Food Chemistry 37 (1990) 81–94

Aluminium Concentrations in Infant Formulae

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(Received 17 April 1989; revised version received and accepted 20 September 1989)

ABSTRACT

Increased loading of aluminium in infants, particularly neonates, is known to be potentially toxic because of their renal immaturity. Any exposure to aluminium from milk replacement formulae may represent a risk. There have been few extensive studies done on the aluminium content of these products and therefore 307 samples from 14 countries were analysed for aluminium by flameless atomic absorption. On a global basis the mean aluminium concentration was found to be 1·40 mg/kg with a 95% interval of 0·17–3·84 mg/kg. In the New Zealand and Australian products, the mean and range of the aluminium levels in infant formulae are statistically the same as in standard whole milk powders despite their more sophisticated processing and their storage in aluminium cans. The aluminium concentration of 55 soya-based (dairy free) milk substitutes from seven countries is much higher, global mean = 18·4 mg/kg, 95% interval 10·4–37·6 mg/kg. Vegetable oil and vitamin/mineral additives were shown to contribute insignificantly to the overall aluminium content of these products.

INTRODUCTION

The toxic and nutritional roles of trace metals have been firmly established in their contribution towards human health and disease. Aluminium was not initially considered important in this regard. The earliest attention paid to this metal concerned industrial exposure to aluminium dust but since then

Food Chemistry 0308-8146/90/$03·50

the biological importance of aluminium has assumed a much greater profile with its implication in a number of pathological conditions (Alfrey, 1984; Leone, 1985; Anon., 1987; Starkey, 1987).

Following the recognition of aluminium as the causative agent of osteopenia and encephalopathy in renal dialysis patients (Wills & Savory, 1982) interest naturally shifted towards the toxicological consequence of exposure in other groups. Logically, accumulation of aluminium can occur either by an increased loading or by a decreased clearance. Aluminium is predominantly excreted by the kidneys and so the risk of toxicity is greatest in renal impairment. Neonates have immature renal function and any increase in the aluminium loading of these infants poses a substantial risk. The most influential studies to date have been the demonstration of aluminium intoxication due to exposure to phosphate binders (Andreoli *et al.*, 1984), intravenous fluids (Sedman *et al.*, 1985) and infant formulae (Freundlich *et al.*, 1985; Fisher *et al.*, 1989). As far as infant nutrition is concerned, the potential for aluminium toxicity in neonates who may depend on infant formulations as their major nutritional source is now reaching the popular medical press. There have been studies carried out to identify potential sources of dietary aluminium in a range of food products (Greger, 1985).

Some surveys on trace metals in dairy products have been comprehensive but have omitted aluminium (Lopez *et al.*, 1985; Mingorance & Lancia, 1985). The same is true for some infant formulae (Cortes *et al.*, 1982; Collip *et al.*, 1983). Some data are available for infant formulae (Freundlich *et al.*, 1985; McGraw *et al.*, 1986) but these have not been very extensive. It is the latter notion that prompted the present study in which a large number of infant formulae were tested in order to determine their aluminium contents.

MATERIALS AND METHODS

All samples were analysed by atomic absorption spectroscopy using electrothermal atomisation. Flame atomisation is frequently not sensitive enough for the levels being measured (detection limit approximately 0·5 mg/kg) and is subject to contamination from the glassware used in the digestion procedures. Aluminium is present in dust and hence the laboratories used in the present study were kept under positive pressure with filtered air.

Sample collection

All infant formulae were canned products obtained randomly throughout a 3-year period from retail outlets. These consumer packs were mostly

believed to be nitrogen flushed. Spray-dried wholemilk powders were obtained directly from factory sources. The samples were shaken with repeated inversion immediately prior to analysis to ensure even distribution of aluminium before testing. Samples were taken under a hood with positive pressure using plastic utensils, thus avoiding all contact with dust or any metallic objects.

Sample preparation

Whole milk powder (0·5 g) infant formulae, or vitamin premix samples were prepared for analysis by suspension in 5 ml water inside 30 ml polypropylene vials. Vegetable oils were dissolved in hexane. These solutions were then ready for injection into the atomic absorption spectrophotometer. The vials and distilled water were free from any traces of aluminium.

Instrumentation

The atomic absorption measurements were carried out at four different locations in New Zealand and Australia. The instruments used were as follows.

At Auckland, two atomic absorption spectrophotometers were used. Initially:

(1) Varian AA775 atomic absorption spectrophotometer (Varian Techtron Pty Ltd, Mulgraves, Victoria, Australia) with an ASD-53 autosampler and a CRA-90 carbon rod atomiser.

Subsequently

(2) Varian SpectrAA 20 atomic absorption spectrophotometer with a GTA-96 graphite thermal analyser. Automated deuterium background correction, standard curve analysis and sample preparation are part of this instrument.

In Australia, the following atomic absorption spectrophotometers were used at three locations.

(3) Varian SpectrAA 30 atomic absorption spectrophotometer with Zeeman background correction and a Furma DS-15 data station.

(4) Perkin–Elmer 2100 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, CT) with a HGA 700 graphite furnace and an AS-70 autosampler.

(5) Perkin–Elmer 503 atomic absorption spectrophotometer with a HGA 500 graphite furnace and an AS-40 autosampler.

The Varian SpectrAA 20 instrument in Auckland was the principal instrument used and so its parameters and settings are described to illustrate the important features of these assays.

The dissolved samples were injected automatically with surfactant into a pyrocoated graphite tube. Nitrogen (3 litre/min) was used as the purge gas through the drying cycles, changing to argon during ashing and atomisation. This avoided the poorer sensitivity given with nitrogen gas due to the formation of aluminium nitrides whilst minimising the excessive consumption of expensive argon gas. The detection wavelength used was 309·3 nm, slit 0·5 nm (lamp current 6 mA). The temperatures and times used for drying, ashing and atomisation are shown in Table 1 and are chosen so as to provide for smooth liquid evaporation without splattering, for efficient ashing and for maximum peak signal.

TABLE 1
Temperatures and Times for Drying, Ashing and Atomisation

Cycle	*Temperature* (°C)	*Time* (s)
Drying (1st stage)	95	1·0
Drying (2nd stage)	105	14·0
Drying (3rd stage)	275	4·0
Ashing (1st stage)	500	5·0
Ashing (2nd stage)	1 300	6·5
Atomisation	2 500	1·7 (read last 1·0 s)
Burn out	2 500	1.0 (gas flow zero)
Cooldown	40	12·3

The atomisation temperature was kept above 2300°C to facilitate the vaporisation of refractory aluminium phosphate. As the graphite tube aged, minor adjustments to the temperature ramps were made. The multiple drying stages were designed to reduce spattering by first evaporating the water at about 100°C and then removing the fat at 275°C. The ashing stages, first at 500°C and then subsequently at 1300°C were effective in reducing the background to a very low level even though deuterium correction was still applied.

The parameters on all the other atomic absorption spectrophotometers were similarly optimised to suit the particular instrument.

Calibration

Calibration was by standard additions with recalibration performed on every sample to remove matrix effects. The stock calibration standard was Spectrosol aluminium chloride (BDH Chemicals, Poole, UK) at a concentration of 1000 mg/litre. The working standard after two stage dilution with 1% nitric acid was 100 μg/litre. 0 μl (for blank), 2 μl or 4 μl

volumes of this solution were injected along with the sample (4 μl), 0·2% Triton X-100 wetting agent (4 μl) and 1% nitric acid to a constant total volume of 12 μl were used to create the calibration graph. The regression line was automatically zeroed on the blank. The low level of aluminium in the blank was subtracted from all readings. All solutions were kept in covered carousels throughout the analyses to prevent any contamination.

Sensitivity, precision and linearity

With the temperature programme as stated, the detection limit for aluminium was 0·001 mg/litre (2 × standard deviation at zero concentration), using peak height measurement over 1·0 s and deuterium background correction. All sample measurements were performed in triplicate and the precision (within-batch coefficient of variation) was normally within 3%. The method was linear over the calibration range. Any departure from linearity would have been apparent from the calibration graph.

The coefficient of variation of the other instruments used in the survey ranged between 3 and 8%. To guard against instrument bias, a common sample was assayed with each batch at each analytical station. The sample used was the standard reference material SRM 1549 (NBS). The measured aluminium levels were 1·51 ± 0·12 mg/kg (mean ± 2 SD).

RESULTS

A preliminary experiment was to test the aluminium content of ten raw milk samples taken prior to pasteurisation or any processing from bulk milk supplies obtained from herds grazed in three geographically distinct New Zealand farming areas. The soils in these areas are predominantly clay-based. They showed a low mean aluminium concentration of 0·0094 mg/litre with a total range of values from 0·004 to 0·015 mg/litre.

Figure 1 illustrates the aluminium levels measured in a selection of 64 spray-dried whole milk powders (25–30% fat) collected from a range of New Zealand and Australian factories. Analysis of the data shows that the distribution of results is skewed (Shapiro–Wilk statistic, 0·918) with a mean of 1·048 mg/kg and a 95% interval of 0·082–3·103 mg/kg (obtained from square root transformed data).

Figure 2 depicts the aluminium content for 307 samples of infant formulae obtained from manufacturing plants in fourteen countries. The scatter diagram shows the untransformed mean of each set and gives an impression of the range of aluminium levels on national bases. Efforts were made to obtain samples spread evenly between the individual countries but

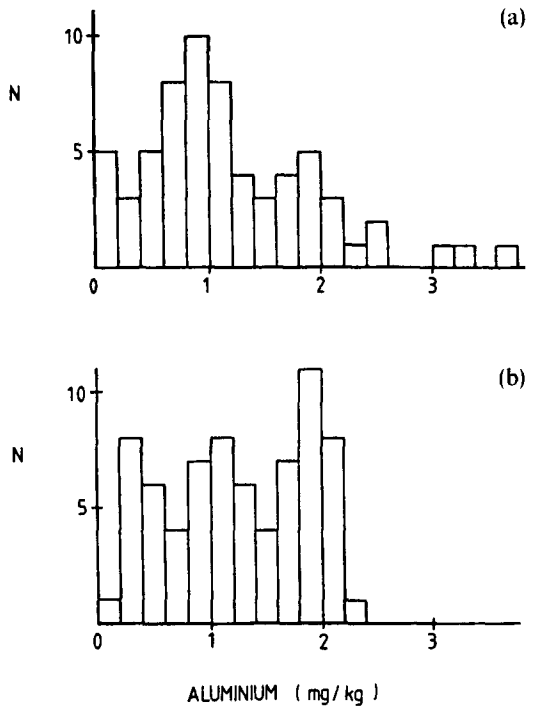

Fig. 1. Comparison of aluminium levels in whole milk powder and infant formulae from Australia and New Zealand. (a) Whole milk powders, (b) infant formulae.

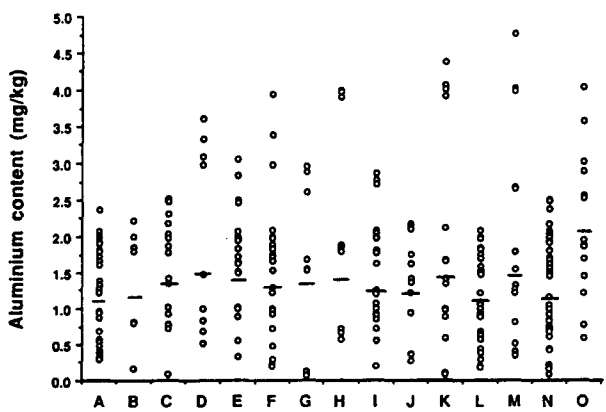

Fig. 2. Aluminium content of canned milk-based infant formulae from different countries. (A) Australia, (B) Brazil, (C) Canada, (D) China, (E) Denmark, (F) England, (G) Finland, (H) France, (I) Holland, (J) Ireland, (K) Japan, (L) New Zealand, (M) Malaysia, (N) USA, (O) Others (includes Chile, Italy, Korea, Mexico, Peru, Taiwan and South Africa—samples were purchased from the named countries but the origin of the contents is uncertain). Means are indicated by (—).

it was not possible to obtain a wide range of samples from some of them. Collectively they give an indication of the aluminium content to be expected in dried infant formulations on a global basis. When the data in Fig. 2 are grouped, a skewed distribution is again evident (Shapiro–Wilk statistic = 0·952) and analysis of the transformed data shows a global mean of 1·40 mg/kg and a 95% interval of 0·165–3·843 mg/kg. It was not a primary intention to over-emphasise differences between each country but a one-way analysis of variance on the ranked data gave a Kruskal Wallis statistic, $H = 16{\cdot}7$ ($p = <0{\cdot}3$). This shows that there is no statistical difference between the median aluminium levels of the various countries.

The data from the New Zealand and Australian 71 milk based infant formulae were collected and compared with the data from the 64 whole milk powders from the same two countries (from Fig. 1). This was done to see if there was any difference in aluminium between dried whole milk and infant formulae. The former are sophisticated blended products made under substantially different conditions from the normal spray drying process. The medians of these two sets of products are 1·27 mg/kg and 1·00 mg/kg, respectively. The two-tailed Mann–Whitney U-test showed there to be no significant statistical difference, $p = <0{\cdot}2$, indicating that during the more extensive processing of infant formulations no appreciable aluminium adulteration is taking place compared with spray-drying. It is curious that our data for New Zealand and Australian infant foods were square waved rather than Gaussian. This gave the impression that there was an 'upper limit' to the aluminium content of these products. It is not known if this is artefactual or is due to a real processing phenomenon.

Figure 3 shows the aluminium content of 55 soya-based infant formulae

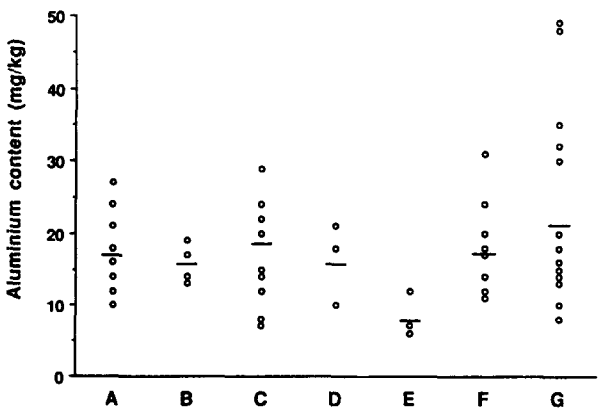

Fig. 3. Aluminium content of soya-based (milk free) infant formulae from different countries. (A) Australia, (B) Denmark, (C) England, (D) France, (E) Holland, (F) New Zealand, (G) United States. Means are indicated by (—).

TABLE 2
Aluminium Content of Additives Used in the Manufacture of Infant Formulae

	N	*Average aluminium concentration (mg/kg)*
Vegetable oils		
sunflower oil	4	0·015
soya bean oil	3	0·017
hydrogenated soya oil	3	0·014
coconut oil	5	0·036
palm oil	5	0·498
butter oil	5	0·029
Vitamin premixes		
fat solubles	4	4·5
water solubles	4	0·045
Mineral premixes	4	1·10

from seven different countries. They contain much higher quantities of aluminium than the milk-based products. On a global basis, the distribution of levels was again skewed (Shapiro–Wilk statistic = 0·877) and the calculated mean and 95% interval from the transformed data were 18·4 mg/kg and 10·4–37·6 mg/kg respectively. The countries were again compared and the Kruskal Wallis statistic, $H = 6{\cdot}86$, indicated no significant difference ($p = < 0{\cdot}5$).

The aluminium concentrations of several oil, vitamin and mineral additives used in the production of infant formulae were tested. The results are summarised in Table 2.

DISCUSSION

Analysis

The analysis of aluminium has received recent attention using many recent techniques such as voltammetry (Van den Berg *et al.*, 1986), fluorimetry (De Pablos *et al.*, 1986), flow injection analysis (Royset, 1987), spectrophotometry (Ohzeki *et al.*, 1988) and HPLC (Haddad & Valeenuwat, 1986; Bond & Nagaosa, 1985). Nevertheless, atomic absorption remains the method of choice particularly with difficult sample materials.

Particular attention was paid in the current study to achieve accurate methodology because of the risks of poor performance of aluminium

analysis at trace level (Parr, 1985). This was assisted by avoiding wet (Bouman *et al.*, 1986) or dry ashing (Sullivan *et al.*, 1987) techniques which are subject to contamination from glassware and reagents. An in-depth study on the various benefits of dispersion techniques for copper in milk powders has been made by Khammas *et al.* (1985) who conclude it presents the best choice of sample preparation for electrothermal atomic absorption. Direct sample dispersion is also used by Mingorance and Lachia (1985) for liquid milk and by De la Guardia *et al.* (1986) for powdered milk using conventional flame atomic absorption and emission techniques. There was no evidence in this study that insoluble forms of aluminium were lost by sedimentation during the analysis. If such material existed in the samples it was maintained in the colloidal environment created by the dissolved powder and by the surfactant (Triton). Acidification of the samples also discouraged aluminium oxides and hydroxides from forming. Conversely, the avoidance of high temperatures during sample preparation discouraged the loss of volatile salts of aluminium.

The analytical performance of the aluminium assays were verified by the normal parameters of reproducibility, repeatability and linearity. Suppression of aluminium ionisation is unnecessary during the assays due to its high ionisation temperature. However, close attention to maximising sensitivity was necessary by careful control of the temperature ramp, particularly at the atomisation stage. An atomisation temperature of 2500°C was deemed sufficient to detect most refractory aluminium compounds. During all analyses it was necessary to pay special attention to maintain the laboratory environment free of dust and other aluminium carriers. Exogenous contamination of samples can be a problem in dirty laboratories. Modern plastics which had been found free of aluminium were used throughout in place of any metallic utensils.

Global aluminium levels

The data obtained in the present study are more extensive than previous reports (Freundlich *et al.*, 1985; McGraw *et al.*, 1986). The global average of aluminium in milk-based infant formulae was found to be 1·40 mg/kg and was not subject to large international variations. These levels agree well with those found recently by Koo *et al.* (1988) and Fisher *et al.* (1989).

Soya-based formulae were much higher in aluminium with a mean value of 18·4 mg/kg which is 13 times higher than their milk based counterparts. This same observation has been noted recently by Prescott (1989). Soya based products are also known to contain 8–15 times more cadmium than milk-based products (Dabeka & McKenzie, 1987). The nutritional safety of these products for infant feeding may come into question.

There is no doubting the clinical consequence of aluminium accumulation in individuals who have renal incompetence. It has been clearly shown that increased aluminium loading of premature infants from parenteral feeding results in increased excretion of this element as compared with breast-fed controls (Sedman *et al.*, 1985). The levels of aluminium in intravenous products as well as some milk formulae have been measured and the daily aluminium loading calculated (McGraw *et al.*, 1986; Broadbent & Pybus, 1986). Of more interest to the present study than clinical exposure to excesses of aluminium by intravenous fluids are the implications of long term oral nutritional supplementation to apparently normal infant populations. The potential for toxicity is seemingly less because of the lower bioavailability as compared with parenteral administration. There is evidence that gastro-intestinal absorption is higher in some infants and also that absorption is influenced by other dietary components such as fruit juices (Slanina *et al.*, 1986). Until the exact mechanism of renal and biliary clearance is known, certain assumptions concerning what constitutes a safe exposure limit cannot be made. Importantly, it is not known if this is exceeded by the consumption of infant formulae or if there is a subclinical state associated with this level of dietary exposure.

Possible sources of aluminium

The question of how aluminium enters into milk powders is undoubtedly of interest. The current work was aimed primarily at surveying the aluminium concentrations found in infant formulae rather than isolating the source of the contamination. Obviously the aluminium could (1) have come from the initial milk source, (2) have been introduced during processing, (3) have been present in the additives or (4) have been taken up from the packaging during storage. Assays of ten bulk raw milk samples from three distinct geographical areas in New Zealand indicated that the milk may not necessarily be the major source of aluminium. Raw milk contains about 10% solids so that drying alone would be expected, on our limited data, to result in aluminium levels of about 0·1 mg/kg. This statement is made with caution because a more detailed investigation is needed. Geological considerations (soil type), water purity, feedstock and herd species will all have an influence on the amount of aluminium finding its way into raw milk.

Processing of modern milk powders is generally performed in stainless steel environments. New Zealand regulations (Ministry of Agriculture & Fisheries, 1989) require the use of stainless steel at all manufacturing stages where fluid milk is in contact with any surface. The quality of water coming into contact with the raw milk, usually at the separators, may influence the aluminium content of the finished product. Once dried, the powder can come

into contact with aluminium-containing equipment. Sacrificial aluminium bearings are present in pumps within some dairy factories but care is usually taken to prevent the worn bearings from contaminating the milk. Aluminium containing pipes and storage vats are also permitted. These same regulations are applied in most western nations although it is not necessarily exclusive to all plants worldwide.

The infant formulae from New Zealand and Australia have a 23-fold range in aluminium content (see Fig. 1). The spray-dried wholemilk powder samples from these two countries have a 38-fold range in aluminium content. Our data cannot disclose if this is due to natural variation or if it represents a variation in aluminium introduced during processing. The samples were obtained from both old and new factories, spanning a production life-time of 55 years. The older factories may have been expected to contribute greater aluminium to the powders but this was not evident from this study probably because of modernisation of their plant and equipment. Clearly there is room for comparative trials between completely stainless steel factories and the ones with aluminium components.

Infant formulae are regularly modified with non-diary products. In particular milkfat is often replaced, in-part or totally, with vegetable oil to increase the linoleic acid content. A selection of oils used for this purpose were assayed for aluminium (Table 2). With the exception of palm oil, the aluminium in the oils is negligible and could not contribute significantly to the overall aluminium content of these formulations, even with total replacement of the butter oil. As a comparison, purified butteroil in New Zealand contained an average of 0·029 mg/kg aluminium which is not notably different from most of the vegetable oils. The reason for the elevated aluminium in palm oil cannot be explained by the authors.

All infant formulae tested were fortified with vitamins and minerals. New Zealand and Australian additives were tested for aluminium, the results being included in Table 1. The fat-soluble vitamin premixes contain about a hundred times more aluminium than the water-soluble premixes, but both products when added to the bulk formulae at the rate of about 1 kg per 1000 kg, evidently would not contribute significantly to the total aluminium of the product. Vitamin premixes from foreign destinations were not available to us for aluminium testing. Four mineral premixes tested for aluminium contents showed low concentrations which contributed minimal contamination to the finished product after thousand-fold dilution during production.

The cans used for infant formulae distribution often contain aluminium alloys. None of the cans involved in the study had a protected (laquered) internal surface. Due to the close contact of the contents with these containers there is a possibility that aluminium could accumulate in the

sample. Four points of note are that (1) aged samples showed no trend towards higher aluminium content compared to freshly packed powders, (2) there was no correlation between the manufacturing date of the infant formulae and its aluminium content, (3) analyses of infant formulae which were left standing undisturbed for three months showed no radial stratification of aluminium and (4) there was no statistical difference between the mean aluminium content of infant formulae from New Zealand and Australia and whole milk powders tested. The infant formulae were canned products whereas the whole milk powders were not. On the basis of these observations, we found no evidence for the contamination of infant formulae from their containers.

Whereas annual and seasonal variations in aluminium have been cited in infant formulations (Prescott, 1989), this could not be confirmed in this study. We did not determine composition of the protein component of our samples by analysis and their identity was not often given by the manufacturer. Therefore, we did not differentiate between whey-, caseinate- or milkpowder-based infant formulae in terms of their aluminium content. The infant formulae made in New Zealand and Australia are almost exclusively whey-based.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the assistance of Dr R. Wells, Australian Government Analytical Laboratories, Sydney; Mr J. Blanche, Nestlés (Australia) and Mr J. Crow, Dairy Technical Services, Melbourne.

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