FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Supporting document 1

Comparative Nutritional Safety Assessment – Application A1074

Minimum L-histidine in Infant Formula Products

Executive summary

This comparative nutritional safety assessment aims to determine whether a reduction in the minimum required amount of L-histidine from 12 mg per 100 kJ to 10 mg per 100 kJ in infant formula products is supported by amino acid composition data in breast milk and whether L-histidine at a level of 10 mg per 100 kJ will support normal growth in formula-fed infants. The approach of the assessment was to review studies analysing amino acid composition of breast milk as a means to benchmark the adequacy of L-histidine levels in infant formula products. In addition, the assessment considered whether the proposed lower level for L-histidine is sufficient to support normal growth of formula-fed infants. It is assumed that the levels of L-histidine present in breast milk support normal infant growth.

Methodologies to determine amino acid concentrations in breast milk were reviewed. The standard method is to measure the concentration of amino acids by automated amino acids analysis against the protein content of the milk sample as determined by nitrogen (Kjeldahl) analysis. The main uncertainty in the method is the determination of protein concentration which relies on linking the nitrogen content with protein. Breast milk contains significant but variable amounts of non-protein nitrogen which can lead to an underestimation of the concentration of available amino acids. There are few studies that have corrected for non-protein nitrogen but it is generally considered that amino acid concentration as reported against the 'crude' protein content is acceptable.

Studies analysing the L-histidine concentration (along with other amino acids) in breast milk were reviewed and relevant studies were used to calculate an average concentration of L-histidine. The average concentration of L-histidine in breast milk was 24 mg/g crude protein, which is comparable to the average concentration reported by several scientific expert panels and international policy organisations. Using appropriate composition data for protein, fat and carbohydrate in breast milk, 24 mg L-histidine/g crude protein is equivalent to 10 mg L-histidine/100 kJ.

The measured level of L-histidine (average of 24 mg/g crude protein) in breast milk is considered sufficient to support normal growth. The adequacy of infant formula products containing approximately 10 mg L-histidine/100 kJ to support infant growth has been assessed in published studies. These studies recorded growth patterns and biochemical data (plasma concentrations of amino acids) in breast and formula-fed infants. All of the studies reported comparable anthropometric data, and the protein concentration in infant formula or breast milk appeared to be the main determinant of plasma amino acid concentrations with higher protein content giving rise to higher plasma amino acid concentrations, including for L-histidine.

Bioavailability of amino acids, as determined by their concentration in plasma, corresponds to the amino acid content of the ingested protein. Given that a reduction in L-histidine content from 12 mg/100 kJ to 10 mg/100 kJ is minor relative to the natural variability in total protein content for breast milk, a small difference in protein digestibility is unlikely to have any physiological importance.

Based on this assessment, it is concluded that the requested lowering of the minimum requirement for L-histidine in infant formula products from 12 mg/100 kJ to 10 mg/100 kJ is appropriate and safe. It represents a reduction in crude protein levels of less than 0.1 mg protein/100kJ and published research studies have shown that 10 mg L-histidine/100 kJ is consistent with levels found in breast milk. In addition, anthropometric measures are comparable between formula-fed infants consuming formula with either 12 mg L-histidine/100 kJ or 10 mg L-histidine/100 kJ, and with breastfed infants, indicating that 10 mg L-histidine is adequate to support normal infant growth.

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1. Introduction

L-histidine is an essential amino acid. Essential amino acids are those that the body cannot synthesise itself and dietary intake is required to maintain protein synthesis. L-histidine is found in breast milk, and in combination with other amino acids plays a critical role in infant nutrition.

1.1 Objective of the Comparative Nutritional Safety Assessment

Standard 2.9.1 – Infant Formula Products of the *Australia New Zealand Food Standards Code* (the Code) prescribes the minimum content of L-histidine required to be present in infant formula products as 12 mg/100 kJ. The Applicant has requested that this level be reduced to 10 mg/100 kJ based on evidence that this level is comparable to the average L-histidine concentration present in breast milk and that this change would permit harmonisation with the relevant Codex Alimentarius standard. The objectives of this comparative nutritional safety assessment are to determine if this requested change in the L-histidine content is consistent with reported levels in breast milk and will support normal growth in formula-fed infants. It is assumed that the amount of L-histidine in breast milk is adequate to meet the requirements of infants for normal growth.

1.2 Approach for the Comparative Nutritional Safety Assessment

The Ministerial Policy Guideline on the *Regulation of Infant Formula Products* (the Policy Guideline) issued in 2011 by the Australia and New Zealand Food Regulation Ministerial Council[1] recommends that the primary reference for the compositional requirements of infant formula and follow-on formula should be breast milk. The policy is consistent with the approach of nutritional scientists and international policy organisations such as the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO) and the Institute of Medicine (IOM), which have sought to characterise the composition of breast milk as a means to define compositional requirements of infant formula products.

Several reports issued since 1970 by scientific advisory groups and consultation panels have reviewed studies on breast milk composition in order to quantify nutrient concentration and thereby issue recommendations on infant formula product composition. In the absence of large, high-quality studies on amino acid composition in breast milk, the approaches undertaken in these reviews were to determine the average breast milk concentration of each amino acid based on combinations of comparable published studies. It is important to note that for amino acid composition of breast milk, a direct comparison of studies is complicated by non-standard sampling protocols and analytical assays, as well as reporting of insufficient details on analytical methods such that an accurate measure of amino acid concentration against a consistent reference value is sometimes not possible.

The approach undertaken in this comparative nutritional safety assessment has been to:

1. Compare the analytical methods and experimental protocols that are used to quantify the amino acid content in breast milk.
2. Consider previous assessments of amino acid composition in breast milk undertaken by other scientific advisory groups.
3. From (2) and additional literature review, select comparable studies which measure L-histidine concentration in breast milk and calculate a mean and range of L-histidine concentrations in breast milk (in mg L-histidine/g crude protein) from the selected

[1] Now the COAG Legislative and Governance Forum on Food Regulation

studies.

4. Determine whether the proposed minimum level of L-histidine of 10 mg/100 kJ corresponds to the mean L-histidine content in breast milk.
5. Review the literature to confirm that nutritional safety issues, particularly in relation to infant growth, do not result from the proposed lowering of minimum L-histidine level in infant formula products.

1.3 Definitions and Terminology

Follow-on formula[2] means an infant formula product represented as either a breast milk substitute or replacement for infant formula and which constitutes the principal liquid source of nourishment in a progressively diversified diet for infants aged from six months.

Infant[2] means a person under the age of 12 months

Infant formula[2] means an infant formula product represented as a breast milk substitute for infants and which satisfies the nutritional requirements of infants aged up to four to six months.

Infant formula product[2] means a product based on milk or other edible food constituents of animal or plant origin which is nutritionally adequate to serve as the principal liquid source of nourishment for infants.

Non-protein nitrogen (NPN) consists mainly of free amino acids, peptides, and urea. In breast milk between 20 to 25% of total nitrogen is present as NPN.

Crude protein is based on all nitrogen-containing substances in breast milk and is calculated from the total nitrogen content of a food multiplied by a conversion factor (usually 6.25 based on the nitrogen content of mixed proteins – see report text)

True protein is based on the all nitrogen-containing substances minus NPN multiplied by an appropriate conversion factor (e.g. 6.38 for milk proteins). However, the calculation excludes nitrogen that may be metabolically available, e.g. amino acids, small peptides, urea, aminosugars, nucleotides, carnitine, and choline.

Adequate Intake (AI)[3] means the average daily nutrient intake level based on observed or experimentally-determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate.

2. Key Assessment Questions

This comparative nutritional safety assessment will address three key questions:

1. Is a reduction in the minimum required amount of L-histidine in infant formula products from 12 mg/100 kJ to 10 mg/100 kJ supported by current available studies on breast milk composition?
2. Is plasma/serum level of L-histidine a marker of dietary intake? If so, is a minimum level of 10 mg/100 kJ of L-histidine in infant formula products adequate to meet the physiological requirements of formula-fed infants compared to breastfed infants?
3. If not, would a minimum level of 10 mg/100 kJ of L-histidine in infant formula products

[2] Source: Standard 2.9.1 – Infant Formula Products of the *Australia New Zealand Food Standards Code*; http://www.comlaw.gov.au/Series/F2008B00658

[3] Source: NHMRC (2006). Nutrient Reference Values for Australia and New Zealand including Recommended Dietary Intakes http://www.nhmrc.gov.au/guidelines/publications/n35-n36-n37

support adequate growth of formula-fed infants compared to breastfed infants?

3. Comparative Nutritional Safety Assessment

3.1 Analysis of L-histidine content in breast milk

Individual amino acids are quantified in breast milk by established analytical methods and the summary below provides a general description of these methods that would be applicable to L-histidine.

3.1.1 Variability of amino acid concentration in breast milk

Proteins are the main source of amino acids in breast milk. Protein content, and therefore amino acid concentration, varies depending on mother's health and nutritional status, delivery time (pre-term versus full term), post-partum sampling period (colostrum versus transitional milk versus mature milk), circadian cycle, and time of collection (foremilk versus hind milk). Although a standard protocol does not exist for sampling breast milk for protein and amino acid determination, generally studies are comparable if mature milk is collected from healthy mothers with infants delivered at full-term and breast milk samples are obtained over a 24 hour period (or at least two time points) and then pooled during a specified period of lactation.

3.1.2 Measurement of amino acids in breast milk

Since the introduction of automated amino acid analysers in the 1970's, quantitation of individual amino acids in biological samples including breast milk has become relatively straight-forward (reviewed in Joint FAO/WHO Expert Consultation, 1991, pg10). Briefly, aliquots of milk are freeze-dried, extracted to remove fat and then hydrolysed (usually by acid treatment) to digest proteins into individual amino acids. Digested amino acids are then separated by ion exchange chromatography, and detected by reaction of individual amino acids with ninhydrin forming a complex which is quantified colorimetrically. The amino acid concentration is calculated in units of mg (or mmol) per 100 mL of milk.

3.1.3 Measurement of protein content in breast milk

To allow comparison of concentrations between different studies and to partially correct for the variability in milk protein levels (as described previously), the amino acid concentration is converted to the amount of amino acid against a reference value, usually the protein content in the sample. Measurement of protein content in breast milk is complex and there are several methods which are either direct or indirect measurements (Table 1). There are various limitations for each method and the lack of a standard accurate measure for 'true' protein concentration is the main source of uncertainty in measuring breast milk amino acid concentration.

Direct measurements include dye-binding assays or colorimetric assays such the Lowry or Bradford methods. Although relatively simple and inexpensive, the main issue with these methods is that the assay requires use of a reference standard and there is no standard that reliably matches the dye-binding properties of the mixture of proteins in breast milk samples. Consequently these methods tend to over-estimate protein content (Lonnerdal et al. 1987).

Table 1: Methods to determine protein content in breast milk[a]

Method	Measure of	Procedure	Limitations
Kjeldahl	Total nitrogen	Digestion then conversion of organic nitrogen to ammonia which is detected by titration	Does not measure true protein content and includes nitrogen from non-protein constituents; different proteins require different conversion factors depending on amino acid composition of the sample protein
Dumas	Total nitrogen	Measures nitrogen released by combustion of milk sample	Requires expensive instrumentation; and as above for Kjeldahl method.
Spectroscopic	Protein	Direct detection by UV spectroscopy at 280 nM	Interference by other milk constituents
Colorimetric	Protein	Interaction of proteins with reagents to form complex which is measured spectrophotometrically	Requires a reference standard that reproduces reagent-binding properties of breast milk proteins
Total amino acids (as measure of protein content)	Sum weight of individual amino acids	Amino acid analysis	Analytically demanding; requires determination of recovery coefficients for each amino acid determination.

[a] Reviewed in Chapter 7 of Greenfield and Southgate (2003) and the EC Scientific Committee on Food report (2003).

Indirect methods for protein determination are the Kjeldahl and Dumas methods with Kjeldahl the most common method used for analysing protein content in breast milk. Both methods measure total nitrogen in a sample which is converted to protein concentration using a conversion factor of 6.25 based on the total nitrogen content in mixed proteins. This amount is generally referred to as the 'crude' protein concentration (Box 1). However, because individual proteins each have different nitrogen content (based on amino acid composition), proteins (or a mixture of similar proteins) will have different conversion factors. A conversion factor of 6.38, which is based on the total nitrogen content in milk proteins, has been used in some studies analysing breast milk amino acids. Calculation of amino acid composition in breast milk using either 6.25 or 6.38 factor gives comparable concentrations (see for example, EC Scientific Committee on Food (EC SCF, 2003) report, page 55). Both are therefore acceptable and for simplicity, the 6.25 factor is generally used by the FAO/WHO in reports on protein content in foods and is more commonly used in recent studies analysing amino acid composition in breast milk[4].

Box 1: Protein – Nitrogen conversion for determination of crude protein concentration

Protein contains 16% Nitrogen
or
1 g Protein (g) yields 0.16 g Nitrogen
or
6.25 g Protein yields 1 g Nitrogen

[4] Note that conversion factors for the purposes of calculating the protein content in the formulation of infant formula (as opposed to estimating protein content in breast milk) is prescribed under Standard 2.9.1 of the Code. For formula composed of milk proteins and their partial protein hydrolysates: protein content = nitrogen content x 6.38; for formula composed of other protein sources: protein content = nitrogen content x 6.25.

The main limitation of nitrogen determination to measure protein content is that it does not correct for non-protein nitrogen (NPN) components. Proteins are the predominate source of nitrogen in breast milk but up to 25% of nitrogen in breast milk is contributed by other nitrogen containing components including free amino acids, peptides, and urea. As a result, protein content measured by the Kjeldahl or Dumas method is sometimes defined as 'crude protein' and amino acid concentration measured is reported as mg/g crude protein.

Various methods to calculate the 'true' protein content - which is the protein content corrected for NPN - have been derived (as reviewed by the 2003 EC Scientific Committee on Food (EC SCF) although none have been adopted as standard methodology. Several studies have calculated protein content as the total sum of anhydrous amino acids quantitatively determined by amino acid analysis to correct for NPN (Davis et al. 1993; Davis et al. 1994). However, the method is limited by the need to determine accurate recovery coefficients for each amino acid and this can be laborious, thus limiting sample numbers.

The Kjeldahl method is accredited by Association of Official Analytical Chemists International for quantitation of protein in food and it is the most common method used in published studies on amino acid composition in breast milk. Furthermore, it has been recommended that individual amino acid concentration be reported against total nitrogen content as standard methodology to avoid confusion around different calculations for protein content (Koletzko et al. 2005). To enable consideration of more studies for this comparative nutritional safety assessment, the analysis in Section 3.3 will mainly focus on those studies measuring L-histidine concentration per gram total nitrogen and then converting to mg L-histidine/g crude protein using the 6.25 conversion factor.

3.2 Comparison of reports issued by scientific advisory groups on breast milk amino acid composition

Regulatory authorities such as Codex Alimentarius and the European Commission (EC) as well as scientific advisory bodies such as the Life Sciences Research Organization (LSRO) and the Institute of Medicine (IOM) have issued reference amino acid profiles for essential amino acids. These profiles are based on the average amino acid composition of breast milk as calculated from studies reported in the scientific literature. Studies considered in each report to calculate an average L-histidine content in breast milk (per gram of crude protein) are listed in Table 2.

The Joint FAO/WHO/UNU Expert Consultation report of 1985 (updated in 1991) provided information on amino acid requirements which were based on breast milk composition data from four published reports[5]. This is the only report which cites research conducted prior to the development of modern analytical techniques giving rise to a higher average L-histidine level.

The LSRO of the American Society for Nutritional Sciences issued a report (Life Sciences Research Office 1998) which referenced four sources to give an average amino acid concentration in breast milk. Two of the referenced studies (Davis et al. 1994; Sarwar et al. 1996) collected milk at only 10 days post-partum and only one study (Picone et al. 1989) provides details on sampling. L-histidine was not included in the list of essential amino acids quantitated in this analysis although the average value in Table 2 was calculated from the four studies cited.

[5] These are as cited in Table 38 on page 121 of the FAO/WHO/UNU (1991) report. A separate chapter in this report (Chapter 4, page 65) cites different studies for the estimates of amino acids in breast milk. However, estimates in the Chapter 4 table are reported in mg/kg/day which cannot be used for comparison.

A report issued by the European Society of Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) in 2005 reviewed eight analytical studies of breast milk amino acid contents (Koletzko et al. 2005). The report concluded that infant formula should contain at a minimum 41 mg of L-histidine/100 kcal (equivalent to 10 mg/100 kJ), which was subsequently adopted by Codex Alimentarius. The ESPGHAN report was preceded by a full report issued by the EC SCF in 2003 analysing the same reference studies excluding Yonekubo et al. (1991), which was not available at the time but the inclusion of this data did not alter the L-histidine content.

The IOM report (IOM Panel on Macronutrients et al. 2005) setting the dietary reference values for nutrient intakes based the Adequate Intake (AI) for infants for L-histidine on four studies. No reason for the selection of these studies is provided although the average value determined is in line with previous reports. The IOM report sets the AI level for L-histidine for infants 0-6 months by multiplying the average L-histidine concentration (converted to mg/L) by the average volume of milk intake per day.

The WHO/FAO/UNU Expert Consultation issued a report in 2007 as part of the WHO Technical Report Series. The report references three studies cited in the IOM report. The L-histidine concentration is lower here since the value is corrected for the non-protein nitrogen, with protein calculated as 75% of total nitrogen.

Table 2: Previous reports by scientific advisory groups on amino acid levels in breast milk

Reference		FAO/WHO (1985 & 1991)	LSRO (1998)	EC SCF (2003)	ESPGHAN (2005)	IOM (2005)	WHO (2007)
1954 Soupart et al		✓					
1965 Lindner et al		✓					
1970 FAO Nutrition Study No 24		✓					
1977 Dep Health Soc Sec		✓					
1985 Lonnerdal and Forsum				✓	✓		
1985 Bindels and Harzer				✓	✓		
1987 Janas et al			✓	✓	✓		
1989 Picone et al			✓				
1991 Heine et al						✓	✓
1991 Yonekubo et al					✓		
1994 Davis et al			✓			✓	✓
1996 Sarwar et al			✓				
1998 Darragh and Moughan				✓	✓	✓	
1998 Villalpando et al				✓	✓	✓	✓
2002 Raiha et al[1]				✓	✓		
L-histidine (mg/g crude protein)[2]	Range	18-36	18-24	18-41	18-41	21-24	18-23
	Mean	26	22	23	23	23	21

[1] Modified from Nayman et al. 1979

[2] Calculated using 6.25 nitrogen conversion factor
Details for each of the studies in Table 2 are summarised in Appendix 1.

Aside from the commonalties between the EC SCF (2003) and ESPGHAN (2005) reports and between the IOM (2005) and WHO/FAO/UNU (2007) reports, there is limited overlap in the studies that were selected for each report and generally no stated reasons for the exclusion or inclusion of specific studies. The EC SCF report questions the reliability of the measurements in studies conducted before 1985, presumably due to improvements in analytical methodology. Nevertheless, averaged values for L-histidine content cited in the reports are similar (approximately 23 mg/g crude protein).

3.3 Comparison of studies that measure L-histidine in breast milk

3.3.1 Summary of research

The table at Appendix 1 summarises the studies that were reviewed for this assessment. The studies include those used for reports shown in Table 2 as well as additional research commonly cited on breast milk amino acid composition or identified though a Medline search using search terms such as breast milk, amino acid and protein. The table indicates some of the issues and information gaps in comparing measurements from different studies.

3.3.2 Average L-histidine in breast milk from selected studies

The amount of L-histidine in breast milk reported in selected studies was assessed to calculate a benchmark for the minimum amount required to be present in infant formula products. Studies from Appendix 1 were selected on the basis that:

- Common analytical methods were used.
- Comparable sampling protocols were used for collecting breast milk.
- Experimentally determined L-histidine concentration was measured against total nitrogen or protein as measured by Kjeldahl analysis. L-histidine reported as µmol/volume of milk can only be used if sufficient data are provided to calculate L-histidine against protein content. In line with current recommendations on analysis of amino acids in breast milk (EC SCF 2003) and for consistency in analysing the various studies, calculation of protein content (from the reported measured nitrogen content) in this assessment uses the 6.25 conversion factor.
- The study was published as a full paper in peer-reviewed scientific literature. Review articles citing work that is less reliable or where the original publication is no longer available were not used.

A summary of the 10 studies selected according to these criteria is shown in Table 3. The studies correspond with those used by Koletzko et al (2005) with the following exceptions:

1. Raiha (2002) was excluded because reported value for L-histidine in breast milk was obtained from earlier reviews without details about analytical methods.
2. Several large Japanese studies have been reported. Yamawaki et al. (2005) and Idota et al. (1991) were used for this assessment instead of Yonekubo et al. (1991) from the ESPGHAN report which was unavailable.
3. Chavalittamrong et al. (1981), Department of Health and Social Security (1977) and Ding et al. (2010) were included because they meet the above selection criteria.

Table 3: Measured L-histidine concentration in breast milk from selected studies

Study	Post-partum sampling (N)	L-histidine			
		mg/100 mL	mg/g total N	mg/g protein[1]	mg/100 kJ
1977 Dep. Health Soc. Sec.	4-6 weeks (96)	31	145	23	11
1981 Chavalittamrong et al.	29-90 days (14)	26	132	23	8.8
1985 Lonnerdal and Forsum	4-16 weeks (3)	23	111	18	7.8
1986 Harzer et al	36 days (10)	44	250	40	15
1987 Janas et al	8 weeks (10)	20	112	18	6.8
1991 Idota et al	31-60 days (>100)	31	144	23	11
1998 Darragh and Moughan	10-14 weeks (20)	27	156	25	9.2
1998 Villalpando et al	4 & 6 months (40+20, 2 groups)	18	135	21	6.1
2005 Yamawaki	21-89 days (40)	35.7	179	29	12.1
2010 Ding et al	7-180 days (40)	36.3	151	24	12.3
Range		18-44	111-250	18-40	6.1-15
Mean of 10 studies (unweighted)		29	148	24	10

[1] Values are per g of crude protein, calculated using the 6.25 conversion factor.

Table 3 shows the L-histidine concentration determined in each of the 10 studies. As indicated , the mean concentration calculated from the 10 studies was 24 mg L-histidine/g crude protein, which is comparable to values determined in previous reports (listed in Table 2). The weighted average, correcting for the variable numbers of samples analysed in each study, was also 24 mg L-histidine/g crude protein. The last column indicates the L-histidine content in mg/100 kJ calculated from the reported value of L-histidine in 100 ml of breast milk in each study and the mean energy value of 295 kJ/100 ml. The mean energy value is based on the composition data for protein, fat and carbohydrate in mature breast milk as reported in the National Health and Medical Research Council Nutrient Reference Values for Australia and New Zealand (NHMRC 2006) and the energy conversion factors according to Standard 1.2.8 – Nutrition Information Requirements of the Code. Averaging the converted amounts gave 10 mg/100 kJ.

The marked overlap in the range of values and comparable means for L-histidine concentration as determined in each of the reports (Table 2) and in this comparative nutritional safety assessment (Table 3)is illustrated in Figure 1. The figure shows values in units of mg/g crude protein to enable comparison between reports.

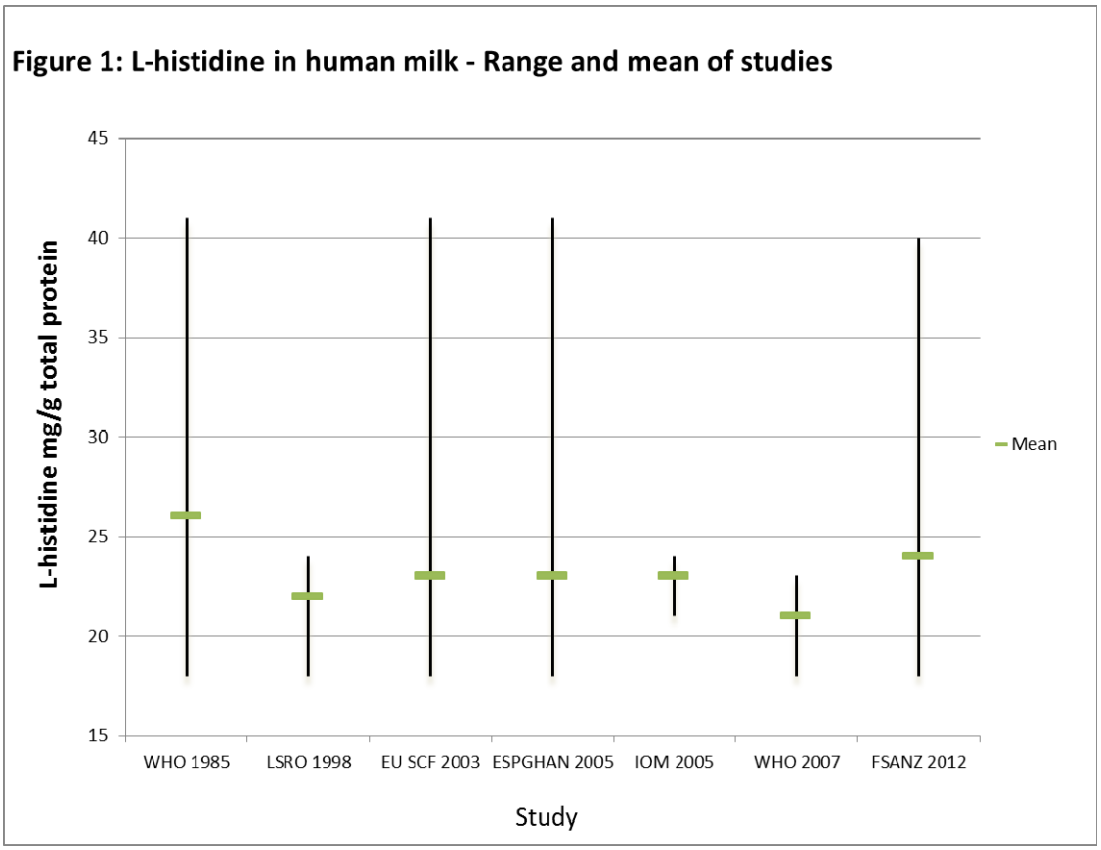

Figure 1: L-histidine content of breast milk

Mean values are shown with the line representing the range of L-histidine values from individual studies cited in each report (described in section 3.2) and from the FSANZ 2012 analysis.

3.4 Comparison of current and proposed minimum levels of L-histidine in infant formula products

The recommended AI for protein for infants aged 0-6 months by the NHMRC and the New Zealand Ministry of Health has been established (NHMRC 2006). However, nutrient reference values for essential amino acids for this age group have not. Therefore, in the absence of a prescribed AI for L-histidine, the proposed lowering of L-histidine content in infant formula products can be considered in light of the requirements under the Code. Current protein requirements under Standard 2.9.1 sets the minimum level of L-histidine of 12 mg/100 kJ and a minimum protein level of 0.45 g /100 kJ. An infant formula product that meets these minimum requirements would contain 27 mg L-histidine/g crude protein. Reducing the minimum requirement for L-histidine to 10 mg/100 kJ with a minimum level of 0.45 g protein/100 kJ would give 22 mg L-histidine/g crude protein. Both values are in the range of L-histidine concentration measured in breast milk, as illustrated in Figure 1.

It should be emphasised that for the purposes of comparison between the various reports, the calculated L-histidine concentrations in breast milk as shown in Figure 1 are based on the 6.25 nitrogen-protein conversion factor. However there is minimal effect if the L-histidine concentration is calculated using the 6.38 conversion factor.

Calculating the L-histidine concentration for the 'FSANZ 2012' assessment (i.e. this assessment) using the 6.38 factor, as would be required under the Code for milk proteins used in infant formula products, the average L-histidine concentration in breast milk (i.e. per gram of 'milk' protein) would be 23 mg L-histidine/g protein, which is not markedly different.

3.5 Nutritional safety issues with the proposed change in L-histidine content

3.5.1 Growth

Five studies were cited in the Application to support the argument that infant formula products with an L-histidine content of ≤ 10.5 mg/100 kJ does not give rise to adverse health effects when compared to formulas with higher L-histidine levels. Two additional studies were identified by searching the Medline database using search terms such as infant formula, protein composition, amino acids, energy and growth, as well as the names of authors who had published relevant studies. The studies are summarised in Appendix 2. In general, the studies reviewed were randomised and controlled, were published in peer reviewed journals and conducted on healthy term infants only. Experimental formulas contained varying levels of L-histidine including amounts ≤ 10 mg/100 kJ and effects on anthropometry were measured. All of the studies compared formula-fed infants to breastfed infants and found that infants given formula containing lower amounts of L-histidine (i.e. containing L-histidine ≤ 10.5 mg/100 kJ) had growth measures comparable to breastfed infants. Also, the studies showed no differences in growth between formula-fed infants consuming products containing either 12 mg/100 kJ or 10 mg/100 kJ of L-histidine.

3.5.2 Plasma amino acid concentration

Lonnerdal and Chen (1990) analysed plasma amino acid concentrations of amino acids in breastfed versus formula-fed infants as a means to evaluate the capacity of formula protein to replicate breast milk protein. Although L-histidine was not analysed in this study, the levels of other amino acids were highest in the plasma of infants fed formula with the highest protein level. In addition, Hernell and Lonnerdal (2003) showed elevated plasma concentrations of certain other amino acids (e.g. phenylalanine, threonine, and methionine) resulting from the higher levels of these amino acids in test formula proteins compared to breast milk. Therefore, the main determinant of plasma amino acid concentration appears to be dietary protein concentration.

Four of the studies listed in Appendix 2 compared plasma concentrations of amino acids between formula-fed and breastfed infants as a measure of the bioavailability (i.e. digestibility and absorption) of amino acids in formulas with different whey/casein ratios, protein levels, or protein sources. In each study the plasma concentration of L-histidine was comparable between formula-fed and breastfed infants. Only a minor increase in plasma L-histidine was detected in formula-fed infants in the study by Trabulsi et al (2011) although this would be expected as a result of the higher L-histidine content (12.2 mg/100 kJ) in the test formula protein.

The limitation of studies listed in Appendix 2 is that the studies have been conducted from birth up to the age of 4-6 months of age, whereas Standard 2.9.1 applies to infant formula products for infants up to 12 months of age. However, the evidence cited by the Applicant is still relevant to the assessment of the safety to infants aged 6-12 months on the basis that the greatest health impact will occur during 0-6 months of age, where formula consumption represents the sole source of nutrition.

4. Conclusion

This comparative nutritional safety assessment has shown that infant formula containing 10 mg L-histidine/100 kJ is comparable to the average amount of L-histidine present in breast milk. This conclusion is based on the best evidence of breast milk composition currently available. The average level of L-histidine in breast milk, measured against crude protein content was calculated by analysis of selected studies and ranged from 18-40 mg L-histidine/g crude protein with an average of 24 mg L-histidine/g crude protein (based on a nitrogen conversion factor of 6.25). This value is consistent with other analyses (Life Sciences Research Office 1998; EC Scientific Committee on Food 2003; Koletzko et al. 2005; WHO/FAO/UNU Expert Consultation 2007) although noting that the studies differed. Converting the average L-histidine content to a per kJ value (using data for protein, carbohydrate and fat compositional date for breast milk and energy conversion data) indicates that this level is equivalent to 10 mg L-histidine/100 kJ (using either 6.25 or 6.38 as the nitrogen conversion factor). Reduction of L-histidine content to 10 mg/100 kJ is equivalent to a reduction in the protein content of less than 0.1 mg protein/100 kJ. It is assumed that levels of L-histidine in breast milk are adequate for normal growth, and this proposed reduction falls well within the range for mature breast milk. There is also published evidence that growth of formula-fed infants consuming a formula containing 10 mg L-histidine/100 kJ is comparable with breastfed infants. These studies measured physiological outcomes (anthropometric measures) and biochemical measures (plasma amino acid concentration) of breastfed and formula-fed infants.

5. References

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Appendices

Appendix 1: Summary of studies measuring the amino acid composition of breast milk

Appendix 2: Infant formula with varying L-histidine concentration: comparison of breastfed infants and formula-fed infants

Appendix 1: *Summary of studies reviewed which measure amino acid composition of breast milk*

Studies selected for this assessment are shaded in grey. See text (Section 3.5) for selection criteria.

Reference	**Type**	**Method**[1]	**L-histidine (mg/g protein)**	**Limitation**
1954 Soupart (cited by Nayman)	Original Research	Method for nitrogen determination not reported	19	Outdated methods sufficient information to calculate L-histidine level at 121 mg /g N or 19 mg/g total protein
1965 Tarjan	Original research	Paper chromatography separation and quantitated by densitometry		Method inconsistent with other studies
1965 Holt and Snyderman	Review	No methods reported	n.a.	L-histidine reported as intake of amino acids/kg body weight/day
1967 Fomon and Filer	Review (book chapter)	No methods reported		
1977 Dep Health Soc Sec	Original research	Standard		
1977 Svanberg	Original research	Standard	23	Amino acid level reported against sum of all amino acids analysed (reported in mg amino acid/g total amino acids)
1979 Nayman (Nayman et al. 1979)	Review	No methods reported	22	Average amino acid concentration based on 4 references including Soupart (1954) and Svanberg (1979). Details on calculation not provided
1981 Chavalittamrong et al	Original research	Standard	23	Average here excludes values 0-22 days post-partum
1983 Renner	Review	No methods reported	26	Average amino acid concentrations based on 21 references. Details on the calculation not provided.
1985 Bindels and Harzer	Conference Report (German)	Standard	41	This study appears to be an early report of the study reported in the following year by Harzer et al.

Reference	Type	Method[1]	L-histidine (mg/g protein)	Limitation
1985 Lonnerdal and Forsum	Original research	Standard	18	
1986 Harzer et al	Original research	Standard	40	
1987 Janas et al.	Original research	Standard	18	
1989 Picone	Original research	Method for amino acid analysis not reported	24	A reference group (N=12) used for sampling but does not indicate at what time of post-partum sampling.
1989 Yonekubo et al	Original research	Standard	24	No translated version available (in Japanese) but see Idota (1991) and Yamawaki (2005) for similar data
1991 Heine et al.	Review	Methods not reported	23	Amino acid composition reported as percentage of milk protein. Data taken from Renner (1983)
1991 Idota et al	Original research	Standard	23	
1992 Hanning et al.	Original research	Breast milk not analysed this study	n.a.	Refers to Atkinson SM et al (1980) which does not measure amino acid composition in breast milk with a useable reference value.
1993 Davis et al.	Original research	Breast milk not analysed this study	26	Breast milk composition refers to values measured by Widdowson 1979 and measured in mg amino acid/ g total amino acids.
1994 Davis et al.	Original research	Liquid chromatography and quantitate by measuring area under the curve.	23	Reference value – amino acid level reported against sum of all amino acids analysed (reported in mg amino acid/g total amino acids)
1996 Dewey et al.	Review	Not reported; breast milk not analysed in this study	26	Values for amino acid content in breast milk taken from Davis et al 1993
1996 Sarwar et al.	Original research	Liquid chromatography and quantitate by measuring area	23	Sampling period 5-10 days post-partum (too early)

Reference	Type	Method[1]	L-histidine (mg/g protein)	Limitation
		under the curve.		
1998 Darragh and Moughan	Original research	Standard	25	
1998 Villalpando et al.	Original research	Standard	18	
2002 Raiha et al.	Original research	Breast milk not analysed in this study (from Nayman 1979)	20	Values for amino acid content in breast milk taken from Soupart 1954 or Svanberg 1977
2003 Hernell and Lonnerdal	Original research	Breast milk not analysed in this study	n.a.	Values for amino acid content in breast milk taken from Hanning et al. 1992 (and references cited within)
2005 Yamawaki et al.	Original research	Standard		
2009 Feng et al	Conference poster	Nitrogen analysed by thermal conductivity; amino acids quantitated by HPLC.	23	No complete paper published to date (although poster provides very complete data for analysis).
2010 Ding et al.	Original research	Proteins were analysed by Chinese Standard GB/T 5009.5-2003 (Kjeldahl)	23	

[1]Standard method means total nitrogen determined by Kjeldahl analysis and amino acids quantified by automated amino acid analysis.

Appendix 2: *Infant formula with varying L-histidine concentration: comparison of breastfed infants and formula-fed infants*

Studies were provided as supporting evidence for the Application. Janas et al (1987) and Picone et al (1989) were identified through Medline search and were also compared. Protein content is listed for each formula to denote the difference between test formulas and is presented in g/100 kcal as cited in the study. Studies are based on healthy term infants exclusively breastfed or formula-fed for the specified periods in the study.

Reference	**Objective**	**Design**	**Formula composition**[a,b]		**Results**
			Protein (g/100 kcal)	**L-histidine (mg/100 kJ)**	
1987 Janas et al.	Compare formulas with varying whey/casein ratios.	Progressive cohort study to 8 weeks	1.8 (EF)	9.5	Weight and length gains comparable between all formula-fed groups and breastfed infants. Plasma histidine concentration similar in all groups.
			1.8 (EF)	9.3	
			1.8 (EF)	8.5	
1989 Picone et al.	Compare whey-based formulas with varying protein concentrations. Breast milk composition data (protein and amino acid concentrations) taken from separate previously published study.	Progressive cohort study to 12 weeks.	1.6 (EF)	10.2	Weight and length gains comparable between formula-fed and breastfed infants. L-histidine concentration similar is all groups.
			2.0 (EF)	12.3	
			2.2 (EF)	13.6	
2002 Raiha et al.	Compare formulas with different protein/energy ratios. Whey/casein ratios modified in EFs.	Progressive cohort study to 120 days	2.2 (SF)	12	Weight and length gains comparable between formula-fed and breastfed infants.
			2.5 (EF)	10.8	
			2.5 (EF)	10.8	
2003 Hernell and Lonnerdal	Compare formulas of different composition (amino acids, carbohydrate, and trace elements)	Progressive cohort study to 6 months	2.0 (SF)	11	Weight and length gains comparable between all formula-fed groups and breastfed infants. Plasma histidine concentration similar in all groups.
			2.8 (EF)	20	
			2.7 (EF)	19	
			2.4 (EF)	12	
2006 Turck et al.	Compare formula with high and low protein content. Whey/casein ratio	Progressive cohort study to 120 days	2.6 (SF)	15	Weight and length gains comparable between formula-fed and breastfed infants.
			1.8 (EF)	10	

	modified in EF.				
2008 Sandstrom et al.	Comparison of formula with differing protein quality: standard formula versus EF containing α-lactalbumin or glycomacropeptide	Progressive cohort study to 6 months (after 4 mo fruit or vegetable puree permitted)	1.96 (SF) 1.96 (EF) 1.96 (EF)	9.8 10.5 10.8	Weight and length gains increased in SF compared to breastfed; EF comparable to breastfed. Plasma histidine concentration similar in all groups.
2011 Trabulsi et al	Compare SF (high protein) to EF (low protein)	Randomised control trial to 120 days. Grouped, double blind at 5-14 days (n=112/ group)	2.1 (SF) 1.9 (EF)	12.2 10.8	Weight gain greater in SF fed infants versus breastfed; EF comparable to breastfed. Plasma histidine levels were within normal range for all groups.

[a] *All values based on 6.25 nitrogen-protein conversion factor except for Janas et al (1987) and Picone et al (1989); Abbreviations: SF, standard formula; EF, experimental formula.*

[b] *Calculated from data provided in paper. For example, L-histidine reported in mg/g protein was converted to mg/100 kJ using the reported protein/energy ratio. Energy conversion 1 kcal = 4.18 kJ (Australia New Zealand Food Standards Code)*