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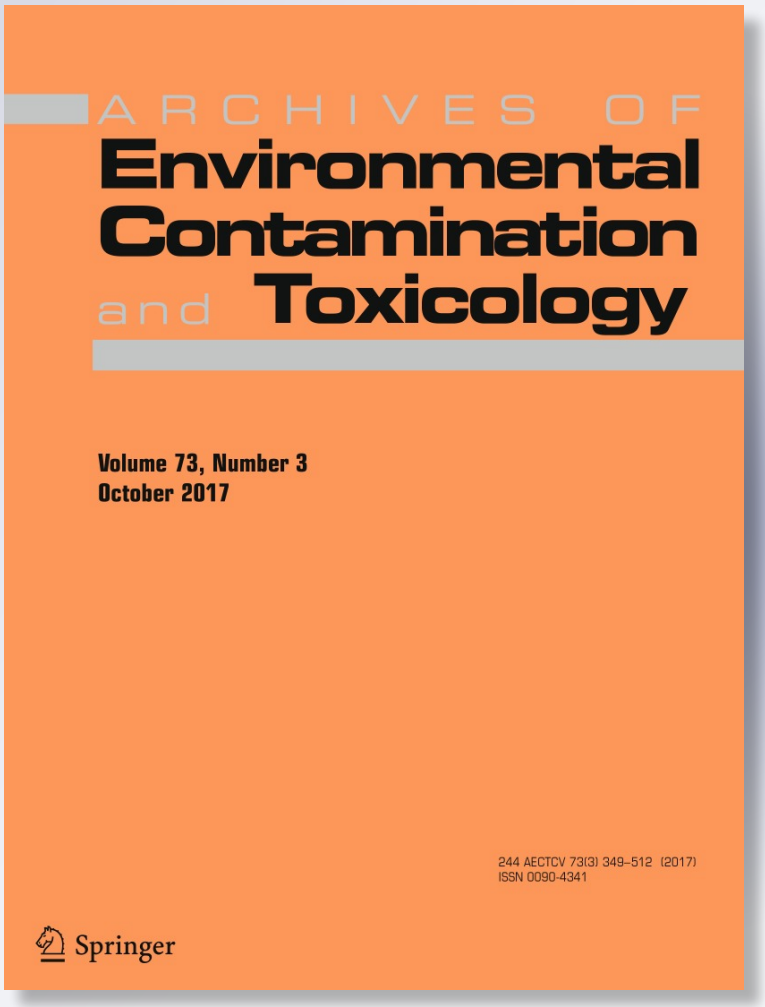

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Urinary Cadmium and Cotinine Levels and Hair Mercury Levels in Czech Children and Their Mothers Within the Framework of the COPHES/DEMOCOPHES Projects

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Abstract The COPHES/DEMOCOPHES twin project was performed in 2011–2012 in 17 European countries to harmonize all steps of the human biomonitoring survey. Urinary cadmium, cotinine, phthalate metabolites, and hair mercury were measured in children ($N = 120$, 6–11 years) and their mothers of reproductive age, living in urban or rural areas. Cadmium in mothers' and children's urine was detected at a geometric mean (GM) concentration 0.227 and 0.109 μg/L, respectively; 95th percentile (P95) was 0.655 and 0.280 μg/L in mothers and children, respectively. No age-related, education-related, or urban versus rural differences were observed within the frame of each population group. Cadmium urinary level in mothers was about twofold compared with children. Higher levels were obtained in all smoking mothers but not in occasionally smoking or mothers and children exposed to environmental tobacco smoke (ETS). Mercury values in mothers were significantly higher in urban than in rural populations but not in children. GM and P95 for mercury in children's hair were 0.098 and 0.439 μg/g and in mothers' hair were 0.155 and 0.570 μg/g. Concentrations for mercury in the Czech samples were lower than European average. Hair mercury increased significantly with consumption of fish or seafood and with number of amalgam tooth fillings (in children). A positive association was found with family educational level. No influence of age was observed. Urinary cadmium and hair mercury levels were lower than health-based guidelines with one exception. High levels of urinary cotinine were found in the 12 smoking mothers (GM approximately 500 μg/L); lower levels in occasionally smoking mothers, $N = 11$ (34.5 μg/L). The mean cotinine levels in nonsmoking mothers who reported daily exposure to ETS was 10.7 μg/L. A similar mean value (10.8 μg/L) was obtained in six children who had daily exposure to ETS. In children without exposure to ETS, the mean cotinine level was 1.39 μg/L urine. Cotinine in the urine of children demonstrates limited protection of the Czech children against exposure to ETS.

Human biomonitoring (HBM) is a well-established method that has been used in the past decade for measuring human exposure to environmental chemicals (Angerer et al. 2011). HBM data that integrate all routes of exposure provide important information about overall exposure, which can be used to guide public health policy and provide important information to public health authorities for enforcement of preventive measures.

HBM has long been used in occupational health as part of preventive strategy to monitor and regulate workplace exposure according to the Biological Exposure Indices (BEI) (Bardodě j et al. 1987; Bernard and Lauwerys 1986). In the mid 1990s, this approach began to be applied more often to monitor and verify exposure of the general population in many countries (Becker et al. 2002; CDC 2001; Ewers et al. 1999; Fiolet et al. 1999), including the Czech Republic (Cerná et al. 1997).

Worldwide increased interest in HBM and its gradual implementation in several European countries has led to its inclusion in the European Environment and Action Plan in the period 2004–2010 (EU Commission 2004). One of the objectives of this plan was to develop a coherent approach

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to HBM in Europe. This event highlighted the importance of HBM and its relevance for the strategy of environment and health; this was followed by initiation and implementation of a project that coordinates HBM activities in European countries. In cooperation with experts from various European countries the ESBIO project was started (Expert team to Support BIOmonitoring). Its results then allowed the creation of the twin COPHES/DEMOCOPHES projects (Casteleyn et al. 2007; Joas et al. 2012).

For implementation of HBM and comparison of results among countries and continents, unification of methodology steps becomes extremely important. Therefore, an international group of experts, associated with the COPHES project, prepared a set of Standard Operational Procedures (SOP) covering all steps necessary to perform HBM (i.e., recruitment of volunteers, sampling of human body fluids and tissues, handling of samples, chemical analysis of biomarkers relevant to environmental exposure to toxic pollutants, QAQC, statistical analysis of data, interpretation of results, and their vertical and horizontal communications). These SOPs were then used in the DEMOCOPHES pilot study with the goal to demonstrate the practical application of harmonized protocols (SOPs) in the 17 participating European countries (Joas et al. 2012). Comparable data were produced on the distribution of biomarkers of selected environmental pollutants, namely mercury in hair, urinary cadmium, cotinine, and phthalate metabolites, including urinary creatinine as a measure for urine dilution.

Summary results of the whole project showed that considerable differences in exposure to selected agents were found among the participating countries (Den Hond et al. 2015). Because the Czech Republic has considerable experience with HBM as a standard part of the nationwide Environmental Health Monitoring System (Cerná et al. 1997; Cerná et al. 2016a, 2016b), we decided to publish the results obtained in the Czech Republic within the DEMOCOPHES project separately as a comparison with already existing Czech data concerning urinary cadmium and mercury in hair.

Materials and Methods

Study Population

Children aged 6–11 years and their mothers, of reproductive age, were selected as representatives of vulnerable populations. Details and rationale for the whole study were reported by Becker et al. (2014). Most countries participating in the survey, including the Czech population, enrolled 120 children and their mothers (60 from urban and 60 from rural areas), which had lived for at least 5 years in the sampling location. Participants were selected based on inclusion/exclusion criteria established for all participating countries (Den Hond et al. 2015). Recruitment was organized through public schools between September 2011 and February 2012. The urban and rural areas were chosen based on population density. The participants defined as the urban population group were selected from four schools in the Capital City of Prague (average population density approximately 2500/km^2), whereas the half defined as the rural population group (population density approximately 170/km^2) was selected from four schools in small towns in the Liberec region of North Bohemia.

The study protocol was approved by the ethics committee of the National Institute of Public Health. All participating mothers gave informed, written consent and received written information regarding the purpose of the study.

Sampling Procedures

Sampling and handling procedures were performed according to unified SOP developed by the COPHES consortium (Becker et al. 2014). Each mother and child collected their first morning urine sample in an appropriate polypropylene vessel previously rinsed with 10% HNO_3, according to written instructions obtained from one of the study fieldworkers. On the day of the urine sampling, a fieldworker met the mother/child pair in their home or alternatively at a prearranged location (usually at school) for a face-to-face interview and hair collection. Two hair strands of approximately 0.2 g were cut close to the head surface from sites in the occipital region using stainless-steel scissors. The hair strands were stored in paper envelopes and transported, under defined conditions, along with urine samples to the analytical laboratory.

The basic standardized questionnaire developed by the COPHES consortium (Becker et al. 2014) consisted of several modules oriented on the residential environment and character of residence, dietary habits (consumption of food commodities potentially associated with exposure), lifestyle, smoking habits and exposure-relevant behavior, the mother's occupation, and the socioeconomic status of the family (see Table 2 in Fiddicke et al. 2015).

Analytical Procedures

All samples from this study were analyzed in the laboratories of the National Institute of Public Health in Prague. The laboratories successfully participated in External Quality Assessment Schemes (EQUAS) and in Interlaboratory Comparison Investigation (ICI) provided by the COPHES Quality Assurance Unit (Schindler et al. 2014). A brief description of the analytical methods follows.

The hair samples used for mercury analysis were washed using the procedure, recommended by WHO/IAEA (i.e., acetone, 3 times demineralized water, acetone), dried, and cut with stainless-steel scissors into pieces <5 mm. Total mercury concentration was determined directly without mineralization: approximately 5 mg of sample was weighed in the boat of an AMA 254 analyzer (Altec), dried, combusted, and decomposed in a stream of oxygen on a catalytic column. After quantitative mercury trapping on the surface of a gold amalgamator, the mercury was completely evaporated at 900 °C into an optical cell and measured at 253.7 nm. Reference material CRM GBW 07601 (reference value 0.36 ± 0.08 μg Hg/g) was used for internal quality control (Čejchanová et al. 2008).

For urinary cadmium analysis, urine samples were diluted tenfold using 1% (v/v) solution of nitric acid. Total cadmium concentrations in urine samples were determined using an inductively coupled plasma mass spectrometer (ICP-MS Elan DRC-e, Perkin Elmer). The concentrations of cadmium in the urine samples were obtained from an external calibration procedure in the range of 0.1–5.0 μg/L. Indium, used as the internal standard, was added to all samples and standards (the total concentration in samples was 10 μg In/L cadmium isotope $^{111}Cd^{+}$ was used for measurement; interference from molybdenum oxide ($^{95}Mo^{16}O^{+}$) was monitored and corrected. Reference material Seronorm Trace Element Urine LOT 0511545 (reference value 4.6 μg/L, $U = 0.4$ μg/L; Sero, Norway) was used for internal quality control).

Urinary cotinine, a metabolite of nicotine, is a commonly used biomarker of active and passive exposure to tobacco smoke. It has a half-life of 16–20 h and therefore reflects tobacco smoke exposure within the past 2–3 days. The biomarker was first monitored in the Czech population with the goal to assess the population's exposure to ETS.

Urinary cotinine was determined following extraction from urine using a gas chromatographic–mass spectrometric method (GC/MS) according to Angerer (Angerer and Schaller 2001) with slight modifications. Urine (2 mL) was spiked with cotinine-d3 as an internal standard, made alkaline with sodium hydroxide, and extracted with dichloromethane. The extract was dried with anhydrous sodium sulphate and the solvent was evaporated to dryness. The residue was dissolved in toluene and analyzed on a DB-5 MS fused silica capillary column (30 m × 0.25 mm × 0.25 μm) at a temperature gradient to 280 °C. The electron impact (EI) ionization detector was set at *m*/*z* values 98 and 101, respectively, for cotinine and cotinine-d3 quantitation.

Urinary creatinine was determined using the HPLC with UV detection (Schneiderka et al. 1993). Urine was diluted with water 1:100 and analyzed (10 μL) on a HEMA Bio 1000 SB Tessek 3 × 150 mm column heated at 30 °C. The mobile phase was 0.016 M ammonium carbonate (pH 8.8–9.1) pumped at 0.4 mL/min. UV detector was set at 234 nm. External calibration was performed using a standard reference, i.e., NIST 914a Creatinine.

Statistics

Statistical analyses were performed using SPSS (version 16.0, Inc., Chicago, IL) and Stata (release 14.1. StataCorp LP, College Station). Levels below the limit of quantification (LOQ) were replaced by half the LOQ value. Urine samples from participants with urinary creatinine content below 300 mg/L or above 3000 mg/L were excluded (WHO 1996). Urinary results are primarily expressed in μg/L of urine and as creatinine-corrected in μg/g of creatinine. Mercury was expressed in μg/g of hair.

Descriptive characteristics included geometric means (GM) and 95% confidence intervals (CI). Between-group comparisons were based on the two-sample *t* test and analysis of variance models applied to natural logarithm transformed data. In case of significant results, Sidak's multiple comparison procedure was used for pairwise comparisons. The degree of association between two continuous variables was measured using Spearman's rank correlation coefficient (*r*). All statistical tests were evaluated as two-sided at a significance level of 0.05.

Results

Demographic characteristics of the population under study are presented in Table 1. Three mothers were excluded from urine analyses, because their urinary creatinine levels did not meet the WHO inclusion criteria. Data for urinary biomarkers were available for 117 mothers (59 from urban and 58 from rural areas) and all 120 children. Hair was analyzed complete for all participants.

The concentrations of mercury in hair, and cadmium and cotinine in urine, for mothers and their children are shown in Table 2 as geometric means and 95% confidence intervals. Cadmium was positively detected (above LOQ = 0.05 μg/L) in 99.1% of mothers ($N = 116$) and 91.7% of children ($N = 110$). The mean cadmium concentration in children was approximately half that of mothers. No differences between urban and rural areas were observed in children or mothers. Also, no age-related differences in urinary cadmium levels were observed in mothers or children; levels of children were not influenced by education levels of mothers.

According to the questionnaire, only 12 mothers were active (i.e., daily) smokers. In their urine, cadmium levels were significantly higher (GM 0.36 μg/L, $p = 0.010$) compared with the pool of occasional smokers, former

Table 1 Characteristics of Czech DEMOCOPHES study population, 2011–2012

	Children			Mothers		
	Urban	Rural	All	Urban	Rural	All
No. of participants	60	60	120	60	60	120
Age (year), mean (SD)	8.4 (1.8)	8.5 (1.7)	8.4 (1.8)	38.6 (3.2)	36.5 (3.6)	37.5 (3.6)
Sex, *n* (%)				–	–	–
Male	29 (48.3)	30 (50.0)	59 (49.2)			
Female	31 (51.7)	30 (50.0)	61 (50.8)			
BMI (body mass index, kg/m^2), mean (SD)	16.2 (2.1)	16.2 (2.3)	16.2 (2.2)	23.2 (3.8)	24.1 (3.9)	23.7 (3.9)
Consumption of fish (all types), *n* (%)						
Several times per week	5 (8.3)	3 (5.0)	8 (6.7)	10 (16.7)	5 (8.3)	15 (12.5)
Once per week or less	55 (91.7)	57 (95.0)	112 (93.3)	50 (83.3)	55 (91.7)	105 (87.5)
Daily and occasionally smokers, *n* (%)	0	0	0	12 (20.0)	12 (20.0)	24 (20.0)
Education, *n* (%)	–	–	–			
Primary education (ISCED 0-2)				1 (1.7)	4 (6.6)	5 (4.2)
Secondary education or postsecondary nontertiary education (ISCED 3-4)				24 (40.0)	37 (61.7)	61 (50.8)
Tertiary education (ISCED 5-6)				35 (58.3)	19 (31.7)	54 (45.0)

smokers, and mothers who had never smoked (GM 0.21 µg/L; Table 3). The differences remained significant after correction for urine density with creatinine. Similar cadmium concentrations were observed in former smokers and mothers who had never smoked but had daily exposure to ETS at home (0.23 µg/L) or elsewhere (0.21 µg/L). In children, there were no substantial differences in creatinine-corrected cadmium levels among the three groups according to ETS exposure at home (Table 4; $p = 0.328$).

The individual urinary cadmium levels of the mothers and children correlated moderately ($r = 0.370$, $p < 0.001$) according to the Spearman correlation coefficient, but only when the values were creatinine corrected. Concentrations of urinary cadmium in group of mothers consuming food from local sources several times a week (GM 0.23 µg/g creatinine) were higher compared with those consuming this type of food once per week or less (GM = 0.19 µg/g creatinine) and showed significant differences for creatinine-corrected cadmium levels ($p = 0.024$).

Comparing urinary cadmium levels with the health-related limits, concentrations in the Czech group did not exceed the health-related limits, e.g., 1 µg/L in mothers and 0.5 µg/L in children (Schulz et al. 2012) with the exception of one boy from an urban area (i.e., Prague) who marginally exceeded the upper urine limit (i.e., 0.59 vs. 0.50 µg/L).

In all hair samples, the mercury levels were above the LOQ (0.014 µg/g). The results (geometric means and 95% CI) are presented in Table 2. Mercury values were higher in the urban population compared with the rural population. The difference was significant in mothers ($p < 0.001$) but not significant in children ($p = 0.078$). GM in mothers was approximately 1.6 times (1.8 in urban and 1.4 in rural areas) higher than in children.

No association was detected between mercury levels and any of the parameters related to exposure registered in the questionnaire except for a positive relationship relative to fish consumption in mothers ($p < 0.001$) and in children ($p < 0.033$) and with number of amalgam teeth fillings in children ($p = 0.043$). Hair mercury content was positively associated with family educational levels for both rural and urban mothers ($p = 0.001$) and children ($p = 0.042$). No age-related influence on hair mercury content in children or mothers was observed.

The levels in the hair of all participants were below the health-based limits of 2.3 µg/g as recommended by JECFA (2004). The mercury levels between mothers and children significantly correlated (Spearman's $r = 0.466$, $p < 0.001$).

The levels of urinary cotinine, in all samples from both population strata, were above the LOQ (0.3 µg/L). The mean urinary cotinine levels in mothers and children are presented in Table 2.

As expected, significantly higher urinary cotinine levels ($p < 0.001$) were found in group of daily smoking mothers ($N = 12$) with a GM of approximately 500 µg of cotinine per L of urine (Table 3). Cotinine concentrations decreased in descending order in 11 occasional smokers (34.5 µg/L), 18 former smokers (2.58 µg/L), and 76 who had never smoked (1.41 µg/L; Table 3). The mean cotinine level in nonsmoking mothers who reported on the questionnaire daily exposure to ETS was approximately 10.7 µg/L. This urinary cotinine level is approximately one-third the values

Table 2 Concentrations of mercury in hair, and cotinine and cadmium in urine from mothers and children in DEMOCOPHES, 2011–2012 in the Czech Republic ($N = 120$ mothers and 120 children) and in all DEMOCOPHES countries

	LOQ	% <LOQ	Czech Republic				All participating countries (Den Hond et al. 2015)
			Urban	Rural	p value*	All	
Children							
Mercury (μg/g hair)	0.014	0.0	0.111	0.086	0.078	0.098	0.145
GM (95% CI)			(0.094, 0.132)	(0.069, 0.109)		(0.083, 0.116)	(0.139, 0.151)
Cadmium (μg/L urine)	0.05	8.3	0.112	0.106	0.668	0.109	0.071
			(0.094, 0.134)	(0.091, 0.124)		(0.096, 0.124)	(0.069, 0.074)
Cadmium (μg/g creatinine)	0.05	8.3	0.112	0.110	0.840	0.111	0.070
			(0.095, 0.133)	(0.097, 0.125)		(0.098, 0.126)	(0.067, 0.072)
Cotinine (μg/L urine)	0.3	0.0	1.530	1.641	0.615	1.585	0.797
			(1.321, 1.773)	(1.306, 2.062)		(1.303, 1.828)	(0.759, 0.837)
Cotinine (μg/g creatinine)	0.3	0.0	1.536	1.699	0.504	1.615	0.774
			(1.271, 1.857)	(1.357, 2.127)		(1.315, 1.984)	(0.736, 0.815)
Mothers							
Mercury (μg/g hair)	0.014	0.0	0.202	0.120	<0.001	0.155	0.225
			(0.172, 0.237)	(0.101, 0.142)		(0.132, 0.182)	(0.216, 0.234)
Cadmium (μg/L urine)	0.05	0.9	0.229	0.225	0.889	0.227	0.219
			(0.194, 0.270)	(0.187, 0.270)		(0.196, 0.263)	(0.211, 0.228)
Cadmium (μg/g creatinine)	0.05	0.9	0.206	0.221	0.411	0.213	0.196
			(0.185, 0.230)	(0.195, 0.250)		(0.189, 0.242)	(0.189, 0.202)
Cotinine (μg/L urine)	0.3	0.0	2.40	6.08	0.023	3.80	2.75
			(1.55, 3.71)	(3.14, 11.78)		(2.54, 5.69)	(2.41, 3.14)
Cotinine (μg/g creatinine)	0.3	0.0	2.16	5.98	0.012	3.58	2.45
			(1.37, 3.40)	(3.15, 11.35)		(2.40, 5.34)	(2.14, 2.80)

Results are presented as geometric means and 95% confidence intervals (in parenthesis)

LOQ level of quantification

* p value for comparison between urban and rural regions of the Czech Republic

seen in women who reported being occasional smokers. Significant differences were seen between urban and rural areas with higher values in mothers living in rural compared with urban areas ($p = 0.023$). Urinary cotinine declined with increased education level in both mothers and children. The highest cotinine values were observed in families with primary or lower secondary educations.

Levels of cotinine in mothers and children expressed both in μg/L and μg/g creatinine correlated significantly (Spearman's $r = 0.568$ and 0.547, $p < 0.001$); however, cotinine levels in most mothers and in all children, were very low (Fig. 1).

All children were assumed to be nonsmokers, which was based on reports by their mothers on the questionnaire and confirmed by analysis of urinary cotinine levels. Unlike mothers, urban and rural children showed no significant difference in exposure to ETS. Substantial daily exposure to ETS at home resulted in similar cotinine concentrations in children as that seen in mothers having daily exposure to ETS (10.8 μg/L). Although only 6 of 120 children could be included in this group (Table 4), the difference from other children was statistically significant ($p < 0.001$). Another six children who were exposed to ETS, but less than daily exposure, also showed a tendency toward increased cotinine levels compared with children never exposed to ETS ($p = 0.086$). Creatinine-corrected cotinine means differed significantly between these two groups ($p = 0.021$). However, the literature cutoff value of 50 μg/L (Jarvis et al. 1987) was not exceeded except for one boy from a rural area with a slightly higher concentration of 53.7 μg/L. The reason for this outlier value could not be determined.

Discussion

The present manuscript reports the Czech results obtained from the DEMOCOPHES project for cadmium and mercury. These biomarkers have long been included in the national survey (CZ-HBM), which has been in operation since 1994 (Cerná et al. 1997), and therefore, the DEMOCOPHES results extend long-term monitoring.

Table 3 Concentrations of cotinine and cadmium in urine from mothers, stratified by smoking status

Smoking status	Mothers, *N* (%)	Cotinine (μg/L)	Cotinine (μg/g creatinine)	Cadmium (μg/L)	Cadmium (μg/g creatinine)
Daily smoker	12 (10.3)	489.15	396.84	0.36	0.29
		(127.78, 1872.44)	(98.49, 1599.00)	(0.30, 0.44)	(0.22, 0.39)
Occasional smoker	11 (9.4)	34.50	29.91	0.22	0.19
		(6.35, 187.55)	(5.90, 151.57)	(0.15, 0.33)	(0.16, 0.24)
Former smoker	18 (15.4)	2.58	2.46	0.21	0.20
		(1.51, 4.39)	(1.41, 4.27)	(0.16, 0.28)	(0.17, 0.24)
Nonsmoker	76 (65.0)	1.41	1.37	0.21	0.21
		(1.23, 1.61)	(1.14, 1.63)	(0.18, 0.25)	(0.19, 0.23)

Results are presented as geometric means and 95% confidence intervals (in parenthesis)

Table 4 Concentrations of cotinine and cadmium in urine from children, stratified by environmental tobacco smoke exposure at home

ETS exposure at home	Children, *N* (%)	Cotinine (μg/L)	Cotinine (μg/g creatinine)	Cadmium (μg/L)	Cadmium (μg/g creatinine)
Daily	6 (5.0)	10.77	7.83	0.180	0.131
		(3.27, 35.45)	(2.55, 24.04)	(0.110, 0.294)	(0.071, 0.242)
Less than daily	6 (5.0)	2.44	3.26	0.061	0.081
		(1.26, 4.71)	(1.54, 6.90)	(0.028, 0.131)	(0.045, 0.148)
Never	108 (90.0)	1.39	1.42	0.110	0.112
		(1.25, 1.55)	(1.25, 1.63)	(0.097, 0.124)	(0.100, 0.125)

Results are presented as geometric means and 95% confidence intervals (in parenthesis)

ETS environmental tobacco smoke

Urinary Cadmium

Cadmium is an environmental pollutant known to accumulate in kidneys where it exhibits chronic adverse effects (Åkesson et al. 2005; Satarug et al. 2017). Therefore, the urinary cadmium has been and continues to be a reliable biomarker of long-term cadmium exposure reflecting cumulative exposure and Cd body burden with the main deposition in kidney as the target organ for toxicity (Horton et al. 2013). Very recently, an overview of human biomonitoring studies of trace elements including cadmium in urine was published by Waseem and Arshad (2016). Environmental exposure occurs mainly from dietary intake and tobacco smoke. According to the Czech Environmental Health Monitoring System, dietary exposure of an average Czech adult citizen to cadmium represent approximately 43 percent of the Tolerable Weekly Intake (TWI) 2.5 μg/kg b.w. (Cerná et al. 2016a, 2016b; Ruprich et al. 1993). TWI corresponds to a value of 1 μg/g creatinine (Berglund et al. 2015). In the DEMOCOPHES study, exposure of the Czech population to cadmium based on urinary levels has been shown to be significantly higher than the European average, especially in children (Den Hond et al. 2015). The higher exposure levels of urinary cadmium also are apparent from previous results obtained for the Czech population. The reasons are not known; higher concentration of cadmium in soil and exposure to ETS are a matter of speculation. Urinary cadmium level (GM and P95) in

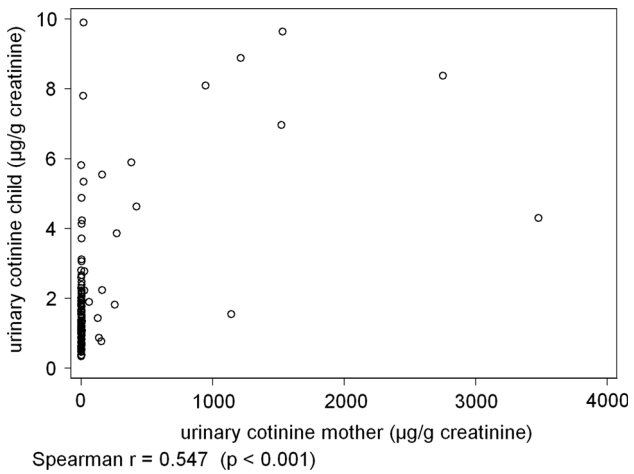

Fig. 1 Relationship of urinary cotinine levels (in μg/g creatinine) between mothers and their children

adult blood donors ($N = 1192$) sampled from 1996–2000 was 0.38 and 2.43 μg/g creatinine. In children ($N = 2008$) aged approximately 10 years, who were sampled and analyzed in the same period, the GM and P95 of cadmium concentrations were 0.32 and 1.52 μg/g creatinine. In both groups (adults and children), slightly, but nonsignificantly higher values were seen in females compared with males (Beneš et al. 2002). Urinary cadmium levels in adults from 2001 to 2003 (GM and P95) were 0.29 and 1.29 μg/g creatinine with significantly higher levels in women than in men ($p < 0.05$) (Batáriová et al. 2006). In our last monitoring period, using urine from adults blood donors (2015, $N = 234$) the GM and P95 was 0.219 g/L and 0.740 μg/L (0.211 and 0.613 μg/g creatinine, females/males, respectively) (Cerná et al. 2016a, 2016b). These values are consistent with the results obtained from Czech mothers in the DEMOCOPHES project. It seems that the exposure of the Czech population to Cd has trended slightly toward lower levels in the past decade.

The urinary cadmium levels did not exceed health-based guidance values HBM-I and HBM II derived by the German Human Biomonitoring Commission based on toxicological and epidemiological data (Schulz et al. 2012). HBM-I values for cadmium in urine of 0.5 μg/L for children and 1.0 μg/L for adults represent the concentration below which, based on the current state of knowledge, there is no risk of adverse health effects (Apel et al. 2016; Schulz et al. 2012) Biomonitoring equivalents (BEs) have similar meaning (Hays et al. 2008). Similar results as in this study were found in Spain's adult population, which was sampled in 2009–2010 with GM = 0.28 and 0.20 μg/g creatinine (Lopez-Herranz et al. 2016) with significantly higher values in females versus males. Higher concentrations of cadmium in the urine of women compared with men are a common finding caused by enhanced gastrointestinal resorption due to low body iron store status. Similar results were obtained in our previous Czech-HBM studies (Batáriová et al. 2006). However, an undeniable fact was that the results of cadmium in the urine of Czech children, in the DEMOCOPHES project, were above the European average (Berglund et al. 2015; Den Hond et al. 2015). It is known that even low-level cadmium exposure represents a public health concern. According to IARC, cadmium is category 1 human carcinogen as well as a known nephrotoxic agent capable of causing rapid kidney damage in the general population even at low urinary Cd levels (Åkesson et al. 2005; Satarug et al. 2017). Therefore, preventive measures are necessary to lower the Cd exposure in the Czech population.

Mercury in Hair

Human hair is considered a suitable matrix for biomonitoring exposure to several contaminants from the environment (Bencko 1995). Mercury concentration in hair is the preferred biomarker for evaluating Hg exposure over extended periods of time. Currently, as well as previously obtained results have confirmed that the Czech population mercury burden is low and does not pose a significant health risk. Results from the DEMOCOPHES study simply confirmed this fact, which is probably related to the relatively low fish consumption (approximately 5 kg per person per year) in the Czech population, unlike coastal countries with higher consumption of fish and simultaneously higher mercury loads (Castaño et al. 2015). Previous studies, oriented on the Czech population burden of mercury, showed that exposure to mercury did not present a health problem for the Czech population. In the CZ-HBM monitoring period 1994–2001 (Beneš et al. 2002), mercury concentrations were measured in the hair of children with an average age of 9.9 years (similar to the age of the children in DEMOCOPHES study). The hair samples were analyzed in 1741 boys and 1815 girls. In addition, mercury concentrations were measured in the blood and urine of the same children. In the children's hair samples, the median value was 0.19 μg/g and the 95th percentile value was below the 1 μg/g, which is considered safe (U.S. EPA 2001). From 2002 to 2006 the median values of mercury in children's hair was successively 0.20, 0.14, and 0.13 μg/g with a slight increase in 2006 to 0.18 μg/g (Puklová et al. 2010). In nonoccupationally exposed adults ($N = 60$), the median and 90th percentile values of mercury in hair were 0.33 and 1.00 μg/g, respectively (Wranová et al. 2009). This means that total hair mercury levels of Czech children are relatively low and the results do not represent a substantial exposure problem to mercury for the general population. Increased concentrations, 0.51 and 4.17 μg/g (median and 90th percentile) were observed in occupationally exposed dentists ($N = 35$). However, the regulation of mercury content in the environment and reducing exposure to mercury in the population is an important measure for preventing developmental neurotoxicity, which brings economic benefits associated with higher IQ levels in the general population (Bellanger et al. 2013).

Urinary Cotinine

ETS is an important environmental toxicant that is mostly found indoors, but it also can occur in outdoor environments. It is a complex mixture of different chemical substances with various and serious adverse effects, including being carcinogenic. Therefore, exposure to ETS can cause serious diseases, particularly in children. Passive smoking in this vulnerable population group has been associated with respiratory illnesses, decreased lung function, asthma, and other health problems (CDC 2006). Safe levels of exposure are not known.

Smoking habits as well as exposure to ETS are most commonly assessed using questionnaires, but the answers of participants can be modified by the subjective attitude of the respondents. Urinary cotinine levels can objectively differentiate between active smokers, passive smokers, and nonsmokers.

Cotinine was monitored in the urine of the Czech population for the first time as part of the DEMOCOPHES study. However, even results obtained in the late 1990s in association with the Teplice Program have highlighted problems associated with high exposure of the Czech children living in polluted areas to ETS (Dostál et al. 2008). Results obtained from 17 participating countries in children of the COPHES/DEMOCOPHES project showed marked differences in urinary cotinine levels with the highest values in Hungary, Romania, and the Czech Republic (Den Hond et al. 2015). Indeed, the average urinary cotinine levels in Czech children were approximately two times higher than the European average.

The home environment appears to be the most important predictor of the cotinine levels in children. The results of several studies found younger children to have higher levels of urinary cotinine than older ones, which might be attributable to the fact that small children spent more time at home and thus may have more exposure to indoor nicotine within their homes (Den Hond et al. 2015). However, in the relatively small group of the Czech children in this study, an inverse relationship between age and urine cotinine levels was not observed.

Smoking in indoor public places is another important source of ETS exposure. Smoking in Czech society is, unfortunately, tolerated. Anti-smoking laws reflecting EU legislation have been repeatedly discussed over the years by the Czech parliament with the goal of banning smoking in public places including restaurants, bars, etc. in an effort to protect the health of the employees of these facilities as well as their clients including children and adolescents. The Czech Republic passed a smoke-free law only in 2016, which comes into force in May 2017. It is possible that the increased exposure of Czech children to ETS reflects the weak anti-smoking legislation in the Czech Republic (Smolders et al. 2015). It is known that anti-smoking legislation pays off. The effectiveness of anti-smoking legislation on health outcomes has been demonstrated at the population level (Cox et al. 2014). However, the most important preventive approach is population education aimed at preventing people from starting to smoke and encouraging smokers, especially, parents who smoke, to quit smoking (Orton et al. 2014).

Conclusions

Urinary cadmium and cotinine and hair mercury levels were measured in Czech children and their mothers participating to the European DEMOCOPHES study. The mercury content in hair was lower than European average and increased with fish consumption and with the number of dental amalgam fillings. The concentrations were considerably below the health-related limits. Urinary cadmium levels were in agreement with results obtained in other studies of the Czech population. Compared with the European levels, the urinary cadmium concentration of the Czech population is above the average value. The mean cadmium concentration in children was approximately half that of the mothers. Urinary cotinine levels were above the European average; in children, they were caused mainly to the exposure to ETS at home; it means that the protection of children against ETS is poor and needs to be enhanced.

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