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ORIGINAL ARTICLE

Heavy Metals in Commercial Food for Infants and Small Children Origin from the Sarajevo Market

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KEYWORDS

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ABSTRACT: The objective of this study was to determine total mercury, lead and cadmium contents in commercial food for infants and small children marketed on the Sarajevo area of Bosnia and Herzegovina (BiH) and to estimate the toxicological risk associated with the consumption of food for infants and small children regarding mercury, lead and cadmium. A total of 30 samples were analysed. The content of lead and cadmium was analysed by graphite furnace atomic absorption spectrometry. Total mercury content was measured with a direct mercury analyser. The limits of cadmium, lead and inorganic mercury for infants and small children was calculated according to the dietary intake limits established by European Food Safety Authority (EFSA) and recommended body weights for European toddlers and infants. Overall, the contents of lead, mercury and cadmium in analyzed commercial food for infants and small children samples were considered quite low. Depending on the frequencies of daily usage the ready for use products for infants and small children there is the some circumstances in which exposure to lead and cadmium appeared to be of health concern.

INTRODUCTION

Infants and small children are highly vulnerable to environmental contaminants exposure due to their little body weight and high growth rate in relation to their exposure.

Environmental contaminants affect organ systems (nervous system, immune system, endocrine system, urogenital system, hematopoietic system, skeletal system, gastrointestinal system) rather than produce specific disease entities. It can be defined at least five classes of environmental contaminants: certain metals, persistent organic pollutants, volatile organic compounds and airborne pollutants. Metals with recorded toxic effects include lead (Pb), mercury (Hg), arsenic (As), cadmium (Cd) and manganese (Mg) [1].

Pb enters into the human organism through the respiratory and gastrointestinal tract [2]. Infants and small children can absorb 30-50% of Pb from the diet, in comparison with 5-15% for adults [3]. In humans, the main target organic system for lead toxicity is the central nervous system. There is considerable evidence demonstrating that an elevated concentration of Pb in children blood is in relation to a reduced score of Intelligence Quotient (IQ) and reduced cognitive functions up to at least seven years of age [4]. Children exposed to the toxic influence of Pb suffer from a reduced intellectual development, subtle growth disorders, hearing disorders and cognitive disorders [5]. The International Agency for Research on Cancer (IARC) has classified lead as possibly carcinogenic to humans (IARC Group 2B) and inorganic compounds of lead as probably carcinogenic to humans (IARC Group

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2A). IARC has not classified organic compounds of lead on the basis of carcinogenicity (IARC Group 3) [4].

Cd is easily absorbed into the human body and it is accumulated in the kidneys and the liver. The absorption of Cd is increased when the diet is poor in calcium and iron and by co-administration of milk [6]. The elimination half-life of Cd is to approximately 30 years and its accumulation is highest during the first three years of human life [7,8]. According to the IARC Cd is the carcinogenic agent for humans [9].

The toxicodynamic and toxicokinetic properties of Hg in humans depend on the chemical form of Hg. Inorganic mercury (iHg) compounds are dominantly absorbed in the gastrointestinal tract but can be absorbed through the skin and respiratory tract, too. Methyl mercury (metHg) is more than 90% and long-chain alkyl derivates are more than 50% absorbed in the gastrointestinal tract. MetHg shows higher mobility in the human organism than iHg compounds [3] and can cross the placental barrier and the blood-brain barrier. MetHg intoxication causes almost exclusively neurological disorders, which are mostly irreversible if they occurred intrauterine [10].

Recent studies shown that the presence of toxic metals in commercial food for infants and small children may represent significant dietary sources of exposure [11].

Considering the potential toxicological risk of Hg, Pb and Cd to human health as well as the lack of data in BiH, this study aimed to determine total Hg, Pb and Cd contents in food for infants and small children labelled as from organic or conventional origin and marketed in the Sarajevo area of BiH and to estimate the toxicological risk associated with the consumption of infant and small children food regarding Hg, Pb and Cd.

MATERIALS AND METHODS

Samples collection

A total of 30 samples of commercial food for infants and small children labelled as from conventional (n=20) or organic (n=10) production. Out of 30 samples 18 samples were processed cereal-based food and 12 samples were fruit-desserts. The samples included 7 imported market brands available in BiH and all registered and labelled as intended for consumption by infants and small children. Samples were randomly collected from supermarkets in Sarajevo in August 2019. All the samples available in packs were collected, mixed thoroughly and stored in plastic containers at -4°C up to analysis.

Reagents and standards

Before analysis, all infant and toddler food samples were homogenized again using a mortar and pestle and mixed to get a uniform texture of analytical portions. Sample preparation for Cd and Pb analysis was done with the standard method of microwave digestion of Institute for Standardization of Bosnia and Herzegovina (BAS EN 13804:2015). Approximately 0.5 g of homogenised samples was microwave-digested in a Microwave Ethos D (Milestone, Sorisole, Italy) oven for 30 min in a closed quartz vessel with 7 mL of 65% HNO_3 (Sigma-Aldrich, Seelze, Germany) and 1 mL of 30% H_2O_2 (Sigma-Aldrich, Seelze, Germany). The microwave oven was programmed at 1500 W and 4 500 000 Pa as maximum power and pressure limit (ramp time 15 min, hold time 15 min and cooling time 20 min). An appropriate blank (7 mL of 65% HNO_3 and 1 mL of 30% H_2O_2) was prepared in the same way. Following digestion, the samples were then transferred into 50 mL vials and were diluted with ultrapure water up to 50 mL.

Analyses were done using the standard method for determination of trace elements in foodstuffs of Institute for Standardization of BiH (BAS EN 13804:2015).

Standards solutions for Pb and Cd (1000 mg L^{-1}±4 mg L^{-1}, Sigma-Aldrich, Seelze, Germany) were used to build up calibration diagrams. Standard dilutions were prepared from a stock solution of 1 g L^{-1} by successive dilution for each metal. All reagents and standard solutions were prepared using ultrapure water (18.2 µS cm^{-1}, Arium611, Sartorius Mechatronics, Hamburg, Germany).

Analyses of total Cd and Pb were performed on the AA-7000F Dual Atomizer System (Shimadzu, Columbia, US) atomic absorption spectrometer, equipped with self-reversal (SR) method background correction, autosampler and graphite furnace. The argon (Ar) flow is used to remove residues from the graphite tube during pyrolysis.

Total Hg content in homogenized samples was measured directly with a direct Hg analyser AMA 254 Mercury Analyzer (Curtage Analyzes Service, Italy). For the analysis, the internal method (IM-OP-5.4-01-1-1-S/AMA) for the determination of Hg in foodstuffs of the

Institute of public health of the Federation of Bosnia and Herzegovina was used. Briefly, the sample was directly weighed (ranged between 0.1 and 0.5 g) into the pre-cleaned sample boat and placed in the instrument and the Hg content was determined using the principle of thermal decomposition, amalgamation and atomic absorption spectrometry at 254 nm.

A standard solution of Hg (1000 mg L^{-1}±2 mg L^{-1}, Merck KGaA, Darmstadt, Germany) was diluted with HNO_3 to obtain working solutions for calibration curves (2.5–30.0 ng and 100–500 ng). All the samples were analysed in triplicate. The blanks and calibration standards were analysed using the same methods.

The content of heavy metals were expressed in milligrams per kilogram of wet weight. Method detection and quantification limits (LoD and LoQ), also expressed in mg kg^{-1} wet weight, were Cd: 0.0266 and 0.0798; Pb: 0.0003 and 0.0011; Hg: 0.004; for LoD and LoQ, respectively.

Data analysis

To obtain the range of heavy metals exposure through consumption of food for infants and small children we have used a similar approach as Gardener and co-workers [5]: the amount of Pb, Cd and Hg were calculated in each food sample among the range of calories (100, 300 and 500 kcal) and servings (1, 2, and 3 servings). Next, the number of food for infants and small children samples that exceeded derived hazard limits within each of the calorie and serving quantities were calculated.

For the calculation of the hazard limits in food for infants and small children for selected toxic metals, we used the data for the tolerable daily intakes (TWI) for Cd and iHg and benchmark dose (lower confidence limit) of Pb for dietary intake value for developmental neurotoxicity ($BMDL_{01}$) which were established by EFSA.

For Cd, EFSA established TWI of 2.5 µg kg^{-1} b.w. [12], for iHg TWI of 4 µg kg^{-1} b. w. and for metHg TWI of 1.3 µg kg^{-1} b. w. The contribution of metHg to total Hg is typically 80-100% in fish and 50-80% in seafood other than fish. In other foods, Hg is presumed to be present as iHg [13].

EFSA was estimated the relation between dietary Pb intake and blood Pb levels in children up to age seven using the Integrated Exposure Uptake Biokinetic (IEUBK) model for Pb in children. Using the IEUBK model, a $BMDL_{01}$ dietary intake value of 0.50 µg kg^{-1} body weight per day for developmental neurotoxicity was derived [4].

In addition, according to the EFSA's Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data [14] for dietary exposure assessment, a body weight of 12 kg was used as a default for European toddlers (aged 1-3 years) and a body weight of 5 kg was used as the default for European infants (aged 0-12 months).

RESULTS AND DISCUSSION

In table 1 the content of the toxic metals among the 30 commercial food items for infants and small children (fruits, cereals, vegetables and meats) most frequently consumed in BiH are shown.

Table 1. Content of heavy metals across the food for infants and small children (N=30)

[µg kg^{-1}]	Lead	Cadmium	Mercury
Max	14.2	79.8	5.6
75th	8.4	5.8	4.2
Median	5.3	2.7	3.4
25th	2.0	1.2	1.1

Overall, the contents of Pb, Hg and Cd in analyzed food samples are considered very low. The results of heavy metals content in our study were in good agreement with the data from EFSA scientific reports about heavy metals dietary exposure in the European population as well as with the result from the other studies.

According to the EFSA report mean Pb content in the ready-to-eat meals for infants and small children (n=969) variated between 7 and 20 µg kg^{-1}. The mean content of Pb in the cereal-based ready-to-eat meals for infants and small children (n=26) was 7 µg kg^{-1}, and for fruit purée (n=196) was 10 µg kg^{-1} [15]. In our study Pb contents in

2 samples (6.7%) were above the mean content of Pb in cereal-based ready-to-eat meals and 4 samples (13.3%) above the mean content of Pb in fruit purée in comparison with 20 EU Member States and Norway [15]. The mean content of Cd in the ready-to-use meals for infants and small children in 20 EU Member States and Norway (n=1350) was 5.69 µg kg^{-1}. In 8 samples (26.7%) analyzed in this study, the content of Cd was above the upper level of mean content on Cd in the ready-to-use meal for infants and small children in 20 EU Member States and Norway [16].

Preliminary analysis of cereal-based food for infants in Spain shown higher content of Pb and Cd in those from organic production (n=17, Pb: 26.07; 21.36–51.63; Cd: 18.52; 16.56–28.50 µg kg^{-1}) than in conventional ones (n=74, Pb: 10.78; 6.43–19.33; Cd: 7.12; 4.40–11.77 µg kg^{-1}) [17]. In the study conducted by Škrbić and co-workers [18] the content of Pb in all analyzed porridge samples origin from Serbia (n=18) was below of the limit of detection, but in the porridge sample origin from Spain (n=28), the mean content of Pb was 9 µg kg^{-1}.

The mean content for total Hg in the ready-to-use meal for infants and small children in 20 EU Member States and Norway (n=834) was 1.6 µg kg^{-1}. In our study in 18 (18%) samples the content of total Hg was above the upper level of mean content of total Hg in the ready-to-use meal for infants and small children in 20 EU Member States and Norway [19].

The European Commission (EC), as well as BiH regulatory, did not set maximum levels for Cd and Hg in the ready-to-use meal for infants and small children [20]. Baring this in mind, it can be concluded that all analyzed samples of commercial food for infants and small children in this study do meet the conditions for presence in the EU and BiH markets.

According to the dietary intake limits for selected heavy metals established by EFSA and recommended body weights for European toddlers and infants, we calculated the limits of Cd, Pb and iHg for toddlers and infants (Table 2).

Table 2. Limits of selected metals for toddlers and infants

Metals	**Limits (µg kg^{-1} b.w. per day)**	
	Toddlers	***Infants***
Cd	0.36	0.36
Pb	0.50	0.50
Hg	0.57	0.57

Tables 3 and 4 show the percentage of food for infants and small children samples that exceeded the EFSA limits for daily Pb and Cd intake, respectively among a range of servings (1-3 servings) and calories (100, 300 and 500 kcal).

Table 3. Percentage of samples which exceed established limits for Cd among the range of servings/calories

Samples	**Exceeds daily limit for 5 kg infants**					
	1 serving	**2 servings**	**3 servings**	**100 kcal**	**300 kcal**	**500 kcal**
All (n=30)	**n=2 (6.7%)**	**n=6 (20%)**	**n=6 (20%)**	**n=2 (6.7%)**	**n=8 (26.7%)**	**n=12 (40%)**
Conventional production (n=20)	**n=2 (10%)**	n=0 (0%)	**n=4 (20%)**	**n=2 (10%)**	**n=8 (80%)**	**n=2 (80%)**
Organic production (n=10)	n=0 (0%)	n=0 (0%)	**n=2 (20%)**	n=0 (0%)	n=0 (0%)	n=0 (0%)
Porridge Cereals-based (n=20)	**n=2 (10%)**	**n=6 (30%)**	**n=6 (30%)**	**n=2 (10%)**	**n=8 (40%)**	**n= 12 (60%)**
Porridge Fruit based (n=10)	n=0 (0%)	n=0 (0%)	n=0 (0%)	n=0 (0%)	n=0 (0%)	n=0 (0%)
	Exceeds daily limit for 12 kg toddlers					
	1 serving	2 servings	3 servings	100 kcal	300 kcal	500 kcal
Samples						

All (n=30)	**n=2 (6.7%)**	**n=2 (6.7%)**	**n=2 (6.7%)**	**n=2 (6.7%)**	**n=2 (6.7%)**	**n=4 (13.3%)**
Conventional production (n=20)	**n=2 (10%)**	**n=2 (10%)**	**n=2 (10%)**	**n=2 (10%)**	**n=2 (10%)**	**n=4 (20%)**
Organic production (n=10)	n=0 (0%)	n=0 (0%)	n=0 (0%)	n=0 (0%)	n=0 (0%)	n=0 (0%)
Porridge Cereals-based (n=20)	**n=2 (10%)**	**n=2 (10%)**	**n=2 (10%)**	**n=2 (10%)**	**n=2 (10%)**	**n=4 (20%)**
Porridge Fruit based (n=10)	n=0 (0%)	n=0 (0%)	n=0 (0%)	n=0 (0%)	n=0 (0%)	n=0 (0%)

Table 4. Percentage of samples which exceed established limits for Pb among a range of servings/calories.

Samples	**Exceeds daily limit for 5 kg infants**					
	1 serving	2 servings	3 servings	100 kcal	300 kcal	500 kcal
All (n=30)	n=0 (0%)	**n=12 (40%)**	**n=18 (60%)**	n=0 (0%)	**n=14 (44.7%)**	**n=22 (73.3%)**
Conventional production (n=20)	n=0 (0%)	**n=12 (60%)**	**n=16 (80%)**	n=0 (0%)	**n=14 (70%)**	**n=14 (70%)**
Organic production (n=10)	n=0 (0%)	n=0 (0%)	**n=2 (20%)**	n=0 (0%)	n=0 (0%)	**n=8 (80%)**
Porridge Cereals-based (n=20)	n=0 (0%)	**n=12 (6%)**	**n=18 (90%)**	n=0 (0%)	**n=14 (0%)**	**n=18 (90%)**
Porridge Fruit based (n=10)	n=0 (0%)	n=0 (0%)	n=0 (0%)	n=0 (0%)	n=0 (0%)	**n=4 (40%)**
	Exceeds daily limit for 12 kg toddlers					
Samples	1 serving	2 servings	3 servings	100 kcal	300 kcal	500 kcal
All (n=30)	n=0 (0%)	n=0 (0%)	**n=2 (6.7%)**	n=0 (0%)	n=0 (0%)	**n=4 (13.3%)**
Conventional production (n=20)	n=0 (0%)	n=0 (0%)	**n=2 (10%)**	n=0 (0%)	n=0 (0%)	**n=4 (20%)**
Organic production (n=10)	n=0 (0%)	n=0 (0%)	n=0 (0%)	n=0 (0%)	n=0 (0%)	n=0 (0%)
Porridge Cereals-based (n=20)	n=0 (0%)	n=0 (0%)	**n=2 (10%)**	n=0 (0%)	n=0 (0%)	**n=4 (20%)**
Porridge Fruit based (n=10)	n=0 (0%)	n=0 (0%)	n=0 (0%)	n=0 (0%)	n=0 (0%)	n=0 (0%)

Two analysed samples (both cereal-based baby food from conventional production) exceeded EFSA Cd daily limit for a 5 kg infants in a single serving, though six samples did so in two servings, and eight in three servings (all of them was cereal-based baby food and two of them were from organic production).

Two food for infants and small children samples exceeded the EFSA Cd daily limit for a 5 kg infants in 100 kcal, 8 in 300 kcal and 12 in 500 kcal (all of them were cereal-based baby food from conventional production).

When the EFSA Cd daily limit for a 12 kg toddlers was considered, 6.7% (n=2) of samples exceeded the limit in a single, two and three servings, and 6.7% (n=2) exceeded the limit in 100 kcal and 200 kcal, increasing to 13.3% (n=4) in 500 kcal. All of the samples that exceeded the EFSA limits were cereal-based baby foods from conventional production.

Twelve samples (all of them cereal-based baby food from conventional production) exceeded EFSA Pb daily limit for a 5 kg infants in two servings, though 18 items did so in three servings (all of them were cereal-based baby food and four of them were from organic production).

Fourteen food for infants and small children samples (46.7%) exceeded the EFSA Pb daily limit for a 5 kg infants in 300 kcal and 22 (73.3%) in 500 kcal (all of them were cereal-based baby food from conventional production).

None of the food for infants and small children samples exceeded the Hg limit across the range of numbers of calories (100, 300 and 500 kcal) and servings (1, 2, and 3 servings).

Depending on the frequencies of daily usage the commercial food for infants and small children there is the same circumstances in which exposure to Pb and Cd appeared to be of health concern.

Our finding underscored the importance of providing infants and small children with a varied diet to avoid overexposing these populations to any contaminant potentially present in food.

Limitation of the study

The presented study is the first of this kind in BiH, even though it has some limitations. Firstly, the samples were collected based on the availability of commercial food for infants and small children in BiH retail stores which does not represent a random representative sample. Secondly, the collected samples do not include all available various categories of commercial food for infants and small children and lastly, the sample sizes across analysed product types were quiet small. (16)

CONCLUSIONS

The studies showed that the toxic metals are very often present in low content in commercial infants and toddler foods particularly those containing cereals and those from conventional production. Depending on the frequency of daily usage of the ready for meal products for infants and small children there is the some circumstances in which exposure to Pb and Cd appeared to be of health concern. Due to the study limitations, these results should be interpreted as preliminary. Further research is needed to understand the level of the exposure to heavy metals and the long-term health effects in infants and small children.

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Conflict of interests

The Author(s) declare(s) that there is no conflict of interest.

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