

Levels of 26 elements in infant formula from USA, UK, and Nigeria by microwave digestion and ICP–OES

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Abstract

The presence of toxic elements in powdered and liquid infant milk may create significant health problems for infants. Babies (0–6 months old) may be more at risk because their only food may be infant formula. In this study, infant formula samples sold in major supermarket chains in Nigeria, the UK and USA were analyzed for their metal contents and estimated daily intakes of these elements were determined. Seventeen brands (three samples per brand) of infant formula samples were analyzed for various essential (Ca, Co, Cu, Cr, Fe, Mg, Mn, Mo, Na, and Zn) and non-essential (Ag, Al, As, Ba, Be, Cd, Hg, Ni, Pb, Sb, Sn, Sr, Ti, Tl, U, and V) elements. Known weights and aliquots of the dry, powdered and liquid infant milk, respectively, were digested on the Ethos Plus microwave labstation. Digests were then analyzed with Perkin-Elmer DV 3300 inductively coupled plasma–optical emission spectrometer (ICP–OES). The observed values of SRM 8435 (Whole Milk Powder) analyzed using the same procedure were in agreement with the NIST certified values. The mean concentrations of the elements in milk-based and soy-based formulas, estimated mean daily intakes through infant formulas, and analysis of variances (ANOVA) across infant formula brands are presented. The results suggest that soy-based powder infant formulas generally had higher element levels than milk-based powder formulations, irrespective of source. The European Union (EU) drinking water maximum admissible concentrations for aluminium and barium and the US EPA standard for thallium were violated in some infant formula brands. Cadmium, lead, nickel and chromium were below their respective limits in drinking water and, also, the estimated daily intakes of Pb and Cd from infant formula were below the FAO/WHO Joint Expert Committee on Food Additives recommended provisional tolerable weekly intakes (PTWI) of 25 and 70 µg/kg body weight, respectively. Some brands had low nutritional contents when compared with the recommended dietary allowances (RDA) and dietary reference intakes (DRI) for use in North America. Only brands 7–9 and 12 (UK), and brands 13, 16 and 17 (USA) met the DRI for zinc. The daily intakes of iron (5.03 mg/day) from brand 2 (UK) and magnesium (23.9 mg/day) from brand 10 (UK) were below their respective recommended intake values. However, all the brands met the calcium DRI value of 210 mg/day. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Infant formulas are liquids or reconstituted powders fed to infants and young children. They serve as substitutes for human milk. Apart from breast milk, infant formulas have a special role to play in the diets of infants because they are the major source of nutrients for infants (Bermejo, Pena, Dominguez, Bermejo, Fraga, & Cocho, 2000; FDA, 1997), and a unique

source of food during the first months of life (Rodriguez Rodriguez, Sanz Alaejos, & Diaz Romero, 2000). They are handy for urban women (Tripathi, Raghunath, Sastry, & Krishnamoorthy, 1999) and many mothers in industrialized countries choose to use commercially manufactured formula to feed their newborn (Guttman & Zimmerman, 2000). Despite the strong endorsement of breastfeeding by the American Academy of Pediatrics (AAP), most infants in the USA are fed some infant formula by the time they are 2 months old (Baker et al., 1999). Also, current breastfeeding rates worldwide are far from optimal, particularly among low-income women, despite mounting evidence about the health,

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psychosocial and societal benefits derived from breastfeeding (Guttman & Zimmerman, 2000). Mothers are sometimes worried about or have been discouraged from breastfeeding because of concerns regarding toxins found in the body and often transferred to the baby through lactation (Walker, 2000). Other reasons for mothers not to breastfeed their babies may include the demands of work or school, life circumstances, such as difficult home situations, and social problems with breastfeeding in public. Some low-income mothers associate breastfeeding with socioeconomic privilege (Guttman & Zimmerman, 2000).

Despite the benefits of infant formulas as a major source of food for infants, the presence of contaminants, such as heavy metals, pesticides and polychlorinated biphenyls (PCBs) in infant formula may pose health risks to children. It has been reported that children are more susceptible to exposure (Tripathi et al., 1999) because of their greater intestinal absorption than adults, and a lower threshold for adverse effects (Cambra & Alonso, 1995). These pollutants may arise from the raw materials used in production, poor quality production processes, adulteration of infant foods and bad practices by mothers as regards infant formulation preparation and handling (Fein & Falci, 1999).

The American Academy of Pediatrics' Committee on Nutrition, since 1969, recommended "the early use of fortified formula which results in augmentation of iron stores which help prevent later development of iron deficiency". Infant formulas have therefore been classified as low-iron or iron-fortified, based on whether they contain less or more than 6.7 mg/l of iron (American Academy of Pediatrics, 1999b). Iron fortified formulas in the USA are up to 12.7 mg/l and within the range 0.2–0.5 mg/l in Europe (Rodriguez Rodriguez et al., 2000).

Most infant formulas are derived from animals or plants and therefore are mostly milk-based or soy-based formulations. Because of the nutritional properties of soybean, dairy-like products, or infant formulas based on soybean, are proposed as one of the most interesting alternatives for adults or children who are allergic to animal proteins (Ramos, Torre, Laborda, & Marina, 1998). Consumption of soybeans is reported to provide immense health benefits (Choi, Suh, Kim, Choue, & Koo, 2001; Glade, 2001) but soybean is known to have high levels of neurotoxins, as well as aluminium and silicon (Walker, 2000). It is also inadequate for premature babies (Tigges, 1997) and unsuitable for low birth weight infants (American Academy of Pediatrics, 1998).

Recently, and in the past, many authors (Baum & Shannon, 1997; Begley, 1997; Bermejo et al., 2000; Fernandez-Lorenzo, Cocho, Rey-Goldar, Couce, & Fraga, 1999; Hawkins, Coffey, Lawson, & Delves, 1994; Hua, Kay, & Indyk, 2000; Krachler & Rossipal, 2000; Lin-Fu, 1982; Ramos et al., 1998; Richmond, Strehlow, &

Chalkley, 1993; Sahin, Aydin, Isimer, Ozalp, & Duru, 1995; Schumann, 1990; Torres, Verdoy, Algeria, Barberá, Farré, & Lagarda, 1999; Tripathi et al., 1999) have worked on infant formulations because of the need to maintain the good health of infants. Some research findings on infant formula report high aluminium content (American Academy of Pediatrics, 1996; Fernandez-Lorenzo et al., 1999; Sahin et al., 1995), inadequate daily intakes of copper and zinc from infant formula consumption (Tripathi et al., 1999) and low selenium content in infant formulations (Torres et al., 1999).

The objective of this research was (1) to determine the levels of both the various essential (Ca, Co, Cu, Cr, Fe, Mg, Mn, Mo, Na, and Zn) and non-essential (Ag, Al, As, Ba, Be, Cd, Hg, Ni, Pb, Sb, Sn, Sr, Ti, Tl, U, and V) elements in infant formula products from three continents and to estimate the daily intakes of these elements from infant formula consumption, and (2) to compare the metal content in infant formula brands and types (soy- and milk-based formulas). Only iron-fortified infant formulas were analyzed in this study because of the increasing use of iron, especially in the developed world (Bermejo et al., 2000), in comparison to low iron formulations.

2. Materials and methods

2.1. Sample collection

Eleven brands of dry powdered infant formulas were purchased from major supermarket chains in London (UK), Auburn (Alabama, USA) and Lagos (Nigeria). Also, three brands each of first milk and follow-on liquid infant formula samples were purchased from London (UK). Samples were bought at various times (between October and December 2000) and from different supermarkets in these cities to allow for randomness. The infant formulas were either milk- or soy-based types. Table 1 presents the sampling locations and sample characteristics. For the precision and accuracy of the method, a standard reference material (SRM 8435: whole milk powder) was analyzed. SRM 8435 was purchased from the National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA.

2.2. Reagents

Nitric acid (trace metals grade; Sp. Gr. 1.40) and LPDE sample bottles were from Fisher Scientific (Suwanee, GA, USA). A mixed standard containing 100 mg/l each of Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sn, Sr, Ti, V, and Zn and other single element standards (1000 mg/l) were supplied by SPEX Certiprep, Inc. (Metuchen, NJ, USA). The calibration standard solutions used were

made by appropriate dilution of the stock standards. Ultra pure deionized water with a resistivity of 18.3 megaohm-cm was prepared by feeding a Nanopure Infinity UV/UF Deionizer (Barnstead Thermolyne, IA, USA) with distilled water.

2.3. Sample digestion and ICP-OES analysis of samples

Ethos Plus microwave labstation with computer-controlled easywave software (Milestone, CT, USA) was used to digest all samples. The labstation provides 100% reliable quality assurance of the analytical sample process through its quality pressure chemical sensor, vent and reseal vessel technology. Between 0.5 and 1.0 g of the infant formula powder samples and 5 ml aliquots of the liquid samples were digested on the Ethos Plus microwave workstation. The conditions for the digestions are presented in Table 2. Duplicate digestions were conducted for each sample. A Perkin-Elmer DV 3300 inductively coupled plasma–optical emission spectrophotometer (ICP–OES) was used to analyze the ele-

ments in digested samples. Levels of various metals in digested samples were determined using the ICP conditions in Table 3. The instrument detection limits (axially viewed) and linear concentration ranges are available on request from Perkin-Elmer Inc., USA.

2.4. Quality control

Washing procedures for sample containers, the microwave digestion vessels, glassware for standards and ICP sample run tubes for metal determinations follow recommended procedures (American Public Health Association, 1998). In brief, sample containers and other glassware were cleaned with metal-free nonionic detergent solution, rinsed many times with tap water, soaked in 50% HNO₃ acid for 24 h and then rinsed many times with metal-free deionized water. All sample containers were rinsed again with deionized water prior to use. Blanks, consisting of deionized water and reagents as well as SRM 8435, were subjected to a similar sample preparation and analytical procedure.

Table 1
Infant formula sample characteristics

Country	Brand code ^a	Sample information	Packaging
Auburn, AL, USA	14, 15 (<i>n</i> =6)	Powder; iron-fortified; milk-based; 0–12 months or from birth	Metal container
	16 (<i>n</i> =3)	Powder; iron-fortified; soy-based; 0–12 months or from birth	Metal container
	13 (<i>n</i> =3)	Powder; iron-fortified; soy-based; 0–12 months or from birth	Paper container
	17 (<i>n</i> =3)	Powder; iron-fortified; 0–12 months; hypoallergenic, for babies with colicky symptoms	Paper container
London, UK	1, 2, 3 (<i>n</i> =9)	Powder; iron-fortified; milk-based; 0–12 months or from birth	Aluminum foil
	4 (<i>n</i> =3)	Powder; iron-fortified; milk-based; 0–12 months or from birth	Paper tin
	7, 8, 9 (<i>n</i> =9)	Liquid; iron-fortified; milk-based; 0–12 months or from birth	Paper
	10, 11, 12 (<i>n</i> =9)	Liquid; iron-fortified; milk-based; follow-on or above 12 months	Paper
Lagos, Nigeria	5, 6 (<i>n</i> =6)	Powder; iron-fortified; milk-based; 0–12 months or from birth	Metal container

^a *n* = number of samples per brand.

Table 2
Ethos Plus microwave labstation digestion conditions^a

Microwave conditions	HNO ₃ digestion of powder samples	HNO ₃ digestion of liquid samples
Sample	0.5–1.0 g	5 ml
Deionized water	3 ml	–
Nitric acid	7 ml	15 ml
Pressure	1500 psi	1500 psi
Temperature ^b	Step 1: 25–170 °C for 10 min at 1000 W; ^c Step 2: 170 °C for 10 min at 1000 W; and cool to room temperature	Step 1: 25–140 °C for 3 min at 1000 W; Step 2: 140–150 °C for 4 min at 1000 W; Step 3: 150 °C for 7 min at 1000 W; Step 4: 150–180 °C for 10 min at 1000 W; Step 5: 180 °C for 20 min at 1000 W
Stirring (200 RPM)	Stirring prevented formation of clumps	None

^a Digestion procedures were adapted from the Milestone Ethos-PLUS microwave labstation manual. The Ethos-PLUS is equipped with a high pressure segmented rotor, internal temperature and pressure monitoring system.

^b Temperature ramping was used to control the exothermic reaction and over pressurization of digestion vessel.

^c The Milestone microwave easywave software automatically delivers the minimum power required to follow the defined temperature profile.

2.5. Statistical analysis

The descriptive statistics that include the minimum, maximum, means and standard deviations of the data were computed using Excel 2000. Milk- and soy-based formulations were compared through *t*-test analysis at 5% level of significance. Also the concentrations of Al, Ba, Ca, Cr, Cu, Fe, Mg, Mn, Mo, Sn, Sr, Na, and Zn, determined in the infant formula samples, were subjected to one-way parametric ANOVA (analysis of variance) to ascertain at 95% confidence level the homogeneity of variances across the brands. These elements were selected and parametric ANOVA adopted because the number of non-detects in the analytical result was less than 15% (McBean & Rovers, 1998).

3. Results and discussion

3.1. SRM 8435 analysis

The result of SRM 8435 analysis is presented in Table 4. The result was generally in agreement with NIST certified values.

3.2. Essential and non-essential element levels in infant formulas

For proper comparison of the element levels in infant formula with drinking water standards, the element levels in all powder formulas analyzed were converted to $\mu\text{g/ml}$, using the specified feeding tables supplied by the infant formula manufacturers. Table 5 suggests that the average levels of essential (calcium, chromium, cop-

per, magnesium, manganese, molybdenum, zinc, and sodium) and non-essential (aluminium, barium, nickel, antimony, thallium, and vanadium) elements in USA soy-based powder formula (brands 13 and 16) were higher than the average values obtained for the USA milk based powder formulas (brands 14, 15 and 17). In addition, essential (copper, manganese, and molybdenum) and non-essential (aluminium, barium, nickel, and vanadium) elements in USA soy-based powder brands (brands 13 and 16) posted average values higher than the values obtained for milk-based brands from the UK (brands 1–4: powder forms; brands 7–9: first liquid; brands 10–12: follow-on) and Nigeria (brands 5 and 6). Table 6 shows that the average levels of most essential and non-essential elements, determined in reconstituted USA soy-based powder formula (brands 13 and 16), were generally higher than the combined average element levels in the milk-based reconstituted infant milk powder from the UK (brands 1–4), Nigeria (brands 5 and 6) and USA (brands 14, 15 and 17).

The elements silver, arsenic, cobalt, mercury and uranium were not detected in any of the samples. Though lead was detectable in UK formulas (brands 1, 2, and 10) and Nigeria formula (brand 6), none of the lead values exceeded the $15 \mu\text{g Pb/l}$ stipulated European union (EU) directive for lead in drinking water. However, the presence of lead in infant milk is of great concern since infants are very sensitive to its toxic effects. Childhood exposure to lead may induce suppression of mental capacity or retardation (Falomir, Algeria, Barberá, Farré, Lagarda, 1999; Hutton & Symon, 1986), aggressive behaviours (Bogden, Oleske, & Louria, 1997) and there is a high negative association between lead exposure and children's intelligence quotient (Schwartz, 1994).

Nickel was detectable in one UK sample (brand 7) and some USA infant formulas (brands 13, 15 and 16). However, the nickel levels in these brands were all below the action level of $50 \mu\text{g/l}$ nickel for drinking water. Brand 13 had the highest nickel value of $11 \mu\text{g/l}$. Cadmium was detectable in three samples from the UK (brand 10: two samples; brand 12: one sample) but none of these samples exceeded the $5 \mu\text{g Cd/l}$ stipulated limit for drinking water (Kiely, 1997). Exposure to cadmium can lead to kidney dysfunction (International programme on food safety, 1992). Chromium was detectable in almost all the samples analyzed and the concentration range obtained was $0.0\text{--}0.027 \mu\text{g/ml}$ with a mean value of $0.010 \mu\text{g/ml}$. All the samples had chromium levels below the action level of $50 \mu\text{g Cr/l}$ limit for drinking water. Chromium is an essential trace element but may create problems above certain concentrations. The recommended dietary intake of chromium for adults is $50\text{--}200 \mu\text{g/day}$ [National Research Council (US), 1989]. Chromium is involved in the metabolism of carbohydrates, lipids, and proteins, mainly by increasing

Table 3

Instrument operating conditions applied for metals determination by ICP-OES

Parameters	
View mode	Axial
View height	15 mm
Gas	Argon
Shear gas	Nitrogen
Gas: plasma	15 l/min
Gas flow: auxiliary	0.5 l/min
Source equilibration time	15 s
Pump speed	18.75 rpm
Pump flow rate	1 ml/min
Detector	Charge array
RF power	1300 W
Nebulizer	0.8 l/min
Sample aspiration rate	1 ml/min
Read	Peak height
Number of replicates	3
Background correction	Manual point correction
Read delay	120 s
Rinse delay	20 s

ICP-OES, inductively coupled plasma-optical emission spectrometer.

the efficiency of insulin. Chromium deficiency affects the maintenance of normal glucose tolerance and healthy lipid profiles (Kobla & Volpe, 2000).

A sample each from brands 7, 10 and 12 (UK) showed values above the drinking water action level of 100 µg/l

for barium (Kiely, 1997; US EPA, 1996). A maximum of 151 µg Ba/l was observed in one of the brand 7 samples from the UK. The concentrations of thallium in some samples from the UK (brands 2–4, and 7–9), Nigeria (brands 5 and 6) and the USA (brands 13–17)

Table 4
Analysis of SRM 8435-whole milk powder

Element	NIST SRM 8435 Certified values (mg/kg)	ICP-OES wavelength	Observed values (mg/kg)
Aluminium	0.9	308.215	0.89±0.01
Arsenic	0.001	188.979	nd
Barium	0.58±0.23	233.527	0.570±0.001
Boron	1.1±0.8	249.678	na
Cadmium	0.0002	214.440	nd
Chromium	0.5	267.716	0.460±0.002
Cobalt	0.003	228.616	0.002±0.001
Copper	0.46±0.23	324.752	0.423±0.005
Fluorine	0.17	–	na
Nickel	0.01	221.648	0.007± 0.002
Tungsten	0.002	–	na
Rubidium	16	–	na
Titanium	4	334.940	3.80±0.002
Iron	1.8±1.1	238.204	1.910±0.002
Lead	0.11±0.05	220.353	0.105±0.003
Manganese	0.17±0.05	257.610	0.150±0.002
Molybdenum	0.29±0.13	202.031	0.240±0.003
Selenium	0.131±0.014	196.026	na
Strontium	4.35±0.50	421.552	3.89±0.004
Zinc	28.0±3.1	213.857	27.0±0.003
Magnesium	814±76	279.077	781±2.12

ICP-OES, inductively coupled plasma–optical emission spectrometer; na, not analyzed; nd, not detected.

Table 5
Mean levels of 26 elements in infant formula samples from the USA, UK and Nigeria

Element	Nigeria milk-based powder formulas (µg/ml) ^a	UK milk-based powder formulas (µg/ml) ^a	UK milk-based liquid first and follow-on formulas (µg/ml)	USA milk-based powder formulas (µg/ml)	USA soy-based powder formula (µg/ml) ^a
Al	0.058±0.022	0.092±0.085	0.101±0.153	0.15±0.12	0.46±0.16
Ba	0.037±0.019	0.023±0.008	0.057±0.037	0.02±0.01	0.05±0.01
Be	nd	nd	0.0011±0.0009	0.0001±0.0002	0.00007±0.00011
Ca	385±34.8	344±53.1	662±151	398±71.1	515±51.6
Cd	nd	nd	0.0003±0.0009	nd	nd
Cr	0.006±0.003	0.005±0.005	0.015±0.004	0.007±0.009	0.011±0.007
Cu	0.41±0.06	0.40±0.14	0.54±0.22	0.49±0.09	0.72±0.11
Fe	8.49±1.21	6.27±2.36	11.30±2.26	9.30±0.46	9.14±0.29
Mg	26.2±1.61	42.2±3.78	60.0±13.3	36.4±9.89	39.2±11.0
Mn	0.06±0.02	0.068±0.024	0.081±0.026	0.09±0.04	0.22±0.04
Mo	0.016±0.005	0.017±0.006	0.028±0.012	0.013±0.003	0.033±0.013
Ni	nd	nd	0.0002±0.0009	0.00002±0.00007	0.002±0.004
Pb	0.0004±0.0010	0.0008±0.0017	0.0008±0.0004	nd	nd
Sb	0.003±0.003	0.015±0.015	0.004±0.009	0.012±0.019	0.014±0.016
Sn	0.024±0.035	0.024±0.026	0.035±0.038	0.085±0.045	0.085±0.051
Sr	0.11±0.01	0.13±0.03	0.214±0.050	0.24±0.06	0.17±0.10
Ti	0.011±0.003	0.012±0.004	0.061±0.053	0.021±0.007	0.034±0.012
Tl	0.033±0.015	0.012±0.016	0.010±0.020	0.04±0.02	0.026±0.013
V	nd	nd	0.001±0.001	0.0002±0.0003	0.003±0.003
Zn	3.49±0.28	3.21±0.89	5.58±1.22	3.66±0.79	5.18±1.16
Na	169±17.1	184±44.6	332±32.1	192±45.9	232±46.7

nd, not detected; Ag, As, Co, U and Hg were not detected in samples.

^a Values of powder samples in µg/ml were derived using feeding tables supplied by infant formula manufacturers.

exceeded thallium drinking water standard of 2 µg/l. The minimum and maximum iron levels were 2.98 µg/ml (brand 1: UK) and 16.7 µg/ml (brand 8: UK), respectively. Adequate iron intake is necessary for growth and development. It is also vital for transporting oxygen in the bloodstream and for the prevention of anemia.

The range obtained for aluminium in this study was 0.002–0.64 µg/ml. All the samples from brands 13 and 16 (soy-based powder milk: USA), brand 1 (milk-based powder: UK), brands 7 and 8 (first liquid: UK) and brand 17 (milk-based powder: USA) analyzed had an aluminium content exceeding the stipulated aluminium guideline (0.2 mg/l) in drinking water. Soy-based and hypoallergenic infant formula brands, both from the USA, had higher aluminium levels than other milk-based brands. High aluminium levels have been found in soy-based, hypoallergenic and some preterm infant formulas in the past (American Academy of Pediatrics, 1998; American Dietetic Association, 2000; Koo, Kaplan, & Krug-Wispe, 1988; Walker, 2000; Weintraub, Hans, Meerklin, & Rosenberg, 1986). A range of 1.21–10.9 µg/g aluminum in infant formula samples, mostly from Europe, was earlier reported (Sahin et al., 1995). Also, previous studies showed that aluminium levels in all infant formulas were higher than those in human milk (American Academy of Pediatrics, 1996). The infant formulas showing the highest levels of alu-

minium are those with additives, such as calcium salts and soy protein, which contain aluminium as a contaminant. There is concern because of the possibility of increased amounts of aluminium being deposited in the brain and the resulting risk of brain dysfunction (Walker, 2000) and aluminium is now being implicated as interfering with a variety of cellular and metabolic processes (American Academy of Pediatrics, 1996). Aluminium may also cause bone disorders but the critical level of aluminium loading that results in bone disorders is not known (American Dietetic Association, 2000). There is evidence that aluminium absorption is greater in infants prior to 6 months of age than after. However, the nature of absorption is not clearly understood (Bishop, 1992).

3.3. Comparison of infant formula types and brands

In general, the average levels of the elements in the milk based first liquid formulas were higher than the corresponding values for the milk-based powder formulas that were reconstituted based on the manufacturers specifications (Table 6). However, this does not suggest that the milk based powder formulas, when appropriately reconstituted, do not meet the minimum nutritional requirements (Section 3.4). The soy-based powder formulas (brands 13 and 16) generally had higher average levels of the elements determined than

Table 6
Mean concentrations of milk-based and soy-based infant formula

Element	Milk-based powder formula brands 1–6, 14, 15, and 17 (µg/ml) ^a	Soy-based powder formula brands 13 and 16 (µg/ml) ^a	Milk-based first liquid formula brands 7–9 (µg/ml)	Milk-based follow-on liquid formula brands 10–12 (µg/ml)
Al	0.10±0.09 ^b	0.46±0.16 ^b	0.168±0.191	0.035±0.011
Ba	0.03±0.01 ^b	0.05±0.01 ^b	0.045±0.042	0.068±0.030
Be	0.0003±0.0003	0.00007±0.0001	0.0002±0.0002	0.0019±0.0002
Ca	371±60.2 ^b	515±51.6 ^b	624±161	700±138
Cd	nd	nd	nd	0.0006±0.0012
Cr	0.006±0.006	0.011±0.007	0.015±0.005	0.015±0.004
Cu	0.43±0.12	0.72±0.11	0.72±0.16	0.36±0.04
Fe	7.78±2.16	9.14±0.29	11.0±3.12	11.6±0.98
Mg	38.9±6.74	39.2±11.0	70.7±9.45	49.4±5.78
Mn	0.07±0.03	0.22±0.04	0.093±0.014	0.069±0.031
Mo	0.015±0.005 ^b	0.033±0.013 ^b	0.027±0.014	0.028±0.010
Na	183±40.1	232±46.7	345±33.4	320±26.7
Ni	7.78E-06±4.04E-05 ^b	0.002±0.004 ^b	0.0004±0.001	nd
Pb	0.0005±0.0013	nd	nd	0.0017±0.005
Sb	0.011±0.015	0.014±0.016	0.008±0.012	nd
Sn	0.044±0.044	0.085±0.051	0.041±0.026	0.029±0.049
Sr	0.16±0.07	0.17±0.10	0.22±0.035	0.21±0.063
Ti	0.015±0.007 ^b	0.034±0.012 ^b	0.015±0.003	0.106±0.034
Tl	0.026±0.021	0.026±0.013	0.020±0.024	nd
V	8.22E-05±0.0002	0.003±0.003	0.0005±0.001	0.002±0.001
Zn	3.42±0.76	5.18±1.16	6.28±0.54	4.88±1.33

nd, not detected; Ag, As, Co, Hg, and U were not detected in samples.

^a Values of powder samples in µg/ml were derived using feeding tables supplied by infant formula manufacturers.

^b *t*-Test statistically significant at *P*<0.05.

the average of the combined values obtained for milk-based powder formulas from the USA, UK and Nigeria (brands 1–6, 14, 15 and 17). The *t*-test, at 5% level of significance, between the infant formula types (soy-based powder: brands 13 and 16 versus milk-based powder: brands 1–6, 14, 15 and 17) suggests significant variation of the concentrations of aluminium, barium, calcium, molybdenum, nickel, and titanium (Table 6). The result of the one-way parametric ANOVA across the brands that were in powder form also suggests significant differences at the 95% confidence level in the variances of aluminium, barium, calcium, chromium, copper, iron, magnesium, manganese, molybdenum, tin, strontium, sodium, and zinc. Similarly, ANOVA result of the liquid infant brands (0–12 months/first term milk) suggest unequal variances of calcium, copper, iron, magnesium, manganese, molybdenum, strontium, sodium, and zinc at the 5% level of significance. This indicates that various brands of commercial infant formula may have different nutrient values and may, as well, cause potentially different levels of exposures to toxic elements.

3.4. Daily intakes of essential and non-essential elements from infant formulas

Estimates of the daily intakes of both essential and non-essential infant formula were calculated, using the feeding tables specified by the manufacturers of the various brands. For first milk liquid formulas (UK), for a baby weighing 7.5 kg and 6 months of age, 900, 960, and 960 ml were specified per day for brands 7, 8, and 9, respectively. Similarly, for the above age and weight, 500, 560 and 800 ml were specified per day for the UK follow-on liquid formula brands 10, 11 and 12, respectively. To estimate the daily intakes of metals from the powdered formulas from the USA (brands 13–17), Nigeria (brands 5 and 6) and the UK (brands 1–4), a 6–12 months old baby would require approximately 5 kg of powdered infant formula every month (Tripathi et al., 1999) and in a year 60 kg. Thus, the daily consumable weight of infant powdered formula would be 164.4 g of infant formula powder. Brand 16 (soy-based milk: USA) recorded the highest daily intakes of aluminium (764 µg/day), calcium (701 mg/day), chromium (21.8 µg/day),

Table 7
Average daily intakes of elements from infant formula from three countries

Element	Nigeria ^a Milk-based (first term powder)	USA ^b Milk-based (first term powder)	USA ^c Soy-based (first term powder)	USA ^d Hypoallergenic (first term powder)	UK ^e Milk-based (first term powder)	UK ^f Milk-based (first term liquid)	UK ^g Milk-based (follow-on liquid)
Al (µg/day)	67	97	573	361	102.8	157	21.7
Ba (µg/day)	42.7	23.8	57.5	29.8	24.3	41.8	42.4
Be (µg/day)	–	–	0.09	0.48	0.67	0.17	1.2
Ca (mg/day)	443	451	645	590	374	586	442
Cd (µg/day)	–	–	–	–	–	–	0.34
Cr (µg/day)	7.35	7.7	14	12.2	5.7	13.9	9.2
Cu (µg/day)	475	609	910	643	422	680	230
Fe (mg/day)	9.77	11.6	11.5	11.83	6.64	10.4	7.2
Mg (mg/day)	41.6	38.1	48.9	60.1	45.94	66.3	30.2
Mn (µg/day)	68.3	80.7	269	182	74.8	87.2	46.9
Na (mg/day)	195	207	291	307	199	325	197
Mo (µg/day)	18.9	18.8	41.9	14.9	18.2	24.9	17
Ni (µg/day)	–	0.05	2.3	–	–	0.4	–
Pb (µg/day)	0.48	–	–	–	0.96	–	0.8
Sb (µg/day)	3	–	18.7	43.9	17.7	7.3	–
Sn (µg/day)	27.9	82.4	108	153	26.8	37.2	16.6
Sr (µg/day)	130	339	221	250	136	205	137
Ti (µg/day)	13.7	21.35	42.7	37.6	13	14.4	67.7
Tl (µg/day)	38.1	47.6	32.2	59.7	12	19.3	–
V (µg/day)	–	0.05	3.5	2.8	–	0.5	1.3
Zn (mg/day)	4.0	4.3	6.5	5.15	3.5	5.9	3.2

Average daily intakes of elements from infant formula types were calculated from the average daily intake values obtained for each brand. The average daily intake of elements from each brand was obtained by relating concentrations in each sample to the quantities of formula consumed per day.

^a Average values for brands 5 and 6.

^b Average values for brands 14 and 15.

^c Average values for brands 13 and 16.

^d Average values for brand 17.

^e Average values for brands 1–4.

^f Average values for brands 7–9.

^g Average values for brands 10–12.

copper (1029 µg/day), manganese (256 µg/day), molybdenum (33.7 µg/day), and tin (145 µg/day). Brand 13, a soy-based milk sample from the USA, equally gave the highest daily intake of zinc (7.76 µg/day). Lead was detected only in brands 1, 2 and 10 (milk based: UK) and brand 6 (milk based: Nigeria). The maximum calculated daily intake of lead was 3.4 µg/day (brand 2: UK). The estimated daily intakes of lead and cadmium from infant formula were below the FAO/WHO Joint Committee on food additives recommended provisional tolerable weekly intakes (PTWI) of 25 µg/kg body weight and 70 µg/kg body weight, respectively (FAO/WHO, 1999).

The calculated daily intakes of essential and non-essential elements from each infant formula brand were compared with recommended dietary allowances (RDA) and dietary reference intakes (DRI) for use in North America (National Academy of Sciences, 1997). The RDA or DRI for 0–6-month-old bottle-fed infants are as follows: iron (6 mg/day), zinc (5 mg/day), calcium (210 mg/day), and magnesium (30 mg/day). Only brands 7–9 and 12 (UK liquid formulas), and brands 13, 16 and 17 (USA powder brands) met the DRI for zinc. In addition, brand 2 (UK powder formula) had an iron level (5.03 mg/day) below the RDI but all the brands met the DRI requirement for calcium. Calcium is critical for the growth and development of bones. Inadequate calcium intake may result in bone fractures, rickets and development of osteoporosis at adulthood (American Academy of Pediatrics, 1999a). Brand 10 (UK liquid follow-on) showed a magnesium daily intake of 23.9 mg/day, which was lower than its DRI value. Studies suggest a positive association between high magnesium intake in humans and increased bone density (Martini & Mayer, 1999). Also, epidemiological and clinical studies on magnesium rich drinking water suggests that it may reduce the frequency of sudden death in humans (Garzon & Eisenberg, 1998).

Table 7 presents the calculated mean daily intakes from the infant formula types across the study areas derived from the mean daily intake values for the infant formula brands from each country. The average daily intake values of most elements from the soy-based powder formulas from the USA were mostly higher than the respective average daily intake values for milk-based samples from the USA, UK and Nigeria and also higher than the average values obtained for hypoallergenic samples mainly from USA. Also milk-based first term liquid samples from the UK had higher daily intake values than the first term powder and follow-on liquid samples, also from the UK.

4. Conclusion

In this study, the levels of 26 elements were determined in liquid and powder infant formula samples

from three countries, USA, UK and Nigeria. The ANOVA results suggests that there were significant variations of some of the element levels across the infant formula brands and this could be attributed to the different manufacturing practices, variations in quality of raw materials, finished products and packaging containers used by infant formula manufacturers. The average levels of almost all essential and non-essential elements determined in reconstituted USA soy-based powder formula brands were generally higher than the average element levels in the milk-based infant milk powder from the UK, Nigeria and USA. Most especially, the content of aluminium in soy-based and hypoallergenic (milk-based) formulas were found to be higher than the values obtained for other milk-based formulas from the UK, Nigeria and USA. The need to further assess the aluminium content of soybeans is crucial because of the problems associated with high aluminium intake.

The nutritional contents of some infant formula brands were found to be lower than the RDA or DRI values for the essential elements zinc, magnesium, and iron. Bottle-fed infants consuming formulations with low levels of essential elements may suffer nutritional deficiencies and consequent health problems. The concentrations of non-essential elements, aluminium, barium, and thallium, exceeded their respective stipulated drinking water values in some cases. However, the concentrations of cadmium, chromium, lead, and nickel in some samples were found to be below their respective recommended limits for drinking water. Also the estimated daily intakes of lead and cadmium from infant formula were below the FAO/WHO Joint Committee on food additives recommended PTWI of 25 and 70 µg/kg body weight, respectively.

In general, the average levels of the elements in the milk based first liquid formulas were higher than the corresponding values for the milk-based powder formulas that were reconstituted based on the manufacturers specifications. However, some of the essential elements in the reconstituted powder formula were still within their nutritional requirement levels.

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