Bull. Environ. Contam. Toxicol. (2002) 69:1–7

DOI: 10.1007/s00128-002-0001-1

Bulletin of Environmental Contamination and Toxicology

Determination of Heavy Metals in the Bones and Livers of Deceased Neonatal Humans

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Received: 15 May 2001/Accepted: 4 March 2002

The research on the determination of various elements in human tissues has been carried out by many scientists, but the metabolism of trace metals in the perinatal period has not been well established yet. Especially interesting seems to be the period in which intensive proliferation of cells and placenta transfer lead to organogenesis. Homeostatic fetal mechanisms are not fully developed in this period and the fetus is susceptible to environmental influence - among other things - to the presence of heavy metals. Not many experiments have been performed in multi-element systems containing heavy metals and these works concern mainly children older than 1 year and adults' tissues. Research have been carried out on bones (Lindh et al. 1980, Drasch et al. 1987, Ericson et al. 1991, Krasnegor 1994, Baranowska et al. 1995, Rosen 1997), liver (Cubells Soriano et al. 1984, Bem et al. 1988), kidneys (Carpenter 1981,Tiran et al. 1995, Fels et al. 1998). Multi-element analysis has not been performed on neonatal tissues.

Bones were chosen as the research material because the skeleton is the main place of heavy metals (and especially lead) accumulation (Samuels et al. 1989,Keinonen 1992, Skerfving et al. 1993). The turnover of lead in bones is very slow what has severe consequences, especially for women whose skeleton becomes a potential endogenic source of lead which may be liberated during pregnancy. Bones are a good indicator of chronic exposure to heavy metals while blood characterizes the dynamics of exposure changes . Liver, the main place of copper and zinc accumulation, was examined as it is most frequently chosen as the research material, which gives the best possibility for the scientists to compare the results obtained with the results of other authors.

The Upper Silesian population, which was our material, is one of the most endangered by heavy metals in Poland. The risk factors are: densely populated cities with heavy traffic, heavy metals melting factories present in big numbers and high lead and cadmium contamination of soil, water and air. The aim of our study was to examine the influence of Silesian environment on heavy metals concentration in children's bones and livers.

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MATERIALS AND METHODS.

In our work bones and livers of new-born children who died in the Upper Silesian Industrial District and whose mothers lived in that ecologically contaminated area were analyzed. In the investigations of fetus exposure to heavy metals we took into account the fact that placenta may act as a protective barrier between mother and fetus.

77 frontal bones and 77 livers of dead new-born children were examined. The samples were taken during autopsy and stored in polyethylene tubes at -20°C until analysis. The neonate state and pregnancy pathology were well documented for all the samples. Digestions of the samples of bones and livers (0.5 - 1.0 g wet weight, the weighing accuracy was 0.1 mg) were performed using microwave irradiation in a MLS 1200 MEGA microwave digestion system Milestone-Italy with the mixture of HNO_3 and $HClO_4$ (3:2) or - for the determination of Hg - with the mixture of HNO_3 and H_2O_2 (5:2). Suprapure HNO_3, $HClO_4$ and H_2O_2 (Merck, Darmstadt, Germany) were used. In this work determination of ten elements was carried out by atomic absorption spectroscopy and differential pulse voltamperommetry.

Metals concentrations were determined with a Perkin Elmer spectrometer Model 5000 equipped with an EDL lamp for mercury and a Carl Zeiss Jena spectrometer Model AAS-30 with deuterium arc background correction and Lvov's platforms. A type MHS-1 unit for generation of hydrides and mercury vapor program HgIII was applied. The GAAS method was used to measure: Pb, Cr, Ni, Mn, Mo and Hg. Mg was determined by FAAS method. Conditions for mercury CVAAS measurements were as follows: λ=253,7 nm, s=0,7 mm, solution volume - 10 mL, reducer volume - 2,5 mL (10% $SnCl_2$ solution in 2M HCl), temperature of quartz tube 200°C. The concentrations of Cd, Cu and Zn were determined with the use of differential pulse anodic Stripping voltammetry. ECO-TRIBO PC-ETP polarograph was used. Quantitative determination was performed by the use of the internal standard method. Cadmium and zinc concentration measurements were carried out using 0.1M CH_3COONa as the electrolyte. Copper was determined in diluted HNO_3 (pH=1). 1 mg/ml standard solutions of metals (Merck Titrisol) were prepared. Water was deionised and then distilled in a quartz apparatus. Automatic pipettes (Plastomel, Poland) with plastic tips were used. All glassware, plastic tips and autosampler cups were cleaned by soaking for 24 hours in 25% (v/v) HNO_3. After cleaning, all the containers were thoroughly rinsed with water.

Standard Reference Material SRM 1577a (bovine liver) and SRM 1486 (bone meal) were used as the certified reference materials. Normal distribution was tested with the Kołmogorov-Smirnov test: $0.05 < p < 0.10$ (STATISTICA v.5.1 for Windows).

RESULTS AND DISCUSSION.

The results of the analysis of the 77 frontal bones and 77 livers of dead new-born children are shown in Table 1.

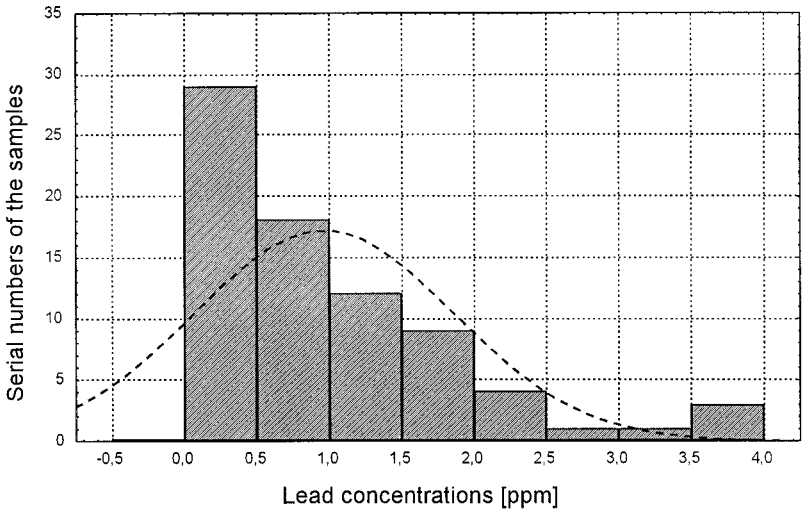

Figure 1. Lead concentration in neonatal bones.

Concentrations of lead in bones is presented in Figure 1. Attention should be paid to the fact that lead was not found in six of the examined bone samples (8% of the samples).

Trace amounts of cadmium were found in only 13% of the examined samples (10 bone samples). Concentration distributions of Cd, Hg and Cr did not correspond with either normal or logarithmic-normal distribution ($p < 0.01$). Concentration distributions of Zn, Cu and Mn (for bones) conformed to logarithmic-normal distribution and for Ni, Mg and Mo the variable distribution was normal and according to the Kołmogorov-Smirnov test $p > 0.20$.

Considering Pb content in the bones and liver samples attention should be paid to the fact that the tissues form deposits of this element early in the fetal period and there is a tendency to accumulate lead in the skeleton - lead content in the bones, on average, is twice as high as the amount of lead found in the liver tissues.

Cadmium occurred only in 5% of the examined liver samples. Similar situation was observed for the bones. These results, in context with our previous research on Cd content in maternal and neonatal (cord) blood (Baranowska 1995), let us assume that placenta acts as an effective barrier against Cd-poisoning of the fetus.

Liver concentration distributions of Pb, Cu, Ni, Mn, Mg and Mo in neonates' livers corresponded with normal distributions, for Zn - with logarithmic-normal distribution and the distribution of Cr concentration did not conform to either of these distributions ($p < 0.05$).

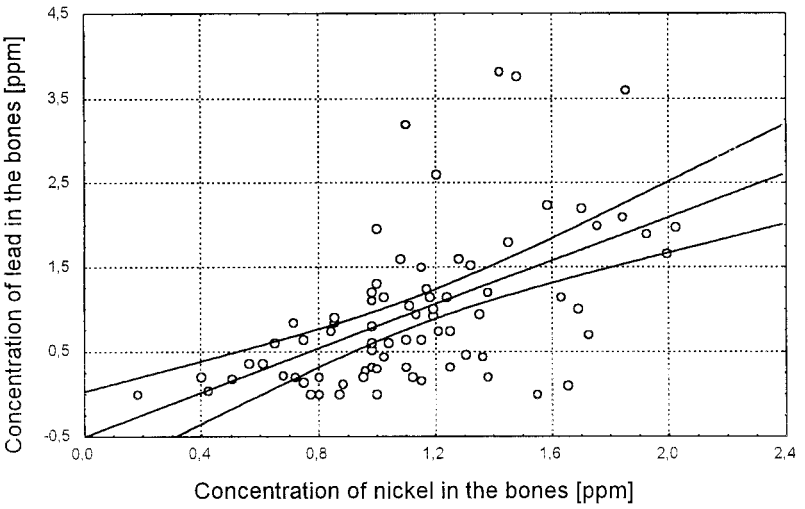

Figure 2. Positive correlation between lead and nickel concentration in the bone samples with 95% confidence intervals. Pearson's R=0.56 $p<0.001$.

The statistical significance of the differences between the average concentrations of metals in neonates' bones and livers is presented in Table 1. Highly statistically significant differences of metal concentrations in livers and bones for 8 examined metals: Pb, Zn, Cu, Cr, Ni, Mn, Mg and Mo were exposed. Pb, Cr, Ni and Mg concentrations were higher in bones than in livers, while livers contained more Mn, Mo, Zn and Cu than bones.

Correlation between concentrations of individual metals in the examined bones and livers as well as correlation between concentrations of different metals in both the kinds of samples were determined. Figure 2 shows an example of a positive correlation between Pb and Ni concentrations in neonates bones. Moreover, in the bones, statistically significant correlations between pairs of metals were observed:
positive for Pb and Cr, Pb and Cu, Zn and Mo and negative for Ni and Mn. Positive correlation between concentrations of two elements may indicate similar source of contamination with these elements or an existence of common, for these metals, activating places in enzymatic or transmission systems in the human body. While negative correlation let us assume an existence of some antagonisms in competision with enzymatic or transmission systems in the human body.
Positive correlation between concentrations of the same metal in both the examined tissues was proven for: Pb, Cr and Ni while concentrations of Cu displayed a negative correlation. Figure 3 presents the correlation between Cr concentrations in the neonates' bones and livers.

So far there has not been publications devoted to the research on the tissue concentrations of metals in dependence on fetal age. In this work relationships

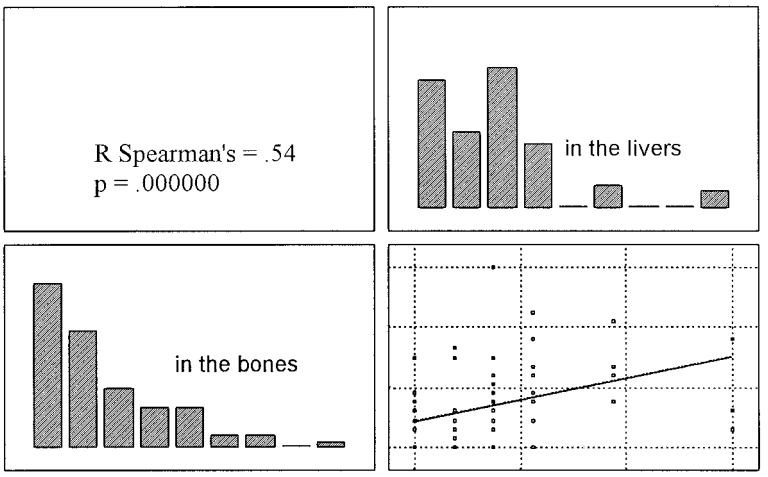

Figure 3. Positive correlation between chromium concentration in the bones and livers samples.

between metals concentrations in bones and livers in dependence on fetal age and pregnancy pathology was determined. It was shown that with the fetus age, the concentrations of examined metals in bones and livers increase.
Statistically significant correlation between the fetal age and metal concentration in bones is shown for Pb (Figure 4). In case of the liver samples such correlations were found for Pb, Zn, Cu and Ni. The exceptions are the concentrations of Mn, Cr, Mg and Mo in both tissues and Cu and Zn in the liver samples which seem to be independent on fetal age. Hg and Cd concentrations in both tissues were too low to define such correlations. In this work the increase of most metals content in tissues within the development of fetal age was shown. From the literature it appears that such a tendency concerns the neonatal age as well.

The comparison of statistical significances of the differences of metals concentrations in dependence on sex was negative.

Statistical analysis conducted with the use of multiple regression method did not display relationships between any of the examined metals and any clinical situation (the kind of delivery, perinatal asphyxia, respiratory distress syndrome, narcotizing enterocolitis, pneumonia, meningitis, infections, intraventricular hemorrhages, anemia, congenital defects). When we considered the corrections related to the fetal age, found correlations lost their significance.

The fact that in cases of severe illnesses in the perinatal period actual contribution of heavy metals was not proven still is not the evidence that the exposure of pregnant

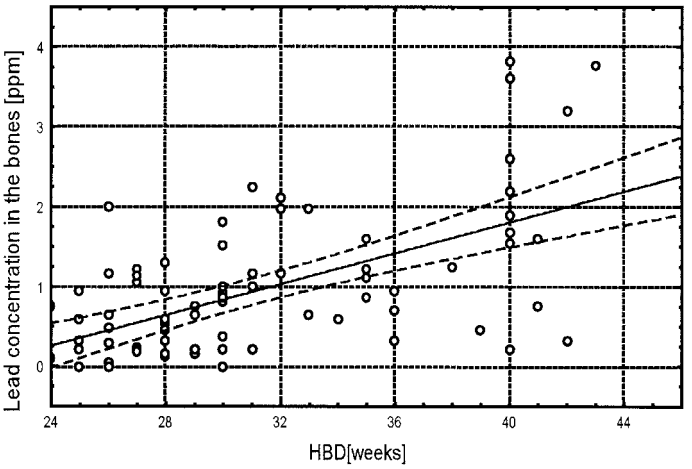

Figure 4. The dependence of bone Pb concentration on fetal age with 95% confidence intervals. R=0.59 $p<<0.05$.

women to these metals is related to low complication risk. As according to the research in the group of older children appears - exposure to even slightly increased concentrations of heavy metals lead to negative neurological effects only after years. Similar situation may occur in cases of intrauterine exposure as well.

Table 1. Statistical significances of the differences between metal concentrations in the bones and in liver samples.

	x (bones) ppm	SD	SEM	x (livers) ppm	SD	SEM	Test
Pb	0.96	0.89	0.10	0.38	0.38	0.040	t
Cd	0.002	0.007	0.0008	0.0			Wilcoxon
Zn	24.82	16.33	1.86	59.10	56.50	6.40	t
							Wilcoxon
Cu	2.11	2.06	0.24	25.82	13.03	1.48	t
							Wilcoxon
Cr	0.045	0.04	0.005	0.018	0.018	0.002	Wilcoxon
Ni	1.13	0.38	0.04	0.20	0.15	0.017	t
Mn	0.51	0.34	0.04	1.32	0.67	0.076	t
							Wilcoxon
Mg	531	12.50	14.30	143	19.31	2.20	t
Hg	0.01	0.0	0.04	0.0			Wilcoxon
Mo	0.35	0.12	0.01	0.67	0.25	0.03	t

n=77, liver and bones concentrations significantly different at $\alpha=0.05$ or better.

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