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Exposure determinants of cadmium in European mothers and their children

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ABSTRACT

The metal cadmium (Cd) is a widespread environmental pollutant with documented adverse effects on the kidneys and bones from long-term environmental exposure, but with insufficiently elucidated public health consequences such as risk of cardiovascular disease, hormone-related cancer in adults and developmental effects in children. This study is the first pan-European human biomonitoring project that succeeded in performing harmonized measurements of Cd in urine in a comparable way in mother–child

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couples from 16 European countries. The aim of the study was to evaluate the overall Cd exposure and significant determinants of Cd exposure.

A study population of 1632 women (24–52 years of age), and 1689 children (5–12 years of age), from 32 rural and urban areas, was examined within a core period of 6 months in 2011–2012. Women were stratified as smokers and non-smokers. As expected, smoking mothers had higher geometric mean (gm) urinary cadmium (UCd; 0.24 μg/g crea; $n=360$) than non-smoking mothers (gm 0.18 μg/g crea; $n=1272$; $p<0.0001$), and children had lower UCd (gm 0.065 μg/g crea; $n=1689$) than their mothers at the country level. Non-smoking women exposed to environmental tobacco smoke (ETS) at home had 14% (95% CI 1–28%) higher UCd than those who were not exposed to ETS at home ($p=0.04$). No influence of ETS at home or other places on UCd levels was detected in children. Smoking women with primary education as the highest educational level of the household had 48% (95% CI 18–86%) higher UCd than those with tertiary education ($p=0.0008$). The same observation was seen in non-smoking women and in children; however they were not statistically significant. In children, living in a rural area was associated with 7% (95% CI 1–13%) higher UCd ($p=0.03$) compared to living in an urban area. Children, 9–12 years had 7% (95% CI 1–13%) higher UCd ($p=0.04$) than children 5–8 years.

About 1% of the mothers, and 0.06% of the children, exceeded the tolerable weekly intake (TWI) appointed by EFSA, corresponding to 1.0 μg Cd/g crea in urine. Poland had the highest UCd in comparison between the 16 countries, while Denmark had the lowest. Whether the differences between countries are related to differences in the degree of environmental Cd contamination or to differences in lifestyle, socioeconomic status or dietary patterns is not clear.

1. Introduction

The metal cadmium (Cd) is a widespread environmental pollutant with documented adverse effects on kidneys and bones in adults from long-term environmental exposure, but with insufficiently elucidated public health consequences such as risk of cardiovascular disease, hormone-related cancer in adults, and developmental effects in children (Jarup and Akesson, 2009; Ciesielski et al., 2012; Tellez-Plaza et al., 2013; Akesson et al., 2014). The International Agency for Research on Cancer (IARC) has classified Cd as a human carcinogen (group 1; IARC, 2012). Cadmium has a very long biological half-life, about 10–30 years, and accumulates in the body with age (Jarup and Akesson, 2009). Approximately 50% of the total body burden is accumulated in the kidneys and urinary Cd is considered a valid biomarker of lifetime (kidney/body) accumulation from overall Cd exposure and thus used in the assessment of Cd-induced health effects (Akerstrom et al., 2013).

Everyone is exposed to Cd via food, which is the main source of exposure in non-smokers. Smokers have a higher body burden of Cd than non-smokers since inhaled tobacco smoke contains Cd, which is absorbed to a large extent in the lungs. In 2009, the European Food Safety Authority (EFSA) assessed the human health risk related to Cd in food (EFSA, 2009). A benchmark dose lower confidence limit (BMDL5), based on renal tubular effects in adults, and adjustment for inter-individual variation led to a value of 1.0 μg Cd/g creatinine in urine, corresponding to a tolerable weekly intake (TWI) of 2.5 μg Cd/kg bw.

This study is part of the COPHES/DEMOCOPHES twin projects which have their origins in the European Environment and Health Action Plan of 2004 with the aim to develop a coherent approach on human biomonitoring (HBM) in Europe (Den Hond et al., 2015; Casteleyn et al., 2015). The project is the first pan-European HBM project that succeeded in performing harmonized biomarker measurements in 17 participating European countries. Within the project, Cd in urine was measured in a comparable way in 16 of these countries and evaluated in relation to lifestyle, socioeconomic status (SES) and environmental exposure factors. An extensive quality control programme for chemical and data analysis was applied, and 14 European laboratories qualified to do the Cd analyses (Schindler et al., 2014).

The aim of this study was to evaluate the overall Cd exposure, assessed by analyses of Cd concentrations in urine in mother–child couples from 16 European countries, and to evaluate significant determinants of Cd exposure based on questionnaire information, considering regional variations on a European level.

2. Materials and methods

A study population of 1724 mother–child couples, from 32 rural and urban areas in 16 European countries, was sampled within a core period of 6 months in 2011–2012. Ethical permission was granted by ethics committees in each country and all mothers and some children gave written informed consent and assent, respectively. All procedures followed the national data protection requirements including notification to the data protection authority (Casteleyn et al., 2015).

2.1. Recruitment and sampling

A harmonized cross-sectional study design was applied, including recruitment of 120 children aged 6–11 years and their mothers aged up to 45 years from each participating country, except in the two smallest countries (Cyprus and Luxemburg) in which 60 mother–child couples were to be recruited (Becker et al., 2014).

The sampling strategy stated that the children should be sampled equally from an urban and a rural area in each country, should be based on equal distribution between gender and age classes and should preferably cover different levels of SES (income, education and occupations). Those eligible for inclusion in the study were healthy children and mothers without metabolic disorders, abnormal urine excretion or liver and kidney disorders, who had a sufficient knowledge of the local language and had been living for at least 5 years in the area. Occupational exposure was not an exclusion criterion. Recruitment was performed via inhabitant registries or schools. The families were visited at home or attended at another location for face-to-face interviews and biological sampling (Fiddicke et al., 2015). In one country, a web-based questionnaire was used in prior to the biological sampling. First-morning urine samples were collected from each mother–child couple for analyses of Cd, creatinine and cotinine.

2.2. Questionnaire information

The mothers answered an extensive questionnaire (developed by COPHES/DEMOCOPHES consortium) covering questions about living environment, food consumption, smoking, lifestyle and sociodemographics and -economics (Becker et al., 2014).

The studied variables were age, gender (for children), smoking habits (verified by urinary cotinine measurements in both smokers and non-smokers), living area (urban/rural), possible industrial emissions, time spent in traffic, use of fossil material for heating, soldering indoors, education (3 categories; highest in the family), Body Mass Index (BMI; kg/m^2), food consumption within the last 4 weeks (consumption frequencies of rice, cereals, mushrooms, offal, game, meat, fish, chocolate, local food), and main drinking water source (private well/commercial products/public water supply).

2.3. Chemical analysis

In order to ensure the quality and comparability of the analytical results, an extensive analytical quality control programme was implemented by COPHES/DEMOCOPHES, including both interlaboratory comparisons and external quality assessment schemes. The laboratories could choose which analytical method they use but only laboratories that completed successfully the quality control programme were qualified to analyse the samples of the study (for details see Schindler et al., 2014). Cadmium in urine (UCd) was determined by ICP-MS, with exception for one laboratory that used atomic absorption spectroscopy. Urinary cotinine was mainly determined by LC–MS/MS and urinary creatinine was mainly analysed by the Jaffe method, but other methods were also used (Schindler et al., 2014). The limit of quantification (LOQ) for Cd in the collected urine samples, reported by the qualified laboratories, ranged between 0.001 and 0.07, except for the laboratory that used AAS, which reported a LOQ of 2 μg/L (Supplemental material).

2.4. Statistical analysis

The information reported through questionnaires was controlled for invalid answers and errors before further analysis by an extensive quality control of the data. Biomarker levels below the respective LOQ were substituted by half the value of the LOQ.

The statistical softwares IBM SPSS version 20 and SAS version 9.3 were used for statistical analyses. The UCd levels were not normally distributed and therefore logarithmic (ln)-transformed values were used for the univariate and multiple analyses.

Univariate and multiple regression models were fitted for evaluation of determinants of Cd exposure. Linear mixed models were used to take into account the clustered design of sampling within member states. Analyses were done separately for children, non-smoking and smoking mothers. First, univariate models were developed for the selected confounders and covariates. Second, multiple regression models were built including those determining factors which were significant at the 0.20 significance level in the univariate analyses and pre-specified confounders (creatinine and age for mothers and children, and additionally gender for children) were forced in the model. By stepwise selection procedures a final model was obtained where the significance level was set at 0.05 for a variable to stay in the model.

The effects of multicollinearity were analysed using variance inflation factors. The assumption of normality was checked with informal diagnostic residual plots and the Kolmogorov–Smirnov test (Neter et al., 1996). Influence diagnostics (e.g. restricted likelihood distance, Cook's D, CovRatio) were used to quantify the influence of one or more observations by computing parameters estimates based on all data points, removing the cases in question from the data, refitting the model, and computing statistics based on the change between full-data and reduced-data estimates.

Analyses of the correlation between the mothers and their children were performed using the non-parametric Spearman's correlation coefficient (rs).

3. Results

Because smoking is a well-known predictor of UCd, women were stratified by smokers (71% daily smokers and 29% occasional smokers; $n=360$) and non-smokers (74% never smokers and 26% former smokers, $n=1272$). The percentage of former smokers in the non-smoking group of women ranged between 16% (Poland) and 42% (Spain). There were significant correlations ($p<0.0001$) between the levels of urinary Cd and smoking (rs=0.16) or urinary cotinine (rs=0.18) as well as between urinary cotinine and smoking (rs=0.65). A cotinine concentration in urine >49.7 μg/L indicates active smoking habits (Jarvis et al., 1987). Thus, women who declared that they were non-smokers but had a cotinine concentration in urine >49.7 μg/L ($n=53$) were excluded from the non-smoking group. None of the children declared that they were smokers, but some of the children ($n=9$) had cotinine levels >49.7 μg/L and were therefore excluded from further analyses. The response rate varied between 4.8% and 66.7% among the countries (Den Hond et al., 2015). Urine samples that were much diluted (<300 mg crea/L) or concentrated (>3000 mg crea/L) were excluded applying the WHO criteria (WHO, 1996). After exclusion of urine samples with creatinine levels <300 mg/L and >3000 mg/L, and cotinine concentrations >49.7 μg/L in non-smokers and children, a total of 1632 women (24–52 years of age; median: 39 years; p25–p75: 35–42 years) and 1689 children (5–12 years of age; median 8 years; p25–p75: 7–10 years) were included in the analysis. In several countries, the inclusion criterion of a maximum age of 45 years of the mothers could not be reached. In total, 44 mothers (2.4% of the study population) above the age of 45 were included in the study. Among the non-smoking and smoking mother–child pairs 51% and 46%, respectively, were living in an urban area.

UCd was positively correlated with urinary creatinine (crea) in both mothers and children. The crea levels were lower in the children (median 1052 mg/L) than in the mothers (medians 1148 and 1296 mg/L in non-smoking and smoking women, respectively). Crea-adjusted UCd concentrations were used in the univariate statistical analyses and for comparisons between countries.

Cadmium was detected above the LOQ in 70% of the children's and in 94% of the mothers' urine samples. In general, smokers had higher geometric mean (gm) UCd than non-smokers ($p<0.0001$) and children, and children had lower gm UCd than their mothers (Table 1). Spearman correlation coefficients were calculated between the individual UCd (μg Cd/g crea) of the mothers and the children. The correlation among non-smoking mother–child pairs (rs=0.31; $n=1248$; $p<0.0001$) was stronger compared to smoking mother–child pairs (rs=0.17; $n=352$; $p=0.0018$; Figure Supplemental material). UCd was significantly increased with age in the mothers ($p<0.001$; Table 1).

3.1. Predictors of cadmium exposure

3.1.1. Univariate analyses

Daily smokers had significantly higher gm UCd ($n=256$) than occasional smokers ($n=104$; $p<0.0001$), and former smokers had significantly higher gm UCd ($n=336$) than never smoking women ($n=936$; $p=0.01$; Table 1). Non-smoking women exposed to environmental tobacco smoke (ETS) at home had significantly higher

Table 1
Cadmium concentrations in urine (UCd; μg/g crea; geometric mean with 95% confidence Intervals) in smoking and non-smoking women, and in children, in relation to various exposure factors (covariates).

	Smoking women, $n=360$	Non-smoking women, $n=1272$	Children, $n=1689$
Overall UCd			
gm (95% CI)	0.24 (0.22; 0.26)	0.18 (0.18; 0.19)	0.065 (0.062; 0.068)
95th-percentile	0.76	0.54	0.23
Age (years)			
5–8			0.066 (0.061; 0.070)
9–12			0.065 (0.061; 0.069)
< 35	0.21 (0.18; 0.24)	0.17 (0.16; 0.19)	
35–40	0.23 (0.21; 0.27)	0.18 (0.17; 0.19)	
> 40	0.31 (0.26; 0.36)	0.20 (0.18; 0.21)	
Smoking			
Daily	0.27 (0.25; 0.30)		
Occasional	0.18 (0.16; 0.21)		
Never		0.18 (0.17; 0.19)	
Former		0.19 (0.18; 0.20)	
Passive smoking			
ETS at home		0.20 (0.18; 0.23)	0.060 (0.052; 0.070)
No ETS at home		0.18 (0.17; 0.19)	0.066 (0.063; 0.070)
Educational level			
Primary	0.35 (0.28; 0.43)	0.22 (0.19; 0.27)	0.066 (0.056; 0.077)
Secondary	0.24 (0.21; 0.27)	0.19 (0.18; 0.21)	0.070 (0.065; 0.075)
Tertiary	0.21 (0.19; 0.24)	0.18 (0.17; 0.18)	0.063 (0.059; 0.066)
Offal consumption			
Several times per month	0.28 (0.23; 0.35)	0.16 (0.13; 0.18)	0.072 (0.060; 0.087)
Once a month or less	0.23 (0.21; 0.25)	0.19 (0.18; 0.19)	0.065 (0.062; 0.068)
Drinking water source			
Public water supply	0.26 (0.24; 0.28)	0.19 (0.18; 0.20)	0.067 (0.064; 0.070)
Commercial products	0.19 (0.15; 0.23)	0.16 (0.15; 0.18)	0.064 (0.057; 0.072)
Well/private water	0.17 (0.09; 0.30)	0.15 (0.13; 0.19)	0.045 (0.036; 0.057)
Area of residence			
Urban	0.25 (0.22; 0.28)	0.18 (0.17; 0.19)	0.062 (0.058; 0.066)
Rural	0.23 (0.21; 0.26)	0.18 (0.17; 0.19)	0.069 (0.064; 0.073)

gm: geometric mean; UCd: urinary cadmium concentration; CI: confidence Interval; and ETS: Environmental Tobacco Smoke

gm UCd ($n=144$) than those who were not ($n=1124$; $p=0.046$; Table 1). No influence of ETS at home or other places on UCd levels was detected in the children.

In smoking women, the levels of UCd increased significantly with decreasing level of education ($p=0.002$; Table 1). The same tendency was seen in non-smoking women and in children, however it was not statistically significant ($p=0.09$ and 0.08, respectively; Table 1).

Smoking women reporting a high consumption of offal (several times per month; $n=55$) had significantly higher gm UCd than those reporting a low consumption ($n=303$; $p=0.04$; Table 1). The same pattern was observed in children; however it was not statistically significant. The opposite was observed in non-smoking women reporting a low consumption of offal (or meat) who had significantly higher gm UCd ($n=1153$) than those reporting a high consumption ($n=111$; $p=0.01$; Table 1). Children reporting a high consumption of chocolate (several times per week) had higher gm UCd (0.067 μg/g crea; $n=1050$) than those reporting a lower consumption (0.062 μg/g crea; $n=634$; $p=0.03$), while children reporting low consumption of fresh water fish (once a month or less) had higher gm UCd than those reporting a high consumption. Interestingly, a reported high consumption of mushrooms (several times per month) was associated with a higher gm UCd in children (0.086 μg/g crea, $n=86$) compared to those with a low consumption (0.064 μg/g crea; $n=1591$), however it was not statistically significant. The same pattern was observed in non-smoking mothers.

Having a public water supply as main drinking water source was associated with a higher gm UCd than having a private well or drinking bottled water, both in non-smoking ($p=0.01$) and smoking women ($p=0.05$). The same pattern was noted in children, however it was not statistically significant ($p=0.07$).

Children living in rural areas had higher gm UCd than children in urban areas ($p=0.01$; Table 1). Also, a reported high consumption of local food (several times per week) was associated with a slightly higher UCd in children ($p=0.12$). Smoking women who spend little time in traffic (1 h per day or less) had higher gm UCd (0.26 μg/g crea; $n=269$) than those who spend more time in traffic (0.19 μg/g crea; $n=77$; $p<0.001$).

No significant associations between reported consumption of cereals or rice, both foods which are possible exposure sources of Cd, and UCd in women or children were detected. Non-smoking mothers having a BMI of 25–30 kg/m^2 (overweight) had slightly higher (non-significant) gm UCd than mothers with a BMI of < 25 kg/m^2 (normal weight) but also slightly higher than obese women (BMI ≥ 30 kg/m^2; $p=0.04$).

3.1.2. Multiple regression models

Significant exposure variables, as evaluated by the univariate analysis, in mothers and children are presented in Table 2. Variables in bold were included in the multiple regression models.

In children, living in a rural area was associated with a 7% higher UCd (Table 3). In non-smoking mothers, higher UCd was associated with former smoking (9% compared to never smoking), ETS at home (14%) and public drinking water source (19%) compared to a private well. In smoking women, higher UCd was associated with daily smoking (41%) compared to occasional smoking and a low education level (48%) compared to tertiary education level (Table 3). As expected, urinary Cd was increasing with age both in smoking and non-smoking women ($p \leq 0.001$), and this was also observed in children ($p=0.04$; Table 3). UCd also increased significantly with increasing concentrations of urinary creatinine ($p<0.001$).

3.1.3. Differences between countries

The gm UCd in children differed by a factor of 7 between the lowest and the highest concentration at the country level (Fig. 1). Children in Denmark and Romania had the lowest gm UCd and United Kingdom and Luxembourg had the highest. In non-smoking and smoking women the difference was 3-fold between the highest and the lowest gm UCd on the country level. Poland had the highest UCd in comparison with the 16 countries, both in smoking and non-smoking women (despite a low percentage of former smokers, 16%, in the non-smoking group), and the third highest in the children (Fig. 1). The second highest for smoking and non-smoking women was Ireland.

It should be noted that those countries that have a high LOQ for UCd, substitution with half LOQ may lead to a higher gm UCd than in countries having a lower LOQ. This could be the case for Cyprus. Only 8% of children´s UCd were above the LOQ of 0.2 μg/L, and 50% and 34% of non-smoking and smoking women´s UCd respectively. In Denmark and Romania, 33% and 5% respectively, of children´s UCd were above LOQ. Still, they were in the lower end of the UCd distribution for all the countries. LOQs for each country are given in the Supplemental material.

Table 2
Variables related to higher urinary cadmium concentrations (UCd μg/g crea) in smoking and non-smoking women, and children; univariate p-values. Variables in **bold** were included in the multiple regression models.

p-Value	Smoking women, $n=360$	Non-smoking women, $n=1272$	Children $n=1689$
$p<0.05$	**Age** **Daily smoking**	**Age** **Former smoking** **ETS at home**	
			Chocolate, high consumption Fresh water fish, low consumption
	Offal, high consumption Game, low consumption	Offal, low consumption Meat, low consumption **Public drinking water**	
	Time spent in traffic, <1 h/day **Educational level, low**		**Rural residence**
$p<0.1$	**Public drinking water** ($p=0.05$)	**Educational level, low** ($p=0.09$)	**Educational level, low** ($p=0.08$) **Public drinking water** ($p=0.07$) No industrial emission ($p=0.08$) **Girl** > **boy** ($p=0.10$)
$p<0.2$	**Rice, high consumption** ($p=0.15$)	Rice, low consumption ($p=0.11$) **BMI, overweight** ($p=0.12$)	**Local food, high consumption** ($p=0.12$) **Soldering at home** ($p=0.15$)

ETS: Environmental Tobacco Smoke; BMI: Body Mass Index.

4. Discussion

For the first time, we have achieved truly comparable measures of Cd in urine on the European level for mother–child couples in 16 countries. As expected, smoking mothers had higher UCd than non-smoking mothers, and children had lower UCd than their mothers. A typical aspect of Cd concentration in urine is the strong influence of age. Since Cd accumulates in the kidney, the Cd concentrations in urine increase with age, which was also observed in this study, both among mothers and children.

Current international health risk assessments are focused on tolerable Cd intake in relation to kidney damage and early signs including proteinuria (EFSA, 2009; WHO, 2011). EFSA suggests a tolerable weekly intake (TWI) of 2.5 μg Cd/kg bw, corresponding to 1.0 μg Cd/g crea in urine. The TWI is set to keep the critical urinary Cd concentration below 1.0 μg Cd/g crea after 50 years of dietary Cd exposure (EFSA, 2009). Based on EFSA's health risk assessment for renal tubular effects, 1.1% of women in the age group 24–52 years (0.6% of non-smoking women and 3.1% of smoking women), and 0.06% of the children 5–12 years, exceeded the TWI. It should be noted here that our data are not representative for the whole European population of women and children of the particular age ranges but merely gives an indication of the exposure situation.

In Germany, the Human Biomonitoring Commission, established in 1992, has defined statistically based reference values (RV_{95}) as well as health related human biomonitoring (HBM) values based on epidemiological data (Schulz et al., 2007). The reference values are ideally based on representative samples of the general population and provide a measure of exposure for the general population (Schulz et al., 2012). The values can then be used for comparison with exposure levels in individuals or population groups. The RV_{95} value appointed by the Commission for UCd in children 3–14 years (0.2 μg/L) was exceeded by 11.5% of the children on the European level, and the RV_{95} value for Cd in non-smoking adults (0.8 μg/L) was exceeded by 3.9% of the non-smoking mothers. The HBM I value for UCd in adults (1 μg/L) corresponds to the EFSA TWI and a low risk for Cd induced proteinuria. The HBM I for children was set to half that value (0.5 μg/L), considering accumulation of Cd with age. In our study, 0.24% of the children exceeded that level. None of the mothers or children in our study exceeded the HBM II values (4 and 2 μg/L, respectively), a level above which there is an increased risk for adverse kidney effects.

Recently, it was suggested that non-renal effects should be considered as critical effects in the health risk assessment of Cd (review by Akesson et al., 2014). It has been questioned if the association between UCd and kidney effect biomarker proteins in urine at very low exposure is due to Cd toxicity, and the clinical significance of slight proteinuria may also be limited (Bernard, 2008; Chaumont et al., 2013). Also, other health effects such as increased risk of osteoporosis and fractures as well as cardiovascular disease and cancer, especially lung cancer and oestrogen-dependent cancers, have been reported at Cd levels that are currently observed in the general population (Tellez-Plaza et al., 2013; Akesson et al., 2014; García-Esquinas et al., 2014). Cross-sectional and prospective studies of decreased bone mineral density and increased risk of osteoporosis and fractures reported associations at UCd levels in the range 0.5–2 μg Cd/g crea (Akesson et al., 2014), demonstrating that the margin between current exposure levels and known as well as suspected health effect levels is quite narrow. In the present study, 16% of smokers, 6.2% of non-smokers and 0.24% of children exceeded 0.5 μg Cd/g crea. Cadmium is also suspected to cause other adverse health effects in humans, also at exposure levels found in the general population, but the results have not been consistent and causality has not been proven. Of particular interest are studies of neurodevelopmental effects in children at low level Cd exposure (Cao et al., 2009; Ciesielski et al., 2012; Kippler et al., 2012a, 2012b).

We evaluated the significance of potential food exposure sources of Cd in smoking and non-smoking women and their children based on questionnaire information. Cadmium is easily taken up by crops such as rice, wheat, vegetables, and potatoes, and widespread low-level Cd contamination of agricultural soil in many areas of the world has led to increased levels in such foods. Cadmium also occurs in high concentrations in shellfish, offal, certain seeds and wild mushrooms (EFSA, 2009; WHO, 2011). Information on Cd occurrence in various foods was collected from 20 European Member States prior to the EFSA health risk assessment of Cd in food. The highest concentrations were detected in seaweed, fish and seafood, chocolate and foods for special dietary uses while the food groups contributing the most, because of high consumption, were cereals and cereal products, vegetables, nuts

Table 3
Determinants of urinary cadmium: multiple regression models in children and mothers.

Parameters	Strata	Estimate (95%CI) for change (multiplicative factor)	*p*-Value	Overall *p*-value
Children				
Number of observations in model: $n=1688$				
Cluster variance: 0.38 ($p=0.004$); residual variance: 0.46; intra-class correlation coefficient: 0.45				
Urinary creatinine level	300–900 mg/L	0.50 (0.46–0.55)	<0.0001	<0.0001
	900–1500 mg/L	0.67 (0.62–0.74)	<0.0001	
	1500–3000 mg/L	1.00	–	
Gender	Boys	0.98 (0.91–1.04)	0.47	0.47
	Girls	1.00	–	
Age	5–8 years	0.93 (0.87–0.998)	0.04	0.04
	9–12 years	1.00	–	
Urban vs rural residency	Urban	0.93 (0.87–0.99)	0.03	0.03
	Rural	1.00	–	
Non-smoking mothers				
Number of observations in model: n=1267				
Cluster variance: 0.09 ($p=0.005$); residual variance: 0.44; intra-class correlation coefficient: 0.17				
Urinary creatinine level	300–900 mg/L	0.33 (0.30–0.37)	<0.0001	<0.0001
	900–1500 mg/L	0.63 (0.58–0.69)	<0.0001	
	1500–3000 mg/L	1.00	–	
Age	$\leq$ 35 years	0.80 (0.72–0.89)	<0.0001	0.0001
	35–40 years	0.89 (0.82–0.97)	0.008	
	$>$ 40 years	1.00	–	
Smoking status	Former	1.09 (1.005–1.19)	0.038	0.038
	Never	1.00	–	
ETS at home	Yes	1.14 (1.01–1.28)	0.036	0.036
	No	1.00	–	
Drinking water source	Public water	1.19 (0.99–1.42)	0.062	0.037
	Commercial water	1.06 (0.86–1.30)	0.574	
	Private well	1.00	–	
Smoking mothers				
Number of observations in model: n=360				
Cluster variance: 0.05 ($p=0.03$); residual variance: 0.48; intra-class correlation coefficient: 0.09				
Urinary creatinine level	300–900 mg/L	0.40 (0.33–0.48)	<0.0001	<0.0001
	900–1500 mg/L	0.63 (0.53–0.75)	<0.0001	
	1500–3000 mg/L	1.00	–	
Age	$\leq$ 35 years	0.68 (0.56–0.83)	0.0001	0.001
	35–40 years	0.78 (0.65–0.93)	0.008	
	$>$ 40 years	1.00	–	
Smoking status	Daily smoker	1.41 (1.18–1.67)	0.0001	0.0001
	Occasional smoker	1.00	–	
Educational level	Primary	1.48 (1.18–1.86)	0.001	0.003
	Secondary	1.15 (0.97–1.36)	0.106	
	Tertiary	1.00	–	

and pulses, starchy roots or potatoes, and meat and meat products (EFSA, 2009).

Questions regarding consumption frequencies of the most common foods were covered in the questionnaire in the current study, however, not in any great detail. Reported consumption of these food groups did not show up as significant determinants of UCd in the multiple regression models. In the univariate analyses, we found a positive association between UCd and a high consumption of offal in smoking women, and a high consumption of chocolate (and mushrooms) in children. Unexpectedly, we found a negative association between UCd and meat consumption in non-smoking women. One explanation could be that a low consumption of meat is a proxy for a diet high in cereals, vegetables, root vegetables and nuts, known to contain Cd, and a diet low in iron, increasing gastrointestinal absorption of Cd because of the concurrent gastrointestinal absorption of Cd and iron in iron deficiency, which is common in women of child-bearing age (Berglund et al., 1994). Another explanation could be related to the excretion of creatinine in urine, which is related to muscle mass but also to meat consumption. Adjusting urinary Cd for creatinine will result in higher UCd in those with a low urinary creatinine concentration (i.e. those who eat less meat) and vice versa. One difficulty in the analyses of UCd data is the strong influence of creatinine excretion and urine dilution (urine volume, sampling period, and timing of sampling) which explain part of the inter-individual variation and give rise to intra-individual variations in UCd (Akerstrom et al., 2014). Despite those difficulties, a standardized sampling time, e.g., a first morning spot urine sample, will take care of part of the inter-individual variations. The variation in creatinine excretion depending on age and sex was further decreased by comparing UCd levels within rather homogenous groups with narrow age spans (i.e. mothers and children).

The only “diet” related variable that was left in the multiple regression models was the use of public water as the main drinking water source, which was associated with higher UCd in non-smoking mothers. The reason for this is not known. Cd in drinking water is the result of natural or anthropogenic contamination or to the release of Cd from joints, pipelines or containers in the drinking water systems. In areas where naturally occurring Cd in the ground is high, or in cases of contamination by agricultural fertilizers, Cd in well water can be a significant source of exposure. In children, rural residency was associated with higher UCd. Factors related to rural living, i.e. time spent in traffic per day (30 min per day or less), no potential environmental Cd contamination from industrial emissions, consumption of local food (several times per week), or using a private well for drinking water were not associated with higher UCd in children. One explanation for the statistically significant association with rural living in children could be exposure to Cd through soil and dust, and/or the use of Cd containing fertilizers, which was not covered by the questionnaire.

Educational level was strongly associated with UCd, and especially in smoking women. Smoking women with primary education (highest level of household) had almost twice as high UCd compared to smoking women with higher education and non-smoking women. Children's UCd was not seemingly affected by the educational level of the household. It is not clear why low education was associated with higher UCd, but possible explanatory factors are related to dietary patterns, SES and living conditions.

There were no obvious differences observed between countries regarding significant exposure predictors, evaluated by the questionnaire, but there were differences in Cd exposure distributions (Fig. 1, Table Supplemental material). Whether these differences are related to differences in national contamination level of the environment or foods available on the market, or to differences in lifestyle or dietary patterns is not clear (Smolders et al., 2015; Sy et al., 2013).

One possible explanation for the higher UCd observed in the Polish study group could be related to a high degree of contamination of agricultural land from the use of fertilizers containing high Cd concentrations. Poland is situated within a region of Europe with naturally low Cd content in soil (<0.05 mg/kg subsoil), sediments and water (FOREGS, 2011). However, a three times higher Cd content in the upper layer of the soil (top soil) than in the deeper layer (subsoil) indicates significant

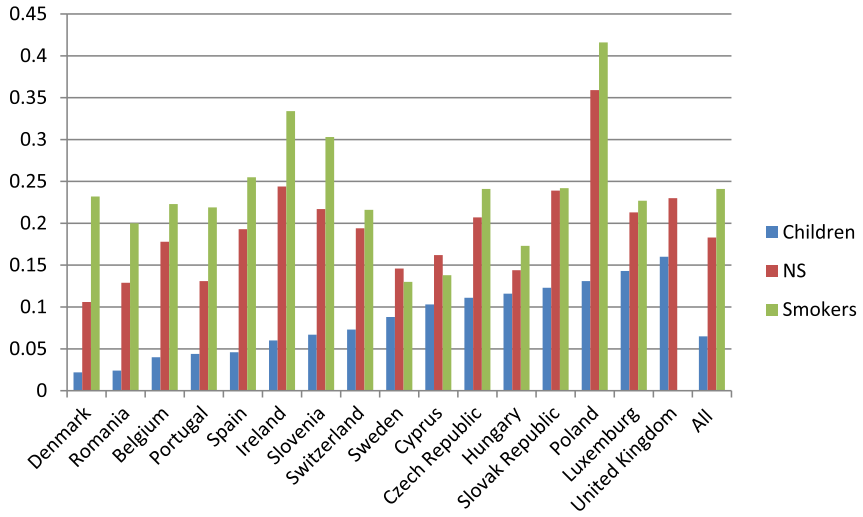

Fig. 1. Urinary Cd (μg/g crea; geometric mean) in smokers, non-smokers (NS) and children in 16 European countries (sorted by children's UCd).

anthropogenic contamination. Indeed, phosphate fertilizers imported from Morocco, containing relatively large amounts of Cd, were used for several years in Poland. The removal of Cd from the environment as well as from the body is very slow, and therefore any addition of Cd to agricultural land has long-lasting consequences for the dietary exposure of the population. Thus, it is important to strictly reduce any further addition of Cd into the food chain.

5. Conclusion

For the first time, we have achieved truly comparable measures of Cd in urine on the European level for mother–child couples in 16 countries. The margin between current exposure levels and known as well as suspected health effect levels is quite narrow. Since food, especially healthy food, is the major Cd exposure source in non-smoking populations, and everyone is exposed via the diet, Cd in food products should be kept as low as possible.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2014.09.042.

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