

1 Article

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# Lead, Cadmium and Arsenic Bioaccessibility of 24- 3 Hour Duplicate Diet Ingested by Preschool Children 4 Attending Day Care Centers in Brazil

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17 #The two first authors deserve the same merit because they had the same level of participation in the data  
18 collection and manuscript writing.

20

21 **Abstract:** Lead, a metal with high neurotoxicity to children; cadmium, a carcinogenic and  
22 bioaccumulative contaminant and arsenic; a class 1 carcinogenic, are toxic elements (TEs) whose  
23 relevant route of exposure may be diet. We determined the bioaccessible fraction of lead, cadmium  
24 and arsenic from the diet of preschool children from 2 day care centers (DCC). A cross-sectional  
25 study was conducted with 64 1–4-year-old children from 2 DCCs, where the 24-hour duplicate diet  
26 samples were collected. The diet samples were analyzed by ICP-MS for lead, cadmium and arsenic  
27 total concentrations (n = 64) and their bioaccessibility were analyzed for a subsample (n = 10). The  
28 dietary intake (DI) mean for lead, cadmium and arsenic were  $0.18 \pm 0.11 \mu\text{g kg}^{-1}\text{bw}$ ,  $0.08 \pm 0.04 \mu\text{g}$   
29  $\text{kg}^{-1}\text{bw}$  and  $0.61 \pm 0.41 \mu\text{g kg}^{-1}\text{bw}$ , respectively. All DI calculated for TEs, considering total intake,  
30 were found lower than the tolerable limits (European Union, EU, or World Health Organization,  
31 WHO, when applicable), except for one child's Pb intake. Bioaccessibilities ranged between 0–93%,  
32 0–103% and 0–69%, for lead, cadmium and arsenic, respectively. Although DI for TEs has been  
33 found lower than TI, these reference values have been recently decreased or withdrawn, as it was  
34 the case for lead and arsenic, whose tolerable limits were withdrawn by WHO.35 **Keywords:** bioaccessibility; 24-hour diet; preschool children; arsenic intake; cadmium intake; lead  
36 intake.

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## 1. Introduction

39 Food is considered an important source of human exposure to some contaminants, such as lead  
40 [1], cadmium [2–3] and arsenic [4]. Cadmium is one of the 11 metals in the list of persistent toxic and  
41 bioaccumulative pollutants, and ingestion of cadmium through contaminated food is the largest  
42 source of this metal exposure for non-smokers [3,5–7]. Cadmium accumulates and damages the  
43 kidneys [2] and is associated with reducing of childhood cognitive ability [3]. Lead and arsenic, on  
44 the other hand, are considered the most toxic elements and are included in the pollutants list set by

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

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

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

## 65 **2. Materials and Methods**

### 66 *2.1 Subjects*

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

76

### 77 *2.2 Sampling of diets and preparation*

78 The sampling of diets was conducted with 64 children attending two DCCs. Daily lead intake  
79 from the diet for each child, considering solid foods and drinks, was estimated using a 24-h duplicate  
80 plate method, including one weekday. The parents and guardians were instructed to maintain the  
81 usual dietary habits of their children and to duplicate the dietary intake as precisely as possible by  
82 observing the amounts that the children really ate and drank. The parents and guardians were asked  
83 to use household measures, such as a tablespoon, teaspoon, or cupful, to approximate the quantities  
84 of children's food ingested. They were also asked to remove the foods' parts that are not normally  
85 eaten, such as bones, skin, and seeds, before storing the duplicate food and drink in containers in a  
86 refrigerator until the researchers collect the 24-h diet samples. For cooked meals, parents were asked  
87 to make a similar plate, with the same portion of the children's plates, and wait until the children  
88 finish the meal and then to add or remove comparable amounts of food from the duplicate plate [21-  
89 22]. The same protocol was accomplished at the DCCs, and the investigators monitored the children  
90 during the whole day, recording the portions. After the samples of duplicate diets had been collected,  
91 they were transported to the laboratory and thoroughly homogenized using a mixer (Arno model  
92 600W, Sao Paulo, SP, Brazil). The weight was recorded (Shimadzu, Barueri, SP, Brazil). Diets were  
93 aliquoted and stored at -22 °C until the chemical analysis was performed for the lead.

94 To avoid contamination, all polypropylene flasks used in the collections were previously cleaned  
95 with a detergent solution, rinsed in  $\text{HNO}_3$  10% overnight, rinsed with deionized water  $18.2\text{M}\Omega\text{cm}$   
96 at  $25^\circ\text{C}$ , dried, and stored in a closed polypropylene container. High-purity water produced by a  
97 Milli-Q water purification system (Millipore, Bedford, MA) was used throughout. A sub-boiling  
98 system (Distilled, Berghof, Germany) was employed to produce high-purity nitric acid.  
99

100 *2.3 Sample preparation*

101 The diet samples were lyophilized by using the lyophilizer (Liopac, L101) at a pressure of  
102  $200\text{ }\mu\text{Hg}$  and checked at  $-50^\circ\text{C}$  for 48 hours. After the lyophilization procedure, the samples were  
103 stored at  $-20^\circ\text{C}$ .

104 *2.4 Acid digestion for metals determination*

105 The elements determination in the diet samples was performed by Inductively Coupled Plasma  
106 Mass Spectrometry (ICP-MS). Firstly, 100 mg of lyophilized sample was weighed (in triplicate) and  
107 1 mL of sub-distilled  $\text{HNO}_3$  was added, which was pre-digested during the night (overnight). The  
108 pre-digestion was followed by a water bath (Solab SL1522L, Brazil) at  $90^\circ\text{C}$  at 4 hours. After cooling,  
109 the volume was made up to 14 mL with deionized water  $18.2\text{ M}\Omega\text{cm}$  at  $25^\circ\text{C}$ . To verify the accuracy  
110 of the procedure, certified reference material (CRM) lobster hepatopancreas (TORT-3, National  
111 Research Council Canada) was used, and was prepared by the same procedure.

112 *2.5 Bioaccessibility*

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

117 *2.6 Preparation of Gastrointestinal Solution*

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

122 *2.7 Preparation of Intestinal solution*

123 Initially, we solubilized ~0.2g of bile salts (0.08 g sodium glycodeoxycholate + 0.05 g sodium  
124 taurodeoxycholate + 0.08g sodium taurocholate hydrate) and 0.5g pancreatin in 100mL of  $\text{NaHCO}_3$   
125 3% w/v. All salts used in the intestinal solution were obtained from sigma Sigma-Aldrich (St. Louis,  
126 USA) [24].

127 *2.8 Gastric digestion simulation*

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

131 *2.9 Intestinal digestion simulation*

132 After gastric digestion, the solution was submitted to the intestinal digestion simulation. For this  
133 purpose,  $\text{NaHCO}_3$  (3% w/v) was added for pH adjustment to 6.8. Then, we added 3mL of intestinal  
134 solution and heated in a water bath at  $37^\circ\text{C}$  during 2h with shaking (50 rpm). Finally, the samples  
135 were cooled to room temperature and centrifuged (SL700, Solab, Brazil) at a rate of 1077g during  
136 20min. The supernatants of this step were separated from the precipitates. Precipitates and

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

139 *2.10 Metals Determination*

140 The determination of the elements total concentration and their bioavailabilities in diet samples  
 141 were carried out by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies,  
 142 7900). An external calibration curve was prepared with standard multielement solution  
 143 (PerkinElmer, Inc.) at concentrations of 0.1; 1; 5; 10; 50; 100; 200; 500 and 1000  $\mu\text{g L}^{-1}$ . Blank solutions  
 144 were also prepared and lobster hepatopancreas reference material (TORT-3, National Research  
 145 Council Canada) prepared using the same protocol for samples. The ICP-MS conditions are presented  
 146 in the Table 1.

147 **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 $\text{min}^{-1}$	15 L $\text{min}^{-1}$
He Flow	5.0 mL $\text{min}^{-1}$	5.0 mL $\text{min}^{-1}$
HeHE	10 mL $\text{min}^{-1}$	10 mL $\text{min}^{-1}$
Nebulizer Gas Flow Rate	0.68 L $\text{min}^{-1}$	1.05 L $\text{min}^{-1}$
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

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 149

150 **3. Results**

151 The anthropometric characteristics of children are presented in Table 2.

152 **Table 2.** Anthropometric characteristics of children from the day care centers (DCC) PS and PF (São  
 153 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 $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Age (years)	3.6 $\pm$ 0.6	3.3 $\pm$ 0.7	2.6 $\pm$ 0.9	3.4 $\pm$ 0.7	3.4 $\pm$ 0.8	3.4 $\pm$ 0.7
Height (cm)	98 $\pm$ 7	95 $\pm$ 6	93 $\pm$ 12	101 $\pm$ 6	97 $\pm$ 8	98 $\pm$ 7
Weight (kg)	17 $\pm$ 4	15 $\pm$ 2	15 $\pm$ 4	17 $\pm$ 3	16 $\pm$ 4	16 $\pm$ 3
BMI* (kg/m <sup>2</sup> )	17 $\pm$ 2	17 $\pm$ 1	18 $\pm$ 1	16 $\pm$ 2	17 $\pm$ 2	17 $\pm$ 2

154 \*Body mass index.

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

161 Lead and cadmium daily ingestions were similar in both DCCs (Table 3). The mean lead intake  
 162 values were below the European Union BMDL for the development of neurotoxic effects in children,  
 163 corresponding to 36% of BMDL for both gender. A child attending the DCC PS ingested a  
 164 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).

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

167  
 168 The bioaccessibility fractions of lead, cadmium and arsenic are presented in Table 4. Even  
 169 though some samples have presented the bioaccessible percentage close to 100%, none of the samples  
 170 reached the values of BMDL, or PTMI, or the withdrawn PTWI, of the potentially toxic elements  
 171 studied. The Bioaccessible Estimated Daily Intake (BEDI), calculated for the sample with higher  
 172 bioaccessibility, corresponds to 34% of Pb BMDL [28], 9.2% of Cd PTMI [27], while for As BEDI is  
 173 equivalent to 59% of the withdrawn PTWI [30].

174 **Table 4.** Percentage range of bioaccessibility (minimum – maximum) for lead, cadmium and arsenic  
 175 in diet, bioaccessibility mean (%) and Children's Bioaccessible Estimated Daily Intake (BEDI) per  
 176 body weight ( $\mu\text{g kg}^{-1}$  bw $^{-1}$  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
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177 \*BEDI calculated for the sample with higher bioaccessibility

#### 178 4. Discussion

179 Values higher than those found in this present study were reported by Kim et al. [31], whom  
 180 investigated the exposure to lead and cadmium of 457 South Korean children aged 0-6 years old  
 181 through a two-day 24-hours recall, a different method. The mean of the lead intake was  $0.46 \mu\text{g kg}^{-1}$   
 182 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  
 183 milk appeared as the main sources of lead exposure. For cadmium, the mean intake was  $0.34 \mu\text{g Cd}$   
 184  $\text{kg}^{-1}$  bw per day. Cereals, fish, shellfish and, algae had a significant contribution to the intake of  
 185 cadmium.

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

191 Pysz et al. [33-34] evaluated lead and cadmium intake of 4-6-year-old children and adolescents  
 192 who lived in orphanages in Krakow, Poland, through 24-hours duplicate diet of four days in each  
 193 season of the year, including weekend days. For the children from the orphanage which has an age  
 194 range equivalent with the children in present study lead and cadmium annual mean intake were  
 195  $11.57$  and  $16.63 \mu\text{g kg}^{-1}$  bw per week, respectively. These values are higher than the ones found in the  
 196 present study and correspond to concentrations 69.75% above BMDL for Pb [28] and 62.42% above  
 197 the Provisional Tolerable Monthly Intake (PTMI) of  $25 \mu\text{g Cd kg}^{-1}$  bw per month [27].

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

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

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

218 The cooking process may influence the elements bioaccessibility [7, 17, 36]. Zhuang et al. [7]  
 219 investigated the lead and cadmium bioaccessibilities in 6 species of vegetables, raw and cooked,  
 220 widely consumed in China, which were purchased in local markets of 9 cities. They found that the  
 221 leafy vegetables had significantly higher concentrations of toxic elements, and the most of those  
 222 concentrations decreased with cooking process. Cadmium had bioaccessible percentage ranging  
 223 from 35 to 65% in raw vegetables and 34 and 64% in cooked vegetables, and for lead, such range were  
 224 20 to 51% and 11 to 48% in raw and cooked vegetables, respectively.

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

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

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

## 242 5. Conclusions

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

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253 Validation F.F.S and B.L.B.; Formal Analysis, I.N.L. and A.P.S.S.F.; Investigation, I.N.L., A.P.S.S.F, F.P.P., T.P.  
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