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Nº of pages: 38

Nº of tables: 7

Nº of figures: 5

Keywords: Aluminium, Infant formula, Drinking water, Daily Intake, Provisional Tolerable Weekly Intake.

Short title: Aluminium content of Spanish Infant Formula

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Abstract

Aluminium toxicity has been relatively well documented in infants with impaired renal function and premature neonates.

The aims of this study were to analyse the concentration of aluminium in the majority of infant formulae sold commercially in Spain, to determine the influence of aluminium content in the tap water in reconstituted powder formulae and to estimate the theoretical toxic aluminium intake in comparison with the PTWI, and lastly, to discuss the possible interactions of certain essential trace elements added to formulation with aluminium according to type or main protein based infant formula.

A total of 82 different infant formulae from 9 different manufacturers were studied. Sample digestion was simulated in a closed acid-decomposition microwave system. Aluminium concentration was determined by atomic absorption spectrophotometry with graphite furnace.

In general, the infant formulae studied provide an aluminium level higher than that found in human milk, especially in the case of soya, preterm or hydrolysed casein-based formulae.

Standard formulae provide lower aluminium intakes amounting to about 4 % PTWI. Specialised and preterm formulae result in moderate intake (11 – 12 % and 8 – 10 % PTWI, respectively). Soya formulae contribute the highest intake (15 % PTWI).

Aluminium exposure from drinking water used for powder formula reconstitution is not considered a clear potential risk.

In accordance with the present state of knowledge about aluminium toxicity, it seems prudent to call for continued efforts to standardise routine quality control and reduce aluminium levels in infant formula as well as to keep the aluminium concentration under 300 $\mu g\ l^{-1}$ for all infant formulae, most specifically those formulae for premature and low birth neonates.

Introduction

Aluminium is the most abundant metallic element, constituting about 8 % of earth's crust. It occurs naturally under several inorganic or organic chemical forms.

Aluminium has an omnipresent character in nature. It is found in the environment both as a result of natural processes and from anthropogenic sources.

Exposure of adults to aluminium occurs through drinking water, food additives and antacids or buffered analgesics. However, aluminium and its compounds appear to be poorly absorbed and it is eliminated effectively in the urine. It has also been reported that neonates are more susceptible to exposure because of their greater intestinal absorption as a consequence of the immature gastrointestinal tract (Sedman et al.1985).

Aluminium toxicity is associated with dementing encephalopathy, osteomalacia and anaemia in patients with renal failure who were receiving dialysis or oral aluminium containing phosphate binders.

In infants, aluminium toxicity has been relatively well documented in the case of neonates with impaired renal function, and ill premature or low birth weight neonates (Sedman et al.1984a, b, Moreno et al. 1994, Puntis et al. 1986, Bishop et al. 1997). High aluminium levels in infant formulae have been implicated in aluminium intoxication in two infants with neonatal uraemia (Freundlich et al. 1985, 1990).

In this respect, it is clear that infants, and especially preterm neonates, display a narrow tolerance to aluminium because of immaturity in the tissues and organs involved in its metabolism. Thus, human milk does represent the pattern of non essential elements most suitable for infants at term. Knowledge of the aluminium content in human milk serves as the basis for formulating appropriate substitutes.

Regardless of the wide variability, the aluminium content in human milk is lower that that found in infant formulae, between 3 and 160 times according to data from bibliographical references (Koo et al. 1988, Baxter et al. 1991, Simmer et al. 1990, Krachler et al. 2000, Coni et al. 1990, Ballabriga et al. 1994, Fernández-Lorenzo et al. 1999). It is thus possible to suggest a reference aluminium range of 3 - 79 $\mu g\ l^{-1}$ to be expected in human milk. Ballabriga et al. (1994) and Fernández-Lorenzo et al. (1999) obtained an aluminium content in Spanish human milk of $6.5 \pm 5.3\ \mu g\ l^{-1}$ (n = 16, range 0.9 – 19.8 $\mu g\ l^{-1}$) and $23.9 \pm 9.6\ \mu g\ l^{-1}$ (n = 45, range 7 - 42 $\mu g\ l^{-1}$), respectively.

In this respect, given the evident toxicological impact of aluminium on neonates it is desirable that infant formulae should be proportionally similar or inferior in aluminium concentrations to those in human milk.

The high aluminium content found in certain infant formulae with complex manufacturing processes, has led to a call for an evaluation of aluminium levels, mainly in both soya and preterm formulae, from international paediatric organisms (AAP 1996).

The aims of this study were to analyse the concentration of aluminium in the majority of infant formulae sold commercially in Spain, to determine the influence of aluminium content in the tap water on final concentration in reconstituted powder formulae, to estimate the theoretical toxic aluminium intake in comparison with the Provisional Tolerable Weekly Intake (PTWI) established by the joint FAO/WHO Expert Committee on Food Additives (WHO 1989), and finally, to discuss the possible interactions of certain essential trace elements added to formulae with aluminium according to type or main protein based infant formula.

Material and Methods

Sample collection

Infant formula

Most infant formulae were purchased directly from manufacturers, and the rest of them in a distribution company in Pamplona (Spain). A total of 82 different infant formulae from 9 different manufacturers were studied. Formulae included both powder (n = 61) and ready-to-use preparations (n = 14), such as those based on cow's milk (n = 68) or soy-based formulae (n = 7). Cow's milk-based formulae included: preterm formula (n = 7), starter formula (adapted (n = 16) and non adapted (n = 4)), follow-up formula (n = 19) and specialised formula (hypoallergenic (n = 12), designed for lactose intolerant (n = 7), or inborn errors of metabolism (n = 10) formulae).

Infant formulae were stored in accordance with the directions on the label. Containers were kept in the in the absence of light at room temperature in a humidity controlled room.

Drinking water

Samples of drinking water were collected in duplicate from the Community of Navarra (Spain) in both urban and rural areas.

Thirty-nine tap water sampling points were selected according to a population census provided by the local office of the National Statistical Institute and piping information obtained from several public water treatment plants in the Community. Eighteen samples were taken from populations in rural areas and twenty one samples in Pamplona (capital of province).

A strict protocol was thus established to carry out tap water sampling.

Sample treatment

Special care was devoted to this phase to minimise the risk of adventitious contamination when handling. Handling rules were adopted to minimise every possible source of contamination from the sampling step onwards, since any mistake in handling would nullify the validity of the results.

All plastic material (low density polyethylene) or implements used which came into contact with the samples (infant formulae or tap water) were cleaned previously in 5 % nitric acid solution (Merck, Darmstadt, Germany) for six days and later rinsed three times with ultrapure water before utilisation.

Infant formula containers were opened in the clean laboratory under flow laminar bench, using vinyl talc-free gloves (Rotiprotect®, Carl Roth, Karlsruhe, Germany) and plastic material (Plastibrand®, Brand, Wertheim, Germany) to perform the sampling.

Infant formula samples were placed in high pressure Teflon digestion bombs and digested with subboiling nitric acid (Merck, Darmstadt, Germany) in a closed acid-decomposition microwave system (Milestone MLS 1200, Millestone s.r.l., Sorisole, Italy).

The solutions obtained were then diluted with ultrapure deionised water and kept in frozen storage at -20 ºC until analysis. Samples were digested in triplicate. Sampling and treatment operations have been described previously in greater detail (Navarro and Alvarez 2002).

In the case of drinking water samples, to avoid flocculation or losses by adsorption to the plastic walls in sampling containers always after measuring pH, 1 mL of subboiling nitric acid per litre was added until approximately pH = 2, and the solution was stored at 4 ºC until analysis.

Analytical methods

Aluminium concentration was determined by graphite furnace atomic absorption spectrometry (GFAAS, GBC GF 2000, Dandenong, Victoria, Australia). The operating parameters and optimising temperature program of the instrument are given in table 1.

[Insert table 1 about here]

Digested samples were diluted in matrix modifier solution. Injections (20 μL) were made in triplicate on L'vov platforms positioned inside pyrolytically coated tubes.

Samples were quantified by reference to a calibration curve obtained for aqueous standards. Working standard solutions (0 to 80 $\mu g\ l^{-1}$) were made up each day by dilution from stock 1000 mg l^{-1} standard solution (Merck, Darmstadt, Germany) in enough subboiling nitric acid to a final acid concentration similar to prepared samples. All solutions were kept in the covered carousel throughout the analyses to prevent any contamination.

Two blanks reagents, an aqueous internal standard, and a replicate of infant formula control, as well as ten infant formula samples were included in each analytical batch to provide on-going quality control information.

The results of the quality control program, repeatability (precision for triplicates with a run) and reproducibility (day to day precision), expressed as coefficient of variation, are presented in table 2.

[Insert table 2 about here]

Analytical blanks were used to correct for any possible contamination during analysis. When expressed in terms of infant formula, aluminium content was $2.3 \pm 0.3\ \mu g\ l^{-1}$. The detection limit was defined as the average of three times the standard deviation of the reagent blanks and corresponded to $3.3\ \mu g\ l^{-1}$ (wet weight) for the infant formulae. In the case of drinking water, blank reagents consisted of ultrapure water which was subjected to the same procedures of treatment, storing and mixing with matrix modifier. Aluminium level in analytical blanks was $0.2 \pm 0.5\ \mu g\ l^{-1}$, resulting in a detection limit of $1.7\ \mu g\ l^{-1}$.

Reference infant formulae were run throughout the course of the study ($174.8 \pm 4.2\ \mu g\ l^{-1}$). The control sample was previously analysed by the standard addition calibration to minimise matrix effects and an aluminium value of $176 \pm 7\ \mu g\ l^{-1}$ was

obtained. Recovery assays of spiked aluminium at different concentrations in this in-house control formula were satisfactory, ranging from 96 to 104 %.

An internal aqueous quality control (20 μg l^{-1}) was run concurrently with analytical samples. The mean ± s.d. of aluminium determined was 20.0 ± 1.1 μg l^{-1} (n = 34), with a range of 18.2 – 22.3. The acceptable range established in the quality program was 17.6 – 22.4 μg l^{-1}.

Given the unavailability of adequate reference material based on milk powder with certified values, IAEA (International Atomic Energy Agency) 155 whey powder was analysed to provide an estimate of accuracy and to guard against instrument bias. The obtained result (43.2 ± 2.6 mg kg^{-1}) shows an acceptable agreement with the information value (non certified) provided from the reference material (53 mg kg^{-1}, ranged 38 – 68).

Iron and zinc concentrations in digested acid solutions were analysed by flame atomic absorption spectrophotometry (FAAS, GBC GF 2000, Dandenong, Victoria, Australia) and inductively coupled plasma atomic emission spectrometry with Meinhard nebulizer (ICP-AES, Jobin Ybon JY 38S Plus Sequential) was used for manganese.

Iron, zinc and manganese calibration curves were accomplished using direct calibration against aqueous standards. Details of measurements, operating parameters and trace element quantification have been described elsewhere (Navarro et al. 1996, Navarro et al. 2000).

The IAEA milk powder A11 was assayed as standard reference material to validate the analytical method. Standard A11 was mineralised and analysed as stated above for infant formulae. The values determined (12 independent analytical runs) were: iron, 3.62 ± 0.09 μg g^{-1}; zinc, 37.10 ± 0.38 μg g^{-1} and manganese, 0.250 ± 0.021 μg g^{-1}; in comparison with certified values (confidence intervals): iron, 3.65 μg g^{-1} (2.89 – 4.41); zinc, 38.9 μg g^{-1} (36.6 – 41.2) and manganese 0.257 μg g^{-1} (0.248 – 0.266).

Chemicals

Suprapure nitric acid was purchased from Merck (Merck, Darmstadt, Germany) and distilled by subboiling before use.

Ultrapure deionised water type Milli Q was used for preparation and/or dilution of treated sample and standard solutions.

Magnesium nitrate was used as matrix modifier for aluminium determination (1,4 g of $Mg(NO_3)_2 \cdot 6H_20$ (Merck, Darmstadt, Germany) and 2 ml of Triton X-100 (Sigma, St. Louis, USA) were diluted in 1 L with ultrapure water).

Statistical Analysis

Statistical analysis was performed with SPSS v.9.0 for windows. Different groups of samples were compared through non parametric Kruskal-Wallis and Mann Whitney U-tests or Wilcoxon test for paired groups, with statistical significance set at $p < 0.05$; and observed tendencies in some trace element contents with regard to aluminium were analysed with the Spearman coefficient.

Results and Discussion

Aluminium contents in infant formula

Table 3 shows the aluminium concentrations for each of the types of infant formulae studied. Aluminium concentration found in powdered infant formula was calculated according to the manufacturer's dilution instructions to express the content in $\mu g\ l^{-1}$.

[Insert table 3 about here]

The wide variability in aluminium content found in some of the formulations included in this study is of special relevance. Because of this, both mean and median values were included in the results table, although we have only used the median value as the most representative parameter.

In this sense, the range of aluminium determined in analysed formulae reflects findings reported in the literature by several researchers listed in table 4.

[Insert table 4 about here]

This notorious variability is a result of the way aluminium is incorporated in infant formulae. Obviously the total aluminium content comes from: a) the source of raw material, that is cow's milk or isolated soy protein; b) it could be included during processing from metallic surfaces of equipment or utensils; c) it could be contributed by the additives or mineral supplements added, and lastly, d) it might be transferred from the

container during storage. Each of these factors could explain by itself the high variability in the aluminium concentrations found in the different infant formulae studied.

In a first view of aluminium levels in different types of infant formula, it is easy to observe the lower aluminium values determined in standard formulae (starter and follow-up formulae) in comparison with specialised, preterm and soya formulae. Subsequently, this fact was verified by the Kruskal-Wallis test ($p = 0.003$).

As previous studies have reported (Koo et al. 1988, Baxter et al. 1991, Simmer et al. 1990, Ballabriga et al. 1994, Bloodworth et al. 1991, Ikem et al. 2002, Dabeka and Mckenzie 1990), the lower aluminium values were detected in milk based formulae, to be more specific in this study, in starter formulae (adapted, 196 ± 152 $\mu g\ l^{-1}$ and non adapted 231 ± 128 $\mu g\ l^{-1}$) and follow-up formulae (272 ± 189 $\mu g\ l^{-1}$). These levels are in agreement with those found by other American and European researchers (Ballabriga et al. 1994, Fernández-Lorenzo et al. 1999, Hawkins et al. 1994, Coni et al. 1993, Ikem et al. 2002). Lower levels found in raw cow's milk show that this by itself is not necessarily the greatest source of aluminium. However, large aluminium ranges observed in standard formulae are indicative of the potential for contamination during manufacture.

It is well known that aluminium is associated with proteins. Table 5 summarises aluminium levels found in different infant formulae, focusing on the main protein contained.

[Insert table 5 about here]

In this respect, there is clear evidence about the involvement of intrinsic aluminium coming from a protein source on the final level found in standard formulae. The highest aluminium content is provided by those formulae based on whole milk (adapted starter formulae: 338 ± 114 $\mu g\ l^{-1}$, non adapted starter formulae: 368 $\mu g\ l^{-1}$ and follow-up formulae: 296 ± 185 $\mu g\ l^{-1}$), followed by skim-milk-based formulae (adapted starter formulae: 190 ± 83 $\mu g\ l^{-1}$, non adapted starter formulae: 136 ± 115 $\mu g\ l^{-1}$ and follow-up formulae: 223 ± 220 $\mu g\ l^{-1}$) and lastly, formulae that include whey proteins (adapted starter formulae: 181 ± 177 $\mu g\ l^{-1}$) or casein (adapted starter formulae: 126 ± 38 $\mu g\ l^{-1}$) in its composition (Table 5).

The present tendency to reduce the protein content, and to replace different proteins with whey protein to be rather like the protein profile found in human milk, may determine the aluminium content in infant formula, which is advantageous in this respect, in the newer formulations.

Nevertheless, the complex manufacturing process of specialised formulae seems to play an important role in the degree of aluminium contamination. The highest aluminium value was determined in formulae designed for inborn errors of metabolism (443 ± 112 μg l^{-1}), an intermediate level was found for formulae without lactose (399 ± 451 μg l^{-1}) and finally, the lowest content was supplied for hypoallergenic formulae (294 ± 945 μg l^{-1}).

The probable source of aluminium contamination might be mainly a consequence of the formula ingredients added (calcium and phosphate salts, vitamins and other minerals) and in second place, the complex processing in which infant formulae come into contact with aluminium-containing surfaces and equipment for the long time that their preparation requires.

A large amount of aluminium in formulae designed for diverse pathologies is an additional source of aluminium for infants fed on these. Although this is unclear, it may represent a health hazard for this risk group of infants. Among the formulae without lactose, mainly casein-based formulae, one formula with a high aluminium level bring out (1439 μg l^{-1}). Similarly, there were two hypoallergenic formulae with aluminium values of 2649 and 2720 μg l^{-1}, both based on hydrolysed protein (casein hydrolysate) and from the same manufacturer (Figure 1).

[Insert figure 1 about here]

If table 5 is carefully observed, it is possible to see that aluminium content in casein hydrolysed hypoallergenic formulae (654 ± 1233 μg l^{-1}) is significantly higher ($p = 0,015$, Mann Whitney U test) than in whey hydrolysed hypoallergenic formulae (190 ± 110 μg l^{-1}). These contents are explained by the necessity for aggressive treatment in order to modify highly the raw material which means that formulae may be exposed to a significant amount of aluminium during manufacture from the chemicals used, machinery and dust particles.

Specialised formulae also contain greater quantities of substances such as calcium, iron, and citrate complexes that may enhance aluminium absorption from the gastrointestinal tract by unknown mechanisms (Sahin et al. 1995). Thus, it is reasonable to understand both the increased bioavailability of aluminium and the elevated plasma concentration noted in the infants fed on casein hydrolysed formulae (Hawkins et al. 1994). These may turn out to be recognised risk factors that must be taken into consideration.

Aluminium content in premature formulae (421 ± 137 μg l^{-1}) is significantly higher than that determined in formulae for infants at term (non adapted starter, $p = 0.042$ and adapted starter, $p = 0.010$). This tendency has been supported by many researchers who provide a similar range of aluminium concentrations (Ballabriga et al. 1994, Hawkins et al. 1994, Coni et al. 1993, Plessi et al. 1997). They try to explain these high levels as a consequence of the type of processing or food additives used which contain aluminium. Aluminium toxicity and the potential impact of these formulae on premature infants' health is one of the main concerns of international paediatric organisations (WHO 1989).

To date, preterm infant formulae are regarded as a potential source of aluminium exposure. The risk of aluminium accumulating in these formulae may be raised especially in preterm infants with renal immaturity linked to a low aluminium clearance due to decreased urine excretion (Sedman et al. 1984b). Moreover, the hypothesis that aluminium overload may be due to an increased intestinal absorption during the first weeks of neonate life has been reported. Both increased absorption and low excretion could explain the high plasma values observed in healthy premature and low birth weight newborns (Sedman et al. 1984b, Puntis et al. 1986, Stockhausen et al. 1990).

Aluminium absorption in infancy has been investigated in a previous study on antacids (Chedid et al. 1991), and the risk of toxicity as a result of exposure to drugs containing aluminium or aluminium contamination of infant formulae has been confirmed. In addition, adverse effects on bone mineralization associated with high plasma aluminium levels in infants have been reported (Bougle et al. 1997). Although direct inhibiting effects of aluminium on bone mineralization have not fully been explained, they seem to be a consequence of interactions with both calcium and phosphorus enhanced by the high aluminium content in premature formulae.

In spite of this, in view of our present knowledge and taking into account the nutritional benefits of premature formulae, it is necessary to keep up to date with these formulations and request from manufacturers the need for a total restructuring of older production processes in order to achieve a further reduction in the aluminium levels in this kind of infant formula.

Concentrations of aluminium in the soya formulae ranged from 313 – 3479 μg l^{-1}, median (573 ± 1132 μg l^{-1}). Undoubtedly, these formulae provide the highest aluminium levels of all studied formulae and as table 3 reveals, the aluminium content is significantly higher than in standard formulae (adapted formulae, $p = 0.001$; non adapted formulae, $p = 0.024$ and follow-up formulae, $p = 0.004$). High levels have also been reported by other

studies (Table 4), calling into question the nutritional safety of soya formulae for neonate feeding.

Aluminium is naturally present in soy beans, and aluminium impurities which are contained in other basic components of the soya formulae or caused by contamination during manufacture represent the most probable reasons for such high aluminium levels in soya formulae.

Regarding the manufacture of soya formulae, its preparation requires crushing, refining and washing procedures to isolate the soy protein from soya bean. A strong treatment with calcium hydroxide, which often contains high amount of aluminium as an impurity, is carried out.

It therefore seems likely that an important part of the final aluminium concentration in soya formulae may be attributed to adventitious contamination during the isolation more than the intrinsic aluminium content of soya beans.

Among the soya formulae, we detected one formula with a very high aluminium value (3479 $\mu g\ l^{-1}$) with respect to other infant formulae. This aluminium level is not surprising if we compare it with values determined in other national or international studies, where similar ranges are found or even several formulae have a higher aluminium concentration of anything up to 5076 $\mu g\ l^{-1}$ (Woollard et al. 1990).

In order to know the influence of the aggregation state on the amount of aluminium found in infant formula, we evaluated statistically the aluminium contents in both powder and ready-to-use liquid formulae.

The industrial production process of milk-based powder formulae involves several operations (warming, centrifugation, drying, homogenising, bleeding, sterilisation, packing, etc.), which bring it into contact it with metallic surfaces of industrial machinery.

In the case of ready-to-use formulae, processing is quite different. From the perspective of this study, the critical process for liquid formulae is the treatment in cationic interchange columns, besides the addition of vitamins or mineral salts.

Considering the 14 pairs of infant formulae with both commercially available formulae (powder and liquid forms), the aluminium content did not differ significantly ($p = 0.060$, Wilcoxon's test) between powder and liquid data groups, although the aluminium

content in ready-to-use formulae had a tendency to be lower than in powder formulae, as in recent studies (Ikem et al. 2002) and in contrast to earlier findings (Gruskin 1991).

Aluminium levels for both powder and liquid formulae over the different types of infant formulae are summarised in table 6. At first glance, the difference found in aluminium could be surprising but we must keep in mind the fact that liquid formulae started to be sold in Spain during the past decade, and were manufactured with a more novel and, in principle, purified process of handling and preparation, in contrast to older powder infant formulae.

[Insert table6 about here]

Manufacturer and determined values

In general, the manufacturing process has been described as an important source of exogenous aluminium contamination.

It is obvious that commercial brands do not include any value for aluminium in the information label. Nevertheless, it is to be expected that this metal should be included in the quality control program and manufacturers should try to obtain the lowest aluminium concentration in infant formula as far as possible.

In order to compare, figure 1 shows concentrations of aluminium determined in the different infant formula belonging to the nine manufacturers evaluated.

The meticulous care shown by manufacturers 7 and 2 (150 ± 90 μg l^{-1}, n = 5 y 142 ± 929 μg l^{-1}, n = 14, respectively) is curious, while manufacturers 3 and 6 (436 ± 67 μg l^{-1}, n = 7 y 416 ± 126 μg l^{-1}, n = 8) show quite the opposite with a large range of aluminium concentration (338 – 541 μg l^{-1} y 261 – 573 μg l^{-1}, respectively). It is also necessary to mention manufacturer 8 which includes numerous (n = 16) and complex formulations in its stock with a discrete aluminium contribution from prepared infant formulae.

When the different infant formulae were considered as a whole, median aluminium content was 340 μg l^{-1} (where percentiles 25 and 75 were 177 and 478 μg l^{-1}, respectively). However, seen separately median aluminium values of different commercial households ranged from 142 to 436 μg l^{-1}.

Considering both points, it would not be imprudent to recommend an upper limit to be set in infant formulae around 300 – 400 μg l^{-1}, which manufacturers could take on without excessive economic cost.

In view of these results and taking into consideration the program to reduce the level of aluminium in infant formula launched by the manufacturing industry in other European countries (Baxter et al. 1991), it seems suitable to call for an effort a) to control as far as possible the critical points of aluminium contamination, b) to modify the industrial handling process in order to obtain formulations in which the concentration of aluminium is less than 300 μg l^{-1} in premature and standard formulae, and 400 μg l^{-1} in the other formulae, a criterion which is a little more restrictive than the recommendation suggested by Simmer et al. (1990), and c) to analyse frequently or routinely the aluminium contents, especially in those formulae with potential impact such as premature or soya formulae.

Estimated dietary aluminium intake

The possibility that aluminium could cause health problems in infants justifies the comparison of estimated dietary intake provided by infant formulae studied with the Provisional Tolerable Weekly Intake (PTWI, 7 μg kg^{-1} of weigh) established by the Joint FAO/WHO Expert Committee on Food Additives (WHO 1989).

Considering that the newborn in each age period observes a similar feeding regimen, in comparison with the PTWI, we have worked out a weekly aluminium intake for infants fed on different types of infant formulae, observing separately the special case of preterm newborns. The daily intake of formulae is estimated according to recommended doses and the feeding tables specified by the manufacturers.

Table 7 shows the daily amount of aluminium supplied by different infant formulae.

[Insert table 7 about here]

Dietary aluminium intake estimated by Dabeka and Mckenzie (1990) through human milk was 2 μg day^{-1} and 3 μg day^{-1} for Canadian infants 0 – 1 and 1 – 3 months old, respectively. If we estimate a human milk reference value from published levels (3 – 79 μg l^{-1}), assuming a daily milk intake of 200 ml kg^{-1}, the range determined implies a daily

aluminium intake of 2.4 – 63.2 μg day^{-1} (0.6 – 15.8 μg kg^{-1} day^{-1}), slightly higher than previous estimated intakes.

The great difference observed in comparison with the aluminium supply from human milk is well known. This fact explains the special interest aroused in Paediatric organisations in reducing the aluminium content in artificial feeding for infants.

Figure 2 compares weekly the aluminium intake (percentage of the PTWI) estimated for each type of infant formulae studied. The differences between standard formulae and soya or specialised formulae observed before, are reflected clearly.

[Insert figure 2 about here]

Starter and follow-up formulae provide the lowest aluminium intake, about 4 % of the PTWI. Specialised formulae, with hypoallergenic formulae in the first place, yield an intermediate intake (11 – 12% of the PTWI). Finally, soya formulae contribute the highest intake (15 % of the PTWI).

Preterm intakes were calculated taking into account the paediatric specifications or manufacturers guidelines and infant weight. Figure 3 contains the estimated weekly aluminium intake by infants fed on preterm formulae.

[Insert figure 3 about here]

These formulae provide a toxic intake similar to special formulae, around 8 – 10 %. This PTWI percentage is far from that supplied by Spanish human milk (0.1 %) and the theoretical value found with the upper limit human milk reference (1.3 %). Once again, the need is highlighted to monitor aluminium contents in this kind of infant formula, because of the risk of developing aluminium toxicity, with the only objective of obtaining a maximum aluminium level similar to the upper limit for human milk. Further studies are therefore required to ensure the safety of formulae for preterm and low weight infants.

Finally, it is possible to evaluate the hypothetical case of an infant formula exceeding the aluminium limit to find the PTWI. This formula should contain an aluminium level ranging from 6000 – 8000 μg l^{-1}. None of the formulae analysed provides more than this interval of values.

If we consider the highest aluminium level determined in the infant formulae analysed (3479 μg l^{-1}, soya formula), daily aluminium intakes would be approximately 45 – 55 % of PTWI.

In spite of everything, it is necessary to be prudent when establishing permissible aluminium intakes, since the absolute calculations must be taken as an approximate estimate, although they are certainly useful to evaluate and make a conservative recommendation about aluminium content in infant formulae.

Complementary contribution to daily aluminium intake from drinking water

Aluminium concentration in natural waters varies significantly depending on numerous physicochemical, mineralogical and geochemical factors.

Water treatment in purifying plants includes a coagulation process using aluminium sulphate to remove organic matter. The beneficial effect of the use of this metal in treated water is recognised. Nevertheless, in spite of good operating conditions, a residual amount is retained and supplied in drinking water. European Community legislation (Directive 80/778/EEC) and WHO guidelines have established an acceptable aluminium concentration of 200 $\mu g\ l^{-1}$ in drinking water (Commission of the European Communities 1980, WHO 1998).

This authorised aluminium level is similar to that found in standard infant formula. As a consequence, it is interesting to evaluate aluminium exposure from tap water and its relative contribution to dietary intake of infants fed on reconstituted powder formulae.

In accordance with the legislation currently in force, taking into consideration the marked daily intake of newborns, tap water used in prepared formulae could supply similar aluminium amounts to those provided by infant formulae on their own.

Aluminium content determined is $22.6 \pm 4.5\ \mu g\ l^{-1}$ (range $4.5 – 172.4\ \mu g\ l^{-1}$). There is no significant difference between aluminium levels found in rural ($24.2 \pm 9.3\ \mu g\ l^{-1}$) and urban groups ($22.3 \pm 2.2\ \mu g\ l^{-1}$). The mean aluminium value was used to establish the influence of aluminium content in drinking water on the final concentration of reconstituted infant formulae.

The amount provided by tap water used in reconstitution of premature formulae is similar to the lower limit found in human milk, approximately 0.05 – 0.06 % of the ISTP. At the same time, aluminium supply to another infant formulae, is also low, about 10 % of standard formulae and 2 – 3 % of special and soya formulae (table 7).

Our results indicate that aluminium exposure from tap water used for formula preparation is not a clear potential risk. Although it is necessary to know that aluminium concentration in drinking water can vary significantly depending on the geographic and geochemical medium where the water supply or spring are sited.

Interactions between aluminium and other trace elements

The levels of trace elements in infant formulae are generally higher than in human milk. In recent years, considerable changes in the levels of these elements in infant formulae have been instituted in the light of improved knowledge of infant requirements.

Trace elements normally added to the raw material (cow milk or soy) are supplied in an inorganic form, sometimes in a high level to compensate for the lower bioavailability of infant formulae (Brätter 1996). There is little information on the impact of these changes and added elements on the final concentration of potential toxic trace elements, including aluminium.

In order to observe the tendency in the levels of certain essential trace elements in relation to the aluminium level in infant formulae, since some toxic metals and nutritionally essential elements share common chemical characteristics, a comparative statistical analysis using the Spearman coefficient between the aluminium and different trace element contents was carried out.

Aluminium behaves similarly to iron in many biological systems (Goyer 1997). As can be observed in figure 4, an inverse correlation was established for follow-up formulae between both concentration values ($p = 0.044$). Cow milk is a very poor source of iron. 14 % of iron occurs in milk fat, about 24 % is bound to casein, while 29 % is bound to whey protein and 32 % is associated with a low molecular weight fraction (Fransson and Lonnerdal 1983). The studied follow-up formulae are mainly based on skim and whole cow milk. This interaction could be attributed to the lower amount of iron added to whole-milk-based formulae, which is linked to a higher aluminium content as was discussed above. On the other hand, for those follow-up formulae based on skim-milk, the iron supplementation to find the requirement is most important but the manufacturing process is simpler.

[Insert figure 4 about here]

Furthermore, the distribution of iron added to infant formula depends on the chemical form of iron supplement used. Supplementation with Fe (II) results in a formula with high iron content in the fat fraction (Hegenauer et al. 1979) whereas if Fe(III) is used, the iron is bound to casein micelles (Demott and Dincer, 1976). Probably, the existing correlation in whole-milk-based formulae ($p = 0.010$) could correspond with the different chemical form used in iron supplementation and the technological process used by manufacturers. The same consideration might be established for formulae designed for inborn errors of metabolism ($p = 0.013$), although the complexity in formulations included in these groups does not allow a clear explanation.

Another negative correlation ($p = 0.004$) was found between zinc and aluminium concentrations in standard infant formulae (figure 5). This corresponds to changes in protein profile by manufacturers, since as in cow milk most of the zinc is in the skim milk fraction and 95 % is associated with casein micelles (Blakeborough et al. 1983, Sing et al. 1989). Lower aluminium and higher zinc concentration were found in casein-based formulae, followed by intermediate values of both elements in whey and skim-milk-based formulae and finally, whole-milk-based formulae provide the highest aluminium and lowest zinc added concentrations. This same tendency is observed for starter and follow-up formulae by themselves with a statistical significance correlation $p = 0.004$ and $p = 0.030$, respectively.

[Insert figure 5 about here]

Finally, a positive correlation was established in hypoallergenic formulae between manganese and aluminium contents ($p = 0.006$). Manganese is distributed in cow milk, 67 % binding to casein, 18 % to low molecular weight proteins and 1 % to lipid fraction (Lonnerdal et al. 1985). This fits with both higher aluminium and manganese contents found in hydrolysed-casein-based formulae and lower values of these elements in hydrolysed-whey-based formulae.

Conclusions

Commercial infant formulae should provide an efficient alternative to human milk, providing a nutritional source that is safe and adequate to neonate needs when the human milk supply is not possible or is insufficient.

In this regard, human milk is still the reference for micronutrients and non-essential trace element contents, given that these data may not be obtained from healthy neonates for ethical reasons.

According to our results, the infant formulae studied provide an aluminium level that is higher than that found in human milk, especially those formulae of high technological complexity (premature, specialised and soya formulae).

International paediatric organisations (AAP 1996) request from manufacturers continued efforts to reduce the aluminium level in infant formulae, bearing in mind mainly soya and preterm formulae. In this sense, specialised formulae based on hydrolysed protein like casein must be incorporated in the risk formulae group, due to their high aluminium content.

High aluminium infant formulae should be evaluated by manufacturers, who should routinely monitor aluminium concentrations, especially in those formulations prepared to feed premature or low birth neonates.

In accordance with the present state of knowledge about aluminium toxicity, it is appropiate to set a maximum guide value for infant formula at 300 $\mu g\ l^{-1}$. Firstly, this level is possible for manufacturers, and in second place, a recent study (Hawkins et al. 1994) indicated that most infants who consumed infant formulae containing more than this limit, had a raised plasma aluminium concentration and so may be at risk of aluminium toxicity.

For these reasons, the safe and adequate aluminium level in infant formulae requires further study. Speciation studies are required in order to establish and characterise the chemical forms in which aluminium is present in human milk and infant formulae (Brätter et al. 1998), with the goal of determining the real toxicity of aluminium compounds and evaluating the true risk of feeding neonates on infant formulae.

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Table 1. Instrumental parameters and optimising furnace program for aluminium determination.

Instrumental parameters					
Wavelength *(nm)*	309.3				
Slit width *(nm)*	1.0				
Lamp current *(mA)*	10				
Sample volume *(μL)*	20				
Measurement Mode	Peak area				
Source	Hollow cathode lamp				
Background correction	Deuterium lamp				
Temperature program					
Step	***Temperature*** *(°C)*	***Ramp*** *(s)*	***Hold*** *(s)*	***Argon flow*** *(ml min^{-1})*	***Read on***
Drying 1	110	5	15	300	-
Drying 2	250	10	15	300	-
Charring	1500	20	5	300	-
Atomization	2500	1	4	300	-
Cleaning	2600	1	5	0	Yes
Cooling	20	5	3	300	-

Table 2. Results of quality control program for aluminium analysis in infant formulae.

Quality program	*n*	*Mean ± SD* ($\mu g\ l^{-1}$)	*Repeatability* (%)	*Reproducibility* (%)
Blank reagents				
Infant formulae	34	2.3 ± 0.3	13.9	15.5
Drinking water	8	0.2 ± 0.5	-	-
Internal Standard*	34	20.0 ± 1.1	1.6	2.6
Infant formula Control[#]	34	174.8 ± 4.2	2.4	2.7
Recovery assay *(%)*				
Infant formula Control	34	96 –104		
Detection limit ($\mu g\ l^{-1}$)[¶]				
Infant formulae	34	3.3		
Drinking water	8	1.7		
IAEA 155 Whey powder ($mg\ kg^{-1}$)				
Determined level	12	43.2 ± 2.6		
Information level		53 (38-68)		

* Aqueous internal standard: 20 µg l^{-1}

[#] Infant formula control: 176 ± 7 (analysed by standard addition method)

[¶] Calculated as $M_b \pm 3$ s.d.$_b$ (M_b: blank mean, s.d.$_b$: standard deviation of blank)

Table 3. Aluminium content in different types of studied infant formulae (μg l^{-1}).

Infant formula	*n*	*Mean*	*Median ± SD*	*Range*
Preterm Formula	7	449	421 ± 137	317 – 726
Starter Formula				
- Non adapted	4	237	231 ± 128	118 – 368
- Adapted	16	252	196 ± 152	68 – 573
Follow-up Formula	19	292	272 ± 189	66 – 788
Specialised Formula				
- Without lactose Formula	7	574	399 ± 451	102 – 1439
- Hypoallergenic Formula	12	687	294 ± 945	105 – 2720
- Inborn errors diet	10	453	443 ± 112	307 – 655
Soya Formula	7	930	573 ± 1132	313 – 3479

Table 4. Aluminium content in infant formulae from different countries.

Reference	*Country*	*Aluminum ($\mu g\ l^{-1}$)*				*Formula*
		n	*Mean*	*Median*	*Range*	
Bloodworth et al., 1991. (19)	Singapore	25			40 - 370	Cow's milk-based
		4			470 - 2500	Soy-based
Simmer et al., 1990 (11)	Australia	17			72 - 1463	Starter and follow-up
		4			184 - 1106	Preterm
		7			62 - 939	Specialized
		4			1192 - 1711	Soy-based
Ballabriga et al., 1989 (14)	Spain	79			202 ± 111*	Adapted
		16			322 ± 164*	Preterm
		26			530 ± 412*	Hypoallergenic
		13			542 ±178*	Without lactose
		10			805 ± 217*	Soy-based
Fdez-Lorenzo et al., 1999 (15)	Spain	47	218.6	160	8 - 1149	Starter
		17	245.8	130	18 - 1129	Follow-up
Biego et al., 1991 (20)	France	6			68 ± 19	-
Baxter et al., 1991 (10)	UK	14			40 - 200	Cow's milk-based
		7			640 - 1340	Soy-based
Hawkins et al., 1994 (21)	UK	24	165		151 - 180	Starter
		7	300		272 - 328	Preterm
		14	161		143 - 180	Fortified
		7	773		632 - 914	Hypoallergenic
		7	534		470 - 598	Soy-based
Coni et al., 1993 (22)	Italy	12			50 - 260	Starter
		4			80 - 410	Preterm
		5			110 - 600	Specialized
		5			390 - 1010	Soy-based
		8			30 - 850	Ready-to-use
Plesi et al., 1997 (23)	Italy	1			450 ± 117*	Starter
		1			479 ± 93*	Follow-up
		1			463 ± 180*	Preterm
		1			2285 ± 232*	Hypoallergenic
		1			1791 ± 275*	Hypoallergenic
		1			1057 ±51*	Soy-based
		1			913 ± 171*	Soy-based
Sahin et al., 1995 (24)	Turkey	10			163 - 1475*	-
Ikem et al., 2001 (25)	Nigeria	6			58 ± 22	Adapted (powder)
	UK	12			92 ± 85	Adapted (powder)
		18			101 ± 37	Adapted (liquid)
	USA	9			150 ± 120	Adapted (powder)
		6			460 ± 160	Soy-based (powder)

Koo et al., 1988 (9)	USA	-			0.014 - 0.453**	Cow's milk-based (liquid)
					0.60 - 2.3**	Soy-based (liquid)
					15 - 108*	Cow's milk-based (powder)
					342 - 1377*	Soy-based (powder)
Dabeka and Mckenzie, 1990 (26)	Canada	6	0.13	0.091	0.010 - 0.36**	Cow's milk-based (liquid)
		4	1.98	0.84	0.40-6.4**	Soy-based (liquid)
		9	97	68	26-336*	Cow's milk-based (powder)
		7	1274	1126	425-2430*	Soy-based (powder)
		9	0.22	0.18	0.017-0.56**	Cow's milk-based (conc.)
		4	1.41	1.21	0.59-2.29**	Soy-based (concentrated)
Woolard et al., 1991 (27)	Various countries	307	189		22-519*	Cow's milk-based
		55	2484		1404-5076*	Soy-based

*Recalculated aluminium concentration (according to dilution 13.5 %) and expressed in μg l^{-1}

** Expressed by authors "as sold" basis in μg g^{-1}

Table 5. Aluminium concentrations in different types of infant formulae with regard to the main protein contained (µg l^{-1}).

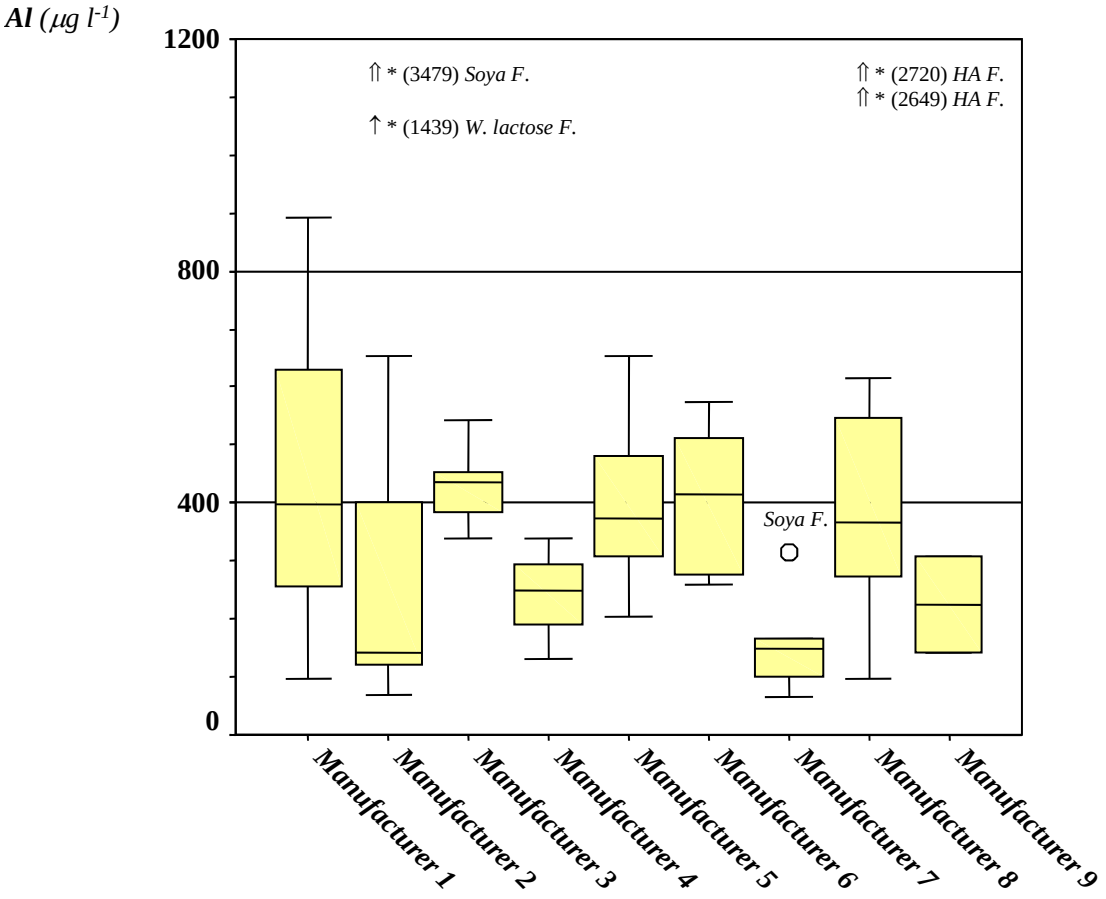
Infant formula	*Aluminium*		
	n	***Median ± SD***	***Range***
Preterm Formula			
Whey-based	4	402 ± 43	339 – 421
Skim-milk-based	1	726	–
Whole-milk-based	1	436	–
Casein hydrolysed	1	317	–
Starter Formula			
- Non adapted			
Skim-milk-based	3	136 ± 115	118 – 325
Whole-milk-based	1	368	–
- Adapted			
Whey-based	6	181 ± 177	158 – 573
Casein-based	3	126 ± 38	68 – 139
Skim-milk-based	2	190 ± 83	132 – 249
Whole-milk-based	5	338 ± 114	261 – 541
Follow-up Formula			
Whey-based	1	334	–
Skim-milk-based	13	223 ± 220	66 – 788
Whole-milk-based	5	296 ± 185	121 – 459
Specialised Formula			
- Without lactose Formula			
Casein-based	6	460 ± 439	309 – 1439
Skim-milk-based	1	102	–
- Hypoallergenic Formula			
Whey hydrolysed	7	190 ± 110	105 – 412
Casein hydrolysed	5	654 ± 1233	298 – 2720
- Inborn errors diet			
Casein-based	1	307	–
Skim-milk-based	1	518	–
Whey hydrolysed	1	655	–
Casein hydrolysed	4	449 ± 98	335 – 529
Free aminoacids	2	401 ± 51	364 – 438
No protein	1	370	-
Soya Formula			
Soy-based	7	573 ± 1132	313 – 3479

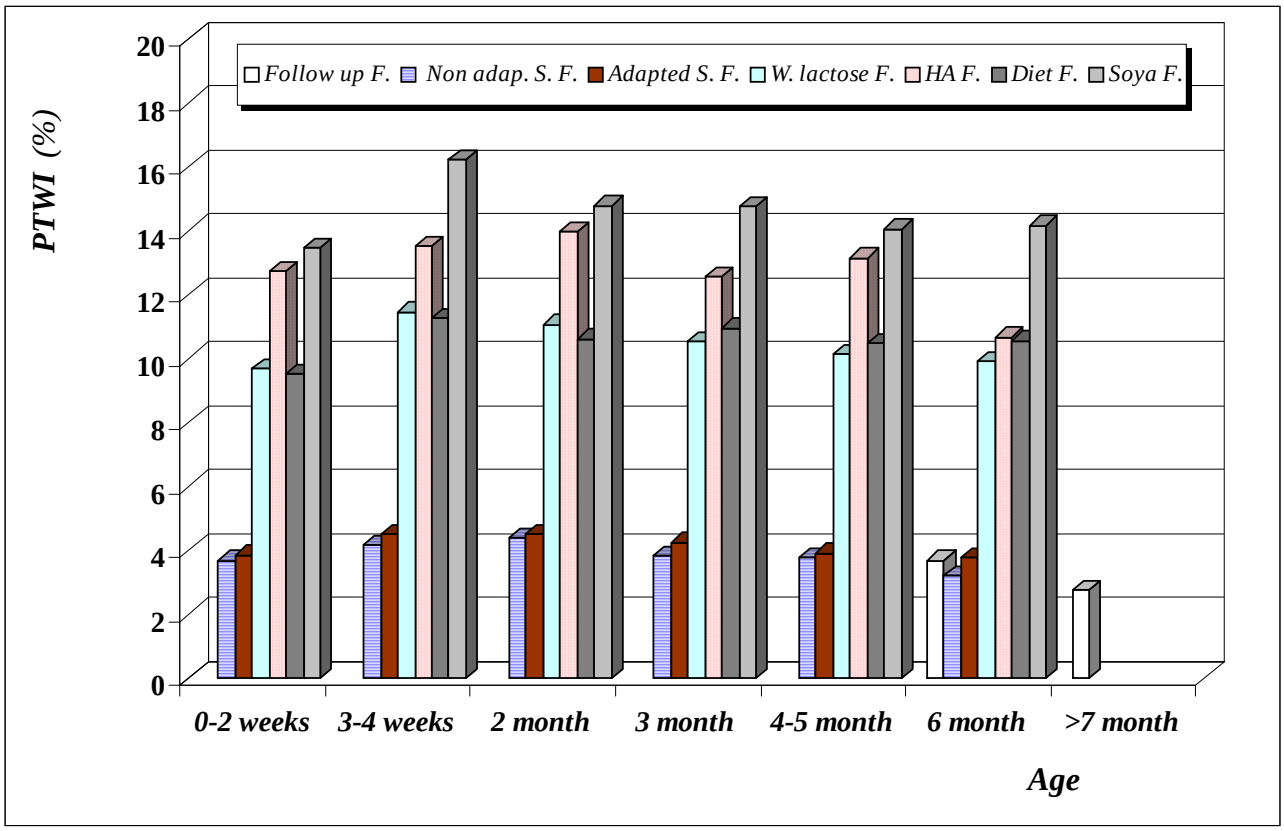
Table 6. Aluminium levels (μg l^{-1}) from different types of infant formulae studied attending to aggregation state (powder or liquid formulae).

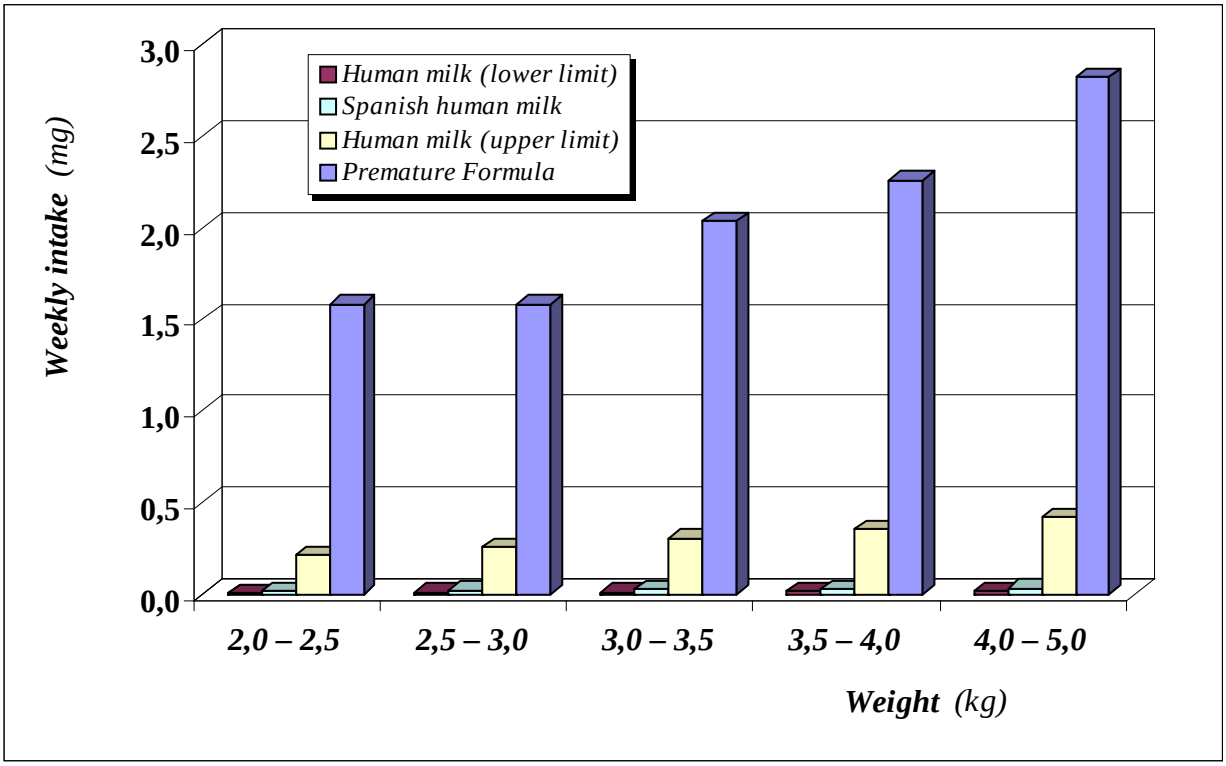
Formula	***Powder***				***Ready-to-use***			
	n	***Mean***	***Median***	***Range***	***n***	***Mean***	***Median***	***Range***
Preterm Formula	6	467	428 ± 20	317 – 726	1	340	–	–
Starter Formula								
Non adapted	3	271	325 ± 18	118 – 368	1	140	–	–
Adapted	12	273	215 ± 26	130 – 573	4	187	172 ± 14	68 – 340
Follow-up Formula	13	313	340 ± 46	66 – 788	6	245	223 ± 14	150 – 460
Specialised Formula								
Hypoallergenic	10	784	338 ± 1014	120 – 2720	2	201	201 ± 19	105 – 300

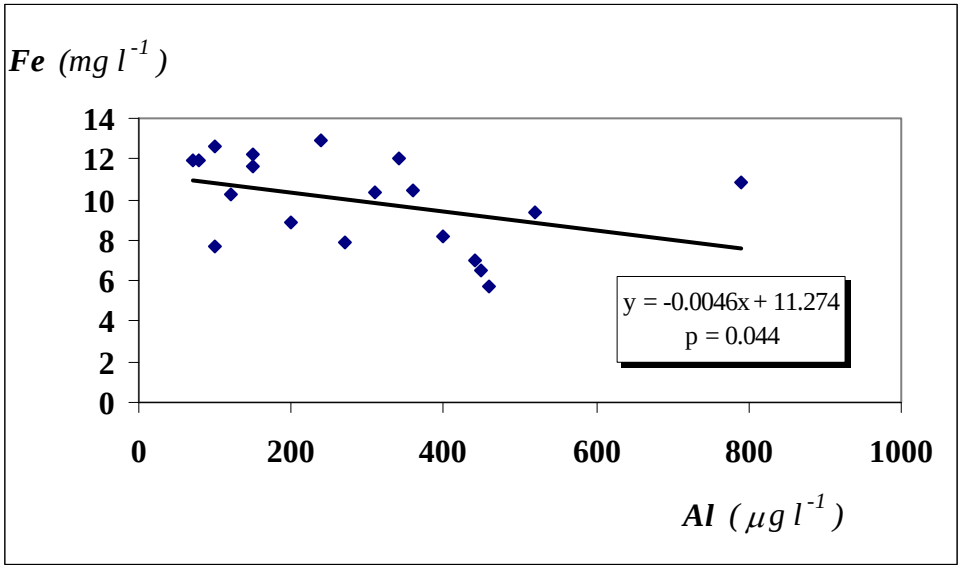
Table 7. Daily intakes of aluminium for infants fed on infant formulae and drinking water used in the reconstitution of powder formulae (µg day^{-1}).

Age	*Starter Formula*		*Follow-up Formula*	*Specialised Formula*			*Soya Formula*	*Drinking water*
	Non adapted	*Adapted*		*Without lactose*	*HA*	*Inborn errors diet*		
0 - 2 weeks	148	155	-	387	510	381	538	12.2
3 - 4 weeks	188	203	-	516	608	508	731	16.2
2 month	238	244	-	597	754	574	798	20.3
3 month	238	264	-	654	778	680	915	20.3
4 - 5 month	283	295	-	761	986	785	1055	23.7
6 month	257	302	296	793	854	846	1133	14.2
> 7 month	-	-	249	-	-	-	-	10.8


Al (μg l-1)
1200
800
400
0
⇑ * (3479) Soya F.
↑ * (1439) W. lactose F.
⇑ * (2720) HA F.
⇑ * (2649) HA F.
Soya F.
Manufacturer 1
Manufacturer 2
Manufacturer 3
Manufacturer 4
Manufacturer 5
Manufacturer 6
Manufacturer 7
Manufacturer 8
Manufacturer 9


PTWI (%)
20
18
16
14
12
10
8
6
4
2
0
Follow up F.
Non adap. S. F.
Adapted S. F.
W. lactose F.
HA F.
Diet F.
Soya F.
0-2 weeks
3-4 weeks
2 month
3 month
4-5 month
6 month
>7 month
Age


Weekly intake (mg)
3,0
2,5
2,0
1,5
1,0
0,5
0,0
Human milk (lower limit)
Spanish human milk
Human milk (upper limit)
Premature Formula
2,0 – 2,5
2,5 – 3,0
3,0 – 3,5
3,5 – 4,0
4,0 – 5,0
Weight (kg)


Fe (mg l-1)
14
12
10
8
6
4
2
0
0
200
400
600
800
1000
y = -0.0046x + 11.274
p = 0.044
Al (µg l-1)

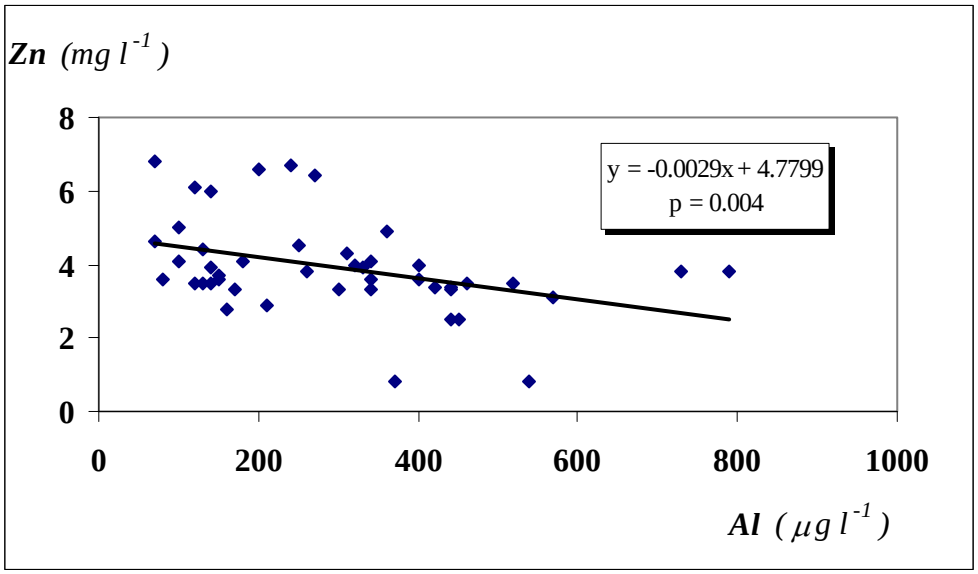

Zn (mg l-1)
8
6
4
2
0
0
200
400
600
800
1000
y = -0.0029x + 4.7799
p = 0.004
Al (µg l-1)

Figure 1. Aluminium distributions in infant formulae provided by different manufacturers (µg l^{-1})

Figure 2. Percentages of PTWI for aluminium estimated from infant formulae.

Figure 3. Weekly dietary aluminium intake for infant fed on premature infant formulae and human milk (mg $week^{-1}$).

Figure 4. Iron versus aluminium in follow-up infant formulae.

Figure 5. Zinc versus aluminium in standard infant formulae (starter and follow-up formulae).

Acknowledgements

Authors wish to thank to Gobierno de Navarra for financial support.