



Toxic Elements in Dried Milk and Evaluation of their Dietary Intake in Infant Formula

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Article History: 20-097 Received: April 10, 2020 Revised: July 04, 2020 Accepted: July 11, 2020

ABSTRACT

Infant's feeding patterns are important for development and growth; therefore babies are very sensitive to toxic elements, mainly through their food, so in the present study, the concentrations and daily intake of some Toxic Elements (TEs); Lead (Pb), Arsenic (As), Cadmium (Cd), Mercury (Hg) and Aluminum (Al) were measured in different and random 60 dried infant foods {30 infant formula (0-6 months) and 30 milk-cereal based infant formula (6 months)} which obtained from various supermarkets and pharmacies. The analysis was done using Inductive Coupled Plasma - Mass Spectrometer (ICP-MS). It could be determined the lead, arsenic, cadmium, mercury and aluminum by mean values of 0.424 ± 0.006 , 0.205 ± 0.003 , 0.014 ± 0.0001 , 0.298 ± 0.007 and 0.464 ± 0.029 mg/kg in the examined infant milk formula samples and a ranged minimum to maximum concentrations of 0.114-0.177, 0.155-0.293, 0.014-0.015, 0.282-0.310 and 0.287-0.437 mg/kg, respectively in the examined milk-cereal based infant formula. Present study indicates that, the greater level of contamination of examined infant formula samples with toxic elements (lead and mercury) surpasses the maximum limit and Provisional Tolerable Daily Intake (PTDI) of these elements. Mercury is over PTDI (0.0005 mg/kg bwt/day) in all milk-cereal based infant formula samples, also arsenic in all examined samples of this type of formula was exceed the maximum limit (0.05 mg/kg) of Indian standard. This investigation shows such types of infant formula need more amendment to set limit of more toxic metals for this sensitive group of population.

Key words: Infant formula; Toxic elements; Milk-cereal based infant formula; Spectrometer.

INTRODUCTION

The World Health Organization (WHO) suggested that; breast milk feeding is the most natural and best source for nutrition of infants. However, when it is not enough and/or possible in some cases, mightily available infant formulas provide a proper substitute (Mehrnia and Bashti, 2014; WHO, 2015). Although the importance of infant milk formula and complementary feeding as a source of nutrition for infants, these are the main root of toxic elements that pose health risks to children (Kazi *et al.*, 2010; Pandelova *et al.*, 2012).

Infants are the most sensitive population group to the harmful effects of toxic metals due to the significant increased absorption of these metals through the digestive tract than adults, an inadequate developed detoxification method, rapid metabolic processes, and higher food intake relative to their body weight (Pandelova *et al.*, 2012).

Heavy metals presence in infant food may be referred to many factors such as; contamination of the original milk, that attributable to consumption of dairy animals to contaminated feed and water or exposure to massive

environmental pollution, species diversity also during manufacture and packaging (Abdelkhalek *et al.*, 2015). The high content of toxic elements as (Lead, Arsenic and Cadmium), in some baby foods may be due to the food additives or other ingredients, especially rice and vegetable (Kazi *et al.*, 2010). Toxic elements could bio-accumulate in vital organs even in low concentrations as due to renal immaturity of children (<12 months); hinders their elimination. These elements get their way in the body through skin, inhalation and ingestion; mostly a high concentration couldn't excrete from infants' body and accumulated in various organs, results in gathering of these elements in the body causing critical disorders such as infertility, neurological, thyroid and autism disorder or even death (Ozbolat and Tuli, 2016).

Monitoring of these toxic elements in infant milk formula is of great health significance to save infants from their acute & chronic toxicity. Appropriate good manufacturing practice and fulfilment of Hazard Analysis and Critical Control Points (HACCP) system are wanted for the maximum safety manufacture (Kazi *et al.*, 2010; Burrell and Exley, 2010; Hafiz *et al.*, 2016). We aim in

this study to define the potential health hazards of toxic elements (Pb, Cd, As, Hg and Al) in infant formula and milk-cereal based infant formula as a source of these metals for Egyptian infants by determination their level and comparing it with permissible limits available in Egyptian standards and several international standards, also we aim to evaluate the intake risk on the basis of the FAO/WHO recommendations; when infants depend on their feeding on these formula and/or milk cereal foods.

MATERIALS AND METHODS

Collection of samples

A total of sixty random samples {30 each of infant formula (0-6-month age) and milk-cereal based infant formula (6-month age)} were obtained from various retail shops, supermarkets and pharmacies located at Giza and Cairo Governorate, Egypt. All samples were keeping in their packages to be examined in the laboratory and were still valid for consumption. All samples were coded and stored at the same conditions like their sources till analysis.

Sample preparation according to (AOAC, 2012)

Into each microwave digestion vessel; a homogenized sample (typically 1g) was weighed and only 0.5ml of deionized water was added to low the nitrous fumes resulting from the matrix digestion in the digestion vessel. Then 8ml of concentrated HNO₃ (Sigma-Aldrich, Germany) (shaking well) and 2 ml of H₂O₂ 30% (Sigma-Aldrich, Germany) were added. The vessels were capped securely and hold into the High-pressure microwave oven (Milestone, Italy) according to the manual instructions. Samples were digested at power of 1800 watt to reach a minimum temperature of 200°C for a minimum time of 15 min, left at the same temperature for other 15min, and then allowed withdrawing for <80°C. After the heating cycle has been completed, the vessels allowed cooling down in water bath for 30min and then vessels were opened carefully. The residual contents of each vessel were poured into an acid-cleaned 50ml volumetric flask and the dilution was done by deionized water to a final volume of 20ml. The samples were stored in polypropylene tubes until be measured by ICP-MS.

Analysis of samples

After complete digestion; all samples were analyzed for their metal contents; lead (Pb), arsenic (As), cadmium (Cd), mercury (Hg) and aluminum (Al) by Inductive Coupled Plasma-Mass Spectrometer (ICP-MS) Method "Perkin-Elmer model optima 2000DV, Waltham, USA" at the trace elements analytical laboratory, Regional Center for Food and Feed (RCFF), Agriculture Research Center, Giza, Egypt.

Calculation of the Estimated Daily Intake

Estimated Daily Intakes (EDI) of these toxic metals was calculated using the following equation:

$$EDI = C \times D / BW$$

Express the mean EDI of toxic elements in mg/kg bwt/day, C: concentration of metals obtained from

samples, D: concentration of daily intake of powder infant formula (g/day) and BW: average Body Weight.

Average daily consumed infant formula powder from feeding dosages recommended by label instructions and growth charts at 6-month-old were 135g/day, while the average body weights of infants were 7.5kg and only 100g/day for milk-cereal based infant formula (Mehmia and Bashti, 2014; Sipahi *et al.*, 2014). PTWI (Provisional Tolerable Weekly Intake) values were divided by 7 to calculate the Provisional Tolerable Daily Intake (PTDI) (Sipahi *et al.*, 2014), while EWI (Estimated Weekly Intake) calculated by multiplying EDI by 7. We comparing the EDI and EWI with PTDI and PTWI set by (JECFA, 2018).

Statistical analysis according to (SPSS version 25)

The analysis of variance (ANOVA) test was conducted to test the possible significance ($P \leq 0.05$) among mean values of heavy metals concentrations using Fishers Least Significance Difference (LSD).

RESULTS

As showing in Table 1, all the examined infant milk formula samples were contaminated with all measured metals; lead, cadmium, aluminium, arsenic and mercury in a ranged minimum to maximum concentrations of 0.384-0.480, 0.014-0.015, 0.296-0.761, 0.174-0.233 and 0.236-0.345mg/kg, respectively, while the mean values for milk-cereal based infant formula samples were 0.145±0.004, 0.014±0.0001, 0.352±0.009, 0.214±0.008 and 0.296±0.014mg/kg, respectively as illustrated in (Table 2).

Table 1: Toxic metals levels in the examined infant milk formula samples (n=30)

Metal	Positive samples		Min.	Max.	Mean±SEM (mg/kg)
	No.	%			
Pb	30	100.00	0.384	0.480	0.424±0.006 ^a
Cd	30	100.00	0.014	0.015	0.014±0.0001 ^b
Al	30	100.00	0.296	0.761	0.464±0.029 ^a
As	30	100.00	0.174	0.233	0.205±0.003 ^a
Hg	30	100.00	0.236	0.345	0.298±0.007 ^a

n=number of examined samples. a and b: significance difference, $P < 0.05$; No.: Number of positive samples.

Table 2: Toxic metals levels in the examined milk-cereal based infant formula samples (n=30)

Metal	Positive samples		Min.	Max.	Mean±SEM (mg/kg)
	No.	%			
Pb	30	100.00	0.114	0.177	0.145±0.004 ^a
Cd	30	100.00	0.014	0.015	0.014±0.0001 ^b
Al	30	100.00	0.287	0.437	0.352±0.009 ^a
As	30	100.00	0.155	0.293	0.214±0.008 ^a
Hg	30	100.00	0.282	0.310	0.296±0.014 ^a

n=number of examined samples. a and b: significance difference, $P < 0.05$; No.: Number of positive samples.

With evaluation the degree of acceptability for infant milk formula (Table 3), all samples weren't acceptable for (Pb, Cd and Al) according to each available standard. In (Table 4); Estimated Daily Intake (EDI) of infant milk formula was tolerable for all measurable metals (Cd, Al and As) with values 0.0003, 0.008 and 0.004mg/kg bwt/day, respectively except lead (0.008mg/kg bwt/day) and mercury (0.005mg/kg bwt/day).

Table 3: Degree of acceptability of the examined infant formula samples based on available standards (n=30)

Metal Standard	Pb			Cd			Al		
	ML	No.	%	ML	No.	%	ML	No.	%
Egyptian Standards (ES: 7136 / 2010)	0.02	0	0.00	NA	----	----	NA	----	----
JECFA (2018)	0.01	0	0.00	NA	----	----	NA	----	----
European Commission (EC) (1881/2006)	0.05	0	0.00	0.01	0	0.00	NA	----	----
FSANZ* (2.9.1/ 2017)	0.02	0	0.00	0.01	0	0.00	0.2	0.00	0.00

n=number of examined samples; No.: Number of acceptable samples; ML=Maximum Limit (mg/kg); NA=Not Available; *FSANZ: Food Standards Australia New Zealand.

Table 4: Comparison of Estimated daily/weekly intake and provisional tolerable daily/weekly intake of toxic elements of infant formula samples according to The Joint Food Agriculture Organization (FAO)/World Health Organization (WHO), 2018 (n=30)

Daily/Weekly intake (mg/kg) Metal	PTWI	PTDI	EWI	EDI	Acceptable samples		Unacceptable samples	
					No.	%	No.	%
Pb	0.025*	0.004	0.05	0.008	0	0.00	30	100.00
Cd	0.006	0.0009	0.002	0.0003	30	100.00	0	0.00
Al	2.00	0.29	0.05	0.008	30	100.00	0	0.00
As	NA	NA	0.03	0.004	-	-	-	-
Hg	0.004	0.0005	0.04	0.005	0	0.00	30	100.00

n=number of examined samples; No.: Number of acceptable or unacceptable samples; PTWI=Provisional Tolerable Weekly Intake; PTDI=Provisional Tolerable Daily Intake; EWI=Estimated Weekly Intake; EDI=Estimated Daily Intake; NA=Not Available; *=Withdrawn at the 73rd meeting (2010), JECFA (The Joint FAO/WHO Expert Committee on Food Additives).

Table 5: Degree of acceptability of the examined milk-cereal based infant formula sample based on available standards (n=30)

Metal Standard	Pb			Cd			As		
	ML	No.	%	ML	No.	%	ML	No.	%
Egyptian Standards (ES: 7136 / 2010)	0.2	30	100.00	0.2	30	100.00	1*	30	100.00
JECFA (2018)	0.2	30	100.00	0.1	30	100.00	NA	----	----
European Commission (EC) (1881/2006)	0.05	0	0.00	0.04	30	100.00	NA	----	----

n=No of examined samples; No.: Number of acceptable samples; ML=Maximum limit; NA=Not available; * Egyptian Standards (ES: 3284/2005).

Table 6: Comparison of Estimated daily/weekly intake and provisional tolerable daily/weekly intake of toxic elements of examined milk-cereal based infant formula samples, JECFA: 2018 (n=30)

Daily/Weekly intake (mg/kg) Metal	PTWI	PTDI	EWI	EDI	Acceptable samples		Unacceptable samples	
					No.	%	No.	%
Pb	0.025*	0.004	0.014	0.002	30	100.00	0	0.00
Cd	0.006	0.0009	0.0013	0.0002	30	100.00	0	0.00
Al	2.00	0.29	0.03	0.005	30	100.00	0	0.00
As	NA	NA	0.02	0.003	-	-	-	-
Hg	0.004	0.0005	0.03	0.004	0	0.00	30	100.00

n=number of examined samples; No.: Number of acceptable or unacceptable samples; PTWI=Provisional Tolerable Weekly Intake; PTDI=Provisional Tolerable Daily Intake; EWI=Estimated Weekly Intake; EDI=Estimated Daily Intake; NA=Not Available; *=Withdrawn at the 73rd meeting (2010), JECFA (The Joint FAO/WHO Expert Committee on Food Additives).

In Table 5, all examined milk-cereal based infant formula samples was acceptable for Pb, Cd and As according to each available standard except for lead concentration which not agreeable (unacceptable) to (European Commission, 2006). The EDI for milk-cereal based infant formula samples was not tolerable for mercury (0.005mg/kg bwt/day) only; as showed in (Table 6), while other metals (Pb, Cd, Al and As) have acceptable EDI 0.002, 0.0002, 0.005 and 0.003mg/kg bwt/day, respectively. All data of EDI and Estimated Weekly Intake (EWI) were judging according to (JECFA, 2018).

DISCUSSION

The majority of metals exist in baby foods naturally or due to incorrect human actions, such as storage, processing, agriculture & industrial activities, increased municipal waste water, formula preparation with low quality water and improper handling by mothers (Joseph *et al.*, 2011). Arsenic, Lead, Mercury, and Cadmium

classified as 1st, 2nd, 3rd, and 4th, respectively in a priority list the “Top 20 Hazardous Substances” in 2001; which complied by the Agency for Toxic Substances and Disease Registry (ATSDR) in collaboration with the U.S. Environmental Protection Agency (Cruz *et al.*, 2009).

Lead

The analytical data for infant milk formula samples in Table 1, were lower than (Pandelova *et al.*, 2012). In Table 2, lead concentration of milk-cereal based infant formula samples was below than findings of (Pandelova *et al.*, 2012; Sipahi *et al.*, 2014). This unforeseen high Pb level in examined baby formula samples could be due to contamination during food processing of various ingredients (Kazi *et al.*, 2010). Lead is one of the most vigorous neurotoxin that has irrevocable effect on infant nervous system development, lead to less learning abilities with negative effects on the intelligence and showing toxicity in children even at minimum levels of exposure, due to its high possible absorption especially in this group of population (US Environmental Protection Agency, 2003).

As shown in Table 3, the lead content of all examined infant milk formula samples were over permissible limit of Egyptian and other international standards. Consequence to high concentration of Pb in infant formula shows high level of daily intake 0.008 mg/kg bw/day compared with PTDI (0.004 mg/kg) which was withdrawn at the 73rd meeting of (JECFA, 2010), due to estimate that previous PTDI is related to reduce children's Intelligence Quotient (IQ) and increase blood pressure in adults. So this PTDI was concluded to be no longer health protective (Table 4). Only according to EC standard (0.05 mg/kg); the lead concentration of milk-cereal based infant formula samples was 100% unacceptable, but acceptable by 100% for all other comparing standards with low and agreeable EDI as shown in Tables 5 and 6.

Cadmium

Cadmium toxicity results in kidney failure, renal stone formation, neurological effects, disturbance in Ca⁺² metabolism, liver disorders, prostate cancer and fetal death (Zaidan *et al.*, 2013; Sipahi *et al.*, 2014).

As shown in Tables 1 and 2, there was a significant difference ($P < 0.05$) between concentrations of cadmium and other toxic elements in all the examined infant formula samples. In Table 1, the cadmium level of infant milk formula samples was elevated than data obtained through (Pandelova *et al.*, 2012; Chekri *et al.*, 2019). The results of milk-cereal based infant formula samples presented in Table 2 were lower than that reported via (Chekri *et al.*, 2019). Permissible limit of cadmium in all type of infant formula need more attention as not mentioned in Egyptian or JECFA standards, as in this study all of examined infant milk formula samples were exceed permissible limit (0.01mg/kg) of EC and FSANZ standards along with passable EDI 0.0003mg/kg bwt/day as revealed in Tables 3 and 4. Cadmium content of all thirty milk-cereal based infant formula samples was acceptable for all mentioned standards (Table 5), with tolerable EDI 0.0002mg/kg bwt/day as mentioned in Table 6.

Aluminium (Al)

This element reported to contaminate baby dried foods and infant formula through equipment used in both processing and storing of bulk products or prepared for selling using Al packaging material (Burrell and Exley, 2010). Because of newborns undeveloped renal system and intestinal barriers, high level of Al would collect in their thyroid gland, brain and vital organs causing sever renal and nervous disorders as Alzheimer's disease (Sipahi *et al.*, 2014; Ahmed *et al.*, 2016).

The data presented in Table 1 in infant formula samples were higher than results obtained by (Chekri *et al.*, 2019), but decreased than findings reported by (Sipahi *et al.*, 2014). Milk-cereal based infant formula contaminated with high Al level (Table 2) and was lower than results reported by (Chekri *et al.*, 2019). All examined infant formula samples were more than maximum limit of FSANZ standard (0.2mg/kg). No more standards mention limit of aluminium for infant formula or even for milk-cereal based infant formula and this need more study due to high content of aluminium in examined baby food samples (Table 3).

In Tables 4 and 6, aluminium EDI of infant milk formula was (0.008mg/kg bwt/day), but was lower intake (0.005mg/kg bwt/day) for milk-cereal based infant formula samples. These results were similar to (Burrell and Exley, 2010), and below than PTDI which set by (JECFA, 2018) (0.29mg/kg bwt/day); so that we need more study as well as determination of its risk in baby foods. Bishop *et al.*, 1997; mentioned that parenteral exposure of preterm infants to 0.055 mg Al/kg bwt/day, which is a level of aluminium that is possible with regular feeding of infant formulas for particular duration resulted in neurodevelopmental effects at 18 months.

Many of researchers have debated inadequacy of emitted acceptable recommended standards for human exposure to aluminium especially newborn children. Therefore; more need for research to minify Aluminium levels in infant formula (Redgrove *et al.*, 2019).

Arsenic

The Agency for Toxic Substances and Disease Registry (ATSDR, 2018) classifies arsenic as number one on its list that pose the highest potential threat to human health. Arsenic belongs to a group of carcinogens that has many chronic effects as several types of cancers, skin lesions, cardiac disease and neurotoxicity. The first solid food for babies (4-6 months) is rice cereal that known to have been contaminated with high quantity of arsenic, as arsenic exposure in infants is about 3 times than adults (Carignan *et al.*, 2016).

It is showed in our study that; the tested samples of infant milk formula were contaminated by arsenic with a mean value of 0.205 ± 0.003 mg/kg (Table 1); which more than level reported via (Chekri *et al.*, 2019), while in milk-cereal based baby food concentrations were with a mean value of 0.214 ± 0.008 mg/Kg (Table 2), this similar to data reported by (Fao *et al.*, 2019), but higher than results obtained by (Chekri *et al.*, 2019).

Referred to Indian Standard, 2006; all infant formula samples exceed 0.05mg/kg permissible limit and only 60% over limit of (National Standards on Food Safety of China, 2010); 0.2mg/kg without any other available standards. The EDI of arsenic for infant milk formula samples exhibited in Table 4 was 0.004mg/kg bwt/day with no available PTDI for total arsenic. In case of examined milk-cereal based baby food samples referred to Egyptian standard; all samples were within legal limit, but exceed limit of Indian Standard, 2006; (0.05mg/kg) with EDI (0.003mg/kg bwt/day) as presented in Tables 5 and 6.

In 1955, infants arsenic poisoning had taken place in Japan from milk powder; this resulted in poisoning of 12131 neonate infants and death of 130. The poisoned infants showed hematopoietic and pancreatic caners and the only six hundred survivors from the previous disaster suffered from neurological diseases and mental retardation effects in their 50 years (Nepalia *et al.*, 2017).

Mercury

All examined infant food samples were polluted with mercury in high levels with a mean value of 0.298 ± 0.007 mg/kg for infant milk formula; these results are similar to (Cruz *et al.*, 2009), and with a mean value of 0.296 ± 0.014 mg/kg for milk-cereal based infant formula (Table 1 and 2), these findings were higher than reported

by (Pandelova *et al.*, 2012). Such high contamination level in infant formula and milk-cereal based infant formula samples with Hg exceed the PTDI set by JECFA (0.0005mg/kg bwt/day) as shown in (Table 4 and 6) with elevated EDI (0.005mg/kg bwt/day) and (0.004mg/kg bwt/day), respectively. Even though no regulatory limit for mercury content in any of mentioned standards for infant formula and milk cereal-based baby food and this need more attention from Egyptian and international authorities. Mercury is excreted firstly after 1–3 months in feces for inorganic Hg and urine for methyl Hg, which is able to cross the Placenta and Blood-Brain, causing neurotoxicity, teratogenicity and brain damage, accordingly mercury in high amount is considering very harmful for infants and has serious consequences on brain, kidney, and lungs (EFSA, 2012).

Conclusion

In our study; infant formula, that especially neonates (0 - 6 month), are highly polluted with unexpected levels of lead, arsenic and mercury as well as not neglectable contamination by cadmium and aluminum; where now the majority of infants all over the world received this type of food. The tolerable values have been established by JECFA for lead and arsenic were withdrawing in 2011 and that mean the tolerable intake values were not considered secure anymore. As our young children are with high sensitivity and vulnerability during growth, they need more attention from authorities. The cereal baby food standards need to be checked as not all elements permissible limit was established. Parents are requiring to be informed about the importance and safety of breast milk especially first 6 month of baby life. Therefore, the infant milk-based food industries need a strict periodical monitoring for levels of potential toxic metals contamination in order to ensure that their products are safe for infants.

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