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Analytical standards for the measurement of nutrients in infant formula

Macronutrients, minerals, carnitine, taurine and nucleotides

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National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

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Abstract

Adequate methods with known performance characteristics for the assessment of the concentration of nutrients in infant and follow-on formula (referred to as “formula”) are essential in the evaluation whether the composition deviates from the compositional provisions as laid down by law. Many standardised analytical methods for the analysis of nutrients in infant formula are internationally available.

Currently, EU regulation regarding formula (Commission Directive 2006/141/EC) does not provide any indication on reference methods to be used in official controls for energy calculation, for the analysis of macronutrients (proteins, fats, carbohydrates and dietary fibre) and for the analysis of minerals, carnitine, taurine and nucleotides in infant formula, nor performance criteria for the selection of such methods of analysis have been set down. Therefore, an overview of the available standardised methods of analysis for the measurement of formula macronutrients as well as minerals, taurine, carnitine and nucleotides in infant formula is provided. Seventy-seven standardised analytical methods from both national and international organizations developing standard analytical methods have been collected and their performance characteristics (i.e. analytical technique, target compound(s), matrixes validated, limit of detection, limit of quantification analytical range, repeatability, reproducibility, accuracy/spike recovery) are described.

It has been noted that for the calculation of the energy content in infant formula according to the EU harmonized criteria the amount of proteins, lipids, dietary fibre, carbohydrates, polyols and organic acids must be determined. Several methods are available for the analysis of proteins, lipids and dietary fibre whereas only one method is available for the determination of carbohydrate content and no method is available for the analysis of polyols and organic acids. As for the minerals, several methods are multi-elemental methods since they are able to measure more than one mineral at once. Most of the standard methods are based on atomic absorption spectroscopy and inductively coupled plasma spectroscopy/spectrometry. Only two standard methods are available for the analysis of fluorine, taurine and nucleotides in formula. The standard methods for the analysis of fluorine are horizontal methods (methods that are applicable to a wide range of food products rather than to a specific one) that have not been validated in infant formula. Finally, only one method is available at present for the analysis of carnitine in formula.

It has been noted that the lower and, in one case, the upper limit of quantification of some methods do not comply with the compositional limits laid down Commission Directive 2006/141/EC. Moreover, the minimum and maximum values are provided per energy content, which is logic from a nutritional point of view. This means that both the uncertainty in the energy calculation and the uncertainty in the measurement of the target nutrient contribute to the total uncertainty in the assessment of the nutrient content when this is provided per energy content.

In absence of relevant European Union instructions, the choice of method of analysis for each of the nutrients here reported in formula should be based on a) the analytical performances of the method (i.e. accuracy/spike recovery, reproducibility, lower and upper limit of quantification) and b) the knowledge of the level of risk associated with an inadequate or excessive nutrient intake.

Keywords: infant formula, methods of analysis, proximate analysis, minerals, taurine, carnitine, nucleotides.

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1 Introduction

Infants that are not fully breast-fed depend completely, or at least partly, on infant formula in the first months of life. Infant formula should therefore completely provide the nutritional need of infants in these first months. At about six months of age, infants will start to consume suited complementary foods in addition to follow-on formula. In the period between 6 and 12 months of age the consumption of follow-on formula will decrease and the consumption of complementary foods will increase.

Because of the large dependence on infant formula and follow-on formula (henceforth together called formula) for the delivery of nutrients required for growth and development it is important that formula indeed deliver an adequate nutrient supply without the risk of excessive intake. Both too low and excessive intake of nutrients may result in adverse health effects. The type and seriousness of these adverse health effects depend on e.g. the type of nutrient, the extent of deviation, the duration, and the age of the infant.

The composition of formula is laid down by law in the EU (1). This legislation lays down which nutrients have to or may be present in formula and in which amounts. However, no reference to specific analytical methods or performance criteria of these methods are provided by law. To estimate and possibly avoid public health risks, the chemical analyses to determine the concentration of a nutrient in formula is essential in the evaluation whether the composition deviates from the legal amounts. For the correct interpretation of the analytical results also some method performance characteristics are important. For the chemical analyses of most nutrients in formula, multiple - but different - standardised analytical methods are internationally available, while for some nutrients such standardised methods are not present.

1.1 Aim of the report

This report provides an overview of the internationally available standardised analytical methods for the determination of selected nutrients in formula. This report focusses on macronutrients, minerals, nucleotides, taurine and carnitine. It follows the report published in 2012 which provided an overview of the standard analytical methods for the analysis of vitamins, inositol and choline in formula (2). Since the energy calculation in formula has to be harmonized with the EU guidelines for nutritional labelling laid down in Council Directive 90/496/EEG and Commission Directive 2008/100/EG, the energy values to be declared shall be calculated from total content of carbohydrates (except polyols), polyols, proteins, lipids, alcohol (ethanol), dietary fibre and organic acids by means of the corresponding conversion factors. The calculation of the energy content is especially important in formula since the compositional provisions provided in Commission Directive 2006/141/EC are given per energy content. For that reason, standard methods of analysis have been inventoried for all the groups of compounds on which the calculation of energy content is based with the exception of ethanol since this is not relevant in infant formula. For the dietary fibre, Commission Directive 2006/141 EC allows only the addition of fructo-oligosaccharides and galacto-oligosaccharides that are soluble fibres. For that reason only methods able to measure soluble dietary fibre or specifically fructo-oligosaccharides and galacto-oligosaccharides have been included in the present report.

For each method the target compound(s) being actually measured, the matrixes wherein the methods have been validated and several method performance characteristics are provided.

2 Approach

In the present study, standardised methods for the analysis of proteins, fats, carbohydrates, dietary fiber, minerals, nucleotides, carnitine and taurine in formula have been collected from international and national organizations developing and setting standardised methods.

All the methods matching the following criteria have been included in the present report:

- Methods that have been validated in infant or follow-on formula (or equivalent category, e.g. food for infants and young children in the Chinese standards)
- Horizontal methods for the analysis of the target compounds
- Methods developed for milk and/or milk products

The sources that were used to search for standardised analytical methods are listed below:

International

- AOAC International (AOAC)
- International Organisation for Standardisation (ISO)
- Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN)
- CODEX alimentarius (FAO/WHO)
- International Dairy Federation (IDF)
- American Association of Cereal Chemists (AACC)

European

- European Committee for Standardisation (CEN)
- European Pharmacopoeia (EP)

USA

- Infant Formula Council (IFC)
- American Oil Chemists' Society (AOCS)

Other national

- Chinese National Standards (GB)
- Russian National Standards (GOST)
- Nordic Committee for food analysis (NMKL)
- Standards Australia (SA)
- Standards New Zealand (NZS)
- Japanese standards (JA)

With exception of CODEX alimentarius and SPIFAN, all are organizations developing standardised methods. CODEX alimentarius periodically publishes lists of recommended standardised methods of analysis and sampling. The SPIFAN is an international expert panel recently established on initiative of AOAC International to establish method requirements, collect and validate new methods of analysis for target nutrients in infant formula.

Information on the methods has been gathered by consulting standard organization official websites and/or official (on-line or hard copy) publications or by directly contacting the organizations. In addition to the method name (reference) and the organisation behind this standardised method, also information is provided on the analytical principle, the target compounds, the matrix in which the method is validated, and several performance characteristics, namely accuracy, spike recovery, limit of Detection (LOD), limit of quantification (LOQ), quantitation/applicability range, relative standard deviation of repeatability (RSD_r), and relative standard deviation of reproducibility (RSD_R).

3 Results and discussion

The standardised analytical methods collected for each nutrient including several characteristics are listed in Appendices II-XXII. In this section the findings will be described and discussed in a broad perspective. For detailed information regarding a specific nutrient or method we would like to refer to the Appendices.

Data sources

Standardised analytical methods matching the criteria listed in section 2 have been published by the following international and national organizations developing standardised methods: International Organisation for Standardisation (ISO), European Committee for Standardisation (CEN), AOAC International (AOAC), International Dairy Federation (IDF), Chinese National Standards (GB), standards Australia (SA) and American Association of Cereal Chemists (AACC). In total, 77 methods have been collected (33 methods for the analysis of macronutrients (proteins, fats, carbohydrates and dietary fibre)), 39 methods for minerals, 2 for taurine, 2 for nucleotides and 1 for carnitine).

The activities of SPIFAN, established on initiative of AOAC International, have recently resulted in the call for methods for the analysis of iodine and carnitine. Methods AOAC 2012.14 and AOAC 2012.15 for the analysis of total iodine by ICP-MS (inductively coupled plasma-mass spectrometry) have been adopted as first action official methods of analysis (methods that have underwent only single-laboratory validation pending results of collaborative studies). Similarly, method AOAC 2012.17 for the analysis of free and total Carnitine in Infant Formula and Adult/Pediatric Nutritional Formula by Liquid Chromatography/Tandem Mass Spectrometry has been recently adopted as first action official method but its details have not been published yet. Other methods that will prove to satisfactorily fulfil the performance requirements established by the expert panel will enter the AOAC official methods SM program and will likely become AOAC official methods within 1 year. In addition, a work item is currently running within an ISO/IDF working group (working item ISO 15151 | IDF 229) for the determination of Ca, Mg, Na, K and P using ICP-OES (inductively coupled plasma-optical emission spectrometry) and for the determination of Cu, Zn, Fe and Mn using ICP-MS in milk and milk products. The method will be likely validated in infant formula as well and will result in an international standard or a technical specification according to whether or not a collaborative study could be performed. The authors are not aware that other standard organisations/panels are currently working on candidate methods to become standardised methods in the near future.

General observations

For most nutrients several standardised methods of analysis are available. As much as 13 standardised methods have been collected dietary fibre, 11 for proteins, 11 for zinc, 9 for fat, iron and copper, 8 for calcium and iodide, 7 for sodium, phosphorous and magnesium, 6 for potassium, 4 for chlorine, selenium and manganese. Conversely, two methods are available for the analysis of fluorine, nucleotides and taurine and only one for carbohydrate and carnitine. Finally, no standard methods for the analysis of organic acids and polyols in formula have been published so far.

According to article 11 of EC Regulation 882/2004 on official controls on foods, “Sampling and analysis methods used in the context of official controls shall comply with relevant Community

rules or, (a) if no such rules exist, with internationally recognised rules or protocols, for example those that the European Committee for Standardisation (CEN) has accepted or those agreed in national legislation; or, (b) in the absence of the above, with other methods fit for the intended purpose or developed in accordance with scientific protocols" (3). Whereas some EU regulations provide official methods of analysis, the Commission Directive on infant formula and follow-on formula (2006/141/EC) does not provide any indication on reference methods to be used in official controls. Again, in some cases, EU regulations refer to a criteria approach for the selection of an appropriate method, by indicating the method performance characteristics that a method has to meet to be suitable for official controls. For instance, performance criteria have been laid down, for the analysis of mycotoxins, nitrates, heavy metals, dioxin, benzo(a)pyrenes. In contrast, no performance criteria have been laid down for the selection of methods to be used for official analysis of nutrients in formula. This means that for each nutrient there is more than one standardised method available to choose from.

Lists of available standard methods of analysis for specific compounds are sometimes published by international organisations such as the Joint Research Centre (JRC)'s Institute for Reference Materials and Measurements (IRMM) and CODEX alimentarius. CODEX alimentarius is an intergovernmental body under the auspices of the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO) of the United Nations. It can be regarded as the most internationally recognized standard organization. Periodically, CODEX alimentarius publishes lists of international standards as recommended method of analysis for nutrients in formula. Methods of analysis are endorsed by CODEX as type I (A method which determines a value that can only be arrived at in terms of the method per se and serves by definition as the only method for establishing the accepted value of the item measured), type II (methods to be recommended in cases of dispute and for calibration purposes), type III (alternative methods) or type IV (tentative methods). All type I and II CODEX endorsed methods for the analysis of the nutrients included in the present report are either European standard CEN methods, ISO/IDF international standards or AOAC official methods (4).

However, it must be pointed out that the most recently approved standardised methods of analysis (AOAC official methods for selenium and nucleotides approved in 2011) have not been yet evaluated by CODEX alimentarius. It is thus possible that in the future, the list of CODEX endorsed methods of analysis will change and updated with the inclusion of newly approved standardised methods.

Analytical principles

The standardised methods of analysis collected are based on a wide range of analytical techniques. The assessment of the total protein content is, in most of the standards, directly or indirectly based on the kjeldahl method but standard methods based on spectrophotometry and mid-infrared spectroscopy have also been published. The kjeldahl method entails the digestion of the sample with sulphuric acid and the titration of the liberated nitrogen. The protein content is then calculated multiplying the measured nitrogen content by a conversion factor and depends on whether total nitrogen or protein nitrogen have been measured. In the former case it is referred to as total protein on a total nitrogen basis. The conversion factor differs according to the protein source (wheat, milk or soy) so that at least the gross formula protein composition has to be known for an accurate determination of the total protein content with the kjeldahl method. In one

standard method the protein content is calculated by difference between the total nitrogen and the non-protein nitrogen both measured by the kjeldahl method.

Fat content is mainly determined by gravimetry after proper solvent extraction. Three basic extraction methods are reported: Rose-Gottlieb, Weibull-Berntrop and Mojonnier which differ for the sample preparation and the extraction solvent. Standard methods for the analysis of fat based on spectrophotometry and turbidimetry have been also published.

The determination of dietary fibre entails in all the cases an enzymatic digestion step of the sample. The single fibre fractions are then determined by gravimetry, LC or spectrophotometry.

Only one standard method is available for the analysis of carbohydrates in formula, method AOAC 986.26. In this method the amount of carbohydrates is not directly measured but rather calculated by difference from the total solid content once protein, fat and ash content have been determined. This implies that, standard methods for the measurement of total solid and ash in formula are required for the implementation of this method. Standard methods for the measurement of total solid and ash in formula are mentioned in AOAC 986.26 but they have not been validated in formula.

Most of the standard methods of analysis for minerals are based on atomic spectrometry: flame atomic absorption spectrometry (FAAS), hydride generation atomic absorption spectrometry (HGAAS), graphite furnace atomic absorption spectrometry (GFAAS) and flame emission spectrometry (FES). In more recent years, standard methods based on inductively coupled plasma based techniques, namely inductively coupled plasma-optical emission spectrometry (ICP-OES) and inductively coupled plasma-mass spectrometry (ICP-MS) have been published. Other analytical techniques include: titration (with potentiometric or precipitation end point determination), spectrophotometry, fluorimetry and liquid chromatography. Finally, standard methods for the analysis of taurine and nucleotides are based on liquid chromatography with UV, fluorescence and mass spectrometry detection.

Matrices validated and target compounds

As for the macronutrients analysis, only 3 out of the 9 standard methods for the analysis of fat listed in Appendix IV have been validated in infant formula. None of the standard methods for the analysis of dietary fibre have been validated in formula, whereas only two standards for the analysis of proteins have been validated in formula (ISO 8968-1/2|IDF 20-1/2) but the results of the collaborative study that has been performed in order to extend the scope of those methods to dried milk products (including infant formula) are not published yet. It is worthwhile noticing that Commission Directive 2006/141/EC defines protein content as nitrogen content multiplied by the proper conversion factor. For that reason both methods based on the measurement of protein nitrogen and total nitrogen have been included. Standard methods for the analysis of dietary fibre can measure either soluble fibre, insoluble fibre or total fibre (sum of soluble and insoluble fibre). However, only fructo-oligosaccharides and galacto-oligosaccharides (soluble fibre) are allowed in formula and their content is regulated according to Commission Directive 2006/141/EC. The methods listed in Appendix V that can measure soluble or total fibre, are able to quantitate fructo-oligosaccharides and galacto-oligosaccharides only to a limited, variable extent. Four methods are specifically developed to measure fructo-oligosaccharides but none can measure the amount of galacto-oligosaccharides.

Concerning the analysis of minerals, most of the methods have been validated in formula. Several are multi-elemental methods i.e. they are able to measure more than one element at once: method AOAC 985.35 for the analysis of Ca, Mg, Fe, Zn, Cu, Mn, Na and K, method AOAC 2011.14 and method AOAC 984.27 for the analysis of Ca, Mg, Fe, Zn, Cu, Mn, Na, P and K, method ISO 8070 | IDF 119:2007 for the analysis of Na, K, Ca and Mg, method GB 5413.21 for the analysis of Ca, Mg, Fe, Zn, Cu, Mn, Na and K. Similarly, methods AOAC 999.10|NMKL 161:1998, AOAC 999.11|NMKL 139:1991 and EN 14084:2003 allow the simultaneous analysis of Cu, Fe and Zn.

The overall mineral content of formula depends on the amount that is supplemented and the fraction naturally occurring in formula ingredients. The compounds that can be used to supplement minerals in formula according to Commission Directive 2006/141/EC are listed in Appendix I. With few exceptions, minerals are supplemented in formula in inorganic form whereas those deriving from formula ingredients are often and mostly in organic form (the element is covalently bound to C, N or O). Depending on the nutrient, the contribution from natural ingredients varies to a different extent. It might be significant, for instance, for Ca and P for milk-based infant formulas. It is safe to assume that Na, K, Ca, Mg, Cl and F occur in formula exclusively in inorganic form. On the other hand, metals such as Fe, Cu, Zn, Mn may occur in formula in both inorganic form and organic form, bound to proteins or coordinated to prosthetic groups.

Analogously, iodine can occur in foods in both inorganic and organic form the latter representing a significant portion of the total iodine in infant formulas. Conversely, selenium always occur in organic form, covalently bound to oxygen in anions selenite SeO_3^{2-} and selenate SeO_4^{2-} or bound to carbon in methylated volatile compounds, selenoamino acids, selenoproteins and other derivatives.

The standardized methods for the analysis of minerals in formula comprise in most of the cases a thorough digestion step (dry ashing, microwave open vessel, microwave closed vessel digestion) that aims to completely mineralise the organic matrix. The collected standard methods are therefore supposed to be able to measure the total amount of minerals in formula. Only exceptions are method ISO 14378:2009 | IDF 167 and AOAC 992.22 that are able to measure only the inorganic forms of iodine and method AOAC 992.24 which does not entail any digestion step prior to the determination of iodine.

Concerning nucleotides, other potential nutritionally important forms are the nucleosides (i.e. the dephosphorylated form of nucleotides) and primary bases (purine and pyrimidines). Nucleosides and primary bases cannot be measured by any of the two standard methods available for the analysis of nucleotides in formula.

Methods performance characteristics

Methods performance characteristics that have been collected comprise the accuracy, spike recovery, limit of detection (LOD), limit of quantification (LOQ), quantitation/applicability range, relative standard deviation of repeatability (RSD_r) and relative standard deviation of reproducibility (RSD_R). These method performance characteristics are reported in Appendices II-XXII. Unfortunately not all the method performance characteristics could be retrieved for each method. Values for LOD and lower LOQ, for instance, are often not reported in the official protocols or must be estimated by the final users according to the specific laboratory conditions and instrumentations. In addition, the upper LOQ values and analytical ranges are rarely reported in the protocols.

This may imply that the method can produce reliable measurements at each concentration above the lower LOQ but this might not always be the case. This might especially important if the nutrients over-supplementation of formula has to be assessed. In some cases only the instrumental analytical range or instrumental LOD, LOQ were available. In addition, the values of LOD, LOQ are given in different units in the official protocols (i.e. /g, /L, /g of solids), whereas the compositional provisions are specified in EU regulation on infant formula per energy content (i.e. per 100 kcal or 100 kJ). To complicate things further, while the regulatory provisions are referred to liquid formula after reconstitution, the LOD and LOQ of some methods are given per mass unit of powdered formula or more in general of dry material. This makes the direct comparison of the provisions with the method performance characteristics not straightforward. For 5 standard methods, it was noticed that the reported analytical range does not comply with the provisions given in EU regulation on infant formula. For instance, the LOQ of sodium based on method EN 15505:2008 (1500 mg/kg powdered formula) is higher than the lower limit set by the EU regulation (125 mg/L) if a dilution factor of 9 is applied for the reconstitution of the powdered formula. Similar observation can be done for the recently approved AOAC 2011.19 for selenium. In this case the LOQ assessed for liquid formula (>10 µg/L) is higher than the limit laid down by regulation (6.25 µg/L). However, the LOQ in powdered formula complies with the regulatory provision. For the analysis of copper, method AOAC 999.11 shows a LOQ (5 mg/kg powdered milk) higher than the minimum amount allowed (210 µg/L), whereas method AOAC 2011.14 shows a LOQ that is slightly higher (220 µg/L considering a dilution factor of 9). Finally the applicability range of method AOAC 992.24 (75-150 µg/L iodide) is outside the minimum-maximum concentration range laid down by Regulation (62.5-354 µg/L).

Data on accuracy/spike recovery have been collected for most of the methods. Accuracy/spike recovery values ranges from 90% to 110% for most of the methods with few exceptions (i.e. method AOAC 983.35 for calcium, recovery=90-129% and the same method for iron, recovery=81%). Data on methods reproducibility (RSD_R) have been collected for almost all the methods. The majority of the established methods of analysis collected have been validated in international collaborative studies, and in those cases reproducibility data are reported. For a few of the recently approved AOAC methods, namely method AOAC 2011.19 and AOAC 2011.19 for nucleotides and method AOAC 2011.21 for selenium, reproducibility data are not yet available pending the results of collaborative studies. For these methods only the intermediate precision (intermediate relative standard deviation of reproducibility, RSD_{IR}) is reported. In the Chinese standards, data on reproducibility are not reported in the official protocols. International standards ISO 12081 | IDF 36 and ISO 6732 | IDF 103 have not been validated in collaborative studies so that only repeatability values are given. Reproducibility values (RSD_R) are in most of the cases <20% even though higher values can also be found especially for the analysis of iron (66% for method AOAC 984.27 and 47% for method EN 14084:2003) and copper (29% for method AOAC 984.27) and some dietary fibre fractions (AOAC 993.19 and AOAC 994.13). For many methods several values of RSD_R are reported when methods have been validated in differently formulated formulas (for example milk- or soy-based infant formula) or at different nutrient concentration. In these cases, a range of RSD_R values is reported in the appendixes. However, as already highlighted in a previous report on the standard methods for the analysis of vitamins, choline and inositol in infant formula (4), since regulation requires the nutrient content to be reported formula per energy content (i.e. per 100kcal or 100kJ), the error in energy determination should be added to the analytical error for the determination of the target nutrient thus inflating the overall uncertainty of the final value.

4 Conclusions and recommendations

Currently, EU regulation does not provide any indication on reference methods to be used in official controls for the analysis of macronutrients, minerals, nucleotides, taurine and carnitine in formula. It neither specifies performance criteria for the selection of such methods, so far. Therefore, it is recommended to fill this gap in the near future and EU regulation could give provisions on reference methods of analysis for nutrients in formula for official controls or set performance criteria for the methods to be used.

The survey has shown that for most of the nutrients studied several standardised methods are available. No methods are at present available for the measurement of polyols, organic acids in formula. For fluorine two standardized methods have been published and only one standardised method is available for carbohydrates and carnitine. For the analysis of minerals, many standardized methods are available for each element. Several of those methods are multi-elemental and most of them have been validated in formula.

As already noted for vitamins, the lower and in one case the upper limits of quantification of some methods do not comply with compositional provisions given in EU regulation for the target nutrients in formula. These provisions are usually given per energy content. From a mere analytical perspective this diminishes the accuracy of nutrients assessment, although there are good reasons to do so from a nutritional point of view. In addition, these provisions refer to the volume of the reconstituted formula whereas the LOD and LOQ values of standard methods are expressed in diverse units and often refer to formula powder. Finally, it has been noted that the upper limit of quantification of the methods is not always clearly reported in the official protocols which is important if the level of nutrients over-supplementation has to be assessed. It would be beneficial if this inconsistency may be overcome in the near future.

In absence of relevant EU rules and of specific protocols/procedures, the choice for any of the several standard methods available for methods for the analysis of macronutrients, minerals, carnitine, taurine and nucleotides in infant formula should take into account the method analytical performances (accuracy/spike recovery, reproducibility, lower and upper limit of quantification). As a general rule of thumb, every standard method which 1) measures the right form(s) of the nutrient with sufficient accuracy, 2) within the provision limits as set down by Commission Directive 2006/141/EC, 3) which measurement uncertainty (expressed as RSD_R) has been established and 4) is <30%, is an acceptable method. For most nutrients, these criteria will not lead to exactly one method. To select a “best” method, a case-by-case approach should be taken. Notably, in such a case expert knowledge of a nutritionist or toxicologist should be combined with the level of uncertainty method presented in this report to judge the risk of under or overconsumption of a nutrient caused by the performance of the method.

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Appendix I
List of substances that may be used in the manufacture of infant formula and follow-on formula in order to satisfy the requirements of macronutrients, minerals, taurine, carnitine and nucleotides. Commission Directive 2006/141/EC (1)

Compound	Formulation
Proteins	Cow's milk proteins
	Protein hydrolysates
	Soy protein isolates
Carbohydrates	Lactose
	Maltose
	Sucrose
	Glucose
Malto-dextrin	Glucose syrup or dried glucose syrup
	Pre-cooked starch (naturally free of gluten)
	Gelatinized starch (naturally free of gluten)
Dietary fibre	Fructo-oligosaccharides
	Galacto-oligosaccharides
Calcium (Ca)	Calcium carbonate
	Calcium chloride
	Calcium salts of citric acid
	Calcium gluconate
	Calcium glycerophosphate
	Calcium lactate
	Calcium salts of orthophosphoric acid
	Calcium hydroxide

Compound	Formulation
Magnesium (Mg)	Magnesium chloride
	Magnesium oxide
	Magnesium salts of orthophosphoric acid
	Magnesium sulphate
	Magnesium gluconate
	Magnesium hydroxide
	Magnesium salts of citric acid
Iron (Fe)	Ferrous citrate
	Ferrous gluconate
	Ferrous lactate
	Ferrous sulphate
	Ferric ammonium citrate
	Ferrous fumarate
	Ferric diphosphate (Ferric pyrophosphate)
	Ferrous bisglycinate
Copper (Cu)	Cupric citrate
	Cupric gluconate
	Cupric sulphate
	Copper-lysine complex
	Cupric carbonate
Iodine (I)	Potassium iodide
	Sodium iodide
	Potassium iodate

Compound	Formulation
Zinc (Zn)	Zinc acetate
	Zinc chloride
	Zinc lactate
	Zinc sulphate
	Zinc citrate
	Zinc gluconate
	Zinc oxide
Manganese (Mn)	Manganese carbonate
	Manganese chloride
	Manganese citrate
	Manganese sulphate
	Manganese gluconate
Sodium (Na)	Sodium bicarbonate
	Sodium chloride
	Sodium citrate
	Sodium gluconate
	Sodium carbonate
	Sodium lactate
	Sodium salts of orthophosphoric acid
	Sodium hydroxide
Potassium (K)	Potassium bicarbonate
	Potassium carbonate
	Potassium chloride
	Potassium salts of citric acid
	Potassium gluconate
	Potassium lactate
	Potassium salts of orthophosphoric acid
	Potassium hydroxide

Compound	Formulation
Selenium (Se)	Sodium selenate
	Sodium selenite
Carnitine	L-carnitine and its hydrochloride
	L-carnitine-L-tartrate
Taurine	Taurine
Nucleotides	Cytidine 5′-monophosphate and its sodium salt
	Uridine 5′-monophosphate and its sodium salt
	Adenosine 5′-monophosphate and its sodium salt
	Guanosine 5′-monophosphate and its sodium salt
	Inosine 5′-monophosphate and its sodium salt

Appendix II
EU provisions for energy content in infant and follow-on formula

Definition of compound (2006/141/EC): Energy.

Limits (2006/141/EC)								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
kJ/100mL		kcal /100mL			kJ/100mL		kcal /100mL	
250	290	60	70		250	290	60	70

As laid down by Council Directive 90/496/EEG and Commission Directive 2008/100/EG, the energy values to be declared shall be calculated from total content of carbohydrates (except polyols), polyols, proteins, lipids, alcohol (ethanol), fibre and organic acids by means of the following conversion factors:

Carbohydrates (excluding polyols)	4 kcal/g (17 kJ/g)
Polyols	2.4 kcal/g (10 kJ/g)
Proteins	4 kcal/g (17 kJ/g)
Fats	9 kcal/g (37 kJ/g)
Alcohol	7 kcal/g (29 kJ/g)
Organic acids	3 kcal/g (13 kJ/g)

Appendix III
EU compositional provisions and overview of the standardised methods of analysis for proteins in infant and follow-on formula

Definition of compound (2006/141/EC): proteins*.

Limits (2006/141/EC): Infant formulae manufactured from cows' milk proteins								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
g / 100kJ		g /L**			g / 100kJ		g /L**	
0.45	0.7	11.25	20.65		0.45	0.8	11.25	23.6

* *Proteins content=nitrogen content*conversion factor (variable according to protein source).*
** *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission Directive 2006/141/EC.*

Limits (2006/141/EC): Infant formulae manufactured from protein hydrolysates								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
g / 100kJ		g /L**			g / 100kJ		g /L**	
0.45	0.7	11.25	20.65		0.56	0.8	14	23.6

* *Proteins content=nitrogen content*conversion factor (variable according to protein source).*
** *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission Directive 2006/141/EC.*

Limits (2006/141/EC): Infant formulae manufactured from soya protein isolates, alone or in a mixture with cows' milk proteins								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
g / 100kJ		g /L**			g / 100kJ		g /L**	
0.56	0.7	14	20.65		0.56	0.8	14	23.6

* *Proteins content=nitrogen content*conversion factor (variable according to protein source).*
** *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission Directive 2006/141/EC.*

Table 1. Overview of the standardised methods of analysis for total proteins in infant and follow-on formula.

Method name	Type of standard	Status	Analytical principle	Matrix validated
AOAC 991.20	AOAC official method	Final action 1994. Revised in 1996	Titrimetry (Kjeldahl on whole sample)	Liquid whole or skimmed milk
AOAC 991.22	AOAC official method	Final action 1994. Revised in 1996	Titrimetry (Kjeldahl on protein fraction)	Liquid whole or skimmed milk
AOAC 991.23[a]	AOAC official method	Final action 1994. Revised in 1996	Calculation (difference between total nitrogen and nonprotein nitrogen content)	Liquid whole or skimmed milk
AOAC 967.12	AOAC official method	Final action 1970	Spectrophotometry	Liquid milk
AOAC 975.17	AOAC official method	First action 1975	Spectrophotometry	Milk
AOAC 972.16	AOAC official method	First action 1972. Revised in 1996	Mid-infrared spectroscopy	Milk
AOAC 955.04	AOAC official method	Final action	Titrimetry (Kjeldah)	Fertilizers[b]
EN ISO 8968-1	European standard	Under revision	Titrimetry (Kjeldahl on whole sample)	Liquid whole or skimmed milk, dried milk products (including infant formula)
EN ISO 8968-2	European standard	Under revision	Titrimetry (Kjeldahl on whole sample)	Liquid whole or skimmed milk, dried milk products (including infant formula)
EN ISO 8968-5:2001	European standard	Under revision	Titrimetry (Kjeldahl on protein fraction)	Liquid whole or skimmed milk
AS 2300.1.2.2-2008	Australian standard	Current	Titrimetry (Kjeldahl on different N fractions)	Milk and milk products

[a] *Methods AOAC 991.20 and AOAC 991.21 are necessary for the implementation of this method.*
[b] *This method (with slight modifications) is mentioned as an alternative method for determination of total protein content in AOAC 986.25, proximate analysis of milk-based infant formula.*

Continued Table 1. Overview of the standardised methods of analysis for total proteins in infant and follow-on formula.

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 991.20	-	-	-	-	0.385%	0.504%
AOAC 991.22	-	-	-	-	0.285%	0.702%
AOAC 991.23	-	-	-	-	0.483%	1.051%
AOAC 967.12	-	-	-	-	-	-
AOAC 975.17	-	-	-	-	-	-
AOAC 972.16	-	-	-	-	-	-
AOAC 955.04	-	-	-	-	-	-
EN ISO 8968-1	-	-	-	-	0.385%	0.504%
EN ISO 8968-2	-	-	-	-	0.385%	0.504%
EN ISO 8968-5:2001	-	-	-	-	0.385%	0.504%
AS 2300.1.2-2: 2008	-	-	-	-	1.4%e	2.9%e

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r =relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*
[e] *Expressed as repeatability limit r and reproducibility limit R respectively. Data are taken from standard: AS 2300.1.2-1: 2008. Determination of nitrogen. Reference kjeldahl method.*

Appendix IV
EU compositional provisions and overview of the standardised methods of analysis for lipids in infant and follow-on formula

Definition of compound (2006/141/EC): lipids.

Limits (2006/141/EC)								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
g / 100kJ		g /L*			g / 100kJ		g /L*	
1.05	1.4	26.25	41.3		0.96	1.4	24	41.3

* *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 2. Overview of the standardised methods of analysis for lipids in infant and follow-on formula.

Method name	Type of standard	Status	Analytical principle	Matrix validated
EN ISO 8381| IDF 123:2008[a]	European standard, codex type I standard. Reference method	Adopted in 2008. Revision scheduled within 2016	Gravimetry (Rose-Gottlieb method)	Liquid, concentrated and dried milk-based infant foods
ISO 8262-1| IDF 124-1: 2005[b]	International standard. Reference method	Adopted in 2005	Gravimetry (Weibull-Berntrop method)	Liquid, concentrated and dried milk-based infant foods
AOAC 989.05	AOAC official method, final action, codex type I standard.	Final action 1995. Revised in 1996	Gravimetry (modified Mojonnier method)	Milk
AOAC 969.16	AOAC official method	First action 1999. Revised in 1996	Turbidimetry	Milk
AOAC 972.16	AOAC official method	First action 1972. Revised in 1996. Revised in 1996	Mid-infrared spectroscopy	Milk
AOAC 945.48[c]	AOAC official method	Final action	Gravimetry (Rose-Gottlieb method)	Evaporated milk (unsweetened)
EN ISO 1211:2010	European standard, reference method for liquid milk	Adopted in 2010. Revision scheduled for 2013	Gravimetry (Rose-Gottlieb method)	Raw and processed milk
EN ISO 1736:2008	European standard, reference method for dried milk and dried milk products	Adopted in 2008. Revision scheduled within 2016	Gravimetry (Rose-Gottlieb method)	Dried milk and dried milk products
GB 5413-3	Chinese standard	Adopted in 2010. Replace GB5413-1985 and GB/T 5413-3:1997	Gravimetry (Rose-Gottlieb method)	Food for infants and young children and dairy products

[a] *The method is not applicable to infant foods with more than a mass fraction of 5% (dry matter) of such added matter as starch, dextrin, vegetables, meat and fruit. It is not applicable to products not or poorly soluble in ammonia and those containing significant amount of free fatty acids.*

[b] *This method is applicable in all those situations for which EN ISO 8381|IDF 123:2008 is not applicable.*

[c] *This method is mentioned for determination of total fat content in AOAC 986.25, proximate analysis of milk-based infant formula.*

Continued Table 2. Overview of the standardised methods of analysis for lipids in infant and follow-on formula.

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
ISO 8381|IDF 123:2008	-	-	-	-	<0.1%	<0.2%
ISO 8262-1 |IDF 124-1: 2005	-	-	-	-	<0.2%	<0.4%
AOAC 989.05	-	-	-	-	0.396%	0.512%
AOAC 969.16	-	-	-	-	<0.04%	-
AOAC 972.16	-	-	-	-	-	-
AOAC 945.48	-	-	-	-	<0.14%	-
EN ISO 1211:2010	-	-	-	-	<0.043%	<0.056%
EN ISO 1736:2008	-	-	-	-	<0.2%e	<0.3%e
GB 5413-3	-	-	-	-	<0.85%	-

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*
[e] *Expressed as repeatability limit r and reproducibility limit R respectively.*

Appendix V
EU compositional provisions and overview of the standardised methods of analysis for carbohydrates in infant and follow-on formula

Definition of compound (2006/141/EC): carbohydrates.

Limits (2006/141/EC)								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
g / 100kJ		g /L*			g / 100kJ		g /L*	
2.2	3.4	55	100.3		2.2	3.4	55	100.3

* *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 3. Overview of the standardised methods of analysis for total carbohydrates in infant and follow-on formula.

Method name	Type of standard	Status	Analytical principle	Matrix validated
AOAC 986.25	AOAC official method	final action 1986	calculation by difference (total solid - (proteins+fats+ash)	Not applicable

Continued Table 3. Overview of the standardised methods of analysis for total carbohydrates in infant and follow-on formula.

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 986.25	-	-	-	-	-	-

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*

Appendix VI
EU compositional provisions and overview of the standardised methods of analysis for dietary fibre in infant and follow-on formula

Definition of compound (2006/141/EC): -

Fructo-oligosaccharides and galacto-oligosaccharides (soluble fibres) may be added to infant and follow-on formula. In that case their content shall exceed: 0.8g/100 mL in a combination of 90% oligogalactosyl-lactose and 10% high molecular weight oligofructosyl-saccharose.

Table 4. Overview of the standardised methods of analysis for dietary fibre in infant and follow-on formula.

Method name	Type of standard	Status	Analytical principle	Target compound	Matrix validated
AOAC 2011.25	AOAC official method. Horizontal method.	First action 2011	LC and gravimetry after enzymatic digestion	Total, soluble and insoluble fiber including fructo-oligosaccharides and galacto-oligosaccharides	Cabbage, mixed grains, chocolate, biscuits, cookies, oat bran and bread
AACC 32-50.01	AACC official method. Horizontal method.	First approval 2011	LC and gravimetry after enzymatic digestion	Total, soluble and insoluble fiber including fructo-oligosaccharides and galacto-oligosaccharides	Cabbage, mixed grains, chocolate, biscuits, cookies, oat bran and bread
AOAC 2009.01	AOAC official method. Horizontal method.	First action 2009	LC and gravimetry after enzymatic digestion	Total, soluble and insoluble fiber including fructo-oligosaccharides and galacto-oligosaccharides	Cabbage, mixed grains, chocolate, biscuits, cookies, oat bran and bread
AACC 32-45.01	AACC approved method. Horizontal method.	Approved in 2009	LC and gravimetry after enzymatic digestion	Total, soluble and insoluble fiber including fructo-oligosaccharides and galacto-oligosaccharides	Cabbage, mixed grains, chocolate, biscuits, cookies, oat bran and bread
AOAC 2001.03[a]	AOAC official method. Horizontal method.	Final action 2004	HPLC and gravimetry after enzymatic digestion	Total, soluble and insoluble fiber including part of fructo-oligosaccharides and galacto-oligosaccharides	Candy, jelly, white bread, juice, soups
AOAC 991.43	AOAC official method. Codex type I method for follow-up formula	Final action 1994. Revised in 2000	Gravimetry (enzymatic digestion)	Total, soluble and insoluble fiber including part of fructo-oligosaccharides and galacto-oligosaccharides	Fruits and vegetables, flour, bran and germ
AOAC 985.29	AOAC official method. Horizontal method	Final action 1986. Revised 2003	Gravimetry (enzymatic digestion)	Total, soluble and insoluble fiber including part of fructo-oligosaccharides and galacto-oligosaccharides	-
AOAC 993.19	AOAC official	Final action	Gravimetry (enzymatic	Soluble fiber including part of fructo-oligosaccharides	Fruits, vegetables and cereal grains

Method name	Type of standard	Status	Analytical principle	Target compound	Matrix validated
	method. Horizontal method	1996. Revised in 2000	digestion)	and galacto-oligosaccharides	
AOAC 994.13	AOAC official method. Horizontal standard	Final action 1999. Revised 2000	Gas chromatography-colorimetry-gravimetry	Total dietary fiber (determined as neutral sugar residues, uronic acid residues and Klason lignin)	Wheat and oat bran, white and rye bread, potex, carrot, apple, green peas
AOAC 997.08	AOAC official method. Horizontal standard	Final action 1999.	High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection after enzymatic digestion	Fructans (oligofructoses, inulin, hydrolyzed inulin, polyfructoses, fructooligosaccharides)	Cheese spreads, chocolate, wine gum, powder mix drink, biscuits
AACC 32-31.01	AACC approved method	Approved in 2001	High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection after enzymatic digestion	Fructans (oligofructoses, inulin, hydrolyzed inulin, polyfructoses, fructooligosaccharides)	Cheese spreads, chocolate, wine gum, powder mix drink, biscuits
AOAC 999.03	AOAC official method. Horizontal standard	Final action 2005	Spectrophotometry after enzymatic digestion	Fructans (oligofructoses, inulin, hydrolyzed inulin, polyfructoses, fructooligosaccharides)	Low fat spread, chocolate, onion, milk powder, stalks, artichokes and vitamin tablets
AACC 32-32.01	AACC approved method	Approved in 2005	Spectrophotometry after enzymatic digestion	Fructans (oligofructoses, inulin, hydrolyzed inulin, polyfructoses, fructooligosaccharides)	Low fat spread, chocolate, onion, milk powder, stalks, artichokes and vitamin tablets

[a] *Not applicable to food containing resistant starch.*

Continued Table 4. Overview of the standardised methods of analysis for fibres in infant and follow-on formula

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 2011.25	-	-	-	-	5.10-18.10% (soluble fiber)	7.53-36.40% (soluble fiber)
AACC 32-50.01	-	-	-	-	5.10-18.10% (soluble fiber)	7.53-36.40% (soluble fiber)
AOAC 2009.01					1.65-12.34%	4.70-17.97%
AACC 32-45.01	-	-	-	-	1.65-12.34%	4.70-17.97%
AOAC 2001.03	-	-	-	-	1.33-6.10%	1.79-9.39%
AOAC 991.43	-	-	-	-	<10.38%	<19.44%
AOAC 985.29	-	-	-	-	<15.83%	<21.92%-
AOAC 993.19	-	-	-	-	<21.93%	<28.28%
AOAC 994.13	-	-	-	-	<58.3%	<56.2%
AOAC 997.08	-	-	-	-	2.9-5.8%	4.8-11.1%
AACC 32-31.01	-	-	-	-	2.9-5.8%	4.8-11.1%
AOAC 999.03	-	-	-	-	2.3-7.3%	5.0-10.8%
AACC 32-32.01	-	-	-	-	2.3-7.3%	5.0-10.8%

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*

Appendix VII
EU compositional provisions and overview of the standardised methods of analysis for sodium in infant and follow-on formula

Definition of compound (2006/141/EC): Sodium.

Potential forms of the nutrient: Inorganic sodium.

Limits (2006/141/EC)								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
mg / 100kJ		mg /L*			mg / 100kJ		mg /L*	
5	14	125	413		5	14	125	413

* *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 5. Overview of the standardised methods of analysis for sodium in infant and follow-up formula.

Method name	Type of standard	Status	Analytical principle	Matrix validated
AOAC 2011.14	AOAC official method. Horizontal method	First action 2011	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Chocolate milk powder, dietetic milk powder, infant cereal, peanut butter, wheat gluten
AOAC 985.35	AOAC official method. Codex type III standard for infant formula	Final action 1988. Revised first action 1997	Flame atomic absorption Spectroscopy (FAAS)	Enteral formula, soy and whey powder, RTF soy-based infant formula
AOAC 984.27	AOAC official method. Codex type III standard for infant formula	Final action 1987	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Infant formula
ISO 8070 | IDF 119:2007	Codex type II standard for infant formula	Adopted in 2007	Flame atomic absorption Spectroscopy (FAAS)	Milk, whey, buttermilk, yogurt, cream, butter, cheese, dried milk, caseinates
GB 5413.21 – part 1	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.21-1997	Flame atomic absorption Spectroscopy (FAAS)	Food for infants and young children, raw milk and dairy products
GB 5413.21 – part 2	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.21-1997	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Food for infants and young children, raw milk and dairy products
EN 15505:2008	European horizontal standard	Adopted in 2008	Flame atomic absorption Spectroscopy (FAAS)	Broccoli, carrots, white bread, saithe fillet, pork and cheese
AOAC 990.23	AOAC official method	First action 1990	Flame emission spectrometry (FES)	Dried milk

Continued Table 5. Overview of the standardised methods of analysis for sodium in infant and follow-up formula.

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 2011.14	-	95-101%	-	100 mg/kg	1.05-3.80%	1.9-4.0%
AOAC 984.27	-	82-105%	-	<100 mg/kg	<10.5%	4.8-11.8%
AOAC 985.35	-	88-110%	-	<100 mg/kg	5.8% (soy-based formula)	3.0-5.8% (soy-based formula)
ISO 8070 | IDF 119:2007	-	-	-	-	4.8% (whole milk powder)	8.6% (whole milk powder)
GB 5413.21 - part1	-	-	1.5 mg/100g	-	5.3%e	-
GB 5413.21 - part2	-	-	1.6 mg/100g	-	3.5%f	-
EN 15505:2008	91.7-95.0%	-	-	1500 mg/kg*	1.9-6.5%	4.2-6.9%
AOAC 990.23	-	-	-	-	2.1%	2.9%

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*
[e] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 15% of the arithmetic mean value.*
[f] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 10% of the arithmetic mean value.*
* *This LOQ is higher than the provisions for the minimum amount of sodium in formula as laid down by Commission Directive 2006/141/EC.*

Appendix VIII
EU compositional provisions and overview of the standardised methods of analysis for potassium in infant and follow-on formula

Definition of compound (2006/141/EC): potassium.

Potential forms of the nutrient: Inorganic potassium.

Limits (2006/141/EC)								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
mg/ 100kJ		mg /L*			mg / 100kJ		mg / L*	
15	38	375	1121		15	38	375	1121

* *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 6. Overview of the standardised methods of analysis for potassium in infant and follow-up formula.

Method name	Type of standard	Status	Analytical principle	Matrix validated
AOAC 2011.14	AOAC official method. Horizontal method	First action 2011	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Chocolate milk powder, dietetic milk powder, infant cereal, peanut butter, wheat gluten
AOAC 984.27	AOAC official method. Codex type III standard for infant formula	Final action 1987	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Infant formula
AOAC 985.35	AOAC official method. Codex type II standard for infant formula	Final action 1988. Revised first action 1997	Flame atomic absorption Spectroscopy (FAAS)	Enteral formula, soy and whey powder, RTF soy-based infant formula
ISO 8070 | IDF 119:2007	Codex type II standard for infant formula	Adopted in 2007	Flame atomic absorption Spectroscopy (FAAS)	Milk, whey, buttermilk, yogurt, cream, butter, cheese, dried milk, casein and caseinates
GB 5413.21 – part 1	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.21-1997	Flame atomic absorption Spectroscopy (FAAS)	Food for infants and young children, raw milk and dairy products
GB 5413.21 – part 2	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.21-1997	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Food for infants and young children, raw milk and dairy products
AOAC 990.23	AOAC official method	First action 1990	Flame emission spectrometry (FES)	Dried milk

Continued Table 6. Overview of the standardised methods of analysis for potassium in infant and follow-up formula.

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 2011.14	-	87-102%	-	200 mg/kg	1.01-2.48%	2.02-7.62%
AOAC 984.27	-	85-111%	-	<100 mg/kg	1.3-6.6%	4.0-12.6%
AOAC 985.35	-	86-100%	-	<100 mg/kg	2.0% (soy-based formula)	2.1-2.7% (soy-based formula)
ISO 8070 | IDF 119:2007	-	-	-	-	4.0% (whole milk powder)	6.4% (whole milk powder)
GB 5413.21 – part 1	-	-	0.2 mg/100g	-	5.3%e	-
GB 5413.21 – part 2	-	-	0.7 mg/100g	-	3.5%f	-
AOAC 990.23	-	-	-	-	1.9%	2.4%

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*
[e] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 15% of the arithmetic mean value.*
[f] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 10% of the arithmetic mean value.*

Appendix IX
EU compositional provisions and overview of the standardised methods of analysis for chloride in infant and follow-on formula

Definition of compound: chloride.

Potential forms of the nutrient: Inorganic chloride.

Limits (2006/141/EC)								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
µg/ 100kJ		mg /L*			µg / 100kJ		mg / L*	
12	38	300	1121		12	38	300	1121

* *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 7. Overview of the standardised methods of analysis for chloride in infant and follow-up formula.

Method name	Type of standard	Status	Analytical principle	Matrix validated
AOAC 986.26	AOAC official method, final action. Codex type III for infant formula	Final action 1988	Potentiometry	Milk-based infant formula
GB 5413.24 - part 1	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.24-1997	Potentiometry	Food for infants and young children, raw milk and dairy products
GB 5413.24 – part 2	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.24-1997	Precipitation titration	Food for infants and young, raw milk children and dairy products
AS 2300.2.8-2008	Australian standard	Current	-	Milk

Continued Table 7. Overview of the standardised methods of analysis for chloride in infant and follow-up formula.

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 986.26	-	-	-	50 mg/kg	-	-
GB 5413.24 – part 1	-	-			0.2%e	-
GB 5413.24 – part 2	-	-	-	-	1.7%f	-
AS 2300.2.8-2008	-	-	-	-	-	-

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*
[e] *The difference between the two parallel measurements on the same sample should be no more than 0.2 g for every 100 g sample.*
[f] *The absolute difference between the two results of repeat measurements should be less than 5% of the mean.*

Appendix X EU compositional provisions and overview of the standardised methods of analysis for calcium in infant and follow-on formula

Definition of compound (2006/141/EC): calcium.

Potential forms of the nutrient: Inorganic calcium.

Limits (2006/141/EC)*								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
mg / 100kJ		mg/L**			mg / 100kJ		mg/L**	
12	33	300	973.5		12	33	300	973.5

* *The calcium:phosphorus ratio shall not be less than 1 nor greater than 2.*

** *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 8. Overview of the standardised methods of analysis for calcium in infant and follow-on formula.

Method name	Type of standard	Status	Analytical principle	Matrix validated
AOAC 2011.14	AOAC official method. Horizontal method	First action 2011	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Chocolate milk powder, dietetic milk powder, infant cereal, peanut butter, wheat gluten
AOAC 984.27	AOAC official method. Codex type III standard for infant formula	Final action 1987	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Infant formula
AOAC 985.35	AOAC official method. Revised first action. Codex type III for infant formula	Final action 1988. Revised first action 1997	Flame atomic absorption Spectroscopy (FAAS)	Enteral formula, soy and whey powder, RTF soy-based infant formula
ISO 8070 | IDF 119:2007	Codex type II standard for infant formula	Adopted in 2007	Flame atomic absorption Spectroscopy (FAAS)	Milk, whey, buttermilk, yogurt, cream, butter, cheese, dried milk, casein and caseinates
ISO 12081 | IDF 36: 2010	International standard	Adopted in 2010. Revises ISO 12081| IDF 36: 1998	Titration	Milk and milk reconstituted from evaporated, condensed and dried milk
GB 5413.21 – part 1	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.21-1997	Flame atomic absorption Spectroscopy (FAAS)	Food for infants and young children, raw milk and dairy products
GB 5413.21 – part 2	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.21-1997	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Food for infants and young children, raw milk and dairy products
AS 2300.2.7-2008	Australian standard			Milk

Continued Table 8. Overview of the standardised methods of analysis for calcium in infant and follow-on formula.

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 2011.14	-	98-100%		150 mg/kg	0.95-3.52%	2.71-6.68%
AOAC 984.27	-	89-104%	-	<150 mg/kg	1.1-8.0%	4.5-14.7%
AOAC 985.35	-	90-129%	-	<100 mg/kg	5.4% (soy-based formula)	10-11% (soy-based formula)
ISO 8070 | IDF 119:2007	-	-	-	-	2.4% (whole milk powder)	5.9% (whole milk powder)
ISO 12081 | IDF 36: 2010	-	-	-	-	0.002%[e]	-
GB 5413.21 - part1	-	-	1.0 mg/100g	-	5.3%[f]	-
GB 5413.21 – part 2	-	-	0.7 mg/100g	-	3.5%[g]	-
AS 2300.2.7-2008	-	-	-	-	-	-

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*
[e] *Expressed as repeatability limit r.*
[f] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 15% of the arithmetic mean value.*
[g] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 10% of the arithmetic mean value.*

Appendix XI
EU compositional provisions and overview of the standardised methods of analysis for phosphorus in infant and follow-on formula

Definition of compound (2006/141/EC): phosphorus.

Potential forms of the nutrient: phosphates.

Limits (2006/141/EC): Infant formulae manufactured from cows' milk proteins or protein hydrolysates*								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
mg/100kJ		mg/L**			mg/100kJ		mg/L**	
6	22	150	649		6	22	150	649

* *The calcium:phosphorus ratio shall not be less than 1 nor greater than 2.*
** *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Limits (2006/141/EC): Infant formulae manufactured from soya protein isolates, alone or in a mixture with cows' milk proteins*								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
mg/100kJ		mg/L**			mg/100kJ		mg/L**	
7.5	25	187.5	737.5		7.5	25	187.5	737.5

* *The calcium:phosphorus ratio shall not be less than 1 nor greater than 2.*
** *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 9. Overview of the standardised methods of analysis for phosphorus in infant and follow-up formula.

Method name	Type of standard	Status	Analytical principle	Matrix validated
AOAC 2011.14	AOAC official method. Horizontal method	First action 2011	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Chocolate milk powder, dietetic milk powder, infant cereal, peanut butter, wheat gluten
AOAC 984.27	AOAC official method. Codex type III standard for infant formula	Final action 1987	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Infant formula
AOAC 986.24	AOAC official method. Codex type II for infant formula.	Final action 1988. Revised first action 1997	Spectrophotometry	Enteral, soy-based infant formula
GB 5413-22	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.22-1997	Spectrophotometry	Food for infants and young children, raw milk and dairy products
ISO 9874 | IDF 42: 2006	International standard	Adopted in 2006. Replace ISO 9874 | IDF 42: 1992	Spectrophotometry	Milk
AOAC 995.11	AOAC official method. Horizontal method	First action 1995	Spectrophotometry	Potato flour, sausage, cold ham, infant formula, cheese, skimmed milk
AS 2300.2.9-2008	Australian standard			Milk

Continued Table 9. Overview of the standardised methods of analysis for phosphorus in infant and follow-up formula.

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 2011.14	-	94-102%	-	100 mg/kg	1.24-2.85%	2.57-3.28%
AOAC 984.27	-	90-110%	-	<100 mg/kg	2.0-7.6%	4.7-15.1%
AOAC 986.24	-	93-100%	-	50 mg/kg	3.0%	3.7% at 526 µg/g; 11% at 447 µg/g
GB 5413-22	-	-	2 mg/100g	-	1.7%e	-
ISO 9874 | IDF 42: 2006	-	-	-	-	0.005% (mass fraction)[f]	0.016% (mass fraction)[f]
AOAC 995.11	-	-	0.05-1.00 g/100g		5.4% (infant formula)	6.1% (infant formula)
AS 2300.2.9-2008	-	-	-		-	-

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*
[e] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 5% of the arithmetic mean value.*
[f] *Expressed as repeatability limit, r and reproducibility limit, R respectively.*

Appendix XII
EU compositional provisions and overview of the standardised methods of analysis for magnesium in infant and follow-on formula

Definition of compound (2006/141/EC): magnesium.

Potential forms of the nutrient: Inorganic magnesium.

Limits (2006/141/EC)								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
mg/100kJ		mg /L*			mg/100kJ		mg /L*	
1.2	3.6	30	106.2		1.2	3.6	30	106.2

* *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 10. Overview of the standardised methods of analysis for magnesium in infant and follow-on formula.

Method name	Type of standard	Status	Analytical principle	Matrix validated
AOAC 2011.14	AOAC official method. Horizontal method	First action 2011	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Chocolate milk powder, dietetic milk powder, infant cereal, peanut butter, wheat gluten
AOAC 984.27	AOAC official method. Codex type III standard for infant formula	Final action 1987	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Infant formula
AOAC 985.35	AOAC official method. Revised first action. Codex type III for infant formula	Final action 1988. Revised first action 1997	Flame atomic absorption Spectroscopy (FAAS)	Enteral formula, soy and whey powder, RTF soy-based infant formula
ISO 8070 | IDF 119:2007	Codex type II standard for infant formula	Adopted in 2007	Flame atomic absorption Spectroscopy (FAAS)	Milk, whey, buttermilk, yogurt, cream, butter, cheese, dried milk, casein and caseinates
GB 5413.21 – part 1	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.21-1997	Flame atomic absorption Spectroscopy (FAAS)	Food for infants and young children, raw milk and dairy products
GB 5413.21 – part 2	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.21-1997	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Food for infants and young children, raw milk and dairy products
EN 15505:2008	European horizontal standard	Adopted in 2008	Flame atomic absorption Spectroscopy (FAAS)	Simulated diets, wheat bran[a], chocolate cake, milk powder, fish muscle, apple

[a] *Not applicable to wheat bran.*

Continued Table 10. Overview of the standardised methods of analysis for magnesium in infant and follow-on formula

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 2011.14	-	95-102%		50 mg/kg	1.27-3.32%	2.92-4.12%
AOAC 984.27	-	85-99%	-	<50 mg/kg	<11.1%	4.4-11.3%
AOAC 985.35	-	84-89%	-	<100 mg/kg	4.4% (soy-based formula)	3.8-5.3% (soy-based formula)
ISO 8070 | IDF 119:2007	-	-	-	-	2.9% (whole milk powder)	3.1% (whole milk powder)
GB 5413.21 – part 1	-	-	0.3 mg/100g	-	5.3%[e]	-
GB 5413.21 – part 2	-	-	0.2 mg/100g	-	3.5%[f]	-
EN 15505:2008	97.5-100% (oyster tissue, wheat flour)	95.5-98.3% (simulated diets)	-	250 mg/kg	2.1% (milk powder)	4.6% (milk powder)

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*
[e] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 15% of the arithmetic mean value.*
[f] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 10% of the arithmetic mean value.*

Appendix XIII
EU compositional provisions and overview of the standardised methods of analysis for iron in infant and follow-on formula

Definition of compound (2006/141/EC): Iron.

Potential forms of the nutrient: Inorganic iron, organic iron (coordinated to organic elements in proteins).

Limits (2006/141/EC): Infant formulae manufactured from cows' milk proteins or protein hydrolysates							
Infant formula				Follow-up formula			
Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
mg/100kJ		mg /L*		mg/100kJ		mg /L*	
0.07	0.3	1.75	8.85	0.14	0.5	3.50	14.75

* *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Limits (2006/141/EC): Infant formulae manufactured from soya protein isolates, alone or in a mixture with cows' milk proteins							
Infant formula				Follow-up formula			
Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
mg/100kJ		mg /L*		mg/100kJ		mg /L*	
0.12	0.5	3.0	14.75	0.22	0.65	5.5	19.175

* *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 11. Overview of the standardised methods of analysis for iron in infant and follow-up formula.

Method name	Type of standard	Status	Analytical principle	Matrix validated
AOAC 2011.14	AOAC official method. Horizontal method	First action 2011	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Chocolate milk powder, dietetic milk powder, infant cereal, peanut butter, wheat gluten
AOAC 984.27	AOAC official method. Codex type III standard for infant formula	Final action 1987	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Infant formula
AOAC 985.35	AOAC official method. Codex type III for infant formula	Final action 1988. Revised first action 1997	Flame atomic absorption Spectroscopy (FAAS)	Enteral formula, soy and whey powder, RTF soy-based infant formula
AOAC 999.10| NMKL 161:1998[a]	AOAC official method. NMKL method. Codex type III for infant formula	First action 1999. Revised 2002	Flame atomic absorption Spectroscopy (FAAS)	Liver paste, mushrooms, fish, wheat bran, simulated diets.
AOAC 999.11|NMKL139:1991	AOAC official method. NMKL method. Codex type II for infant formula	First action 1999. Revised 2002	Flame atomic absorption Spectroscopy (FAAS)	Liver paste, milk powder, apple sauce, minced fish, wheat bran, simulated diets.
GB 5413.21 – part 1	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.21-1997	Flame atomic absorption Spectroscopy (FAAS)	Food for infants and young children, raw milk and dairy products
GB 5413.21 – part 2	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.21-1997	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Food for infants and young children, raw milk and dairy products
EN 14084:2003[b]	European horizontal standard	Adopted in 2003	Flame atomic absorption Spectroscopy (FAAS)	composite diets, cereals, fish, bovine muscle, milk powder, wheat bran and mushroom.
EN 14082:2003	European horizontal standard	Adopted in 2003	graphite furnace atomic absorption spectrometry (GFAAS)	composite diets, cereals, fish, fruit, liver, milk
ISO 6732 | IDF 103: 2010	International standard	Adopted in 2010. Reference method	Spectrophotometry	milk, evaporated, sweetened, dried and condensed milk; whey, dried whey, buttermilk and dreid buttermilk; yogurt and skimmed yogurt; cream and butter; anhydrous butterfat, butteroil, ice-cream; cheese; caseins, and caseinates

[a] *Method is not applicable to foods with a fat content ≥ 40%.*
[b] *The method is not applicable to oils, fats and other extremely fatty products.*

Continued Table 11. Overview of the standardised methods of analysis for iron in infant and follow-up formula.

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 2011.14		91-102%	-	10 mg/kg	1.81-4.17%	3.12-12.45%
AOAC 984.27	-	82-99%	-	<10 mg/kg	1.6-63.9%	5.7-66.3%
AOAC 985.35	-	81%	-	<10 mg/kg	4.1% (soy-based formula)	4.1% (soy-based formula)
AOAC 999.10| NMKL 161:1998	102.5%-107.5%		1.6 mg/kg [e]	7 mg/kg	3.2-5.4%	5.9-17%
AOAC 999.11| NMKL139:1991	-	-	0.8 mg/kg	4 mg/kg	11% (milk powder)	14% (milk powder)
GB 5413.21 – part 1	-	-	0.02 mg/100g		5.3%[f]	
GB 5413.21 – part 2	-	-	0.003 mg/100g		3.5%[g]	
EN 14084:2003	102.5%-107.5%	-	1.6 mg/kg [h]	7 mg/kg	3.2-5.4%	47% (milk powder)[i]
EN 14082:2003	-	-	- [l]	- [l]	-	35% (milk powder)
ISO 6732 | IDF 103	-	-	-	-	0.02-0.2 mg/kg [m]	-

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*
[e] *Calculated for a 0.5 g sample.*
[f] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 15% of the arithmetic mean value.*
[g] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 10% of the arithmetic mean value.*
[h] *Calculated for a 0.5 g sample.*
[i] *At concentration below the limit of quantification of the method.*
[l] *LOD and LOQ should be estimated taking into account the SD found in the long term evaluation.*
[m] *The difference between the results of two measurements under repeatability conditions will not be greater than the reported value.*

Appendix XIV
EU compositional provisions and overview of the standardised methods of analysis for zinc in infant and follow-on formula

Definition of compound (2006/141/EC): Zinc.

Potential forms of the nutrient: Inorganic zinc, organic zinc (coordinated to organic elements in proteins).

Limits (2006/141/EC)								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
mg/100kJ		mg /L*			mg/100kJ		mg /L*	
0.12	0.36	3	10.62		0.12	0.36	3	10.62

* *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 12. Overview of the standardised methods of analysis for zinc in infant and follow-up formula.

Method name	Type of standard	Status	Analytical principle	Matrix validated
AOAC 2011.14	AOAC official method. Horizontal method	First action 2011	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Chocolate milk powder, dietetic milk powder, infant cereal, peanut butter, wheat gluten
AOAC 984.27	AOAC official method. Codex type III standard for infant formula	Final action 1987	Inductively coupled plasma atomic emission spectro-scopy (ICP-AES)	Infant formula
AOAC 985.35	AOAC official method. Codex type II for infant formula	Final action 1988. Revised first action 1997	Flame atomic absorption Spectroscopy (FAAS)	Enteral formula, soy and whey powder, RTF soy-based infant formula
AOAC 999.10| NMKL 161:1998[a]	AOAC official method. NMKL method. Codex type III for infant formula	First action 1999. Revised 2002	Flame atomic absorption Spectroscopy (FAAS)	Liver paste, mushrooms, fish, wheat bran, simulated diets.
AOAC 999.11|NMKL139:1991	AOAC official method. NMKL method. Codex type II for infant formula	First action 1999. Revised 2002	Flame atomic absorption Spectroscopy (FAAS)	Liver paste, milk powder, apple sauce, minced fish, wheat bran, simulated diets.
GB/T 5413.21 – part 1	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.21-1997	Flame atomic absorption Spectroscopy (FAAS)	Food for infants and young children, raw milk and dairy products
GB/T 5413.21 – part 2	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.21-1997	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Food for infants and young children, raw milk and dairy products
EN 14084:2003[b]	European horizontal standard	Adopted in 2003	Flame or graphite furnace atomic absorption Spectroscopy (FAAS)	composite diets, cereals, fish, bovine muscle, milk powder, wheat bran and mushroom.
EN 14082:2003	European horizontal standard	Adopted in 2003	graphite furnace atomic absorption spectrometry (GFAAS)	composite diets, cereals, fish, fruit, liver, milk
ISO 11813 | IDF 156	International standard	Current	Flame atomic absorption Spectroscopy (FAAS)	Milk and milk products
AOAC 986.15	AOAC official method	Final action 1988. Revised 1996	hydride generation atomic absorption spectrometry (HGAAS)	Chicken and applesauce
AOAC 969.32	AOAC official method	Final action 1971	Flame atomic absorption Spectroscopy (FAAS)	Sucrose solutions, soya meal and wheat flour

[a] *Method is not applicable to foods with a fat content ≥ 40%.*
[b] *The method is not applicable to oils, fats and other extremely fatty products.*

Continued Table 12. Overview of the standardised methods of analysis for zinc in infant and follow-up formula.

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 2011.14	-	92-105%	-	5 mg/kg	1.95-3.96%	3.44-6.69%
AOAC 984.27	-	90-98%	-	<5 mg/kg	1.7-13.0%	6.5-15.9%
AOAC 985.35	-	100-105%	-	<5 mg/kg	6.7% (soy-based formula)	6.9-7.9% (soy-based formula)
AOAC 999.10| NMKL 161:1998	100-103.5%	-	0.24 mg/kg [e]	4 mg/kg	1.6-4.0%	9.3% (milk powder)
AOAC 999.11|NMKL139:1991	-	-	0.057 mg/kg	0.7 mg/kg	-	8.0% (milk powder)
GB/T 5413.21 – part 1	-	-	0.02 mg/100g	-	5.3%[f]	-
GB/T 5413.21 – part 2	-	-	0.002 mg/100g	-	3.5%[g]	-
EN 14084:2003 [c]	100-103.5%	-	0.24 mg/kg [h]	4 mg/kg	1.6-4.0%	9.3% (milk powder)
EN 14082:2003	-	-	-[i]	-[i]	-	8.0% (milk powder)
ISO 11813 | IDF 156	-	-	25-70 mg/kg (validation range)		2.5%	4 mg/kg (dry product) [l]
AOAC 986.15	-	96.5%	-	-	5.6%	5.4%
AOAC 969.32	-	98-102%	-	-	1.2%	<2.7%

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*
[e] *Calculated for a 0.5 g sample.*
[f] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 15% of the arithmetic mean value.*
[g] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 10% of the arithmetic mean value.*
[h] *Calculated for a 0.5 g sample.*
[i] *LOD and LOQ should be estimated taking into account the SD found in the long term evaluation.*
[l] *Reproducibility limit R.*

Appendix XV
EU compositional provisions and overview of the standardised methods of analysis for copper in infant and follow-on formula

Definition of compound (2006/141/EC): Copper.

Potential forms of the nutrient: Inorganic copper, organic copper (coordinated to organic elements in proteins).

Limits (2006/141/EC)								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
µg/100kJ		µg /L*			µg/100kJ		µg /L*	
8.4	25	210	737.5		8.4	25	210	737.5

* *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 13. Overview of the standardised methods of analysis for copper in infant and follow-up formula.

Method name	Type of standard	Status	Analytical principle	Matrix validated
AOAC 2011.14	AOAC official method. Horizontal method	First action 2011	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Chocolate milk powder, dietetic milk powder, infant cereal, peanut butter, wheat gluten
AOAC 984.27	AOAC official method. Codex type III standard for infant formula	Final action 1987	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Infant formula
AOAC 985.35	AOAC official method. Codex type II for infant formula	Final action 1988. Revised first action 1997	Flame atomic absorption Spectroscopy (FAAS)	Enteral formula, soy and whey powder, RTF soy-based infant formula
AOAC 999.10| NMKL 161:1998[a]	AOAC official method. NMKL method. Codex type III for infant formula	First action 1999. Revised 2002	Flame atomic absorption Spectroscopy (FAAS)	Liver paste, mushrooms, fish, wheat bran, simulated diets.
AOAC 999.11|NMKL139:1991	AOAC official method. NMKL method. Codex type II for infant formula	First action 1999. Revised 2002	Flame atomic absorption Spectroscopy (FAAS)	Liver paste, milk powder, apple sauce, minced fish, wheat bran, simulated diets.
GB 5413.21 – part 1	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.21-1997	Flame atomic absorption Spectroscopy (FAAS)	Food for infants and young children, raw milk and dairy products
GB 5413.21 – part 2	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.21-1997	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Food for infants and young children, raw milk and dairy products
EN 14084:2003[b]	European horizontal standard	Current	Flame atomic absorption Spectroscopy (FAAS)	composite diets, cereals, fish, bovine muscle, milk powder, wheat bran and mushroom.
EN 14082:2003	European horizontal standard	Adopted in 2003	graphite furnace atomic absorption spectrometry (GFAAS)	composite diets, cereals, fish, fruit, liver, milk
ISO 5738 | IDF 76	International standard	Revised version adopted in 2004	Spectrophotometry	Milk, evaporated and sweetened condensed milk, milk powders, cream, butter, buttermilk, ice-creams, cheese and cheese products, casein and caseinates

[a] *Method is not applicable to foods with a fat content ≥ 40%.*
[b] *The method is not applicable to oils, fats and other extremely fatty products.*

Continued Table 13. Overview of the standardised methods of analysis for copper in infant and follow-up formula.

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 2011.14	-	95-101%	-	2 mg/kg*	1.57-4.98%	4.77-11.72%
AOAC 984.27	-	83-116%	-	<2 mg/kg	2.6-14.9%	8.2-29%
AOAC 985.35	-	90-95%	-	<1 mg/kg	7.0% (soy-based formula)	11-13% (soy-based formula)
AOAC 999.10| NMKL 161:1998	101%-109.5%	-	0.098 mg/kg[e]	0.2 mg/kg	1.5-4.3%	3.8-39%
AOAC 999.11|NMKL139:1991	-	-	0.108 mg/kg	5 mg/kg*	6.9% (milk powder)	6.9% (milk powder)
GB 5413.21 – part 1	-	-	0.0045 mg/100g	-	5.3%[f]	-
GB 5413.21 – part 2	-	-	0.002 mg/100g	-	3.5%[g]	-
EN 14084:2003	101%-109.5%	-	0.098 mg/kg[h]	0.2 mg/kg	1.5-4.3%	27% (milk powder)
EN 14082:2003	-	-	-[i]	-[i]	-	47% (milk powder)
ISO 5738 | IDF 76	-	-	-	-	2.2-5.9 % (milk powder)	4.4-11.8% (milk powder)

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*
[e] *Calculated for 0.5 g sample.*
[f] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 15% of the arithmetic mean value.*
[g] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 10% of the arithmetic mean value.*
[h] *Calculated for 0.5 g sample.*
[i] *LOD and LOQ should be estimated taking into account the SD found in the long term evaluation.*
* *This LOQ is higher than the provisions for the minimum amount of copper in formula as laid down by Commission Directive 2006/141/EC.*

Appendix XVI
EU compositional provisions and overview of the standardised methods of analysis for iodine infant and follow-on formula

Definition of compound (2006/141/EC): Iodine

Potential forms of the nutrient: Iodide, organoiodine compounds, oxides of iodine

Limits (2006/141/EC)								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
µg/100kJ		µg /L*			µg/100kJ		µg /L*	
2.5	12	62.5	354		2.5	12	62.5	354

* *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 14. Overview of the standardised methods of analysis for iodine in infant and follow-up formula.

Method name	Type of standard	Status	Analytical principle	Matrix validated
AOAC 2012.14	AOAC official method	First action 2012	ICP-MS	Infant formula and adult nutritional products
AOAC 2012.15	AOAC official method	First action 2012	ICP-MS	Infant formula and adult nutritional products
AOAC 992.24	AOAC official method. Codex type II for infant and follow-up formula.	final action	Ion-selective potentiometry	Milk-based Infant formula
GB 5413-23	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.23-1997	Spectrophotometry	formulated foods and milk powder for infants and young children
EN 15511:2008	European horizontal standard	Adopted in 2008	Inductively coupled plasma mass spectrometry (ICP-MS)	Pig kidney, ready to serve pudding, soy products, cod muscle, iodised salt, marine algae
ISO 14378:2009 | IDF[a] 167	International standard	Adopted in 2009	HPLC	Whole milk, dried skim milk
AOAC 992.22[a]	AOAC official method	First action 1992	HPLC	Whole milk, dried skim milk
AS 2300.2.3-2008	Australian standard	Current	-	milk

[a] *Only free ionic iodide (not organically bound) is measured.*

Continued Table 14. Overview of the standardised methods of analysis for iodine in infant and follow-up formula.

Method name	Method performance characteristics				
	Accuracy	Spike recovery	Range of applicability	RSD_r[a]	RSD_R[b]
AOAC 2012.14	101-108%	92-105%	LOQ[c]=50 ng/g in liquid formula and 250 ng/g in powdered formula	0.5-3.0%	<6% (RSD_{IR}[e])
AOAC 2012.15	94.2-100% (non-fat milk powder, multivitamin/ multielement tablet)	-	LOD[d]=0.25 ng/g (powdered formula); LOQc=25-50 ng/g (powdered formula)	2.75%	-
AOAC 992.24	-	-	Applicable to RTF liquid milk-based IF containing 75-150 µg/L iodide*	4.0-11.4%	13.5-18.2%
GB 5413-23	-	-	-	1.7%[f]	-
EN 15511:2008	88-108% (pig kidney, cod muscle)	101% (ready to serve pudding and soy products)	LOQ=0.1 mg/kg [g]	0.7-7.8%	6.2-19%
ISO 14378:2009 | IDF 167	-	91%	0.03 – 1 µg/g (milk); 0.03 – 10 µg/g (dried skim milk)	9.0% (powdered skim milk)	12.7% (powdered skim milk)
AOAC 992.22	-	91%	0.03 – 1 µg/g (milk); 0.03 – 10 µg/g (dried skim milk)	9.0% (powdered skim milk)	12.7% (powdered skim milk)
AS 2300.2.3-2008	-	-	-	-	-

[a] *RSD_r=relative standard deviation of repeatability.*
[b] *RSD_R=relative standard deviation of reproducibility.*
[c] *LOQ=limit of quantification.*
[d] *LOD=limit of detection.*
[e] *RSD_{IR}=Intermediate relative standard deviation of reproducibility.*
[f] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 5% of the arithmetic mean value.*
[g] *The value is given for information and calculated by using 250 mg of sample. Every laboratory has to calculate its own LOQ according to specific laboratory conditions and instrumentation.*
* *This analytical range is outside the provisions for the minimum and maximum amount of copper in formula as laid down by Commission Directive 2006/141/EC.*

Appendix XVII
EU compositional provisions and overview of the standardised methods of analysis for selenium in infant and follow-on formula

Definition of compound: Selenium.

Potential forms of the nutrient: Inorganic selenium, organoselenium compounds.

Limits (2006/141/EC)								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
µg/100kJ		µg /L*			µg/100kJ		µg /L*	
0.25	2.2	6.25	64.9		0.25	2.2	6.25	64.9

* *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 15. Overview of the standardised methods of analysis for selenium in infant and follow-up formula

Method name	Type of standard	Status	Analytical principle	Matrix validated
AOAC 2011.19	AOAC official method	First action 2011	Inductively coupled plasma mass spectrometry (ICP-MS)	Infant formula, adult and medical nutritional
EN 14627:2005	European horizontal standard. Codex type II for infant formula	Adopted in 2005	hydride generation atomic absorption spectrometry (HGAAS)	Wholemeal wheat flour, celery, spinach, pig kidney, white clover, rice
AOAC 974.15	AOAC official method	Final action 1976. Revised 1996	Fluorimetry	Corn, skim milk powder, meat, fish
AOAC 986.15	AOAC official method	Final action 1988. Revised 1996	hydride generation atomic absorption spectrometry (HGAAS)	Chicken and applesauce

Continued Table 15. Overview of the standardised methods of analysis for selenium in infant and follow-up formula.

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 2011.19	101.8%	96.4-105.5%	-	10 ng/g (liquid)*; 50 ng/g (powder)[e]	-	1.0-3.4% (RSD_{IR})[f]
EN 14627:2005	97.3-108%	-	0.005 mg/kg[g]	-	2.4-12%	7.2-16%
AOAC 974.15	96%	101%	10 ng Se[h]	-	2.5-6.7%	-
AOAC 986.15	-	95.1%	-	-	13.2%	16.5%

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*
[e] *Sample Practical limit of quantification (PLOQ) that is equivalent to the lower linear limit of the calibration curve multiplied by the dilution factor.*
[f] *Intermediate precision calculated on data measured by two analysts on 6 separate days.*
[g] *The value is given for information and calculated by using 0.5 g of sample and a volume of dilution of 20 mL.*
[h] *LOD is related to the blank level. The value reported refers to a blank level= 25ng.*
* *This LOQ is higher than the provisions for the minimum amount of selenium in formula as laid down by Commission Directive 2006/141/EC.*

Appendix XVIII
EU compositional provisions and overview of the standardised methods of analysis for manganese in infant and follow-on formula

Definition of compound (2006/141/EC): Manganese.

Potential forms of the nutrient: Inorganic manganese.

Limits (2006/141/EC)								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
µg/100kJ		µg /L*			µg/100kJ		µg /L*	
0.25	25	6.26	737.5		0.25	25	6.26	737.5

* *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 16. Overview of the standardised methods of analysis for manganese in infant and follow-up formula.

Method name	Type of standard	Status	Analytical principle	Matrix validated
AOAC 2011.14	AOAC official method. Horizontal method	First action 2011	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Chocolate milk powder, dietetic milk powder, infant cereal, peanut butter, wheat gluten
AOAC 984.27	AOAC official method Codex type III standard for infant formula	Final action 1987	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Infant formula
AOAC 985.35	AOAC official method. Codex type II for infant formula	Final action 1988. Revised first action 1997	Flame atomic absorption Spectroscopy (FAAS)	Enteral formula, soy and whey powder, RTF soy-based infant formula
GB 5413.21 – part 1	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.21-1997	Flame atomic absorption Spectroscopy (FAAS)	Food for infants and young children, raw milk and dairy products
GB 5413.21 – part 2	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.21-1997	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Food for infants and young children, raw milk and dairy products

Continued Table 16. Overview of the standardised methods of analysis for manganese in infant and follow-up formula.

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 2011.14	-	90-99%	-	0.05 mg/kg	1.75-6.79%	4.89-8.82%
AOAC 984.27	-	82-103%	-	<0.03 mg/kg	<18.9%	2.5-19.4%
AOAC 985.35	-	86-87%	-	<0.01 mg/kg	4.8% (soy-based formula)	8.0-14% (soy-based formula)
GB 5413.21 – part 1	-	-	0.001 mg/100g	-	5.3%[e]	-
GB 5413.21 – part 2	-	-	0.005 mg/100g	-	3.5%[f]	-

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*
[e] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 15% of the arithmetic mean value.*
[f] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 10% of the arithmetic mean value.*

Appendix XIX
EU compositional provisions and overview of the standardised methods of analysis for Fluoride in infant and follow-on formula

Definition of compound (2006/141/EC): Fluorine.

Potential forms of the nutrient: Fluoride.

Limits (2006/141/EC)							
Infant formula				Follow-up formula			
Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
µg/100kJ		µg /L*		µg/100kJ		µg /L*	
-	25	-	737.5	-	25	-	737.5

* *Minimum Values /L have been calculated taken into account the minimum and provision for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 17. Overview of the standardised methods of analysis for fluoride in infant and follow-on formula.

Method name	Type of standard	Status	Analytical principle	Target compound	Matrix validated
AOAC 954.05	AOAC official method. Horizontal method	First action 1954	Fluorescence quencing	Soluble fluorides	-
AOAC 905.03	AOAC official method. Horizontal method	Final action	Hydrofluoric acid test	Soluble fluorides	-

Continued Table 17. Overview of the standardised methods of analysis for fluorine in infant and follow-on formula.

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 954.05	-	-	-	-	-	-
AOAC 905.03	-	-	-	-	-	-

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*

Appendix XX
EU compositional provisions and overview of the standardised methods of analysis for taurine in infant and follow-on formula

Definition of compound (2006/141/EC): Taurine.

Potential forms of the nutrient: Taurine.

Limits (2006/141/EC)								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
mg /100kJ		mg/L*			mg /100kJ		mg/L*	
-	2.9	-	85.55		-	2.9	-	85.55

* *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 18. Overview of the standardised methods of analysis for taurine in infant and follow-up formula.

Method name	Type of standard	Status	Analytical principle	Matrix validated
AOAC 997.05[a]	AOAC official method	Final action 2001. Revised 2002	Liquid chromatography with UV or fluorescence detection	Soy-based and milk-based infant formula
GB 5413.26-part 1	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.26-1997	Liquid chromatography with fluorescence detection	Food for infants and young children, raw milk and dairy products
GB 5413.26-part 2	Chinese standard	Adopted in 2010. Replace GB 5413-1985 and GB/T 5413.26-1997	Liquid chromatography with UV or fluorescence detection	Food for infants and young children, raw milk and dairy products

[a] *Applicable to determination of taurine (endogenous and supplemental) in powdered milk and powdered infant formula containing 5–100 mg/100 g solids. Method is not applicable to protein-hydrolysed materials.*

Continued Table 18. Overview of the standardised methods of analysis for taurine in infant and follow-up formula.

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 997.05	-	96-103%	-	1 mg/100g	3.46-10.15%	4.54-11.79%
GB 5413.26-part 1	-	-	0.5 mg/100g	-	3.5%[e]	-
GB 5413.26-part 2			5 mg/100g (UV); 0.1 mg/100g (fluorescence)	-	3.5%[e]	-

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*
[e] *The absolute difference between two independent measurements performed under repeatability conditions should not exceed 10% of the arithmetic mean value.*

Appendix XXI
EU compositional provisions for for carnitine in infant and follow-on formula

Definition of compound (2006/141/EC): carnitine.

Potential forms of the nutrient: carnitine.

Limits (2006/141/EC)								
Infant formula					Follow-up formula			
Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
mg /100kJ		mg/L*			mg /100kJ		mg/L*	
0.3	-	7.5	-		0.3	-	7.5	-

* *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 19. Overview of the standardised methods of analysis for taurine in infant and follow-up formula.

Method name	Type of standard	Status	Analytical principle	Matrix validated
AOAC 2012.17	AOAC official method	Final action 2012	Liquid Chromatography/Tandem Mass Spectrometry	infant formula and adult/pediatric nutrition formula

Continued Table 19. Overview of the standardised methods of analysis for taurine in infant and follow-up formula.

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 2012.17 [e]	-	-	-	-	-	-

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*
[e] *Performance characteristics of the method are not available yet since the method has not been published yet.*

Appendix XXII
EU compositional provisions and overview of the standardised methods of analysis for nucleotides in infant and follow-on formula

Definition of compound: Nucleotides.

Potential forms of the nutrient: cytidine 5-monophosphate, uridine 5-monophosphate, adenosine 5′-monophosphate, guanosine 5-monophosphate, inosine 5-monophosphate, nucleosides, primary bases.

	Limits (2006/141/EC)*								
	Infant formula					Follow-up formula			
	Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum
	mg /100kJ		mg /L**			mg /100kJ		mg /L**	
Cytidine 5′-monophosphate	-	0.60	-	17.7		-	0.60	-	17.7
Uridine 5′-monophosphate	-	0.42	-	12.39		-	0.42	-	12.39
Adenosine 5′-monophosphate	-	0.36	-	10.62		-	0.36	-	10.62
Guanosine 5′-monophosphate	-	0.12	-	3.54		-	0.12	-	3.54
Inosine 5′-monophosphate	-	0.24	-	7.08		-	0.24	-	7.08

* *The total concentration of nucleotides shall not exceed 1,2 mg/100 kJ (35.4 mg/L**).*
** *Minimum and Maximum Values /L have been calculated taken respectively into account the minimum and the maximum provisions for energy as reported in annex I and II of EU Commission directive 2006/141/EC.*

Table 20. Overview of the standardised methods of analysis for nucleotides in infant and follow-up formula.

Method name	Type of standard	Status	Analytical principle	Matrix validated
AOAC 2011.20	AOAC official method	First action 2011	HPLC with UV detection	milk and milk-based pediatric and adult nutritional products
AOAC 2011.21	AOAC official method	First action 2011	hydrophilic interaction liquid chromatography with tandem mass spectrometry (HILIC-MS/MS).	infant formula and adult/pediatric nutritional formula

Continued Table 20. Overview of the standardised methods of analysis for nucleotides in infant and follow-up formula.

Method name	Method performance characteristics					
	Accuracy	Spike recovery	LOD[a]	LOQ[b]	RSD_r[c]	RSD_R[d]
AOAC 2011.20	-	92 - 101%	0.06-0.19 mg/kg according to nucleotides	Upper limit of quantification=17.6 mg/kg	1.0–2.3 %	3.8–8.6% (RSD_{IR})
AOAC 2011.21	-	98.1 to 108.9%[e]	0.005 (AMP, GMP, CMP, and IMP) and 0.01 mg/mL (UMP) µg/mL[f]	0.01 (AMP, CMP), 0.02 (UMP), 0.025 (GMP), and 0.03 (IMP) µg/mL[f]	1.1–4.5%	0.7–5.4% (RSD_{IR})

[a] *LOD=limit of detection.*
[b] *LOQ=limit of quantification.*
[c] *RSD_r=relative standard deviation of repeatability.*
[d] *RSD_R=relative standard deviation of reproducibility.*
[e] *Inter-day recovery, assessed by the recovery test of 2 times/1 day for 3 days.*
[f] *Instrumental LOD and LOQ.*

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