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Appropriateness of Essentials Trace Metals in Commonly Consumed Infant Formulae in Nigeria

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Abstract

BACKGROUND: Mothers who feed their children with infant food have increased have over the years leaving a large percentage of children who consume other types of liquid food for supplementation or as alternatives.

AIM: Determining the levels of essential trace metals in these formulae with the aim of ascertain their appropriateness is considered important.

METHODS: Iron, zinc, manganese, chromium and cobalt in 26 infant formulae purchased from Port Harcourt city, Nigeria were determined by Atomic Absorption Spectrophotometry. The estimated daily intake EDI and percentage of EDI to the recommended daily allowance of these essential trace metals were used in the exposure assessment.

RESULTS: The highest mean concentration of Mn, Cr and Co was found in the milk based (0.15 ± 0.09 mg/kg), (0.61 ± 0.70 mg/kg), (0.12 ± 0.32 mg/kg) compared to the cereal based and cereal mix based but the differences was also not significant. The EDI of chromium in the infant formulae exceeded the RDA.

CONCLUSION: Infant formulae may add to the chromium body burden of infants in Nigeria.

Introduction

Breast milk is proven to be the best source of infant nutrition especially for the first six-months of life and has been so recommended. According to World Health Organization less than 40% of 6-month-old infants are exclusively breast fed in developing countries [1].

Ideally breast milk substitutes or alternatives provide adequate supplies of energy and nutrients to support the rapid growth rate during the infant's first 6 months of life as failure to do so can directly affect infant growth and can have long-term consequences on organ development and function, which may result in adverse health effects later in life [2]. Whereas there have been considerable efforts in the understanding of infant formulae composition on protein and energy content and a few nutrients and vitamins [3], most essential trace metals present in infant formulae have received very little attention. In other to meet specific nutritional infant requirements, the formulae are fortified with essential metals which have crucial roles in several body functions [4], [5], [6]. However, some of the essential metals are required in smaller quantities because excess of any of the essential trace metals at an early stage of life may induce developmental adverse health effects such as diabetes, hypertension and obesity in adult life [6].

According to the American Academy of Pediatrics (AAP) iron and zinc are critical nutrients, therefore ensuring adequate intake of iron and zinc can help reduce the risk of developing severe deficiencies such as iron deficiency anemia [7], [8] or impaired growth, such as rickets [9]. Usually chromium is present in foods mainly in its trivalent state chromium Cr (III) with very low toxicity by the oral route [10]. Trivalent chromium an essential trace element in that it has been postulated to be necessary for the efficacy of insulin in regulation of the metabolism of carbohydrates, lipids and proteins.

Intake of these essential trace metals in amounts exceeding cut off points such as the tolerable upper intake level (UL) could lead to adverse effects. Childhood nutrition intake can inform Pediatricians about key nutrient intakes during a critical growth period, as well as provide a basis for population level estimates. The present study has therefore estimated the levels of essential trace metals in commonly consumed infant formulae in Nigeria with a view understanding their appropriateness in adherence to the Recommended Dietary Allowance RDA.

Materials and Method

Sampling

Twenty six samples of different brands of infant formulae, representing most kinds of powdered milk and cereal for infants with age range from birth up to first year of life and above, were purchased from stores in Port-Harcourt, Rivers state, Nigeria and divided into three groups with codes as (milk based infant formula coded as: M1 to M9, Cereal based coded as: C1 to C7 and Cereal mix based coded as: CM1 to CM10

Products under analysis were infant formula and follow-on formula samples; soy-based infant formula; milk and rice-based products for infants; rice gruel, wheat gruel and mixed cereal for infant (all products sold as powder) vegetable meals and fruit-based desserts.

Infant formulae preparation

The infant formulae samples (1–2 g) were weighed with plastic materials to prevent contamination with metals and digested using the hot-block digestion as in our previous procedure [11]. Briefly, approximately 9 mL of 65% concentrated nitric acid (HNO_3) and 3 mL of perchloric acid was added in a ratio of 3: 1 prior to heating and the solution was transferred to a hot plate and heated to a temperature of 120°C for about 5 h. The sample was introduced into an oven under a temperature that was gradually increased by 10°C every 60 min until the final temperature of 450°C was attained with white ashes obtained after 18 h. Afterwards the samples were left to cool, and the white ash was dissolved in 5 mL of 1.5% nitric acid (HNO_3) and a final volume of 25 mL was made by addition of deionized water. The resulting solution was filtered using a Whatman filter paper no. 42 fitted into a Buchner funnel to avoid residues from getting into the beaker before transferring it into a tightly sealed plastic container. Metal concentrations were assayed with atomic absorption spectroscopy (Model 205, Buck Scientific, East Norwalk, CT, USA). Samples were analyzed in triplicates [11].

Quality control

The instrument was recalibrated after every ten runs. The analytical procedure was checked using the spike recovery method (SRM). A known standard of the metals was introduced into already-analyzed samples and re-analyzed. The results of the recovery studies for Cr, Zn, Mn, Fe and Co were more than 97% [11]. The relative standard deviation between replicate analyses was less than 4%. The limit of detection (LOD) for Cr, Al, Zn, Mn, Fe and Co were 0.005 ppm, with blank values reading as 0.00 ppm for all the metals in deionized water with an electrical conductivity value of less than 5 µS/cm. The limit of quantification (LOQ) for Cr, Mn, Fe and Co was 0.04 and 0.06 ppm for Zn and Fe.

Risk Assessment

The health risks of each essential trace metal *via* consumption of infant formulae were assessed based on the comparism of estimated daily intake EDI with the RDA. The estimated daily intake EDI was calculated as the following

equations (1) $$EDI = \frac{C \times IR}{BW} \quad 1$$

...where *EDI* is the estimated daily intake of heavy metals (mg/kg/d) *C* is the mean concentration of heavy metals

in infant formula samples (mg/kg) and *IR* is the intake of infant formula per kg body weight per day BW is the body weight. The estimated daily intakes EDI (mg/kg bw/day) of iron (Fe), zinc (Zn), manganese (Mn), chromium (Cr) and cobalt (Co) in different groups of infant formula in different age groups (0-12 months) of body weights 3.5 – 10kg and daily intake rates (DIR) of 0.075-0.135kg [12].

Statistical Analysis

Statistical analysis was carried out using the Graphpad prism version 6.5. All results were expressed as mean value ± standard deviation (SD).

The data were analyzed using one-way analysis of variance (ANOVA) and turkey post hoc test at 95% confidence level. $P < 0.05$ was considered as statistically significant. Principal component analysis (PCA) was applied to reduce the number of variables extracting as much information as possible.

Results

The concentrations of iron (Fe), zinc (Zn), manganese (Mn), chromium (Cr) and cobalt (Co) in the 26 analyzed infant formulae as shown on Table 1 varied from 2.01-8.02 mg/kg, 2.39-10.41 mg/kg, 0.02-0.34 mg/kg 0.002-2.08 mg/kg and 0.001-0.96 mg/kg respectively. The highest concentrations of Fe, Zn, Mn Cr and Co were detected in the milk-based infant formulae M8 Cowbell Tina (8.02 mg/kg), Cowbell Tina M8 (10.41 mg/kg), Nutristart M5 (0.34 mg/kg), Peak baby M3 (2.08 mg/kg) and Nutristart M5 (0.96 mg/kg) respectively. Milk-based formulae sample M2 (2.01 mg/kg) had lowest iron and lowest zinc level was detected in cereal mix based formula sample Cm8 (2.09 mg/kg).The lowest manganese level was observed in cereal mix based formulae samples Cm3 and Cm5 (0.02 mg/kg), whereas lowest chromium level was found in cereal based formulae sample C4 (0.002 mg/kg) and lowest cobalt level was detected in cereal mix based formulae samples Cm3 and Cm7 (0.001mg/kg).

Table 1: Concentration of essential trace metals (mg/kg) in different brands of Infant Formulae

Sample Code	Brand Name of Infant Formula	Class	Manufacturer	Fe	Zn	Mn	Cr	Co
M_1	Pre NAN	Milk based	Nestle	3.30	5.31	0.06	0.13	0.004
M_2	Sma	Milk based (starter powdered milk)	Wyeth nutritionals	2.01	4.58	0.05	0.56	0.002
M_3	Peak Baby	Milk based (starter powdered milk)	Friesland campina	2.42	8.82	0.17	2.08	0.02
M_4	Lactogen 1	Milkbased (starter powdered milk)	Nestle	2.11	4.83	0.15	0.41	0.01
M_5	Nutristart	Milk based (follow-on milk)		4.04	5.57	0.34	0.61	0.96
M_6	Sma Pro	Milk based follow-on milk)	Nestle	5.24	7.59	0.17	0.06	0.01
M_7	Sma Pro	Milk based (starter milk)	Wyeth nutritionals	4.12	5.07	0.04	1.43	0.03
M_8	Cowbell Tina	Milk based (follow-up milk)	Cowbell	8.02	10.41	0.17	0.20	0.04
M_9	My Boy Eldorin	Milk based		5.46	8.22	0.19	0.04	0.003
C_1	Nestle Cerelac	Cereal based	Nestle	2.59	8.39	0.15	0.05	0.002
C_2	Nestum Baby Cereal	Cereal based	Nestle	4.13	6.40	0.03	1.51	0.03
C_3	Golden Country Baby Cereal	Cereal based	Sun mark Ltd.	5.14	5.93	0.10	0.95	0.004
C_4	Friso Gold	Cereal based	Friso Gold	3.52	5.18	0.04	0.002	0.003
C_5	Cerelac Infant Cereal	Cereal based		2.40	5.10	0.06	0.003	0.01
C_6	Pediasure: Grow And Gain	Cereal based		4.99	7.51	0.20	0.02	0.003
C_7	Aptamil: Organic Rice	Cereal based		3.18	4.25	0.06	0.01	0.05
Cm_1	Nutribom	Cereal based (Mix)	Nutrimental	3.74	5.16	0.17	0.26	0.02
Cm_2	Ridielac (Vina Milk)	Cereal based (Mix)	Vietnam dairy products	4.09	5.12	0.04	0.10	0.01
Cm_3	Nutriben	Cereal based (Mix)	Alter farmacia	3.48	10.10	0.02	0.40	0.001
Cm_4	Ninolac	Cereal based (Mix)	Ninolac maroc SARL	5.69	10.25	0.12	0.42	0.01
Cm_5	Gerber	Cereal based (Mix)	Nestle	6.97	8.66	0.02	0.83	0.002
Cm_6	Heinz Dinners	Cereal based (Mix)	Heinz	4.25	7.33	0.29	0.40	0.004
Cm_7	Gerber Oatmeal	Cereal based (Mix)	Nestle	3.20	9.19	0.09	1.17	0.001
Cm_8	Heinz Summer Fruits	Cereal based (Mix)	Heinz	6.96	2.39	0.04	0.02	0.02
Cm_9	Nutrilac Infant Cereal	Cereal based (Mix)		2.34	7.82	0.03	0.02	0.01
Cm_{10}	Cerelac Infant Cereal	Cereal based (Mix)		4.23	5.40	0.14	0.32	0.01

The mean concentrations of iron, zinc, manganese, chromium and cobalt in three different groups of infant formulae is shown on Table 2.

Table 2: Mean concentration(mg/kg) of essential metals in three different groups of infant formulae

Groups	Fe	Zn	Mn	Cr	Co
Milk based (n = 9)					
Mean ± SD	4.08 ± 1.95	6.71 ± 2.10	0.15 ± 0.09	0.61 ± 0.70	0.12 ± 0.32
Range	2.01-8.02	4.58-10.41	0.04-0.34	0.06-2.08	0.002-0.96
Cereal based (n = 7)					
Mean ± SD	3.71 ± 1.09	6.11 ± 1.45	0.09 ± 0.06	0.36 ± 0.62	0.01 ± 0.02
Range	2.40-5.14	4.25-8.39	0.03-0.20	0.002-1.51	0.002-0.05
Cereal mix based (n = 10)					
Mean ± SD	4.49 ± 1.56	7.14 ± 2.56	0.10 ± 0.08	0.40 ± 0.36	0.01 ± 0.01
Range	2.34-6.97	2.39-10.25	0.018-0.29	0.02-1.17	0.001-0.02

The mean iron concentration in the cereal mix-based formulae (4.49 ± 1.56 mg/kg) was higher than milk-based. The mean level of zinc 7.14 ± 2.56 mg/kg in cereal mix based infant formulae was higher than the level found in milk-based and cereal based but the differences were not significant. The highest mean concentrations of manganese, chromium and cobalt were found in the milk-based 0.15 ± 0.09 mg/kg, 0.61 ± 0.70 mg/kg, 0.12 ± 0.32 mg/kg respectively compared to the cereal based and cereal mix based but the differences were also not significant.

Table 3 shows the EDI of iron, zinc, manganese, chromium and cobalt in milk-based infant formula ranged from 0.055-0.97, 0.091-0.16, 0.002-0.004, 0.008-0.015 and 1.6E-3-2.9E-3 mg/kg bw/day respectively. The EDI of iron, zinc, manganese, chromium and cobalt in cereal based infant formula ranged from 0.05-0.088, 0.082-0.145, 0.001-0.002, 0.005- 0.009 and 1.4E-4-2.4E-4 mg/kg bw/day respectively. The EDI of iron, zinc, manganese, chromium and cobalt cereal mixed based infant formula ranged from 0.061-0.107, 0.096-0.17, 0.001-0.002, 0.005-0.01 and 1.4E-4-2.4E-4 mg/kg bw/day respectively.

Table 3: Estimated daily intake EDI (mg/kg bw/day) of essential trace metals in different groups of infant formulae

Milk based							
Age	DIR (kg)	Bw (kg)	Fe	Zn	Mn	Cr	Co
0-2 weeks	0.075	3.5	0.087	0.144	0.003	0.013	2.6E-3
2-4 weeks	0.1	4.2	0.097	0.16	0.004	0.015	2.9E-3
2 months	0.11	4.7	0.095	0.157	0.004	0.014	2.8E-3
4 months	0.145	6.5	0.091	0.15	0.003	0.014	2.7E-3
6 months	0.135	7.5	0.073	0.121	0.003	0.011	2.2E-3
6-12 months	0.135	10	0.055	0.091	0.002	0.008	1.6E-3
Cereal based							
Age	DIR (kg)	Bw (kg)	Fe	Zn	Mn	Cr	Co
0-2 weeks	0.075	3.5	0.08	0.131	0.002	0.008	2.1E-4
2-4 weeks	0.1	4.2	0.088	0.145	0.002	0.009	2.4E-4
2 months	0.11	4.7	0.087	0.143	0.002	0.008	2.3E-4
4 months	0.145	6.5	0.083	0.136	0.002	0.008	2.2E-4
6 months	0.135	7.5	0.067	0.11	0.002	0.006	1.8E-4
6-12 months	0.135	10	0.05	0.082	0.001	0.005	1.4E-4
Cereal mix based							
Age	DIR (kg)	Bw (kg)	Fe	Zn	Mn	Cr	Co
0-2 weeks	0.075	3.5	0.096	0.153	0.002	0.009	2.1E-4
2-4 weeks	0.1	4.2	0.107	0.17	0.002	0.01	2.4E-4
2 months	0.11	4.7	0.105	0.167	0.002	0.009	2.3E-4
4 months	0.145	6.5	0.1	0.159	0.002	0.009	2.2E-4
6 months	0.135	7.5	0.081	0.129	0.002	0.007	1.8E-4
6-12 months	0.135	10	0.061	0.096	0.001	0.005	1.4E-4

Table 4 shows the percentage of iron, zinc, manganese, chromium and cobalt in the infant formulae to the RDA. The highest and lowest percentages of iron to RDA in age groups 2-4 weeks and 6-12 months were 39.6% and 22.5% respectively, whereas the highest and lowest percentages of zinc to the RDA were in age groups 2-4 weeks (85%) and 6-12 months (48.2%). The highest and lowest percentages of manganese to RDA were in age groups 2-4 weeks and 6-12 months with values of 0.6% and 0.34% respectively. The highest and lowest percentage of chromium to the RDA were in age groups 2-4 (264.1%) weeks and 6-12 (149.7%). The highest and lowest percentage of cobalt to the RDA were in age groups 2-4 weeks and 6-12 months with values of 23.8% and 13.5% respectively.

Table 4: Percentage of Adequate Intake or RDA of essential trace metals

	Fe	Zn	Mn	Cr	Co
RDA (µg/kg)					12
Adequate intake (µg/kg)	270	200	600	5.5	-
Age% Adequate intake/RDA					
0-2 weeks	35.6	76.5	0.54	237.7	21.4
2-4 weeks	39.6	85	0.6	264.1	23.8
2 months	38.9	83.6	0.59	259.6	23.4
4 months	37.1	79.6	0.56	247.4	22.3
6 months	29.9	64.3	0.45	199.6	18
6-12 months	22.5	48.2	0.34	149.7	13.5

Principal Component Analysis (PCA) for Fe, Zn, Mn, Co and Cr.

Given the 5 metal variables, iron, zinc, manganese, chromium and cobalt, and the three infant formulae, the PCA outputs were as follow: Figure 1 is the biplot of F1 against F2, in which Fe and Zn lie on the horizontal axis (abscissa) demarcating the 1st and 4th quadrants which coincided with the plotting position of the cereal based formulae. This further indicates that Fe and Zn were dominant in the cereal based formulae as compared to the other infant formulae. However, Fe and Zn are found in both in the cereal based (mix) and milk based infant formulae given their positions in the 1st and 4th quadrants.

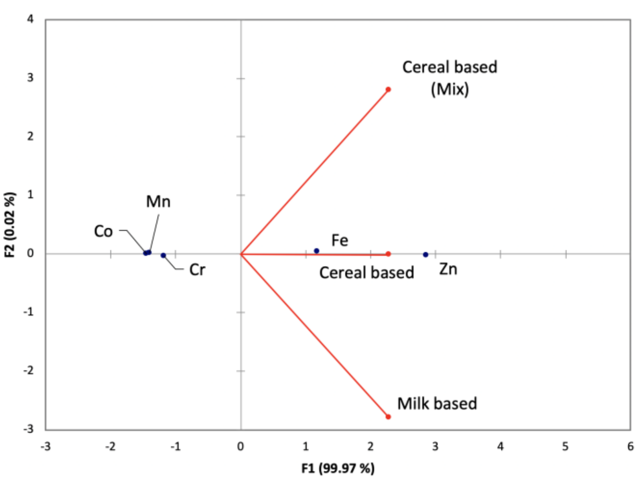

Figure 1: Biplot for PCA on five metal variables (Principal Component Analysis (PCA) for Fe, Zn, Mn, Co and Cr)

The corresponding eigenvalue of 2.9992 confirmed just one principal factor (F1) (given that a factor with eigenvalue greater than one '> 1' is considered a principal component) with a variability of 99.97 % (see Table 5). Furthermore, Mn, Co and Cr seem to play the role of trace elements in the infant formulas (in other words, minor factors whereas Fe and Zn represents the major factor).

Table 5: Eigenvalues

	F1	F2	F3
Eigenvalue	2.9992	0.0007	0.0001
Variability (%)	99.9743	0.0224	0.0032
Cumulative (%)	99.9743	99.9968	100.0000

Discussion

Mothers who feed their children with infant food have increased over the years with only about 34.8% of babies placed on exclusive breast feeding within the first six months of life [13]. Hence a larger percentage of children consume other types of liquid food for supplementation. Determining essential elements from these formulae is considered important more so as children eat more food in proportion to their body weight compared to adults to ascertain the appropriateness or otherwise of these essential trace elements. To bridge this knowledge gap on infant exposure to trace elements, the present study has assessed the concentrations of a range of essential elements in infant formulae intended for consumption during the first 0-12 months of life.

In this study zinc level was significantly higher than other essential trace metals with the overall mean levels of iron, manganese, chromium and cobalt in the following decreasing order of Zn > Fe > Cr > Mn > Co. The highest mean levels of manganese, chromium and cobalt were found in milk-based infant formulae. Zinc and chromium in this study exceeded their permissible limits of 3.28 and 0.3 mg/kg respectively [14], [15]. Cobalt is a key component of cyanocobalamin B12 vitamin with diverse functions related to the brain and nervous system [16]. Milk-based infant formulae had the highest level of cobalt 0.12 ± 0.32 mg/kg (range 0.002-0.96 mg/kg). The fortification of some cereal products with vitamin B12 may account for the presence of cobalt in infant formulae too [17].

According to manufacturer's quotations on the labels, the levels of iron and manganese ranged from 1-70 mg and 10-130 µg respectively. About 62 percent did not indicate the levels of manganese while 12 percent did not indicate levels of iron. More than 98 percent of the infant formulae in this study were packaged in aluminum foil. Since manganese are often added to aluminum to improve its strength properties [18], aluminum packaging may impact on the manganese levels in these infant formulae. Infant milk formulae sold in Nigeria consist not only milk, but other ingredients of plants origin as also reported of Tanzanian labeled baby milk formulae [19]. Plant ingredients impact on the manganese levels in the cereal samples since cereals contain higher manganese than products of animal [19]. However, in this study higher manganese levels were seen in the milk-based 0.15 ± 0.09 mg/kg than the cereal based infant formulae 0.09 ± 0.06 mg/kg. The lowest mean concentrations of iron and zinc in the infant formulae were 3.71 ± 1.09 (cereal based) and 6.11 ± 1.45 (cereal based).

Cows' milk is not only known to be deficient in iron, but its high casein and calcium levels hamper iron absorption which is very vital for growing infants and young children [20]. This may discourage cows' milk alone intake by infants and young children because of risk of iron deficiency and attendant health challenges [20]. In apparently healthy children, cows' milk induces intestinal occult blood loss which exacerbate iron deficiency [20]. Several variables namely breed of cow, lactation status, season, herd health and nutrition are known to account for the variations in the macro- and micronutrient contents of milk [21], [22], [23].

Whereas EFSA has set thresholds for intake levels for potentially toxic metals lead, cadmium and mercury [24], no explicit thresholds have yet been set trace metals, zinc, chromium, iron, manganese and cobalt. For these essential trace metals levels that lead to deficiencies and chronic or acute poisonings are known and some Nutrition Societies notably of Germany, Austria and Switzerland have established various threshold values [25]. In view of the differences in the requirements, the DACH joint recommendations for Germany, Austria and Switzerland have established reference values for babies between 0 and 4 months, 5 – 12 months and children 1 – 4 years of age [25] with recommended daily intakes of iron and zinc as 1 mg [25]. The highest estimated daily intake of iron and zinc in this study were 0.107 and 0.17 mg/kg bw/day respectively at 2-4 weeks for cereal mix infant formulae. The mainstay of the American Academy of Pediatrics AAP recommendations [26] is to ensure adequate dietary zinc intake for babies and young children especially during the transition from breast milk to complementary foods. Some studies have shown that over half of children 12 – 23 months have zinc intakes that exceed the upper limit UL [27], [28]. Cereal based formulae may be fortified with zinc and have been shown to lead to higher zinc intakes [28]. Childhood zinc deficiency is a common nutritional problem in sub-Sahara Africa with growth retardation, impairment of cognitive function and high susceptibility to infectious diseases as the cardinal features [29]. Zinc-fortified formulae are known to improve growth and immune function in children recovering from malnutrition higher linear growth together with increased salivary concentrations of immunoglobulin have been reported [30]. It is feared that increased dietary intake of iron through food fortification programs may hamper absorption of zinc. Since studies have shown that when dietary ligands are present, there is little or no interaction between the absorption of zinc and iron [31] (Davidson et al., 1995), in practice therefore, when infant formulas contain 7 mg of iron per liter, the ratio of iron to zinc should be 1: 1 provided there is satisfactory satisfactory hematologic indexes and the exclusion of possible interactions between these minerals [32].

High concentrations of iron in infant formulae may be rationalized by both concern for iron deficiency and poor bioavailability in infants though formulae fortification has improved the bioavailability to near breast milk levels [33]. The risk for iron deficiency at 0-6 months of age in healthy term infants is low due to enough iron stores at birth [34]. However, since infants aged 0-6 months lack the capacity to control iron absorption because of absence of divalent metal transporter 1 (DMT1) which regulates iron absorption, high iron levels in infant formulae may predispose infants at this age to excessive absorption of iron [35]. Uncontrolled iron intake in humans is of delicate public health concern since man does not seem to have a defined system of iron excretion [36], [37]. Excessive iron ingestion in infants has been associated with impaired growth and increased morbidity [38], impaired cognitive development and immune function [39], [40]. A formulae fortification level of 2 mg/l in milk-based formulas has been proposed sufficient for healthy term infants [2], [33] in the absence of an established optimal iron intake in infants [37]. The iron levels in milk-based formulae 4.08 ± 1.95 and range 2.01-8.02 mg/kg, and in cereal mixed formulae 4.49 ± 1.56 and range 2.34-6.97 mg/kg exceed the proposed fortification level of 2 mg/l.

Low levels of manganese have been found in infant formulae in Nigeria [41]. Infant formulae can be fortified with manganese. Deficiency of manganese is virtually unknown in humans but there is a growing evidence of its toxicity [42], [43]. Manganese exists in its divalent form in infant formulae [44] but in breast milk it is in the trivalent form [45]. Like iron, DMT1 is also responsible for the absorption of manganese [46]. Like iron too, there can be manganese overload following consumption of infant formulae with high levels of manganese due to under expression of DMT1 in infancy. Breast milk contains only very few micrograms of manganese, therefore the guidance upper limit of manganese in infant formulae is set at the value that did not consider the increasing evidence of neurotoxicity in children [47] as 670 μg/l [48]. The mean concentrations of manganese in milk-based, cereal and cereal mixed infant formulae were 0.15 ± 0.09, 0.09 ± 0.06 and 0.10 ± 0.08 mg/kg respectively. Neuropsychological effects like poorer cognitive outcome and hyperactivity have been seen in children with elevated postnatal exposure from different sources including infant formulae [43]. It is estimated that one out of every 125-150 children may be living with autism in Nigeria [49]. There remains a knowledge gap on the impact of dietary manganese on childhood autism in Nigeria.

Owing to the low absorption of cow's and soy milk, the minimum level for manganese in infant formulae based on the 3 – 4 µg/l mean concentration in breast milk is about 7µg/l[2] (SCF, 2003). Increasingly there have been indications of significant associations between ingestions of relatively low manganese and lower IQ scores [50], suggesting that the manganese levels of some of the infant formulae in this study may not be safe for infants.

The estimated daily intake EDI for all age groups of infants for iron, manganese, cobalt and zinc were below the adequate daily intake ADI of (0.27 mg/day, 600 ug/day, 12 ug/day and 200 ug/day) respectively. Whereas the EDI of chromium at age group 6-12 months for cereal based and cereal mix base were below the ADI of 5.5 ug/day, the EDI of other age groups in all infant formulae exceeded the permissible ADI. These essential trace metals namely iron, zinc, manganese, chromium and cobalt are required in smaller amounts but perform numerous physiological functions in the organism. Iron forms part of hemoglobin necessary for the transport of oxygen [7], [51]. These are usually added to infant formulae to standardize their concentrations with the breast milk. Zinc and manganese play important roles as cofactors in enzymes intervening in multiple metabolic reactions [52].

Some infant formulae can provide more than the recommended adequate intake amounts of the essential trace metals like manganese and chromium, but none exceeded the tolerable upper intake concentration of manganese [53] or permitted daily exposure of chromium [54]. Similarly, some infant formulae with the highest cobalt concentration can deliver slightly more than the permitted daily exposure of cobalt [54]. The chromium concentration in cereal-based infant formula in this study ranged from 0.002-1.51 with a mean of 0.36 ± 0.62 mg/kg. The chromium level in cereal based infant formulae from Brazil ranged from 3.9 to 35.2 µg/kg, 5.4 – 14.9 µg/kg, 5.2 – 36.1 µg/kg and LOD – 12.6 µg/kg in Brazil, Mexico, Germany and Canada respectively [55].

A likely limitation of the present study is that, the results/data may be conservative since they were obtained after mixing infant formulae with deionized water given that some essential trace metals can occur in water [56]. Nonetheless, this study has that the estimated daily intake of chromium in the infant formulae exceeded its RDA. Since the ultimate goal of infant feeding is to ensure that concentrations of essential trace metals in infant formulae are equivalent to or lower than those found in human milk, regulatory bodies in developing countries should periodically conduct targeted analyses of infant formulae types from different manufacturers to ensure that no processes or ingredients have been inadvertently introduced to compromise quality.

In conclusion, it is desirable that infant formulae should supply the growing infant with adequate amounts of essential trace metals at levels devoid of excessive exposure. The present study has shown that infant formulae may contribute higher chromium than breast milk. With respect to evidence of cognitive impairments and other childhood health effects associated with these essential trace elements more research on the bioavailability and role of speciation is urgently needed. Also, the wide range of variations in the infant formulae in this study cast some doubts on the knowledge of infant's requirement of these elements, and the need for their fortification to such high concentrations.

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