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Research Article

Analysis of Heavy Metals and Hydrocyanic Acid in Selected Infant Formula in Abuja, Federal Capital Territory of Nigeria

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Abstract: Infant formula mimics breast milk as closely as possible and can be advantageous for the proper growth and development of children as adequate nutrition during infancy is essential for lifelong health and wellbeing. However, there are many possible risks associated with the use of artificial formula due to the presence of contaminants .In this study the presence of heavy metals and hydrocyanic acid which long-term exposure could have serious implication on children's health were assessed in some infant formula in federal capital territory, Abuja, Nigeria. The estimated daily intake of these heavy metals (copper, iron, lead, chromium and arsenic) and hydrocyanic acid were also computed from the result. It was observed that all products contain heavy metals (both essential and toxic elements) within the tolerable limit for infants and very low amount of hydrocyanic acid. Therefore, infant formula produced in or imported to Nigeria can serve adequately as complementary food for infants because they are adequate in essential trace elements, contain the toxic elements within safety limits and low hydrocyanic acid concentration that cannot lead to cyanide poisoning.
Keywords: Infant formula, heavy metals, breast milk, hydrocyanic acid, contaminants

INTRODUCTION

Breast milk is the best natural source of nutrition for infants; it contains optimal balance of fats, carbohydrates, proteins and immune factors that help infants to combat infections [1, 2]. It is recommended as the sole source of infant nutrition for the first 6 months of life. However, less than 35% of the world's infants are exclusively breast fed at this age[3] due to many factors, complementary feeding may therefore becomes necessary[4-6], thus the need for infant formula[3,7].

Complementary food is defined as all foods and fluids other than breast milk given to the infant, of which infant formula are the commonest. Infant formula has a special role to play in the diets of Infant because they are the major source of nutrients. They are primarily derived from animals or plants and are therefore mostly milk-, soy- or cereal-based. They mimic breast milk as closely as possible, although it is difficult to produce a formula equal in all respects to breast milk [6, 8-10]. The use of formula can be advantageous for the proper growth and development of children. However, they are rated second best to natural breast milk and possible risks associated with the use of artificial formulae like development of immune system disorders, diabetes, food allergies, obesity, and coronary heart disease in later life have been observed[11,12].

Many elements are present in foods naturally or through human activities, such as processing, storage, farming activities, industrial emission, use of poor quality water for formula preparation, improper handling of formula by mothers or through intentional addition[6,13,14].Some of these elements are necessary for the maintenance of the physiological and metabolic processes of the body of which appropriate intakes are required: deprivation can lead to diseases [6]; whereas, excessive intake may leads to serious health implications[4,9,15-17].In addition to these beneficial elements, toxic elements are also present; these do not have nutritional values. Consequently, they are not essential or required in human foods. Their presence in foods or food products is as a result of possible contamination during industrial food processing, metal contaminated raw materials, contaminated food preservative or leakage of metals from packaging material.[4,6,9,18,19]. Monitoring of these elements in infant formula is of considerable health importance in a bid to protect the infants from their acute and chronic toxicity on infants. Infant formula may also contain toxic substances such as hydrocyanic acid (HCN) released during the hydrolysis of cyanogenic glucosides[20-23].Hydrocyanic acid is toxic to humans eliciting reactions similar to cyanogenic glucosides like vomiting, exacerbate goitre, cretinism in iodine-deficient regions, nausea, dizziness, stomach pains, weakness, headache, diarrhea, paralysis, neurological disorders, Tropical Ataxic Neuropathy (TAN), stunting of children and occasionally, death [24-26].

The aim of this study was to test for the presence of hydrocyanic acid and selected heavy metals (Pb,Cr,As,Fe,Cu), in selected infant formula in Abuja,Nigeria.

EXPERIMENTAL SECTION

Experiment site

The survey and the analyses were carried out in the chosen markets in Abuja and Department of Biochemistry, Bingham University Karu Nasarawa state, Nigeria, respectively.

Survey and collection of samples

A survey of infant formula commercially available in Abuja was carried out in supermarkets, departmental stores, open market, shopping malls and various sales outlets. Ten different samples were selected for this study. The selection was made to reflect brands that are readily available in the market and frequently utilized for infant. Selected samples have the same packaging materials (tin) and close manufacturing date. They are shown below and represented by their NAFDAC number except a sample without a NAFDAC number.

S/N	NAFDAC Number	Manufacture Date	Age Range (In months)	Manufacturer
1	A1-0525	30-05-2012	0-6	Nestle France SAS
2	01-7770	10-2012	6 onward	Friesland Campina, Holland
3	01-4391	12-2012	12-36	frieslandCampina WAMCO Nigeria PLC
4	B1- 2780	22-02-2012	6-12	Wyeth Nutritionals Ireland
5	01-9543	06-08-12	6-12	Nestle France SAS
6	01-0672	08-20212	6 onward	Nestle Nigeria PLC
7	015831	05-2012	0-12	Abbott Laboratory B.V.
8	Cerelac (NAFDAC number not indicated)	Not indicated	6 onward	Nestle Spain
9	B1-1721	21-09-11	6 onward	Nutricia Cuijk B.V.
10	01-0317	04-2012	0 onward	Abbott Ireland

Chemicals

Potassium cyanide (Sigma chemical Co, St Louis, USA), Picric acid (BDH, England), Sodium hydroxide(Hopkins and Williams Ltd, England), Nitric acid (Sigma chemical Co, St Louis, USA),Sulphuric acid (Sigma chemical Co, St Louis, USA),Hydrogen perioxide (Sigma chemical Co, St Louis, USA)

Methods

Determination of heavy metals in the samples

The samples were digested following the procedure described by Iyaka[27],briefly, 20ml HNO_3 was added to 10.0g of the sample and allowed to stand for 15min. The mixture was heated until the liquid reduced to 5ml. After cooling, 20ml HNO_3, 10ml H_2SO_4 and 8mlH_2O_2 were added and the contents were evaporated to 5ml. After cooling, to eliminate residual acid, 10ml deionized H_2O was added and the mixture was boiled for 10min (this was repeated twice). After cooling the digest was filtered into 25ml volumetric flask and made up to mark with deionized H_2O. The infant formula filtrates' were subsequently analyzed for the presence of heavy metals (Pb, Cd, Cu, As and Cr) by atomic absorption spectrophotometer (Model GBC Pal 3000 Auto sampler device).

Determination of hydrocyanic acid in the samples

The hydrocyanic acid of the samples was determined using the alkaline picrate method with modifications [28].1.0 g of the sample was weighed out from each bread or wheat flour sample in an electronic weighing balance. This was transferred into a 100ml volumetric flask. Ten milliliter (10 ml) of distilled water was added; the mixture was shaken and allowed to stand for 30 mins at 28 ± 5°C. The supernatant was collected and measured with measuring cylinder and was used for cyanide analysis. For this analysis, 1ml of the sample was pipette into test tube followed by 1ml of 0.04M picric acid and 1ml of 0.75M NaOH .The solution in the test tube was mixed and incubated for 15 minutes at room temperature. In this method, hydrocyanic acid (HCN) released during hydrolysis of the cyanogenic glycosides reacts with picric acid to produce a yellow colored solution. Sodium hydroxide preserves the HCN released. Colour intensity was measured spectrophotometrically at 540nm.The same process above was used for all other samples and the reference standard.

Calculation:

$$\mathbf{CONC_{HCN}} = \frac{\mathbf{O.D_{TEST}}}{\mathbf{O.D_{STD}}} \times \frac{\mathbf{CONC_{STD} \times Volume\ obtained}}{\mathbf{Volume\ of\ sample\ used}} \times \mathbf{1000}$$

O.D = Optical Density/Absorbance, STD= standard, Conc= Concentration

RESULT AND DISCUSSION

The heavy metal contents and estimated daily intake of infant formula analyzed are shown in Tables 1and 2 respectively. The estimation of daily intake by infants through infant formula was calculated based on the consumption of 60 kg/year of formula by a 6-12 months old baby with average body weight of 7.0 kg [4,29] Thus, the average daily consumable weight of powdered infant formula would be 164.4 g. The estimated daily intake of elements was then calculated by multiplying the overall average concentration of the elements obtained in the formulae (Table 2) by average daily consumable weight of the formula.

The lead (Pb) contents of infant formula analyzed ranged between 0.000 to 0.864ppm. The lead levels in all the formulae analysed were significantly less than the Food and Agriculture Organization and World Health Organization maximum value of 25 µg/kg[9]. The source of the small amount of Pb detected in the formulae may possibly be due to food contamination during industrial food production, presence in the raw materials or leakage from packaging materials. Lead ingestion in infants may lead to suppression of mental capacity or retardation (a High negative correlations between Pb exposure and children's intelligent quotient have been reported)[30-33] and aggressive behavior[34]. The levels of Lead detected in the formula samples are low and poses no danger to infants. With the average body weight of 7kg for a 6-12 months old baby, the lead contents(Table2) of all the samples are lower than 25 µg/kg Provisional Tolerable Weekly Intake (PTWI) recommended by FAO/WHO joint committee on food additives[9,29,35]**.**

Chromium contents of the infant formula examined ranged from 0.00 to 0.062 ppm with samples (01-4391 and 01-9543) having undetectable amount and sample (B1- 2780) having the highest(Table 1).This is in contrast with the findings of Olu-Owolabi et al.[4],who reported zero level of chromium in some infant formula in Nigeria but agrees with Al Khalifa and Dilshad Ahmad[9],who reported various level of chromium in infant formula marketed in Saudi Arabia. Chromium plays some important roles in the body,it is essential for normal glucose, lipids and protein metabolism.The daily intake of 50-200 µg chromium has been recommended for adults by US National Academy of Sciences. Too little chromium in the diet may lead to insulin resistance. However, there is still no standard against which chromium deficiency can be established.[36,37]. The toxic effects of Chromium intake include skin rashes, nose irritations, bleeding, stomach upset, kidney and liver damage, nasal itch, insulin resistance and lungs cancer[38].

Arsenic is ranked as one of the top three most hazardous substances in the priority list of toxic metals compiled by Environmental Protection Agency, the Agency for Toxic Substances and Disease Registry (ATSDR) in 2001[39].Arsenic was detected in five of the samples analysed(Table 1) and the estimated daily intake for an infant weighing 7kg is shown in (Table2).These quantities are lower than the values obtained by Al Khalifa and Ahmad[9]. Continuous exposure to lower levels of exposure to arsenic may cause nausea, vomiting, diarrhea, decreased production of red and white blood cells, abnormal heart rhythm, blood vessel damage, a "pins and needles" sensation in hands and feet, shock, coma, convulsions, irritation, inflammation, ulceration of mucous membranes and skin. Chronic toxic effects could lead to fatigue, loss of energy, gastrointestinal disturbance, nasal septum perforation, ulceration in folds of skin, increased pigmentation of skin, appearance of small "corns" or "warts" on the palms, soles, and torso; exfoliative dermatitis, rashes, muscular paralyses, atrophy, sensory disturbances, visual disturbances and blindness, degeneration of liver (cirrhosis) and kidneys, damage to upper respiratory tract, lung cancer, immunosuppression and death.[36,40-44].

The copper contents and estimated daily intake by of infants are shown in Tables 1 and 2 respectively. Copper is an essential dietary trace element required for proper development and general repair of body tissues. They are found in many metalloproteins and essential enzymes controlling cellular activities; it plays a key role in bone formation, skeletal mineralization and in maintaining the integrity of the connective tissues. Hence, they are usually added in trace amounts to human foods or taken as food supplements. Deficiency of copper causes low white blood cell count and poor growth. However, at elevated concentrations, they could have serious health implications for human. Excess intake of copper can cause vomiting, nervous system disorder , Wilson's diseases, liver and kidney damage, brain damage, intestinal discomfort, dizziness and headaches, while excess accumulation of copper in liver may result in hepatitis or cirrhosis and in a hemolytic crisis[4,45-48] .Joint FAO/WHO Expert Committee on Food Additives [49] has set the provisional tolerable daily intake of copper at 3 mg.

All formulas examined were fortified with iron and the quantity detected in the samples ranged from 10ppm to 37.9ppm (Table1). Iron is required in trace amounts for proper body development and for general repair of body tissues and are considered essential elements. They are found in many metalloproteins and essential enzymes controlling cellular activities. Iron plays many key roles in biological systems, including oxygen transport (hemoglobin and myoglobin), respiration and energy metabolism (cytochromes and iron-sulphur proteins), destruction of hydrogen peroxides (hydrogen peroxidase and catalase), and DNA synthesis (ribonucleotide reductase). The deficiency of iron results in anemia which is recognized by its symptom such as low blood iron level, small red

blood cells and low blood hemoglobin values [50].The high level of iron in infant formula as observed in this study has been justified by low bioavailability in breast milk as well as concern for iron deficiency in infants. The health concern however, was that infants lack the ability to regulate iron absorption[51,52] as a result, iron may be absorbed at levels above those required, resulting in iron toxicity. Excessive iron intake has been associated with impaired growth, increased morbidity, impaired immune and cognitive development function[51-57].The optimal Fe intake for infants has not yet been established[53],the values obtained does not exceed the upper the limit set by EC Regulation 446/2001 and far below the values obtained in Saudi Arabia by Al Khalifa and Dilshad Ahmad[9].

Table 1: Heavy metal contents of the infant formula

Samples	Heavy Metals (ppm)				
	Cu	Fe	Pb	Cr	As
01-4391	1.66± 0.04	27.5±0.51	0.515±0.013	N.D*	0.045±0.005
B1-1721		28.4±0.42	0.224±0.012	0.035±0.005	0.045±0.005
01-9543	4.62±0.10	29.2±0.80	0.099±0.004	N.D	N.D
B1- 2780	5.04±0.06	37.9±0.42	N.D	0.062±0.021	N.D
01-0317	3.48±0.07	32.4±0.40	0.160±0.010	0.039±0.003	0.020±0.002
01-7770	2.47±0.02	10.4±0.40	0.188±0.006	0.033±0.003	N.D
01-0672	4.41±0.02	36.9±0.51	N.D	0.017±0.002	N.D
015831	2.37±0.06	29.0±1.00	0.864±0.012	0.055±0.005	0.019±0.003
Cerelac (NAFDAC number not indicated)	1.297±0.04	33.8±0.81	0.474±0.013	0.031±0.001	0.012±0.003
A1-0525	2.71±0.09	33.9±0.80	0.3197±0.010	N.D	N.D

The values are mean ± standard deviation of triplicates, ND= not detected

Table2: Estimated daily intake of heavy metals from infant formulae

Samples	Heavy Metals (mg)				
	Cu	Fe	Pb	Cr	As
01-4391	0.273	4.521	0.085	N.D	0.007
B1-1721	0.363	4.669	0.037	0.006	0.007
01-9543	0.760	4.800	0.016	N.D	N.D
B1- 2780	0.823	6.231	N.D	0.010	N.D
01-0317	0.572	3.847	0.026	0.006	0.003
01-7770	0.406	1.710	0.031	0.005	N.D
01-0672	0.725	6.066	N.D	0.003	N.D
015831	0.390	4.468	0.142	0.009	0.003
Cerelac (NAFDAC number not indicated)	0.213	5.557	0.078	0.005	0.002
A1-0525	0.446	5.573	0.053	N.D	N.D

The hydrocyanic acid (HCN) contents of the samples analyzed were in the range 0.21 to 0.62mg/1000g and 0.035 to 0.102mg estimated as daily intake of hydrocyanic acid (HCN) from the infant formula (Tables 3 and 4). The result showed a low hydrocyanic acid (HCN) content in the samples.High hydrocyanic acid (HCN) intake could results in release of large quantity of cyanide that can inhibit cellular oxidation and the activity of vitamin K dependent carboxylase of the liver in addition to exacerbate goitre , cretinism, nausea, dizziness, stomach pains, weakness, headache, diarrhea , paralysis, neurological disorders, Tropical Ataxic Neuropathy (TAN), stunting of children and occasionally death associated to its consumption in large quantity through food[58,59,60,61]. Consumption of infant formula analysed will not lead to the lethal dose of 35 mg hydrocyanic acid per kg body weight reported by Eneobong[19] .

Table 3: Hydrocyanic Acid (HCN) Contents Of The Samples

Infant formula	Hydrocyanic acid ((ppm)
01-4391	0.5±0.03
B1-1721	0.62±0.03
01-9543	0.30±0.0006
B1- 2780	0.24±0.03
01-0317	0.39±0.004
01-7770	0.28±0.03
01-0672	0.22±0.04
015831	0.40±0.001
(NAFDAC number not indicated)	0.21±0.002
A1-0525	0.304±0.002

The values are mean ± standard deviation of triplicates

Table 4: Estimated Daily Intake Of Hydrocyanic Acid From Infant Formula

Infant formula	Hydrocyanic acid (mg)
01-4391	0.082
B1-1721	0.102
01-9543	0.049
B1- 2780	0.039
01-0317	0.064
01-7770	0.046
01-0672	0.036
015831	0.066
(NAFDAC number not indicated)	0.035
A1-0525	0.050

The values are mean ± standard deviation of triplicates

CONCLUSION

The results of this research work showed that infant formula produced in or imported to Nigeria can serve adequately as complementary foods for infants because they are adequate in essential trace elements, contain the toxic elements within safety limits and low level of hydrocyanic acid that cannot lead to cyanide poisoning. However there is a need to investigate the source(s) the of contaminant in these infant formula.

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