Democophes Hu – A Human Biomonitoring Study for the Assessment of Environmental Exposures of School-Aged Children and their Mothers in Hungary

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Abstract

In the frame of the DEMOCOPHES study, which aimed to create a basis for a uniform European Union Human Biomonitoring (HBM) System by determining the mercury concentration of the hair samples and the concentrations of cadmium, cotinine, and a number of phthalate metabolites in the urine samples of 6 to 11 year-old children and their mothers from 17 countries, samples from 120 - 120 children and their mothers were measured in Hungary. Half of the participants lived in a district of Budapest with high traffic density and the other half in a rural area. The mercury, cadmium, and phthalate metabolite concentrations of the Hungarian samples did not exceed the guidance values used in this study. However, the high concentration of cotinine in urine suggested that a large number of children in Hungary were still exposed to environmental tobacco smoke.

Key Words: COPHES, DEMOCOPHES, human biomonitoring, mercury, cadmium, phthalates, cotinine

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INTRODUCTION

Human biomonitoring (HBM) studies include, among others, collecting and analysis of human tissue samples in order to ascertain environmental exposure. Such studies have been conducted for decades, but these always focused on a particular country or region or on certain target groups, and had different protocols and analytical backgrounds.

This is why the European Council decided in 2004, within the framework of action 3 of the European Environment and Health Action Plan 2004-2010, to support the development of a coherent and harmonized human biomonitoring system throughout Europe by means of commonly developed protocols, strategies and scientific tools ensuring reliable and comparable data, whilst also leading to a more effective use of sources.

That was the goal of the COPHES Project (COnsortium to Perform Human biomonitoring on a European Scale), which began with the support of the European Union in 2009 and with the participation of 27 European countries, including Hungary. The COPHES developed those guidelines that served as the basis of the European scale HMB study conducted in the framework of the DEMOCOPHES Project (DEMOnstration of COPHES, Becker k. et al., 2014.). The goal of the DEMOCOPHES pilot study was to test the method itself and to establish the European reference values for the measured substances. In order to disclose the environmental sources of exposures, and the way in which the materials may enter the human body, a detailed questionnaire survey was also part of the DEMOCOPHES project.

This paper reports exclusively on the Hungarian results of measurements and associations with their exposure sources revealed in the frame of the DEMOCOPHES study.

MATERIALS AND METHODS

Study population

In Hungary, as in most countries, 120 mother-child pairs were included in the study.The participants were recruited from September 2011 to March 2012.

The two sampling locations were District 8 of Budapest as a highly urbanized (hereafter: urban) area, and Rétság with less than 5000 inhabitants (hereafter: rural) area. The selection of the participants was done through the schools.

Based on the participation criteria, the upper age-limit for the mothers was set at 45 years. The children were selected from the 6-11 age group approximately evenly by years of age and gender. The mother-child pairs had to have lived for at least 5 years together in the same household in the study site before sampling. Metabolic disturbances, abnormal diuresis, and kidney diseases were exclusion criteria. Only one child per mother was included in the study group.

Recruitment, interviews, and collection of samples was done by the staff members of the relevant local public health institutes (Balassagyarmat, Rétság, Szécsény Subregional Public Health Institute and the District Offices VI-VII-VIII of the Budapest Public Health Institute).

The interviews and the collection of samples were performed in the homes of the participants by public health inspectors. Training of the interviewers was centrally organized. The interviewers used paper questionnaires with questions on the indoor and outdoor residential environment, nutrition, smoking behaviour, use of make-ups and toiletries, amalgam tooth fillings, car use, use of mercury-containing devices, the mothers' occupation and socio-demographic characteristics of the families.

Analysis of the selected biomarkers

Mercury content of the hair samples, cotinine and cadmium concentrations, as well as concentrations of eight phthalate metabolites in the urine samples were analysed in the course of the Hungarian study. First morning voids were collected and the measured concentrations in the urine were expressed in the context of creatinine concentration.

The analysis of the mercury content of hair and the cadmium concentrations in urine was performed by the chemical laboratory of the former National Institute of Occupational Health, (at the time of the study reorganized as the Occupational Safety and Labour Affairs Directorate of the National Labour Office, and recently: Occupational Health Directorate of the National Public Health Institute) using inductively coupled plasma mass spectrometry (ICP-MS). Gas chromatography (GC) to measure cotinine and high performance liquid chromatography mass spectrometry (HPLC-MS) of phthalates were performed in the laboratory of the National Institute of Public Health of the Czech Republic in Prague (Department for the Assessment of Occupational Exposure to Chemicals). The laboratories that performed the analyses were chosen through a strict quality assurance procedure, which consisted of Inter-laboratory Comparison Investigations (ICI), and external quality assessment schemes (EQUAS) (Schindler et al., 2014, Esteban et al., 2015).

Limit of quantification (LOQ) was an important factor because lower levels were to be expected in case of the general population than in the case of occupational exposures generally measured by the laboratory. First morning urine samples with creatinine levels < 300 mg/l or > 3000 mg/l were excluded (WHO, 1996).

Data processing, statistical evaluation

Data processing and assessment of the correlations were performed by using the STATA/SE 10.0 programme package. In order to compare the samples from the two testing sites Mann-Whitney test and ANOVA and to assess the associations between the concentrations and the possible sources of exposure linear regression procedure were used and Spearman correlation was also examined.

Ethical considerations

All participants provided informed consent. The study was approved by the Scientific and Research Committee of the Medical Research Council. [43/PI/2012.(2687/2012/EKU)].

RESULTS

Participation rate was low at both testing sites, in Budapest it was even lower than in the countryside *(Table I)*.

Table I.

Response rate in the two sampling sites

Location	Total number of invitation letter	Participation refused	Total number of participants (mother/child pair)	Response rate (%)
Urban (Budapest)	558	498	60	10.75
Rural (Rétság)	210	150	60	28.57
Total	768	648	120	15.63

Mercury content of the hair samples is shown in *Table II*. The geometric mean concentrations were significantly higher in the samples of mothers (0.038 µg/g hair) than among the children (0.026 µg/g; p<0.0001).

Table II.

Total mercury content of hair in the studied population (µg/g hair)

Participant group	Location	n	>LOQ (0.015 µg/g) %	GM	CI GM	min.	max.	P50	P95
Mothers	urban	60	81.7	0.055*	0.040 - 0.076	0.008	0.864	0.064	0.338
	rural	60	55.0	0.027	0.019 - 0.037	0.008	0.499	0.023	0.163
	total	**120**	**68.3**	**0.038**	**0.030 - 0.049**	**0.008**	**0.864**	**0.045**	**0.279**
Children	urban	59	67.8	0.040**	0.028 - 0.058	0.008	0.664	0.050	0.416
	rural	60	35.0	0.016	0.012 - 0.022	0.008	0.242	0.008	0.136
	total	**119**	**51.3**	**0.026**	**0.020 - 0.033**	**0.008**	**0.664**	**0.016**	**0.320**

n=total number; %>LOQ=percentage of participants with a value above LOQ; GM=geometric mean; CI GM=95% confidence interval of GM; min.=minimum; max.=maximum; P50=50th percentile; P95=95th percentile; *p=0.0019; ** p=0.0002

Comparison according to study sites showed that both the mothers (p=0.019) and the children (p=0.0002) had significantly higher mercury concentrations in the urban hair samples than in the rural ones.

According to the Spearman correlation calculation, the mercury levels in hair of the mothers and the children showed strong, significant correlation (r=0.668; p<0.0001) *(Figure 1)*.

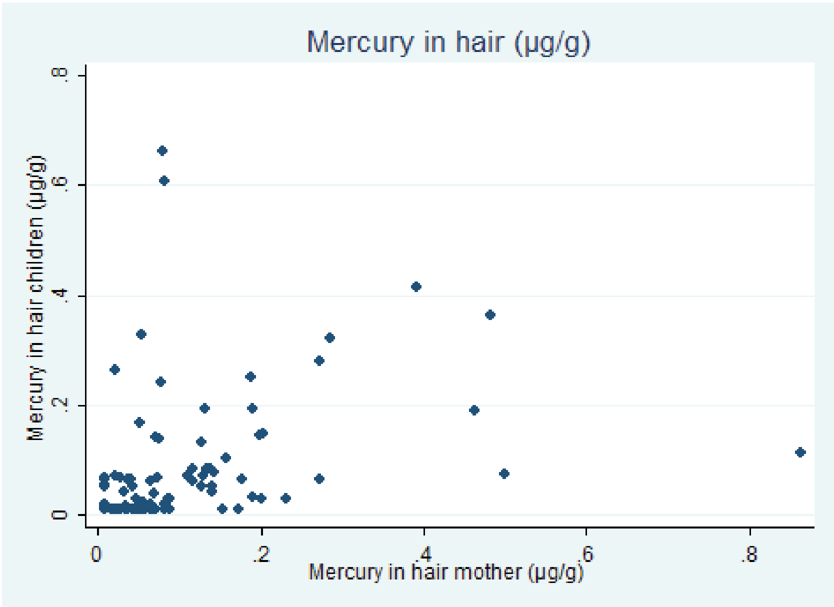


Figure 1. Correlation between mercury content of the mothers' and the children's hair samples

As far as the possible sources of mercury exposure are concerned, fish consumption was a significant (p<0.01) determinant of the mercury content of the mothers' but not the children's hair samples *(Figure 2)*. Non-parametric trend analysis also confirmed a significant association (p<0.001) between the frequency of mothers' fish consumption and mercury content of their hair samples. Wine consumption of the mothers, as well as their everyday use of make-ups also turned out to be significant ways of mercury exposure *(Figures 3 and 4)*. Interestingly enough, mercury content of hair samples not only of the mothers but of their children was increased if the mother used make-up every day *(Fig. 4)*.

However, we did not find elevated mercury level in the hair samples of the Hungarian participants in relation to broken thermometers (n=45) or energy saving lamps (n=13). Amalgam was not used at all for filling the teeth of children but more than half of the mothers (n=69) reported having teeth with amalgam filling. However, their hair samples did not contain elevated level of mercury, either.

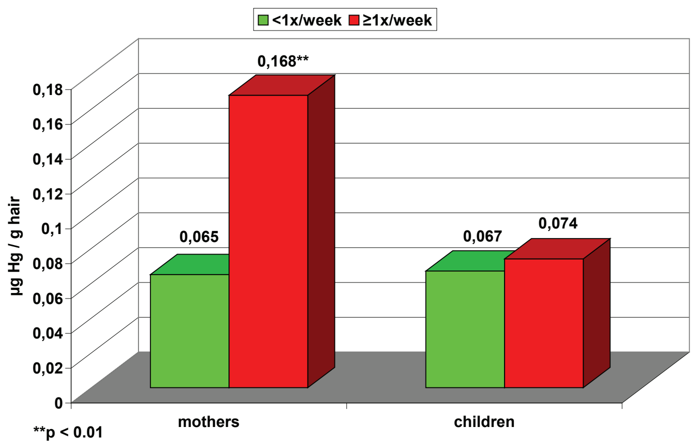


Fig. 2. Association between mean mercury contents of the Hungarian hair samples and fish consumption

Fig. 3. Association between mean mercury concentrations in the hair samples of Hungarian mothers and their wine consumption

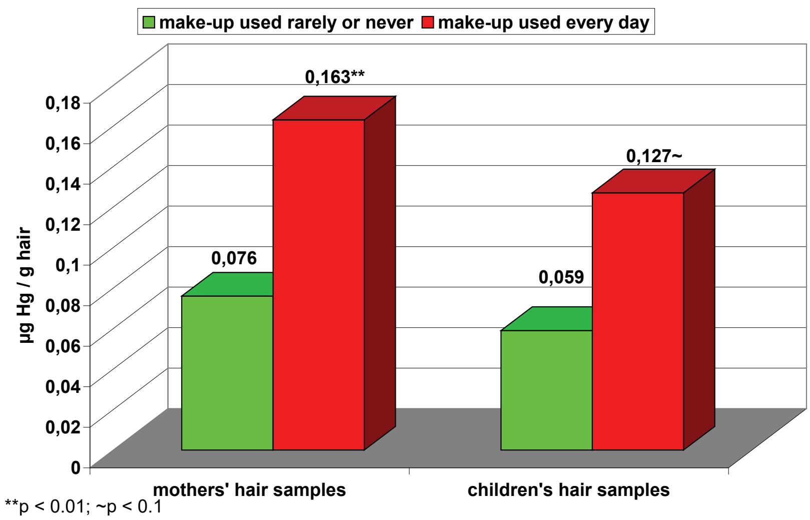


Fig. 4. Association between mean mercury concentrations in the Hungarian hair samples and frequency of make-up use by the mothers

Geometric mean concentration of cadmium in the mothers' urine samples was 0.153 µg/g creatinine and that among the children was 0.117 µg/g creatinine. There were no significant differences according to the study sites in the case of the mothers. The children living in the rural area, however, had a significantly higher cadmium concentration (p=0.018) than the children in the urban area. The cadmium concentration in the urine of mothers was significantly higher than that measured in the urine of children, especially in the case of Budapest (p<0.001) *(Table III.)*

Table III.

Urinary cadmium concentrations of the study population (µg/g creatinine)

Participant group	Location	n	>LOQ (0.10 µg/l) %	GM	CI GM	min.	max.	P50	P95
mothers	urban	59	84.8	0.163	0.139 - 0.191	0.042	0.799	0.170	0.444
	rural	56	76.8	0.144	0.122 - 0.169	0.040	0.537	0.150	0.445
	total	**115**	**80.9**	**0.153**	**0.137 - 0.172**	**0.040**	**0.799**	**0.159**	**0.444**
children	urban	59	64.4	0.101	0.083 - 0.122	0.029	0.410	0.104	0.336
	rural	58	74.1	0.137*	0.117 - 0.160	0.032	0.353	0.148	0.307
	total	**117**	**69.2**	**0.117**	**0.104 - 0.133**	**0.029**	**0.410**	**0.131**	**0.320**

n=total number; %>LOQ=percentage of participants with a value above LOQ; GM=geometric mean; CI GM=95% confidence interval of GM; min.=minimum; max.=maximum; P50=50th percentile; P95=95th percentile; *p=0.018

There was a positive and significant correlation between the cadmium concentrations of the mothers and the children (p=0.0002), the Spearman correlation coefficient was 0.3423 *(Figure 5.)*.

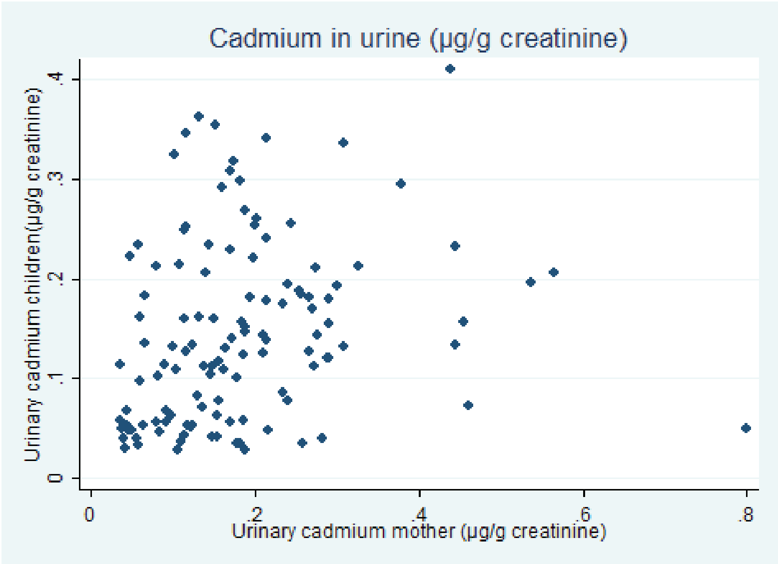


Figure. 5 Correlation between urinary cadmium concentrations of the mothers and the children

Smoking contributed a lot to the cadmium concentration of the mothers' but not the children's urine samples *(Fig. 6)*. There was a significant (p=0.044) trend between the number of cigarettes smoked daily by the mothers and the cadmium level of their urine samples.

Overall passive smoking (environmental tobacco smoke produced all persons smoking in the flat) had a significant impact on the urinary cadmium concentration. There was an increasing trend between cadmium and cotinine concentrations of the children's urine samples *(Figure 7)*. Though the overall trend between the cotinine and cadmium concentrations was statistically not significant, the difference in the cadmium concentrations between the lower and the upper thirds of the cotinine concentrations was significant (p=0.032).

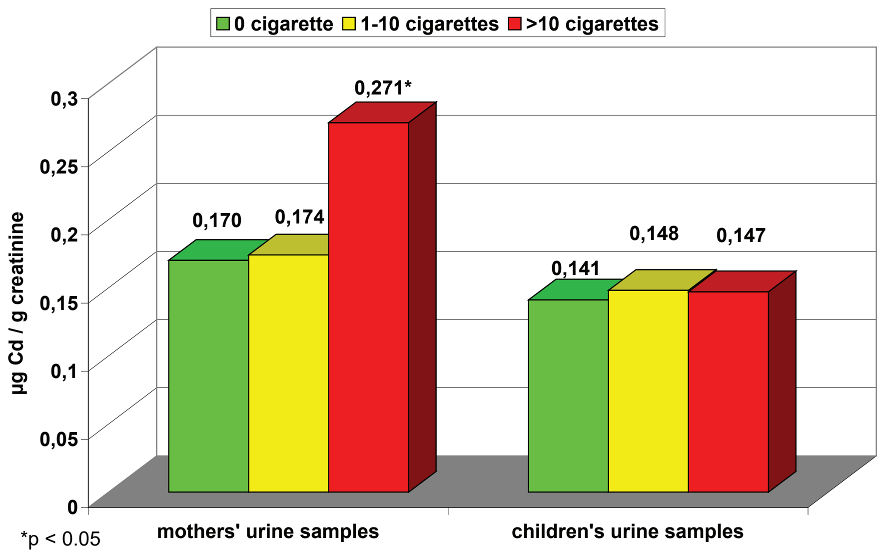


Figure 6. Number of cigarettes smoked daily by the mothers and cadmium level of their own and their children's urine samples

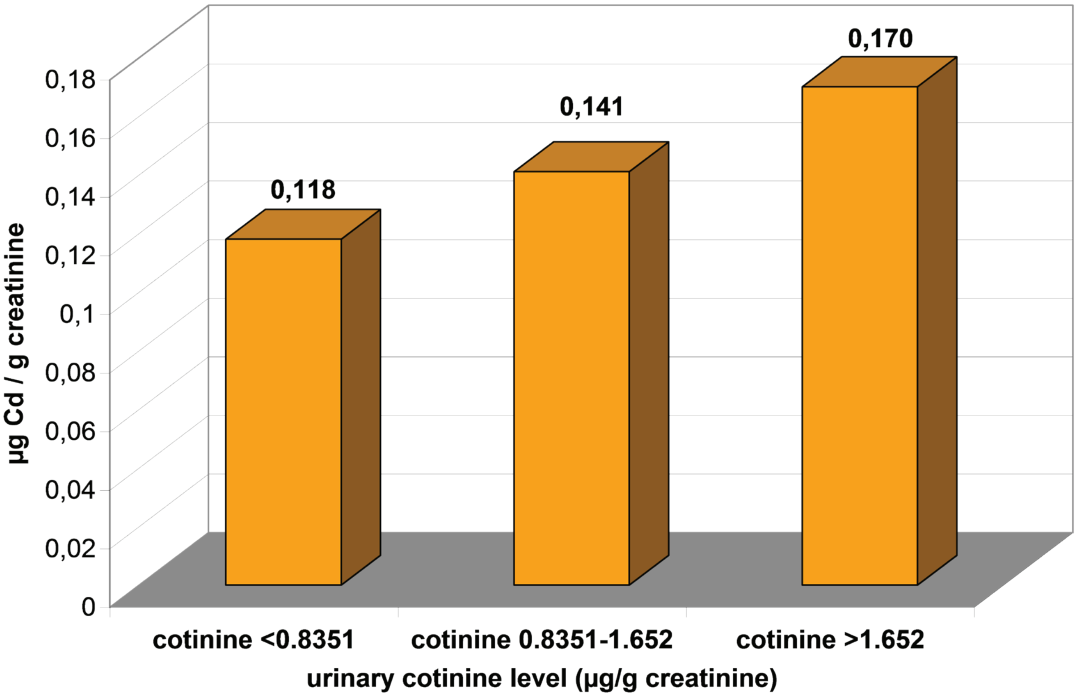


Figure 7. Associations between cadmium and cotinine concentrations of the children's urine samples

In the case of cotinine, 100% of the samples were over the LOQ value. Geometric mean of the cotinine concentration of smoking mothers was 363.6 µg/g creatinine (P50=987.4 µg/g creatinine, P95=2661 µg/g creatinine), while it was 1.17 µg/g creatinine (P50=0.75 µg/g creatinine, P95=48.23 µg/g creatinine) among the non-smoking mothers. The difference was highly significant ($p<0.0001$). There was no active smoker among the children. The cotinine concentrations were significantly higher in the mothers' than in the children's urine samples ($p<0.001$) *(Table IV)*.

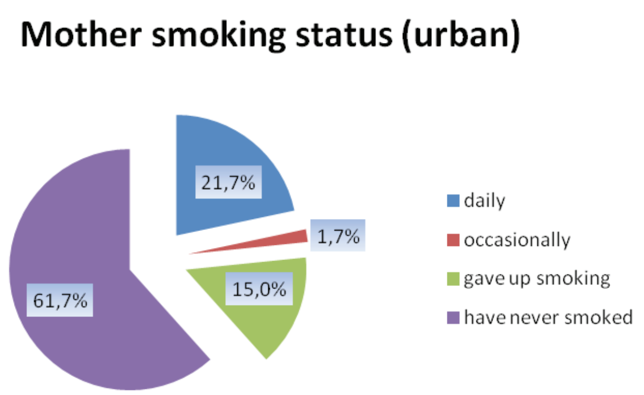
Table IV.

Urinary cotinine concentrations of the study population (µg/g creatinine)

Participants	Location	n	>LOQ (0.30 µg/l) %	GM	CI GM	min.	max.	P50	P95
Mothers	Urban	59	100.0	4.57	2.07 - 10.08	0.27	2661	1.26	1740
	Rural	56	100.0	9.11	3.63 - 22.86	0.22	2849	1.39	2260
	Total	**115**	**100.0**	**6.40**	**3.51 - 11.64**	**0.22**	**2849**	**1.30**	**2165**
Children	Urban	59	100.0	1.44	1.14 - 1.82	0.40	25.97	1.16	11.52
	Rural	58	100.0	1.74	1.27 - 2.39	0.29	33.37	1.14	25.28
	Total	**117**	**100.0**	**1.58**	**1.30 - 1.92**	**0.29**	**33.37**	**1.15**	**24.17**

n=total number; %>LOQ=percentage of participants with a value above LOQ; GM=geometric mean; CI GM=95% confidence interval of GM; min.=minimum; max.=maximum; P50=50th percentile; P95=95th percentile

Although the number of smoking mothers was higher at Rétság, in the rural area *(Figure 8.)*, there was no significant difference in the children's cotinine concentration between the study locations. The Spearman correlation of the urine cotinine concentrations of the children and the mothers showed a strong, significant association ($r=0.6182$; $p<0.0001$). The high values belong to the heavy smoker mothers *(Figure 9)*. This association is more clearly illustrated in *Figure 10,* showing that the intensity of environmental tobacco smoke (ETS) exposure is explicitly reflected in the children's urinary cotinine concentration.



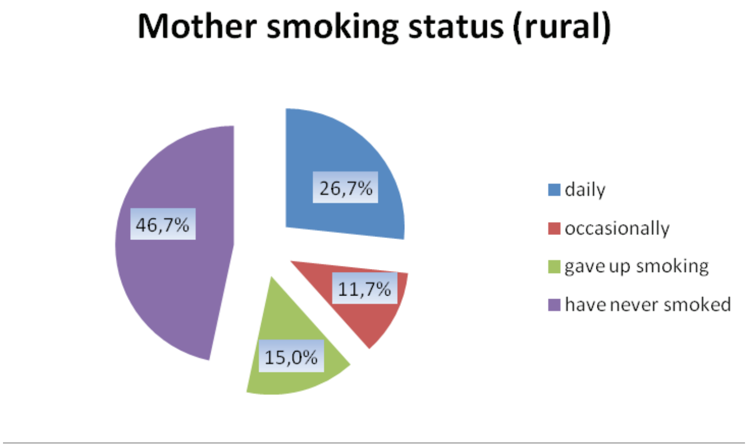


Figure 8. Smoking status of the urban and the rural mothers

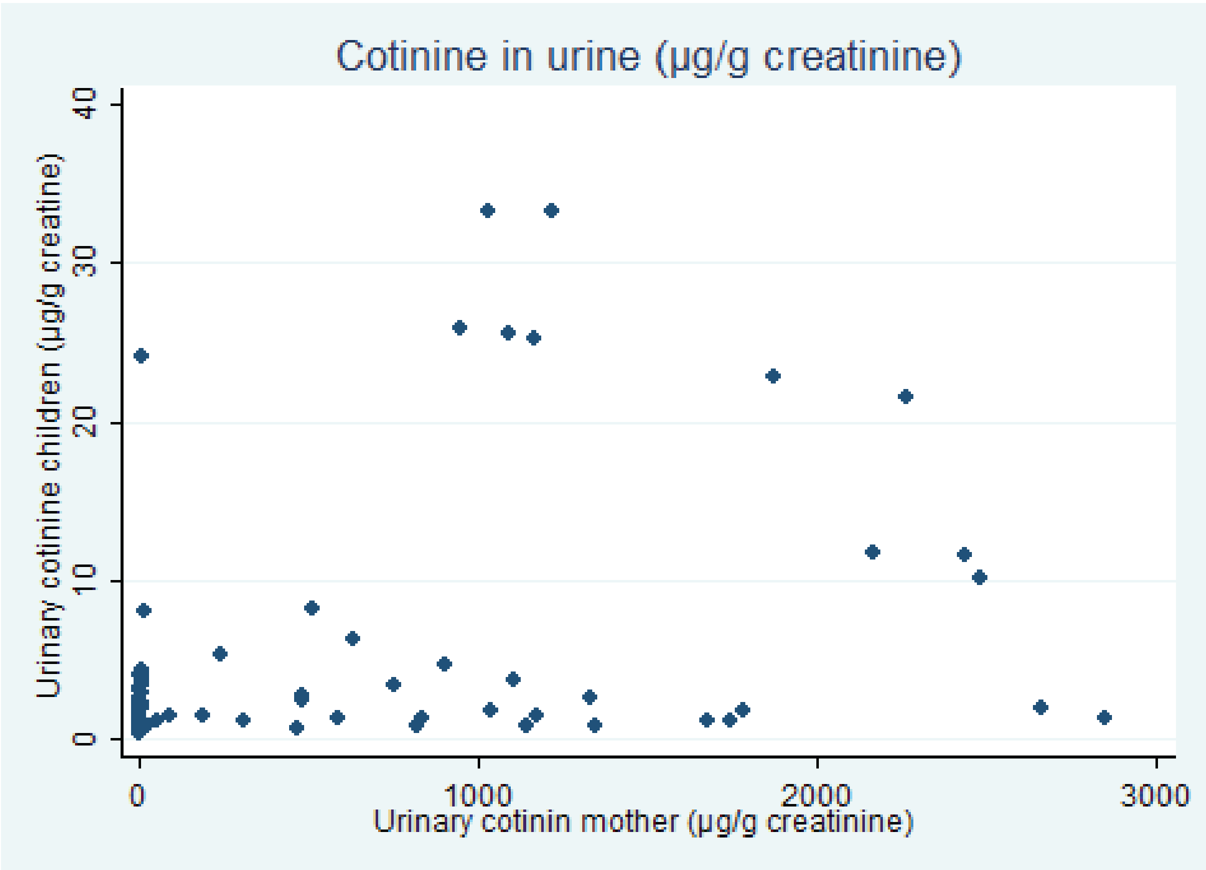


Figure 9. Correlations between urinary cotinine concentrations of the mothers and the children

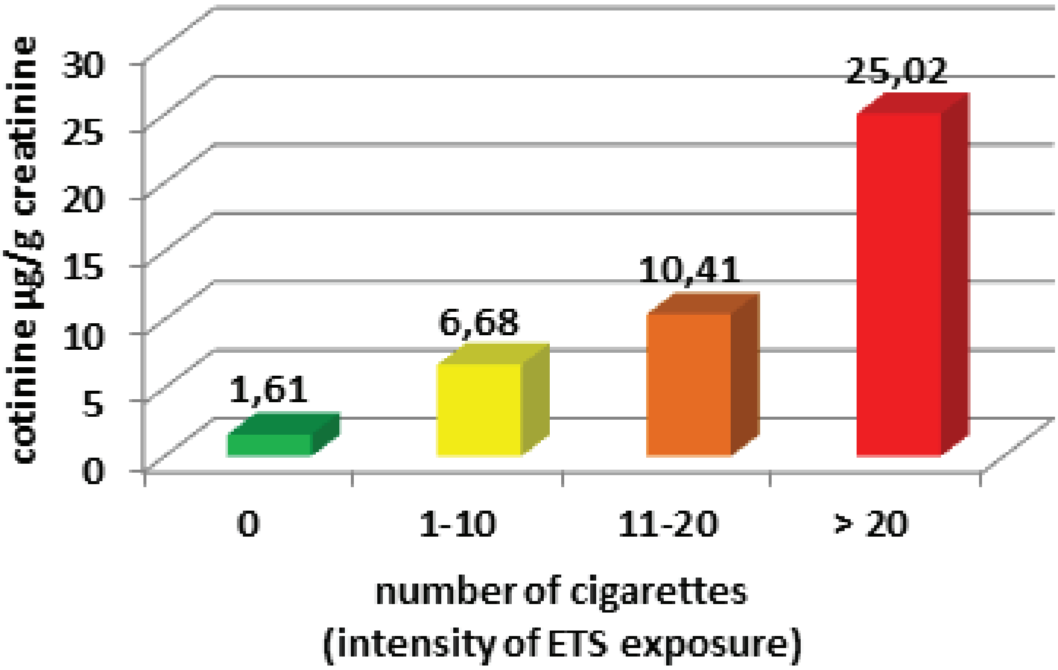


Figure 10. Associations between the daily number of cigarettes smoked in the children's indoor environment and the urinary cotinine concentrations of the Hungarian children

Urine concentrations of the following di(2-ethylhexyl) phthalate (DEHP) metabolites were measured: mono(2-ethylhexyl) phthalate (MEHP), 5hydroxy-mono(2-ethylhexyl) phthalate (5OH-MEHP), and 5oxo-mono(2-ethylhexyl) phthalate (5oxo-MEHP). The geometric mean of DEHP was 29.48µg/g creatinine among the mothers and 59.39 µg/g creatinine among the children. *Table V.* presents the concentrations of the phthalate metabolites in the urine samples of the mothers and the children. With the exception of mono-ethyl phthalate (MEP), all the measured phthalate metabolite concentrations were significantly higher in the children's urine samples than in those of the mothers ($\sum p<0.001$).

Table V.

Urinary MEHP, 5OH-MEHP, 5oxo-MEHP, MMP, MEP, MCHP, MBzP and MnBP concentrations of the study population (µg/g creatinine)

Phthalate metabolites	Partici-pants	Location	n	> LOQ %	GM	CI GM	min.	max.	P50	P95
MEHP (mono(2-ethyl-hexyl) phthalate) (µg/g creatinine) LOQ=2 µg/l	mothers	urban	59	76.3	2.70	2.18 - 3.33	0.43	16.16	2.76	11.77
		rural	56	73.2	3.51	2.70 - 4.57	0.43	53.02	3.89	16.90
		total	115	74.8	3.07	2.60 - 3.63	0.43	53.02	3.45	13.81
	children	urban	59	69.5	2.70	2.17 - 3.35	0.60	33.54	2.86	15.10
		rural	58	81.0	3.82*	3.09 - 4.72	0.48	27.46	3.98	17.16
		total	117	75.2	3.20	2.75 - 3.73	0.48	33.54	3.25	15.90
5OH-MEHP 5-hy-droxy-mono(2-ethyl-hexyl phthalate) (µg/g creatinine) LOQ=0.61 µg/l	mothers	urban	59	100.0	14.31	11.91 - 17.20	2.09	77.26	14.98	38.07
		rural	56	100.0	16.95	14.07 - 20.41	3.33	104.86	14.99	61.37
		total	115	100.0	15.54	13.65 - 17.69	2.09	104.9	14.98	54.70
	children	urban	59	100.0	26.33	22.13 - 31.32	6.09	184.1	25.31	86.02
		rural	58	100.0	33.21	27.55 - 40.04	7.02	239.6	28.01	126.80
		total	117	100.0	29.54	26.01 - 33.55	6.09	239.6	27.03	99.35

Phthalate metabolites	Partici-pants	Location	n	> LOQ %	GM	CI GM	min.	max.	P50	P95
5oxo-MEHP (5oxo-mono(2-ethylhexyl phthalate) (µg/g creatinine) LOQ=0.24 µg/l	mothers	urban	59	100.0	9.76	8.14 - 11.69	1.53	49.69	10.68	29.88
		rural	56	100.0	11.06	9.24 - 13.23	2.72	66.65	9.80	38.16
		total	115	100.0	10.40	9.14 - 11.76	1.53	66.65	9.90	37.33
	children	urban	59	100.0	18.44	15.29 - 22.25	4.28	114.9	21.15	70.10
		rural	58	100.0	21.97	18.36 - 26.28	4.40	107.3	18.95	99.81
		total	117	100.0	20.11	17.68 - 22.88	4.28	114.9	19.58	70.10
MMP (monomethyl phthalate) (µg/g creatinine) LOQ=4.73 µg/l	mothers	urban	59	32.2	3.92	2.78 - 5.53	1.00	601.0	2.98	104.47
		rural	56	32.1	4.80	3.22 - 7.16	0.98	544.9	2.75	72.38
		total	115	32.2	4.33	3.34 - 5.61	0.98	601.0	2.75	72.38
	children	urban	59	50.9	4.94	3.71 - 6.59	0.97	189.3	4.18	49.88
		rural	58	43.1	5.48	3.83 - 7.85	1.13	451.4	3.27	120.06
		total	117	47.0	5.20	4.15 - 6.52	0.97	451.4	3.64	54.48
MEP (monoethyl phthalate) (µg/g creatinine) LOQ=0.64 µg/l	mothers	urban	59	100.0	55.44**	40.12 - 76.61	2.39	1762	60.71	462.35
		rural	56	100.0	34.73	27.06 - 44.58	3.24	264.2	30.37	194.28
		total	115	100.0	44.15	35.89 - 54.31	2.39	1762	42.26	261.14
	children	urban	59	100.0	44.91	34.41 - 58.62	2.98	408.3	46.46	203.95
		rural	58	100.0	37.82	28.05 - 51.00	2.43	1289	33.65	271.54
		total	117	100.0	41.25	33.85 - 50.26	2.43	1289	37.38	206.61

Phthalate metabolites	Partici-pants	Location	n	> LOQ %	GM	CI GM	min.	max.	P50	P95
MCHP (monocyclo-hexyl phthalate) (µg/g creatinine) LOQ=0.22 µg/l	mothers	urban	59	3.4	0.09	0.08 - 0.11	0.04	1.29	0.09	0.22
		rural	56	0.0						
		total	115	1.7	0.09	0.08 - 0.10	0.04	1.29	0.09	0.22
	children	urban	59	1.7						
		rural	58	0.0						
		total	117	0.9	0.10	0.09 - 0.11	0.04	1.71	0.10	0.22
MBzP (mono-benzyl phthal-ate) (µg/g creatinine) LOQ=1.2 µg/l	mothers	urban	59	93.2	3.93	3.19 - 4.85	0.38	42.81	3.43	17.17
		rural	56	96.4	3.70	3.02 - 4.54	0.63	43.57	3.27	18.94
		total	115	94.8	3.82	3.31 - 4.41	0.38	43.57	0.34	17.17
	children	urban	59	100.0	5.73	4.60 - 7.13	0.54	60.93	6.14	24.33
		rural	58	98.3	7.73	5.62 - 10.63	0.64	602.82	6.42	191.84
		total	117	99.2	6.65	5.49 - 8.05	0.54	602.82	6.14	28.12
MnBP (mono-n-butyl phthal-ate) (µg/g creatinine) LOQ=1.51 µg/l	mothers	urban	59	100.0	29.84	25.06 - 35.53	2.64	214.35	29.43	67.96
		rural	56	100.0	35.65	30.95 - 41.06	10.11	162.66	33.11	126.09
		total	115	100.0	32.54	29.08 - 36.41	2.64	214.35	51.74	162.11
	children	urban	59	100.0	43.05	36.21 - 51.20	12.13	152.83	44.51	119.92
		rural	58	100.0	61.50***	51.18 - 73.90	13.90	339.08	67.63	236.48
		total	117	100.0	51.38	45.18 - 58.42	12.13	339.08	52.70	162.11

N=total number; %>LOQ=percentage of participants with a value above LOQ; GM=geometric mean; CI GM=95% confidence interval of GM; min.= minimum; max.= maximum; P50=50th percentile; P95=95th percentile.

*p=0.024 **p=0.025 ***p=0.006

In the case of MEHP, we found that the mothers in Rétság had a higher geometric mean than those in Budapest, but the difference was not statistically significant. Among the children, however, we measured significantly higher concentrations at Rétság (p=0.024). MEHP concentrations in urine samples of both the children and their mothers were significantly higher if their flats were redecorated in the last 12 months *(Figure 11)*.

In the case of 5OH-MEHP, the values of all samples were above the LOQ. When comparing the results from rural and urban areas, we did not find any significant difference between the two sites either among the mothers or the children.

The 5oxo-MEHP concentrations were also above the LOQ in all samples. There were no significant differences between the concentrations of samples from the two locations.

There was no significant difference in the urine concentrations of monomethyl-phthalate (MMP) between the study sites, although the averages at Rétság were somewhat higher.

The MEP concentrations of the mothers were significantly higher in Budapest than in Rétság (p=0.025). We could not find such differences in the case of children. The mothers' urinary MEP concentrations were significantly higher than those of their children (p<0.001). The MEP concentrations in the urine samples of mothers using make-ups at least once a week were significantly higher than of those not using make-ups at all. Slightly elevated MEP concentrations could also be observed among the children whose mother regularly used make-ups but the difference was statistically not significant *(Figure 12)*. None of the children used make-ups.

The monocyclohexyl-phthalate (MCHP) concentrations exceeded the LOQ values in three cases: in the case of a mother-child pair living in Budapest (1.29 μg/g creatinine and 1.71 μg/g creatinine) and in the case of a mother in Budapest (0.26 μg/g creatinine).

In the case of monobenzyl-phthalate (MBzP) we found three unusually high values among the children from Rétság, but there were no significant differences in the mean values between the study sites either for the mothers, or for the children. Significantly higher concentrations of MBzP were found in the urine samples of children living in flats with PVC flooring than of those without PVC floored flats. Similar trend could be observed in the case of mothers, too, but the difference was much smaller and statistically not significant *(Figure 13)*.

The mono-n-butyl-phthalate (MnBP) was present in 100% of the samples in an assuredly detectable amount. There were no significant differences between the study sites in the case of the mothers, but the children in Rétság had significantly higher concentrations than those living in Budapest (p=0.006).

In the case of all measured phthalate metabolites there was a significant correlation between the urine concentrations of the mothers and the children according to the Spearman correlation analysis *(Table VI)*.

Table VI.

Correlations of the various phthalate metabolite concentrations measured in the mothers' and the children's urine samples

BIOMARKER (µg/g creatinine)	**Spearman correlation coefficient**
MEHP	r=0.379***
5OH-MEHP	r=0.425***
5oxo-MEHP	r=0.419***
MMP	r=0.533***
MEP	r=0.433***
MBzP	r=0.554***
MnBP	r=0.480***

*** p<0.0001

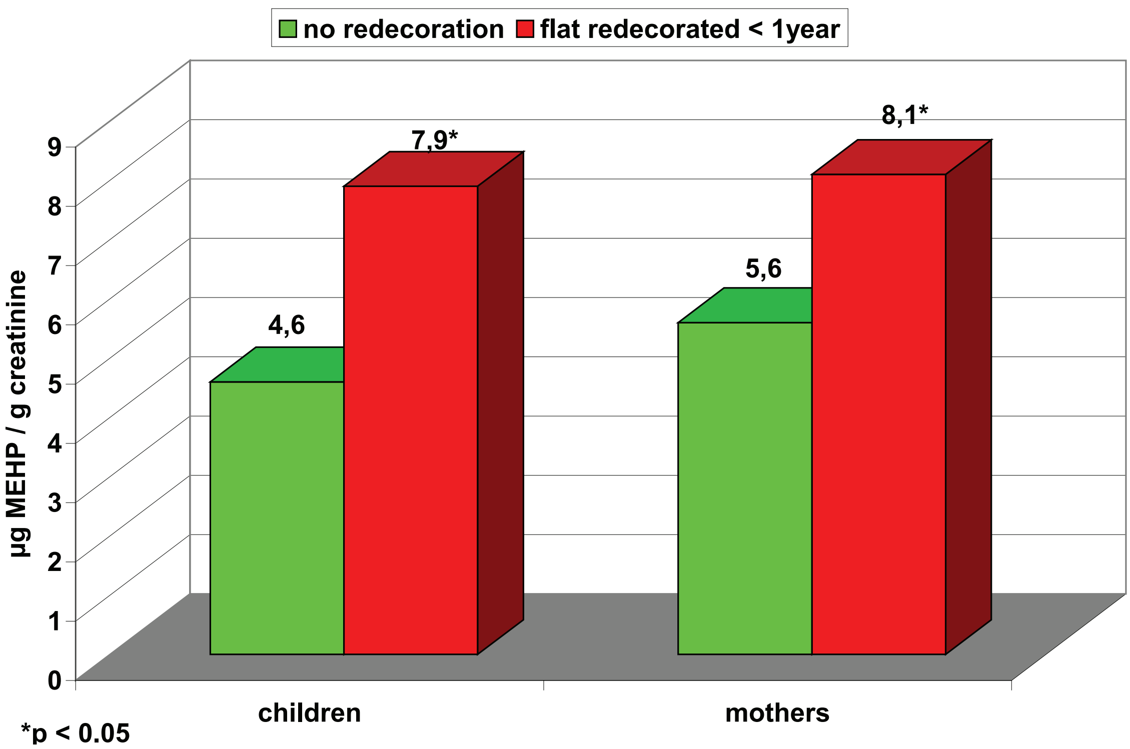


Figure 11. Mono-ethyl-hexyl phthalate (MEHP) concentrations measured in urine samples of mothers and their children living in flats redecorated or not during the last 12 months

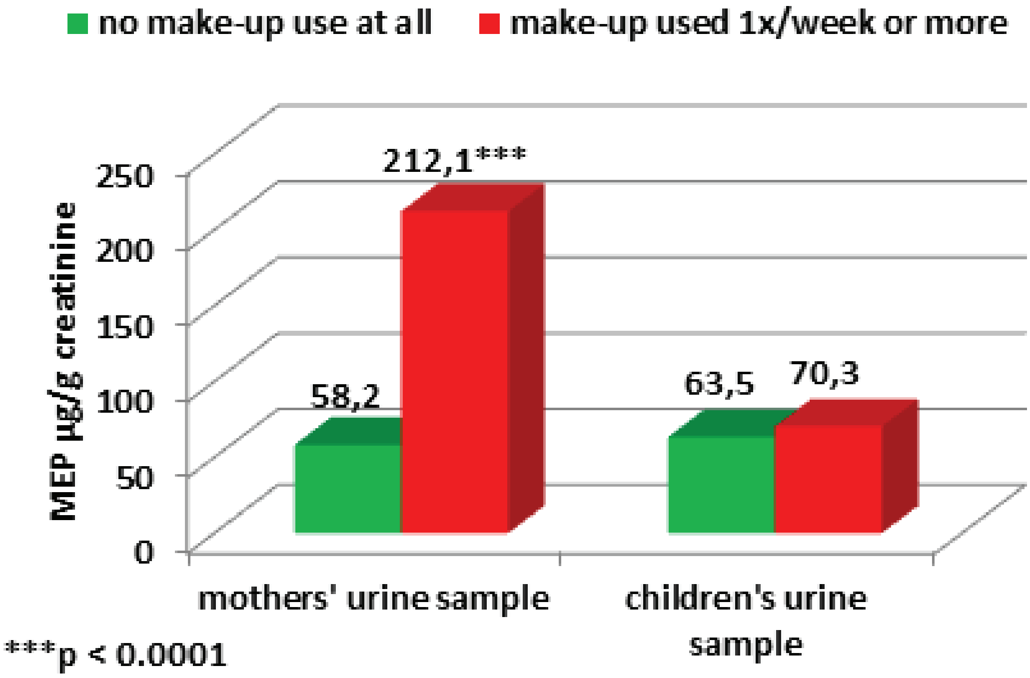


Figure 12. Mono-ethyl phthalate (MEP) concentrations measured in urine samples of 6-11 year old children and their mothers using make-ups at least 1x/week or not at all

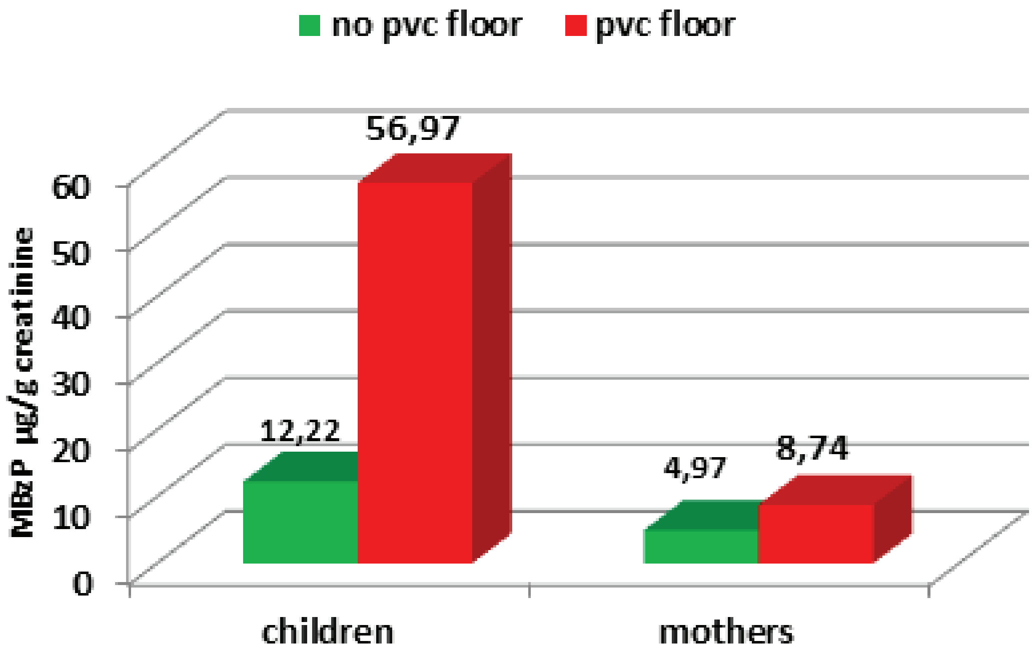


Figure 13. Mono-benzyl phthalate (MBzP) concentrations measured in urine samples of mothers and their children living in flats with or without PVC flooring

DISCUSSION

There is no official reference value for the total *mercury* content of hair, therefore in the DEMOCOPHES we used the value suggested by WHO/JECFA (2004) for hair (2.3 µg/g). This value was not reached by any of the study subjects. However, considering the latest value suggested by the WHO (0.58 µg/g) taking into account the DEMOCOPHES results as well (Bellanger et al., 2013), these were exceeded in case of one mother in Budapest (0.864 µg/g) and two children also from Budapest (0.609 µg/g and 0.664 µg/g).

In the case of mothers, the geometric mean of the total mercury content of hair samples was considerably lower than the average of the participating countries (EU-GM = 0.225 µg/g), which was also true for the children ($p<0.001$, EU-GM=0.145 µg/g, Elly Den Hond et al., 2015). The Hungarian hair samples contained the lowest mercury level in the study. Although we could not find measurement results in the Hungarian literature for the mercury content of hair, there are many examples in the international literature. In the Czech Republic, the geometric mean of the total mercury content in the hair samples of children between the ages of 8 and 10 studied between 1994 and 2001 was 0.20 µg/g (Benes et al., 2003). In the 2006-2007 French biomonitoring study the geometric mean was 0.37 µg/g among the 3-17 year olds and 0.59 µg/g among the 18-74 year olds (Frery et al., 2010). The US National Health and Nutrition Examination Survey (NHANES, 1999-2000) found a geometric mean of 0.12 µg/g among 1-5 year old children and 0.20 µg/g among adult women (McDowell et al., 2004). Wranová et al. (2009) compared the mercury content in dentists' hair to that of a control group. In the case of the exposed dentists the median was 0.51 µg/g, while the non-exposed control group had a median value of 0.33 µg/g. Results of earlier studies referred to in the summary guidelines published by the WHO–UNEP (2008) showed similar values. The mercury concentrations measured in this study are almost an order of magnitude smaller than the concentrations measured in other countries, this could be due to the low consumption of sea food. Mercury accumulates through the course of one's life, which explains the differences between the mothers and the children. The higher values in Budapest can be explained by the higher consumption of sea food. Fish and wine consumption of the mothers, as well as their everyday use of make-ups showed significant associations with mercury content of the hair samples.

In the case of *cadmium*, we used the values for reference defined by the German Human Biomonitoring Commission (children: HBM I=0.5 µg/L, HBM II=2 µg/L, adults: HBM I=1 µg/L, and HBM II=4 µg/L) (Schulz et al., 2012). The Hungarian concentrations were below these values in every case. The cadmium concentration of urine was studied in Hungary in 2011-2012 in connection with the "red sludge catastrophy". In the case of children living in the exposed area, a geometric mean of 0.11 µg/g creatinine was measured. There were no significant differences between the exposed and the control area (Rudnai et al., 2011). In the NHANES study between 2009–2010 the geometric mean of the cadmium concentration in the urine of children aged 6–11 was 0.08 µg/g creatinine and for women it was 0.23 µg/g creatinine (CDC, 2013). The GerES III. study in Germany found a geometric mean of 0.18 µg/g creatinine for adults (Becker et al., 2003), it was 0.29 µg/g creatinine for Czech adult blood donors (Batáriova et al., 2006), while the human biomonitoring study in France found a geometric mean of 0.29 µg/g creatinine (Frery et al., 2010). The Hungarian concentrations measured in the DEMOCOPHES study fit well to these values. The geometric mean of the aggregated concentration of the countries in the study was 0.20 µg/g creatinine (0,219 µg/L) for the mothers and 0.07 µg/g creatinine (0,071 µg/L) for the children (Elly Den Hond et al., 2015).

There is no official reference value for *cotinine.* According to Riboli et al. (1995), a person can be considered a smoker or a strongly exposed non-smoker if the cotinine concentration of his or her urine exceeds 50 µg/g creatinine, therefore we chose this concentration as our reference value. In our study 26.7% of the urine samples of the mothers had concentrations exceeding this value, while none of the samples from the children contained such a high concentration. In the questionnaire survey, 24.2% of the Hungarian mothers said they smoked daily and an additional 6.7% said that they were occasional smokers. There was no active smoker among the children. Based on the aggregated European data, the geometric mean of the cotinine concentration was 2.45 µg/g creatinine (2.75 µg/L) among the mothers and 0.80 µg/g creatinine (0.77 µg/L) among the children (Elly Den Hond et al. 2015), thus the Hungarian values were significantly higher both for the children and the mothers than the European average ($p=0.001$).

However in the GerES IV study 2.5 µg/L cotinine concentration was measured in the urine samples of 3-14 year old children (Becker et al., 2008), this value in the DEMOCOPHES study was 1.8 µg/L. The regulation for the protection of non-smokers' health was introduced in Germany earlier, while in Hungary it became stricter only in 2012.

With the exception of MEP, we found higher *phthalate* metabolite concentrations in the children's urine samples compared to the mothers' ones. A possible explanation for the phenomenon is the higher exposure of children, which can be due to that children put their hands and their plastic toys in their mouth more frequently or, as it may be the case regarding MBzP, they have closer contact with plastic floor and phthalate containing dust. Also, children have a higher food intake in relation to their body weight. MEP is the metabolite of diethyl-phthalate (DEP), which is common in hygiene products and in cosmetics that are primarily used by women, which could also explain the higher concentrations in their case.

In the DEMOCOPHES study we used the 5-OH-MEHP and 5-oxo-MEHP common concentrations as suggested by the German Human Biomonitoring Commission (Schulz et al., 2012). For children aged 6-13 years this was 500 µg/L and for women of reproductive age it was 300 µg/L. These were exceeded in Hungary by one child (0.9%) and one mother (0.9%).

The European geometric mean of the DEHP (MEHP+5OH-mehp+5oxo-MEHP) concentration was 26.00 µg/g (29,2 µg/L) for the mothers and 46.25 µg/g in the context of creatinine concentration (47,6 µg/L) among children (Elly Den Hond et al., 2015). The Hungarian values were significantly higher for both the mothers and the children ($p<0.05$).

In 2007 during a study among children in Frankfurt (Koch et al., 2011) they measured 7.3 µg/g creatinine for MEHP, 29.2 µg/g creatinine for 5OH-MEHP, and 24.3 µg/g creatinine geometric mean in the case of 5oxo-MEHP.

In the 2009-2010 NHANES study (CDC, 2013), the geometric mean of the measured urinary concentrations among children was even lower: 2.13 µg/g creatinine for MEHP, 19.6 µg/g creatinine for 5-OH-MEHP, and 12.7 µg/g creatinine in the case of 5oxo-MEHP.

Kasper-Sonnerberg et al. in their Duisburg study (2012) measured 3.7 µg/g creatinine geometric mean for adult women in the case of MEHP, which was close to the Hungarian value, while the geometric mean of MEHP in the 2009-2010 NHANES study was 1.68 µg/g creatinine. In the Duisburg study (Kasper-Sonnerberg et al., 2012) the geometric mean of the 5-OH-MEHP concentration was 14.1 µg/g creatinine in adult women, while in the NHANES

(2009-2010) study this value was 13.3 µg/g creatinine (CDC, 2013).

The geometric mean of 5-oxo-MEHP for women was 10.0 µg/g creatinine in the Duisburg study (Kasper-Sonnerberg et al., 2012), while it was 8.6 µg/g creatinine in the NHANES (2009-2010) (CDC, 2013). The geometric mean measured in this present study was between the above mentioned two values.

We only measured the MMP and MCHP concentrations in the Hungarian samples. In the Duisburg study (Kasper-Sonnerberg et al., 2012), in the case of MMP, they found lower concentrations (children: 4.8 µg/g creatinine, 1.1 µg/g creatinine) than the geometric mean measured in Hungary (children: 5.2 µg/g creatinine, mothers: 4.33 µg/g creatinine).

They found even lower MMP geometric mean concentration in children in the NHANES (2009-2010) study (2.77 µg/g creatinine) (CDC, 2013).

There is little data available in the literature about MCHP. The geometric mean of the MCHP concentrations was below the LOD (0.402 µg/L) both for women and children in the NHANES (2009-2010) study (CDC, 2013). For children the P95 was 1.80 µg/g creatinine.

The MEP concentrations of the Hungarian samples were similar to those of the aggregated European samples: the geometric mean for mothers of the aggregated European samples was 42.94 µg/g creatinine (48.2 µg/L), while it was 33.37 µg/g creatinine (34.4 µg/L) for children (Elly Den Hond et al. 2015). The results of the Duisburg study (Kasper-Sonnerberg et al., 2012) were similar for the mothers (42.3 µg/g creatinine) and children (38.1 µg/g creatinine). The geometric means in NHANES (2009-2010) were higher: 81.9 µg/g creatinine for women, and 45.9 µg/g creatinine for 6-11 year old children (CDC, 2013).

The aggregated European geometric mean MBzP concentrations were 4.02 µg/g creatinine (4,5 µg/L) for mothers and 6.94 µg/g creatinine (7,1 µg/L) for children (Elly Den Hond et al. 2015). The Hungarian values were similar. Earlier studies measured higher concentrations. In the Duisburg study (Kasper-Sonnerberg et al., 2012) the mothers' MBzP geometric mean concentration was 5.5 µg/g creatinine, while it was 12.2 µg/g creatinine for children. In the NHANES (2009-2010) study they measured 7.29 µg/g creatinine for the mothers and 15.1 µg/g creatinine for the children (CDC, 2013).

The metabolite of dibutyl-phthalate is MnBP. The geometric mean of the aggregated European MnBP concentration was 21.48 µg/g creatinine (23.9 µg/L) for mothers and 33.98 µg/g creatinine (34.8 µg/L) for children (Elly Den Hond et al. 2015). Based on the 95% CI values, the Hungarian values were significantly higher ($p<0.05$) than the European means for both the mothers and the children. Data from the international literature show great deviation. Xibiao et al., (2008) measured 67.1 µg/g creatinine in pregnant women in Rotterdam, the Duisburg study (Kasper-Sonnerberg et al., 2012) measured 27.5 µg/g creatinine, while the NHANES (2009-2010) study (CDC, 2013) measured 17.8 µg/g creatinine for women. The concentrations for the children are higher than those of the mothers, but they are lower than the Hungarian values. In the Duisburg study, the geometric mean of MnBP in the 6-7 year old children's urine was 46.9 µg/g creatinine, the same for 6-11 year old children was 28.3 µg/g creatinine in the NHANES (2009-2010) study (CDC, 2013).

CONCLUSIONS

One hundred twenty mother-child pairs were studied in Hungary and 1844 mother-child pairs in total in Europe within the framework of the COPHES/DEMOCOPHES projects. Although the Hungarian sample was not representative, this was the first occasion where we received completely comparable data concerning biomarkers from 17 European countries at the same time that could be compared to each other and to data from other international HBM studies. The COPHES/DEMOCOPHES projects successfully laid the groundwork for a continuous European human biomonitoring programme, that can be extended with the study of other materials and that can be a continuous source of information about certain environmental exposures, thereby substantiating the necessary interventions.

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