Essential and Toxic Metals in Infant Formula from the European Community

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Abstract

Infant formulas are intended for children aged 6 - 12 months who, for one reason or another, cannot be breastfed by their mother. These products are made to meet the nutritional needs of children and are therefore, required to be a source of essential metals. However, they may also contain non-essential and toxic metals from the raw material which they are made with and this may be a health risk to children. For this reason, the content of 19 metals (Na, K, Ca, Mg, Fe, Cu, Zn, Cr, B, Ba, Ni, Li, V, Sr, Mo, Mn, Al, Cd, and Pb) were determined in a total of 30 infant formula samples from 15 different brands using inductively coupled plasma atomic emission optical spectroscopy (ICP-OES) with the objective of evaluating the nutritional profile and the toxicological risk derived from the consumption of these products. Ca was the major macroelement with a mean concentration of 4544mg/kg wet weight. As regards trace elements, Fe stands out (55.9mg/kg ww). The mean concentration found for Pb (0.07mg/kg ww) exceeds the maximum limit established in European legislation. The analyzed formulas cover the daily requirements of almost all essential elements. The intake of Pb from the consumption recommended by the manufacturer’s means that lead contributes 233% of the Tolerable Daily Intake (TDI). Infant formulas meet the children’s nutritional needs, although the concentration of Pb may pose a risk to children’s health.

Keywords: Infant formula; Toxic metals; Dietary intake; Toxicological assessment; Toxic risk

Introduction

Breastfeeding is the main source of nutrients for infants since it meets nutritional needs, facilitating the growth and proper development of infants [1,2]. The composition of breast milk confers numerous benefits to the newborns, reducing the risk of diseases such as enterocolitis or sepsis and strengthening the immune system of infants [2].

Although breast milk is the basis for infant feeding, there may be a number of situations that hinder breastfeeding, such as work, social reasons, medical contraindications or pharmacological treatments that cannot be replaced or withdrawn and pose a risk to the infant breast-feeder [3]. When these situations arise, an alternative diet should be used to meet the nutritional needs of infants, such as infant formulas or preparations. In particular, infant formulas are dairy milk substitutes for the mother’s milk intended for children aged 6 to 12 months [4], which is a period when a supplementary feeding is started, and therefore these infant formulas do not need to completely meet the infants’ nutritional requirements because this supplementary diet are also a source of nutrients [5].

The macroelements (Na, K, Ca, Mg), are among the essential minerals required for a correct nutrition and are necessary in large quantities, as they play an important role in the human organism. For example, calcium (Ca), which is the major constituent of bone [6,7] or magnesium (Mg), a metal of much importance for maintaining electrolytic balance [8]. On the other hand, trace elements or microelements (Fe, Cu, Cr, Zn, Mn, Mo and Co) are required in smaller amounts but perform numerous physiological functions in the organism. As in the case of iron (Fe), which forms part of the hemoglobin necessary for the transport of oxygen [6,9], it is also added to infant formula to equalize its concentration with the mother’s milk, or copper (Cu), which plays an important role as a cofactor in enzymes intervening in multiple metabolic reactions [10].

Although infant formulas are beneficial and safe formulas, they may be a source of contaminants, such as trace elements with no function in the human organism (Sr, Ni, Li, B, Ba and V) or toxic metals (Al, Cd and Pb), which tend to accumulate and are a health risk to children [11,12], who are a more vulnerable
group as their excretory capacity is lower than that of an adult, and their body weight is less and they have a weaker immune system [13].

High intakes of non-essential trace elements have toxic effects on health, such as strontium (Sr) for example, which can interfere in numerous biological processes which calcium participates in, due to its affinity for this element, which can lead to childhood rickets [14]. Besides which, Sr can produce insoluble compounds with phosphorus (P) leading to phosphorus deficiency [15,16]. However, there are no known cases of intoxication from these metals arising from diet.

Toxic metals tend to accumulate in the human organism causing multiple toxic effects. Aluminum (Al), a neurotoxic agent, has an affinity for the brain where it accumulates and can cause damage to the central nervous system (CNS). Numerous studies have linked high concentrations of Al in the brain with memory loss and neurodegenerative diseases such as Alzheimer’s or Parkinson’s [17,18]. Cadmium (Cd) and lead (Pb) are characterized by a high half-life and by their tendency to accumulate in different parts of the body. Excessive intake of Cd and Pb can lead to cardiovascular disease, damage to the nervous system and the bones [19-21].

Since infant formulas are intended for growing children, they need to be safe and meet the nutritional needs of the target population. Therefore, the objectives of this study are to determine the content of macronutrients (Na, K, Ca and Mg) and essential elements (Fe, Cu, Zn, Cr, Mn and Mo) in infant formulas to evaluate the nutritional profile as well as the content of non-essential metals (B, Ba, Li, Ni, V and Sr) and toxic metals (Al, Cd and Pb) in order to evaluate the toxicological risk derived from the consumption of these products, taking into account the maximum intake limits and the established legislation.

**Materials and methods**

**Samples**

**Table 1:** Characteristics of analyzed the powered infant formula samples.

| Identification | Class              | Protein source | Age group     | No. Samples | Format | Type of container |
|----------------|--------------------|----------------|---------------|-------------|--------|-------------------|
| M1             | Enriched formula   | Cow’s milk     | 6-12 months   | 2           | 800 g  | Paper bag         |
| M2             | Enriched formula   | Cow’s milk     | 6-12 months   | 2           | 400 g  | Aluminium tin     |
| M3             | Enriched formula   | Cow’s milk     | 6-12 months   | 2           | 800 g  | Aluminium tin     |
| M4             | Enriched formula   | Cow’s milk     | 6-12 months   | 2           | 800 g  | Aluminium tin     |
| M5             | Enriched formula   | Cow’s milk     | 6-12 months   | 2           | 800 g  | Aluminium tin     |
| M6             | Enriched formula   | Cow’s milk     | 6-12 months   | 2           | 800 g  | Aluminium tin     |
| M7             | Enriched formula   | Cow’s milk     | 6-12 months   | 2           | 800 g  | Aluminium tin     |
| M8             | Enriched formula   | Cow’s milk     | 6-12 months   | 2           | 800 g  | Aluminium tin     |
| M9             | Enriched formula   | Cow’s milk     | 6-12 months   | 2           | 800 g  | Aluminium tin     |
| M10            | Enriched formula   | Cow’s milk     | 6-12 months   | 2           | 800 g  | Aluminium tin     |
| M11            | Enriched formula   | Cow’s milk     | 6-12 months   | 2           | 800 g  | Aluminium tin     |
| M12            | Enriched formula   | Cow’s milk     | 6-12 months   | 2           | 800 g  | Aluminium tin     |
| M13            | Soya based formula | Soy            | 6-12 months   | 2           | 400 g  | Aluminium tin     |
| M14            | Hydrolyzed formula | Extensively hydrolysed | 6-12 months | 2 | 800 g | Aluminium tin |
| M15            | Hypoallergenic formula | Extensively hydrolysed | 6-12 months | 2 | 800 g | Aluminium tin |

A total of 30 samples of different types of powdered infant formulas with 15 different brand names, for infants aged between 6 and 12 months, were analyzed. Table 1 shows the characteristics of the samples analyzed.

The samples were purchased in pharmacies on the island of Tenerife (Canary Islands, Spain) between December 2016 and March 2017. They were stored in their original containers until they were opened.

**Treatment of samples and analysis**

Five grams of each previously homogenized sample were weighed in triplicate in porcelain crucibles (Staatlich, Germany).
They were dried in an oven (Nabertherm, Germany) for 24 hours at 70-80 °C. They were then subjected to acid digestion with 65% HNO₃ of reagent purity (Honeybell Fluka, Germany). After digestion, the capsules were placed in a muffle furnace (Nabertherm, Germany) with a time-temperature program of 425 °C-24 hours, with a gradual rise in temperature of 50 °C/hour. The white or greyish white ashes were dissolved in 1.5% nitric acid (HNO₃) and transferred to sterile, hermetic polyethylene containers [7,10,22]. The determination of the metals was performed in a period of less than two weeks after their preparation.

The metal content was determined by inductively coupled plasma atomic emission optical spectroscopy (ICP-OES) using a Thermo Scientific iCAP 6000 series spectrometer (Waltham, MA, USA). The instrumental conditions were as follows: approximate RF power of 1.2kW, gas flow (nebulizer flow, auxiliary flow) of 0.5L/min, pump speed of 50rpm, stabilization time of 0 seconds, wavelength (nm) of each metal: Al (167), B (249.7), Ba (455.4), Ca (317.9), Cd (226.3), Cr (267.7), Cu (257.6), Mo (257.6), Na (589.6), Ni (231.6), Pb (220.3), Sr (407.7), V (310.2), Zn (206.2).

The quantification limits of the metals were calculated as ten times the standard deviation (SD) obtained from the analysis of 15 targets under reproducible conditions [23], and were as follows: 0.012mg/L (Al), 0.012mg/L (B), 0.005mg/L (Ba), 1.995mg/L (Ca), 0.001mg/L (Cd), 0.008mg/L (Cr), 0.012mg/L (Cu), 0.009mg/L (Fe), 1.884mg/L (K), 0.013mg/L (Li), 1.943mg/L (Mg), 0.008mg/L (Mn), 0.002mg/L (Mo), 3.655mg/L (Na), 0.003mg/L (Ni), 0.001mg/L (Pb), 0.003mg/L (Sr), 0.005mg/L (V), 0.007mg/L (Zn).

In order to ensure the accuracy and precision of the analytical method, a quality control was carried out based on the recovery rate obtained after subjecting the certified reference materials, similar to the matrix under study, to the same treatment procedure as the samples. The reference materials used were BCR-150 and BCR-063R Skim Milk Powder, from the Institute for Reference Materials and Measurements (IRMM), and 1549 Non-Fat Milk Powder from the National Institute of Standards and Technology (NIST). All recovery rates were higher than 98%, and no significant differences (p = 0) were found between the certified concentration and the concentration obtained.

**Statistical analysis**

The statistical analysis was conducted with the IBM Statistics SPSS software for Mac™. First, a normality study was carried out to determine whether the data followed a normal distribution or not by applying the Kolmogorov-Smirnov and Shapiro-Wilk tests [24]. The data which did not follow a normal distribution were analysed by nonparametric tests using the Kruskal-Wallis test [25].

These studies were carried out to study the possible existence of significant differences in the metal content among the different brands of infant formula studied. Values of p<0.05 were considered significantly different.

**Results**

Table 2: Mean metal content and standard deviation found in the analyzed samples without differentiating by brand.

| Metal | Mean concentration (mg/kg wet weight)±SD |
|-------|---------------------------------------|
| **Macroelements** |                                      |
| K     | 4107±264                               |
| Mg    | 441±16.5                               |
| Na    | 1668±88.7                              |
| Ca    | 4544±318                               |
| **Trace elements** |                                    |
| Cr    | 0.13±0.02                              |
| Cu    | 3.75±0.40                              |
| Fe    | 55.9±7.55                               |
| Zn    | 34.2±1.09                              |
| Mn    | 1.00±0.23                              |
| Mo    | 0.14±0.01                              |
| V     | 0.28±0.16                              |
| Ni    | 0.07±0.04                              |
| Li    | 0.96±0.44                              |
| Sr    | 2.47±0.95                              |
| Ba    | 1.20±0.42                              |
| B     | 1.59±0.21                              |
| **Toxic metals** |                                    |
| Al    | 4.02±2.01                              |
| Cd    | 0.01±0.00                              |
| Pb    | 0.07±0.02                              |

Table 2 shows the mean concentrations (mg/kg wet weight) of each metal studied and their obtained standard deviations for all samples without taking the brand into account.

Ca is the major macroelement, with a mean concentration of 4544mg/kg ww wet weight, the remaining macroelements follow the sequence K (4107mg/kg ww)> Na (1668mg/kg ww)> Mg (441mg/kg ww). As for the essential trace elements, the concentrations of Fe (55.9mg/kg ww) and Zn (3.42mg/kg ww) are noteworthy. The mean concentration of Sr at 2.47mg/kg ww is worth mentioning in the case of non-essential trace elements.

Al is the toxic metal with the highest mean concentration of 4.02mg/kg ww, followed by Pb (0.07mg/kg ww) and Cd (0.01mg/kg ww). It should be noted that the mean level of Pb...
found was above the maximum limit of 0.02mg/kg fresh weight for powdered infant formula established by Regulation (EC) No 1881/2006 of 19 December 2006 which sets the maximum content of certain contaminants in foodstuffs [26].

In addition, Regulation (EU) No 488/2014 amending Regulation (EC) No 1881/2006 sets a maximum limit of 0.01mg/kg fresh weight for powdered infant formula made from protein obtained from cows’ milk or from protein hydrolysates, and of 0.02mg/kg fresh weight for infant formula prepared from soy protein either alone or in combination with cow’s milk [27]. Therefore, the mean Cd concentration found reaches the maximum limit established in the legislation.

Studies conducted by Kazi et al. [28] and by Winiarska-Mieczan [13] report Pb and Cd concentrations in infant formulas similar to the results obtained here. Although Pandelova et al. [29] found higher Cd levels than those in the present study.

It is noteworthy that both the Pb and Cd levels found in human milk are lower than those recorded in the infant formula studied [30]. However, levels of contaminants in breast milk depend on diet, environment, and habits, with higher levels of both metals being found in female smokers or in those living in contaminated areas [31].

Metal content obtained in each brand studied

Table 3 shows the concentrations of macroelements and essential trace elements (mg/kg wet weight) and their standard deviations obtained depending on the infant formula brand studied.

| Sample | Ca±Standard Deviation | K±Standard Deviation | Mg±Standard Deviation | Na±Standard Deviation | Fe±Standard Deviation | Cu±Standard Deviation | Cr±Standard Deviation | Mn±Standard Deviation | Mo±Standard Deviation | Zn±Standard Deviation |
|--------|-----------------------|----------------------|-----------------------|-----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| M1     | 3286±281              | 3052±26.6            | 398±12.7              | 398±12.7              | 41.9±10.4            | 2.76±0.54            | 0.11±0.02            | 0.84±0.15            | 0.10±0.02            | 28.9±4.87           |
| M2     | 4559±257              | 4520±137             | 454±18.9              | 454±18.9              | 67.4±7.22            | 4.01±0.44            | 0.14±0.01            | 2.02±0.07            | 0.23±0.01            | 38.1±0.97           |
| M3     | 4773±455              | 4306±360             | 417±6.07              | 417±6.07              | 62.8±12.3            | 4.02±0.37            | 0.14±0.02            | 0.43±0.03            | 0.07±0.01            | 38.0±1.14           |
| M4     | 5221±348              | 4403±131             | 457±21.1              | 457±21.1              | 57.5±10.8            | 4.38±0.28            | 0.12±0.01            | 0.55±0.03            | 0.09±0.01            | 38.1±0.84           |
| M5     | 5117±149              | 4411±166             | 581±15.8              | 581±15.8              | 60.1±1.02            | 3.01±0.03            | 0.16±0.01            | 0.96±0.02            | 0.19±0.00            | 35.5±0.40           |
| M6     | 3043±92.0             | 4091±9.9             | 405±11.6              | 405±11.6              | 35.6±0.86            | 3.56±0.10            | 0.08±0.01            | 0.87±0.01            | 0.04±0.01            | 36.4±0.52           |
| M7     | 4341±198              | 3650±106             | 314±35.4              | 314±35.4              | 51.2±6.13            | 3.41±0.17            | 0.13±0.02            | 0.46±0.01            | 0.14±0.00            | 27.4±0.99           |
| M8     | 5527±387              | 4352±295             | 419±21.4              | 419±21.4              | 41.1±6.89            | 3.61±0.29            | 0.13±0.01            | 0.36±0.02            | 0.07±0.01            | 33.2±0.22           |
| M9     | 4628±493              | 4162±178             | 369±2.98              | 369±2.98              | 48.0±9.76            | 3.61±0.77            | 0.12±0.02            | 0.48±0.05            | 0.07±0.01            | 37.2±1.49           |
| M10    | 4981±260              | 4462±320             | 448±44.2              | 448±44.3              | 53.5±3.38            | 3.72±0.38            | 0.13±0.03            | 1.04±0.10            | 0.11±0.01            | 34.5±1.22           |
| M11    | 4375±208              | 3425±240             | 548±17.7              | 548±17.7              | 53.9±1.50            | 3.85±0.31            | 0.10±0.01            | 0.96±0.13            | 0.19±0.01            | 37.3±0.89           |
| M12    | 4483±471              | 4314±412             | 577±10.4              | 577±10.4              | 65.5±12.6            | 4.08±0.62            | 0.13±0.04            | 0.72±0.05            | 0.17±0.02            | 42.8±0.61           |
| M13    | 4454±543              | 4391±598             | 502±11.5              | 502±11.5              | 68.9±14.0            | 2.99±0.53            | 0.18±0.03            | 2.20±0.41            | 0.35±0.05            | 27.0±0.91           |
| M14    | 5417±158              | 4060±195             | 302±9.75              | 302±9.75              | 65.9±12.1            | 4.66±0.78            | 0.13±0.01            | 1.27±1.11            | 0.26±0.01            | 27.1±0.28           |
| M15    | 3960±465              | 4005±497             | 419±7.57              | 419±7.57              | 65.5±14.3            | 4.58±0.45            | 0.11±0.02            | 1.79±1.24            | 0.06±0.01            | 32.1±0.96           |

As for macroelements, the highest concentrations of Ca found in the branded formulas were in M8 (5527mg/kg ww) and M14 (5417mg/kg ww). The highest level of K was in M2, with a concentration of 4520mg/kg ww. Whereas the highest Mg and Na concentrations were observed in M5 (581mg/kg ww) and M13 (2061mg/kg ww), respectively.
As regards the essential trace elements, the Fe level determined in the samples of the M1368.9mg/kg ww is noteworthy as are the Zn contents in the M2 and M4 samples, with both being 3.81mg/kg ww.

Table 4 shows the mean concentrations (mg/kg wet weight) and standard deviations of the non-essential trace elements and toxic metals found for each analyzed brand.

Sr is the most notable trace element with an average concentration of 5.26mg/kg ww, with the highest concentration being found in M10. Furthermore, it is worth mentioning the mean levels of B and Li found in the M14 were 3.21mg/kg ww and 2.84mg/kg ww, respectively. The statistical analysis confirmed the existence of significant differences in the vanadium content between the different samples analyzed, with the highest mean concentration being determined in M2.

Regarding toxic metals, M7 had the highest level of aluminium which was 7.05mg/kg ww. Besides, the Pb concentration found in M1 is noteworthy, with a mean concentration of 0.09mg/kg ww. The Cd levels, however, were similar in all the analyzed brands, with the exception of M13, whose Cd concentration was the highest with a mean value of 0.02mg/kg ww. This higher concentration of Cd can be explained by the fact that M13 corresponds to the soybean-based infant formula, and that the soybean has the capacity to absorb toxic metals from the soil [32].
The Recommended Daily Intake (RDI) values for macroelements and essential trace elements for children aged 7-12 months, established by FESNAD (the Spanish Federation of Nutrition, Food and Dietetic Societies), are as follows: 525 mg Ca/day, 700 mg K/day, 75 mg Mg/day, 370 mg Na/day, 8 mg Fe/day, 4 mg Zn/day, 0.3 μg Cu/day, 0.6 mg Mn/day, 3 μg Mo/day [33], and for chromium set at 5.5 μg Cr/day by the Institute of Medicine, Food and Nutrition Board [6].

In view of the results presented in Table 4, the contribution of macroelements from the consumption of the infant formulas studied meets the nutritional requirements of infants from 7 to 12 months of age, with the noteworthy contribution of calcium, which accounts for 129% of RDI, followed by Mg, K and Na, with contribution rates of 88.1%, 88.0% and 68.0%, respectively. As regards the essential trace elements, the nutritional needs of infants are met, exceeding 100% of the RDI for all metals, with the exception of Mn, whose contribution is 25% of the recommended daily intake. However, it must be taken into account that at these ages, the infants start other foods that contribute to nutritional requirements.

The content of macroelements (Na, K, Ca and Mg) and toxic metals (Al, Cd and Pb) were determined in 30 samples of 15 different brands of infant formula by ICP-OES. The study carried out shows that infant formulas meet the macroelement and essential element nutritional needs of infants aged between 6-12 months. The concentration of Pb found in each of the analyzed samples was higher than the maximum limit established in European legislation. The consumption of these products can weight/day, for barium 0.02 mg Ba/kg body weight/day [38] and for strontium of 0.13 mg Sr/kg body weight/day [39].

3. The Spanish Agency for Food Safety and Nutrition (AESAN) has suggested a lead TDI of 0.5 μg/kg body weight/day [40], adapted from BMDL01 (Benchmark dose modelling) dosage set by the EFSA [41].

The contribution percentages obtained for non-essential trace elements and for toxic metals (Table 5) are below tolerable daily and admissible weekly daily intake values, with the exception of barium and lead. In the case of barium, a percentage of contribution that is 100% of the TDI was observed, in spite of this, no cases of intoxication from the intake of barium from the diet have been found.

However, in the case of lead, the percentage of contribution is 233% of the TDI far exceeding the tolerable daily intake value. This is an alarming finding, as lead has multiple toxic effects because it is a carcinogenic, mutagenic metal that damages the developing nervous system [41]. Infants, who these products are intended for, are a highly vulnerable group because they are growing, which is why lead can cause irreversible harmful effects [13]. Although some studies have shown that calcium, due to its similar chemical characteristics, interferes with the absorption of lead at the gastrointestinal level [42], it is necessary to introduce greater controls on these products.

Conclusion

The content of macroelements (Na, K, Ca and Mg), trace elements (Fe, Cu, Cr, Zn, Mn, Mo, Ni, Li, B, Ba, V, and Sr) and toxic metals (Al, Cd and Pb) were determined in 30 samples of 15 different brands of infant formula by ICP-OES. The study carried out shows that infant formulas meet the macroelement and essential element nutritional needs of infants aged between 6-12 months. The concentration of Pb found in each of the analyzed samples was higher than the maximum limit established in European legislation. The consumption of these products can
carry serious risks from a high intake of Pb, which far exceeds the Tolerable Daily Intake (TDI). Therefore, the consumption of these products is not safe. This alarming finding needs to be acted on by the relevant authorities by increasing the controls both on the final products and on the raw materials which are used to make them.

**Conflict of interest**

The authors declare that they have no conflicts of interests.

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