



Blood cadmium, mercury, and lead in children: An international comparison of cities in six European countries, and China, Ecuador, and Morocco[☆]

Františka Hrubá^{a,*}, Ulf Strömberg^b, Milena Černá^c, Chunying Chen^d, Florencia Harari^e, Raúl Harari^e, Milena Horvat^f, Kvetoslava Koppová^a, Andreja Kos^g, Andrea Krsková^h, Mladen Krsnikⁱ, Jawhar Laamech^j, Yu-Feng Li^d, Lina Löfmark^b, Thomas Lundh^b, Nils-Göran Lundström^k, Badiia Lyoussi^j, Darja Mazej^f, Joško Osredkarⁱ, Krystyna Pawlas^l, Natalia Pawlas^l, Adam Prokopowicz^l, Gerda Rentschler^b, Věra Spěváčková^h, Zdravko Spiric^g, Janja Tratnik^f, Staffan Skerfving^b, Ingvar A. Bergdahl^k

^a Regional Authority of Public Health, Banská Bystrica, Slovakia

^b Division of Occupational and Environmental Medicine, University Hospital, Lund, Sweden

^c Charles University, Third Faculty of Medicine, Prague, Czech Republic

^d Institute of High Energy Physics and National Center for Nanoscience and Technology, Chinese Academy of Sciences, Beijing, China

^e Institute for the Development of Production and the Work Environment (IFA), Quito, Ecuador

^f Department of Environmental Sciences, Institut Jožef Stefan, Ljubljana, Slovenia

^g Oikon Ltd, Zagreb, Croatia

^h National institute of Public Health, Prague, Czech Republic

ⁱ University Clinical Centre Ljubljana, Ljubljana, Slovenia

^j Laboratory of Physiology, Pharmacology and Environmental Health, Fez Atlas, Morocco

^k Department of Public Health and Clinical Medicine, Occupational and Environmental Medicine, Umeå University, Umeå, Sweden

^l Institute of Occupational Medicine and Environmental Health, Sosnowiec, Poland

ARTICLE INFO

Article history:

Received 19 January 2011

Accepted 11 December 2011

Available online 16 January 2012

Keywords:

Cadmium
Mercury
Lead
Children
Exposure
Biomonitoring

ABSTRACT

Children's blood-lead concentration (B-Pb) is well studied, but little is known about cadmium (B-Cd) and mercury (B-Hg), in particular for central Europe. Such information is necessary for risk assessment and management. Therefore, we here describe and compare B-Pb, B-Cd and B-Hg in children in six European, and three non-European cities, and identify determinants of these exposures. About 50 school children (7–14 years) from each city were recruited (totally 433) in 2007–2008. Interview and questionnaire data were obtained. A blood sample was analyzed: only two laboratories with strict quality control were used. The European cities showed only minor differences for B-Cd (geometric means 0.11–0.17 µg/L) and B-Pb (14–20 µg/L), but larger for B-Hg (0.12–0.94 µg/L). Corresponding means for the non-European countries were 0.21–0.26, 32–71, and 0.3–3.2 µg/L, respectively. For B-Cd in European samples, traffic intensity close to home was a statistically significant determinant, for B-Hg fish consumption and amalgam fillings, and for B-Pb sex (boys higher). This study shows that European city children's B-Cd and B-Pb vary only little between countries; B-Hg differs considerably, due to varying tooth restoration practices and fish intake. Traffic intensity seemed to be a determinant for B-Cd. The metal concentrations were low from a risk perspective but the chosen non-European cities showed higher concentrations than the cities in Europe.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Toxic metals have been studied for centuries. The general population is exposed to lead (Pb) mainly from petrol, industrial emissions,

paint and ceramics (Skerfving and Bergdahl, 2007), to mercury (Hg) from fish intake and dental amalgam fillings (Berlin et al., 2007) and to cadmium (Cd) from cereals and vegetables due to contamination of soil from fertilizers and industrial emissions (EFSA, 2009; Nordberg et al., 2007).

The margin is small between the level of exposure and toxic effects. Therefore, for risk assessment and management and for follow-up of time trends, there is a need for adequate information on exposure. For the toxic metals Cd, Hg and Pb, their concentrations in blood are relevant biomarkers of exposure, though the demands on analytical quality are high, especially for meaningful comparisons between geographic areas and over time periods.

[☆] Disclaimer/Competing interests declaration: The paper reflects only the authors' views; the European Union is not liable for any use that may be made of the information. The authors have no competing interests.

* Corresponding author at: Regional Authority of Public Health, Cesta k nemocnici 1, 975 56 Banská Bystrica, Slovakia. Tel.: +421 905 338 309.

E-mail addresses: frantiska.hrubka@vzbb.sk, fhruba@gmail.com (F. Hrubá).

¹ Present address: Oskarsrogatan 5/2tr, 17153 Solna, Sweden. Tel.: +46 708 626 314.

Levels of Pb in blood (B-Pb) have been extensively measured (Smolders et al., 2010). However, for some areas of Europe, there is only limited information and Hg and Cd have been studied only to a limited extent. International comparisons with directly comparable data are in principle lacking.

Hence, we here present data on concentrations in blood of Pb (B-Pb), Hg (B-Hg) and Cd (B-Cd), and a number of their determinants, in children from six European and three non-European cities. Strict control of analytical quality was performed to assure international comparability and we believe this is the first precise multinational study of these metals, since the 1980s (Vahter and Slorach, 1991).

2. Materials and methods

2.1. Study areas and subjects

In each country one school in an urban location (Table 1) was selected. Contact was established first with the school management and then with parents and children. In each city we aimed at recruiting 50 children of age 7–11 years. The study was approved by the local ethics committee in each country where sampling took place. Written consent was obtained from a parent of each child. Oral consent was obtained from the child before sampling.

2.2. Blood sampling

A nurse took venous blood samples from the arm after cleaning with an ethanol swab. We used evacuated plastic tubes with heparin for sampling. After evaluating a number of different brands, we chose Greiner Vacuette 4 mL Lithium Heparin tubes (Greiner-Bio One GmbH, Frickenhausen, Germany). The levels of all three metals in these tubes were below 0.03 µg/L at leaching tests with 4 mL of 2% nitric acid. In order to avoid false international differences due to differences in sampling equipment, we shipped sampling tubes and needles from one laboratory to all partners, using one and the same production batch of sampling tubes. All samples were stored in freezer at –20 °C direct after sampling, transported to the laboratories on dry ice and again stored at –20 °C until analysis. The samples had

been thawed for local analysis before shipment to the central laboratory, with the exception of those from Ecuador, Slovakia and Sweden. Total storage time until analysis was about 2 years.

2.3. Questionnaire and interview

Information on individual factors of potential concern for metals exposure was obtained through three sources: Questionnaire to the school, questionnaire to the parents, and interview/examination of the child. From the parents' questionnaire, information on their education (basic, middle, high), anyone smoking at home (yes/no), source of water and heating, traffic near the home (less than one vs one or more cars per minute), and the child's intake of offal (less than one vs one or more meals per month), fish (<1, 1–2, ≥3 meals per month), and shellfish (<1 vs ≥1 meals per month) was obtained. Information on number of amalgam fillings, use of chewing gum and child's attempts to smoke was obtained at the examination.

2.4. Chemical analyses

All analyses were carried out on whole blood. B-Pb and B-Cd were determined at Department of Occupational and Environmental Medicine, University Hospital, Lund, Sweden, by inductively coupled plasma mass spectrometry (ICP-MS; Thermo X7, Thermo Elemental, Winsford, UK). A sample volume of 250 µL was diluted 10 times with an alkaline solution (Bárány et al., 1997). Using this solution as a carrier/rinsing fluid, the samples were introduced in a segment-flow mode and analyzed in peak-jumping mode, 75 sweeps and 1 point per peak, 30 ms dwell time for ¹¹⁴Cd and ¹¹⁸Sn, 20 ms for ²⁰⁶, ²⁰⁷, ²⁰⁸Pb (summed) and 10 ms for the internal standards ¹¹⁵In, ²⁰⁵Tl and ²⁰⁹Bi. Interference corrections were made for ¹¹⁴Cd for the spectral overlap of Sn. The detection limits for Cd and Pb, calculated as 3 times the standard deviation (SD) of the blank (based on all blanks in one analytical batch), varied slightly from day to day and was in average 0.01 (range: 0.01–0.04) µg/l for Cd and 0.06 (range: 0.05–0.10) µg/L for Pb. All samples were prepared in duplicate, and the method imprecision (calculated as the coefficient of variation for all duplicate preparations measurements) were for Cd and Pb 9.3 and 6.8%, respectively, with both preparation and analyses made

Table 1
Background characteristics of the study populations.

Country	Croatia	Czech Republic	Poland	Slovakia	Slovenia	Sweden	China	Ecuador	Morocco
City	Koprivnica	Prague	Wroclaw	Banska Bystrica	Ljubljana	Landskrona	Guiyang	Camilo Ponce Enríquez	Fez, Sefrou
Population	31,000	1100,000	635,000	80,000	300,000	30,000	4000,000	17,000	1000,000
Sampling date	Jun 07	May, Jun 07	Jun 08	May 07	Jun 07	May 07	Sep 07	Aug 07	Feb 08
No. of children (girls/boys)	52 (27/25)	21 (7/14)	30 (8/22)	57 (35/22)	45 (26/19)	41 (19/22)	29 (18/11)	69 (38/31)	39 (18/21)
Participation rate %	– ^a	26%	– ^a	81%	78%	63%	48% ^b	95%	– ^a
Age	8.7	8.4	8.0	8.9	9.0	9.1	8.2	7.2	10.2
[mean (range)]	(8–10)	(7–10)	(7–8)	(7–11)	(7–11)	(8–11)	(7–10)	(7–10)	(7–14)
Smoking at home (%)	50	19	23	14	9	29	52	16	51
Parental education (%)									
Primary school	6	5	3	0	0	10	7	46	5
Secondary	58	71	54	60	28	72	69	51	59
Higher	36	24	43	40	72	18	24	3	36
Amalgam fillings (%)									
0	32	60	93	21	37	93	90	98	92
1–2	58	10	7	33	20	7	7	1	8
≥3+	10	30	0	46	43	0	3	1	0
Fish intake [meals/month (%)]									
<1	10	43	7	33	25	17	35	1	8
1–3	59	33	37	37	45	44	48	20	61
>3	31	24	56	30	30	39	17	79	31
Shellfish intake [>1 meals/month (%)]	4	10	3	2	24	15	45	99	0
Traffic density [>1 cars/min (%)]	53	67	33	51	8	31	52	59	46

^a The full participation rate could not be calculated for three cities, only the participation rate among those who responded positively to the first invitation could be calculated for these cities. It was: Koprivnica, Croatia: 91%; Wroclaw, Poland: 68%; Fez, Sefrou 80%.

^b In Guiyang, China 83% left a blood sample but only 48% had remaining volumes for the re-analysis in a central laboratory.

pair wise. The analytical accuracy was checked against reference material: For Seronorm Trace elements whole blood (Lot. MR4206, SERO AS, Billingstad, Norway), the results obtained were for Cd 0.61 ± 0.03 (mean \pm SD; $n = 51$; recommended 0.68–0.80) $\mu\text{g/L}$, and for Pb 26.7 ± 1.0 ($n = 21$; recommended 26.2–29.0) $\mu\text{g/L}$. For human blood reference samples from Centre de Toxicologie du Quebec, International Comparison Program, Quebec, Canada, the obtained values for Cd (Lot C0515) was 0.76 ± 0.14 , ($n = 51$; recommended 0.79 ± 0.23) and for Pb (Lot L0608) 31.8 ± 1.1 (31.1 ± 4.7) $\mu\text{g/L}$.

Mercury was determined either at the Jozef Stefan Institute, Ljubljana, Slovenia (samples from Slovenia and Croatia) or Lund University (all other countries). The two laboratories use different methods but have shown excellent agreement in inter-laboratory comparison: The deviation was in average less than 5% for blood samples (using the same sampling tubes as for the children) containing 0.88 to 1.3 $\mu\text{g Hg/L}$: The results obtained were for Lund 1.22 ± 0.05 , 1.64 ± 0.08 and 0.88 ± 0.01 $\mu\text{g Hg/L}$ vs. Ljubljana 1.28 ± 0.07 , 1.64 ± 0.03 and 0.96 ± 0.03 $\mu\text{g Hg/L}$.

In Lund, Hg was determined in acid-digested samples by cold vapor atomic fluorescence spectrophotometry (Sandborgh-Englund et al., 1998). The detection limit was in average 0.07 (range: 0.03–0.13) $\mu\text{g/L}$. The method imprecision was 7.6%. The analytical accuracy for Hg in Seronorm Trace elements whole blood (Lot. MR4206 and 0512627, SERO AS) was 2.1 ± 0.15 ($n = 115$; recommended 2.0–2.4) and 15.7 ± 1.3 , ($n = 115$; 16.1–19.7) $\mu\text{g/L}$.

In the Josef Stefan Institute, total Hg in blood was determined by thermal combustion, amalgamation and atomic absorption spectrometry using Direct Mercury Analyser (DMA-80) at 254 nm (EPA Method 7473). The method imprecision was 6%. The detection limit was 0.1 $\mu\text{g/L}$. The accuracy was checked by the use of reference material obtained from Seronorm: Trace Elements Whole Blood 1 (Lot no. MR4206x). The result obtained was 2.2 ± 0.1 $\mu\text{g/L}$, based on 8 independent determinations (recommended value: 2.2 ± 0.2 $\mu\text{g/L}$).

We express the concentrations as $\mu\text{g/L}$. 1 $\mu\text{g Cd/L} = 0.0091$ $\mu\text{mol/L}$; 1 $\mu\text{g Pb/L} = 0.0050$ $\mu\text{mol/L}$; 1 $\mu\text{g Hg/L} = 0.0050$ $\mu\text{mol/L}$.

3. Calculation

We included measurement values below detection limits in the statistical analyses, not to bias data distribution, but individual results below detection limits are not reported in tables or text. First, the effects of country (all and European only) on blood metal concentrations (natural logarithm transformed) were examined by log-linear models, and then each potentially influential categorical variable (sex, age, fish and shellfish intake, amalgam fillings, traffic density, parental smoking and education) for the European children, in analyses stratifying on country. An added variable was considered as influential if $p < 0.05$. The final model for a blood metal concentration included all influential variables. Possible interactions between country and influential variables were also evaluated. Finally, we evaluated each potentially influential variable for the non-European children.

“Statistically significant” denotes $p < 0.05$ (two-sided). The Stata 10 statistical package was used for all statistical analyses.

4. Results

Background data for the 433 children are presented in Table 1.

B-Cd and B-Pb showed only marginal differences between the European cities, with a range of about 1.5 times between the lowest and the highest geometric mean (Table 2; Fig. 1). The lowest and highest B-Cd and B-Pb in children in the same city ranged 1.8–5.9 times, with the most narrow range in Prague, the Czech Republic and largest in Banska Bystrica, Slovakia.

In contrast, B-Hg varied by a factor of eight between the European cities (Table 2; Fig. 1). Also, the variation within cities was large (8–140 times), smallest in Ljubljana, Slovenia and largest in Wroclaw, Poland.

Children from Camilo Ponce Enríquez in Ecuador and Fez and Sefrou in Morocco had higher B-Cd and B-Pb than the European children (Table 2; Fig. 1). For B-Hg, children from Guiyang, China and Camilo Ponce Enríquez, Ecuador were higher, but not those from Fez and Sefrou, Morocco.

In Europe, country was an important determinant for B-Hg, explaining 52% of the variance, whereas it only marginally explained the variance for B-Cd (12%) and B-Pb (16%; Table 3).

Among the other potentially influential factors, traffic intensity outside the house in which the child lived was statistically significantly related to B-Cd (Table 3), but neither shellfish or offal consumption, nor parental smoking at home was related to B-Cd. The relation to traffic intensity was consistent for all the European countries.

B-Hg was related to amalgam fillings and fish consumption (Table 3), and B-Pb was higher in boys than in girls (Table 3). An association to shellfish consumption was seen, in spite of the fact that the intake was low. For non-European cities, we did not find any determinants of exposure.

5. Discussion

5.1. Strengths and weaknesses of our study

We choose to recruit the children through one school in each city, instead of, e.g., a random sample of the population in the whole country or the city. This affects the possibility to generalize, but we gave priority to simplicity. Since a major finding was the remarkably small difference in B-Cd and B-Pb between countries, the sampling strategy did probably not cause much bias. For B-Hg we are less certain that there are no socioeconomic differences between schools that may have affected the results.

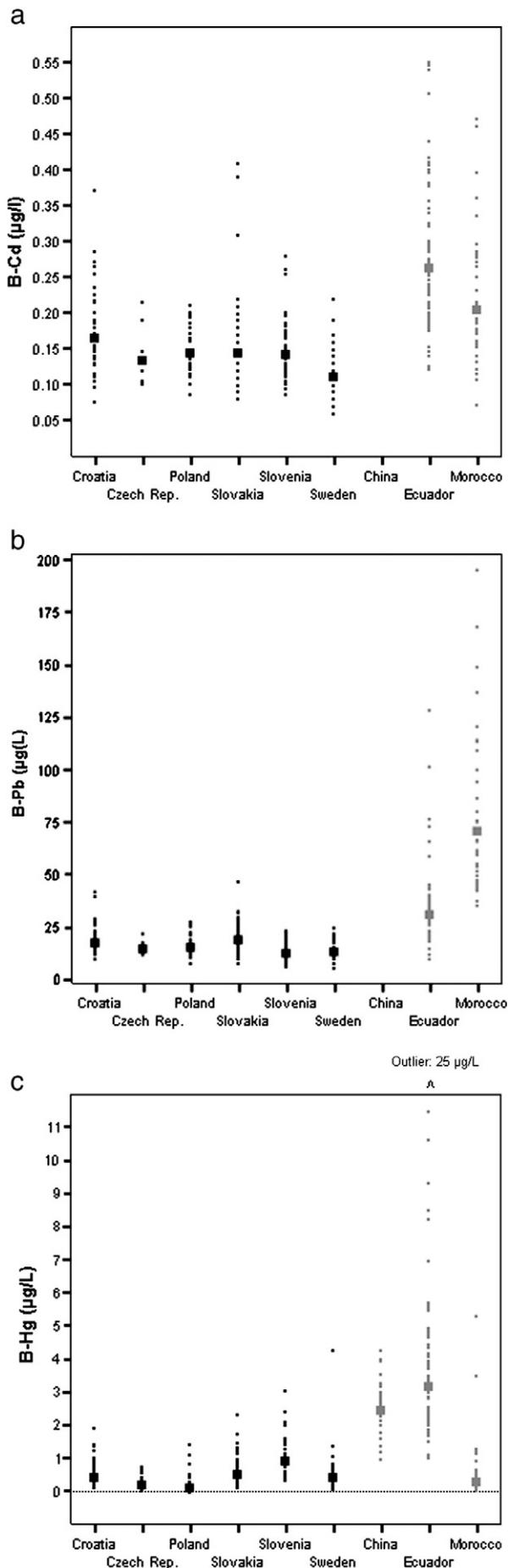
The number of children sampled was small. Despite this, because of the limited variation, the number is not too meager for B-Cd and B-Pb. For B-Hg, a larger sample would be valuable.

We choose to use blood concentrations as the biomarker. A disadvantage is the invasive sampling, a fact to consider when examining children. On the other hand, blood concentrations are relevant for exposure and risk for all the three metals, and the risk of an external contamination is smaller than for urine and hair. Also, the analytical procedure is simpler than for urine, and there is no need to adjust for differences in dilution. There is no simple relationship between metal levels in blood and other biological media (Skerfving et al., 1999). Hence, the mercury in blood is composed of elemental and methylmercury mainly in blood cells, and mercuric mercury in plasma, while hair mercury is mainly used for monitoring of methylmercury and urinary mercury for

Table 2

Metal concentrations ($\mu\text{g/L}$) in blood of city children by country. N = number of children examined. GM = geometric mean.

City, Country	Cadmium			Lead			Mercury		
	N	GM	Range	N	GM	Range	N	GM	Range
Koprivnica, Croatia	46	0.17	0.08–0.37	46	17.9	10–42	52	0.44	0.14–1.9
Prague, Czech Republic	8	0.13	0.10–0.22	8	15.5	12–22	21	0.21	<0.07–0.75
Wroclaw, Poland	27	0.15	0.09–0.21	27	16.3	8.0–28	30	0.12	<0.07–1.4
Ban. Bystrica, Slovakia	57	0.14	0.08–0.41	57	19.4	8.0–47	57	0.52	0.12–2.3
Ljubljana, Slovenia	42	0.14	0.09–0.28	42	13.4	6.9–24	45	0.94	0.36–3.0
Landskrona, Sweden	41	0.11	0.06–0.22	41	14.0	6.0–25	41	0.43	0.10–1.4
Guiyang, China	0	–	–	0	–	–	29	2.45	0.99–4.3
Camilo Ponce Enríquez, Ecuador	69	0.26	0.12–0.55	69	31.7	10–130	69	3.23	1.0–25
Fez, Sefrou, Morocco	39	0.21	0.07–0.47	39	71.0	36–200	39	0.31	<0.07–5.3



inorganic. As a consequence, though the concentrations in the different media correlate, the ratios between concentrations in different media vary depending upon the relative variation in exposure pattern of the population to the mercury species (Berlin et al., 2007). Blood cadmium reflects recent uptake, as well as the body burden, which reflects the long-term accumulation, while urinary cadmium is mainly determined by the kidney concentration (Nordberg et al., 2007), though its pattern may be more complex at low exposure (Bernard, 2008). For lead, blood concentration is nowadays used almost exclusively (Skerfving and Bergdahl, 2007).

We made considerable efforts to make the results comparable between countries. One step was to use the same sampling tubes and needles in all locations. In order to find the best sampling tubes, we investigated the level of contamination of different brands of evacuated sampling tubes. It turned out that today's evacuated plastic tubes can be very clean, compared to glass tubes. The tubes we found to be cleanest were not certified for metal contamination levels, but we decided that we should use those tubes, since the certificates for 'metal sterile' did allow much higher contamination levels. By choosing to use tubes of one single production batch, we wanted to minimize differences in contamination levels.

Initially, we performed the chemical analyses in laboratories in different countries, but then we realized that the international differences in concentrations were so small that systematic interlaboratory differences of $\pm 10\%$ would seriously bias any evaluation of international differences for Cd and Pb. Very few interlaboratory comparisons can prove that strong coherence between laboratories at the low concentrations in the present study. It thus became obvious that characterizing these small differences would require centralized chemical analyses. Therefore, we decided to re-analyze the blood samples. Then, cadmium and lead was determined in one single laboratory, while mercury was determined in two laboratories that had shown excellent agreement in an inter-laboratory comparison. Hence, the results from the different countries are fully comparable. This is a major strength of the study, but at the same time, the use of centralized chemical analyses may complicate future follow-ups of time trends in the different countries. In the re-analysis, the sample size decreased for several countries due to lack of remaining blood for the re-analyses. However we did not find a significant difference in means for re-analyzed and not-re-analyzed subsamples, when comparing original values.

One of the major experiences from this study is the great importance, even today, of careful analytical quality control, in spite of the fact that biomonitoring of metals has a long record. Hence, to enable adequate international comparisons, we had to centralize the analyses. Thus, one of the major conclusions from this study is the crucial need for advanced quality control.

A weakness of the present study is the simple classifications of potential determinants of exposure, such as food intake, traffic intensity, etc. We made the choice to use simple questions, in order to make future follow-ups of time trends easy to carry out. However, we may not have identified all determinants.

5.2. Cadmium

There was not much variation between European cities in B-Cd, in spite of the fact that there has been concern about industrial emissions in central European countries after expansion of the heavy industry after world war two.

There was a surprising association between B-Cd and traffic density near the home. There are only minor amounts of Cd in vehicle

Fig. 1. Metal concentrations in blood [Part A: cadmium (B-Cd); Part B: lead (B-Pb); Part C: mercury (B-Hg)] among children from six European (black dots) and three non-European cities (gray dots). City-specific geometric means are indicated (filled squares). Data on B-Cd and B-Pb from China are lacking. There were significant differences in all metal concentrations across all cities ($p < 0.001$), as well as across the European cities ($p < 0.001$).

Table 3

Associations between metal concentrations in blood in children from European cities and potential determinants. R² = explained variance. n.s. = not significant (p < 0.05). CI = confidence interval.

Metal	Model including country R ² (%)	Variable	Model including country and other determinants				
			Relative change (point estimate)	95% CI	p-value	R ² (%)	Interaction country/determinant
Cadmium	12	Traffic density					
		≤ 1 car/min	1.00	Reference	0.02	17	n.s.
		> 1	1.11	1.02–1.22			
Lead	16	Sex					
		Boys	1.00	Reference	0.001	23	n.s.
		Girls	1.14	1.05–1.24			
		Shellfish intake					
		< 1 meals/month	1.00	Reference	0.003	n.s.	
		≥ 1	1.25	1.08–1.44			
Mercury	52	Amalgam fillings					
		0–1	1.00	Reference	<0.001	55	n.s.
		> 1	1.51	1.20			
		Fish intake					
		< 1 meals/month	1.00	Reference	0.026	n.s.	
		1–3	0.96	0.76–1.21			
		> 3	1.27	0.99–1.64			

exhaust. However, tires containing zinc contaminated by Cd (Hjortenkrans et al., 2007) may explain the finding. There was no sex difference, although it is well established that adult females have higher B-Cd than males, but that is due to higher gastrointestinal absorption in individuals with low iron status, common in young and middle-aged women as a consequence of menstruation, which is of course not of relevance for young children. We found no association between B-Cd and exposure to environmental tobacco smoke, in spite of the fact that tobacco contains Cd, and that active smoking is a major determinant of B-Cd. The lack of association with passive smoking is in accordance with earlier reports (Skerfving et al., 1999). Shellfish intake was not a determinant of B-Cd, in spite of the fact that it contains Cd. However, the intake was low.

We used blood to estimate the exposure and risk. B-Cd reflects both the recent exposure and the body burden (Nordberg et al., 2007). The levels were low, in parity with those reported earlier in some studies of children from Czech Republic, Germany and Sweden (Batárióvá et al., 2006; Beneš et al., 2000; Link et al., 2007; Skerfving et al., 1999). On the contrary many surveys in those countries have showed higher levels, but often these reports show less strict analytical quality control, and we therefore suspect that the results are falsely high. For the US only little NHANES data on B-Cd in children has been reported. A major reason appears to be that the concentrations have been too low for accurate determinations. For children 12–36 months, the geometric mean (GM) appears to be at or below 0.2 µg/L (Cao et al., 2009), not suggestive of large discrepancies between the US and Europe, though a large uncertainty remains.

The B-Cd in Morocco and Ecuador were considerably higher. Hence, there must be sources of Cd exposure in those countries. Probably, the explanation is higher intake of rice, which is well known to absorb Cd from soil (Nordberg et al., 2007).

In spite of the low B-Cd in Europe, the situation is far from comforting. Thus, since Cd accumulates in the body over time, even the low levels observed in children may result in high concentrations in kidney in the elderly, in particular in females. Such accumulation means that a large fraction of the adult female European population is under risk to develop effects on kidney (Akeson et al., 2005; EFSA, 2009) and bone (Akeson et al., 2006). In fact, kidney effects have been seen already in teenagers (Bernard et al., to be published).

Hence, the B-Cd is a matter of concern. Also, there is reason to believe that the exposure is relatively constant over time (Link et al., 2007; Wennberg et al., 2006), in spite of the fact that the emissions have decreased (WHO, 2009), probably because the concentration in contaminated agricultural soil declines very slowly. Thus, further

pollution must be minimized, and means to decrease the transfer of Cd from soil and through the food chain to consumption should be further investigated.

5.3. Mercury

Of the three studied metals, B-Hg was the one that differed most between the city children from different countries. The concentrations in the European cities and in Fez, Morocco were lower than previously reported from many countries (Batárióvá et al., 2006; Beneš et al., 2000; Skerfving et al., 1999), possibly because B-Hg has decreased over time, but in general agreement with present levels in German (Schulz et al., 2009) and US children (Caldwell et al., 2009). The levels in children from the two cities in China and Ecuador were much higher. One should be careful in generalizing these levels to the whole countries, especially for such a large country as China, and Ecuador has gold-mining areas where much Hg is locally used.

In spite of the present low B-Hgs, the well known (Berlin et al., 2007; Schulz et al., 2009) determinants of B-Hg, in terms of methyl-Hg from fish intake and elemental Hg from dental amalgam fillings were present, and may explain most of the variation between cities, since these factors differed much. In Sweden and Poland, amalgam use in children has been phased out, while the intake of fish, in spite of the fact that it is relatively low, is still larger than in the other countries. In the other end of the range, Slovakian and Slovenian children had a high number of fillings, while the intake of fish was near this study's average. The high B-Hgs in China and Ecuador was associated with high intakes of fish; amalgam fillings were infrequent.

The interpretation of B-Hg in terms of toxic risk is complicated by the fact that it is a mixture of methyl-Hg and inorganic Hg, which have very different toxicology and sources (Berlin et al., 2007). In cases that require more precise estimates of exposure to inorganic Hg vapor or methyl-Hg other biomarkers than total B-Hg should be used. However, all the present mean B-Hgs are much lower than those suspected to cause health effects. The critical one is impact of methyl-Hg from fish on the fetal brain, resulting in impairment of cognitive function in children, at B-Hgs in maternal and cord blood of > 20 µg/L; (WHO, 2009). Only occasionally did children in Ecuador reach such concentrations.

5.4. Lead

The B-Pb differed only marginally between the European cities. Since the exposure to Pb has decreased dramatically after the phasing

out of petrol-Pb (Stroh et al., 2009), it is only meaningful to compare to recent studies, which are few. Then, the present concentrations are in accordance with other data from e.g. Sweden (Stroh et al., 2009), Germany (Schulz et al., 2009), and the US (Jones et al., 2009).

There was no impact of living close to traffic. This suggests that the effect of petrol-Pb has faded away after the phasing out (Stroh et al., 2009). The cities in Ecuador and Morocco were higher than the European cities. The source is unknown, but may be water or food contamination or a remaining contamination from leaded petrol. Local observations do not indicate lead-glazed ceramics as a source, though that possibility cannot be completely ruled out.

The impact of shellfish intake on B-Pb was surprising and has not been reported before. It might be a chance finding, but the observation may be because shellfish contains Pb. Other exposure sources may have made this influence difficult to observe in previous studies, but now with a decrease in exposure from the major sources, also sources with smaller impact may become visible.

The European children were only occasionally in the range presently suspected to cause health effects (slight impairment of cognitive functions at $> 50 \mu\text{g/L}$; Skerfving and Bergdahl, 2007). However, a fraction of the children from Ecuador, and more than half of those from Morocco had concentrations, that urges preventive actions, firstly elimination of any petrol-Pb if such is still in use (officially or on a black market).

6. Conclusions

This study shows that B-Cd and B-Pb in children were quite similar in six European cities, while for B-Hg there were considerable differences. B-Pb in Fez, Morocco is much higher, to some extent also in Camilo Ponce Enriquez, Ecuador. B-Hg in children from the cities in Ecuador and China were higher than in Europe.

In spite of the low levels in Europe, B-Hg displayed that it was associated with amalgam fillings and fish intake, B-Cd with closeness to traffic.

When related to risk, B-Pb was low in Europe, but in Morocco on a level that is suspected of causing effects. Some of the Chinese B-Hgs were at a level which is suspected to cause risk for a fetus if persisting into women's adulthood. B-Cd was low, but a persisting exposure on that level may still cause health effects late in life.

There is a need for continuous follow-up of the exposure patterns in children in Europe. For cadmium and lead this study indicates that international differences should not be the main concern, but 'hot spots' of particular exposures may still need to be focused and these have not been studied herein. Obviously, other parts of the world should be monitored.

Blood is a suitable biomonitoring tool, but it is a necessity to standardize the sampling procedure, to have a strict quality control, and be very cautious if considering use of multiple laboratories when differences in exposure are to be investigated.

Acknowledgment

Grants were supplied from the European Union (Sixth Framework Programme; PHIME; FOOD-CT-2006-016253) and a long series of funding agencies in the participating countries. Technical assistance was given by Ms. Anna Akantis and Mr. Giovanni Ferrari. We thank

the participating children and the personnel that helped with recruitment in each country.

References

- Akesson A, Lundh T, Vahter M, Bjellerup P, Lidfeldt J, Nerbrand C, et al. Tubular and glomerular kidney effects in Swedish women with low environmental cadmium exposure. *Environ Health Perspect* 2005;11:1627–31.
- Akesson A, Bjellerup P, Lundh T, Lidfeldt J, Nerbrand C, Samsioe G, et al. Cadmium-induced effects on bone in a population-based study of women. *Environ Health Perspect* 2006;6:830–4.
- Barany E, Bergdahl IA, Schütz A, Skerfving S, Oskarsson A. Inductively coupled plasma mass spectrometry for direct multi-element analysis of diluted human blood and serum. *J Anal At Spectrom* 1997;12:1005–9.
- Batárióvá A, Spěváčková V, Beneš B, Čejchanová M, Šmíd J, Černá M. Blood and urine levels of Pb, Cd and Hg in the general population of the Czech Republic and proposed reference values. *Int J Hyg Environ Health* 2006;209:359–66.
- Beneš B, Spěváčková V, Šmíd J, Čejchanová M, Černá M, Šubrt P, et al. The concentration levels of Cd, Pb, Hg, Cu, Zn and Se in blood of the population in the Czech Republic. *Cent Eur J Public Health* 2000;8:117–9.
- Berlin M, Zalups RK, Fowler BA. Mercury. In: Nordberg GF, Fowler BA, Nordberg M, Friberg LT, editors. *Handbook on the toxicology of metals*. Elsevier: Academic Press; 2007. p. 675–727.
- Bernard A. Biomarkers of metal toxicity in population studies: research potential and interpretation issues. *J Toxicol Environ Health A* 2008;71:1259–65.
- Caldwell KL, Mortensen ME, Jones RL, Caudill SP, Osterloh JD. Total blood mercury concentrations in the U.S. population 1999–2006. *Int J Hyg Environ Health* 2009;212:588–98.
- Cao Y, Chen A, Radcliffe J, Dietrich KN, Jones RL, Caldwell K, et al. Postnatal cadmium exposure, neurodevelopment, and blood pressure in children at 2, 5, and 7 years of age. *EFSA J* 2009;980:1–139.
- EFSA. Cadmium in food – scientific opinion of the Panel on Contaminants in the Food Chain. *Eur Food Safety Auth* 2009.
- Hjortenkrans DS, Bergbäck BG, Häggerud AV. Metal emissions from brake linings and tires: case studies of Stockholm, Sweden 1995/1998 and 2005. *Environ Sci Technol* 2007;41:5224–30.
- Jones RL, Homa DM, Meyr PA, Brody DJ, Caldwell KL, Pirkle JL, et al. Trends in blood lead levels and blood lead testing among US children aged 1–5 years, 1988–2004. *Pediatrics* 2009;123:376–85.
- Link B, Gabrio T, Piechotowski I, Zollner I, Schwenk M. Baden-Wuerttemberg Environmental Health Survey (BW-EHS) from 1996 to 2003: toxic metals in blood and urine of children. *Int J Hyg Environ Health* 2007;210:357–71.
- Nordberg GR, Nogawa K, Nordberg M, Friberg LT. Cadmium. In: Nordberg GF, Fowler BA, Nordberg M, Friberg LT, editors. *Handbook on the toxicology of metals*. Elsevier: Academic Press; 2007. p. 445–86.
- Sandborgh-Englund G, Elinder CG, Langworth S, Schütz A, Ekstrand J. Mercury in biological fluids after amalgam removal. *J Dent Res* 1998;77:615–24.
- Schulz C, Angerer J, Eweres U, Heudorf U, Wilhelm M. Revised and new reference values for environmental pollutants in urine or blood of children in Germany derived from the German Environmental Survey on Children 2003–2006 (GerES IV). *Int J Hyg Environ Health* 2009;212:637–47.
- Skerfving S, Bencko V, Vahter M, Schütz A, Gerhardsson L. Environmental health in the Baltic region – toxic metals. *Scand J Work Environ Health* 1999;25(Suppl. 3):40–64.
- Skerfving S, Bergdahl IA. Lead. In: Nordberg GF, Fowler BA, Nordberg M, Friberg LT, editors. *Handbook on the toxicology of metals*. Elsevier: Academic Press; 2007. p. 599–643.
- Smolders R, Alimonti A, Černá M, Den Hond E, Kristiansen J, Palkovičová L, et al. Availability and comparability of human biomonitoring data across Europe: a case-study on blood-lead levels. *Sci Total Environ* 2010;408:1437–45.
- Stroh E, Lundh T, Oudin A, Jakobsson K, Skerfving S, Strömberg U. Yearly blood-lead measurements in Swedish children during 1978–2007. *BMC Public Health* 2009;9:225–39.
- Vahter M, Slorach S. Exposure monitoring of lead and cadmium. An international pilot study within the UNEP/WHO human exposure assessment location (HEAL) project. Technical report. Nairobi: WHO; 1991.
- Wennberg M, Lundh T, Bergdahl IA, Hallmans G, Jansson J-H, Stegmayr B, et al. Time trends in burdens of cadmium, lead, and mercury in the population of northern Sweden. *Environ Res* 2006;100:330–8.
- WHO. Health risks of heavy metals from long-range transboundary air pollution. Joint WHO/Convention Task Force on the Health Aspects of Air Pollution. Europe: WHO; 2009.