Original article DOI: 10.1515/aiht-2015-66-2686

Bioaccessibility of lead in sand intended for playground sandboxes in Slovenia: a preliminary study

Rok Fink[1], Andreja Kukec[2], and Ivan Eržen[2,3]

University of Ljubljana Faculty of Health Sciences[1], University of Ljubljana Faculty of Medicine[2], National Institute of Public Health[3], Ljubljana, Slovenia

[Received in June 2015; CrossChecked in June 2015; Accepted in November 2015]

Slovene press has recently expressed concern for the health of children who ingest playground sand contaminated with lead. However, current assessments may be overestimating the health risk, as they assume that human body absorbs the whole amount of a contaminant. The aim of our study was to see how much lead in sand intended for playground sandboxes in Slovenia really is absorbed and what health risk it may present. To do that, we ran bioaccessibility tests to simulate metabolism and see how digestion affects lead levels. Our results show low bioaccessibility of lead from sand (0.01-36.84 %). Taking into account lead bioaccessibility, we ran several scenarios of sand ingestion in children and established that in general the risk is negligible, except for pica behaviour with extremely high (20 g) ingestion of sand with highly mobilised lead (2.69 μg g^{-1}). Further research should assess real-life exposure to lead from playground sand in children and perhaps update these preliminary bioaccessibility data.

KEY WORDS: *exposure scenarios; intake;* in vitro *model; risk assessment; toxic metal*

Children playing in sandboxes often expose themselves to lead in the sand (1-8). In small children this exposure is the highest through unintentional or intentional ingestion (the so called pica behaviour) (9). On average, children can ingest between 50 and 200 mg of sand per day. In case of pica behaviour, ingestion soars to between 1 and 20 g per day (10-12).

However, not all of the ingested toxic metal content will be absorbed by the human body. Absorption will depend on age, stomach content, chemical compound containing the metal, and its bioaccessibility (13). In simple terms, *bioaccessibility* is the absorption potential - that fraction of a substance that is dissociated from soil (or sand for that matter) by gastrointestinal juices and available for absorption. An even more accurate parameter of exposure is *bioavailability* or the potential of a substance to reach circulation. However, determining it requires complex, expensive, and time-consuming *in vivo* analysis, which raises practical and ethical issues when it comes to humans and animals. Bioaccessibility, in turn, is a much simpler *in vitro* method generally simulating gastric and small intestinal digestion, which, depending on the specific method used, correlates with bioavailability (14).

The primary aim of our study was to analyse lead concentrations in sand intended for playground sandboxes in Slovenia and to assess its bioaccessibility. Our secondary aim was to simulate different scenarios of oral sand intake in children and compare our exposure assessment to current tolerable intake values.

Correspondence to: Rok Fink, University of Ljubljana Faculty of Health Sciences, Zdravstvena pot 5, SI-1000 Ljubljana, Slovenia;
e-mail: *rok.fink@zf.uni-lj.si*

METHODS

First we identified the producers of sand intended for playgrounds; then we assessed lead bioaccessibility in sand samples by simulating metabolism *in vitro* and analysing the effects of digestion on absorption; and finally we included our findings in different ingestion scenarios to assess the risk of exposure.

Producers of sand intended for sandboxes

In 2014, the Health Inspectorate of the Republic of Slovenia analysed sand samples from 75 kindergarten sandboxes as part of the regular monitoring across the nation. From kindergarten books the Inspectorate identified five suppliers of sand of different types (three limestone and two quartz).

Our bioaccessibility and risk assessment were based on 26 sand samples obtained from these five producers, all providing sand originating in Slovenia.

Sample preparation and data analysis

To assess the bioaccessibility of lead in sand samples, we simulated sand metabolism *in vitro* to see how digestion affects lead absorption. The experiment followed the standards ISO/TS 17924 (15) and DIN 19738 (16) under realistic worst-case conditions. It was based on the

properties of the human digestion process, the contaminants, and geochemistry and mineralogy of soils. At first 2 g of sampled sand were treated with synthetic saliva (50 mg L^{-1} NaCl, 15 mg L^{-1} NaSCN, 55 mg L^{-1} Na_2SO_4, 15 mg L^{-1} $NaHCO_3$, 45 mg L^{-1} KCl, 60 mg L^{-1} KH_2PO_4, 15 mg L^{-1} $CaCl_2x2H_2O$, 75 mg L^{-1} mucin, 25 mg L^{-1} α-amylase, 15 mg L^{-1} urea, and 1 mg L^{-1} uric acid) for 30 minutes. In the next step, synthetic stomach juices were added to suspension (290 mg L^{-1} NaCl, 70 mg L^{-1} KCl, 27 mg L^{-1} KH_2PO_4, 100 mg L^{-1} pepsin, 300 mg L^{-1} mucin and HCl for pH correction at 2.0) and mixed with an agitator at 4,500 *g* for 2 hours. Whole milk powder (10 g L^{-1}) was added to stomach juices to simulate the influence of food on the mobilisation of lead. After that, we added synthetic intestinal juices (30 mg L^{-1} KCl, 50 mg L^{-1} $CaCl_2x2H_2O$, 20 mg L^{-1} $MgCl_2x6H_2O$, 100 mg L^{-1} $NaHCO_3$, 30 mg L^{-1} trypsin, 900 mg L^{-1} pancreatin, 900 mg L^{-1} lyophilized bile, and 30 mg L^{-1} urea) and set the pH to 7.5 using a phosphate buffer. Digestion continued for another 6 hours. The temperature was controlled by means of a water bath at 37 °C.

With digestion completed, the samples were centrifuged at 7000 *g* for 10 minutes, after which the supernatant was decanted. The residual pellet was stirred in 30 mL of distilled water for 20 min, centrifuged again, and the supernatant decanted. The decanted solutions were combined for analysis. Figure 1 shows a detailed flow chart of the experiment.

We also wanted to see if particle size had any influence on the amount of lead, so we analysed sand particles of five different sizes (less than 100 μm; 100-200 μm; 200-315 μm; 315-500 μm; 500-630 μm).

Lead concentration was analysed with the inductively coupled plasma mass spectrometer (ICP-MS) Agilent 7500a (Agilent Technologies Inc., Palo Alto, CA, USA) before and after digestion. This method allows direct measurements in the supernatant, without any further preparation and manipulation. In sampling, analysis, traceability of results, and determination of the boundaries we followed the DIN 32645 (17) and ISO 17025 (18) standards. Each series of digestion experiments was controlled with a digestion tube without soil to exclude the presence of lead in the test solution, and none of the control measurements exceeded the detection limit for lead.

The amount of mobilised lead (w_{mob}) was calculated as follows:

$$w_{i,mob} = \frac{\rho . V}{m} \quad (1)$$

where $w_{i,mob}$ is the mass fraction of mobilised lead (μg g^{-1}), ρ is the concentration of lead in test solution (μg L^{-1}), V is the volume of test solution (L), and m is the mass of soil sample (g).

Bioaccessibility of lead was calculated using following equation:

$$R_i = \frac{w_{i,mob} . 100}{w_{i,total}} \quad (2)$$

where Ri is bioaccessibility (%), $w_{i,mob}$ is the mass fraction of mobilised lead (μg g^{-1}), and $w_{i,total}$ is the total amount of lead in the original sample before digestion (μg g^{-1}).

Simulation of sand ingestion

Having obtained the results for bioaccessibility, we simulated several ingestion scenarios in which children ingest between 50 mg and 200 mg of sand per day (average ingestion) and between 1 and 20 g (in pica behaviour). Then we compared the simulation findings with the current provisional tolerable lead intake (36 μg per day) for children weighing 10 kg (19).

RESULTS AND DISCUSSION

Lead concentrations in sand and bioaccessibility

Table 1 shows lead levels before digestion and those obtained with the gastrointestinal simulation model for different particle sizes and sand producers (Table 1). We found large variability in lead concentrations (4.90-19.20 μg L^{-1}) between the five sand suppliers for kindergartens. Our results also indicate that the amount of lead is not related to particle size. One of the reasons may be that the sand particles we analysed were relatively coarse, as several studies with fine particles reported results contrary to ours: that heavy metal concentration in soil is related to particle size (20-22).

Our bioaccessibility test with the ingestion model has shown that the relation between the amount of mobilised lead and lead levels in soil is not linear. Take the outstanding example of sand samples from Limestone sand producer 2, whose mass fraction was as high as 10,685 μg g^{-1} (10.69 mg g^{-1}!), yet only a small amount of lead (2.69 μg g^{-1} or 0.03 %) was mobilised. Limited bioaccessibility of metals may be related to their solubility (23). This suggests that the health risks from poorly soluble soil contaminants are in many cases overestimated because bioaccessibility has not been taken into account.

Several *in vitro* gastrointestinal extraction protocols have been used to evaluate the bioaccessibility of toxic elements in soils. Oomen et al. (24) analysed the bioaccessibility of lead under controlled laboratory conditions, where artificial standard soil OECD medium was contaminated with 530 mg kg^{-1} of lead, and found that only 23 % of lead was bioaccessible. Our results have shown a wider range of lead bioaccessibility from sand (0.01-36.84 %), but all these findings confirm that it is the amount of mobilised contaminant that counts in any risk assessment.

Table 1 *Results of the experiment using gastrointestinal simulation model for lead*

Source of sand	Pb in original sample ($w_{i,total}$) [µg g^{-1}]	Fraction (µm)	Concentration of Pb in the test solution (ρ) [µg L^{-1}]	Amount of mobilised Pb ($w_{i,mob}$) [µg g^{-1}]	Bioaccessibility (R_i) (%)
Limestone sand producer 1	4.10	100-200	7.70	1.44	35.21
		200-315	7.30	0.78	19.14
		315-500	6.60	0.87	21.33
		500-630	10.60	1.51	36.84
		630-1000	11.00	1.29	31.52
Limestone sand producer 2	10,685	100	5.40	0.90	0.01
		100-200	19.20	2.69	0.03
		200-315	19.20	2.54	0.02
		315-500	4.90	0.71	0.01
		500-630	18.10	2.35	0.02
		630-1000	8.00	1.06	0.01
Limestone sand producer 3	12.00	100	4.90	0.87	7.25
		100-200	4.90	0.66	5.51
		200-315	4.90	0.59	4.88
		315-500	4.90	0.65	5.41
		500-630	4.90	0.72	6.02
Quartz sand producer 1	2.90	100	4.90	0.56	19.43
		100-200	4.90	0.55	19.01
		200-315	4.90	0.60	20.70
		500-630	4.90	0.69	23.66
Quartz sand producer 2	13.20	100	8.00	1.34	10.15
		100-200	4.90	0.65	4.92
		200-315	4.90	0.70	5.29
		315-500	4.90	0.66	5.01
		500-630	4.90	0.66	5.01
		630-1000	4.90	0.64	4.83

$w_{i,total}$ is the total amount of lead in the original sample, based on pooled measurement before digestion (µg g^{-1})

Simulation of sand ingestion taking into the account bioaccessibility of lead

Table 2 shows lead intake scenarios based on bioaccessibility results (amount of mobilised lead) for limestone and quartz sand, taking into account current tolerable intake. Our findings suggest that sand ingestion in normal behaviour would not lead to increased risk of exposure to lead, except in the worst case scenario with highest mobilised Pb levels and pica intake of 20 g of sand per day.

CONCLUSIONS

Toxic metal pollution of playgrounds has received wide public interest, and recent studies suggest that pollution and children's exposure to metals in these areas are of high concern. Our study is important because it shows that current assessments of risk from exposure to lead in children playing with sand, even those with pica disorder, may be overblown because they do not take into account bioaccessibility. The results of our study suggest that bioaccessibility is more related to lead concentration in test solution (ρ) than to its particle size. Moreover, only a small fraction of lead dissolves in digestive juices. Exposure of children via sand ingestion is therefore negligible in most scenarios.

There are several limitations to this preliminary study. Firstly, we did not do any statistical analysis due to too few samples. Secondly, our measurements start from the assumption that children are exposed to lead in sand as delivered by producers, which may exclude input from playground environment such as precipitation, leaching, etc. These can affect lead's bioaccessibility. Our future research will therefore address this shortcoming, and we will try to establish lead bioaccessibility in real-life conditions.

Acknowledgements

This study was conducted as a part of the project entitled "Preparation of guidelines to limit values for pollutants in playgrounds soil", which was supported by the Ministry of Health, Chemical Office of the Republic of Slovenia.

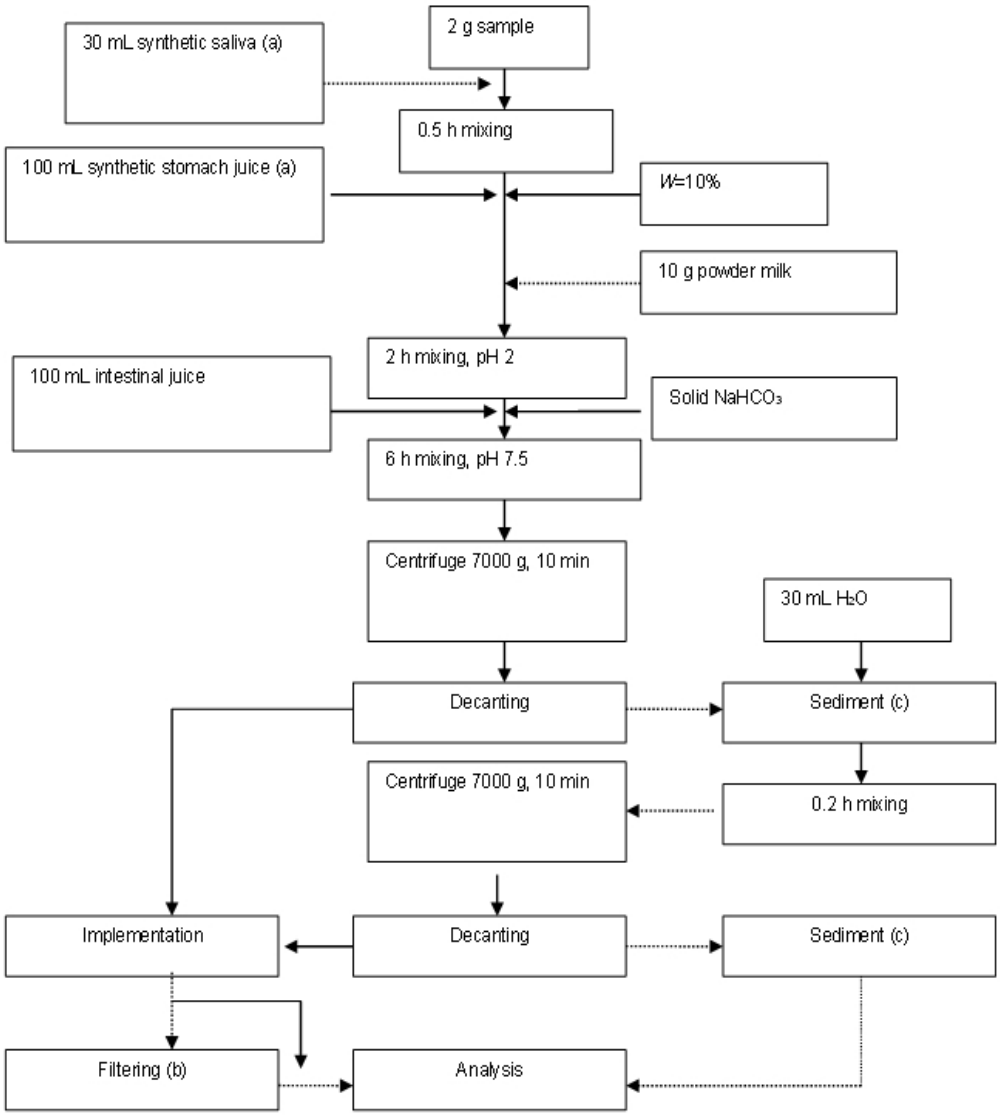

Figure 1 *Flow chart of the experiment conducted to assess the bioaccessibility of lead in sand samples in simulated digestion conditions following the DIN ISO 11464 norm*
a) The volume of synthetic stomach juice is reduced after adding synthetic saliva to 70 mL. Therefore, adding 30 mL of water is necessary; b) if applicable; c) measures for quality

Table 2 *Simulation of lead intake based on bioaccessibility of limestone and quartz sand and predicted sand intake for children*

Scenarios	Amount of mobilised Pb ($w_{i,mob}$) µg g^{-1}	PTI[a] µg day^{-1}	Ingestion of sand in children[b]				Ingestion of sand in children with pica behaviour[b]			
			50 mg day^{-1}		200 mg day^{-1}		1000 mg day^{-1}		20,000 mg day^{-1}	
			Pb dose µg day^{-1}	% of PTI	Pb dose µg day^{-1}	% of PTI	Pb dose µg day^{-1}	% of PTI	Pb dose µg day^{-1}	% of PTI
Limestone sand										
Lowest	0.59	36	0.03	0.083	0.12	0.3	0.6	1.6	11.7	32.7
Highest	2.69	36	0.13	0.37	0.54	1.5	2.7	7.5	53.8	**149.5**
Quartz sand										
Lowest	0.55	36	0.03	0.083	0.12	0.3	0.6	1.6	11.7	32.5
Highest	1.34	36	0.07	0.194	0.27	0.7	1.3	3.6	26.8	74.4

a - Provisional tolerable intake for 10-kg children. This is the limit above which adverse health effects are expected (19)
b - Young children have an oral intake between 50 and 200 mg day^{-1}. About 5 % of all children display pica behaviour; these children have an oral intake of soil between 1 and 20 g day^{-1} (10, 12)

REFERENCES

1. Ottesen RT, Alexander J, Langedal M, Haugland T, Høygaard E. Soil pollution in day-care centers and playgrounds in Norway: National action plan for mapping and remediation. Environ Geochem Health 2008;30:623-37. doi: 10.1007/s10653-008-9181-x
2. Acosta JA, Cano AF, Arocena JM, Debela F, Martínez-Martínez S. Distribution of metals in soil particle size fractions and its implication to risk assessment of playgrounds in Murcia City (Spain). Geoderma 2009;149:101-9. doi: 10.1016/j.geoderma.2008.11.034
3. Eržen I. An assessment of lead exposure among children attending kindergartens in Celje. Zdrav Var 2011;50:113-20. doi: 10.2478/v10152-010-0034-5
4. Mielke HW, Covington TP, Mielke Jr PW, Wolman FJ, Powell ET, Gonzales CR. Soil intervention as a strategy for lead exposure prevention: The New Orleans lead-safe childcare playground project. Environ Pollut 2011;159:2071-7. doi: 10.1016/j.envpol.2010.11.008
5. Taylor MP, Camenzuli D, Kristensen LJ, Forbes M, Zahran S. Environmental lead exposure risks associated with children's outdoor playgrounds. Environ Pollut 2013;178:447-54. doi: 10.1016/j.envpol.2013.03.054
6. Zhong MS, Jiang L, Han D, Xia TX, Yao JJ, Jia XY, Peng C. Cadmium exposure via diet and its implication on the derivation of health-based soil screening values in China. J Expo Sci Environ Epidemiol 2015;25:433-42. doi: 10.1038/jes.2015.5
7. Chen H, Teng Y, Lu S, Wang Y, Wang J. Contamination features and health risk of soil heavy metals in China. Sci Total Environ 2015;512-513:143-53. doi: 10.1016/j.scitotenv.2015.01.025
8. De Miguel E, Iribarren I, Chacón E, Ordoñez A, Charlesworth S. Risk-based evaluation of the exposure of children to trace elements in playgrounds in Madrid (Spain). Chemosphere 2007;66:505-13. Doi: 10.1016/j.chemosphere.2006.05.065
9. Elom NI, Entwistle JA, Dean JR. How safe is the playground? An environmental health risk assessment of As and Pb levels in school playing fields in NE England. Environ Chem Lett 2013;11:343-51. doi: 10.1007/s10311-013-0413-7
10. Sedman RM, Mahmood RJ. Soil ingestion by children and adults reconsidered using the results of recent tracer studies. J Air Waste Manage Assoc 1994;44:141-4. doi: 10.1080/1073161X.1994.10467242
11. Calabrese EJ, Stanek EJ. Soil pica: Not a rare event. J Environ Sci Health Part A: Environ Sci Engin Toxicol 1993;28:373-84. doi: 10.1080/10934529309375884
12. Calabrese EJ, Stanek EJ, James RC, Roberts SM. Soil ingestion: a concern for acute toxicity in children. Environ Health Perspect 1997; 105 (12): 1354-1358. Doi: 10.1289/ehp.971051354
13. Ciobanu C, Slencu BG, Cuciureanu R. Estimation of dietary intake of cadmium and lead through food consumption. Rev Med Chir Soc Med Nat Iasi 2012;116:617-23. PMID: 23077963
14. Li J, Li K, Cave M, Li HB, Ma LQ. Lead bioaccessibility in 12 contaminated soils from China: Correlation to lead relative bioavailability and lead in different fractions. J Hazard Mater 2015; 295:15:55-62. doi:10.1016/j.jhazmat.2015.03.061
15. ISO/TS 17924:2007. Soil quality -- Assessment of human exposure from ingestion of soil and soil material -- Guidance on the application and selection of physiologically based extraction methods for the estimation of the human bioaccessibility/bioavailability of metals in soil. 16 pages.
16. DIN 19738:2004-07. Soil quality - Absorption availability of organic and inorganic pollutants from contaminated soil material. 20 pages.
17. DIN 32645:2008-11. Chemical analysis - Decision limit, detection limit and determination limit under repeatability conditions - Terms, methods, evaluation. 28 pages.
18. ISO/IEC 17025:2005. General requirements for the competence of testing and calibration laboratories. 28 pages.
19. Lead. Evaluation of health risk to infants and children. International Programme on Chemical Safety. http://www.inchem.org/documents/jecfa/jecmono/v21je16.htm
20. Ljung K, Selinus O, Otabbong E, Berglund M. Metal and arsenic distribution in soil particle sizes relevant to soil ingestion by children. Appl Geochem 2006;21:1613-24. doi: 10.1016/j.apgeochem.2006.05.005
21. Wang DF, Wu HJ, Chen J, Jiao SX, Dai XM. Assessment of heavy metal pollution of urban roadside soil with set pair analysis and geoaccumulation index. Advanced Materials Research 2010;113-116:960-4. doi: 10.4028/www.scientific.net/AMR.113-116.960
22. Al-Rajhi MA, Al-Shayeb SM, Seaward MRD, Edwards HGM. Particle size effect for metal pollution analysis of atmospherically deposited dust. Atmos Environ 1996;30:145-53. doi: 10.1016/1352-2310(95)00164-T
23. Oomen AG, Rompelberg CJM, Kamp EVD, Pereboom DPKH, Zwart LLD, Sips AJAM. Effect of bile type on the bioaccessibility of soil contaminants in an in vitro digestion model. Arch Environ Contam Toxicol 2004;46:183-8. doi: 10.1007/s00244-003-2138-2
24. Oomen AG, Rompelberg CJM, Bruil M, Dobbe C, Pereboom DPKH, Sips AJAM. Development of an in vitro digestion model for estimating the bioaccessibility of soil contaminants. Arch Environ Contam Toxicol 2003;44:281-7. doi: 10.1007/s00244-002-1278-0

Biološka dostopnost svinca v mivki, ki je namenjen otroškim peskovnikom v Sloveniji

V zadnjem obdobju so se v Sloveniji pojavili številni pomisleki o varnosti mivke, ki je namenjena za otroške peskovnike. Ocena tveganja otrok, ki so izpostavljeni svincu temelji na predpostavki, da je celotna količina toksične kovine biološko dostopna. Zato je bil namen raziskave analizirati biološko dostopnost svinca v mivki, ki je namenjen za peskovnike otroških igrišč. Z simulacijo prebavnega trakta smo analizirali vpliv prebavnih sokov za izplavljanje svinca iz mivke. Rezultati kažejo na majhno biodostopnost svinca v mivki (0,01–36,84 %) slovenskih proizvajalcev. Na podlagi biološke dostopnosti smo simulirali različne scenarije vnosa in ugotovili, da je tveganje zanemarljivo, razen v primeru pica sindroma (zaužitje 20 g mivke) ter ob predpostavki največje dokazane biološke dostopnosti (2,69 μg g^{-1}). Raziskave v prihodnosti bodo vključevale realno izpostavljenost otrok svincu iz peskovnikov in bodo morda nadgradile preliminarno raziskavo.

KLJUČNE BESEDE: in vitro *model; ocena tveganja; scenariji izpostavljenosti; toksična kovina; vnos*