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# Lead, Cadmium and Arsenic Bioaccessibility of 24-Hour Duplicate Diet Ingested by Preschool Children Attending Day Care Centers in Brazil

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**Abstract:** Lead, a metal with high neurotoxicity to children; cadmium, a carcinogenic and bioaccumulative contaminant and arsenic; a class 1 carcinogenic, are toxic elements (TEs) whose relevant route of exposure may be diet. We determined the bioaccessible fraction of lead, cadmium and arsenic from the diet of preschool children from 2 day care centers (DCC). A cross-sectional study was conducted with 64 1–4-year-old children from 2 DCCs, where the 24-hour duplicate diet samples were collected. The diet samples were analyzed by ICP-MS for lead, cadmium and arsenic total concentrations ( $n = 64$ ) and their bioaccessibility were analyzed for a subsample ( $n = 10$ ). The dietary intake (DI) mean for lead, cadmium and arsenic were  $0.18 \pm 0.11 \mu\text{g kg}^{-1}\text{bw}$ ,  $0.08 \pm 0.04 \mu\text{g kg}^{-1}\text{bw}$  and  $0.61 \pm 0.41 \mu\text{g kg}^{-1}\text{bw}$ , respectively. All DI calculated for TEs, considering total intake, were found lower than the tolerable limits (European Union, EU, or World Health Organization, WHO, when applicable), except for one child's Pb intake. Bioaccessibilities ranged between 0–93%, 0–103% and 0–69%, for lead, cadmium and arsenic, respectively. Although DI for TEs has been found lower than TI, these reference values have been recently decreased or withdrawn, as it was the case for lead and arsenic, whose tolerable limits were withdrawn by WHO.

**Keywords:** bioaccessibility; 24-hour diet; preschool children; arsenic intake; cadmium intake; lead intake.

## 1. Introduction

Food is considered an important source of human exposure to some contaminants, such as lead [1], cadmium [2–3] and arsenic [4]. Cadmium is one of the 11 metals in the list of persistent toxic and bioaccumulative pollutants, and ingestion of cadmium through contaminated food is the largest source of this metal exposure for non-smokers [3,5–7]. Cadmium accumulates and damages the kidneys [2] and is associated with reducing of childhood cognitive ability [3]. Lead and arsenic, on the other hand, are considered the most toxic elements and are included in the pollutants list set by

United States Environmental Protection Agency (USEPA) [6]. Lead exposure in childhood is associated with deficits in attention, concentration, intelligence, learning, psychomotor skills and aggressiveness [8-11]. Arsenic is also associated with children cognitive deficits [12-13]. Food contributes with up to 93% of the arsenic total intake [14] and can contribute on average 83% of lead intake [15].

Children are the most vulnerable group to the effects of potentially toxic elements, as they present higher gastrointestinal absorption, faster metabolic processes, detoxification system in development and higher food consumption by body weight compared to adults. In addition, the blood-brain barrier is not fully developed yet at this stage of life, which allows toxic elements to accumulate in the brain, causing dysfunction in the central nervous system [9,16].

The concentration of potentially toxic elements in foods is not proportional to the bioaccessible concentration [17]. The fraction of a contaminant that is released from the food matrix into the digestive fluid and thus available for intestinal absorption is the bioaccessible fraction of that component [7]. The bioaccessibility determination of contaminants ingested through food allows a better evaluation of the potential health risks, avoiding overestimation [18-19]. Therefore, the aim of this study was to determine, through 24-h duplicate diet method, the bioaccessible fraction of lead, cadmium and arsenic from the diet of preschool children attending 2-day care centers (DCC) in Sao Paulo, Brazil. The Bioaccessible Estimated Daily Intake (BEDI) results were then compared with the Benchmark Dose Level (BMDL) by European Food Safety Authority (EFSA) for lead, and with the Provisional Tolerable Monthly Intake (PTMI) by World Health Organization (WHO) for cadmium.

## 2. Materials and Methods

### 2.1 Subjects

This study was conducted with 64 1–4-year-old children from 2 DCCs, one of them located in the East Zone (coded PF) and the other one in the South Zone (coded PS) of the city of Sao Paulo, Brazil, where had been found high blood lead levels (BLLs) in a previous study [20]. Children included in this study spend approximately 10 hours/day at day care centers. This study was reviewed and approved by the Institutional Review Board (IRB) of the School of Public Health of the University of Sao Paulo, Brazil (Protocol #1.127.698). The children's parents and guardians were invited to a meeting with the investigators to discuss the potential sources of lead exposure and its health effects. All children whose parents/guardians signed an informed consent form were included in the study.

### 2.2 Sampling of diets and preparation

The sampling of diets was conducted with 64 children attending two DCCs. Daily lead intake from the diet for each child, considering solid foods and drinks, was estimated using a 24-h duplicate plate method, including one weekday. The parents and guardians were instructed to maintain the usual dietary habits of their children and to duplicate the dietary intake as precisely as possible by observing the amounts that the children really ate and drank. The parents and guardians were asked to use household measures, such as a tablespoon, teaspoon, or cupful, to approximate the quantities of children's food ingested. They were also asked to remove the foods' parts that are not normally eaten, such as bones, skin, and seeds, before storing the duplicate food and drink in containers in a refrigerator until the researchers collect the 24-h diet samples. For cooked meals, parents were asked to make a similar plate, with the same portion of the children's plates, and wait until the children finish the meal and then to add or remove comparable amounts of food from the duplicate plate [21-22]. The same protocol was accomplished at the DCCs, and the investigators monitored the children during the whole day, recording the portions. After the samples of duplicate diets had been collected, they were transported to the laboratory and thoroughly homogenized using a mixer (Arno model 600W, Sao Paulo, SP, Brazil). The weight was recorded (Shimadzu, Barueri, SP, Brazil). Diets were aliquoted and stored at -22 °C until the chemical analysis was performed for the lead.

To avoid contamination, all polypropylene flasks used in the collections were previously cleaned with a detergent solution, rinsed in HNO<sub>3</sub> 10% overnight, rinsed with deionized water 18.2MΩ.cm at 25 °C, dried, and stored in a closed polypropylene container. High-purity water produced by a Milli-Q water purification system (Millipore, Bedford, MA) was used throughout. A sub-boiling system (Distilled, Berghof, Germany) was employed to produce high-purity nitric acid.

### 2.3 Sample preparation

The diet samples were lyophilized by using the lyophilizer (Liotop, L101) at a pressure of 200 μHg and checked at -50 °C for 48 hours. After the lyophilization procedure, the samples were stored at -20 °C.

### 2.4 Acid digestion for metals determination

The elements determination in the diet samples was performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Firstly, 100 mg of lyophilized sample was weighed (in triplicate) and 1 mL of sub-distilled HNO<sub>3</sub> was added, which was pre-digested during the night (overnight). The pre-digestion was followed by a water bath (Solab SL1522L, Brazil) at 90° C at 4 hours. After cooling, the volume was made up to 14 mL with deionized water 18.2 MΩ.cm at 25 °C. To verify the accuracy of the procedure, certified reference material (CRM) lobster hepatopancreas (TORT-3, National Research Council Canada) was used, and was prepared by the same procedure.

### 2.5 Bioaccessibility

In vitro bioaccessibility assessment was performed according to Bertin et al. [23] and the United States Pharmacopoeia [24]. The assay was performed in two steps: the first, using gastric solution and the second, using intestinal solution. In this phase, the same CRM was used to verify the accuracy of the procedure.

### 2.6 Preparation of Gastrointestinal Solution

For the gastric solution, 0.32g of pepsin (Sigma-Aldrich, St. Louis, USA) was dissolved in ultra-pure water (~80 mL, Millipore RiO-DITM, Massachusetts, USA). After, we added 0.7mL of sub-boiled HCl (36% v/v, Synth, Brazil) and the volume made up to 100 mL. Then, the pH was adjusted to 1.2 using 0.1M HCl [24].

### 2.7 Preparation of Intestinal solution

Initially, we solubilized ~0.2g of bile salts (0.08 g sodium glycodeoxycholate + 0.05 g sodium taurodeoxycholate + 0.08g sodium taurocholate hydrate) and 0.5g pancreatin in 100mL of NaHCO<sub>3</sub> 3% w/v. All salts used in the intestinal solution were obtained from sigma Sigma-Aldrich (St. Louis, USA) [24].

### 2.8 Gastric digestion simulation

Samples (200mg) were weight in conical tubes (50 mL) (Falcon®, Corning, Tamaulipas, Mexico). After, we added 3 mL of gastric solution. Then, samples were placed in water bath (SL1522L, Solab, Brazil) at 37 °C during 2 h. The samples were gently shaken every 20 minutes [23].

### 2.9 Intestinal digestion simulation

After gastric digestion, the solution was submitted to the intestinal digestion simulation. For this purpose, NaHCO<sub>3</sub> (3% w/v) was added for pH adjustment to 6.8. Then, we added 3mL of intestinal solution and heated in a water bath at 37°C during 2h with shaking (50 rpm). Finally, the samples were cooled to room temperature and centrifuged (SL700, Solab, Brazil) at a rate of 1077g during 20min. The supernatants of this step were separated from the precipitates. Precipitates and

supernatant were digested following the same procedure previously described for totals element quantification [23].

2.10 Metals Determination

The determination of the elements total concentration and their bioavailabilities in diet samples were carried out by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies, 7900). An external calibration curve was prepared with standard multielement solution (PerkinElmer, Inc.) at concentrations of 0.1; 1; 5; 10; 50; 100; 200; 500 and 1000 µg L<sup>-1</sup>. Blank solutions were also prepared and lobster hepatopancreas reference material (TORT-3, National Research Council Canada) prepared using the same protocol for samples. The ICP-MS conditions are presented in the Table 1.

Table 1. Operational conditions for ICP-MS in total diets analyses and bioaccessibility

Parameter	Diet	Bioaccessibility
Radio Frequency Power	1600 W	1550 W
Argon Flow Rate	15 L min <sup>-1</sup>	15 L min <sup>-1</sup>
He Flow	5.0 mL min <sup>-1</sup>	5.0 mL min <sup>-1</sup>
HeHE	10 mL min <sup>-1</sup>	10 mL min <sup>-1</sup>
Nebulizer Gas Flow Rate	0.68 L min <sup>-1</sup>	1.05 L min <sup>-1</sup>
Collision Cell	Helium (purity >99.99%)	Helium (purity >99.99%)
Nebulizer Chamber	Scott (double pass)	Scott (double pass)
Interface	Nickel cones	Nickel cones
Sampling Cone	1 mm	1 mm
Skimmer	0.45 mm	0.45 mm

3. Results

The anthropometric characteristics of children are presented in Table 2.

Table 2. Anthropometric characteristics of children from the day care centers (DCC) PS and PF (São Paulo, 2015)

	PS DCC		PF DCC		Total	
	Male (n = 26)	Female (n = 15)	Male (n = 8)	Female (n = 15)	Male (n = 34)	Female (n = 30)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Age (years)	3.6 ± 0.6	3.3 ± 0.7	2.6 ± 0.9	3.4 ± 0.7	3.4 ± 0.8	3.4 ± 0.7
Height (cm)	98 ± 7	95 ± 6	93 ± 12	101 ± 6	97 ± 8	98 ± 7
Weight (kg)	17 ± 4	15 ± 2	15 ± 4	17 ± 3	16 ± 4	16 ± 3
BMI* (kg/m <sup>2</sup> )	17 ± 2	17 ± 1	18 ± 1	16 ± 2	17 ± 2	17 ± 2

\*Body mass index.

According to the Joint Expert Committee on Food Additives (JECFA), the provisional tolerable weekly intake (PTWI) for inorganic arsenic was withdrawn [25], as well as PTWI for lead [26]. The provisional tolerable monthly intake for cadmium is 25  $\mu\text{g kg}^{-1}$  bw per month [27]. The European Union has the Benchmark Dose Level (BMDL) for lead, which is 0.5  $\mu\text{g Pb kg}^{-1}$  bw [28]. However, for cadmium [29] and arsenic [30] the values were also withdrawn. That information is essential to consider properly the results presented in Tables 3 and 4.

Lead and cadmium daily ingestions were similar in both DCCs (Table 3). The mean lead intake values were below the European Union BMDL for the development of neurotoxic effects in children, corresponding to 36% of BMDL for both gender. A child attending the DCC PS ingested a concentration 37.5% (0.8  $\mu\text{g Pb kg}^{-1}$  bw) higher than the BMDL (0.5  $\mu\text{g Pb kg}^{-1}$  bw).

**Table 3.** Children’s daily and weekly ingestion of lead (Pb), arsenic (As) and cadmium (Cd) ( $\mu\text{g kg}^{-1}$  bw<sup>-1</sup> day or week) from two day care centers of São Paulo (PS DCC and PF DCC), 2015.

	PS DCC		PF DCC		Total	
	Male	Female	Male	Female	Male	Female
	(n = 26)	(n = 15)	(n = 8)	(n = 15)	(n = 34)	(n = 30)
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Pb daily intake	0.18 $\pm$ 0.15	0.20 $\pm$ 0.08	0.18 $\pm$ 0.08	0.15 $\pm$ 0.05	0.18 $\pm$ 0.14	0.18 $\pm$ 0.07
Pb weekly intake	1.25 $\pm$ 1.05	1.42 $\pm$ 0.58	1.29 $\pm$ 0.54	1.03 $\pm$ 0.36	1.26 $\pm$ 0.95	1.23 $\pm$ 0.51
As daily intake	0.70 $\pm$ 0.35	0.79 $\pm$ 0.49	0.54 $\pm$ 0.55	0.35 $\pm$ 0.19	0.66 $\pm$ 0.40	0.57 $\pm$ 0.43
As weekly intake	4.91 $\pm$ 2.43	5.53 $\pm$ 3.41	3.78 $\pm$ 3.83	2.47 $\pm$ 1.34	4.65 $\pm$ 2.80	4.00 $\pm$ 2.98
Cd daily intake	0.08 $\pm$ 0.03	0.10 $\pm$ 0.05	0.08 $\pm$ 0.03	0.07 $\pm$ 0.03	0.08 $\pm$ 0.03	0.09 $\pm$ 0.04
Cd weekly intake	0.54 $\pm$ 0.21	0.71 $\pm$ 0.37	0.59 $\pm$ 0.20	0.49 $\pm$ 0.18	0.55 $\pm$ 0.20	0.60 $\pm$ 0.30

The bioaccessibility fractions of lead, cadmium and arsenic are presented in Table 4. Even though some samples have presented the bioaccessible percentage close to 100%, none of the samples reached the values of BMDL, or PTMI, or the withdrawn PTWI, of the potentially toxic elements studied. The Bioaccessible Estimated Daily Intake (BEDI), calculated for the sample with higher bioaccessibility, corresponds to 34% of Pb BMDL [28], 9.2% of Cd PTMI [27], while for As BEDI is equivalent to 59% of the withdrawn PTWI [30].

**Table 4.** Percentage range of bioaccessibility (minimum – maximum) for lead, cadmium and arsenic in diet, bioaccessibility mean (%) and Children’s Bioaccessible Estimated Daily Intake (BEDI) per body weight ( $\mu\text{g kg}^{-1}$  bw<sup>-1</sup> day), São Paulo, 2015

Parameter	Lead	Cadmium	Arsenic
Range of Bioaccessibility (%)	0-93	0-100	0-69
Bioaccessibility mean (%)	4%	0%	9%



BEDI* ( $\mu\text{g kg}^{-1}$ bw per day)	0.17	0.04	1.26
*BEDI calculated for the sample with higher bioaccessibility			

4. Discussion

Values higher than those found in this present study were reported by Kim et al. [31], whom investigated the exposure to lead and cadmium of 457 South Korean children aged 0-6 years old through a two-day 24-hours recall, a different method. The mean of the lead intake was  $0.46 \mu\text{g kg}^{-1}$  bw per day, with 35% of children exceeding the BMDL value of  $0.5 \mu\text{g Pb kg}^{-1}$  bw per day. Fruits and milk appeared as the main sources of lead exposure. For cadmium, the mean intake was  $0.34 \mu\text{g Cd kg}^{-1}$  bw per day. Cereals, fish, shellfish and, algae had a significant contribution to the intake of cadmium.

Watanabe et al. [32] reported the exposure to lead and cadmium through the 24-hours duplicate diet and urine evaluation of 108 children (4-6 years old) from 4 DCC located in Seoul city and Jeju Island, Korea. They found a geometric mean for Cd intake of  $0.58 \mu\text{g kg}^{-1}$  bw per day, 7 times higher than our finds (Table 3) and, 19.5% of which was attributed to rice consumption. For Pb it was found a geometric mean of  $0.27 \mu\text{g Pb kg}^{-1}$  bw per day, 1.5 times higher than our finds (Table 3).

Pysz et al. [33-34] evaluated lead and cadmium intake of 4-6-year-old children and adolescents who lived in orphanages in Krakow, Poland, through 24-hours duplicate diet of four days in each season of the year, including weekend days. For the children from the orphanage which has an age range equivalent with the children in present study lead and cadmium annual mean intake were 11.57 and  $16.63 \mu\text{g kg}^{-1}$  bw per week, respectively. These values are higher than the ones found in the present study and correspond to concentrations 69.75% above BMDL for Pb [28] and 62.42% above the Provisional Tolerable Monthly Intake (PTMI) of  $25 \mu\text{g Cd kg}^{-1}$  bw per month [27].

A duplicate diet approach was also applied in a study performed in Jinhu area, China, to estimate the arsenic dietary intake for thirty children (2-5 years old) and thirty adults (29-55 years old). The diet collection period were 3 days wherein one of them was a weekend day. The mean arsenic intake for the children was similar of our findings (Table 3),  $0.6 \text{ mg kg}^{-1}$  bw day<sup>-1</sup> [1].

The bioaccessibility fractions of lead, cadmium and arsenic are presented in Table 4. Even though some samples presented the bioaccessible percentage close to 100%, none of the samples reached the values of BMDL, or PTMI, or the withdrawn PTWI, of the potentially toxic elements studied. The Bioaccessible Estimated Daily Intake (BEDI), calculated for the sample with higher bioaccessibility, corresponds to 34% of Pb BMDL [28], 9.2% of Cd PTMI [27], while for As BEDI is equivalent to 59% of the withdrawn PTWI [30].

As far as we know, the present study was the first one to determine the bioaccessibility of metals in a 24-hours total duplicate diet. Previous studies evaluated only the elemental bioaccessibility in food, raw and/or cooked, not considering the total diet. Hu et al. [35] determined lead and cadmium bioaccessibility in vegetables cultivated in Hong Kong, and they found a range of bioaccessibility from 20 to 68% and from 21 to 96%, respectively. Fu and Cui [17] also verified the bioaccessibility for lead and cadmium in vegetables, evaluating the differences in the gastric and intestinal phases and in raw and cooked. They found that cadmium is more bioavailable in the gastric phase; lead, in the intestinal phase, and that cooked vegetables presented lower concentrations of these bioaccessible elements. For lead, bioaccessibility mean was 9.4% in raw vegetables, and 3.2% in cooked, while for cadmium was 11.2% in raw vegetables and 6.1% in cooked.

The cooking process may influence the elements bioaccessibility [7, 17, 36]. Zhuang et al. [7] investigated the lead and cadmium bioaccessibilities in 6 species of vegetables, raw and cooked, widely consumed in China, which were purchased in local markets of 9 cities. They found that the leafy vegetables had significantly higher concentrations of toxic elements, and the most of those concentrations decreased with cooking process. Cadmium had bioaccessible percentage ranging from 35 to 65% in raw vegetables and 34 and 64% in cooked vegetables, and for lead, such range were 20 to 51% and 11 to 48% in raw and cooked vegetables, respectively.

For children, chemicals exposure is a huge threat due to the children's health impact on organs, systems and functions, because their developmental process and growth [37]. The diet is considered

a relevant source for some toxicants; however, it is not the unique one and it is required a global approach to control the chemical exposure. Children are exposed to numberless toxicants at home, at school, in the playground and, other places [37-42]. The maximum limits for toxicants are continuously in decreasing by regulation agencies beyond the implementation of specific regulations for children's items [43]. The Canada Consumer Product Safety Act (CCPS) regulates the children's jewelry items, which contain lead and cadmium [44]. Furthermore, the children's cosmetics items such as fragrances, makeup, nail polish, face paint and similar items are regulated by Health Canada under the Cosmetic Regulations of the Food and Drugs Act [45]. Similar regulations of the lead concentration in paint or surface coating on children's toys have been established in European Union and Australia [10,46].

The present study brings important data related to children's exposure to highly potentially toxic elements as lead, cadmium and arsenic through diet. However, some limitation might be considered. We evaluated one weekday, future studies may include more days to consider the variety of the meals consumed by children. Taking into account the weekend days, the diet can be different once the children not attend the day care centers.

5. Conclusions

Our findings showed that Brazilian preschool children's diet did not contain high arsenic, cadmium and lead levels compared to data from other countries. Even though our findings indicate that children's dietary exposure to arsenic, cadmium and lead is not very high, the bioaccessibility range of the elements had a large variability and the safe reference limits have been decreased or withdrawn. Considering a possible overall exposure, with other further exposure sources and routes, our findings suggest that the children may be at considerable risk of lead and arsenic exposure through diet. Currently, especially for arsenic, neither WHO nor EFSA has benchmarks considered safe for its ingestion. We believe the same should be purposed for lead. There is no safe level for lead exposure.

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