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Assessment of some element content and potential health risks in infant formulas available in Turkish markets

Kubra Ozturk[1], Cigdem Er Caliskan[2*], Zehra Akinci[3], Harun Ciftci[4,5]

[1]Kırşehir Ahi Evran University, Project Coordinator, 40100, Kırşehir, Türkiye
[2] Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Field Crops, 40100, Kırşehir, Türkiye
[3] Fırat University, Faculty of Engineering, Department of Bioengineering, 23119, Elazığ, Türkiye
[4] Kırşehir Ahi Evran University, Faculty of Medicine, Department of Medical Biochemistry, 40100, Kırşehir, Türkiye
[5] Çankırı Karatekin University Rectorate, 18100, Çankırı, Türkiye

Abstract

Accurately determining the composition of essential and toxic elements in commercial infant formulas is critical to ensuring safe nutrition for infants. In this study, the concentrations of essential and toxic elements (Pb, Ni, Cd, Al, Cr, Cu, Fe, Mn, Zn, Co) in infant formulas were determined using high-resolution continuum-source flame atomic absorption spectrometry (HR-CS FAAS) and have been evaluated for health risk. The measured values for concentrations from lowest to highest were (in mg/kg): 36.38–77.45 (Zn), 6.28–12.88 (Al), 2.37–4.91 (Cu), 22.01–51.64 (Fe), and 0.55–2.06 (Mn). The highest concentrations of Ni and Cd were 0.18 and 0.09 mg/kg, respectively, while the lowest concentrations for these metals were below the detection limit. The Cr, Co and Pb levels were below the detection limits in all samples. According to the risk assessment conducted for infants aged 0–24 months, which involved calculating the estimated daily intake (EDI), the estimated weekly intake (EWI), the target hazard quotient (THQ), and the hazard index (HI), it was found that the THQ values range from 0.00 to 0.06 for Ni, from 0.00 to 0.69 for Cd, and from 0.17 to 0.22 for Al. However, since the HI for all age groups is less than 1, it can be concluded that there is no health concern for the elements Ni, Cr, Cd, Pb, Al, and Co.

Keywords: Infant food, element, FAAS, health risks

1. Introduction

Nowadays, the intense involvement of women in business life is increasing the demand for commercial infant formulas. Thanks to the development of food technology, this high demand has increased production and consumption, as well as an increase in the quality and variety of infant formulas [1]. According to the 2018 data from the Turkey Demographic and Health Survey, infant formula consumption is 59% for infants aged 0–1 months and 45% for infants aged 2–3 months, but this rate decreases rapidly with age, falling to 14% for infants aged 4–5 months [2]. In addition to environmental contaminants, agrochemical residues and natural toxins, contaminants introduced during food processing, packaging and storage pose safety concerns for infant formula [3]. This has also become a matter of growing concern within society.

Providing a growing baby with an appropriate diet is critical for healthy growth and development. Low intake or reduced bioavailability of nutrients can lead to deficiencies and impaired body functions [4]. Recent clinical research has shown that certain metals and metalloids, such as iron, copper, zinc, and selenium, which are essential micronutrients, play a crucial role in various biochemical functions in all living organisms, including infants. These functions include bone mineralization, enzymatic reactions, redox reactions, hormone secretion, as well as protection of cells and lipids in biological membranes [5]. However, excessive exposure to these metals can cause toxicity and lead to adverse health effects, such as neurotoxicity, developmental delays, and impaired immune function in infants. Therefore, it is essential to understand the

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***Author of correspondence:** cigdemer86@gmail.com, cigdem.ercaliskan@ahievran.edu.tr
Tel: +90 (386) 280 58 50
Fax: +90 (386) 280 48 15
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balance between the benefits and risks of these metals in infant formulas to ensure optimal infant health [6].

Improper nutrition during infancy can directly affect infant growth, as well as lead to long-term adverse health effects on organ development and function. Several studies have shown that formula feeding is associated with several health risks. These include immune system disorders, food allergies, obesity, diabetes, and coronary heart disease later in life [7]. Parents are becoming more conscious about the healthy nutrition of their children and are demanding more information about the contents of commercial baby foods [8]. In recent years, numerous studies have been conducted on the mineral profile of infant formula samples [9]. Most of these studies have focused on comparing breast milk with cow's milk-based formula substitutes [8]. A review of the literature shows that studies on infant feeding are available in both developing and industrialized countries [4,10–13]. Chajduk et al. (2018) analyzed the basic contents of 16 formulas for infants aged 0 to 8 months in Poland [8]. Mohamed et al. (2021) determined the concentrations of seventeen elements (Al, Br, Ca, Cl, Co, Cu, Fe, I, K, Mg, Mn, Na, Rb, Sb, Sc, Se, and Zn) in infant formulas of 34 different brands collected from pharmacies and local markets in Egypt [14]. Kazi et al. (2010) conducted studies on a total of eight different infant formula products in Pakistan [15], while Basaran (2022) determined the levels of toxic metals in 36 infant formula samples in Turkey [10]. It has been observed that research on trace element analysis of commercially available baby foods is limited in our country.

The most widely published techniques for elemental analysis of infant formula are inductively coupled plasma optical emission spectrometry (ICP/OES) [16], electrothermal atomic absorption spectrometer (ETAAS) [17], flame and graphite furnace atomic absorption spectrometry (GF-AAS) [18,19], and inductively coupled plasma mass spectrometry (ICP-MS) [10,20,21]. Furthermore, elemental levels in milk powder formulations for infants and cereals have been successfully determined by energy dispersive X-ray fluorescence (EDXRF), and wavelength dispersive X-ray fluorescence (WDXRF) [22,23]. The main advantages of plasma techniques are high sensitivity and the ability to determine multiple elements. However, these techniques can be expensive, and high organic matrix content, particularly in the case of ICP-MS, can affect results through spectral and/or non-spectral interferences. [24]. High-resolution continuum source flame atomic absorption spectrometry (HR-CS FAAS) offers several advantages over other techniques, including low cost of analysis, a high resolution monochromator, and the ability to determine multiple elements using a single light source (xenon arc) [25,26]. In addition, HR-CS FAAS allows the analysis of a wide range of elements, enabling consistent methodology across different application areas. HR-CS FAAS was therefore selected as the method of choice for this study.

The nutritional values, mineral, and vitamin content of ready-to-eat infant formulas may not have enough data on their labeling. Toxic elements could be present due to raw materials used in the preparation of infant formulas and foods, contamination, or food processing. Finding micronutrients is important to know if infants are getting the right nutrients during this critical developmental period. This study aims to determine the levels of some essential and toxic elements in commercial infant formulas and their contribution to the dietary intake of infants. Additionally, the risk of toxic elements, estimated daily intake (EDI), target hazard quotient (THQ), and hazard index (HI) were calculated for infants (0 to 24 months), and estimated weekly intake (EWI) values were compared with provisional tolerated weekly intake (PTWI) values.

2. Materials and Method

2.1. Sample Collection

In this study, a total of 36 samples, 2 product from each brand with different production dates (6 different brands × 3 different age groups × 2 different production date), were purchased from supermarkets and pharmacies in the province of Kırşehir, Turkey, in 2022. The samples were analyzed before their expiration date.

2.2. Chemicals

During the process of determining the levels of essential and toxic elements in infant formulas aged 0–24 months, it was ensured that all chemicals used were of analytical reagent quality. Concentrated nitric acid (65% HNO_3) and hydrogen peroxide (30% H_2O_2) were purchased from Merck Co. (Darmstadt, Germany). Additionally, ultrapure deionized water (18.3 MΩ cm, Millipore, Corporation, MA, USA) was used to prepare the standards and sample solutions.

2.3. Preparation of Samples

For infant food samples (0.25–0.30 g), 100 mL containers made of polytetrafluoroethylene (PTFE) containers with screw caps resistant to microwave oven pressure and temperature were used. To each sample placed in the PTFE containers, 5 mL of 65% HNO_3 (w/w) was added, followed by 1 mL of 30% H_2O_2 (w/w) solution. The mixture was incubated for 30 min at room temperature. The sealed lids of the PTFE containers were closed and solubilized in a microwave oven using 3 different programs. The digestion program was applied

Table 1. HR-CS FAAS device variables and limit of detection

Variables	Pb	Ni	Cd	Al	Cr	Cu	Fe	Mn	Zn	Co
Wavelength, nm	217.00	232.00	228.80	396.15	359.34	324.75	248.32	279.48	213.85	240.72
N_2O-C_2H_2 flow rate, L/h	0	0	0	215	0	0	0	0	0	0
C_2H_2-air flow rate L/h	65	55	50	55	100	55	60	80	60	60
Burner height, mm	8	7	6	7	7	6	5	8	8	6
Evaluation Pixels, pm	3	3	3	3	3	3	3	3	3	3
*LOD (μg/L)	5	1.2	0.4	22	5	1	1	1	1	2

* 3σ, 11 repetitions

as 250 W for 5 min; 450 W for 5 min and 800 W for 10 min. After the PTFE containers were cooled to room temperature, their lids were carefully opened in a fume hood and the volume of the obtained clear mixture (undigested samples had the microwave digestion process applied again) was made up to 10 mL with 0.1 M HNO_3 solution. The same procedures were applied to the blank solution, which did not contain the food sample [27]. Analysis of the samples was carried out by making three parallel readings. In the study, the HR-CS FAAS device model Analytik Jena ContrAA 300 (GLE, Berlin, Germany) was used for the metal detection. The detection limits for the elements studied (Catalog Analytikjena, 2008) are given in Table 1. Additionally, an air-acetylene flame was used for the determination of Pb, Ni, Cd, Cr, Cu, Fe, Mn, Zn, and Co and a nitrous oxide-acetylene flame for Al under optimized conditions as specified in Table 1.

2.4. Human Health Risk Assessment

The risk of toxic elements for infants (0 to 24 months) was assessed by calculating EDI, THQ, and the HI. The EDI was calculated according to Equation 1 [28].

$$EDI = \frac{M_c \, x \, DI}{BW} \qquad (1)$$

Where EDI is the estimated daily intake (mg/kg/day), M_c is the elemental content of infant formula (mg/kg dry weight), and DI is the daily infant formula intake (kg); 120 g/day for (0–6 months) infants, 160 g/day for (6–12 months) infants, and 200 g/day for (12–24 months) infants [11]. Where BW is the reference body weight (kg); 5.9 kg for (0–6 months) infants, 9.3 kg for (6–12 months) infants, and 12.2 kg for (12–24 months) infants [11].

The THQ value was used to assess the non-carcinogenic risk (Equation 2);

$$THQ = \frac{EDI}{RfD} \qquad (2)$$

The reference dose (RfD) values for metals are given in Table 2 [29,30]. The value given as Total THQ (T THQ) or HI, is calculated as the total THQ values of all elements investigated. A TTHQ greater than 1 indicates non-carcinogenic health risks to the consumer [31]. When consuming food, people are exposed not only to one metal but also to multiple metals contained in that food. Therefore, the HI was calculated by summing the THQ values of each metal in the study (Equation 3). HI < 1 indicates no health risk concern, while HI ≥ 1 indicates a potential health concern.

$$\sum THQ(HI) = (THQ_1 + THQ_2 + \ldots\ldots + THQ_n) \qquad (3)$$

Table 2. Reference dose (RfD) values of metals

Metal	RfD (mg/kg/day)
Pb	0.004
Ni	0.02
Cd	0.001
Al	1.00
Cr	1.5
Cu	0.04
Fe	0.7
Mn	0.14
Zn	0.3

3. Results and Discussion

As infant formula is often the primary source of nutrition for infants and children, it is essential that the quality and nutritional content of these foods are appropriate for their health due to their low body weight in the face of high nutrient needs, infants are particularly vulnerable to exposure to nutritional products with the wrong ingredients [32]. Metals such as Pd, Cd, Hg and As are considered toxic and cause adverse effects even at low levels. To demonstrate the adequacy of infant formula products, it is important to determine whether the macro- and micro-element content of these products is within the range of reliable reference values.

The measured values for concentrations from lowest to highest were (in mg/kg): 36.38–77.45 (Zn), 6.28–12.88 (Al), 2.37–4.91 (Cu), 22.01–51.64 (Fe), and 0.55–2.06 (Mn). The highest concentrations of Ni and Cd were 0.18 and 0.09 mg/kg, respectively, while the lowest concentrations for these metals were below the detection limit. The concentrations of Cr, Co and Pb were below the limit of detection in all samples (Table 3). The accuracy of the method was determined using the standard addition method. The recoveries were calculated above 90%. EDI and EWI of various metals for infants are shown in Table 4. PTWI is the maximum amount of heavy metals in the weekly and daily diet that does not pose a risk to human health. Therefore, according to the FAO/WHO (2004) recommendation, the heavy metal data in infant formulas were compared with the PTWI. This was performed to determine the safe level for weekly consumption of infant formul.

Zinc (Zn) is an essential part of the activity of more than 200 enzymes involved in digestion, metabolism, reproduction and wound healing [33]. Zinc deficiency leads to deterioration of the immune system and cognitive disorders. However, high zinc intake can cause toxicity and acute zinc poisoning has been associated with non-specific gastrointestinal symptoms such as abdominal pain, diarrhea, nausea and vomiting [34]. In this study, the mean Zn levels of infant formula samples Nos. 1, 2 and 3 were 57.47 ± 2.87, 56.23 ± 2.34 and 62.15 ± 2.33 mg/kg, respectively. In all infant formula samples, the mean Zn level was approximately 58.62 (36.38–77.45) mg/kg (Table 3). In the literature studies, Zn levels in infant formula and follow-on formula were determined to be 1.61–2.02 mg/kg [11], 4.69–11.34 mg/kg [35], 27.88–71.55 mg/kg [36], 32.53–42.98 mg/kg [37], 6.82–17.19 mg/kg [38], 36.2–52.3 mg/kg [39], 0.92–37.2 mg /kg [19], and 23.6–53.9 mg/kg [32]. The PTWI for Zn was been determined by FAO/WHO as 7000 µg/kg body weight/week [40]. When the EWI values given in Table 4 were compared with the PTWI value, it was observed that the EWI values were above the limit value in infant formulas number 1 and 3.

Although nickel (Ni) is an essential micronutrient source for the organism at low concentrations, it is a toxic element at high concentrations. It is known to act as a cofactor in the activation of certain enzymes involved in the breakdown or usage of glucose. Nickel may aid in the production of prolactin and therefore may be involved in human breast milk production [41]. It has been shown that acute exposure of the human body to nickel can cause various health problems such as liver, kidney, spleen, brain tissue damage, vesicular eczema and lung [42]. In this study, the mean Ni levels in infant formula samples Nos. 2, and 3 were 0.068 ± 0.002 and 0.065 ± 0.003 mg/kg, respectively (Table 3). The highest Ni concentration was found in infant formula number 3 (Brand 4). In literature studies, commercial infant formulas in the Egyptian market did not contain detectable amounts of Ni [6], while in another study, the Ni levels in infant formula and follow-on formula were determined to be 0.219–2.23 mg/kg [11]. The PTWI for Ni was been determined by the FAO/WHO as 35 µg/kg body weight/week [40]. When the EWI values given in

Table 3. Macro and microelement concentrations of infant foods (mg/kg)

Mark	N	Zn	Ni	Cr	Cd	Pb	Al	Co	Cu	Fe	Mn
					Number 1						
Brand 1*	2	36.38 ± 1.82	BDL	ᵇBDL	BDL	BDL	7.28 ± 0.36	BDL	2.37 ± 0.17	37.15 ± 1.86	0.88 ± 0.04
Brand 2*	2	75.85 ± 3.79	BDL	BDL	BDL	BDL	12.88 ± 0.99	BDL	3.00 ± 0.15	43.45 ± 2.17	0.88 ± 0.04
Brand 3*	2	52.83 ± 2.64	BDL	BDL	BDL	BDL	11.55 ± 0.68	BDL	3.73 ± 0.19	22.01 ± 1.10	1.56 ± 0.08
Brand 4*	2	59.77 ± 2.99	BDL	BDL	BDL	BDL	11.71 ± 0.69	BDL	3.34 ± 0.17	41.84 ± 2.09	0.55 ± 0.03
Brand 5*	2	66.08 ± 3.30	BDL	BDL	BDL	BDL	11.83 ± 0.69	BDL	4.61 ± 0.23	44.18 ± 2.21	0.80 ± 0.04
Brand 6*	2	53.89 ± 2.69	BDL	BDL	BDL	BDL	9.16 ± 0.46	BDL	3.87 ± 0.19	42.62 ± 2.13	0.60 ± 0.03
					Number 2						
Brand 1**	2	42.12 ± 1.45	BDL	BDL	BDL	BDL	6.28 ± 0.53	BDL	4.37 ± 0.18	39.15 ± 1.28	0.95 ± 0.06
Brand 2**	2	75.36 ± 3.16	BDL	BDL	0.030 ± 0.002	BDL	11.25 ± 1.02	BDL	3.25 ± 0.12	40.25 ± 1.29	0.96 ± 0.05
Brand 3**	2	49.23 ± 2.25	0.12 ± 0.02	BDL	0.040 ± 0.002	BDL	12.23 ± 1.56	BDL	4.53 ± 0.19	36.03 ± 1.02	1.96 ± 0.18
Brand 4**	2	55.18 ± 2.23	0.17 ± 0.02	BDL	BDL	BDL	11.31 ± 1.62	BDL	3.63 ± 0.17	51.64 ± 2.06	0.63 ± 0.04
Brand 5**	2	65.01 ± 2.88	0.12 ± 0.01	BDL	BDL	BDL	10.20 ± 1.09	BDL	4.83 ± 0.24	48.16 ± 2.02	0.90 ± 0.06
Brand 6**	2	50.52 ± 2.10	BDL	BDL	BDL	BDL	10.16 ± 0.96	BDL	3.57 ± 0.15	42.62 ± 2.13	0.72 ± 0.04
					Number 3						
Brand 1***	2	52.20 ± 1.65	BDL	BDL	0.03 ± 0.01	BDL	6.58 ± 0.90	BDL	4.37 ± 0.20	40.55 ± 2.01	0.89 ± 0.03
Brand 2***	2	77.45 ± 2.63	BDL	BDL	0.03 ± 0.01	BDL	12.72 ± 1.08	BDL	3.15 ± 0.15	43.35 ± 2.10	0.96 ± 0.05
Brand 3***	2	62.83 ± 2.25	0.11 ± 0.02	BDL	0.070 ± 0.003	BDL	11.55 ± 1.23	BDL	2.93 ± 0.14	23.56 ± 1.63	2.06 ± 0.08
Brand 4***	2	58.70 ± 2.62	0.18 ± 0.02	BDL	0.090 ± 0.001	BDL	10.22 ± 1.02	BDL	3.56 ± 0.16	43.84 ± 1.62	0.61 ± 0.03
Brand 5***	2	65.35 ± 2.54	0.10 ± 0.02	BDL	BDL	BDL	10.60 ± 1.50	BDL	4.91 ± 0.21	45.08 ± 2.26	0.87 ± 0.04
Brand 6***	2	56.38 ± 2.28	BDL	BDL	BDL	BDL	9.23 ± 1.00	BDL	4.07 ± 0.18	43.15 ± 1.56	0.73 ± 0.03

x ± SD (Mean ± Standard deviation), (N=3)
*: 0–6 months, **: 6–12 months, ***: 12–24 months
ᵇBDL: Below the Limit of Detection
N: Number of samples (in powder form)

Table 4 were compared with the PTWI value, it was observed that it was lower than the limit value.

Chromium (Cr) mainly appears in two forms: trivalent Cr (Cr^{3+}) and hexavalent Cr (Cr^{6+}). Cr^{6+} is reported to be toxic, mutagenic, carcinogenic and death of apoptotic cells, oxidative stress, changed gene expression and can induce DNA damage. On the other hand, Cr^{3+} is included among essential trace elements for the maintenance of effective glucose, lipid, and protein metabolism in living things [43]. In this study, Cr concentrations in all analyzed samples were below the detectable values of the device. In literature studies, Cr levels in infant formula and follow-on formula were determined to be <0.007–0.053 mg/kg [32], 2.51–83.80 µg/kg) [27], 100–1450 µg/kg [18], and <LOD–6.29 × 10^{-2} mg/kg [11]. The PTWI for chromium was determined as 23.3 µg/kg body weight/week by FAO/WHO [40].

High levels of exposure to toxic pollutants such as Cd found in foods can accumulate in the kidney and liver due to its long biological half-life, leading to kidney toxicity, cancer, kidney stone formation, neurological effects, and serious damage to calcium metabolism [6]. The highest Cd concentration was found in infant formula number 3 (0.090 ± 0.001). Some values found were above the Cd limits (0.005–0.02 mg/kg) set by the European Commission for infant formula and follow-on formula [44]. The FAO/WHO and the Joint Expert Committee on Food Additives (JECFA) have determined the PTWI for cadmium as 7 µg/kg body weight/week [40]. When the EWI values given in Table 4 were compared with the PTWI values, it was determined that the Cd values of all infant formulas analyzed were low. In the literature studies, Cd levels in infant and follow-on formulas were determined to be 0.13–3.58 µg/kg [27], <0.000–0.015 mg/kg [45], 0.038–0.476 mg/kg [28], 0.01 mg/kg [46], 0.012 mg/kg [47], and 0.005–0.017 mg/kg [6], and < LOD–1.64 × 10^{-2} mg/kg [11].

Lead (Pb) is a potent neurotoxin in its alkyl form, and ingestion with milk increases its absorption. Lead exposure in infants affects the nervous system and normal development of the brain, causing learning difficulties and anemia [48]. In this study, Pb concentrations in all analyzed samples were below the detectable values of the device. The allowable limit for lead in infant formula has been determined by the Codex Alimentarius Commission (CAC) as 10 µg/kg [49] and by the European Union as 50 µg/kg [50]. In studies conducted in infant and follow-on formulas in different countries in the literature, the Pb range was determined to be (0.36–5.57 µg/kg) in China) [27], <LOD–0.01 mg/kg in Iran [11], (31.0–1040 µg/kg) in Lebanon [28], (25.7–45.5 µg/kg) in Spain [51],[36] (16.0–103 µg/kg) in Ethiopia, [35] (0.14–2.46 µg/kg), and [52] (0.55–24.9 µg/kg) in Turkey. The PTWI for lead was been determined by the FAO/WHO as 25 µg/kg body weight/week [40].

The presence of Al, a toxic element, in infant formula is a matter of great public health importance and should be treated with caution. There are epidemiological data that aluminum causes Alzheimer's disease. These data also raise doubts that exposure to aluminum may have neurological, skeletal, hematopoietic, immunological, and other adverse health effects [53]. The US Food and Drug Administration (FDA) has reported that this metal can accumulate in the central nervous system in the case of higher than 4–5 µg/ kg/day of aluminum in premature infants with impaired kidney function [54]. In this study, the mean Al levels in infant formula samples Nos. 1, 2 and 3 were 10.74 ± 0.65, 10.23 ± 1.13 and 10.15 ± 1.12 mg/kg, respectively, and the mean Al level in all infant formula samples was approximately 10.37 (6.28–12.88) mg/kg (Table 3). The highest Al concentration was found in infant formula number 1 (Brand 2). In literature studies, Al levels in infant formula and follow-on formula were determined to be 189–653 µg/kg [55], 1.3–17.1 mg/kg [56], and 718–6987 µg/ kg [10]. As a joint decision, FAO/WHO/JECFA determined the PTWI value for Al as 2 mg/kg bw [57]. On the other hand, the European Food Safety Authority (EFSA) has set a TWI (Tolerable Weekly Intake) of 1 mg/kg bw for Al in all food sources [58]. The Committee observed that the PTWI may be exceeded in some demographics, particularly in children who regularly consume foods containing Al-based additives or ingredients [59]. When the EWI values given in Table 4 were compared with the PTWI value, it was observed that all infant formulas were below the limit value.

Cobalt (Co) is an essential metal found in the active site of vitamin B-12 and is central to the biochemical reactions of life. Overexposure has caused various adverse health effects in animals and humans, such as vasodilation, flushing, and cardiomyopathy [60]. In this study, Co concentrations in all analyzed samples were below the detectable values of the device. In the literature studies, Co levels in infant formula and follow-on formula were determined to be 0.018–0.036 mg/kg [32], and 9.00 × 10^{-4}–4.90 × 10^{-3} mg/kg [11].

Copper (Cu) deficiency can decrease leukocytes, anemia, and osteoporosis in infants and children. In addition, excessive copper intake can cause acute poisoning, transient gastrointestinal disturbances with symptoms such as vomiting, nausea, and abdominal pain, and even liver toxicity resulting in death [61]. The mean Cu levels in infant formula samples Nos.1, 2 and 3 were 3.49 ± 0.18, 4.03 ± 0.17 and 3.83 ± 0.17 mg/kg, respectively, and the mean Cu level in all infant formula samples was approximately 3.78 (2.37 – 4.91) mg/kg (Table 3).

Table 4. Estimates of daily intake (EDI) and weekly intake (EWI) of various metals

Month		Element									
		Zn	Ni	Cr	Cd	Pb	Al	Co	Cu	Fe	Mn
EDI (mg/kg bw/day)	0–6 month[a]	1.17	0.00	0.00	0.00	0.00	0.22	0.00	0.07	0.78	0.02
	6–12 month[b]	0.97	0.00	0.00	0.00	0.00	0.18	0.00	0.07	0.74	0.02
	12–24 month[c]	1.02	0.00	0.00	0.00	0.00	0.17	0.00	0.06	0.65	0.02
EWI (mg/kg bw/week)	0–6* month	8.18	0.00	0.00	0.00	0.00	1.53	0.00	0.50	5.49	0.13
	6–12** month	6.77	0.01	0.00	0.00	0.00	1.23	0.00	0.49	5.17	0.12
	12–24*** month	7.13	0.01	0.00	0.00	0.00	1.16	0.00	0.44	4.58	0.12

[a]Mean bw: 5.9 kg *: Number 1
[b]Mean bw: 9.3 kg **:Number 2
[c]Mean bw: 12.2 kg ***:Number 3

The highest copper concentration was determined in the number 3 (Brand 5) infant formula. In the literature studies, Cu levels in infant and follow-on formulas were determined to be 0.11–2.37 mg/kg [35], 0.41–6.35mg/kg [32], and 1.24×10^{-3}– 1.87×10^{-2} mg/kg [11]. For copper, PTWI was been determined as 3500 µg/kg /week by FAO/WHO [40]. When the EWI values given in Table 4 were compared with the PTWI value. It was determined that copper, which is considered a critical cofactor in the effective use of many elements, especially iron [62], was lower than the standard value.

Iron is involved in various metabolic processes in living organisms, such as electron transfer, substrate oxidation-reduction, hormone synthesis, oxygen transport and storage, DNA replication-repair and cell cycle control, nitrogen fixation, and protection from reactive oxygen species [63,64]. The decrease in the concentration of this element, which is important in terms of metabolism, increases cadmium absorption in the body [64]. Therefore, sufficient iron must be taken to reduce cadmium absorption. In this study, the mean Fe levels in infant formula samples Nos. 1, 2, and 3 were 38.54 ± 1.93, 42.97 ± 1.63 and 39.92 ± 1.86 mg/kg, respectively. The mean Fe level in all infant formula samples was approximately 40.476 (22.01–51.64) mg/kg (Table 3). The highest iron concentration was found in infant formula number 2 (Brand 4). In the literature studies, Fe levels in infant formula and follow-on formula were determined to be 1.33–7.54 mg/kg [35], 3.6–77.8 mg/kg [32], and 1.49–3.01 mg/kg [11]. For iron, PTWI has been determined as 5600 µg/kg body weight/week by FAO/WHO [40]. When the EWI values given in Table 4 were compared with the PTWI value, it was observed that it was lower than the limit value.

Manganese (Mn)is a vital element and cofactor of many key enzymes and acts as a component of metalloenzymes such as manganese superoxide dismutase (MnSOD), which is mainly responsible for scavenging Reactive Oxygen Species (ROS) in mitochondrial oxidative stress. However, excessive exposure to manganese causes toxicity in the central nervous, heart, respiratory, and reproductive systems [61,65]. In this study, the mean manganese levels in infant formula samples Nos. 1, 2 and 3 were 0.88 ± 0.26, 1.02 ± 0.07 and 1.02 ± 0.04 mg/kg, respectively. The mean Mn content in all infant formula samples was approximately 0.97 (0.55–2.06) mg/kg (Table 3). The highest manganese concentration was determined in an infant formula suitable for use number 3 (Brand 3). When the results were compared with other studies, Mn levels in infant and follow-on formulas were determined to be 0.01–0.07 mg/kg [35], 0.157–0.796 mg/kg [32], and 0.0426–0.0803 mg/kg [11]. The PTWI for manganese has been determined by the FAO/WHO as 980 µg/kg body weight/week [66]. When the EWI values given in Table 4 were compared with the PTWI value, it was found that it was lower than the limit value.

Table 5. THQ and HI values of heavy metals in infants due to consumption of infant formula

Age groups	THQ						HI
	Ni	Cr	Cd	Pb	Al	Co	
0–6 months	0.00	0.00	0.00	0.00	0.22	0.00	0.22
6–12 months	0.06	0.00	0.69	0.00	0.18	0.00	0.92
12–24 months	0.05	0.00	0.66	0.00	0.17	0.00	0.88

The THQ and HI (ΣTHQ) values calculated for each heavy metal are given in Table 5. The THQ values ranged from 0.00 to 0.06 for Ni, from 0.00 to 0.69 for Cd, and from 0.17 to 0.22 for Al. HI <1 was found for all age groups. Because of the evaluations, it can be said that there are no health concerns for the elements of Ni, Cr, Cd, Pb, Al, and Co.

4. Conclusions

Foods used for the healthy development of infants must have sufficient and appropriate content. Contaminated raw materials and/or contaminated equipment during the manufacturing process may result in toxic metal contamination of infant formula. Limited data on metal contamination in infant formula, especially considering the toxic effects of metals on this sensitive population, prompted the need for our study. The levels of the elements Zn, Ni, Cr, Cd, Pb, Al, Co, Cu, Fe, and Mn in infant formula sold on the market in our country were determined and their THQ values were calculated. Because of the evaluations, it was found that the levels of carcinogenic elements in the infant formula samples studied were relatively low and the THQ and HI values were < 1. These results are consistent with similar studies reported in the literature and further support the safety of these products. It is important to emphasize that although the levels of heavy metals in the infant formulas studied were found to be safe, minimizing heavy metal exposure should remain a priority. Overall, this study contributes to the existing literature by providing significant data on the levels of various elements in infant formulas in our country and confirming their safety. The findings support the importance of ongoing monitoring and quality control efforts to ensure the healthy development of infants and to minimize potential risks associated with heavy metal exposure.

References

[1] WHO, WHO recommendations on postnatal care of the mother and newborn 2014, World Health Organization, Geneva.
[2] TDHS, 2018 Türkiye Demographic and Health Survey, Ankara, Türkiye, 2019.
[3] Ş. Saçmacı, M. Saçmacı, Determination of Arsenic (III) and Total Arsenic at Trace Levels in Baby Food Samples via a New Functionalized Magnetic Graphane Oxide Nanocomposite, Biol Trace Elem Res, 199, 2021, 4856–4866.
[4] N. Zand, B.Z. Chowdhry, F.B. Zotor, D.S. Wray, P. Amuna, F.S. Pullen, Essential and trace elements content of commercial infant foods in the UK, Food Chem, 128, 2011, 123–128.
[5] L.J. Taylor, M. Gallagher, F.S. McCullough, The role of parental influence and additional factors in the determination of food choices for pre-school children, Int J Consumer Stud, 28, 2004, 337–346.
[6] M.M. Ghuniem, M.A. Khorshed, M.M. Khalil, Determination of some essential and toxic elements composition of commercial infant formula in the Egyptian market and their contribution to dietary intake of infants, Int J Environ Anal Chem, 100, 2020, 525–548.
[7] A. Barclay, L. Weaver, Feeding the normal infant, child and adolescent, Medicine, 34, 2006, 551–556.
[8] E. Chajduk, M. Pyszynska, H. Polkowska-Motrenko, Determination of trace elements in infant formulas available on polish market, Biol Trace Elem Res, 186, 2018, 589–596.
[9] L. Herreros-Chavez, A. Morales-Rubio, M. Cervera, Green methodology for quality control of elemental content of infant milk powder, LWT, 111, 2019, 484–489.
[10] B. Başaran, An assessment of heavy metal level in infant formula on the market in Turkey and the hazard index, J Food Compos Anal, 105, 2022, 104258.
[11] A. Kiani, M. Arabameri, M. Moazzen, N. Shariatifar, S. Aeenehvand, G.J. Khaniki, M. Abdel-Wahhab, S. Shahsavari, Probabilistic health risk assessment of trace elements in baby food and milk powder using ICP-OES method, Biol Trace Elem Res, 200, 2022, 2486–2497.
[12] V. Sirot, T. Traore, T. Guérin, L. Noël, M. Bachelot, J.-P. Cravedi, A. Mazur, P. Glorennec, P. Vasseur, J. Jean, French infant total diet study: exposure to selected trace elements and associated health risks, Food Chem Toxicol, 120, 2018, 625–633.
[13] C. Vella, E. Attard, Consumption of minerals, toxic metals and hydroxymethylfurfural: Analysis of infant foods and formulae, Toxics, 7, 2019, 33.
[14] G.Y. Mohamed, M. Soliman, S.A. Issa, N.M. Mohamed, M. Al-Abyad, Trace elements assessment and natural radioactivity levels of infant formulas consumed in Egypt, J Radioanal Nucl Chem, 330, 2021, 1127–1136.
[15] T.G. Kazi, N. Jalbani, J.A. Baig, M.B. Arain, H.I. Afridi, M.K. Jamali, A.Q. Shah, A.N. Memon, Evaluation of toxic elements in baby foods commercially available in Pakistan, Food Chem, 119, 2010, 1313–1317.
[16] S. Bağdat, E. Köse Baran, F. Tokay, Element fractionation analysis for infant formula and food additives by inductively coupled plasma optical emission spectrometry, Int J Food Sci Technol, 49, 2014, 392–398.
[17] T.G. Kazi, N. Jalbani, J.A. Baig, H.I. Afridi, G.A. Kandhro, M.B. Arain, M.K. Jamali, A.Q. Shah, Determination of toxic elements in infant formulae by using electrothermal atomic absorption spectrometer, Food Chem Toxicol, 47, 2009, 1425–1429.
[18] F. Salah, I. Esmat, A. Mohamed, Heavy metals residues and trace elements in milk powder marketed in Dakahlia Governorate, International Food Research Journal, 20, 2013, 1807–1812.
[19] S. Saracoglu, K.O. Saygi, O.D. Uluozlu, M. Tuzen, M. Soylak, Determination of trace element contents of baby foods from Turkey, Food Chem, 105, 2007, 280–285.
[20] K. Ljung, B. Palm, M. Grandér, M. Vahter, High concentrations of essential and toxic elements in infant formula and infant foods–A matter of concern, Food Chem, 127, 2011, 943–951.
[21] H. Chen, Y. Yao, C. Zhang, J. Ping, Determination of Heavy Metal Ions in Infant Milk Powder Using a Nanoporous Carbon Modified Disposable Sensor, Foods, 12, 2023, 730.
[22] T. Gunicheva, Advisability of X-ray fluorescence analysis of dry residue of cow milk applied to monitor environment, X-Ray Spectrometry: An International Journal, 39, 2010, 22–27.
[23] L. Perring, J. Blanc, Faster measurement of minerals in milk powders: comparison of a high power wavelength dispersive XRF system with ICP-AES and potentiometry reference methods, Food Anal Method, 1, 2008, 205–213.
[24] L. Balcaen, L. Moens, F. Vanhaecke, Determination of isotope ratios of metals (and metalloids) by means of inductively coupled plasma-mass spectrometry for provenancing purposes—A review, Spectrochim. Acta B: At. Spectrosc., 65, 2010, 769–786.
[25] B. Welz, High-resolution continuum source AAS: the better way to perform atomic absorption spectrometry, Anal Bioanal Chem, 381, 2005, 69–71.
[26] R.R. Gamela, E.G. Barrera, Á.T. Duarte, W. Boschetti, M.M. da Silva, M.G.R. Vale, M.B. Dessuy, Fast sequential determination of Zn, Fe, Mg, Ca, Na, and K in infant formulas by high-resolution continuum source flame atomic absorption spectrometry using ultrasound-assisted extraction, Food Analytical Methods, 12, 2019, 1420–1428.
[27] C. Su, N. Zheng, Y. Gao, S. Huang, X. Yang, Z. Wang, H. Yang, J. Wang, Content and dietary exposure assessment of toxic elements in infant formulas from the Chinese market, Foods, 9, 2020, 1839.
[28] J. Elaridi, H. Dimassi, O. Al Yamani, M. Estephan, H.F. Hassan, Determination of lead, cadmium and arsenic in infant formula in the Lebanese market, Food Control, 123, 2021, 107750.
[29] EPA, Regional Screening Level (RSL) Summary Table 2011.
[30] EPA, Integrated Risk Information System 2016.
[31] EPA, Regional screening levels (RSLs) equations.
[32] M. Sager, C. McCulloch, D. Schoder, Heavy metal content and element analysis of infant formula and milk powder samples purchased on the Tanzanian market: International branded versus black market products, Food Chem, 255, 2018, 365–371.
[33] M. Aliasgharpour, Zn Status in gastroenteritis children under five years old, Int. J Med Investig, 4, 2015, 180–182.
[34] M.J. Kwon, M.I. Boyanov, J.-S. Yang, S. Lee, Y.H. Hwang, J.Y. Lee, B. Mishra, K.M. Kemner, Transformation of zinc-concentrate in surface and subsurface environments: Implications for assessing zinc mobility/toxicity and choosing an optimal remediation strategy, Environ Pollut, 226, 2017, 346–355.
[35] G. Lutfullah, A.A. Khan, A.Y. Amjad, S. Perveen, Comparative study of heavy metals in dried and fluid milk in Peshawar by

atomic absorption spectrophotometry, Sci World J, 2014, 2014, 1–5.

[36] T. Eticha, M. Afrasa, G. Kahsay, H. Gebretsadik, Infant exposure to metals through consumption of formula feeding in Mekelle, Ethiopia, Int J Anal Chem, 2018, 2018, 1–5.

[37] A. Alemu, Levels of Essential and Toxic Metals in Commercial Powdered Infant Formulas, Addis Ababa University, 2008.

[38] O. Aguzue, S. Kakulu, S. Thomas, Flame atomic absorption spectrophotometric determination of heavy metals in selected infant formula in the Nigerian Market, Arch Appl Sci Res, 6, 2014, 128–132.

[39] M. Pandelova, W.L. Lopez, B. Michalke, K.-W. Schramm, Ca, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn contents in baby foods from the EU market: Comparison of assessed infant intakes with the present safety limits for minerals and trace elements, J Food Compos Anal, 27, 2012, 120–127.

[40] FAO/WHO, Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) 1956–2003 (First through Sixty-First Meetings), FAO and WHO, 2004.

[41] A. Singh, R.S. Kumar, Role of Nickel in animal performance: A review, The Pharma Innovation Journal, SP 10, 2021, 643–646.

[42] A. Mehri, Trace elements in human nutrition (II)–an update, I J Preven Medic, 11, 2020, 1–17.

[43] M. Shahid, S. Shamshad, M. Rafiq, S. Khalid, I. Bibi, N.K. Niazi, C. Dumat, M.I. Rashid, Chromium speciation, bioavailability, uptake, toxicity and detoxification in soil-plant system: A review, Chemosphere, 178, 2017, 513–533.

[44] EFSA, Statement on tolerable weekly intake for cadmium, 2011.

[45] B. Başaran, An assessment of heavy metal level in infant formula on the market in Turkey and the hazard index, Journal of Food Composition and Analysis, 105, 2022, 104258.

[46] A. Domínguez, S. Paz, C. Rubio, A. Gutiérrez, D. González-Weller, C. Revert, A. Hardisson, Essential and toxic metals in infant formula from the European Community, Open Access J. Toxicol, 2, 2017, 555585.

[47] M. Mania, M. Wojciechowska-Mazurek, K. Starska, M. Rebeniak, T. Szynal, A. Strzelecka, J. Postupolski, Toxic Elements in Commercial Infant Food, Estimated Dietary Intake, and Risk Assessment in Poland, Polish Journal of Environmental Studies, 24, 2015, 2525–2536.

[48] K. Schümann, The toxicological estimation of the heavy metal content (Cd, Hg, Pb) in food for infants and small children, Z Ernahrungswiss, 29, 1990, 54–73.

[49] CAC, General Standard for Contaminants and Toxins in Food and Feed. CXS193-1995 amended in 2019., 2019.

[50] EU, Commission Regulation (EU) 2015/1005 of 25 June 2015 Amending Regulation (EC) No 1881/2006 As Regards Maximum Levels of Lead in Certain Foodstuffs 2015.

[51] R. Moreno-Rojas, P. Sánchez-Segarra, C. Cañal-Ruiz, M. Amaro-López, G. Zurera-Cosano, Lead content in Spanish market infant formulas and toxicological contribution, Food Addit Contam, 19, 2002, 241–245.

[52] H. Sipahi, A. Eken, A. Aydın, G. Şahin, T. Baydar, Safety assessment of essential and toxic metals in infant formulas, Turkish J Pedıatr, 56, 2014, 385–391.

[53] S.C. Bondy, Low levels of aluminum can lead to behavioral and morphological changes associated with Alzheimer's disease and age-related neurodegeneration, Neurotoxicology, 52, 2016, 222–229.

[54] G.L. Klein, A.M. Leichtner, M.B. Heyman, Aluminum in large and small volume parenterals used in total parenteral nutrition: response to the Food and Drug Administration notice of proposed rule by the North American Society for Pediatric Gastroenterology and Nutrition, J Pediatr Gastr Nutr, 27, 1998, 457–460.

[55] R. Chekri, E. Le Calvez, J. Zinck, J.-C. Leblanc, V. Sirot, M. Hulin, L. Noël, T. Guérin, Trace element contents in foods from the first French total diet study on infants and toddlers, J Food Compos Anal, 78, 2019, 108–120.

[56] A.O. de Souza, C.C. Pereira, A.I. Heling, E.Q. Oreste, S. Cadore, A.S. Ribeiro, M.A. Vieira, Determination of total concentration and bioaccessible fraction of metals in infant cereals by MIP OES, J Food Compos Anal, 77, 2019, 60–65.

[57] FAO/WHO, Evaluation of certain food additives and contaminants: seventy-fourth report of the Joint FAO/WHO Expert Committee on Food Additives, 2011, pp. 136–136.

[58] F. Aguilar, H. Autrup, S. Barlow, L. Castle, R. Crebelli, W. Dekant, K. Engel, N. Gontard, D. Gott, S. Grilli, Safety of aluminium from dietary intake scientific opinion of the panel on food additives, flavourings, processing aids and food contact materials (AFC), EFSA J, 754, 2008, 1–34.

[59] C. Ibrahim, Z. Kammouni, M. Barake, M. Kassir, A. Al-Jawaldeh, J. Matta, Y. Sacre, L. Hanna-Wakim, J. Haddad, M. Hoteit, Pediatric Health Risk Assessment for Exposure to Aluminum from Infant Formulas and Children under the Age of Five's Food Products among Arab Infants: Experience from Lebanon, Foods, 11, 2022, 2503.

[60] C.H. Watanabe, A.S.C. Monteiro, E.S.J. Gontijo, V.S. Lira, C. de Castro Bueno, N.T. Kumar, R. Fracácio, A.H. Rosa, Toxicity assessment of arsenic and cobalt in the presence of aquatic humic substances of different molecular sizes, Ecotox Envıron Safe, 139, 2017, 1–8.

[61] S.C. Izah, N. Chakrabarty, A.L. Srivastav, A review on heavy metal concentration in potable water sources in Nigeria: human health effects and mitigating measures, Expos Health, 8, 2016, 285–304.

[62] M.A. Khan, Nutritional adequacy of commercial infant milk formulas, Ecol Food Nutr, 47, 2008, 188–204.

[63] M.M. Ghuniem, M.A. Khorshed, M. Reda, S.M. Mahmoud, G. Hammad, Assessment of the potential health risk of heavy metal exposure from the consumption of herbal, black and green tea, Biomed J Sci & Tech Res, 16, 2019, 11810–11817.

[64] C. Verma, K. Tapadia, A.B. Soni, Determination of iron (III) in food, biological and environmental samples, Food Chem, 221, 2017, 1415–1420.

[65] V.A. Lemos, C.G. Novaes, M.A. Bezerra, An automated preconcentration system for the determination of manganese in food samples, J Food Compos Anal, 22, 2009, 337–342.

[66] EPA, Manganese compounds 2014.