HUMAN BIO-MONITORING STUDY – TOXIC ELEMENTS IN BLOOD OF WOMEN

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SUMMARY

The article describes bio-monitoring study concerning recent and long-term exposure of Czech women to toxic metals. Blood cadmium, mercury and lead levels were measured in groups of women with various life styles, etc., to have comparable results in the course of a long study period; a strong emphasis was laid on the quality control of the whole process during the study. Higher cadmium level was found in smokers compared to non-smokers, lower mercury level was found in the group of women who never eat fish. A slight increase of blood lead level with age was observed. No significant differences were found between localities in a given period. Our results do not differ from those gained in other European countries.

Key words: bio-monitoring, women, cadmium, mercury, lead

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INTRODUCTION

At the Fifth Intergovernmental Forum on Chemical Safety held in 2006 in Budapest, problems of cadmium, mercury and lead health risks in human populations were discussed (1). National and local human bio-monitoring studies have been recommended in addition to the global ones.

In the Czech Republic, the Human Bio-monitoring Project (CZ – HBM) started as a part of the Environmental Health Monitoring System (EHMS) as far back as in 1994 (2). The aim was to determine the exposure of various groups of the Czech general (occupationally non-exposed) population (adults, children) to environmental contamination. The EHMS project includes eight subsystems influencing the health status of the general population. Significant items, monitored within EHMS were ambient air, drinking water and diet. Details of the organization of CZ – HBM are described elsewhere (3–5)

The first two years of the CZ – HBM sub-project (1994–1995) were devoted to the Pilot study. Some details as sampling methods, choice of experimental conditions and suggestion of QA/QC parameters were verified. On the basis of this Pilot study, a standard operation protocol has been elaborated. Since 1996, CZ – HBM subproject is in full operation.

The first period of bio-monitoring (1996–2003) was limited to the urban areas of Plzeň and Ústí nad Labem (industry, traffic), and Benešov and Žďár nad Sázavou (more rural areas). The results obtained within this sub-project in the years 1996–1998 were published (6–8) as well as the results pertaining to years 2001–2003 were published (3). On the basis of these results, reference values (95th percentile of the measured species in the given matrix) (9) for the Czech population have been defined (3, 10). Data obtained in this period confirmed differences between men and women (11, 12).

In 2005, a second period started. Prague and Ostrava were chosen as localities with traffic and industry, localities Liberec and Zlín as more rural areas. Review of the history, current status and future development of CZ – HBM within the EHMS project and revised reference values were published (13). Rolling results are periodically published in annual reports on the web site www.szu.cz.

From 2006 till 2010, the National Institute of Public Health participated in the large European project PHIME (Public Health Impact of a long-term, low level Mixed Elements in susceptible population – www.phime.org), aimed at toxicology, epidemiology, working and environmental exposure and biochemistry. Altogether thirty one countries from Europe, Africa, Asia and South America participated in this project. One part of this project concerned women exposure (vulnerable group of population) to toxic elements and platinum metals (14). Whole blood was chosen as a matrix reflecting recent exposure.

Women, especially in the fertile age, are considered to be a susceptible group of population more influenced by the environment than men (11, 12). In our paper, the results of blood toxic elements in women from both periods of CZ – HBM were discussed. As the CZ PHIME samples were collected in the same locality (Prague) and under the same conditions as one part of CZ – HBM samples, data of both groups were evaluated together. Special emphasis was given on the compatibility of the results collected over the fourteen year period.

MATERIALS AND METHODS

Sampling

In the first period (1996–2003), altogether 857 and in the second period (2005–2009) 494 women participated in the CZ – HBM project (blood donors, age 18–58 y).

In the EU PHIME project, altogether 50 Czech women, aged 55 to 59 years living in Prague were recruited in 2009.

Both projects have been approved by the ethical committee of the National Institute of Public Health in Prague.

Informed consent has been obtained from all participants; information on age, length of stay in the locality, smoking, diet habits and occupational exposure were recorded in the questionnaire. Since 2005, the consumption of fish and the number of amalgam tooth fillings have also been included.

Summary statistics is presented in Table 1.

A standard operation protocol elaborated for sampling, transport and storage of samples in the Pilot study was respected throughout the study.

For sampling, two types of heparinised tubes designated for the determination of trace elements in whole blood were chosen:

- CZ – HBM project – Sarstedt S-Monovette für die metal-Analytik (sampling in the first period) and Trace Element Tubes, Greiner bio-one (in the second period);
- PHIME: Trace Element Tubes, Greiner bio-one, 2009.

To test the purity of tubes and needles, leaching tests were carried out with 2% nitric acid and/or with the whole blood reference material with known concentration of elements under study (simulation of ligands in blood). Results did not show any significant contamination for Cd, Hg and Pb, and proved the availability of sampling devices in the project.

After blood collection, samples were coded, frozen and stored at −18°C until analysis.

Analytical Procedure

For the determination of Cd and Pb, all blood samples were mineralised with mixture of concentrated $HNO_3 + H_2O_2$ in a microwave oven MEGA 1200 (Milestone) equipped with an evaporation rotor FAM 40 which diminished the possibility of external contamination. After mineralization, the solution was evaporated to the volume of approximately 0.1 mL and diluted by demineralized water to a final volume 5 mL. Measurements were done by the method of atomic absorption spectroscopy (AAS). Perkin-Elmer AAS 4100 ZL atomic absorption spectrometer was used for determination of Cd and Pb (electrothermal atomization technique).

Determination of mercury was carried out in all samples directly without mineralization using a single purpose spectrometer AMA 254 (Advanced Mercury Analyser, Altec Praha, CZ).

Limits of detection (LOD), calculated for original sample as a 3 times standard deviation of blank, were 0.2 (0.3 till 2003) $\mu g.L^{-1}$ for Cd, 3.5 $\mu g.L^{-1}$ for Pb, and 0.05 $\mu g.L^{-1}$ for Hg.

Deionised water (Millipore, 18 $M\Omega.cm^{-1}$), Suprapur purity reagents nitric acid, hydrogen peroxide (Merck) and matrix modifiers for AAS (Merck) were used.

Statistics

All results and sample characteristics (e.g. age and life style) were used for descriptive statistic analyse. Data were processed using Unistat 5.1 and Statistica version 7. Medians of concentration levels were presented (log-normal distribution of results). Kruskal-Wallis test was used for statistical analysis, a probability value <0.05 was regarded as significant. For statistical evaluation, the results below LOD were replaced by a value equal to one-half of the limit of detection – LOD (about 2% of results for cadmium).

QA/QC

To guarantee compatibility of results for a long-term study, strict rules of QA/QC have to be fulfilled. To avoid seasonal changes, sampling has been performed only at spring time (April – June). All blood samples were analysed in the laboratory of the Institute of Public Health by the same operators on the same devices. Laboratory is accredited by the Czech Institute of Accreditation.

All sampling devices (tubes, needles) have been purchased and distributed centrally. Blanks and certified reference materials were used throughout the study (whole blood – Seronorm Trace Elements Whole Blood L-1, L-2); Shewhart's control charts (regulation diagrams) were constructed to assure the stability control of the whole system (Fig. 1–3).

The laboratory participated regularly in Round Robin Tests organized by Ekocentrum Ostrava (EC), IAEA and University of Erlangen; results of these comparisons are summarized in Tables 2 and 3.

RESULTS AND DISCUSSION

- Results obtained for the whole monitored population in the period 1996–2003 have been published previously (3, 5). This paper completed previous results of toxic metals concentration levels in blood of women with results found in the period 2005 to 2009.

Table 1. *Summary statistics of participating women*

	1996	1997	1998	1999	2000	2001	2002	2003	2005	2007	2009	PHIME
N	134	104	81	101	96	112	104	106	170	161	155	50
N smoking	41	34	19	30	37	30	29	26	42	46	34	26
Age avg	32	34	33	32	34	33	32	32	34	34	34	56
Age min	18	18	18	18	18	18	19	18	18	19	18	47
Age max	46	51	55	52	53	54	51	58	56	57	64	59

N - Number of participating women, avg - average

Table 2. *Results of Round Robin tests organized by the University of Erlangen-Nurnberg: G-EQUAS - blood, environmental level; results, tolerance range. (μg.L^{-1})*

Organizer	Cd A	Cd B	Hg A	Hg B	Pb A	Pb B
EQUAS 35; 2005	0.4	1.25	1.12	2.97	56.88	105.3
	0.23–0.51	0.77–1.44	0.76–1.77	1.91–3.61	45.9–64.84	93.59–126.62
EQUAS 37; 2006	0.5	1.50	3.89	6.79	55.3	92.55
	0.23–0.65	1.13–1.82	2.36–5.14	4.83–8.8	43.11–61.65	77.33–104.63
EQUAS 39; 2007	0.9	1.62	2.22	4.75	73.23	129.5
	0.45–0.95	1.06–1.74	1.36–3.09	3.16–5.96	68.66–93.44	117.8–150.86
EQUAS 43; 2009	0.75	1.15	1.05	2.30	53.0	66.1
	0.34–0.76	0.66–1.20	0.61–1.57	1.77–3.45	50.06–65.72	65.21–85.07
EQUAS 46; 2010	0.55	0.94	0.48	2.11	19.73	65.92
	0.27–0.57	0.71–1.19	0.27–0.75	1.47–2.67	17.01–26.55	63.15–85.41

Table 3. *Z-score for elements under study in interlaboratory comparison tests*

Organizer	Cd	Hg	Pb
EC XIV/2-96; 1996	−1.96	1.36	0.07
IAEA 140; 1997	0.6	−0.2	−0.8
EC XIX/2; 2001	−0.744	*	−1.29
IAEA 407; 2003	0.47	1.73	−0.67

* not determined or demanded

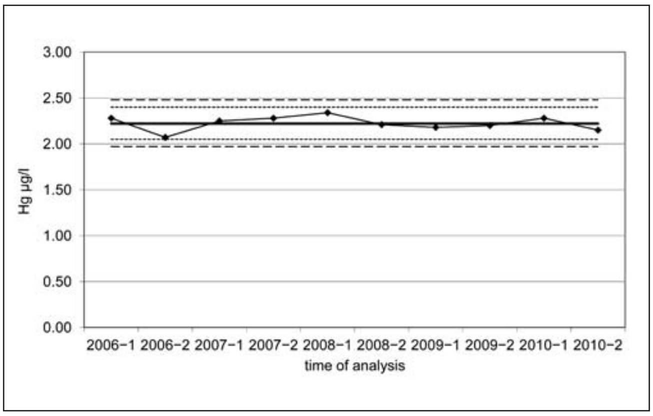

Fig. 2. *Shewhart´s control chart – Hg in blood: Seronorm WB I, period 2006–2010 (There are approximately 6 months intervals between individual points) dashed lines – upper and lower control limits; dotted lines – upper and lower warning lines; full line – center line.*

Cadmium

Puklová et al. described the cadmium exposure pathways (diet, drinking water, ambient air and soil) investigated in the first period 1994−2003 (15).

Results for blood cadmium level in women from the second period (2005–2009) and PHIME project confirm differences

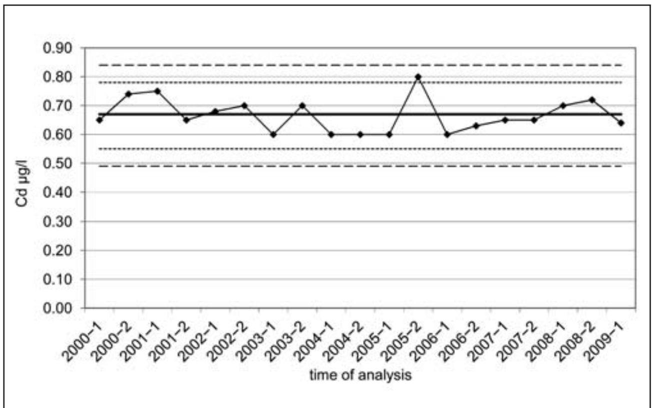

Fig. 1. *Shewhart´s control chart – Cd in blood: Seronorm WB I, period 2000–2009 (There are approximately 6 months intervals between individual points) dashed lines – upper and lower control limits; dotted lines – upper and lower warning lines; full line – center line.*

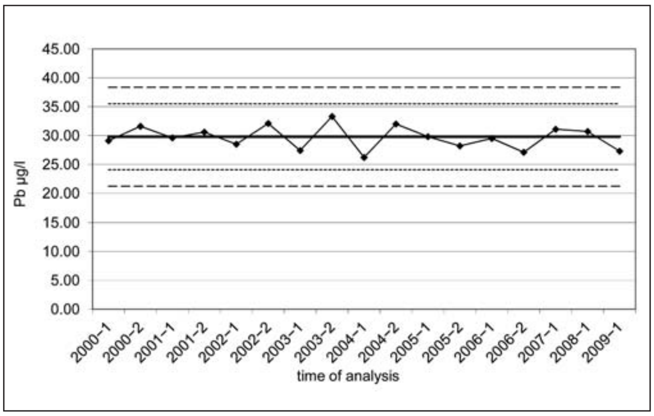

Fig. 3. *Shewhart´s control chart – Pb in blood: Seronorm WB I, period 2000–2009 (There are approximately 6 months intervals between individual points) dashed lines – upper and lower control limits; dotted lines – upper and lower warning lines; full line – center line.*

between smokers and non-smokers. No significant differences were found between men and women, no significant difference was age related.

Decreasing time trends over both periods in blood cadmium level in women can be seen in Fig. 4, influence of women smoking habits in the second period is shown in Fig. 5.

Mercury

The difference between men and women with higher values in women found in the first period (3) was confirmed also in the second period (16). Time trends in blood mercury levels in women over both periods can be seen in Fig. 6.

In the second period and PHIME project, the dependence of the blood mercury level in women versus fish consumption has been found (Fig. 7), no significant difference was age related.

Lead

Lower blood lead levels in women (compared to men) found previously were confirmed also in the second period (17). Decreasing trends in women lead blood levels were found in both periods (Fig. 8). Slight increase (p=0.07) of blood lead level in women with growing age was observed (Fig. 9).

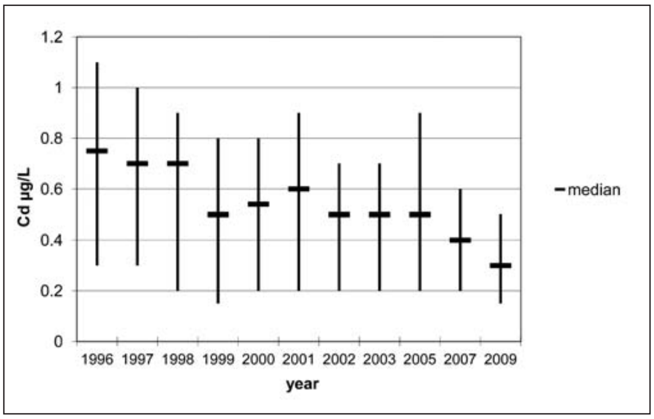

Fig. 4. Time trend of blood cadmium level in women – non smokers.

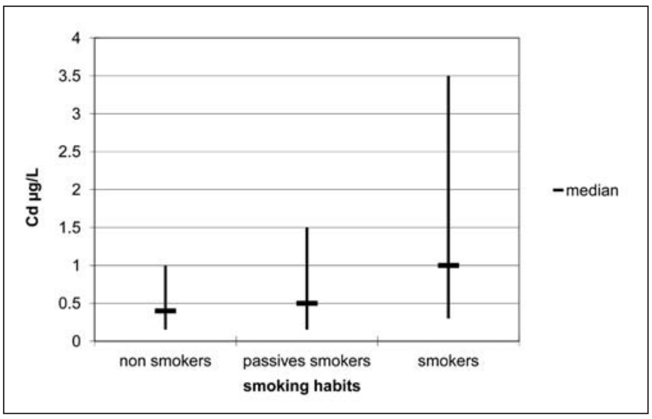

Fig. 5. Influence of smoking habits on Cd blood level in women (2005–2009).

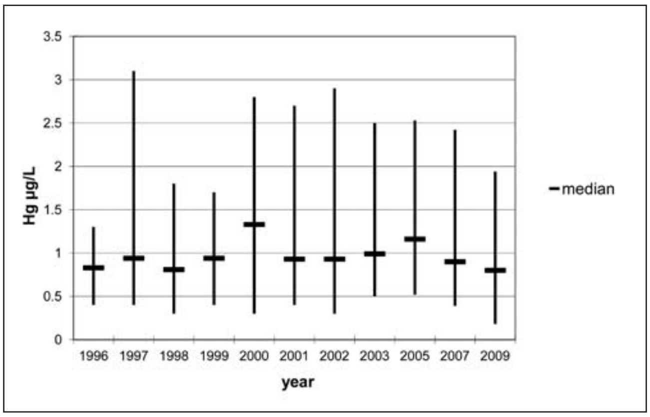

Fig. 6. Time trend of blood mercury level in women.

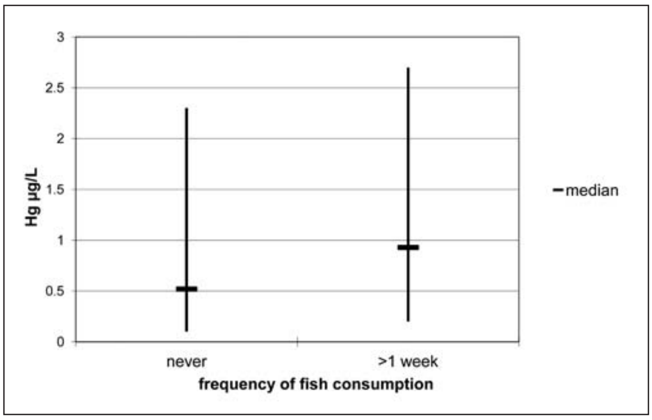

Fig. 7. Influence of fish consumption on Hg blood level in women.

Legend to Fig. 4–7

The lines represent the interval between the 10th to 90th percentile in all cases.

CONCLUSIONS

From the results obtained it follows that:

- The blood cadmium level in women depends strongly on smoking habits. Medians throughout the whole study for women smokers were in the range of 1.1–1.3 µg.L^{-1}, for non-smokers 0.3–0.7 µg.L^{-1}. The time trend (only non-smokers taken into account) showed a slight decrease. About 2% of results were under LOD, 2% of samples exceeded the reference value proposed for the Czech adult population (3µg.L^{-1}) (3).
- The concentration of mercury in blood was significantly higher in women consuming fish at least 2 times a week in comparison to those not consuming fish at all or sporadically (median 1.0 µg/L vs. 0.5 µg.L^{-1}). No significant change was observed in the time trend for the period 1996–2003 (except for the year 2000, when the highest median was found for which no explanation could be offered so far); a slight downward trend was observed in the period 2005–2009. About 0.5% of samples were in the range of 4 to 5 µg.L^{-1} (i.e. higher than proposed Czech reference value (3) 4 µg.L^{-1} but lower than the control value HBM I (5 µg.L^{-1}), defined as a "concentration in the body matrix of a substance bellow which no adverse health effect should be expected") (9, 18). Approximately 2% of results exceeded the HBM I value.

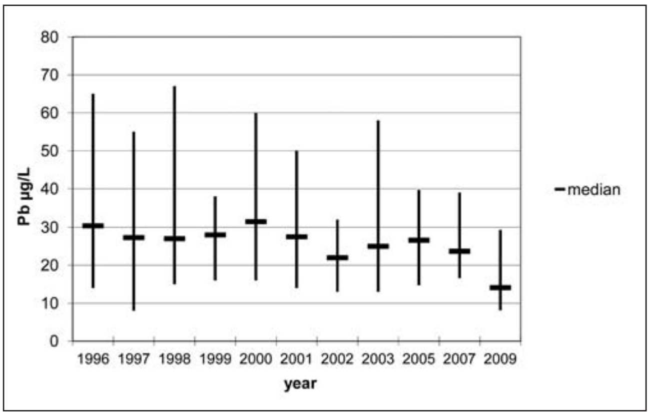

Fig. 8. *Time trend of lead blood level in women.*

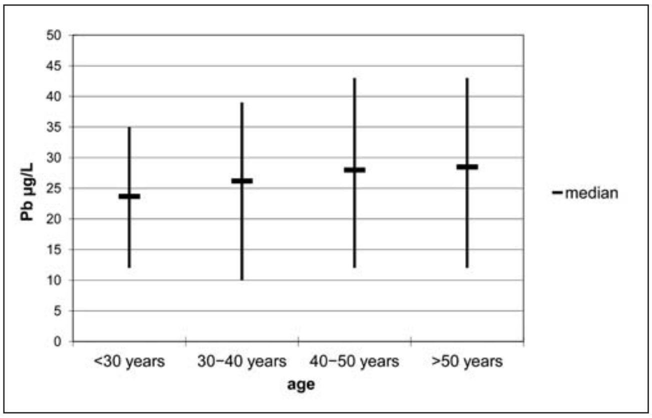

Fig. 9. *Age-related blood lead level.*

Legend to Fig. 8–9
The lines represent the interval between the 10th to 90th percentile in all cases.

- Blood lead concentration in the second period confirmed a significantly decreasing trend in the first period and was positively related with age. About 1.5% of the values exceeded the proposed Czech reference value (3) for the adult women population (65 µg.L^{-1}), 0.8% exceeded HBM I limit for women of age <45 years (100 µg.L^{-1}), and none of the values was higher than 150 µg.L^{-1} (HBM I for men and women of age >45 years).
- Generally, we can conclude that trends and differences found in the CZ HBM system are in agreement with those found in other countries (19, 20).
- Comparison of the results in Czech women population with results of samples from different European and non-European countries analysed in 2009 during the PHIME project will be also published elsewhere (14).

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