All follow-on formula consumed by infants contained **1.6 g total protein/100 kcal (0.38g/100kJ)**;

[25] By adding appropriate amounts of free amino acids and micronutrients during the manufacturing process, manufacturers will ensure that the amino acid and micronutrient profiles of the proposed follow-on formula will comply with Standard 2.9.1.

2. The energy content of the formulas did not change;

3. Protein intake from other sources (infant formula, complementary foods) did not change[26].

The results of EFSA's analysis are summarised in Table 5.1.4.3-1 below.

Table 5.1.4.3-1 Protein Intakes in European Non-breastfed Infants 6 to 12 Months of age, assuming a Protein Content in Follow-on Formula of 1.6 g Total Protein/100 kcal, as Estimated by the EFSA (EFSA, 2017)[a]

Country	n	Original Survey			Re-calculation of Protein Intakes by the EFSA assuming Follow-on Formula Contained 1.6 g protein/100 kcal		PRI (g/day) for Protein for European Infants 6 to 12 mo of age[b]	IOM's RDA (g/day) for protein for infants 7 to 12 mo of age
		Mean Energy Intake (kcal/day)	Mean Protein Intake (g/day)	5^{th}, 2.5^{th} Percentiles of Protein Intake (g/day)	Mean Protein Intake (g/day)	5^{th}, 2.5^{th} Percentiles of Protein Intake (g/day)		
Bulgaria	343	859	27.4	14.1, 11.9	27.2	13.7, 11.9	Girls: 8 (6 mo) to 10 (12 mo) Boys: 9 (6 mo) to 11 (12 mo)	11.0
Denmark	473	933	30.0	16.2, 14.7	29.4	15.8, 13.8		
United Kingdom	1,029	790	25.2	13.0, 11.7	24.4	12.6, 11.2		

EFSA = European Food Safety Authority; IOM = Institute of Medicine; mo = months; n = number of infants; PRI = Population Reference Intake; RDA = Recommended dietary allowance; WHO = World Health Organization.
[a] Parts of this table were replicated or adapted from EFSA (2017).
[b] The PRIs were generated by the EFSA as follows: "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 mo and a PRI of 11 g protein per day for girls and 12 g protein per day for boys aged 12 mo were established (EFSA, 2012). The use of the 50th percentile of the WHO Growth Standards (WHO, 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 mo and a PRI of 10 g protein per day for girls and 11 g protein per day for boys aged 12 mo (EFSA, 2013)".

The following can be concluded from an analysis of the data provided in Table 5.1.4.3-1:

- Older infants (6 to 12 months of age) are consuming, on average, an excess of protein – mean dietary protein intakes of this age group (25.2 to 30.0 g/day) far exceed the EU's Population Reference Intakes (PRIs) (8 to 11 g protein/day) and the IOM's RDA (11 g protein/day).

- With a reduction in protein in follow-on formula to 1.6 g total protein/100 kcal (0.38g/100kJ), the re-calculated daily protein intakes for infants 6 to 12 months of age were estimated by the EFSA to be 24.4 to 29.4 g protein/day across Bulgaria, Denmark or the United Kingdom, which represent decreases of 0.2 to 0.8 g/day from the mean daily protein intakes from the original surveys (27.4, 30.0, and 25.2 g/day, respectively).

- With a reduced protein minimum in follow-on formula to 1.6 g total protein/100 kcal (0.38g/100kJ), the re-calculated protein intakes at the 5^{th} and 2.5^{th} percentiles for infants 6 to 12 months of age living in Bulgaria, Denmark or the United Kingdom decreased by 0 to 0.9 g/day from their estimations as per the original surveys.

[26] Table 9 in EFSA (2017) lists 17 categories of foods that contributed to the daily protein intake in infants 6 to 12 months of age, living in Bulgaria, Denmark, and the United Kingdom. Of the 17 categories and not including the category of "infant formula and follow-up formula", the groups most greatly contributing to protein intakes were: "milk and dairy products"; "grains and grain-based products"; "foods for infants and small children"; and, "meat and meat products".

- With a reduced protein minimum in follow-on formula to 1.6 g total protein/100 kcal (0.38g/100kJ), for infants 6 to 12 months of age and living in Bulgaria, Denmark or the United Kingdom, protein intakes at the 2.5th percentile (*i.e.,* 11.2 to 13.8 g/day) meet and exceed the upper end of the PRIs established for European infants 6 to 12 months of age (*i.e.,* 11 g/day) and meet and exceed the RDA[27] established by the IOM for infants 7 to 12 months of age (*i.e.,* 11.0 g/day) (IOM, 2005).

To put the above analyses into a context that is relevant to infants living in Australia and New Zealand, dietary protein reference values in this jurisdiction need to be considered. In Australia and New Zealand, the AI for protein established for infants 7 to 12 months of age is <u>14 g/day</u> or 1.60 g/kg body weight/day (NHMRC, 2014). This AI was established by considering (i) the mean protein content of human milk at 7 to 12 months of lactation; (ii) the average volume of breast milk consumed during this period; and (iii) the mean protein intake from complementary foods for breastfed infants aged 7 to 12 months[28]. The Australian Government's NHMRC calculated the AI for protein for infants 7 to 12 months of age as 13.7 g/day, rounded up to 14 g/day, by adding the protein intake from human milk (6.6 g/day) and complementary foods (7.1 g/day). However, the applicant believes that the IOM's RDA for infants 7 to 12 months of age (11.0 g/day) is more representative of protein requirements in this age group as compared to the AI established by the Australian Government's NHMRC (14 g/day), since the RDA is based on "estimated average requirements" (EAR), which considers the variation in maintenance needs and the variation in the rate of protein deposition (protein for growth)[29]. Indeed, Lioret *et al.* (2013), who tracked the dietary intakes (including protein intakes) of children living in Australia at 9 and 18 months of age, acknowledge that "nutrient adequacy based on the AIs should also be considered carefully, as the evidence base for AI is weaker than for EARs".

In light of the intake modelling conducted by the EFSA described above, that a reduced protein minimum in follow-on formula to 1.6 g total protein/100 kcal generated protein intakes of 11.2 to 13.8 g/day for infants 6 to 12 months of age at the 2.5th percentile is noteworthy since these intakes, although not exceeding the Australian Government's NHMRC's AI of 14 g/day, do meet and exceed the IOM's protein RDA for infants 7 to 12 months of age (*i.e.,* 11.0 g/day) and, indeed, the latter can be defended as a more appropriate reference value. Using IOM's RDA for protein for infants 7 to 12 months of age (11.0 g/day), it is expected that a reduction in the protein minimum in follow-on formula to 1.6 g total protein/100 kcal will meet the protein intake requirements for 97.5% of infants aged 6 to 12 months who live in Australia/New Zealand.

Notwithstanding that the Australian Government NHMRC's AI for protein of 14 g/day is not met by infants at the 5th (based on data from the United Kingdom) or 2.5th (based on data from the United Kingdom and Bulgaria) percentiles of protein intakes (see Table 5.1.4.3-1), with a reduction of protein in

[27] The RDA is defined as covering the intake requirements of 97.5% of the age group (IOM, 2005).
[28] Specifically, the Australian Government's NHMRC used the following estimations to calculate the AI (i) the mean protein content of human milk at 7 to 12 months of lactation is estimated at <u>11 g/L</u> (Dewey *et al.,*1984, Mitoulas *et al.,* 2002, Nommsen *et al.,* 1991), (ii) the average volume of breast milk consumed during this period is estimated at 0.6 L/day (Heinig *et al.,* 1993 – in IOM, 2005), and (iii) the mean protein intake from complementary foods for breastfed infants aged 7 to 12 months is estimated at 7.1 g protein/day (U.S. NHANES III data – IOM, 2002). Thus, the NHMRC calculated the AI as: 11 g protein/L human milk x 0.6 L human milk consumed/day = 6.6 g protein/day (human milk) + 7.1 g protein/day (complementary foods) = **13.7 g protein/day, rounded up to 14 g/day** and, assuming a reference body weight of 9 kg = 1.6 g/kg body weight/day (NHMRC, 2014).
[29] The RDA of 11.0 g protein/day or 1.2 g protein/kg/day for infants 7 to 12 months was calculated by the IOM using EAR for older infants, which takes into account the variation in maintenance needs and the variation in the rate of protein deposition (protein for growth) (IOM, 2005). The IOM estimated the EAR by the factorial method by considering the median (110 mg nitrogen/kg/d, equivalent to 688 mg protein/kg/d) of the nitrogen intake for nitrogen equilibrium, the efficiency of protein utilisation for growth, and the mean protein deposition for boys and for girls. The resulting mean protein requirement was estimated as 1.0 g/kg body weight/d for boys and for girls (IOM, 2005).

a follow-on formula to 1.6 g total protein/100 kcal, the daily protein intakes of the majority of infants 6 to 12 months of age would nevertheless meet the NHMRC's AI.

While there are no Australian or New Zealand NNSs on the likely consumption of protein (from all dietary sources) in infants 6 to 12 months of age, dietary surveys informing on the protein intakes of 9-month old infants were conducted in Australia in 1999 to 2001 (Conn *et al.,* 2009) and 2008 to 2009 (Lioret *et al*., 2013). From these 2 studies, the mean (±SD) protein intakes of 9-month old infants were **29.0±10.9 g protein/day for girls and boys** (Lioret *et al.,* 2013; **n=177**; breastfed or not breastfed + complementary foods); **26±8 g protein/day for girls** (Conn *et al.,* 2009; **n=161**; breastfed or not breastfed + complementary foods); and, **29±8 g protein/day for boys** (Conn *et al.,* 2009; **n=180**; breastfed or not breastfed + complementary foods). Given the mean daily protein intakes (~26 to 29 g/day) and also the median daily protein intakes (25 to 29 g/day) across both studies, which were conducted a decade apart, it appears that protein intakes have remained quite stable over a span of 10 years. **These data also indicate that, at the mean and median, 9-month old infants living in Australia (Melbourne or Adelaide) are far exceeding (by about 2-fold) the Australian Government NHMRC's AI (14 g/day) and also the IOM's RDA (11.0 g/day) established for dietary protein for older infants (7 to 12 months).** Protein intakes in excess of metabolic requirements have public health implications, as discussed earlier[30]. Indeed, dietary intake analyses conducted by Campbell *et al.* (2017), who report on the same study cohort as described by Lioret *et al.* (2013), provide evidence that protein intakes at 9 and 18 months can predict intakes at 5 years – *i.e.,* the residualised protein intakes at 9 months were significantly associated with intakes at 18 months (p=0.007) and 5 years (p=0.006). Lowering the currently excessive protein intakes in older infants who are not breastfed could result in potential health benefits in the local Australian-New Zealand context, namely a reduced risk of excessive protein intakes during and after infancy, and a reduced risk of overweight/obesity later in life.

In establishing the AI for protein for infants 7 to 12 months of age, the NHMRC considered the mean protein intake from complementary foods for breastfed infants aged 7 to 12 months to be 7.1 g protein/day (U.S. NHANES III data – IOM, 2005; NHMRC, 2014), with 6.6 g protein/day delivered by human milk. In the absence of human milk contributing to protein intakes, it is of interest to assess whether infants consuming the LPFs in 2 pivotal studies (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015) averaged protein intakes of at least 6.6 g/day. In Ziegler *et al.* (2015), actual average intakes of the LPF were 917 mL/day at 6 months of age, 850 mL/day at 8 months of age, 810 mL/day at 10 months of age, and 719 mL/day at 12 months of age. Given the composition of the LPF was 1.08 g protein per 100 mL, this corresponds to a daily protein intake from the principal milk source of 9.9 g/day at 6 months, 9.18 g/day at 8 months, 8.75 g/day at 10 months, and 7.77 g/day at 12 months. The intake data from the LPF group in Inostroza *et al*. (2014) were similar, with 980 mL/day (10.2 g protein/day) consumed at 6 months of age, 896 mL/day (9.3 g protein/day) consumed at 9 months of age, and 854 mL/day (8.8 g protein/day) consumed at 12 months of age (EFSA, 2017)[31]. **Thus, in Ziegler *et al.* (2015) and Inostroza *et al. (*2014), from 6 to 12 months of age, the average daily protein intakes of infants that was consumed from their principal milk source (LPF) ranged from 7.77 to 10.2 g protein/day, levels which are above 6.6 g protein/day – the amount of protein typically consumed by breastfed infants 7 to 12 months of age.**

[30] Epidemiological evidence exists that links high protein intakes in infancy to overweight (including obesity) in childhood (Ohlund *et al.,* 2010; Rolland-Cachera *et al.,* 1995; Gunnarsdottir and Thorsdottir, 2003; Günther *et al.,* 2007), contributing to the 'early protein hypothesis' – *i.e.,* that high early protein intakes in excess of metabolic requirements enhance weight gain in infancy and increase later obesity risk (Koletzo *et al.,* 2005).

[31] It should be noted that the formula intakes reported in the supplementary tables in Inostroza et al. (2014) are not correct; however, they are correctly reported in Table 12 in EFSA (2017).

5.1.4.4 *The percentage of the food group to which the use of the nutritive substance is proposed or the percentage of the market likely to use the nutritive substance*

The proposed reduction of minimum protein to 1.6 g/100 kcal (0.38 g/100 kJ) is only applicable to follow-on formula for infants 6 to 12 months of age as regulated by Standard 2.9.1.

In deriving the estimated percentage of the food group (*i.e.,* follow-on formula) in which a protein level of 1.6 g/100 kcal (0.38g/100kJ) will be used, it can be assumed, as the *most conservative* measure, that this level of protein will be included in all follow-on formula products marketed in Australia and New Zealand – *i.e.,* all manufacturers will implement this lower protein level. The current total market size in volume (kg of product sold) for follow-on formula (all brands) in Australia and New Zealand based on scan sales is 9,001,872 kilos (9002 MT) based on 7,965,490 kilos (for Australia) and 1,036,382 kilos (for New Zealand) (Neilsen MAT 4.03.2018, AU; 8.04.2018, NZ).

In reality, however, it is unlikely that follow-on formula with a protein level of 1.6 g/100 kcal (0.38g/100kJ) will have 100% market penetration across all industry players. At minimum, market penetration of a follow-on formula with a protein level of 1.6 g/100 kcal (0.38g/100kJ) will be initiated by the Applicant *via* 1 specific brand of a follow-on formula. As such, at minimum, the percentage of the market and corresponding volume of product sold are represented in Table 5.1.4.4-1 below, based on scan sales data.

Table 5.1.4.4-1 Market Data in Australia and New Zealand for 1 Brand of a Follow-on Formula

Follow-on Formula	Volume of Sales (kg of product sold)	% Market Share
Australia		
Brand A	967,000	11.3%[a]

[a] Source: AC Nielsen MAT to 04.03.2018

5.1.4.5 *Information relating to the use of the nutritive substance in other countries*

Australia/New Zealand and the EU have standards for both follow-on formula (from 6 months onward) and infant formula. Similarly, Codex has recommendations regarding the composition of both follow-on formula and infant formula. As described in Section 5.2.4, Codex is currently updating its *Standard for Follow-Up Formula* (Codex Alimentarius, 2017a – CX/NFSDU 17/39/4 Rev.1). In the below Table 5.1.4.5-1, standards for follow-on formula are summarised, as they apply to Australia/New Zealand and the EU or as recommended by Codex.

Table 5.1.4.5-1 Details regarding the Protein Composition of Formula Intended for Use by Infants 6 to 12 Months of Age as Specified in Standards across Key Jurisdictions or as Recommended by Codex

Jurisdiction or Authority	Pertinent Regulation or Standard	Pertinent Definition	Permitted Protein Use Levels in Formula		Specifics on Type/Quality of Protein
			Minimum	Maximum	
Australia and New Zealand	Standard 2.9.1 (FSANZ, 2017)	'Follow-on formula' means an infant formula product that: (a) is represented as either a breast-milk substitute or replacement for infant formula; and (b) is suitable to constitute the principal liquid source of nourishment in a progressively diversified diet for infants from the age of 6 months.	0.45 g/100 kJ (1.88 g/100 kcal)	1.3 g/100 kJ (5.4 g/100 kcal) or 1.4 g/100kJ (5.85 g/100 kcal) (for IFPSDU protein substitutes)	Protein content must be calculated in accordance with the equation set out in Section S29—3, Schedule 29 (FSANZ, 2016)[32]. Based on milk or other edible food constituents of animal or plant origin[33]. L-amino acids may be added[34].
EU	Commission Directive 2006/141/EC (EC, 2006)	'Follow-on formulae' means foodstuffs intended for particular nutritional use by infants[35] when appropriate complementary feeding is introduced	0.38 g/100 kJ (1.6 g/100 kcal)	0.6 g/100 kJ (2.5 g/100 kcal	Cow's or goat's milk protein.[37]
			0.56 g/100 kJ (2.25 g/100 kcal)	0.8 g/100 kJ (3.5 g/100 kcal)	Protein hydrolysates

[32] S29-3 states that the protein content (PC) of infant formula product must be calculated in accordance with the following equation: PC=NC x F, where NC is the nitrogen content of the infant formula product and F is 6.38 (for milk proteins and their partial protein hydrolysates) or 6.25 otherwise (FSANZ, 2016).

[33] In Standard 2.9.1, Section 2.9.1-3 (Definitions), an "infant formula product" is defined as "a product based on milk or other edible food constituents of animal or plant origin which is nutritionally adequate to serve by itself either as the sole or principal liquid source of nourishment for infants, depending on the age of the infant (Australian Government, 2017a).

[34] Division 3 of Standard 2.9.1, Section 2.9.1-10 (Infant formula and follow-on formula – protein – further requirements) states "(1) The L-amino acids listed in the table to Section S29—6 must be present in infant formula and follow-on formula at a level no less than the corresponding minimum level specified in the table and (2) Despite subsection (1), L-amino acids listed in the table to section S29—6 may be added to infant formula or follow-on formula only in an amount necessary to improve protein quality" (Australian Government, 2017a). The table in S29-6 lists the following L-amino acids and their minimum amount per 100 kJ as: histidine (10 mg), isoleucine (21 mg), leucine (42 mg), lysine (30 mg), cysteine and cysteine total (6 mg), cysteine, cystine, and methionine total (19 mg), phenylalanine (17 mg), phenylalanine and tyrosine total (32 mg), threonine (19 mg), tryptophan (7 mg), valine (25 mg) (FSANZ, 2016).

[35] In the European Union, "infants" means children under the age of 12 months (Commission Directive 2006/141/EC – EC, 2006).

[37] Article 4 in Commission Directive 2018/561 /EC (EC, amending Delegated Regulation (EU) 2016/127 with regard to protein requirements for follow-on formula) states the following: "On request from the Commission, the European Food Safety Authority issued a scientific opinion on 5 April 2017 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 (4). The European Food Safety Authority concluded that the use of follow-on formula, based on intact protein from cow's milk or from goat's milk, with a protein content of 1,6 g/100 kcal (0,38 g/100 kJ) and otherwise complying with the requirements of the relevant Union rules, is safe and suitable for healthy infants living in Europe with an intake of complementary foods of a sufficient quality. On the basis of that opinion, and in order to foster the development of innovative products, the minimum protein content required under Delegated Regulation (EU) 2016/127 for follow-on formula based on cow's milk or goat's milk protein should be lowered to 1,6 g/100 kcal."

Table 5.1.4.5-1 Details regarding the Protein Composition of Formula Intended for Use by Infants 6 to 12 Months of Age as Specified in Standards across Key Jurisdictions or as Recommended by Codex

Jurisdiction or Authority	Pertinent Regulation or Standard	Pertinent Definition	Permitted Protein Use Levels in Formula		Specifics on Type/Quality of Protein
			Minimum	Maximum	
		and constituting the principal liquid element in a progressively diversified diet of such infants[36].	0.56 g/100 kJ (2.25 g/100 kcal)	0.8 g/100 kJ (3.5 g/100 kcal)	Soya protein isolates, alone or in a mixture with cows' milk proteins For formula containing any of the above 3 protein sources, amino acids may be added[38].
Codex	CODEX STAN 156-1987 (Codex Alimentarius, 2017b)	'Follow-up formula' means a food intended for use as a liquid part of the weaning diet for the infant from the 6th month on and for young children. [Note relevant definition being revised – relevant age scope relating to a footnote permission for protein at a minimum of 1.6 g/100 kcal is the 6 to 12 month older infant]	3.0 g/100 kcal – *currently being revised to 1.8 g/100 kcal with an accompanying footnote[39].*	5.5 g/100 kcal – *currently being revised to 3.0 g/100 kcal*	The minimum value applies to cows' and goats' milk protein. For follow-up formula based on non-cows' or non-goats' milk protein, other minimum values may need to be applied. For follow-up formula based on soy protein isolate, a minimum value of 2.25 g/100 kcal (0.54 g/100 kJ) applies. Isolated amino acids may be added to follow-up formula only to improve protein quality, only in amounts necessary for that purpose.

[36] The European Union's definition of "follow-on formula" does not specify a minimum age for when follow-on formula can be used; however, Article 13 of Commission Directive 2006/141/EC does state that "in the case of follow-on formulae, a statement to the effect that the product is suitable only for particular nutritional use by infants over the age of six months, that it should form only part of a diversified diet, that it is not to be used as a substitute for breast milk during the first 6 months of life" (Commission Directive 2006/141/EC – EC, 2006).

[38] Point 2 in Annex II in Commission Directive 2006/141/EC (EC, 2006) states that "amino acids may be added to follow-on formulae solely for the purpose of improving the nutritional value of the proteins, and only in the proportions necessary for that purpose" (EC, 2006).

[39] The footnote to read as follows: *"A lower minimum protein level between 1.6 and 1.8 g/100 kcal (0.38 and 0.43 g/100kJ) in follow-up formula based on non-hydrolysed milk protein can be accepted. Such follow-up formula and follow-up formula based on hydrolysed protein should be evaluated for their safety and suitability and assessed by a competent national and/or regional authority based on clinical evidence".*

Table 5.1.4.5-1 Details regarding the Protein Composition of Formula Intended for Use by Infants 6 to 12 Months of Age as Specified in Standards across Key Jurisdictions or as Recommended by Codex

Jurisdiction or Authority	Pertinent Regulation or Standard	Pertinent Definition	Permitted Protein Use Levels in Formula		Specifics on Type/Quality of Protein
			Minimum	Maximum	

EC=European Commission; EU=European Union.

5.1.4.6 *For foods where consumption has changed in recent years, information on likely current food consumption*

No national Australian or New Zealand nutrition survey data exist for children <2 years of age; as such, whether there has been a significant change in recent years in the consumption of protein in this demographic, and in the more specific demographic of infants 6 to 12 months of age, is not known. The best available data on current intakes of protein in infants 6 to 12 months of age, living in Australia, are summarised in Section 5.1.4.3 above. The best available data on changes in dietary intakes of protein in infants 6 to 12 months of age should follow-on formula be re-formulated to include a lower level of minimum protein (1.6 g/100 kcal) also are summarised in Section 5.1.4.3.

In the best available evidence on the protein intakes in older infants (9 months of age) living in Australia (Conn *et al.,* 2009; Lioret *et al.,* 2013), the dietary surveys were conducted a decade apart (*i.e.,* 1999 to 2001 in Conn *et al.,* 2009 and 2008 to 2009 in Lioret *et al.,* 2013). Across both studies, mean and median protein intakes of 25 to 29 g protein/day for 9-month old infants were reported, suggesting that protein intakes have remained relatively stable over time; see Section 5.1.4.3.

5.1.5 Information Related to the Nutritional Impact of a Vitamin or Mineral

This application is not related to a vitamin or mineral; as such, the application requirements stated under E.1 to E.2 in Section 3.3.3 (Substances Used for a Nutritive Purpose) of FSANZ's *Application Handbook* are considered "not applicable".

5.1.6 Information Related to the Nutritional Impact of a Nutritive Substance other than Vitamins and Minerals

5.1.6.1 *Information related to the nutritional purpose of the use of the substance in each food*

The target population for the use of the nutritive substance (protein) at a lower level (minimum 1.6 g total protein/100 kcal; 0.38g/100kJ) in follow-on formula is infants 6 to 12 months of age.

Data to demonstrate that follow-on formula containing protein at a lower minimum level (*i.e.,* 1.6 g total protein/100 kcal or 0.38g/100kJ) can contribute to the "nutritional purpose" (*i.e.,* adequate growth and development) of formula-fed infants 6 to 12 months of age are discussed in Section 5.2.1.3.

Data to demonstrate that the recommended protein minimum of 1.6 g/100 kcal (0.38g/100kJ) is more closely aligned to the reference food (*i.e.,* breast milk) for infants 6 to 12 months of age are discussed in Section 5.2.1.3.1.

5.1.7 Information Related to the Potential Impact on Consumer Understanding and Behaviour

5.1.7.1 *Information to demonstrate the level of consumer awareness and understanding of the nutritive substances in the food(s)*

Since this application relates to lowering the amount of a nutritive substance (protein) that is already permitted in follow-on formula used by infants 6 to 12 months of age, effects of this change on consumer awareness and understanding are not considered an applicable concern.

In any event, the presence of a lower protein level will be made known to consumers only by the nutrition information panel on the label. Infant formula products (including follow-on formula for infants 6 to 12 months of age) must comply with the Food Standards Code 1.2.7 'Nutrition, health and related claims', which prohibits nutrition content claims and health claims on infant formula products.

The reason for lowering the amount of protein to infant formula products is to make its composition closer to that of human breast milk, which is considered the gold-standard of infant feeding. It is anticipated that such products may be recommended by health care professionals and in turn will be well-received by consumers. Industry will inform and educate health care professionals about the lowering of the amount of protein in follow-on formula.

In addition, it is recommended in the evidence-based Infant Feeding Guidelines by the NHMRC that "it is preferable to use a formula with a lower protein level" (NHMRC, 2012a).

5.1.7.2 Information on the actual or potential behaviour of consumers in response to proposed food(s)

This section is not applicable since this application relates to lowering the amount of a nutrient that is already permitted in follow-on formula. Lowering the protein level required in follow-on formula will not lead to the substitution or avoidance of foods that promote healthy eating or the addition of foods that are inconsistent with Australia's or New Zealand's nutrition policies or guidelines.

The authorisation of lowering the amount of protein in follow-on formula in Australia and New Zealand is anticipated to result in more consumer choice and the eventual adoption of lower protein levels by other infant formula manufacturers, providing a health benefit for consumers and better alignment with Australia and New Zealand's nutrition guidelines. The anticipated behaviour of Australian and New Zealand consumers in response to the market entry of lower protein levels in follow-on formula is expected to be positive due to health care professional recommendations on the health benefit of lower protein follow-on formula.

5.1.7.3 Information to demonstrate that the consumption of food(s) containing the nutritive substance will not adversely affect any population groups (e.g., particular age or cultural groups)

Information to demonstrate that decreasing the protein level in follow-on formula to 1.6 g total protein/100 kcal will not adversely affect the protein intakes of infants 6 to 12 months of age is discussed in Sections 5.1.4.3. Additional data to support that this compositional change will not adversely affect the target group (infants aged 6 to 12 months) is discussed in Section 5.2.1.3.1 below.

5.2 Application to Vary the Standards Related to Special Purpose Foods – Infant Formula Products

5.2.1 Information Related to Composition

5.2.1.1 Purpose of the compositional change

The target population of the proposed compositional change is infants 6 to 12 months of age. The primary purpose of the compositional change is to more closely align the protein quantity in follow-on formula used by infants 6 to 12 months of age with the level of protein found in human milk from the 6^{th} to the 12^{th} month of lactation so that older infants receive adequate, but not excess, protein during a period when protein intakes are complemented by a progressively diversified healthy diet.

5.2.1.2 General data requirements for supporting evidence

In general, the data that are cited in this application to support the proposed reduction in the minimum protein content of follow-on formula encompass:

- Two randomised, double-blind and controlled infant intervention studies wherein the effects, on growth, of a LPF (1.61 or 1.65 g/100 kcal; 0.39g/100kJ) were compared to a HPF (2.15 or 2.7 g/100 kcal; 0.51 or 0.65g/100kJ respectively) and to a reference group comprised of breastfed infants (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015).

- The EFSA's thorough scientific assessment of the safety of infant follow-on formula containing a total protein content of 1.6 g/100 kcal (0.38g/100kJ) (EFSA, 2017), which included pertinent dietary intake modelling should a follow-on formula with a lower total protein content (1.6 g/100 kcal; 0.38g/100kJ) be marketed and which summarised pertinent study results from the 2 infant intervention studies mentioned above (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015). Importantly, EFSA's opinion includes results of Ziegler *et al.* (2015) and Inostroza *et al.* (2014) that were not reported in the original publications, such as the mean "absolute" length, weight, and head circumference of infants in the intervention groups (and the mean absolute difference between groups) at 3, 6, and 12 months, for study completers and for the per protocol population, and the mean daily protein intakes at 4, 6, 8/9, and 12 months from formula alone.

- The total protein content in the breast milk of mothers from the 6th to the 12th month of lactation, as reported in 3 pivotal studies, 2 conducted in the U.S. (Dewey *et al.,* 1984; Nommsen *et al.,*1991) and 1 in Australia (Mitoulas *et al.,* 2002). Importantly, the Australian Government's NHMRC used these 3 studies in estimating the mean protein content of human milk (11 g protein/L) at 7 to 12 months of lactation.

- Protein intakes of 9-month old infants living in Australia (Melbourne and Adelaide; mean protein intakes of 26 to 29 g/day and median protein intakes of 25 to 29 g/day), which suggest higher-than-necessary protein intakes within this population group (Conn *et al.,* 2009; Lioret *et al.,* 2013[40]) – *i.e.,* the mean and median intakes are exceeding (by 2-fold) the AI for protein for infants 7 to 12 months of age of 14 g/day established by the Australian Government's NHMRC.

Regulatory precedence by other credible national or regional authorities, namely EFSA who recognise the safety and suitability of a follow-on formula for older infants (6 to 12 months of age) with a lower protein minimum – *i.e.,* 1.6 g total protein/100 kcal or 0.38g/100kJ (EFSA, 2017), provided sufficient complementary foods in the diet. A minimum protein level of 1.6g /100 kcal is currently permitted in the EU. Codex is also supportive of a lower minimum protein level between 1.6 and 1.8g/100kcal (0.38 and 0.43g/100kJ), under set conditions (i.e. '…*should be evaluated for their safety and suitability and assessed by a competent national and/or regional authority based on clinical evidence*'). (Codex Alimentarius, 2017a – CX/NFSDU 17/39/4 Rev.1).

[40] Lioret *et al.* (2013) reported data from a cluster randomised controlled trial – The Melbourne InFANT Program. Specifically, they reported the dietary intakes of 9- and 18-month old infants living in Melbourne, Australia. Campbell *et al.* (2017) reported the dietary intakes of this same cohort at 9 and 18 months of age, but also at 3.5 and 5.5 years of age.

5.2.1.3 Specific information requirements for the nutritional safety, tolerance and efficacy of the proposed compositional change: Nutritive substance (including energy or macronutrient), novel food, or novel food ingredient

5.2.1.3.1 Characterisation of proposed substance or the comparable substances in breast milk

True *versus* Total Protein in Human Breast Milk

Protein levels in breast milk can be estimated in 2 different ways – by either including or excluding "non-protein nitrogen", such as urea, amino acids and other nitrogen-containing compounds (SCF, 2003; FAO/WHO/UNU, 2007 cited in EFSA, 2017). The inclusion of non-protein nitrogen estimates **"total protein"** (calculated from total nitrogen, with a 6.25 conversion factor)[41] and the exclusion of non-protein nitrogen estimates **"true protein"** (total nitrogen minus non-protein nitrogen, with a 6.25 conversion factor) (Hibberd *et al.,* 1982; Lemons *et al.,* 1982; Butte *et al.,* 1984a,b,c; Gidrewicz and Fenton, 2014; EFSA, 2017). Approximately 25% of the nitrogen in human milk is provided as non-protein nitrogen (Donovan and Lönnerdal, 1989). Based on studies reviewed by Gidrewicz and Fenton (2014), protein estimated as total protein *versus* true protein can vary quite substantially. More specifically, in a comparison of studies wherein protein levels in mothers' breast milk[42] were measured as "true protein" (g/100 mL) (11 studies[43]) *versus* "total protein" (g/100 mL) (17 studies[44]), the latter values were higher. More specifically, Gidrewicz and Fenton (2014) calculated the percent difference between these protein estimates to range from 1 to 37% in the first 3 months of lactation; the difference appreciably increased from 1% in the first 3 days of lactation to 24% by the 4th day, reaching 37% at weeks 7 to 9, and 20% at Weeks 10 to 12[45].

Changing Protein Levels in Breast Milk throughout Lactation

Most recently, Lönnerdal *et al.* (2017) visually depicted the **"true protein"** content (g/100 mL) in breast milk during the 1st year of lactation, based on a review of 26 original articles published between 1973 and 2011 and conducted in Australia (n=2; Arnold *et al.,* 1987 and Mitoulas *et al.,* 2002), the U.S. (n=11); Argentina (n=1); Finland (n=1); France (n=2); Germany (n=2); UK and Germany (n=1); Spain (n=1); Sweden (n=1); Peru (n=1); Japan (n=1); Pakistan (n=1); Israel (n=1); see Figure 5.2.1.3.1-1 below. The 26 articles provided 130 data points during the 1st year of lactation. Importantly, 70% of the data points corresponded to true protein levels for the first 3 months of lactation, which is apparent by the density of data points during this time period in Figure 5.2.1.3.1-1. Indeed, the paucity of data on the true (or total) protein levels in breast milk for the 1st full year of lactation has been noted by the EFSA (EFSA, 2017).

Although Lönnerdal *et al.* (2017) state that "data were extracted from studies that reported "**true protein content,** protein-bound amino acids, and bioactive proteins", it does appear that some studies were included wherein **"total protein"** was measured (*e.g.,* Mitoulas *et al.,* 2002; Nommsen *et al.,* 1991,

[41] Total protein is calculated by multiplying total nitrogen by 6.25 (Gidrewicz and Fenton, 2014). Total protein reflects the nitrogen found in protein and also non-protein nitrogen, such as urea, amino acids and other nitrogen-containing compounds (SCF, 2003; FAO/WHO/UNU, 2007 cited in EFSA, 2017).
[42] Mothers of "healthy term infants".
[43] Anderson *et al.,* 1983; Britton, 1986; Butte *et al.,* 1984b,c, 1990; Hibberd *et al.,* 1982; Lemons *et al.,* 1982; MIchaelsen *et al.,* 1994; Montagne *et al.,* 1999; Nommsen *et al.,* 1991; Sanchez-Pozo *et al.,* 1986.
[44] Anderson *et al.,* 1983; Arnold *et al.,* 1987; Butte *et al.,* 1984b,c, 1990; Corvaglia *et al.,* 2008; Cregan *et al.,* 2002; Ferris *et al.,* 1988; Gross *et al.,* 1980; Hibberd *et al.,* 1982; Hosoi *et al.,* 2005; Lepage *et al.,* 1984; Lemons *et al.,* 1982; Motil *et al.,* 1997; Reinken and Docyx, 1985; Saarela *et al.,* 2005; Yamawaki *et al.,* 2005.
[45] At 10 to 12 weeks, Gidrewicz and Fenton (2014) estimated the difference between true protein (1.0 g/100 mL; 0.35g/100kJ) and total protein (1.2 g/100 mL; 0.42g/100kJ) to be 0.2 g/100 mL, a 20% difference, which was statistically significant at P<0.00001.

and perhaps others)[46]. However, notwithstanding this ambiguity, the analyses conducted by Lönnerdal *et al.* (2017) will be discussed below, since their publication on the protein content of breastmilk is the most recent. **Further, the main point of highlighting the data by Lönnerdal *et al.* (2017) is to observe the "trend" in the changes of the protein levels in breast milk throughout the 1st year of lactation, namely their decrease over time.** The protein content of breast milk reported on by Lönnerdal *et al.* (2017) will be referred to as "true protein", to align with how Lönnerdal *et al.* (2017) refers to the protein content of breast milk in their analyses.

As seen in Figure 5.2.1.3.1-1 below, based on their review of 26 original articles, Lönnerdal *et al.* (2017) observed **a <u>decline</u> in the "true protein" content of breast milk (g/100 mL) <u>over the 1st year of lactation</u>.** Other investigators have similarly observed a reduction in the protein levels in breast milk throughout lactation (Michaelsen *et al.,* 1990; Allen *et al.,* 1991; Nommsen *et al.,* 1991; Mitoulas *et al.,* 2002; Saarela *et al.,* 2005; Hester *et al.,* 2012; Gidrewicz and Fenton, 2014; Lönnerdal *et al.,* 2017), with appreciable decreases (≥25%) occurring by the 6th month of lactation (Hytten, 1954; Prentice *et al.,* 1981; Butte *et al.,* 1984a; Allen *et al.,* 1991). **Based on the data represented in Figure 5.2.1.3.1-1, from the 6th to the 12th months of lactation, the level of "true protein" in breast milk remains steady at levels between 1.0 and 1.13 g/100 mL (visually inferred from black solid line; 0.35 – 0.40g/100kJ).**

Lönnerdal *et al.* (2017) also visually depicted the concurrent decreases in the true protein level in breast milk throughout lactation (as estimated from their review of 26 original articles) and the protein needs of infants during the 1st year as calculated by Dewey *et al.* (1996) by superimposing these data; see Figure 5.2.1.3.1-2 below. **Indeed, as shown in Figure 5.2.1.3.1-2, and as noted by Lönnerdal *et al.* (2017), the changes in true protein content closely parallel changes in infant protein requirements, both of which decrease throughout the 1st year of lactation.** Based on the data represented in 5.2.1.3.1-2, at 91 to 360 days (3 to 12 months), the protein requirement of infants average ~1.3 g/day/kg body weight (visually inferred from dashed black line; 0.46g/100kJ).

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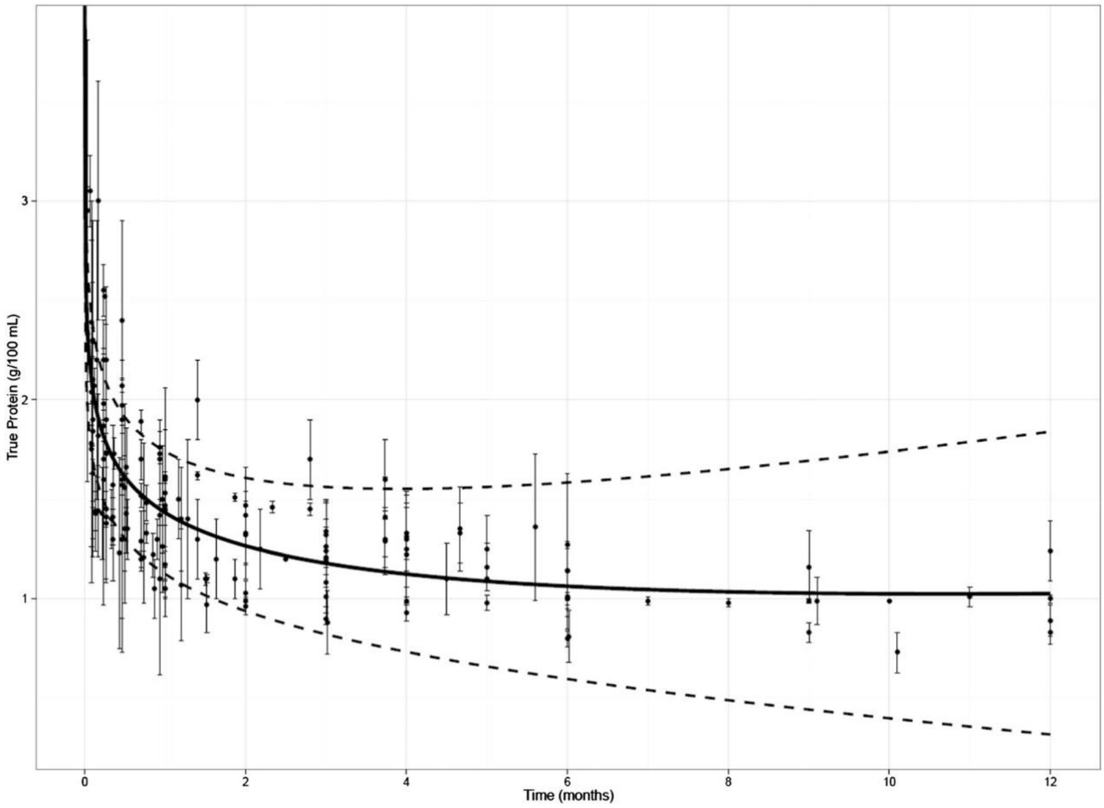
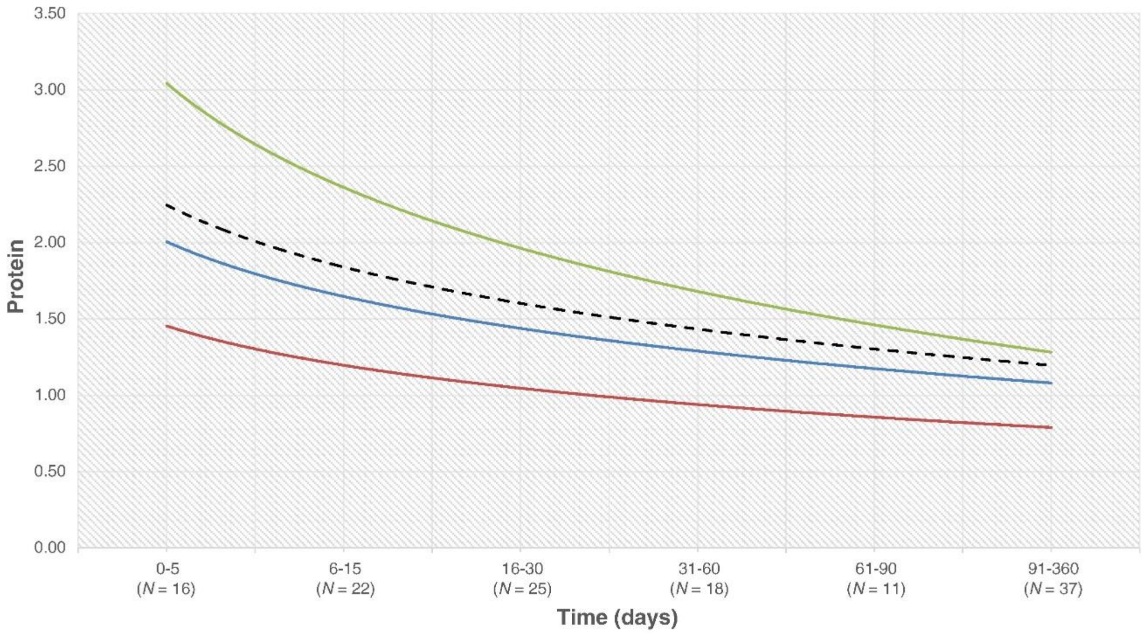
[46] For example, in Mitoulas *et al.* (2002), although the investigators do not specifically identify their measurement of protein in breast milk as total or true protein, a commercial protein assay kit manufactured by Bio-Rad Laboratories was used, which is the same assay kit used by Cregan *et al.* (2002) and Cregan *et al.* specifically refer to the protein estimate derived using this assay as "total protein". Further, in Mitoulas *et al.* (2002), the Kjeldahl procedure was used to estimate the protein concentration of the protein standard, which was mature breast milk and, indeed, the Kjeldahl procedure estimates "total nitrogen" (*i.e.,* total protein). In Nommsen *et al.* (1991), the investigators specifically refer to the protein measured in breast milk as "total protein".

Figure 5.2.1.3.1-1 A Visual Depiction of Changing True Protein Levels in Breastmilk over the 1st Year of Lactation



Taken from Lönnerdal *et al.* (2017). A linear regression of the **true protein** content (g/100 mL) in breast milk over the 1st year of lactation (solid line). Since the protein data exhibited a logarithmic decay, a linear regression model was fitted to the data and specified as true protein. The percentage of variation explained by the model (adjusted R2) was 0.581. Upper and lower 95% confidence limits (dashed lines) were constructed to account for the variation. Data points taken from the 26 original articles correspond to mean values and standard deviations (represented by error bars).

Figure 5.2.1.3.1-2 A Visual Depiction of Changing True Protein Levels in Breastmilk and Estimated Protein Requirements During the 1st Year of Infancy



Taken from Lönnerdal *et al.* (2017). The estimated evolution of infant protein requirements (in g per day per kg of body weight; dashed black line) and **true protein** content in human milk (in g per 100 mL; minimum, solid red line; median solid light blue line and maximum solid green line). Logarithmic regressions were calculated from the human milk protein concentration dataset (Figure 5.2.1.3.1-1 above) and from the dietary protein requirements dataset from Dewey *et al*. (1996).

Data on "Total Protein" in Breast Milk During the 6th to 12th Months of Lactation

Since the proposed compositional change relates to a reduction in the minimum of **"total protein"** in follow-on formula to be used in infants 6 to 12 months of age, the existing evidence on the **"total protein" level in breast milk** during the **6th to 12th of lactation** will be discussed herein.

There are 3 pivotal studies, 2 conducted in the U.S. (Dewey *et al.,* 1984; Nommsen *et al.,*1991) and 1 conducted in Australia (Mitoulas *et al.,* 2002) that provide the best estimates of "**total protein"** in breast milk from the 6th to the 12th months of lactation. Importantly, the Australian Government's NHMRC used these 3 studies in estimating the mean protein content of human milk (11 g protein/L) at 7 to 12 months of lactation. Across these 3 studies, the average **"total protein"** in breast milk for the relevant period of lactation (6th to 12th month) was as follows:

- **Dewey *et al.* (1984) – U.S. study:** 1.91 g total protein/100 kcal (0.46g/100kJ) for the 7th to the 11th months of lactation (27 breast milk samples)[47].

- **Nommsen *et al.* (1991) – U.S. study:** 1.67 g total protein/100 kcal[48] (0.40g/100kJ) for the 6th to the 12th months of lactation (21 to 45 women).

- **Mitoulas *et al.* (2002) – Australian study:** 1.26 g total protein/100 kcal[49] (0.30g/100kJ) for the 6th to the 12th months of lactation (5 to 8 women; 10 to 16 breast milk samples).

Thus, based on the findings of Dewey *et al.* (1984), Nommsen *et al.* (1991) and Mitoulas *et al.* (2002), the average total protein levels in breast milk from the 6th to the 12th months of lactation range from 1.26 to 1.91 g/100 kcal (0.30 – 0.46g/100kJ). Of these 3 studies, however, the data from Nommsen *et al.* (1991) and Mitoulas *et al.* (2002) are considered more complete and accurate estimates of grams total protein/100 kcal in breast milk during the specified periods of lactation since, in these 2 studies, for each month of lactation (*e.g.,* 6th, 9th, 12th month), both the total protein amount and gross energy of breast milk were reported per litre of breast milk. Importantly, for each timepoint, the protein and energy levels were derived from the same breast milk samples.

In contrast, in Dewey *et al.* (1984), a single estimate of total protein was reported for the 7th to 11th months of lactation (*i.e.,* 1.24 ±0.22 g/100 mL; 0.46g/100kJ) and thus protein levels in breast milk were not provided for each specific month. Further, a corresponding gross energy of the breast milk samples which were used to quantify total protein during the 7th to 11th months of lactation was not provided. Rather, the authors stated that 65 kcal/100 mL could be considered to be the energy contribution of breast milk to the total daily caloric intakes of infants. Indeed, the applicant used this energy estimate to derive the protein to energy levels in breast milk for the 7th to 11th months of lactation for Dewey *et al.* (1984). However, it should be noted that the basis for the gross energy estimation (65 kcal/100 mL) provided by Dewey *et al.* (1984) is unclear and, importantly, it does not appear to have been derived

[47] Dewey *et al*. (1984) reported the total protein content of breast milk (27 breast milk samples from women who were producing at least 500 mL/day – *i.e.,* full lactation) as 1.24 ± 0.22 g protein/100 mL, for the 7th to 11th months of lactation. The energy contribution of breast milk was stated by the authors to be 65 kcal/100 mL. Thus, the amount of total protein per 100 kcal = 1.91 g/100 kcal (0.46g/100kJ).

[48] The average of the amount of total protein per 100 kcal for months 6 (1.61 g/100 kcal), 9 (1.64 g/100 kcal) and 12 (1.76 g/100 kcal) was taken (see Table 5.2.1.3.1-1).

[49] The average of the amount of total protein per 100 kcal for months 6 (1.28 g/100 kcal), 9 (1.24 g/100 kcal) and 12 (1.25 g/100 kcal) was taken (see Table 5.2.1.3.1-1).

from the same breast milk samples that were used to generate total protein levels (*i.e.,* 1.24 ±0.22 g/100 mL).

Given the above-mentioned limitations of the data reported by Dewey *et al.* (1984), the applicant believes that of the 3 studies (Dewey *et al.,* 1984; Nommsen *et al.,* 1991; Mitoulas *et al.,* 2002), the total protein to energy estimates of breast milk derived from the data reported by Mitoulas *et al.* (2002) and Nommsen *et al.* (1991) – *i.e.,* <u>1.26 to 1.67 g total protein/100 kcal (0.30 – 0.40g/100kJ)</u>, are more accurate estimates of the levels of total protein occurring in breast milk during the 6th to 12th months of lactation.

An important point regarding the studies conducted by Mitoulas *et al.* (2002) and Nommsen *et al.* (1991) relates to the methods and protein standards used in estimating the total protein quantity in breast milk (see Table 5.2.1.3.1-1). Mitoulas *et al.* (2002) used a commercial protein assay kit to measure total protein and mature <u>human milk</u> was used as the protein standard. In contrast, Nommsen *et al.* (1991) used a modified Lowry assay, and <u>bovine serum albumin</u> was used as the protein standard. Importantly, Nommsen *et al.* (1991) acknowledge that the modified Lowry assay "tends to result in slightly elevated values for total protein" because human milk has a greater proportion of aromatic amino acids than does bovine serum albumin. They defended their choice of protein standard (bovine serum albumin) by stating that human milk protein standards are not stable and thus not practical for use in longitudinal studies. **Thus, although across both studies (Mitoulas *et al.,* 2002; Nommsen *et al.,* 1991), the total protein ranged from 1.26 to 1.67 g/100 kcal (0.30 – 0.40g/100kJ) during the 6th to 12th months of lactation, it should be noted that the upper end of this range (1.67 g total protein/100 kcal or 0.40g/100kJ), estimated by Nommsen *et al.* (1991), is a slight overestimate of actual total protein occurring in breast milk during this time period because of the methods used by Nommsen *et al.* (1991) to measure total protein.**

Findings from Mitoulas *et al.* (2002), a study conducted in Australia, warrant a greater discussion since these data most closely reflect the jurisdiction of interest for the proposal made in this Application. Indeed, as shown in Table 5.2.1.3.1-1 and Figure 5.2.1.3.1-3, at the 6th, 9th, and 12th months of lactation, the "total protein" levels of breast milk from women living in Australia (Mitoulas *et al.*, 2002), averaged 0.80±0.04 g/100 mL (=<u>1.28 g/100 kcal; 0.31g/100kJ</u>), 0.83±0.05 g/100 mL (=<u>1.24 g/100 kcal; 0.30g/100kJ</u>), and 0.83±0.06 g/100 mL (=<u>1.25 g/100 kcal; 0.30g/100kJ</u>), respectively, in contrast to levels of 1.05±0.04 g/100 mL (=1.62 g/100 kcal; 0.39g/100kJ) in the 1st month of lactation[50]. Importantly, Mitoulas *et al.* (2002) reported that the total protein content of breast milk at the 6th and 9th months of lactation was statistically significantly lower than at the 1st, 2nd, and 4th months of lactation ($p<0.05$).

Ziegler *et al.* (2015) state that an adequate protein content of formulas fed after 3 months could be assumed to be 1.30 g protein/100 kcal (0.31g/100kJ), "as long as the protein is of high quality" (Ziegler *et al.*, 2015); although not explicitly stated, it is assumed that Ziegler *et* al. (2015) was referring to "total protein" rather than "true protein". Interestingly, this value aligns with the average total protein levels estimated for the 6th to 12th months of lactation in Mitoulas *et al.* (2002) – *i.e.,* 1.26 g total protein/100 kcal (0.30g/100kJ). Furthermore, the protein levels occurring in the breast milk of Australian mothers

[50] Protein levels in g/100 kcal were derived using data from Table 2 in the study by Mitoulas *et al*. (2002), wherein the protein level (g/L) and energy content (KJ/mL) of breast milk were reported for the 1st, 2nd, 4th, 6th, 9th, and 12th months of lactation. In the 6th month of lactation, the mean total protein (g/L) and energy (KJ/mL) contents ± standard error of 16 breast milk samples from 8 mothers were 8.03±0.38 g/L and 2.62±0.09 KJ/mL, respectively. In the 9th month of lactation, the mean total protein and energy contents of 12 breast milk samples from 6 mothers were 8.34±0.45 g/L and 2.81±0.09 KJ/mL, respectively. In the 12th month of lactation, the mean total protein and energy contents of 10 breast milk samples from 5 mothers were 8.34±0.57 g/L and 2.79±0.14 KJ/mL, respectively. In contrast, in the 1st month of lactation, the mean protein and energy contents of 18 breast milk samples from 9 mothers were 10.5±0.4 g/L and 2.72±0.06 KJ/mL, respectively.

from the 6th to the 12th month of lactation are lower than the minimum required amount in follow-on formula as per the Code (0.45 g protein/100 kJ, which is equivalent to 1.88 g protein/100 kcal).

Based on data from Mitoulas *et al.* (2002), the proposed minimum protein quantity recommended in this application for follow-on formula for use by infants aged 6 to 12 months of 1.6 g total protein/100 kcal (0.38g/100kJ) more closely aligns with, and still exceeds, the levels of protein occurring in the breast milk of Australian mothers during the 6th and 12th months of lactation (an average of 1.26 g total protein/100 kcal, equivalent to 0.30g/100kJ).

Table 5.2.1.3.1-1 "Total Protein" Levels in Breast Milk from Australian or American Mothers of Healthy Term Infants[51]

Reference	Description of Mothers and Infants Country	Assay for Protein Analysis	Time Period of Lactation (No. of Mothers Providing Breast Milk Samples)	Total Protein (g/100 mL); Mean±SEM or SD[52]	Total Protein (g/100 kcal); Mean (g/100KJ in brackets)
Mitoulas *et al.* (2002)	5 to 9 mothers (18 to 35 yrs of age) of healthy term infants[53] Australia	Commercial protein assay kit (Bio-Rad Laboratories); protein concentration in mature *human milk* calculated by Kjeldahl procedure and used as protein standard.	1st mo (n=9)	*1.05±0.04[a] [54]*	*1.62[55] (0.39)*
			2nd mo (n=9)	*0.96±0.04[a]*	*1.61 (0.39)*
			4th mo (n=9)	*0.93±0.04[a]*	*1.51 (0.36)*
			6th mo (n=8)	***0.80±0.04[b]***	***1.28 (0.31)***
			9th mo (n=6)	***0.83±0.05[b,c]***	***1.24 (0.30)***
			12th mo (n=5)	***0.83±0.06[a,c] [56]***	***1.25 (0.30)***
Nommsen *et al.* (1991)	21 to 58 mothers (mean age of 30.4±4.6 yrs) of healthy infants U.S.	Modified Lowry assay with bovine serum albumin as the standard.	3rd mo (n=58)	*1.21±0.15*	*1.74[57](0.42)*
			6th mo (n=45)	***1.14±0.15***	***1.61 (0.39)***
			9th mo (n=28)	***1.16±0.18***	***1.64 (0.39)***
			12 mo (n=21)	***1.24±0.15***	***1.76 (0.42)***

mo = months; n = number; No. = number; SD = standard deviation; SEM = standard error of mean; U.S. = United States; yrs = years.
Mean values with unlike superscripts were significantly different at p<0.05.

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[51] Shaded and bolded data are considered the most relevant data for the purposes of this application. This is because the shaded cells provide the protein quantity of breast milk at periods of lactation most comparable to the time-period during which the follow-on formula (proposed for a compositional change in this application) would be used (*i.e.,* 6 to 12 months postpartum). Italicized values calculated by the applicant using data from the publications. Italicized values calculated by the applicant using data from the publications. Mean values with unlike superscripts were significantly different at p<0.05.

[52] Mitoulas *et al.* (2002) showed variability as SEM; Nommsen *et al.* (1991) used SD.

[53] One infant was preterm (born at 31 weeks).

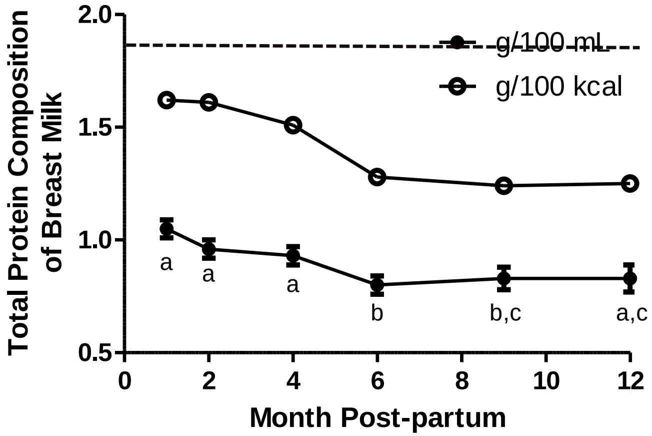
[54] In Mitoulas *et al.* (2002), values were converted from g/L to g/100 mL.

[55] The energy content (KJ/mL) was reported for breast milk collected at 1, 2, 4, 6, 9, and 12 months of lactation, which was used to convert protein content from g/L to g/100 kcal; see Table 2 in Mitoulas *et al.* (2002).

[56] The protein content of breast milk at 12 months, although not significantly different from its level in breast milk at 9 months (as indicated by very comparable mean protein values between 9 and 12 months) was also not significantly different from levels of protein in breast milk at the 1st, 2nd, and 4th months of lactation (even though the mean protein level at 12 months was lower than the mean levels of protein during the first 4 months of lactation). This could have been because of the higher standard error of the mean at 12 months as compared to the standard error of the mean in the first 4 months of lactation and subject attrition (n=5 at 12 months *versus* n=9 at the 1st, 2nd, and 4th month).

[57] The energy content (kcal/L) was reported for breast milk collected at 3, 6, 9, and 12 months of lactation, which was used to convert protein content from g/L to g/100 kcal; see Table 2 in Nommsen *et al.* (1991).

Figure 5.2.1.3.1-3 Total Protein in Breast Milk (g/100 kcal) from Australian Mothers over the 1st Year of Lactation (Mitoulas *et al.,* 2002).



Dashed line represents the current total protein minimum for follow-on formula in Australia/New Zealand (*i.e.,* 1.88 g total protein/100 kcal; 0.45g/100kJ).

5.2.1.3.2 Nutritional safety and tolerance of the proposed compositional change

Two infant intervention studies (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015) provide clinical evidence that the proposed compositional change is safe and well tolerated. In these 2 pertinent studies, the effects on anthropometric parameters (length, weight, head circumference) of a LPF containing 1.61 or 1.65 g total protein/100 kcal (0.39g/100kJ), that aligns with the proposed compositional change of a minimum 1.6 g total protein/100 kcal (0.38g/100kJ), was compared to a HPF (2.15 or 2.7 g total protein/100 kcal; 0.51 – 0.65g/100kJ) and a reference group comprised of breastfed infants. The formulas were fed to infants from 3 to 12 months of age; thus, anthropometric changes were monitored for a minimum period of 2 months within the relevant age range of infants for the use of a follow-on formula (6 to 12 months), as required by FSANZ. The key characteristics and results of both these studies are further described below.

It should be noted that EFSA's evaluation of these 2 studies, as provided in their published scientific opinion (EFSA, 2017), includes additional pertinent information on study results that is not provided in the publications* (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015), *such as, the mean "absolute" length, weight, and head circumference of infants in each group (and the mean absolute difference in these outcomes between groups) at 3, 6, and 12 months, for study completers and for the per protocol population, and the mean daily protein intakes at 4, 6, 8/9, and 12 months from formula alone. As such, EFSA's scientific opinion should additionally be reviewed, since the results on the above-mentioned outcomes, while discussed herein, were not transcribed in detail in this application (see Appendices A through D in EFSA, 2017).

Overview and Results of Inostroza et al. (2014) – Conducted in Chile

Table 5.2.1.3.2-1 below summarizes the key study characteristics of Inostroza *et al.* (2014). The study was a randomized, double-blind, controlled, parallel-arm study conducted in Chile. Healthy term infants[58] who were "predominantly formula fed" were randomised at 3 months of age to receive either a LPF (1.65 g total protein/100 kcal; 0.39g/100kJ; n=86) or HPF (2.70 g total protein/100 kcal; 0.65g/100kJ; n=86) until 12 months of age. The LPF contained probiotics [*Lactobacillus* PR and *Bifidobacterium lactis* (Bb12)] that were not present in the HPF; however, this difference is not expected to have confounded the observed effects of the LPF on anthropometric parameters.

From 3 to 6 months of age, the infants were to be exclusively fed a LPF or HPF[59], and from 6 to 12 months of age, the infants continued consuming their formula but were also permitted complementary foods. A reference group comprised of breastfed infants (n=76) who were "predominantly breastfed" (*i.e.,* no more than 1 formula feeding per day) was also included in the study. Breastfed infants were exclusively breastfed from study enrolment to 6 months of age and from 6 to 12 months of age could consume follow-on formula (2.4 g/100 kcal; 0.57g/100kJ) and complementary foods. Although the formulas were consumed up to 12 months of age, growth was monitored up to 24 months of age.

Table 5.2.1.3.2-1 lists all the outcomes that were measured and statistically analysed by the investigators, as reported in the publication, which included weight gain (g/day) – the primary outcome[60], weight-for-age z-score, length-for-age z-score, head circumference z-score, body mass index (BMI)-for-age z-score, body composition (fat mass, lean mass) and serum biomarkers. Results on the aforementioned variables are not fully described in the publication; for example, quantitative and

[58] A birthweight of >2,500 g and <4,800 g and a gestational age ≥37 weeks and <42 weeks.
[59] As stated by EFSA (2017), although the protocol intended for infants to be exclusively fed the formulas until 6 months of age, complementary foods in amounts >4 teaspoons per day were introduced before 6 months of age in 66 infants (28 in the LPF group, 24 in the HPF group, and 14 in the BF group), who were not excluded from the statistical analyses.
[60] The primary outcome of weight gain (g/day) was for the period of 3 to 6 months when the LPF or HPF were to be exclusively fed to infants.

statistical data (within and between groups) are missing for length-for-age z-score, head circumference z-score, and BMI-for-age z-score. Importantly, in EFSA (2017), results are provided on changes in absolute length, weight, and head circumference at 3, 6, and 12 months.

Of the 172 formula-fed infants who were randomized, 142 completed the study to 6 months of age (n=66 in LPF group, and n=76 in HPF group) and 120 to 12 months of age (n=54 in LPF group and n=66 in HPF group). Of the 76 breastfed infants enrolled, 65 completed the study to 6 months of age, and 61 infants to 12 months of age. Infant attrition rates were 19.7% in the breastfed group, 23.3% in the HPF group, and 37.2% in the LPF group, with reasons for withdrawal provided. The statistical analyses of changes in the growth parameters (*i.e.,* z-scores of anthropometric outcomes), as reported in the publication, were done on the aforementioned "completers". In EFSA (2017), results on the changes in anthropometric variables for both completers and the per protocol population are provided.

Notable results, as reported in the publication (Inostroza *et al.,* 2014), are:

- Weight gain (g/day) from 3 to 6 months of age, the primary outcome of the study, was not significantly different between infants consuming a LPF (1.65 g/100 kcal) *versus* breastfed infants, with a mean difference between groups in weight gain of -0.72 g/day (95% CI = -2.46 to 1.01); P=0.411.

- Weight gain (g/day) from 6 to 12 months of age, which is the exposure period of interest for the follow-on formula proposed for a compositional change in this application, was not significantly different between infants consuming a LPF (1.65 g/100 kcal; 0.39g/100kJ) *versus* breastfed infants. The mean difference between these groups in weight gain was 0.77 g/day (95% CI=-0.50 to 2.05; P=0.233), with infants in the LPF group experiencing a greater (but not significant) mean daily weight gain than breastfed infants. During this same time period, no significant differences were observed in daily weight gain between infants consuming the LPF *versus* the HPF [-0.88 g/day (95% CI = -2.10 to 0.35); P=0.159]; the mean daily weight gain of infants in the HPF group was greater (+0.88 g/day) than in the LPF group.

- At 6 and 12 months of age, the difference in weight-for-age z-scores between infants consuming the LPF (1.65 g/100 kcal; 0.39g/100kJ) *versus* breastfed infants was not significant [6 mo: -0.07 (95% CI = -0.26 to 0.13); p=0.508]; 12 mo: 0.16 (95% CI = -0.05 to 0.36); P=0.128].

- At 12 months of age, body composition, as measured by fat mass (kg), fat mass (%), lean mass (kg), lean mass (%), bone mineral content (g), bone mineral content (% lean mass), did not significantly differ between the LPF and breastfed groups. Compared to the HPF group, the LPF had significantly lower bone mineral content (g) (p<0.006), with all other parameters related to body composition comparable between the LPF and HPF groups.

- In considering the changes in serum biomarkers (blood urea nitrogen, insulin growth factor-1, insulin, C-peptide, ghrelin, leptin) between infants consuming a LPF (1.65 g/100 kcal; 0.39g/100kJ) *versus* breastfed infants[61], at 12 months of age, no significant differences were observed between these groups in all biomarkers except leptin, which was significantly lower in infants receiving a LPF *versus* breastfed infants (P=0.009). At 6 months of age, blood urea nitrogen was significantly higher in the LPF group *versus* the breastfed group, with no significant difference observed at 12 months (at 12 months, blood urea nitrogen levels were higher in the LPF group as compared to breastfed infants). However, as asserted by the EFSA (2017), the blood urea nitrogen remained within the normal range in the LPF group throughout the study.

[61] These analyses were based on per protocol populations. The group sizes were: LPF (6 mo: n=55; 12 mo: n=47), HPF (6 mo: n=68; 12 mo: n=60), breastfed reference group (not reported).

Although not reported in the publication, serum albumin was also measured and, as noted by EFSA (2017), its levels remained within the normal range throughout the study for all the groups, including the LPF group.

- At 6 months of age, the plasma concentrations of 9 of 10 essential amino acids were higher in the LPF group *versus* the breastfed group (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, tyrosine, valine) with the exception of threonine, which was lower in the LPF group *versus* the breastfed group. Furthermore, the higher levels of essential amino acids in the LPF group *versus* the breastfed group reached statistical significance at $p<0.05$ for 7 amino acids (isoleucine, lysine, methionine, phenylalanine, tryptophan, tyrosine, valine); higher levels of leucine in the LPF group were not statistically different from the breastfed group ($p=0.09$) and statistical results were not reported for histidine and threonine. Data for plasma levels of essential amino acids were not provided for the 12th month timepoint.

- There was a statistically significant difference in the intake of protein (mean±SD), from formula alone, between formula-fed infants, with infants in the LPF group consuming 6.24 g/day less protein at 9 months of age (95% CI=-7.54 to -4.94) and 4.46 g/day less protein at 12 months of age (95% CI= -5.85 to -3.07) *versus* infants in the HPF group ($p<0.001$)[62]. Daily protein intakes (mean±SD), from formula alone, for LPF and HPF infants at 9 months of age were 9.3±2.6 g/day and 15.5±4.3 g/day, respectively, and at 12 months, 8.8±3.4 g/day and 13.2±3.9 g/day, respectively. As reported by EFSA (2017), at 6 months of age, daily protein intakes were 10.2±2.6 g/day and 17.0±3.0 g/day for LPF and HPF infants, respectively; whether the intakes between the LPF and HPF groups were significantly different at 6 months of age was not reported in EFSA (2017).

Overview and Results of Ziegler et al. (2015) – Conducted in the U.S.

Ziegler *et al.* (2015) conducted a randomized, double-blind, controlled, parallel-arm study in the U.S. At or before 3 months of age (84±4 days), healthy term infants[63] who been fed formula for ≥2 weeks were randomly assigned to receive either a LPF (1.61 g/100 kcal; 0.39g/100kJ; n=97) or HPF (2.15 g/100 kcal; 0.51g/100kJ; n=97) until 12 months of age. From 3 to 4 months of age, the infants were exclusively fed the LPF or HPF; from 4 to 6 months, the infants continued their formula but could also consume complementary foods in small amounts[64]; from 6 to 12 months, the infants continued on their formula but could also consume complementary foods in unrestricted amounts. A reference group comprised of breastfed infants (n=112) who were breastfed at 3 months and whose mothers indicated their intent to breastfeed for at least 6 months was also enrolled. Like formula-fed infants, breastfed infants could consume complementary foods in small amounts from 4 to 6 months, and in unrestricted amounts from 6 to 12 months; parents could also use a standard formula for breastfed infants from 6 to 12 months.

Of the 194 formula-fed infants who were randomized, 183 completed the study to 6 months of age (n=92 in LPF group, and n=91 in HPF group) and 174 to 12 months of age (n=87 in LPF group and n=87 in HPF group). Of the 112 breastfed infants enrolled, 109 completed the study to 6 months of age, and 105 infants to 12 months of age. Compared to Inostroza *et al.* (2014), in Ziegler *et al.* (2015), infant attrition rates were lower, overall, for all 3 groups and comparable between the LPF and HPF groups: LPF group (10.3%); HPF group (10.3%); breastfed group (6.3%).

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[62] The protein intakes reported for the 6th and 9th month timepoints in the publication apply to the 9th month and 12th month timepoints, respectively, in EFSA (2017). Indeed, formula and protein intakes are correctly reported in EFSA (2017).

[63] A birthweight of ≥2,500 g and ≤4,500 g and a gestational age ≥37 weeks.

[64] Up to 3 teaspoons per day of dry cereal could be provided, and up to 2 tablespoons per day of single-ingredient first or second foods beginning at 5 months of age.

Table 5.2.1.3.2-1 lists all the outcomes that were measured and statistically analysed by investigators, as reported in the publication, which included weight gain (g/day) – the primary outcome[65], weight-for-age z-score, length-for-age z-score, head circumference z-score, BMI-for-age z-score, and serum biomarkers. Importantly, in EFSA (2017), results are provided on changes in absolute length, weight, and head circumference at 3, 6, and 12 months of age. The statistical analyses of changes in the growth parameters (*i.e.,* z-scores of anthropometric outcomes), as reported in the publication, were done on "completers". In EFSA (2017), results on the changes in the anthropometric variables for both completers and the per protocol population are provided.

Notable results, as reported in the publication (Ziegler *et al.,* 2015), are:

- Weight gain (g/day), from 3 to 6 months of age, the primary outcome of the study, was significantly greater in infants consuming a LPF (1.61 g/100 kcal; 0.39g/100kJ) *versus* breastfed infants with a mean difference between groups of 2.04 g/day (95% CI = 0.66 to 3.43; P=0.004) and not significantly different between the LPF and HPF groups with a mean difference between groups of -0.84 g/day (95% CI = -2.25 to 0.57; P=0.2426).

- Weight gain (g/day) from 6 to 12 months of age, which is the exposure period of interest for the follow-on formula proposed for a compositional change in this application, was significantly greater in infants consuming a LPF (1.61 g/100 kcal; 0.39g/100kJ) *versus* breastfed infants. The mean difference between these groups in weight gain was 1.08 g/day (95% CI= 0.16 to 2.00; P=0.0213). During this same time period, no significant differences were observed in daily weight gain between infants consuming the LPF *versus* the HPF [-0.77 g/day (95% CI = -1.70 to 0.17); P=0.1063]; the mean daily weight gain of infants in the HPF group was greater (+0.77 g/day) than in the LPF group.

- At 6 and 12 months of age, there was a statistically significant difference in weight-for-age z-scores between the LPF (1.61 g/100 kcal; 0.39g/100kJ) and breastfed infants with the mean difference above zero: 6 mo: 0.24 (95% CI = 0.09 to 0.40; P=0.0023) and at 12 mo: 0.32 (95% CI = 0.16 to 0.48; P=0.0001). No significant differences were observed in weight-for-age z-scores between the LPF and HPF groups at 6 months [-0.06 (95% CI = -0.22 to 0.11); P=0.4928] but a significant difference was observed at 12 months [-0.21 (95% CI = -0.37 to 0.05); P=0.0125].

- At 6 and 12 months of age, there was a statistically significant difference in length-for-age z-scores between the LPF infants (1.61 g/100 kcal; 0.39g/100kJ) *versus* breastfed infants with the mean difference above zero: 6 mo: 0.32 (95% CI = 0.17 to 0.47; P=0.0000) and at 12 mo: 0.49 (95% CI = 0.34 to 0.64; P=0.0000). No significant differences were observed in length-for-age z-scores between the LPF and HPF groups at 6 months [-0.05 (95% CI = -0.20 to 0.11); P=0.5517] and at 12 months [-0.10 (95% CI = -0.26 to 0.05); P=0.1965].

- At 6 and 12 months of age, the BMI-for-age z-scores between the LPF infants (1.61 g/100 kcal; 0.39g/100kJ) *versus* breastfed infants were not significantly different [6 mo: 0.11 (95% CI = -0.09 to 0.30); P=0.2763 and at 12 mo: 0.10 (95% CI = -0.10 to 0.29); P=0.3429]. Also, no significant differences were observed between the LPF and HPF groups at 6 and 12 months [6 mo: -0.04 (95% CI = -0.24 to 0.17); P=0.7312 and at 12 mo: -0.19 (95% CI = -0.39 to 0.01); P=0.0647].

- In considering the changes in serum biomarkers (blood urea nitrogen, insulin growth factor-1, albumin, C-peptide) between infants consuming a LPF (1.61 g/100 kcal; 0.39g/100kJ) *versus*

[65] The primary outcome of weight gain (g/day) was for the period of 3 to 6 months of age when the LPF or HPF were the primary sources of nutrition for the infants.

breastfed infants[66], at 12 months of age, statistically significant differences were observed only for blood urea nitrogen (lower for the LPF group *versus* breastfed infants) and insulin growth factor-1 (higher in the LPF group *versus* breastfed infants). Changes in these biomarkers were acknowledged by the investigators as not being indicative of protein deficiency; rather, the study authors stated that these data confirm that protein intakes were meeting the needs of individual infants. Further, EFSA (2017) also noted that levels of blood urea nitrogen and serum albumin remained within the normal range for all the groups.

- At 6 months of age, based on formula intake alone, the median protein intake (g/kg/day) in the LPF group was 1.50 g/kg body weight/day [interquartile range [(IQR): 1.31 to 1.65] *versus* 1.89 g/kg body weight/day (1.72 to 2.17) in the HPF group. In EFSA (2017), daily protein intakes for the LPF *versus* HPF groups, from formula alone, were reported at 4 months (9.8±2.3 *vs*. 12.4±2.5 g/day), 6 months (9.9±2.5 *vs*. 12.5±2.6 g/day), 8 months (9.2±2.3 *vs*. 11.9±2.5 g/day), and 12 months (7.8±2.6 *vs*. 10.1±3.3 g/day), respectively; whether differences in intakes between groups were significant was not reported in EFSA (2017).

[66] These analyses were based on per protocol populations; group sizes were not reported for the PP analyses.

Table 5.2.1.3.2-1 Key Characteristics of Studies Comparing the Effects of a Lower- *versus* a Higher-Protein Formula[67]

Reference	Study Objective	Study Design, Duration, Country	Study Power	Study Population	Intervention	Measured Outcomes and Assessment Methods	Statistically Analysed Outcomes[68]	Dietary Intakes	Analysis Populations	Strengths (Confounders Accounted For) and Limitations
Inostroza *et al.* (2014)	To investigate whether a LPF (1.65 g/100 kcal; 0.39g/100kJ) leads to slower growth between 3 and 6 months *versus* a HPF (2.70 g/100 kcal; 0.65g/100kJ) and whether the former can support normal growth.	Design: R, DB, C, P, BF reference group included Formula-fed infants **Birth to 3 mo:** A starter formula (1.8 g/100 kcal; 0.43g/100kJ) **3 to 6 mo:** A LPF (1.65 g/100 kcal; 0.39g/100kJ) or HPF formula (2.70 g/100 kcal; 0.65g/100kJ), exclusively; however complementary foods were consumed by some infants[69] **6 to 12 mo:** A LPF or HPF with complementary foods Breastfed infants **Birth to 6 mo:** Exclusive breastfeeding **6 to 12 mo:** Breastfeeding + follow-up formula (2.4 g/100 kcal; 0.57g/100kJ) + complementary foods. Growth monitored until 24 months of age. Country: Chile	Sample size of 182 FF infants and 91 BF infants was estimated based on a mean difference in weight gain of 2 g/day that is clinically relevant[70]	Healthy term infants[71] Birth: n= 305 infants enrolled 3 mo: 172 FF infants randomized to LPF (n=86) or HPF (n=86); 76 BF infants[72] 6 mo: n=66 analysed (LPF); n=76 analysed (HPF); n=65 analysed (BF) 12 mo: n=54 analysed (LPF); n=66 analysed (HPF); n=61 analysed (BF) 24 mo: n=50 analysed (LPF); n=64 analysed (HPF); n=56 analysed (BF)	LPF: 1.65 g protein/100 kcal (0.39g/100kJ) + probiotics [2x10^7 CFU/g formula of *Lactobacillus PR* and *Bifidobacterium lactis Bb12*) HPF: 2.70 g protein/100 kcal (0.65g/100kJ) & (no probiotics) Protein was provided by intact bovine milk protein with a whey to casein ratio of 60:40 Levels of vitamins + minerals + trace elements in formula met recommendations stated by CODEX Alimentarius Complementary foods could start at 6 mo	• **Weight**: measured in duplicate (average used); without clothes; to the nearest 10 g using calibrated electronic scales • **Length:** measured in duplicate (average used); 2 measurers; to nearest 1 mm using a measuring board with fixed headboard and movable foot board • **Head circumference:** measured in duplicate (average used); to nearest 1 mm using a non-stretchable measuring tape • **Body composition** (fat mass, lean mass, bone mineral content): DXA Lunar Prodigy Advance with software Encore version • **Plasma biomarkers**: Analyses conducted at laboratories or using standardized assays or kits	• Weight gain (g/day) • Weight-for-age z score • Length-for-age z score • Head circumference z score • BMI (kg/m^2) • BMI-for-age z score • Body weight (kg) • Fat mass (% and kg) • Lean mass (% and kg) • Bone mineral content (g and % lean mass) • BUN (mg/dL) • IGF-1 (μg/L) • Insulin (mU/L) • C-peptide (pmol/L) • Ghrelin (plasma) (ng/L) • Leptin (μg/L) • Essential amino acids[73] (plasma; μmol/L)	For the LPF and HPF groups, formula intake (mL/day), energy intake (kcal/day – from formula alone), and protein intake (g/day – from formula alone) analysed at 4, 6, and 9 mo[74].	ITT and PP	**Strengths** • R, DB, C study • Study powered for a clinically relevant outcome (weight gain) • Appropriate inclusion/exclusion criteria which accounted for gestational age, birthweight, and health of infants • Randomisation[75] stratified for infant gender, ethnicity, pre-pregnancy maternal BMI (25 to 30 or >30 kg/m^2), and type of feeding between 1.5 to 3 mo[76] • Anthropometric outcomes measured in duplicate • Covariates considered in statistical analyses[77] • Baseline comparability of infants and mothers across the 3 groups provided[78] • Formula and protein intakes assessed throughout the study. **Limitations** • Probiotics included in LPF but not in HPF • Quantitative and statistical results not

[67] Abbreviations: ANCOVA = analysis of covariance; BF = breastfed; BMI = body mass index; BUN = blood urea nitrogen; C = controlled; CFU = colony forming units; DB = double-blind; DXA = dual-energy x-ray absorptiometry; EFSA = European Food Safety Authority; FF = formula-fed; HPF = higher protein formula; ITT = intention-to-treat; IGF-1 = insulin-like growth factor 1; kcal = kilocalorie; LPF = lower protein formula; mo = month; NR = not reported; P = parallel; PP = per protocol; R = randomised; U.S.= United States; wk = week; yrs = years
[68] As reported in the publications.
[69] As stated in EFSA (2017), although the protocol intended for infants to be exclusively fed the formulas until 6 months of age, complementary foods in amounts >4 teaspoons per day were introduced before 6 months of age in 66 infants (28 in the LPF group, 24 in the HPF group, and 14 in the BF group), who were not excluded from the statistical analysis.
[70] Other assumptions made for the sample size calculation conducted by Inostroza *et al.* (2014) were: type 1 error = 5% (2-sided test); power=80%; and, drop-out rate=30%. Since attrition was <30%, enrollment was stopped when it could be expected that at least 64 infants per group would reach 6 months. **The primary study outcome was weight gain (g/day) from 3 to 6 months because during that period, the study formulas were the near-exclusive source of nutrients.**
[71] Inclusion criteria: A birthweight of >2500 g and <4800 g and a gestational age ≥37 weeks and <42 weeks. Exclusion criteria: weight <5th percentile for gestational age, maternal diabetes (including gestational onset diabetes), >5 cigarettes per day during pregnancy, use of illicit drugs, or presence of chronic inflammatory condition, congenital illness or malformations that could affect growth, hospitalisation for <2 days.
[72] BF infants were "predominantly breastfed" and received no more than 1 formula feeding per day.
[73] Ten essential amino acids were analyzed: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine, and valine.
[74] Intake of formula (quantitative) and consumption of complementary foods (semi-quantitatively) recorded by parents on "hand-held diaries" for 3 days before the visits at 3, 4, 6, and 9 mo. However, as stated in EFSA (2017), the calculation of protein and energy from complementary foods at any timepoint was not possible with the information provided by the studies. Table 12 in EFSA (2017) provides the best available evidence on daily protein intakes in the formula groups (from formula alone) at 4, 6, 9, and 12 months.
[75] An Internet randomisation system used, Trial Sys.
[76] Type of feeding was "exclusively formula" or "formula and breast".
[77] Primary variable (weight gain from 3 to 6 mo) was analyzed by ANCOVA with correction for infant weight at 3 mo, infant gender, ethnicity, maternal BMI, antibiotic use, and complementary foods before 6 months. Anthropometric data converted to z-scores and fixed effects were maternal BMI, infant gender, and ethnicity.
[78] Variables statistically compared at baseline were: Mothers –age (years); weight (kg); height (cm); BMI (kg/m^2); smoking in pregnancy (%); alcohol in pregnancy (%); caesarean delivery (%); Caucasian ethnicity (%). Infants – gender; weight (kg); length (cm); head circumference (cm); BMI at birth (kg/m^2). Differences between groups were not statistically difference except that mothers of breastfed infants were more likely to consume alcohol and less likely to smoke than mothers of formula-fed infants. There were no differences between infants between the 3 groups.

Table 5.2.1.3.2-1 Key Characteristics of Studies Comparing the Effects of a Lower- *versus* a Higher-Protein Formula[67]

Reference	Study Objective	Study Design, Duration, Country	Study Power	Study Population	Intervention	Measured Outcomes and Assessment Methods	Statistically Analysed Outcomes[68]	Dietary Intakes	Analysis Populations	Strengths (Confounders Accounted For) and Limitations
										provided for all relevant outcomes (*e.g.,* head circumference z-score; length-for-age z-score; BMI-for-age z-score; body composition)
Ziegler *et al.* (2015)	To establish the adequacy (safety) of a formula with a protein content of 1.61 g/100 kcal (0.39g/100kJ), and to determine whether the LPF may decrease the proportion of infants who were growing rapidly.	Design: R, DB, C, P, BF reference group included Formula-fed infants **84±4 days (~3 mo) to 4 mo**[79]: LPF (1.61 g/100 kcal; 0.39g/100kJ) or HPF (2.15 g/100 kcal; 0.51g/100kJ) **4 to 6 mo:** LPF or HPF + complementary foods in small amounts[80] **6 to 12 mo:** LPF or HPF + complementary foods in unrestricted amounts Breastfed infants **3 to 4 mo:** Exclusive breastfeeding **4 to 6 mo:** Breastfeeding + complementary foods in small amounts **6 to 12 mo:** Breastfeeding + complementary foods in unrestricted amounts +	Sample size of 88 infants/group was calculated based on a mean difference in weight gain of 2 g/day that is clinically relevant[81]	Healthy term infants[82] Birth: n= 344 infants enrolled 3 mo: 194 FF infants randomized to LPF (n=97) or HPF (n=97); 112 BF infants 6 mo: n=92 analysed (LPF); n=91 analysed (HPF); n=109 analysed (BF) 12 mo: n=87 analysed (LPF); n=87 analysed (HPF); n=105 analysed (BF)	LPF: 1.61 g/100 kcal (0.39g/100kJ) HPF: 2.15 g/100 kcal (0.51g/100kJ) Protein was provided by intact bovine milk protein with a whey to casein ratio of 60:40[83] Levels of vitamins + minerals + trace elements in formula met recommendations stated by CODEX Alimentarius	• **Weight**: measured to the nearest 10 g using "established methods". • **Length:** measured to nearest 1 mm using "established methods" • **Head circumference:** measured to nearest 0.5 cm using a non-stretchable measuring tape • **Body composition:** no details provided • **Plasma biomarkers**: Analyses conducted at laboratories using standardized assays or methods	• Weight gain (g/day) • Weight-for-age z score • Length-for-age z score • Head circumference z score • BMI (kg/m²) • BMI-for-age z score • BUN (mg/dL) • IGF-1 (μg/L) • Albumin (g/L) • C-peptide (pmol/L)	For the LPF and HPF groups, formula intake (mL/day) at 3, 4, 6, 8, 10, 12 mo. and protein intake (g/kg/day – formula alone) at 4 and 6 mo analysed[84].	ITT (nutritional parameters)[85] and PP (biomarkers)[86]	**Strengths** • R, DB, C study • Study powered for a clinically relevant outcome (weight gain) • Randomization stratified for study centre, gender of infant, pre-pregnancy maternal BMI (<25; 25 to <30; ≥30)[87] • Appropriate inclusion/exclusion criteria which accounted for gestational age, birthweight, and health of infants • Covariates considered in statistical analyses[88] • Baseline comparability of infants and mothers across the 3 groups provided[89] • Formula and protein intakes assessed every 1 to 2 months **Limitations** • Quantitative and statistical results not provided for all relevant

[79] Infants who had been fed formula for ≥2 weeks were randomly assigned to a formula group.
[80] Up to 3 teaspoons per day of dry cereal could be provided, and up to 2 tablespoons per day of single-ingredient first or second foods beginning at 5 months.
[81] Other assumptions for the sample size calculation were: a standard deviation of 4 g/day, a type I error (α) of 5%, and a power of 90%. The additional recruitment of 22 infants/group was planned to compensate for an expected 20% dropout rate. **The primary study outcome was weight gain (g/day) from 3 to 6 months because during that period, the study formulas were the near-exclusive source of nutrients.**
[82] Inclusion criteria: Infants born ≥37 weeks of gestation with birth weight ≥2,500 g and ≤4,500 g were enrolled at or before 3 months of age. Exclusion criteria: multiple births, infants with illnesses or malformations that could affect growth, infants with suspected or confirmed allergy to cows' milk protein, and infants who participated in another clinical trial.
[83] HPF contained unmodified bovine milk proteins with a whey:casein ratio of 60:40. LPF contained bovine whey proteins modified by removal of caseinoglycomacropeptide, resulting in higher tryptophan and lower threonine content. LPF also contained essential and branched-chain (insulinogenic) amino acids in amounts close to mature breast milk.
[84] A feeding questionnaire completed at each visit (visits occurred every 28±4 days until age 168 days, after which visits occurred within 14 days of ages 240, 300, and 360 days) to determine the amount of formula and complementary foods that the infant consumed during the preceding 2 days. However, as stated in EFSA (2017), the information provided by this study did not allow for the calculation of energy and protein intake from complementary foods at any timepoint. The best available evidence on daily protein intakes in the formula groups (from formula alone) at 4, 6, 8, and 12 months is summarised in Table 12 in EFSA (2017).
[85] Results on the following nutritional parameters were based on ITT analysis: weight gain and z-scores for anthropometric outcomes.
[86] Results on the following biomarkers were based on PP analyses: BUN; IGF-1; Albumin; C-peptide.
[87] Random allocation sequences were generated with the Nestlé Trial Balance application using secure access *via* the Internet.
[88] Primary variable (weight gain from 3 to 6 mo) was analyzed by ANCOVA with correction for infant weight gain between 0 and 3 mo, infant gender, and maternal pre-pregnancy BMI. Anthropometric data converted to z-scores and fixed effects were values at 3 mo, infant gender, and maternal BMI.
[89] Variables statistically compared at baseline were: Infants – % male; % Caucasian; weight at birth (kg); length at birth (cm); BMI at birth (kg/m²); gestational age (wk); vaginal delivery. Mothers – age (yrs), weight (kg), length (cm), BMI (kg/m²), cigarettes/day during pregnancy. There were no differences between infants in the 3 groups; however, mothers of breastfed infants had significantly lower weight and BMI (mean of 64.0 kg and 23.2 kg/m2, respectively) than mothers of infants fed LPF (73.5 kg and 25.8 kg/m², respectively) or HPF (70.3 kg and 25.5 kg/m², respectively). There were no differences between infants between the 3 groups.

Table 5.2.1.3.2-1 Key Characteristics of Studies Comparing the Effects of a Lower- *versus* a Higher-Protein Formula[67]

Reference	Study Objective	Study Design, Duration, Country	Study Power	Study Population	Intervention	Measured Outcomes and Assessment Methods	Statistically Analysed Outcomes[68]	Dietary Intakes	Analysis Populations	Strengths (Confounders Accounted For) and Limitations
		'standard formula' (if requested by parents) Country: U.S.								outcomes (*e.g.,* head circumference z-score); however, reported in EFSA (2017)

ANCOVA = analysis of covariance; BF = breastfed; BMI = body mass index; BUN = blood urea nitrogen; C = controlled; CFU = colony forming units; DB = double-blind; DXA = dual-energy x-ray absorptiometry; EFSA = European Food Safety Authority; FF = formula-fed; HPF = higher protein formula; ITT = intention-to-treat; IGF-1 = insulin-like growth factor 1; kcal = kilocalorie; LPF = lower protein formula; mo = month; NR = not reported; P = parallel; PP = per protocol; R = randomised; U.S.= United States; wk = week; yrs = years.

Appraisal of Studies

As stated earlier, both Inostroza *et al.* (2014) and Ziegler *et al*. (2015) investigated the effects of a LPF containing a protein to energy ratio of 1.61 or 1.65 g total protein/100 kcal (0.39g/100kJ) that aligns with the proposed compositional change of minimum 1.6 g total protein/100 kcal (0.38g/100kJ). A control group of infants consuming a HPF (2.15 or 2.7 g total protein/100 kcal; 0.51 or 0.65g/100kJ) and a reference group comprised of breastfed infants were included in both studies.

Both Inostroza *et al.* (2014) and Ziegler *et al.* (2015) used robust study designs (randomized, double-blind and controlled) and the studies were powered for a clinically relevant outcome (weight gain from 3 to 6 months), which is also an outcome indicative of the safety and tolerance of a lower-protein follow-on formula which is the subject of this application.

Both studies followed a similar timeline for introduction of the study formulas. Across both studies, the formulas (LPF or HPF) were the near exclusive source of nutrients from 3 to 6 months, and from 6 to 12 months, the infants continued consuming their formulas (LPF or HPF) but also consumed complementary foods in unrestricted amounts.

As outlined in Table 5.2.1.3.2-1, investigators of both studies attempted to reduce the effects of potential confounders and bias and increase the rigor of their studies by:

- Using established and valid methods to measure anthropometric variables (weight, length, head circumference) (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015).
- Using WHO standards to establish z-scores for anthropometric outcomes (length-for-age, weight-for-age, head circumference-for-age) (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015).
- Considering infant characteristics (infant gestational age, birth weight, and overall health) [Inostroza *et al.,* 2014; Ziegler *et al.,* 2015)] and maternal characteristics (Inostroza *et al.,* 2014) in the inclusion/exclusion criteria used for infant enrolment.
- Stratifying the randomization of infants to the formula groups based on infant and maternal characteristics [infant gender, ethnicity, pre-pregnancy maternal BMI (25 to 30 or >30 kg/m^2), and type of feeding between 1.5 to 3 months in Inostroza *et al.* (2014); and, study centre, gender of infant, and pre-pregnancy maternal BMI (<25; 25 to <30; ≥30) in Ziegler *et al.* (2015)] which was **successful in producing no significant differences in infant characteristics across the 3 groups at baseline** (LPF, HPF, breastfed) in each study (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015).
- Including covariates in the statistical analyses of results which, for the analysis of the primary variable of weight gain were: infant weight at 3 months, infant gender, ethnicity, maternal BMI, antibiotic use, and complementary foods before 6 months in Inostroza *et al.* (2014); and, infant weight gain between birth and 3 months, infant gender, and maternal pre-pregnancy BMI in Ziegler *et al.* (2015). For the analysis of anthropometric data converted to z-scores, the fixed effects included were maternal pre-pregnancy BMI, infant gender, and ethnicity in Inostroza *et al.* (2014) and outcome values at 3 months, infant gender, and maternal pre-pregnancy BMI in Ziegler *et al.* (2015).
- Assessing formula intakes throughout the study period (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015).

Overall Conclusions

Across 2 pertinent studies (Inostroza *et al.,* 2014 and Ziegler *et al.,* 2015) wherein, in combination, 141 infants received a LPF and 166 infants were breastfed[90], at 6 and 12 months of age, as compared to breastfed infants, there were no adverse effects in infants receiving the LPF on infant weight, length, and head circumference. More specifically, as summarized in Appendices A to D in EFSA (2017), across both studies for 'completers' and the 'per protocol population', at 6 and 12 months, the LPF led to **mean increases** in weight (kg), length (cm), and head circumference (cm) as compared to the breastfed reference group; **mean increases** in weight gain (g/day) and weight change (kg) were also observed with the LPF compared to the breastfed reference group during this time period (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015)[91]. Indeed, in both studies for 'completers' and the 'per protocol population', from 6 to 12 months, weight gain (g/day) and weight change (kg) in the LPF *versus* the breastfed reference group were more similar as compared to the HPF *versus* the breastfed reference group (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015). Furthermore, as asserted by EFSA (2017), with intake of the LPF, mean weight-for-age z-scores were, at all ages, at or above the median of WHO Growth Standards across both studies.

Regarding changes in anthropometric outcomes, statistically significant[92] findings were observed only for length and head circumference in Inostroza *et al.* (2014) at 3 months, which resolved by 6 months (*i.e.,* at 6 months, there were no statistically significant unfavourable differences between infants on LPF *versus* breastfed infants in length or head circumference for both the per protocol (PP) population and completers).

Regarding changes in blood urea nitrogen, across the 2 studies (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015), an inconsistent effect of the LPF was observed on blood urea nitrogen. Specifically, in Inostroza *et al.* (2014), at 12 months, blood urea nitrogen was higher in the LPF group *versus* breastfed infants (10.58±15.14 *versus* 8.84±4.82 mg/dL, respectively) albeit levels were not significantly different between groups (p=0.833). In contrast, in Ziegler *et al.* (2015), at 12 months, blood urea nitrogen was statistically significantly lower in the LPF group *versus* breastfed infants (11.3 *versus* 12.66 mg/dL, respectively; p=0.006). Importantly, as noted by EFSA (2017), in both studies, blood urea nitrogen remained within the normal range in the LPF group.

Regarding changes in plasma levels of essential amino acids, which were only measured in 1 of the 2 studies (Inostroza *et al.,* 2014), at 6 months of age, mean plasma concentrations of 9 of 10 essential amino acids were higher in the LPF group as compared to the breastfed group (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, tyrosine, valine) with the exception of threonine which was lower in the LPF group *versus* the breastfed group. Furthermore, the higher levels of essential amino acids in the LPF group *versus* the breastfed group reached statistical significance at $p<0.05$ for 7 amino acids (isoleucine, lysine, methionine, phenylalanine, tryptophan, tyrosine, valine); higher levels of leucine in the LPF group were not statistically different from the breastfed group (p=0.09) and statistical results were not reported for histidine and threonine. Data for plasma levels of essential amino acids were not provided for the 12th month timepoint.

As such, based on an assessment of the effect of a LPF *versus* breastfeeding on anthropometric variables and other blood or plasma measures, the 2 pertinent studies (Inostroza *et al.,* 2014 and Ziegler *et al.,* 2015) provide evidence that a follow-on formula made with a lower total protein level of

[90] Number of infants analyzed across both studies, in combination, at 12 months.

[91] The difference in weight gain achieved with intake of a LPF *versus* infants in the breastfed reference group from 6 to 12 months was 1.04 g/day (95% CI=0.12 to 1.95) for 'completers' in Ziegler *et al.,* (2015) and 0.77 g/day (95% CI=-0.50 to 2.05) for 'completers' in Inostroza *et al.* (2014).

[92] An unfavourable difference is meant to indicate a net decrease in an anthropometric parameter when comparing the LPF group *versus* the breastfed group.

1.61 (0.39g/100kJ) (Ziegler *et al.,* 2015) or 1.65g/100 kcal (0.39g/100kJ) (Inostroza *et al.,* 2014) is safe and results in normal growth and development.

5.2.1.3.3 Efficacy of the proposed compositional change

The following lines of evidence highlight the public health implications associated with protein intakes during infancy that are in excess of metabolic requirements. The below-mentioned findings justify the 'efficacy' of a lower-protein follow-on formula as proposed in this Application, in that it could reduce the risk of excessive weight gain during and after infancy and thus decrease the risk of overweight/obesity in later life.

- Compared to breastfed infants, formula-fed infants have greater body weight gains in infancy (Dewey, 1998; Kramer *et al.,* 2004; Victora *et al.,* 1998 cited in Koletzko *et al.,* 2009) and evidence exists to indicate that rapid weight gain in infancy is associated with an increased risk of overweight in children (Péneau *et al.*, 2011; Weng *et al.,* 2012).

- Different intakes of metabolizable substrates, specifically protein (Koletzko *et al.,* 2005), between formula-fed and breastfed infants may explain the greater weight gain in formula-fed infants. Indeed, protein intake per kilogram of body weight is estimated to be 55 to 80% higher in formula-fed than breastfed infants (Alexy *et al.,* 1999 cited in Koletzko *et al.,* 2009). This observation is supported by the study by Conn *et al.* (2009), wherein the food and nutrient intakes of 9-month old infants (n=341; 180 boys and 161 girls; median weight of 9.2 kg; median length of 71.2 cm) living in Australia were measured from 1999 to 2001. Based on dietary intake data (that reflected consumption patterns 'over the past month') ascertained through structured open-ended questions and with a food frequency format, the mean±SD protein intake reported for 'breastfed infants' (n=121)[93] and 'not breastfed infants' (n=220) was 23±7 g protein/day and 30±8 g protein/day, respectively, a difference which reached statistical significance (p<0.001). Based on these nutrient intakes generated for 9-month old infants living in Australia, it appears that breastfed infants have about a 30% lower daily protein intake as compared to infants who are not breastfed. An analysis of dietary data generated from the Melbourne (Australia) InFANT wherein dietary data for children at ages 9 and 18 months, and 3.5 and 5 years were collected using three 24-hour dietary recalls (Lioret *at al.,* 2013; Campbell *et al.,* 2017) showed that at 9 months, both the earlier introduction of solids and the primary milk source being formula/dairy or mixed (as opposed to it being breast milk), were associated with the consumption of significantly more protein per 1, 000 kcal (Campbell *et al.,* 2017).

- Epidemiological evidence exists that links high protein intakes in infancy to overweight (including obesity) in childhood (Ohlund *et al.,* 2010; Rolland-Cachera *et al.,* 1995; Gunnarsdottir and Thorsdottir, 2003; Günther *et al.,* 2007), contributing to the 'early protein hypothesis' – *i.e.,* that high protein intakes in excess of metabolic requirements early in life enhance weight gain in infancy and increase obesity risk later in life (Koletzo *et al.,* 2005). In a study by Inostroza *et al.* (2014) wherein infants were fed a LPF (1.65 g/100 kcal; 0.39g/100kJ) or a HPF (2.7 g/100 kcal; 0.65g/100kJ) from 3 to 12 months (with complementary foods introduced in an unrestricted fashion from 6 to 12 months), at 6 and 12 months of age, the percentage of infants whose weight was >90th percentile of the WHO standards was significantly lower among infants fed the

[93] Regarding the contribution of breast milk to daily nutrient intakes, Conn *et al.* (2009) stated the following: "The quantity of breast milk consumed was estimated from information on the frequency of feeding only, as descriptions of the duration of feeds were often too variable or vague to be useful in this regard. The volume of breast milk per feed was calculated from the data of Dewey *et al.* (1984). Where breast-feeding occurred 6 or more times daily, the assigned volume was 130 mL per feed, with 4 or 5 feeds per day assigned 101 mL per feed and up to 3 feeds per day assigned 55 mL per feed. The nutritional content of breast milk was obtained from published values (Department of Health and Social Security, 1977)".

LPF *versus* the HPF (10.6% *versus* 22.4%, respectively, at 6 months[94] and 18.5% *versus* 31.8%, respectively, at 12 months[95]). The lower protein intake achieved by breastfed *versus* formula-fed infants may be among the reasons why breastfed infants are at lower risk of overweight/obesity later in life (Weng *et al.,* 2012; Arenz *et al.,* 2004; Owen *et al.,* 2005; Harder *et al.,* 2005; Hester *et al.,* 2012).

- Dietary surveys informing on the protein intakes of 9-month old infants were conducted in Australia (Melbourne or Adelaide) in 1999 to 2001 (Conn *et al.,* 2009) and 2008 to 2009 (Lioret *et al.,* 2013). From these 2 studies, the mean (±SD) protein intakes of 9-month old infants were 29.0±10.9 g protein/day for girls and boys (Lioret *et al.,* 2013; n=177; breastfed or not breastfed + complementary foods); 26±8 g protein/day for girls (Conn *et al.,* 2009; n=161; breastfed or not breastfed + complementary foods); and, 29±8 g protein/day for boys (Conn *et al.,* 2009; n=180; breastfed or not breastfed + complementary foods). The mean daily protein intakes (~26 to 29 g/day) and also the median daily protein intakes (25 to 29 g/day) across both these studies indicate that 9-month old infants living in Australia (Melbourne or Adelaide) are far exceeding (by about 2-fold) the Australian Government NHMRC's AI for dietary protein (14 g/day) for older infants (7 to 12 months of age) and the IOM's RDA for dietary protein for older infants (7 to 12 months of age). Protein intakes in excess of metabolic requirements have public health implications, as discussed above. Indeed, dietary intake analyses conducted by Campbell *et al.* (2017), who report on the same study cohort as described by Lioret *et al.* (2013), provide evidence that protein intakes at 9 and 18 months of age can predict intakes at 5 years – *i.e.,* the residualised protein intakes at 9 months were significantly associated with intakes at 18 months (p=0.007) and 5 years (p=0.006). **Lowering the currently excessive protein intakes in older infants (6 to 12 months of age) who are not breastfed could result in potential health benefits in the local Australian-New Zealand context, namely a reduced risk of excessive protein intakes during and after infancy and possibly a reduced risk of overweight/obesity later in life.**

The above findings highlight the potential adverse effects associated with excess dietary protein intake. However, it should be noted that dietary protein is, indeed, an essential component of the diet, supplying the body with nitrogen and amino acids (EFSA, 2017), which are needed for the synthesis of nucleic acids, hormones and vitamins (IOM, 2005). Proteins are the major structural components of all cells in the body (IOM, 2005) and are essential in growth and development (Dupont, 2003), including the development of the brain and bones (Bonjour *et al.,* 2001). Proteins also function as enzymes and transport carriers (IOM, 2005).

Proteins play a particularly important function in infancy (*i.e.,* birth to 12 months), when growth and brain development are at their peak. In fact, during the 1st year of life, ~87% of protein intake over and above that used for maintenance is utilized for tissue synthesis (Dewey *et al.,* 1996 cited in Dupont, 2003). It is estimated that protein synthesis in infants averages 6.9 g/kg/day (Young *et al.,* 1975; IOM, 2005). Many proteins in human milk have demonstrated roles beyond nutrition, providing enzymatic activity, enhancing nutrient absorption, stimulating growth, modulating the immune system and defending against pathogens by inhibiting bacterial adhesion (Lönnerdal *et al.,* 2017).

The applicant is aware of the FSANZ review of evidence undertaken in 2016 as part of the Nutrition Assessment for Proposal P1028. Whilst acknowledging that all studies have limitations, the applicant is of the view that the large CHOP study (Koletzko et al, 2009; Weber et al, 2014) has several methodological strengths and is exactly the type of infant feeding study that health professionals and regulators alike have long been requesting (Koletzko et al 2012; ESPGHAN Committee on Nutrition, 2001; ESPGHAN Committee on Nutrition, 2003). It was independent of industry funding, a prospective

[94] Odds ratio = 5.3, 95% CI =1.2 to 23.5.
[95] Odds ratio = 3.6; 95% CI =1.1 to 11.2.

double-blind randomised trial completed in several countries and with a sample size in excess of 1000, with long-term follow up. In all of these regards, it can be considered a 'model' clinical trial. Indeed the conclusions of the 6-year follow up noted that it provides ***"strong evidence"*** (Weber et al, 2014). Furthermore, since the 2016 review of FSANZ, we note the positive review of EFSA (2017) on protein level of 1.6g/100kcal (0.38g/100kJ), & Koletzko (2017) where it is stated that there is ***"conclusive evidence for programming effects of infant protein supply".*** The applicant also notes that the CHOP study has been instrumental in amending the protein levels in regulations including the EU and Codex (Koletzko, 2017). Clinical evidence also continues to build with further published clinical trials (Oropeza-Ceja et al, 2018; Liotto et al, 2018) which support the notion that infants fed lower protein infant formula products grow more similarly to their fully breastfed counterparts, when compared to infants fed higher protein formulations. It is noted that these outcome measures are aligned to the policy principles of the Ministerial Council, who state that follow-on formula "…must strive to achieve as closely as possible the normal growth and development…of healthy full term breastfed infants at the appropriate age" (Australian and New Zealand Food Regulation Ministerial Council, 2011). Taken all together, the applicant is of the view that the reduction in protein is safe, and that the available evidence is supportive of the link between early protein intake and metabolic programming.In 2 randomised, double-blind, controlled intervention studies conducted in Chile (Inostroza *et al.,* 2014) and in the U.S. (Ziegler *et al.,* 2015), the effects, on infant growth (weight, length, head circumference), of a LPF (1.61 or 1.65 g/100 kcal, equivalent to 0.39g/100kJin Ziegler *et al.,* 2015 and Inostroza *et al.,* 2014, respectively) were compared to a HPF (2.15 g/100 kcal or 2.70 g/100 kcal in Ziegler *et al.,* 2015 and Inostroza *et al.,* 2014, respectively – equivalent to 0.51 and 0.65g/100kJ) and also to a breastfed reference group. The formulas were administered from 3 to 12 months of age[96]. Across both studies (Inostroza *et al.,* 2014 and Ziegler *et al.,* 2015) wherein, in combination, 141 infants received a LPF and 166 infants were breastfed[97], at 6 and 12 months of age, as compared to breastfed infants, there were no adverse effects on infants receiving the LPF on infant weight, length, and head circumference. As summarized in Appendices A to D in EFSA (2017), across both studies for 'completers' and the 'per protocol population', at 6 and 12 months, the LPF led to mean increases in weight (kg), length (cm), and head circumference (cm) compared to the breastfed reference group; mean increases in weight gain (g/day) and weight change (kg) were also observed with the LPF compared to the breastfed reference group during this time period (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015)[98]. Indeed, in both studies for 'completers' and the 'per protocol population', from 6 to 12 months, weight gain (g/day) and weight change (kg) in the lower-protein group *versus* the breastfed reference group were more similar as compared to the higher-protein group *versus* the breastfed reference group (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015). **These findings demonstrate that a LPF (1.61 or 1.65 g total protein/100 kcal; 0.39g/100kJ) used by infants 6 to 12 months of age contributes to healthy rates of growth and thus a follow-on formula with a lower protein level can achieve its intended outcomes in older infants 6 to 12 months of age.**

Interestingly, in Inostroza *et al.* (2014), at 6 and 12 months of age, the percentage of infants whose weight was >90th percentile of the WHO standards was significantly lower among infants fed the LPF *versus* the HPF (10.6% *versus* 22.4%, respectively, at 6 months[99] and 18.5% *versus* 31.8%, respectively, at 12 months[100]). **Thus, a LPF can not only lead to healthy rates of growth in infants 6 to 12 months of age, but it may also have public health implications with respect to effects on risk for overweight (including obesity) later in life.**

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[96] In both studies (Inostroza *et al.,* 2014; Ziegler *et al.,* 2015), complementary foods were unrestricted from 6 to 12 months.
[97] Number of infants analyzed across both studies, in combination, at 12 months.
[98] The difference in weight gain achieved with intake of a LPF *versus* infants in the breastfed reference group from 6 to 12 months was 1.04 g/day (95% CI=0.12 to 1.95) for 'completers' in Ziegler *et al.,* (2015) and 0.77 g/day (95% CI=-0.50 to 2.05) for 'completers' in Inostroza *et al.* (2014).
[99] Odds ratio = 5.3, 95% CI =1.2 to 23.5.
[100] Odds ratio = 3.6; 95% CI =1.1 to 11.2.

5.2.2 Information Related to the Dietary Intake or Dietary Exposure

5.2.2.1 Data to enable the dietary intake or exposure of the target population to be estimated

Section 5.1.4.3 includes a comprehensive discussion of the estimated dietary protein intakes of infants 6 to 12 months of age should a follow-on formula be reformulated to contain a lower minimum total protein of 1.6 g/100 kcal (0.38g/100kJ). Section 5.1.4.3 also includes dietary data on the protein intakes of 9-month old infants living in Australia (Melbourne or Adelaide) (Conn *et al.,* 2009; Lioret *et al.,* 2013; Campbell *et al.,* 2017).

5.2.2.2 Data on the recommended level of formula consumption for the target population

The recommended level of formula consumption can differ between manufacturers (different feeding tables, reconstituted volumes, and scoop weights) and between recipes.

The Applicant (Nestlé) has standardised feeding tables by age, as does the rest of industry. The following information required by 5.2.2.2 relates to a typical follow-on formula for The Applicant (Nestlé):

(i) the capacity of the product scoop (in grams of product) – 4.5 g
(ii) the number of scoops required per feed – 7 scoops
(iii) the volume of water required per feed – 210 mL
(iv) total volume of the made-up feed – 238 mL

(v) recommended number of feeds per day relevant to each age group in the relevant target population – 4-3 feeds for infants 6-9 months of age, 3 feeds for infants 9-12 months of age

In the context of this Application to request a protein minimum of 1.6g/100kcal (0.38g/100kJ) for follow-on formula, we have also calculated estimated daily protein intake based on 1.6g/100kcal (0.38g/100kJ) protein and the scoop weight and feeding tables of the 5 major manufacturers and brands on the Australian and New Zealand market representing the substantial market share of the total market:

Based on Protein at 1.6g/100kcal (assume same energy and reconstitution instructions, and total volume of feeds per day)								
BRAND A (Applicant; Manufacturer #1)	per 100ml	per 100kcal	Reconstitution					
Protein (g)	1.07	1.60	1 scoop + 30 ml =	34	ml			
Energy (kcal)	67							
Age of infant	Scoops per serve	Volume	no. of feeds per day	**total protein per day**	**% AI (from breast milk 6.9g/day)**	no. of feeds per day	**total protein per day**	**% AI (from breast milk 6.9g/day)**
6-9 months	7	238	3	**7.7**	**110.9%**	4	**10.2**	**147.9%**
Over 9 months	7	238	3	**7.7**	**110.9%**	4	**10.2**	**147.9%**
BRAND B (Manufacturer #2)	per 100ml	per 100kcal	Reconstitution					
Protein (g)	1.09	1.60	1 scoop + 50ml =	56	ml			
Energy (kcal)	68							
Age of infant	Scoops per serve	Volume	no. of feeds per day	**total protein per day**	**% AI (from breast milk 6.9g/day)**	no. of feeds per day	**total protein per day**	**% AI (from breast milk 6.9g/day)**
6-9 months	5	280	3	**9.1**	**132.5%**	4	**12.2**	**176.6%**
9-12 months	4	224	3	**7.3**	**106.0%**	4	**9.7**	**141.3%**
BRAND C (Manufacturer #3)	per 100ml	per 100kcal	Reconstitution					
Protein (g)	1.04	1.60	1 scoop + 60 ml =	66.7	ml			
Energy* (kcal)	65							
Age of infant	Scoops per serve	Volume	no. of feeds per day	**total protein per day**	**% AI (from breast milk 6.9g/day)**	no. of feeds per day	**total protein per day**	**% AI (from breast milk 6.9g/day)**
6-12 months	3	200	4	**8.3**	**120.6%**	5	**10.4**	**150.8%**
BRAND D (Manufacturer #4)	per 100ml	per 100kcal	Reconstitution					
Protein (g)	1.18	1.60	1 scoop + 50 ml =	56	ml			
Energy* (kcal)	74							
Age of infant	Scoops per serve	Volume	no. of feeds per day	**total protein per day**	**% AI (from breast milk 6.9g/day)**	no. of feeds per day	**total protein per day**	**% AI (from breast milk 6.9g/day)**
6-9 months	5	280	3	**9.9**	**144.1%**	4	**13.3**	**192.2%**
9-12months	4	224	3	**8.0**	**115.3%**	4	**10.6**	**153.7%**
BRAND E (Manufacturer #5)	per 100ml	per 100kcal	Reconstitution					
Protein (g)	1.04	1.60	1 scoop + 60 ml =	66.7	ml			
Energy* (kcal)	65							
Age of infant	Scoops per serve	Volume	no. of feeds per day	**total protein per day**	**% AI (from breast milk 6.9g/day)**	no. of feeds per day	**total protein per day**	**% AI (from breast milk 6.9g/day)**
6-12 months	4	267	3	**8.4**	**121.2%**	4	**11.2**	**161.6%**
***Energy converted from kJ to kCal. Conversion factor 4.18. Ref FSC Std 1.2.8 Clause 1 (4)**								

The data above demonstrates, that along with a sufficient complementary diet, older infants are consuming sufficient protein. It is also worthwhile to mention, that these calculations are based on a conservative and restrictive assumption, given it is not technically feasible to manufacture a recipe to target 1.6g/100kcal (0.38g/100kJ), and the actual recipe protein target will actually be higher than the regulatory minimum, in order to account for raw material, manufacturing, and analytical variability.

5.2.2.3 Information relating to the substance

The older infant (6 to 12 months of age) should be consuming a progressively diversified diet which, in addition to principal milk sources of protein (*i.e.,* breast milk, formula, dairy), will contain complementary food sources of protein.

No national Australian or New Zealand nutrition survey data exist for children <2 years of age to understand the likely consumption of protein from "complementary foods" that infants are likely to consume from 6 to 12 months of age. The best available data on <u>mean (±SD) daily protein intakes</u> of older infants (*i.e.,* 9 months of age) living in Australia (Melbourne or Adelaide) were reported by Lioret *et al.* (2013)[101] and Conn *et al.* (2009)[102]. Across both studies, mean daily protein intakes ranged from 26 to 29 g/day: <u>29.0±10.9 g protein/day</u> for girls and boys (Lioret *et al.,* 2013; n=177; breastfed or not breastfed + complementary foods) and <u>26±8 g protein/day for girls</u> (Conn *et al.,* 2009; n=161; breastfed

[101] In Lioret *et al.* (2013), the infants' dietary intakes were assessed by trained nutritionists by telephone-administered dietary recalls with parents. Booklets were provided to parents to aid in the estimation of portion sizes. Two or 3 non-consecutive days of dietary data were collected for each infant, including 1 weekend day. Calls were unscheduled where possible. Nutrient intakes were evaluated using the 2007 Australian Food, Supplement and Nutrient (AUSNUT) Database (FSANZ, 2008).
[102] In Conn *et al.* (2009), the infants' dietary intakes were ascertained *via* face-to-face interviews using structured open-ended questions about consumption patterns 'over the past month' with a food frequency format (checklists with commonly-eaten foods were incorporated as standard prompts). Photographs were used to aid in the estimation of portion sizes. Most of the nutritional information was obtained from NUTrient data TABle for use in Australia (NUTTAB95).

or not breastfed + complementary foods) and 29±8 g protein/day for boys (Conn *et al.,* 2009; n=180; breastfed or not breastfed + complementary foods).

More specifically, Lioret *et al.* (2013) reported that in 9-month old infants (breastfed or not breastfed), foods represented, on average, 40.5% of total food/beverage intake (in grams) and the percentage of infants who consumed the following food groups at high levels (*versus* at intermediate or low levels of consumption) was: water (96.0%), cereal-based products (96.6%), meat and poultry (81.4%), animal products (meat products, meat and poultry, fish and egg products) (89.3%), fruits (94.9%), vegetables (94.9%), and dairy foods (88.1%), baby foods in jars (89.8%), breast milk (46.3%), infant or toddler formula (71.2%). In Conn *et al.* (2009), wherein the intake of foods was stratified for "breastfed" or "not breastfed" infants, in "not breastfed" infants, the percentage of 9-month old infants consuming the following foods was as follows: formula (92%), cows' milk (49%), water (95%), other drinks (62%), cereals (100%), dairy foods (excluding cows' milk) (96%), meat or poultry (66%), fish (27%), eggs (20%), fruit (92%), vegetables (89%), infant dinners (64%), fats and oils (54%), sugar and products (39%), miscellaneous (68%).

In considering the findings of Lioret *et al.* (2013) and Conn *et al.* (2009), it is apparent that infants 9 months of age, including non-breastfed infants, living in Australia, are consuming complementary foods and, further, the majority are consumers of food groups containing foods that are good sources of protein, such as meat and/or poultry, dairy foods, and cereals.

Corroborating the above findings are data compiled by the EFSA showing the protein intakes of infants 6 to 12 months of age from Bulgaria (n=343), Denmark (n=473) and the United Kingdom (n=1029) from 17 different food groups[103] (EFSA, 2017 – see Table 9). Across all 3 countries, of 16 food groups (excluding the food group of "infant/follow-on formula"), the food groups most greatly contributing to the daily protein intakes of infants 6 to 12 months of age are "milk and dairy products" (contributing 4.4 to 9.2 g protein/day), "grains and grain-based products" (contributing 2.4 to 5.3 g protein/day), and "meat and meat products" (contributing 2.9 to 5.2 g protein/day). Taking into account the daily protein contributions of all the food groups listed in Table 9 in EFSA (2017) and excluding the contribution of infant/follow-on formula to daily protein intakes, across Bulgaria, Denmark, and the United Kingdom, **complementary foods alone contribute 24.6, 23.7 and 17.7 g protein/day, respectively. Importantly, based on these data, the daily protein consumed by infants 6 to 12 months of age (from Bulgaria, Denmark, or the United Kingdom) from complementary foods alone exceeds the Australian Government NHMRC's recommended mean protein intake from complementary foods which, for infants 7 to 12 months of age, is 7.1 g protein/day (NHMRC, 2014; U.S. NHANES III data – IOM, 2005), and also exceeds the NHMRC's AI for protein for older infants 7 to 12 months of age (14 g/day) and the IOM's RDA for protein for older infants 7 to 12 months of age (11.0 g/day). Thus, based on these mean data, during the period of infancy from 6 to 12 months of age, the protein requirements of infants are met by complementary foods alone.**

[103] The 17 food groups are: animal and vegetable fats and oils; composite food (including frozen products); eggs and egg products; fish and other seafood; food for infants and small children; fruit and fruit products; fruit and vegetable juices; grains and grain-based products; herbs, spices, and condiments; legumes, nuts, and oilseeds; meat and meat products; milk and dairy products; snacks, desserts, and other foods; starch roots and tubers; sugar and confectionary; vegetables and vegetable products (EFSA, 2017).

5.2.3 Information Related to Labelling Requirements Under Part 2.9 of the Code

5.2.3.1 Information related to safety or nutritional impact of the proposed labelling change

It is anticipated that a follow-on formula formulated with a lower protein content as proposed in this application (0.38 g/100 kJ or 1.6 g/100 kcal) will have a declared average protein content per 100 mL (as consumed) in the nutrition information panel on the product label.

No other labelling changes are expected on a follow-on formula formulated with a lower protein content (*i.e.*, 1.6 g/ 100 kcal or 0.38g/100kJ) and used by infants 6 to 12 months of age (*e.g.,* as related to warning or advisory statements, directions for use, or conditions).

5.2.3.2 Information to demonstrate that the proposed labelling change will be understood and will assist consumers

The reason for lowering the minimum amount of protein in infant follow-on formula products is to make its composition closer to that of human breast milk, which is considered the gold-standard of infant feeding. Follow-on formula manufacturers implementing this change will likely inform and educate health care professionals regarding the reasons for lowering protein levels in follow on formula. It is anticipated that such products may be recommended by health care professionals who can therefore help educate consumers about this change.

Consumer understanding could be better facilitated by providing information on product packs regarding the lowering of protein levels and associated benefits. However, as nutrient content and health claims are prohibited for infant formula products (Standard 1.2.7), this is not currently an avenue to assist consumers in making informed choices.

5.2.4 Information Related to Internationally Recognised Standards, Codes of Practice, Recommendations and Guidelines

5.2.4.1 The Australian Government's National Health and Medical Research Council

In 2012, the Australian Government's NHMRC published a literature review titled "Literature Review: Infant Feeding Guidelines" (NHMRC, 2012b) wherein the NHMRC acknowledged that formula-fed infants grow at a different rate than breastfed infants and the former are heavier at 12 months of age and have a slightly increased risk of later obesity (WHO European Region 2007 cited in NHMRC, 2012b). The NHMRC highlighted the findings of Koletzko *et al.* (2009)[104], also stating that "a lower protein intake in infancy might diminish the later risk of overweight and obesity". Additionally, due to the potential for excess protein to increase obesity, in the NHMRC's "Eat for Health: Infant Feeding Guidelines – Information for health workers" (NHMRC, 2012a), it is stated that "it is preferable to use a formula with a lower protein level".

5.2.4.2 Codex Alimentarius Commission – Committee on Nutrition and Foods for Special Dietary Uses

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[104] In Koletzko *et al.* (2009), 1138 healthy, formula-fed infants were randomly assigned to receive either cow milk-based infant and follow-on formula with lower (**1.77** and 2.2 g protein/100 kcal, respectively) or higher (2.9 and 4.4 g protein/100 kcal, respectively) protein contents for the 1st year; a breastfed reference group was also included (n=619). Weight, length, weight-for-length and BMI were determined at 3, 6, 12, and 24 months of age. This study was <u>excluded</u> by the applicant and was not considered a "pertinent study" to support the proposed composition change in the protein quantity of follow-on formula since the LPF in Koletzko *et al.* (2009) did not align with our compositional requirement of 1.61±0.05 g protein/100 kcal.

The Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) is currently drafting a revised *Standard for Follow-Up Formula* (CODEX STAN 156-1987 – Codex Alimentarius, 2017b)[105], which will specify a minimum protein content of 1.8 g/100 kcal (for cows' and goats' milk protein), with an associated footnote 6 permitting that '*A lower minimum protein level between 1.6 and 1.8 g/100kcal (0.38 and 0.43 g/100kJ) in follow-up formula based on non-hydrolysed milk protein can be accepted. Such follow-up formula and follow-up formula based on hydrolysed protein should be evaluated for their safety and suitability and assessed by a competent national and/or regional authority based on clinical evidence*' (CXS 156-1987 – for adoption at Step 5).

5.2.4.3 Scientific Committee on Food

In 2003, the EU's *Scientific Committee on Food* published a "Report of the Scientific Committee on Food on the Revision of Essential Requirements of Infant Formulae and Follow-on Formulae" (SCF, 2003). Included in this report were relevant comments related to modifying follow-on formula beyond established standards, as follows:

- "The Committee is aware of continuing improvements in the understanding of the complex composition of human milk, in dietary effects on physiological outcomes in the infant, and in food technology, which have led *and will continue to lead to innovative modifications of infant formulae and follow-on formulae*".
- "The addition of new ingredients or of *established ingredients in newly determined amounts that deviate from the established guidance on formula composition*, the reduction or elimination of current constituents, or any other modification of formula composition *should be made possible if the benefit, suitability and safety for particular use by infants have been established by generally accepted scientific data and this is overseen and evaluated by an independent scientific body prior to the introduction of such modified products into the market*".

5.2.4.4 European Food Safety Authority

As discussed throughout this application, in 2017, the EFSA published a 29-page scientific opinion on the safety of an infant follow-on formula with a protein content of 1.6 g/100 kcal equivalent to 0.38g/100kJ (EFSA, 2017). Their assessment was initiated by an application to the EC, submitted by Nestlé, to market a follow-on formula with a new minimum protein content of 1.61 g/100 kcal (0.39g/100kJ). For their assessment, the EFSA considered: (i) the dietary protein requirements of infants in the 2nd half of their 1st year of life; (ii) the protein content of breast milk during the 1st year of lactation; (iii) the dietary protein intake of infants in Europe from breast milk, formula, and complementary foods; (iv) the overall contribution that a follow-on formula with a protein content of 1.6 g/100 kcal (0.38g/100kJ) could make towards meeting the protein requirements in the target population, assuming an intake of complementary foods of sufficient quality; and (v) results of 2 human intervention studies in healthy term infants (Ziegler *et al.,* 2015; Inostroza *et al.,* 2014). **Overall, the EFSA concluded that a follow-on formula with a protein content of at least 1.6 g/100 kcal (0.38g/100kJ) from either intact cows' milk protein or intact goats' milk protein is safe and suitable for healthy infants living in Europe with an intake of complementary foods of sufficient quality.**

[105] CODEX STAN 156-1987 states that follow-up formula (suitable for infants aged 6 months on and for young children) contain "Not less than **3.0 g per 100 available calories** (or 0.7 g per 100 available kilojoules) of protein of nutritional quality equivalent to that of casein or a greater quantity of other protein in inverse proportion to its nutritional quality. The quality of the protein shall not be less than 85% of that of casein. The total quantity of protein shall not be more than 5.5 g per 100 available calories (or 1.3 g per 100 available kilojoules)".

6.0 The EU regulation (*EU COMMISSION DELEGATED REGULATION (EU) 2018/561 of 29 January 2018 amending Delegated Regulation (EU) 2016/127)* reflecting the new minimum protein level, has been published, and products can be placed on market from 5th May, 2018 onwards. ASSESSMENT PROCEDURE

The applicant considers the "General Procedure", specifically a Level 2 assessment, to be the appropriate procedure to be adopted in assessing this application since it relates to "changing a compositional requirement for a food" and the assessment is considered of "average complexity" by the applicant.

7.0 CONFIDENTIAL COMMERCIAL INFORMATION

8.0 See separate document marked as confidential. OTHER CONFIDENTIAL INFORMATION

9.0 EXCLUSIVE CAPTURABLE COMMERCIAL BENEFIT

This Application is not requesting for an Exclusive Capturable Commercial Benefit.

10.0 INTERNATIONAL AND OTHER NATIONAL STANDARDS

10.1 International Standards

Section 5.1.4.5 and Table 5.1.4.5-1 and Section 5.2.4.2 summarize pertinent information related to existing Codex standards regarding the composition of follow-on formula (and infant formula) and the outcomes of recent decisions by Codex regarding the lowering of the recommended minimum protein content in follow-on formula to 1.6 g/100 kcal. The lowering of the minimum protein content in follow-up formula for older infants (6 to 12 months of age) was the 1st of 37 recommendations made by an EWG who led the initiative to redraft and further discuss the Codex *Standard for Follow-Up Formula* (CODEX STAN 156-1987 – Codex Alimentarius, 2017b)[106]. The current agreed-upon draft of the *Standard for Follow-Up Formula* specifies a minimum protein content of 1.8 g/100 kcal, equivalent to 0.43g/100kJ (for cows' and goats' milk protein) with an associated footnote 6 permitting that formula based on non-hydrolysed milk protein and containing 1.6- 1.8 g of protein per 100 kcal (0.38-0.43g/100kJ) should be evaluated for their safety and suitability and assessed by a competent national and/or regional authority based on clinical evidence. (Codex Alimentarius, 2017a – CX/NFSDU 17/39/4 Rev.1).

10.2 Other National Standards or Regulations

Section 5.1.4.5 and Table 5.1.4.5-1 outline the required (Australia/New Zealand, EU) or recommended (*i.e.,* Codex) minimum and maximum levels of protein in follow-on formula.

[106] CODEX STAN 156-1987 states that follow-up formula (suitable for infants aged 6 months on and for young children) contain "Not less than **3.0 g per 100 available calories** (or 0.7 g per 100 available kilojoules) of protein of nutritional quality equivalent to that of casein or a greater quantity of other protein in inverse proportion to its nutritional quality. The quality of the protein shall not be less than 85% of that of casein. The total quantity of protein shall not be more than 5.5 g per 100 available calories (or 1.3 g per 100 available kilojoules)".

11.0 STATUTORY DECLARATION

A signed statutory declaration by an authorised senior officer is attached separately.

12.0 CHECKLISTS

12.1 Checklist for General Requirements

12.2 Checklist for General requirements

This Checklist will assist you in determining if you have met the mandatory format and information requirements as detailed in Guideline 3.1.1 – General requirements. All applications **must** include this Checklist.

General requirements (3.1.1)		
Check	**Page No.**	**Mandatory requirements**
☑		A Form of application ☑ *Application in English* ☑ *Executive Summary (separated from main application electronically)* ☑ *Relevant sections of Part 3 clearly identified* ☑ *Pages sequentially numbered* ☑ *Electronic copy (searchable)* ☑ *All references provided*
☑		B Applicant details
☑		C Purpose of the application
☑		D Justification for the application ☑ *Regulatory impact information* ☑ *Impact on international trade*
☑		E Information to support the application ☑ *Data requirements*
☑		F Assessment procedure ☑ *General* ☐ *Major* ☐ *Minor* ☐ *High level health claim variation*

N/A	G Confidential commercial information *N/A CCI material separated from other application material* *N/A Formal request including reasons* *N/A Non-confidential summary provided*
N/A	H Other confidential information *N/A Confidential material separated from other application material* *N/A Formal request including reasons*
N/A	I Exclusive Capturable Commercial Benefit *N/A Justification provided*
☑	J International and other national standards ☑ *International standards* *N/A Other national standards*
☑	K Statutory Declaration
☑	L Checklist/s provided with application ☑ *3.1.1 Checklist* ☑ *All page number references from application included* ☑ *Any other relevant checklists for Chapters 3.2–3.7*

12.3 Checklist for Applications for Substances Added to Food – Substances Used for a Nutritive Purpose

Checklist for applications for substances added to food

This Checklist is in addition to the Checklist for Guideline 3.1.1 and will assist you in determining if you have met the information requirements as specified in Guidelines 3.3.1–3.3.3.

Substances used of a nutritive purpose (3.3.3)		
Check	**Page No.**	**Mandatory requirements**
☑		A.1 Purpose of the use of the substance
☑		A.2 General data requirements for supporting evidence
N/A		B.1 Identification
N/A		B.2 Chemical and physical properties
N/A		B.3 Impurity profile
N/A		B.4 manufacturing process
N/A		B.5 Specification for identity and purity
N/A		B.6 Analytical method for detection
N/A		B.7 Proposed food label
N/A		C.1 Toxicokinetics and metabolism, degradation products and major metabolites
N/A		C.2 Animal or human studies
N/A		C.3 International safety assessments
☑		D.1 List of food groups or foods likely to contain the nutritive substance
☑		D.2 Proposed maximum levels in food groups or foods
☑		D.3 Likely level of consumption
☑		D.4 Percentage of food group to use nutritive substance
☑		D.5 Use in other countries (if available)
☑		D.6 Where consumption has changed, information on likely consumption
N/A		E.1 Need to permit addition of vitamin or mineral
N/A		E.2 Demonstrated potential to address deficit or health benefit
☑		F.1 Nutritional purpose (other than vitamins and minerals)
☑		G.1 Consumer awareness and understanding
N/A		G.2 Actual or potential behaviour of consumers
☑		H.3 Demonstration of no adverse effects on any population groups

12.4 Checklist for Applications for Special Purpose Foods – Infant Formula Products

Special purpose foods – Infant formula products (3.6.2)		
Check	**Page No.**	**Mandatory requirements**
☑		A.1 Purpose of compositional change
☑		A.2 Data for supporting evidence
☑		A.3 Specific information requirements ☑ *Characterisation of proposed substance in breast milk* ☑ *Nutritional safety and tolerance* ☑ *Efficacy of proposed compositional change* ☑ *Tolerance of proposed compositional change*
☑		B.1 Dietary intake or exposure of target population
☑		B.2 Level of consumption
☑		B.3 Information relating to the substance
☑		C.1 Safety or nutritional impact of labelling change
☑		C.2 Demonstrated consumer understanding of labelling change
☑		D Internationally recognised codes of practice and guidelines on labelling

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