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Technical Note

Investigation of heavy metal concentrations and determination of estimated daily intake and health risk index infant formula and baby foods in Zahedan in 2020

Aram DOKHT KHATIBI[1], Maryam BAZZI[2], Davoud BALARAK[1,*]

[1]*Department of Environmental Health, Health Promotion Research Center, Zahedan University of Medical Sciences, Zahedan, 98167-43463, Iran*
[2]*Student Research Committee, Zahedan University of Medical Sciences, Zahedan, 98167-43463, Iran*

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ABSTRACT

Despite the importance of breastfeeding, complementary or complete feeding with infant formula is performed worldwide. Metal contaminants, especially lead and cadmium, have many toxic and adverse effects, especially in children. Therefore, this work was conducted to investigate the levels of lead and cadmium in infant formula and baby foods. This study was performed on 18 samples of infant formula and 7 samples of baby food. Random sampling was performed among the best-selling and most consumed brands available in the Zahedan market, and elements were measured by atomic absorption spectroscopy using a graphite furnace. Moreover, Estimated Daily Intake (EDI) and health risk index (HRI) were calculated for the average of the total data. The mean concentrations of lead in infant formula and baby food samples were 14.7±0.98 and 13.77±1.51 µg/kg, respectively; lead was observed in all samples, although the difference between the amount of lead in samples no. 2 and no. 9 and its standard amount was significant ($p<0.05$). The average concentrations of cadmium in infant formula and baby food samples were 0.097±0.016 and 0.705±0.12 µg/kg, respectively; it was observed in 22% of infant formula samples and 57% of baby food samples, but the difference in cadmium concentrations in the samples was not significant compared to the standard concentration ($p>0.05$). The highest EDI for lead in infant formula for ages of 0 to six months was 0.22 µg/kg.day.bw. Also, the HRI for all samples was much less than one, which indicates that the baby's food and infant formula are healthy in terms of daily intake of cadmium and lead. Finally, it can be concluded that the amount of cadmium and lead in the infant formula and baby food samples, according to some indicators, is less than the standard level.

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***Corresponding author.**
*E-mail address: dbalarak2@gmail.com

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INTRODUCTION

Industrial and chemical pollutants have directly and indirectly endangered human life, and heavy metals are one of these pollutants. Among the heavy metals, lead and cadmium can enter the body through food and, if contamination persists, accumulate in the body and cause acute and chronic poisoning in humans [1]. Some other metals, such as lead and cadmium, can cause the poisoning of living organisms in high concentrations [2]. High concentrations of lead in the blood and its accumulation in muscles and joints cause pain in these areas as well as the impaired transfer of iron molecules and eventually anemia. The main source of industrial cadmium is phosphate fertilizers, which are absorbed by plants and accumulated in tissues, such as the liver and kidneys, and cause anemia and increased blood pressure, bone deformation, bone fractures, and short stature [3, 4]. Among all the factors that affect the growth and development of infants, nutrition has the highest position [5]. Despite the importance of breastfeeding for the optimal growth, mental development, and health of the child, complementary or complete feeding with infant formula is performed worldwide [6]. Infant formula and baby food, which are used as a complementary food or main food for children, contain essential nutrients that are beneficial for the health of children and infants. Due to the importance and sensitivity of the nutritional value of infants and children, raw materials in the preparation of this product has a very desirable position and quality [7]. Infant formula has all the basic needs of children. It has been found to be one of the most popular dairy products due to its high durability, and its use is important in the production of many other products such as ice cream, cheese as well as a component in many bakery products, confectionery, etc. [8]. The amount of infant formula consumed depends on different conditions according to the amount of energy and minerals needed by children at different ages [8]. The composition of infant formula and baby food is different from the foods that make up the general diet; thus, it is necessary to know the amount of many metals and elements in this food group. Essential elements are usually added to infant formula during production to meet nutritional requirements, so it is important to control the amount of these added elements because the high levels of them can act as a potentially dangerous source [9]. Newborns are sensitive to toxins, especially metals, due to the formation of body structure, high intestinal absorption, and higher energy consumption in the first year of life. Numerous studies have shown that infants are vulnerable to contact with heavy metals due to underdevelopment of the renal system and low tolerance to these contaminants [10, 11].

Severe exposure to lead and cadmium in infant formula can damage kidney function and reduce kidney function, especially in preterm infants [12]. Exposure of fetuses and infants to lead has devastating effects on the development of the nervous system and causes learning disabilities and reduced IQ in later stages of life [13]. According to the Alimentarius Codex and the Iranian standard, the permissible amounts of lead in infant formula and baby food are 0.01 mg/kg and 0.02 mg/kg, respectively, and the amount of cadmium is zero [14, 15]. In a study conducted in Shakur, Libya, Elbagermi et al. [16] determined the amount of essential and non-essential elements in some commercial baby foods and observed that essential and non-essential elements and toxic elements were in the safe range. Also, in a study entitled "determination of Heavy Metals Remaining in Dry Milk in Mansoura, Egypt", Abdelkhalek et al. [17] reported that most of the samples contained lead and cadmium, and their levels were higher than allowable level. Malakoutian et al. [18] conducted a study in different cities of Iran to measure the cadmium and lead levels in infant formula; they detected that the amount of these elements is higher than the standard and global limits, which may be due to the high concentration of pollutants in raw materials.

In recent years, when people are exposed to different types of diseases, prevention will be a better solution than treatment. Unfortunately, the importance of this issue in developing countries is ignored, and this is the fundamental difference between developed countries and developing countries. Sistan and Baluchestan is one of the poorest provinces of Iran; the importance of using complementary nutrition in this province is very high due to the large number of children in a family. Determining the levels of heavy metals in infant formula and baby food samples and collecting them from the market will prevent serious diseases in the future; these issues can reduce the immune system in the face of other diseases and ultimately increase treatment costs.

Assessing the level of heavy metals in infant formula and baby food has been done in Iran and other parts of the world. The results of conducted studies have shown that it is still important to measure these compounds in samples on the market due to introducing new products and high variety and high consumption of these products. Therefore, the aim of this study was to investigate the amount of heavy metals in infant formula and baby food offered in Zahedan in 2020.

MATERIALS AND METHODS

This is a descriptive cross-sectional; it was conducted in the analysis laboratory of Zahedan University of Medical Sciences in 2020. Hydrochloric acid and nitric acid were used to digest samples, and standard solutions of heavy metals lead and cadmium (a concentration of 1000 mg/L) were prepared by Merck, Germany. High purity distilled water was taken from the Milli Q device, Millipore instrument. The atomic absorption device with graphite furnace used to measure heavy metals was Perkin Elmer Model A Analyst 700 (made in USA). Nebertherm electric oven (made in Germany) was used for producing ash.

During the experiments, we employed analytical grade reagents. To prepare the standard solutions, stock standard (1000 mg/L) was used. Drawing the calibration curves was done by consideration of five points (r2 = 0.998 to 0.9996). By the addition of metal standard solutions to a certain amount of sample, the matrix spike recovery was accomplished. After that, digestion of spiked samples was done, and their analysis was then considered (recovery percentages were from 95.5% to 104.6%). In this study, the limit of detection (LOD) was estimated as three times the pooled standard deviation of six runs of blank measurements. LOD values for Pb and Cd were obtained to be 0.008 and 0.0096 mg/kg, respectively. Also, the preparation of blank samples was done by employing the procedures used for the samples.

18 different brands of infant formula and 7 brands of baby food were collected in a census from Zahedan supermarkets between March and April 2019. Infant formula and baby food were broadly existing on the market and were employed to consume by infants from six months to one year. Metal containers were employed for all products, including infant formula and baby food. In order to easy identification of samples, the codes were considered for them. Sample containers and all glassware, after placing in 10% nitric acid for 24 h, were thoroughly washed with distilled deionized water.

For the experiment, the initial weight of the crucibles that have been washed with acid and dried was first determined. Then, 5 g of the sample (infant formula/baby food) was added to the crucibles. After weighing all the samples, the crucibles were transferred to the kiln, and the samples were ashed by gradually increasing the temperature to 500°C. The samples were placed in the kiln for 8 hours. After cooling, 10 ml of 6 M hydrochloric acid was added to the ash samples and placed on a heater at 100°C for 1 hour to dry. The samples were then mixed with 10 ml of 0.1 M nitric acid solution, and finally, the amount of lead and cadmium was determined by graphite furnace by atomic absorption spectroscopy.

The FAO/WHO Joint Committee on Food Additives tried to assess the potential and chronic hazards of toxic elements by introducing parameters such as Tolerable Daily Intake (TDI) and Provisional Tolerable Weekly Intake (PTWI) and measuring the estimated daily intake (EDI). Estimated Daily Intake (EDI) was calculated by multiplying the element concentration by the daily consumption and dividing it by the infant's body weight.

For estimation of the health risk index (HRI), mean daily intake values of heavy metals were divided by their safe limits to assess risk exposure to these metals in the baby formula.

According to Tables and dosages, which have been suggested by manufacturers, the average daily consumed powder infant formula from feeding at 6 and 7−12 months were 200 and 100 g/day; however, according to literature, the mean body weights of infants at both ages were 6.5 kg and 10 kg, respectively [16].

Analysis of data were performed using one-way analysis of variance (ANOVA) and Tukey post hoc test at 95% confidence level. SPSS version 20.0 was employed for statistical analysis.

RESULTS AND DISCUSSION

According to the British Ministry of Agriculture, Fisheries, and Food in 1999, the concentration of cadmium in infant formula was usually 1 ng/g [19]. According to the results of our study, the average cadmium was 0.097±0.016 in infant formula and was 0.705±0.12 in baby food (Table 1). The highest amount of cadmium was 1.19 ng/g in product No.4 for infant formula and was 1.8 ng/g in product No.5 for baby food. By comparing the average of all samples with the values of other countries, we can see the low level of cadmium in infant formula and baby food offered in Zahedan. The standard limit for cadmium in conventional liquid milk is $10 \mu g.l^{-1}$ according to Codex standard [1] and 10 micrograms per gram according to the American Public Health Association standard. The authority states that the daily limit of cadmium intake for infants is 10 ng/kg.bw. d.

According to published studies, the range of cadmium infant formula in some studied countries was as follows: UK (1-3 ng/g) [20], India (0.1-0.07 ng/g) [20], Saudi Arabia (7 ng/g) [13], Spain (6.8 ng/g) [21], and Poland (0.4-2 ng/g) [22]. In a study by Ikem et al. [2], it was found that cadmium ranged from indistinguishable values in Nigeria and the United States to 0.3 ng/g in the United Kingdom, which was the lowest value compared to other studies.

In the present study, the levels of Cd in the infant formula and Infant cereals were observed to be in the range of 0.000097 mg/kg to 0.000705; this was in accordance with the results of another study [27]. Nevertheless, in other studies accomplished in different countries, such as Addis Ababa, Ethiopia [23], Nigeria [24], and Türkiye [28, 29], minor levels of Cd were detected in baby formulas. The types and origins of raw materials, manufacturing processes, and adulteration of the products are the factors that are effective on the levels of the metals in infant feeding.

Table 2 compares concentrations of desired elements in infant formulas and infant cereals evaluated with data obtained in the previous studies. In the present study, the mean of lead in the taken samples was observed to be 0.147 mg/kg; this result was in agreement with the results observed in other studies [11−15]. In the study conducted by Aguzue et al. [24] in Abuja, Nigeria, the lead levels in infant formula were reported to be 0.23-0.08 mg/kg. However, lower amounts of lead (0.00375−0.0249 mg/kg) were observed in study performed in Türkiye [16]. Moreover, the levels of lead in the powdered infant formulas in Addis Ababa, Ethiopia, were detected to be lower than the detection limit.

One of the reasons for the higher concentration of lead in infant formula and baby food can be due to the high

Table 1. Statistical analytical results of heavy metals in Infant milk formula and Infant cereals samples

sample	Pb (μg/kg)	positive samples=100%	Cd (μg/kg)	positive samples=22%
Infant milk formula	Mean±S.D		Mean±S.D	
1	12.74 ±1.42	∑Mean±S.D=14.7±0.98	not detected	∑Mean±S.D=0.097±0.016
2	49.73±0.85	($p<0.05$)	ND	($p<0.05$)
3	15.01±1.46		ND	
4	12.91±0.96		1.19± 0.14	
5	8.27± 0.79		ND	
6	6.14± 0.52		ND	
7	5.92±0.41		0.22±0.08	
8	11.16± 0.53		ND	
9	45.27± 3.27		ND	
10	8.33±0.73		0.11±0.07	
11	10.15±0.95		ND	
12	15.65±0.72		ND	
13	10.92±0.64		ND	
14	6.11±0.47		ND	
15	15.20±1.23		0.24±0.12	
16	16.78±1.46		ND	
17	6.54±0.71		ND	
18	8.55±0.53		ND	
Infant cereals	Mean±S.E		Mean±S.E	positive samples=57%
1	14.52± 1.75	positive samples=100%	1.32±0.32	
2	18.16± 2.27		1.74±0.41	∑Mean±S.D=0.705±0.12
3	15.20± 1.89	∑Mean±S.D=13.77±1.51	0.26±0.08	
4	10.33± 1.03		ND	
5	8.51± 0.89		1.8±0.04	
6	15.46± 1.39		ND	
7	14.22± 1.37		ND	

Table 2. A comparison of metals levels (mg/kg) with reported data in the literature

Origin/market site	Cd	Pb	Ref
Ethiopia	ND	ND–0.103	[23]
Nigeria	0.05–0.4	0.08–0.23	[24]
Pakistan	0.0042–0.0123	0.0287–0.097	[25]
Kenya	ND	0.018–0.059	[26]
EU market	0.00033–0.00045	0.0082–0.043	[27]
Turkey	ND-0.00749	0.00375–0.0249	[28]
Zahedan	*0.000097±0.000016	*0.0147±0.00098	This study
	**0.0007±0.00012	**0.0137±0.0015	

*Infant milk formula, **Infant cereals

concentration of contaminants in raw materials [30]. For example, in a study conducted by Tajkarimi et al., it was testified that the average concentration of lead in raw milk entering 15 milk factories in different parts of Iran was 7.9 ng/ml [32]. Therefore, it is necessary to pay more attention to the processing and packaging processes of infant formula by monitoring the quality of raw milk imported to the factory to prevent contamination of the product. Due to the nutritional importance of calcium and zinc and their inhibitory properties in the absorption of lead and cadmium,

Table 3. Average estimated daily intake of elements in formula and infant formula and comparison with permitted levels

	Age (months)	Pb	Cd	P Pb	P Cd
TDI (μg/kg.day.bw)	0-6	1.8	1	-	-
	6-12	3.6	0.5	-	-
PTWI (μg/kg.week.bw)	0-6	25	7	-	-
	6-12	12.5	3.5	-	-
EDI (μg/kg.day.bw)	0-6	0.226	0.001	0.035	0.056
For Infant milk formula	6-12	0.147	0.0009	0.034	0.075
EDI (μg/kg.day.bw)	0-6	0.211	0.01	0.046	0.035
For Infant cereals	6-12	0.137	0.007	0.044	0.085
HRI	0-6	0.12	0.1	-	-
For Infant milk formula	6-12	0.04	0.09	-	-
HRI	0-6	0.117	0.1	-	-
For Infant cereals	6-12	0.038	0.7	-	-

measures should be taken to enrich infant formula with these elements [31].

The reason for increasing Pb level in infant formula may be the original contaminated cow‘s milk employed for producing these products with Pb. The excessive exposure of lactating cows to environmental Pb from heavy traffic and consumption of contaminated feeding stuff and water may be the factors of the contamination of original cow's milk. Furthermore, contamination of raw milk may also be due to metallic Pb from Pb soldered cans. There is an association between Pb with caseins in cow's milk, and freezing or heating has no significant effect on the distribution of Pb in cow's milk and milk products. Nonetheless, the desiccation process leads to a substantial increase in the concentration of Pb [32-33].

Generally, there is a very low concentration of Cd in milk and dairy products unless dairy animals consume contaminated feeds and water. In addition, another source of Cd in milk and other dairy products is the contamination during storage, marketing, and leaching from containers. After heat treatment of milk, the distribution change of Cd occurs; description of this event may be performed by the formation of complexes between the whey proteins and the metal or to the desegregation of the Cd bound to casein micelles [34-35].

As can be seen in Table 3, the highest EDI value obtained from lead is 0.226 μg/kg.day.bw, which is less than the standard value, so there is no problem in that the daily and weekly standard equations are 1.8 and 12.5, respectively. Of course, it is necessary to mention that the average of all metals was used to obtain EDI.

According to a one-way analysis of variance, significant differences could not be detected between various brands of infant formula products for their lead contents ($p<0.05$). According to our obtained results, the amount of cadmium was not significant for any of the brands. Conversely, a suggestively higher mean value of lead was distinguished in products no.2 ($p=0.046$) and no.9 ($p=0.044$) for infant formula.

Examination of EDI in different age groups with the standard value for both metals showed that there is no significant difference between the standard value and the average EDI value (shown in Table 3).

Eventually, several factors, e.g., differences between species, features of the manufacturing practices, and probable contamination caused by the equipment during the process, are considered to be effective on heavy metal concentrations. Some parameters such as pH, quality of raw materials of containers, and equipment affect the oxidation of containers and equipment, which increase in this process leads to rising the metal contents of samples. Subsequently, the metal levels in milk powder are affected by the metal contents of the original milk.

CONCLUSION

In this study, the concentration of lead and cadmium were investigated in 18 brands of infant formula and 7 brands of baby food consumed in Zahedan. EDI and HRI indices were also calculated for comparison with TDI and PTWI standards. The results showed that lead was present in all samples and the highest amount was in samples no. 2 and no. 9, which were equal to 49.73±0.85 and 45.27±3.27μg/kg. Also, out of 18 brands of infant formula, cadmium was found only in 4 brands; its average value in them was equal to 0.097±0.016 μg/kg, while its average value in baby food was 0.705±0.12 μg/kg. EDI levels for lead and cadmium for both age ranges and both infant formula and infant food samples were lower than the standard TDI and PTWI indices. Moreover, for both investigated metals, the health risk indices were obtained to be less than the threshold of 1 at mean exposure; this denotes that consumption of the formula feeding to the general infants is associated with low health risks caused by these metals.

Nevertheless, since infants are a more sensitive population, a regular assessment of infant formula products in terms of toxic heavy metals is necessary.

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CONFLICT OF INTERESTS

No competing interests are declared by the authors.

AUTHOR CONTRIBUTION

The conception and design of the study are the result of the contributions of all the authors. A. D. Khatibi and M. Bazzi prepared the material, collected the data, and performed the analyses. Davoud Balarak was responsible for writing the first draft of the manuscript. All the authors provided their comments on the earlier versions of the manuscript. The final manuscript was read and approved by all the authors.

DATA AVAILABILITY STATEMENT

In order to request the datasets utilized and analyzed in the present study, the corresponding author may be contacted.

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CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

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