PHIME WPIII:1
Sources of exposure, geographical patterns and time trends: Exposure to elements in children and women

Blood cadmium, mercury, and lead in children from Croatia, Czech Republic, Poland, Slovakia, Slovenia, Sweden, China, Ecuador, and Morocco

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METALS

LEAD
Lead is a well-known neurotoxin. Lead exposures have developmental and neurobehavioural effects on fetuses, infants and children. Food is the predominant source of lead uptake in the general population. Ingestion of contaminated soil, dust and old lead-based paint due to hand-to-mouth activities may also be important regarding lead intake in children. When tap-water systems with leaded pipes are used, lead intake via drinking-water can be an important source (1,2).

MERCURY
Chronic exposure to low levels of inorganic mercury vapour is associated to adverse effects on the central nervous system, kidneys and thyroid.
Metylmercury is a potent neurotoxic chemical. Exposure to methylmercury is more dangerous for young children than for adults, because of the lower thresholds for neurological effects from methylmercury and the higher levels of distribution of methylmercury to the developing brains. Emissions of mercury to the air from both anthropogenic and natural sources are in inorganic forms that can be converted biologically to methylmercury in soil and water. Methylmercury bioaccumulates and enters the human body readily via the dietary route. Children are the most susceptible population group, the exposure being mainly from fish in the diet. The major route of non-occupational exposure to inorganic mercury is via dental amalgam fillings (1,2).

CADMIUM
Cadmium exposures are associated with kidney and bone damage. Cadmium has also been identified as a potential human carcinogen, causing lung cancer. Food is the main source of cadmium exposure in the general population (representing >90% of the total intake in non-smokers). Dust resuspension can constitute a substantial part of the crop contamination and exposures via inhalation and digestion (1,2).

SPECIFIC OBJECTIVES

Systematic approach to human biomonitoring of level of contaminants in blood for sensitive populations. Geographical patterns, regional differences, and determinants of exposure for children population.

MATERIAL AND METHODS

Study areas and subjects
In each country schools in urban, rural, and industrial locations were selected. Contact was established first with the school management and then with parents and children. In each country 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.

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.

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), child's intake of offal (less than one *vs* one and 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.

Chemical analyses
First round of analyses of B-Pb, B-Cd, and B-Hg were done in experienced laboratories from the participating countries, by the following table:

Country	Laboratory	Country	Laboratory
Croatia	JSI, Slovenia	Slovenia	JSI, Slovenia
Czech Republic	NIPH, Czech Republic	Sweden	LU, Sweden
Poland	IOMEH, Poland	China	China
Slovakia	LU, Sweden	Ecuador	LU, Sweden
		Morocco	LU, Sweden

A subset of blood samples of children from urban areas was re-analysed in one single laboratory in Sweden, though with the exception that Hg data was used also from the laboratory JSI Slovenia that had shown excellent agreement in inter-laboratory comparisons.
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 (3). 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 ^{114}Cd and ^{118}Sn, 20 ms for $^{206, 207, 208}$Pb (summed) and 10 ms for the internal standards ^{115}In, ^{205}Tl and ^{209}Bi. Interference corrections were made for ^{114}Cd for the spectral overlap of Sn. The detection limits for Cd and Pb, calculated as 3 times the standard deviation (SD) of the blank were 0.01 and 0.06 µg/L, respectively. All samples were prepared in duplicate, and the method imprecision (calculated as the coefficient of variation for duplicate preparations measurements) were for Cd and Pb 9.3 and 6.8%, respectively. 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±SD; n=51; recommended 0.68–0.80) µg/L, and for Pb 26.7±1.0 (n=21; recommended 26.2–29.0) µg/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) µg/L.
In Lund, Hg was determined in acid-digested samples by cold vapour atomic fluorescence spectrophotometry (Sandborgh-Englund et al., 1998). The detection limit was 0.07 µg/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) µg/L.
We express the concentrations as µg/L. 1 µg Cd/L=0.0091 µmol/L; 1 µg Pb/L=0.0050 µmol/L; 1 µg Hg/L=0.0050 µmol/L.

Statistical analyses
Data for children from urban areas: First, the effects of country 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.
Data for children from urban, rural, and industrial areas, original measurements: These data were used for assessment of regional differences. As country was an effect modifier, regional differences were studies by log-linear models separately for each country.
"Statistically significant" denotes p<0.05 (two-sided). The Stata 10 statistical package was used for all statistical analyses.

CONCLUSIONS AND IMPLICATIONS

Biomonitoring
- This study shows that B-Cd and B-Pb in children were quite similar in six European countries, while for B-Hg there were considerable differences. B-Pb in Morocco is much higher, to some extent also in Ecuador. B-Hg in Ecuador and China were higher than in Europe.
- In spite of the low levels, 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 the fetus if persisting into 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. Then, international differences should not be the main concern, but 'hot spots' of particular exposures should be focussed upon. Obviously, other parts of the world should be monitored.
- Actions should be taken to decrease human exposure in industrially contaminated areas where biomonitoring shows that a significantly increased exposure occurs.

Methodological issues
- Blood was a suitable biomonitoring tool, but it is a necessity to standardize the sampling procedure, to have a very strict quality control, and be very cautious if considering use of multiple laboratories when international differences in exposure are to be investigated.
- When a multi-centre study is planned and a choice has to be made between employing central or local chemical analyses, we would recommend that the expected difference in exposures between the centres is considered in relation to the expected systematic differences between laboratories. The latter need to be characterized at the concentration levels of interest and these may of course be very low if the general population is studied. In the case of metals, we see an overwhelming risk of failure if separate laboratories are used for characterization of international differences. There are however several advantages of a local strategy, e.g. comparability with other local studies, local commitment to the study aims, simplification of future time-trend studies. Then, a compromise between central and local analyses may be to employ the combined strategy we here present. The requirements of carefully chosen statistical methods of analysis may seem difficult to meet. However, these requirements mainly stem from the multidimensional character of a study that incorporates different determinants of exposure. Therefore, skilled statisticians are required, regardless of whether local or centralized chemical analyses are employed.

RESULTS

Table 1: Background characteristics of the study populations

Country City	Croatia Koprivnica	Czech Republic Prague	Poland Wroclaw	Slovakia Banska Bystrica	Slovenia Ljubljana	Sweden Lands-krona	China Guiyang	Ecuador Camilo Ponce Enríquez	Morocco Fez, Sefrou
Population	31,000	1100,000	635,000	80,000	300,000	30,000	?	?	?
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)	71 (36/35)	39 (18/21)
Age [mean (range)]	8.7 (8-10)	8.4 (7-10)	8.0 (7-8)	8.9 (7-11)	9.0 (7-11)	9.1 (8-11)	8.2 (7-10)	7.2 (6-10)	10.2 (7-14)
Smoking									
at home (%)	50	19	23	14	9	29	52	16	51
Parental education (%)									
Primary school Secondary Higher	6 58 36	5 71 24	3 54 43	0 60 40	0 28 72	10 72 18	7 69 24	46 51 3	5 59 36
Amalgam fillings (%)									
0 1-2 ≥3+	32 58 10	60 10 30	93 7 0	21 33 46	37 20 43	93 7 0	90 7 3	98 1 1	92 8 0
Fish intake[meals/month (%)]									
<1 1-3 >3	10 59 31	43 33 24	7 37 56	33 37 30	25 45 30	17 44 39	35 48 17	1 20 79	8 61 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

Table 2: Metal concentrations (µg/L) in blood of city children by country. N=number of children examined. GM=geometric mean

Country	Cadmium			Lead			Mercury		
	N	GM	Range	N	GM	Range	N	GM	Range
Croatia	46	0.17	0.08-0.37	46	17.9	10-42	52	0.44	0.14-1.9
Czech Republic	8	0.13	0.10-0.22	8	15.5	12-22	21	0.21	0.02-0.75
Poland	27	0.15	0.09-0.21	27	16.3	8.0-28	30	0.12	0.001-1.4
Slovakia	57	0.14	0.08-0.41	57	19.4	8.0-47	57	0.52	0.12-2.3
Slovenia	42	0.14	0.09-0.28	42	13.4	6.9-24	45	0.94	0.36-3.0
Sweden	41	0.11	0.06-0.22	41	14.0	6.0-25	41	0.43	0.10-1.4
China	0	-	-	0	-	-	29	2.45	0.99-4.3
Ecuador	69	0.26	0.12-0.55	69	31.7	10-130	69	3.23	1.0-25
Morocco	39	0.21	0.07-0.47	39	71.0	36-200	39	0.31	0.05-5.3

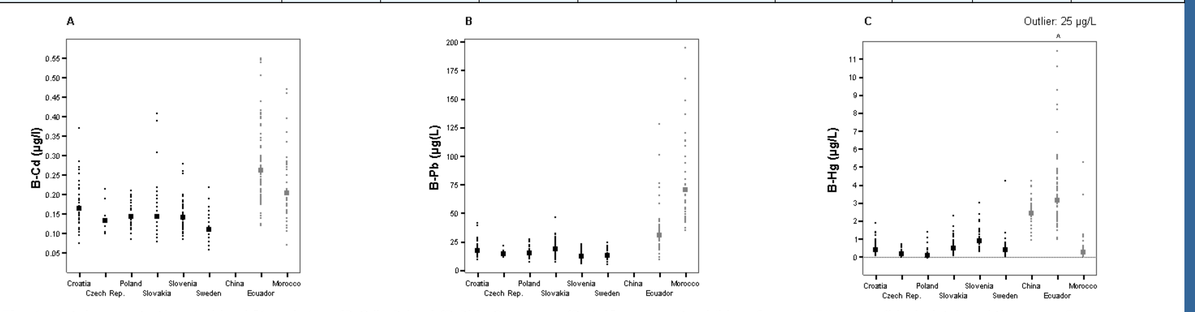

Fig. 1. Metal concentrations in blood [A:cadmium (B-Cd); B:lead (B-Pb); C:mercury (B-Hg)] among city children from six European (black dots) and three non-European countries (grey dots). Country-specific geometric means are indicated (filled squares). Data on B-Cd and B-Pb from China are lacking. There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

Table 3: Associations between metal concentrations in blood in city children from European countries 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	1.00 1.11	Reference 1.02-1.22	0.02	17	n.s.
Lead	16	**Sex**					
		Boys Girls	1.00 1.14	Reference 1.05-1.24	0.001	23	n.s.
		Shellfish intake					
		<1 meals/month ≥1	1.00 1.25	Reference 1.08-1.44	0.003		n.s.
Mercury	52	**Amalgam fillings**					
		0-1 >1	1.00 1.51	Reference 1.20	<0.001	55	n.s.
		Fish intake					
		<1 meals/month 1-3 >3	1.00 0.96 1.27	Reference 0.76-1.21 0.99-1.64	0.026		n.s.

Table 4: Metal blood levels for children living in rural and industrial areas (percent change) compared to children living in urban areas (GM) in 8 countries

Country Area	B-Cd		B-Pb		B-Hg	
	GM (µg/L)	Multipl.effect	GM (µg/L)	Multipl.effect	GM (µg/L)	Multipl.effect
CROATIA						
Urban	**0.17*/0.26$**	1.00	**17.9*/19.4$**	1.00	**0.44***	1.00
Rural		1.17 (1.07-1.27)		1.13 (0.96-1.33)		0.82 (0.66-1.01)
Industrial		1.13 (1.03-1.23)		1.18 (0.99-1.40)		0.65 (0.51-0.81)
CZECH REPUBLIC						
Urban	**0.13*/0.18$**	1.00	**15.5*/24.3$**	1.00	**0.21*/0.42$**	1.00
Rural		1.20 (1.03-1.42)		0.62 (0.51-0.77)		1.01 (0.75-1.37)
Industrial		1.60 (1.37-1.86)		0.95 (0.78-1.16)		0.96 (0.72-1.28)
POLAND						
Urban	**0.15*/0.20$**	1.00	**16.3*/14.2$**	1.00	**0.12*/0.31$**	1.00
Rural		1.20 (0.91-1.60)		1.40 (1.18-1.67)		0.70 (0.48-0.99)
Industrial (Pb)		2.13 (1.61-2.81)		2.28 (1.92-2.70)		0.57 (0.40-0.82)
SLOVAKIA						
Urban	**0.14***	1.00	**19.5***	1.00	**0.52***	1.00
Rural		0.87 (0.77-0.99)		1.12 (0.99-1.26)		0.52(0.40-0.68)
Industrial		0.99 (0.87-1.12)		1.82 (1.61-2.05)		0.57(0.44-0.74)
SLOVENIA						
Urban	**0.14*/0.27$**	1.00	**13.4*/20.2$**	1.00	**0.94***	1.00
Rural		1.23 (1.09-1.38)		1.10 (0.96-1.26)		0.76 (0.63-0.92)
Industrial (Hg)		0.92 (0.82-1.04)		0.93 (0.81-1.07)		0.97 (0.80-1.17)
SWEDEN						
Urban	**0.11***	1.00	**13.9***	1.00	**0.46***	1.00
Rural		0.84 (0.74-0.95)		0.81 (0.69-0.95)		1.40 (1.10-1.80)
Industrial (Pb)		1.01 (0.89-1.13)		1.03 (0.88-1.21)		2.09 (1.65-2.65)
CHINA						
Urban (Pb)	**./0.75$**	1.00	**./64.2$**	1.00	**2.45*/2.23$**	1.00
Rural		0.95 (0.90-1.01)		0.72 (0.62-0.84)		1.19 (1.07-1.34)
Industrial (Hg)		0.99 (0.93-1.05)		0.76 (0.64-0.90)		3.07 (2.71-3.47)
ECUADOR						
Urban (Pb?)	**0.26***	1.00	**31.6***	1.00	**3.19***	1.00
Rural		1.21 (1.05-1.40)		1.26 (1.08-1.46)		0.91 (0.74-1.12)
Industrial (Hg)		1.04 (0.88-1.22)		0.95 (0.80-1.12)		1.50 (1.18-1.89)

* Geometric mean (GM) from data of (re)analyzed samples in one centralized laboratory
$ Geometric mean (GM) from original analyses of the same children.

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