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Abstract

Background: For Europe as a whole, data on internal exposure to environmental chemicals do not yet exist. Characterization of the internal individual chemical environment is expected to enhance understanding of the environmental threats to health.

Objectives: We developed and applied a harmonized protocol to collect comparable human biomonitoring data all over Europe.

Methods: In 17 European countries, we measured mercury in hair and cotinine, phthalate metabolites and cadmium in urine of 1844 children (5-11 years) and their mothers. Specimens were collected over a 5 month period in 2011-2012. We obtained information on personal characteristics, environment, and life style. We used the resulting database to compare concentrations of exposure biomarkers within Europe, to identify determinants of exposure, and to compare exposure biomarkers with health-based guidelines.

Results: Biomarker concentrations showed a wide variability in the European population. However, levels in children and mothers were highly correlated. Most biomarker concentrations were below the health-based guidance values.

Conclusions: We have taken the first steps to assess personal chemical exposures in Europe as a whole. Key success factors were the harmonised protocol development, intensive training and capacity building for field work, chemical analysis and communication, as well as stringent quality control programs for chemical and data analysis. Our project demonstrates the feasibility of a European-wide human biomonitoring framework to support the decision-making process of environmental measures to protect public health.

Introduction

Human biomonitoring (HBM) measures the levels of environmental chemicals or their metabolites in easily accessible body fluids and tissues (Angerer et al. 2006), and reflects all routes of uptake - oral, dermal, inhalative - and all relevant sources. The power of HBM to identify spatial and temporal trends in human exposures has contributed successfully to initiate policy measures and to focus on protection of susceptible populations such as children and pregnant mothers. The ban of lead from gasoline was triggered by elevated blood lead levels in the National Health and Nutrition Examination Survey (NHANES) (Pirkle et al. 1994). Results of the German Environmental Survey (GerES) led to recommendations to avoid mercury containing amalgam teeth fillings in children (Becker et al. 2013) and contributed to restriction of phthalate use in plastics (Goen et al. 2011). Increasing levels of polybrominated diphenylethers (PBDEs) in maternal milk samples of Sweden have lead to the gradual phasing out of lower PBDEs (Meironyte et al. 1999).

Experience with human biomonitoring in the general population differs among European countries, with long standing traditions in countries such as Germany (Becker et al. 2008), France (Frery et al. 2012), the Czech Republic (Cerna et al. 2012), Belgium (Flanders)(Schoeters et al. 2011), Spain (Perez-Gomez, 2013) while other countries have no experience at all.

The 'European Environment and Health Action Plan' (Communication from the Commission, 2004) prioritized the need to harmonize HBM in Europe to allow comparison of data among countries and provide tools for follow-up of temporal and spatial trends in chemical exposures. The preparation of the protocol, including the selection of chemicals, study populations, started in 2005 with the Expert team to Support BIOmonitoring in Europe (ESBIO) project. With the

funding of the Consortium to Perform Human Biomonitoring on a European Scale (COPHES) and its demonstration project DEMOCOPHES, the feasibility of a harmonized HBM approach was tested(Human Biomonitoring in Europe, 2012). COPHES designed the final protocol and made justified choices for exposure biomarkers, sample size and recruitment strategy. DEMOCOPHES allowed 17 European countries to put this protocol into practice. Selected chemicals included phthalates that are present in some consumer products and foodpackaging (Koch and Calafat 2009), mercury and cadmium as ubiquitous developmental toxicants of concern (Grandjean and Landrigan 2006), urinary cotinine (Avila-Tang et al. 2013) as a biomarker for exposure to cigarette smoke; urinary creatinine was included as a measure for urine dilution. Young children and mothers of childbearing age were selected as vulnerable age groups. Mercury in hair (Budtz-Jorgensen et al. 2004) and urinary cadmium (Akerstrom et al. 2013) are markers of chemicals that accumulate in the body over a longer time period, urinary phthalate metabolites (Wittassek et al. 2011) and cotinine (Avila-Tang et al. 2013) measured in spot urine samples represent short-time exposure.

Methods

Study design and participants

The cross-sectional survey was designed to include 120 children (5-11 years) and their mothers in each country, with 60 mother-child pairs in Cyprus and Luxembourg due to the countries' smaller populations. We sampled the children and mothersbetween September 2011 and February 2012, either through schools or population registries. These were convenience samples with equal shares in an urban and a rural location as defined according to regional standards. We included only healthy children and mothers (no metabolic disturbances), who had sufficient knowledge of the local language and had been living at least for 5 years at the sampling location.

Details and rationale for the study design are reported by Becker et al. (Becker et al. 2014). The sample size allowed us to estimate preliminary country specific reference values (Poulsen et al. 1997) and a minimally important difference in mean biomarker values of 30% between countries (α= 0.05, β= 0.80). Field workers from the national study centers were trained, instructions were provided centrally and adapted at national level to the language, cultural conventions, ethical and legal requirements. Information on characteristics of the study population and potential determinants of internal exposure were obtained through personalised interviews using questionnaires. Standard Operation Procedures (SOPs) to collect hair and morning urine samples were implemented (Becker et al. 2014). The study was approved by ethics committees in each country (list of ethics committees per country: see Supplemental Material, Table S1); mothers and children gave written informed consent or assent, respectively. All procedures followed the national data protection requirements including notification to data protection authorities.

Chemical analysis

We established a Quality Assurance Program to guarantee the quality and comparability of analytical results among laboratories (Schindler et al. 2014). Each participating laboratory received SOPs for sampling, sample conservation and chemical analysis (Becker et al. 2014; Schindler et al. 2014). We organized two Interlaboratory Comparison Investigations and two External Quality Assessment Schemes (ICI/EQUAS) with native control material (hair, urine) sent to all laboratories willing to participate. For the evaluation of the ICIs we calculated consensus values as the mean of the results of the participating laboratories (after exclusion of outliers). For the evaluation of the EQUAS, we calculated assigned values (target values) from the results of experienced, renowned reference laboratories. Laboratories were defined as 'qualified laboratories' if they participated successfully in at least one ICI and one EQUAS

round or in two EQUAS rounds (Schindler et al. 2014). The number of laboratories that qualified for each analyte was: mercury, 15; cotinine, 9; cadmium, 14; phthalate metabolites (MEHP, 5OH-MEPH, 5oxo-MEHP, MEP, MBzP, MnBP, MuBP), 7 and creatinine, 14.

Database management and statistical analysis

National data centers applied uniform rules for database construction by using one centrally developed code book with pre-defined variable names, unities, formats and coding rules. Quality controls on the data were performed with centrally developed programs (SAS or SPSS). These strict and uniform rules for database construction allowed us to pool all country-specific data into one central European database. We used SAS software, version 9.3 (SAS Institute Inc.) for analysis of the central database. We replaced values below the LOQ by ½LOQ and transformed biomarker data to natural log-transformed concentrations (ln). We excluded samples with creatinine concentrations below 300 mg/L or above 3000 mg/L from statistical analysis (WHO,1996). We calculated weighted geometric means (GM) (95% confidence interval, 95%CI) and 90th percentiles (P90) (95% CI) so that the countries were equally represented except for Cyprus and Luxembourg that contributed only half. Using multiple mixed regression models with country as random factor, we identified determinants of exposure biomarkers by including pre-specified confounders and significant covariates ($P<0.25$ from univariate model to enter and $P<0.05$ to stay) in a stepwise model. We expressed urinary biomarkers in µg/L with urinary creatinine included as confounder. We expressed results as % change (95% CI) of biomarker concentration for change of the determinant, after adjustment for all other variables in the model. Detailed methodology and full models are given in the Supplemental Material ("Identification of determinants of exposure; Comparison of results between countries" and Table S2).

To compare biomarker values among countries, we compared the GM of a country with the European GM by mixed linear regression analysis, after adjustment for pre-specified confounders (Figure 1). To visualize similarity between the biomarker levels and between different countries and/or mothers and children from the same country a heat map was generated using the clustergram function (Matlab, The MathWorks Inc. Massachusetts, USA) (Figure 2). Hierarchical clustering with Euclidean distance metric and average linkage was used to generate the hierarchical tree. Prior to analysis the GM of each country was divided by the European GM. The ratio was calculated for mothers and children separately and was logarithmically transformed ($\log_2$ base) to obtain symmetry around 0 (= $\log_2(1)$). The nearest-neighbor method was applied to impute missing data.

To put the results in a health risk context, we calculated the proportion of individuals with levels above health-based guidance values (Aylward et al. 2009a;Aylward et al. 2009b;Hays et al. 2008;Joint Expert Committee on Food Additives 2003;Schulz et al. 2012).

Results

Determinants of biomarker concentrations

Descriptive statistics of 1844 children and mothers included in the study are given in Table 1. Participants were equally recruited according to predefined strata of gender, age and sampling area in each country. Descriptive statistics of the biomarkers and multiple regression models are given in the Supplemental Material (Tables S3-S19).

Fish consumption was the major predictor of mercury levels in hair, both in children and in mothers (Supplemental Material, Tables S4 and S5). Consumption of sea fish, shellfish or fresh water fish in the past four weeks independently contributed to mercury levels in the body. In

multiple regression models, frequent (several times/week) compared to sporadic (once/week or less) sea fish consumption was associated with 46% (95% CI: 26-69%) higher mercury levels in children and 51% (34-71%) in mothers; shellfish with 56% (35-79%) in children and 38% (24-55%) in mothers, fresh water fish with 23% (8-39%) in children and 23% (11-37%) in mothers. The GM mercury levels of mothers were higher than those of the children (Table 2), but levels of mothers and children were higly correlated (Spearman's r = 0.72, $p<0.001$, n= 1833). Older mothers had 15% (5-24%) higher levels compared to the youngest age group (Supplemental Material, Table S5). Younger children of 5-8 years showed 8% (0-17%) higher levels compared to the older group of 9-11 years (Supplemental Material, Table S4). Participants from families with a higher educational level (tertiary vs. primary education) had 19% (4-31%) higher levels of mercury in children and 25% (13-36%) in mothers

Cadmium levels in mothers were significantly higher in active smoking mothers and this was independent of age. The GM were higher in mothers than in children (Table 2). Older mothers had 25% (18-32%) higher levels than younger mothers (Supplemental Material, Table S9). Levels in mothers and children showed a low but significant correlation (Spearman's r = 0.24, $p<0.001$, n= 1660). After adjustment for age and smoking, mothers from families with a tertiary education had 34% (17-54%) lower levels compared to those with a primary education. In children, except for age and creatinine, no significant determinants were identified (Supplemental Material, Table S8).

The urinary levels of MEHP, 5OH-MEHP and 5oxo-MEH were highly correlated (Pearson's $r>0.70$), and thus their sum was used in the analyses. The GM of urinary phthalate metabolites (except MEP, related to PCP use) were higher in children than in mothers (Table 2). Phthalate levels of mothers and children were significantly correlated ($p<0.001$): Spearman's r ranged

between 0.40 and 0.49. Multiple regression models (Table 3) showed that younger children of 5-8 years showed higher levels compared to the older group of 9-11 years. Participants from families reporting to have PVC floors or walls, had significantly increased levels of MBzP and MiBP in children and mothers and of MnBP in children (Table 3 and 4). A small effect of recent renovation works on MiBP was seen in mothers who reported renovation in the house in the past two years. Frequent use of personal care products (PCP) increased urinary MEP levels in mothers and children and urinary MiBP levels in children. Unexpectedly, urinary levels of DEHP metabolites and MnBP in mothers were lower in high PCP users. High consumption of ice cream was associated with higher urinary levels of DEHP metabolites and MBzP levels in children and with higher MnBP and MBzP levels in mothers. High consumption of chewing gum was related to higher urinary levels of DEHP metabolites in children and to higher MEP levels in mothers. After adjustment for confounders and significant covariates, educational level was still a predictor of phthalate biomarkers, i.e. significantly higher urinary levels were found for DEHP metabolites in mothers from families with a primary education, for MiBP (mothers) and MEP (children) in families with secondary education and for MnBP (children) in families with tertiary education.

In mothers, the effect of active smoking on cotinine levels was dominant (Supplemental Material, Table S7). Levels in mothers and children correlated strongly (Spearman's $r = 0.71$, $p<0.001$, $n= 1777$). The younger children of 5-8 years showed 16% (8-25%) higher levels compared to the older group of 9-11 years (Supplemental Material, Table S6). In children, environmental tobacco smoke (ETS) at home was the strongest predictor. Compared to children who were never exposed to ETS at home, children with daily exposure had 5 times higher values (+504% (429-593%)) and children with less than daily exposure had almost double values

(+181% (155-211%)). Exposure to ETS in other places than home resulted in 19% (10-29%) higher values. In comparison with children from families with a tertiary education, those with a secondary education had 20% (10-30%) higher cotinine levels in urine and those with primary education had 49% (29-72%) higher values.

The geographical aspect

Residence in urban or rural area did not show up as a significant determinant of internal exposure at the EU level. Only mercury in hair showed, independently of fish consumption, higher levels in urban areas compared to rural areas: 35% (23-47%) higher in children and 30% (19-41%) in mothers (Supplemental Material, Table S4 and S5).

The average biomarker concentrations varied significantly among the European countries. This holds for the unadjusted data (Supplemental Material, Table S20-S35) and for data after adjustment for age, gender and weighing for equal group sizes (Figure 1). The average biomarker concentrations of mercury in hair of Spanish and Portugese children were respectively 6 and 7 times higher than the European average. Cadmium varied less among the countries: average urinary cadmium levels in Polish and Slovak mothers were respectively 1.9 and 1.7 times higher than the European average. In Romania and Hungary average cotinine levels were respectively 2.4 and 2.2 times higher than the European average reflecting the weak anti-smoking legislation in these countries. Swedish children had on average 3 times higher urinary MBzP levels than the average European value. Slovak children had almost twice the average European biomarker concentrations of DEHP metabolites, while Polish children showed the highest average levels of MnBP and MiBP. Average MEP levels in Spain were 6 times higher than the European average. The heat map (Figure 2) showed that biomarker data from mothers and children clustered

together except in Czech Republic and Slovakia. Overall the biomarker clustering followed geographical grouping. The South European countries (ES, PT) clustered separately from the other countries; Eastern European countries (RO, HU, PL, CZ, SK) formed a further cluster; West European countries (DE, BE, LU, DK) also showed fairly good resemblance.

Although the sampling frame of the European biomonitoring program differs from that of the US national program, the geometric means and P90 of COPHES/DEMOCOPHES are well in line with the results obtained in NHANES (Center for Disease Control (CDC) 2013; McDowell et al. 2004) (Table 2). For MiBP, higher values were observed in Europe compared to the US (factor 3-4), both in mothers and children (Table 2). Differences for other biomarkers were modest with a trend in Europe for lower biomarker concentrations of MBzP and MEP, higher concentrations of MnBP and DEHP and similar levels for cadmium and mercury.

Available health-based guidance values allow to put the observed biomarker concentrations in a risk context. Few participants exceeded these values (Table 2). The P90 of the biomarker values are far below the guidance values, only for urinary cadmium P90 of mothers and children were within a factor two of the concentration below which no risk for adverse health effects is expected (Schulz et al. 2012) and for mercury they are below a factor three.

Discussion

This first Europe-wide program provides biomarker data from mothers and children of 17 European countries. Since we recruited in one rural and one urban area per country, our sample was not representative for the EU population.Yet, the recruited sample had a similar smoking behavior as the average European population (Currie 2010). Also, the countries ranked for their reported fish consumption according to national statistics (FAO,2008). The educational level of

the participants was skewed towards a higher educational level. The study design allowed to conclude that exposure to mercury, cadmium, phthalates, and nicotine is widespread in the European population.

Differences in environment and life style influenced individual biomarker values and country specific averages. If we compared average levels between countries, the biomarker patterns varied according to geographic trends. Yet, few study participants exceeded the available health-based guidance values. The major strength of our study is comparable data from 17 European countries produced through a harmonized process including the use of a commonly developed protocol, intensive training and capacity building for field work, chemical analyses, reporting and communication, as well as stringent quality control programs for chemical and data analysis. This allowed us to measure both well-known pollutants such as cadmium, cotinine or mercury and new emerging chemicals such as phthalates.

Our current study identified younger children as more exposed to phthalates (except MEP), cotinine and mercury. These results are in line with US data for exposure to phthalates (Silva et al.,2004) and ETS (Bernert et al.,2010). The underlying reasons cannot be derived from this study but may be explained by higher exposure relative to body size through inhalation of dust or food intake; by typical exposure patterns in children, e.g. contact with toys, more time spent on the floor, more frequent hand-mouth contact; or by differences in metabolism. Additionally, the higher cotinine levels in younger children might be due to the fact that they spend more time at home, and thus may be more exposed to nicotine, since smoking in public buildings is much more controlled than in private homes. We observed a significant influence of social class (represented by the highest educational level within the family) on each of the biomarker levels even after adjustment for confounders and significant covariates: mercury level in hair increased

in children and mothers if social class was higher, while cotinine, cadmium, phthalate metabolites were lower with increasing educational level of the family. Possibly, underlying life-style factors that vary with socio-economic status, and were not considered in the questionnaires, may account for these findings. These associations between social class and biomarker concentrations are in line with US data (Tyrrell et al. 2013) and may partly be mediated by smoking, occupation and diet (fish consumption, local food, convenience food). Our findings, like others, thus indicate that public health remediation measures to decrease environmental exposure and disease burden within a society should be stratified according to age groups and social strata within the population.

Fish consumption and social status were identified as important and independent determinants of mercury levels, both in mothers and children. This is in line with results from several populations with moderate to high fish consumption (Deroma et al. 2013). Mercury levels in children and in women of childbearing age are important parameters to monitor since pre- and postnatal mercury exposure, even at low levels, has adverse neurodevelopmental effects (Karagas et al. 2012). Although several high fish consuming countries such as France, Finland, Lithuania, Malta and Italy are not participating in DEMOCOPHES, at present, 1.4% of the children and 3.4% of the mothers in our study population had mercury levels above the JECFA/WHO provisional threshold value of 2.3 µg/g hair. This proportion differs considerably by country with 0% of participants exceeding the threshold in most northern and central European countries and up to 33% of the mothers with levels above the safe dose in countries with high fish consumption with implications for loss of IQ points and costs (Bellanger et al. 2013). If these data urge policy makers to take actions, current biomarker concentrations can be used as baseline for follow-up, both for the exposure of the population and the environment. The major exposure route for

DEHP is food (Koch and Calafat 2009). Therefore, we were not surprised to find an association between DEHP metabolites with chewing gum and ice cream consumption. Most probably, these two food items are not specific sources, but rather represent predilection for flavored, packed, or processed food, and thus may be proxies for convenience food. The association between urinary MBzP and PVC materials in the home is in accordance with recent findings in children (Carlstedt et al. 2012). Although high molecular weight phthalates like DEHP are the major phthalates used in PVC, no association was found between the presence of PVC at home and urinary DEHP metabolites. Given the facts that DEHP exposure is dominated by foodstuff (Koch et al. 2013) and that DEHP house dust does not correlate with DEHP body burden (Becker et al. 2004), a significant correlation was not really expected. The lower levels of DEHP metabolites and MnBP in mothers that were high PCP users was not expected and may relate to cross correlation with other personal habits. The relative levels of phthalate metabolites differ substantially among countries which points to different sources, products on the market or behavior characteristics. Despite legal restrictions on the use of DEHP, di-*n*-butyl phthalate, and diisobutyl phthalate as imposed by EU directives, these compounds are still ubiquitous in Europeans. They are short-lived in the body, implying that exposures to these compounds are still part of current daily life. Diethylphthalate, one of the principal phthalates in cosmetic products (Koch and Calafat 2009), is not yet restricted. High levels of its metabolite MEP were found.

The health impact of cigarette smoking is well documuented (U.S.Department of Health and Human Services 2004). The home environment appears to be the most important predictor of the cotinine levels in children. Further awareness of parents therefor is needed. The importance of anti-smoke legislation pays off as countries with a stronger legislation that was longer in place

showed the lowest cotinine levels (http://ec.europa.eu/health/tobacco/docs/tobacco_overview2011_en.pdf). The effectiveness of anti-smoke legislation on health outcomes has been demonstrated on a population level (Cox et al. 2013).

Conclusion

This HBM study presents the first steps, for Europe as a whole, to register internal chemical exposures at individual level. Although the sampling protocol is not yet reprentative for the geographivcal distribution of the population in the country, the results show remarkable differences in the biomarker concentration profiles by country residence. Personal habits and life style are strong determinants of internal exposure. The harmonized protocols and stringent quality control measures ensure that these are true differences, not related to variability in protocols, analytical measurements, or interpretation. These data offer policy makers direct means to evaluate whether implementation of protective measures and legislations related to chemicals are adequate to protect health of the entire population or whether they need to be adjusted.

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Table 1. Descriptive statistics of the study population.

	Children			Mothers		
	N	**Median (P25-P75)**	**Min.-max.**	**N**	**Median (P25-P75)**	**Min.-max.**
Age (years)	1844	8 (7,10)	5-12	1844	39 (35,42)	24 -52
Urinary creatinine (mg/L)	1842	1053 (784,1426)	10-3120	1839	1163 (781,1618)	57-3670
Body height (cm)	1819	135 (127,145)	98-170	1836	166 (161,170)	145-191
Body weight (kg)	1820	30 (25,36)	14-81	1836	64 (58,72)	35-186
Body-mass index (kg/m²)	1811	16.3 (14.9,18.2)	10.0-36.1	1833	23.2 (21.1,26.3)	14.7-62.2
	Children			**Mothers**		
	N	**Categories**	**N (%)**	**N**	**Categories**	**N (%)**
Gender	1844	Boy Girl	912 (49.5%) 932 (50.5%)	1844	Woman	1844 (100%)
Area of residence	1844	Rural Urban	923 (50.1%) 921 (49.9%)	1844	*as in children*	
Highest educational level of the family	1843	Primary (ISCED 0-2) Secondary (ISCED 3-4) Tertiary (ISCED 5-6)	166 (9.0%) 607 (32.9%) 1070 (58.1%)	1843	*as in children*	
Smoking habits	1844	Smoker	0 (0%)	1844	Daily smoker	283 (15.3%)
		Non-smoker	1844 (100%)		Occasional smoker	106 (5.7%)
					Former smoker	401 (21.7%)
					Never smoker	1054 (57.2%)
ETS at home (non-smokers only)	1842	Daily Less than daily Never	179 (9.7%) 130 (7.1%) 1533 (83.2%)	1450	Yes No	162 (11.2%) 1288 (88.8%)
ETS elsewhere (non-smokers only)	1842	Yes No	775 (42.1%) 1067 (57.9%)	1455	Yes No	827 (56.8%) 628 (43.2%)
ETS in last 24 hours (non-smokers only)	1840	Yes No	232 (12.6%) 1608 (87.4%)	1450	Yes No	164 (11.3%) 1286 (88.7%)

	Children			Mothers		
	N	**Categories**	**N (%)**	**N**	**Categories**	**N (%)**
Fish consumption (all types)	1844	Several times/week Once a week or less	442 (24.0%) 1402 (76.0%)	1844	Several times/week Once a week or less	483 (26.2%) 1361 (73.8%)
Consumption of seafish	1840	Several times/week Once a week or less	283 (15.4%) 1557 (84.6%)	1840	Several times/week Once a week or less	294 (16.0%) 1546 (84.0%)
Consumption of shellfish	1820	Several times/week Once a week or less	194 (10.7%) 1626 (89.3%)	1826	Several times/week Once a week or less	355 (19.4%) 1471 (80.6%)
Consumption of fresh water fish	1815	Several times/week Once a week or less	248 (13.7%) 1567 (86.3%)	1818	Several times/week Once a week or less	298 (16.4%) 1520 (83.6%)
Consumption of sea food products	1811	Several times/month Once a month or less	94 (5.2%) 1717 (94.8%)	1811	Several times/month Once a month or less	154 (8.5%) 1657 (91.5%)
Consumption of ice cream	1821	Several times/week Once a week or less	185 (10.2%) 1636 (89.8%)	1829	Several times/month Once a month or less	536 (29.3%) 1293 (70.7%)
Consumption of chewing gum	1662	Several times/week Once a week or less	578 (34.8%) 1084 (65.2%)	1675	Several times/week Once a week or less	626 (37.4%) 1049 (62.6%)
Use of personal care products[a]	1816	High or moderate Low	822 (45.3%) 994 (54.7%)	1806	High Moderate or low	861 (47.7%) 945 (52.3%)
PVC in house	1773	PVC in floors or walls No PVC	342 (19.3%) 1431 (80.7%)	1773	*as in children*	

P25: 25th percentile; P75: 75th percentile; N: number; ISCED: International Standard Classification of Education; ETS: environmental tobacco smoke; PVC: polyvinyl chloride

[a]Use of personal care products (PCP) is calculated as a score based on the frequency (never to daily) of 9 PCP groups (make-up, eye make-up, shampoo, hair styling products, body lotions and creams, fragrances, deodorant, massage oil and nail polish).

Table 2. European exposure values in children and mothers in COPHES/DEMOCOPHES study.

Biomarker of exposure	**COPHES/DEMOCOPHES study**					**NHANES**[d]			
	N	**% >LOQ**[a]	**GM (95% CI)**[b]	**P90 (95% CI)**[b]	**N (%) exceeding guidance value**[c]	**Period**	**N**	**GM (95% CI)**	**P90 (95% CI)**
Mercury in hair (µg/g)	1836	85.9%	0.145 (0.139,0.151)	0.800 (0.698,0.917)	JECFA: N=25 (1.4%)	1999-2000	838	0.12 (0.10,0.12)	0.41
Urinary cotinine (µg/L)	1818	57.6%	0.80 (0.76,0.84)	4.90 (3.90,6.16)	-	-	-	-	-
Urinary cadmium (µg/L)	1698	70.1%	0.071 (0.069,0.074)	0.220 (0.209,0.232)	HBM-I: N=6 (0.4%) HBM-II: N=0 (0.0%) BE: N=0 (0.0%)	2009-2010	415	0.057 (0.053,0.061)	0.130 (0.120,0.160)
Urinary DEHP metabolites (µg/L) (5)	1816	85.6%	47.6 (46.0,49.3)	137 (126,150)	HBM-I: N=12 (0.6%) BE: N=53 (2.9%)	2009-2010	415	MEHP: 1.64 (1.45,1.85) 5OH-MEHP: 15.0 (13.2,17.1) 5oxo-MEHP: 9.87 (8.72,11.0) ∑(GM)=26.5	
Urinary MEP (µg/L)	1816	98.0%	34.4 (32.8,36.0)	159 (138,183)	BE: N=0 (0.0%)	2009-2010	415	35.2 (31.2,39.8)	151 (114,207)
Urinary MBzP (µg/L)	1816	95.2%	7.1 (6.8,7.5)	27.8 (25.2,30.6)	BE: N=0 (0.0%)	2009-2010	415	11.6 (9.51,14.1)	63.9 (47.4,76.8)
Urinary MnBP (µg/L)	1355	99.9%	34.8 (33.5,36.2)	95.5 (87.3,104.5)	-	2009-2010	415	21.7 (19.0,24.8)	83.8 (59.6,121)
Urinary MiBP (µg/L)	1355	99.8%	45.4 (43.6,47.3)	131 (117,147)	-	2009-2010	415	10.2 (9.10,11.4)	35.7 (28.8,46.9)
Mercury in hair (µg/g)	1839	90.5%	0·225 (0·216,0·234)	1.200 (1.068,1.349)	JECFA: N=62 (3.4%)	1999-2000	1726	0.20 (0.16,0.24)	1.11
Urinary cotinine (µg/L)	1800	62.4%	2.75 (2.41,3.14)	1182 (974,1434)	-	-	-	-	-

Biomarker of exposure	COPHES/DEMOCOPHES study					NHANES[d]			
	N	% >LOQ[a]	GM (95% CI)[b]	P90 (95% CI)[b]	N (%) exceeding guidance value[c]	Period	N	GM (95% CI)	P90 (95% CI)
Urinary cadmium (µg/L)	1685	93.8%	0.219 (0.211,0.228)	0.620 (0.580,0.663)	HBM-I: N=49 (2.9%) HBM-II: N=0 (0.0%) BE: N=26 (1.5%)	2009-2010	1450	0.188 (0.172,0.206)	0·740 (0·620,0·880)
Urinary DEHP metabolites (µg/L) [e]	1800	81.6%	29.2 (28.1,30.3)	91 (84,100)	HBM-I: N=19 (1.0%) BE: N=28 (1.5%)	2009-2010	1350	MEHP: 1.39 (1.21,1.60) 5OH-MEHP: 11.0 (9.58,12.8) 5oxo-MEHP: 7.09 (6.17,8.14) ∑(GM)=19.5	
Urinary MEP (µg/L)	1800	95.2%	48.2 (45.6,51.0)	252 (221,287)	BE: N=0 (0.0%)	2009-2010	1350	67.8 (60.3,76.4)	548 (392,675)
Urinary MBzP (µg/L)	1800	91.8%	4.5 (4.3,4.7)	17·7 (16.1,19.5)	BE: N=0 (0.0%)	2009-2010	1350	6.04 (5.38,6.77)	29.3 (24.5,36.9)
Urinary MnBP (µg/L)	1347	99.4%	23.9 (23.0,24.9)	66.2 (60.5,72.4)	-	2009-2010	1350	14.7 (13.1,16.5)	57.7 (52.7,63.9)
Urinary MiBP (µg/L)	1347	99.4%	30.1 (28.9,31.4)	88 (81,96)	-	2009-2010	1350	7.50 (6.68,8.43)	29.1 (25.3,33.5)

LOQ: limit of quantification; N: number; GM: geometric mean; 95% CI: 95% confidence interval; P90: 90th percentile; DEHP: di(2-ethylhexyl)phthalate; MEP: mono-ethyl phthalate; MBzP: mono-benzyl phthalate; MnBP: mono-n-butyl phthalate; MiBP: mono-iso-butyl phthalate; JECFA: Joint FAO/WHO Expert Committee on Food Additives; HBM-I human biomonitoring value I; HBM-II: human biomonitoring value II; BE: biomonitoring equivalent; ∑(GM): sum of geometric means of MEHP, 5OH-MEHP and 5oxo-MEHP.

[a]Limits of quantification (LOQs) ranged between 0.001 to 0.137 µg/g for mercury in hair, 0.1-1.2 µg/L for urinary cotinine, 0.001-0.2 µg/L for urinary cadmium, 0.3-3.9 µg/L for urinary MEHP, 0.1-9.2 µg/L for urinary 5OH-MEHP, 0.1-6.2 µg/L for urinary 5oxo-MEHP, 0.5-11 µg/L for urinary MEP, 0.2-5 µg/L for urinary MBzP, 0.5-4.4 µg/L for urinary MnBP and 0.5-4.9 µg/L for urinary MiBP. [b]Geometric means and 90th percentiles are weighed but not adjusted for confounders (see methods). [c]Health-based exposure values are available for mercury: JECFA guideline = 2.3 µg/g (Joint Expert Committee on Food Additives 2003); cadmium: HBM-I in children = 0.5 µg/L; HBM-II in children = 1 µg/L;

HBM-I in adults = 1.0 µg/L; HBM-II in adults = 4.0 µg/L (Schulz et al. 2012); BE in children and in mothers = 1.2 µg/L (Hays et al. 2008); phthalate metabolites: HBM-I value for DEHP metabolites are based on the sum of 5OH-MEHP and 5oxo-MEHP and equal 500 µg/L in children and 300 µg/L in adults (Schulz et al. 2012); BE's for DEHP metabolites are based on the sum of MEHP, 5OH-MEHP and 5oxo-MEHP and equal 260 µg/L in children and in mothers (Aylward et al. 2009a); BE for MEP in mothers and children = 18 mg/L (Aylward et al. 2009b); BE for MBzP in children and adults = 3.8 mg/L (Aylward et al. 2009b). [d]NHANES: data of urinary cadmium and urinary phthalate metabolites from 'The Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, March 2013' (Center for Disease Control (CDC) 2013); data of mercury in hair: from McDowell et all. (McDowell et al. 2004) Data of COPHES/DEMOCOPHES children are compared with NHANES subgroup 'Age group 6-11 years'; data of COPHES/DEMOCOPHES mothers are compared with NHANES subgroup 'Females'. [e]Urinary DEHP metabolites: sum of MEHP, 5OH-MEHP and 5oxo-MEHP.

Table 3. Determinants of exposure to urinary phthalate metabolites (µg/L): multiple regression models in children.

		Estimate (95% CI) for change (multiplicative factor)				
Parameters	**Strata**	**DEHP**	**MEP**	**MBzP**	**MnBP**	**MiBP**
Age[b]	5-8 years 9-11 years	1.19 (1.11, 1.27) 1.00	1.15 (1.04, 1.26) 1.00	1.15 (1.06, 1.26) 1.00	1.15 (1.07, 1.24) 1.00	1.19 (1.10, 1.28) 1.00
Gender[b]	boys girls	ns	ns	ns	0.91 (0.85, 0.98) 1.00	0.92 (0.85, 0.99) 1.00
Urinary creatinine level[b]	300-900 mg/L 900-1500 mg/L 1500-3000 mg/L	0.46 (0.42,0.51) 0.75 (0.69, 0.83) 1.00	0.41 (0.36, 0.47) 0.68 (0.61, 0.77) 1.00	0.41 (0.37, 0.47) 0.69 (0.62, 0.78) 1.00	0.45 (0.41, 0.50) 0.73 (0.66, 0.81) 1.00	0.45 (0.40, 0.50) 0.72 (0.65, 0.80) 1.00
Urine sampling period	<10 hours 10-11 hours ≥11 hours	ns	1.20 (1.06, 1.35) 1.14 (1.02, 1.29) 1.00	ns	ns	ns
Morning urine	yes no	ns	ns	1.98 (1.17, 3.36) 1.00	ns	ns
Educational level of the family	primary secondary tertiary	ns	0.91 (0.81, 1.03) 0.89 (0.82, 0.97) 1.00	ns	0.91 (0.81, 1.03) 0.89 (0.82, 0.97) 1.00	ns
Use of personal care products[a]	moderate to high use low use	ns	1.24 (1.13, 1.37) 1.00	ns	ns	1.13 (1.03, 1.23) 1.00
Ice cream consumption	several times/week once/week or less	1.12 (1.01, 1.25) 1.00	ns	1.18 (1.02, 1.36) 1.00	ns	ns
Gum consumption	several times/week once/week or less	1.10 (1.02, 1.18) 1.00	ns	ns	ns	ns
PVC in floors/walls	yes no	ns	ns	1.50 (1.34, 1.68) 1.00	1.19 (1.08, 1.32) 1.00	1.22 (1.09, 1.35) 1.00

[a]Use of personal care products (PCP) is calculated as a score based on the frequency (never to daily) of 9 PCP groups (make-up, eye make-up, shampoo, hair styling products, body lotions and creams, fragrances, deodorant, massage oil and nail polish). [b]The confounders urinary creatinine level, gender, and age were forced in the multiple regression models, even if not significant.

Table 4. Determinants of exposure to urinary phthalate metabolites (µg/L): multiple regression models in mothers.

		Estimate (95% CI) for change (multiplicative factor)				
Parameters	**Strata**	**DEHP**	**MEP**	**MBzP**	**MnBP**	**MiBP**
Age[b]	≤35 years 35-40 years >40 years	ns	ns	ns	0.81 (0.73, 0.89) 0.93 (0.86, 1.01) 1.00	ns
Body-mass index	normal weight overweight obese	ns	ns	ns	1.15 (1.02, 1.29) 1.09 (0.96, 1.24) 1.00	ns
Urinary creatinine level[b]	300-900 mg/L 900-1500 mg/L 1500-3000 mg/L	0.35 (0.32, 0.38) 0.62 (0.57, 0.68) 1.00	0.32 (0.28, 0.37) 0.63 (0.55, 0.72) 1.00	0.33 (0.30, 0.37) 0.59 (0.54, 0.65) 1.00	0.35 (0.32, 0.38) 0.60 (0.55, 0.66) 1.00	0.38 (0.35, 0.41) 0.61 (0.56, 0.67) 1.00
Urine sampling period	<7 hours 7-9 hours ≥9 hours	0.87 (0.79, 0.97) 0.97 (0.88, 1.06) 1.00	ns	ns	ns	ns
Educational level of the family	primary secondary tertiary	1.20 (1.05, 1.37) 1.04 (0.96, 1.13) 1.00	ns	ns	ns	1.09 (0.97, 1.23) 1.11 (1.02, 1.21) 1.00
Use of personal care products[a]	high use moderate to low use	0.91 (0.84, 0.98) 1.00	1.40 (1.25, 1.56) 1.00	ns	0.92 (0.86, 0.99) 1.00	ns
Ice cream consumption	several times/month once/month or less	ns	ns	1.13 (1.03, 1.24) 1.00	1.10 (1.01, 1.19) 1.00	ns
Gum consumption	several times/week once/week or less	ns	1.19 (1.06, 1.34) 1.00	ns	ns	ns
PVC in floors/walls	yes no	ns	ns	1.32 (1.19, 1.47) 1.00	ns	1.15 (1.04, 1.26) 1.00
Renovation in house	yes no	ns	ns	ns	ns	1.08 (1.00, 1.16) 1.00

ns: not significant; abbreviations biomarkers: see Table 2.

[a]Use of personal care products (PCP) is calculated as a score based on the frequency (never to daily) of 9 PCP groups (make-up, eye make-up, shampoo, hair styling products, body lotions and creams, fragrances, deodorant, massage oil and nail polish). [b]The confounders urinary creatinine level and age were forced in the multiple regression models, even if not significant

Figure legends

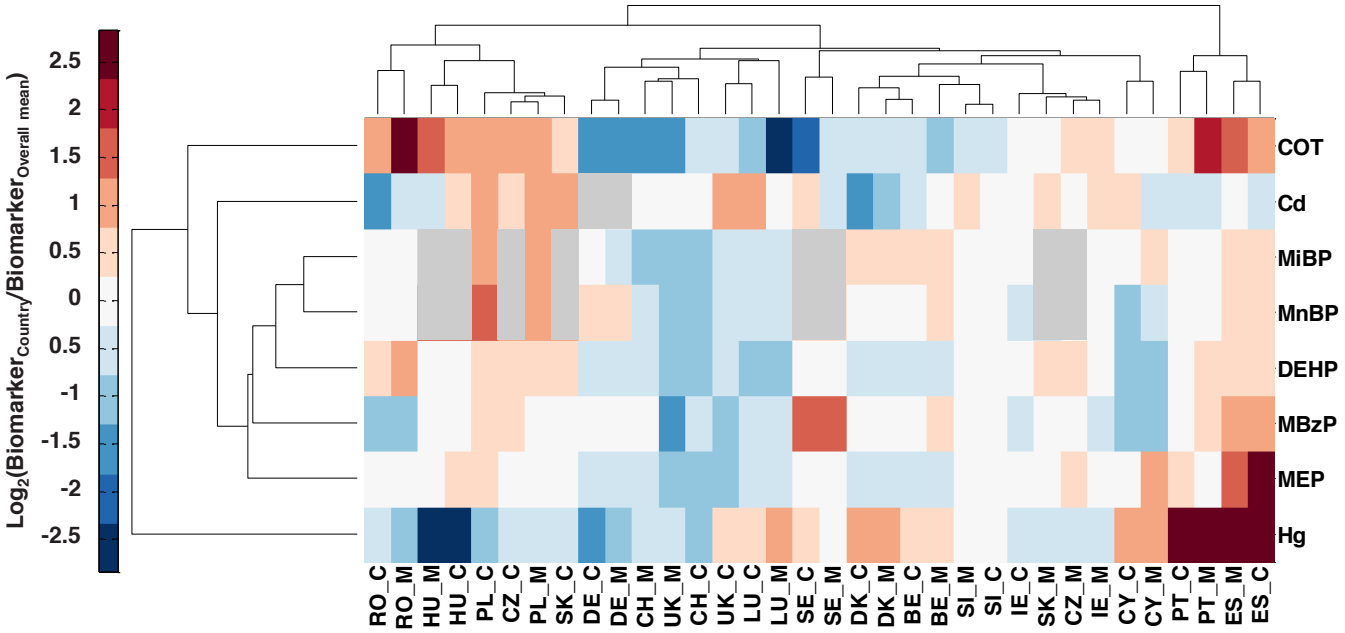
Figure 1. Overview of geometric means (95% CI) of biomarker concentrations (μg/L) in children and mothers of the participating countries. Country codes: Belgium (BE); Switzerland (CH), Cyprus (CY), Czech Republic (CZ), Germany (DE), Denmark (DK), Spain (ES), Hungary (HU), Ireland (IE), Luxembourg (LU), Poland (PL), Portugal (PT), Romania (RO), Sweden (SE), Slovenia (SI), Slovak Republic (SK) and United Kingdom (UK). Abbeviations biomarkers: see Table 2.All data in children are adjusted for age and gender; urinary metabolites are additionally adjusted for urinary creatinine; all data in mothers are adjusted for age; urinary metabolites are additionally adjusted for urinary creatinine; urinary cadmium is additionally adjusted for smoking. Light grey: GM of country significantly below European GM. Dark grey: GM of country is significantly above European GM. White: no significant difference between GM of country and European GM. NA: no biomarker data available. European GMs: see Table 2.

Figure 2. Heat map showing clustering of biomarkers (dendrogram to the left side) and clustering of countries (dendrogram at the top). Red and blue intensities indicate fold increases respectively decreases (expressed as log2) in country specific biomarker concentrations adjusted for age and gender relative to the European geometric mean. Country codes: see Figure 1. Country codes followed by M present concentrations in mothers, countrycodes followed by C present concentrations in children. White rectangles: missing data.

Figure 1.

CHILDREN	BE	CH	CY	CZ	DE	DK	ES	HU	IE	LU	PL	PT	RO	SE	SI	SK	UK
Mercury	0.204 (0.172, 0.241)	0.076 (0.065, 0.090)	0.326 (0.257, 0.413)	0.098 (0.083, 0.116)	0.055 (0.046, 0.065)	0.250 0.211, 0.295)	0.884 (0.747, 1.046)	0.025 (0.021, 0.029)	0.097 (0.082, 0.114)	0.181 (0.142, 0.229)	0.070 (0.060, 0.083)	1.033 (0.873, 1.222)	0.085 (0.072, 0.101)	0.181 (0.153, 0.214)	0.169 (0.142, 0.200)	0.092 (0.078, 0.109)	0.192 (0.163, 0.228)
Cadmium	0.046 (0.040, 0.052)	0.081 (0.071, 0.092)	0.114 0.096, 0.261)	0.117 (0.104, 0.133)	NA	0.024 (0.021, 0.027)	0.047 (0.041, 0.053)	0.129 (0.113, 0.146)	0.068 (0.060, 0.077)	0.154 (0.129, 0.184)	0.134 (0.118, 0.152)	0.045 (0.039, 0.051)	0.026 (0.023, 0.029)	0.090 (0.079, 0.103)	0.077 (0.068, 0.087)	0.144 (0.127, 0.163)	0.167 (0.147, 0.191)
Cotinine	0.629 (0.517, 0.766)	0.508 (0.418, 0.619)	0.842 (0.638, 1.111	1.602 (1.316, 1.950)	0.305 (0.251, 0.371)	0.658 (0.541, 0.801)	1.485 (1.219, 1.810)	1.776 (1.460, 2.161)	0.708 (0.582, 0.862)	0.397 (0.301, 0.524)	1.568 (1.288, 1.909)	1.093 (0.897, 1.333)	1.942 (1.943, 1.597)	0.202 (0.165, 0.246)	0.529 (0.434, 0.644)	1.085 (0.892, 1.320)	0.661 (0.542, 0.806)
DEHP	37.3 (32.9, 42.2)	28.1 (24.9, 31.9)	25.0 (21.0, 29.8)	71.1 (62.7, 80.5)	39.5 (34.9, 44.8)	40.9 (36.1, 46.4)	73.4 (64.7, 83.2)	58.7 (51.8, 66.5)	59.6 (52.6, 67.5)	25.8 (21.7, 30.8)	76.4 (67.4, 86.6)	48.2 (42.5, 54.6)	74.0 (65.4, 83.8)	49.9 (43.9, 56.6)	46.3 (40.8, 53.4)	82.7 (73.0, 93.7)	37.5 (33.0, 42.5)
MEP	26.7 (22.3, 32.0)	19.7 (16.5, 23.6)	41.2 (31.9, 53.1)	34.4 (28.7, 41.1)	23.1 (19.3, 27.7)	22.1 (18.5, 26.5)	208.3 (173.7, 249,8)	45.4 (37.9, 54.3)	42.5 (35.5, 50.9)	26.8 (20.8, 34.6)	46.9 (39.1, 56.2)	50.2 (41.8, 60.2)	34.8 (29.1, 41.7)	33.3 (27.8, 40.0)	40.2 (33.5, 48.1)	37.5 (31.3, 44.9)	16.9 (14.1, 20.3)
MBzP	9.0 (7.5, 10.6)	5.1 (4.3, 6.1)	3.7 (2.9, 4.7)	9.1 (7.6, 10.8)	6.6 (5.5, 7.8)	8.0 (6.7, 9.4)	14.6 (12.3, 17.3)	7.3 (6.2, 8.7)	5.9 (5.0, 7.0)	5.2 (4.1, 6.6)	9.3 (7.9, 11.1)	8.1 (6.8, 9.6)	4.1 (3.5, 4.9)	23.1 (19.4, 27.5)	7.9 (6.6, 9.4)	7.9 (6.6, 9.4)	4.2 (3.5, 4.9)
MnBP	39.4 (34.9, 44.6)	20.1 (17.8, 22.8)	20.6 (17.3, 24.6)	NA	46.4 (41.0, 52.5)	33.6 (29.7, 38.1)	52.7 (46.5, 59.7)	NA	28.5 (25.2, 32.2)	28.2 (23.7, 33.6)	90.4 (80.0, 102.3)	33.3 (29.4, 37.7)	43.2 (38.2, 48.8)	NA	38.0 (33.5, 42.9)	NA	26.4 (23.3, 29.9)
MiBP	59.2 (51.9, 67.5)	20.5 (18.0, 23.4)	51.8 (43.0, 62.3)	NA	41.4 (36.3, 47.2)	62.2 (54.5, 71.0)	63.8 (55.9, 72.9)	NA	45.4 (39.8, 51.7)	36.9 (30.6, 44.4)	108.3 (95.0, 123.5)	40.3 (35.3, 46.1)	51.1 (44.8, 58.3)	NA	55.0 (48.2, 62.7)	NA	30.3 (26.5, 34.6)
MOTHERS	**BE**	**CH**	**CY**	**CZ**	**DE**	**DK**	**ES**	**HU**	**IE**	**LU**	**PL**	**PT**	**RO**	**SE**	**SI**	**SK**	**UK**
Mercury	0.368 (0.313, 0.431)	0.153 (0.131, 0.180)	0.462 (0.369, 0.578)	0.156 (0.133, 0.183)	0.107 (0.092, 0.126)	0.391 (0.333, 0.458)	1.486 (1.267, 1.744)	0.039 (0.033, 0.045)	0.162 (0.139, 0.190)	0.387 (0.308, 0.485)	0.135 (0.116, 0.159)	1.200 (1.023, 1.406)	0.100 (0.085, 0.117)	0.252 (0.215, 0.295)	0.255 (0.217, 0.299)	0.132 (0.112, 0.154)	0.153 (0.130, 0.180)
Cadmium	0.224 (0.197, 0.255)	0.224 (0.197, 0.255)	0.183 (0.153, 0.219)	0.259 (0.228, 0.295)	NA	0.132 (0.116, 0.150)	0.212 (0.187, 0.241)	0.183 (0.161, 0.207)	0.296 (0.261, 0.336)	0.249 (0.208, 0.298)	0.453 (0.399, 0.514)	0.186 (0.164, 0.211)	0.187 (0.164, 0.212)	0.175 (0.154, 0.199)	0.289 (0.255, 0.329)	0.306 (0.269, 0.348)	0.267 (0.234, 0.304)
Cotinine	1.257 (0.736, 2.147)	0.844 (0.493, 1.446)	2.825 (1.329, 6.002)	3.773 (2.211, 6.441)	1.005 (0.588, 1.717)	1.871 (1.091, 3.209)	9.586 (5.597, 16.42)	7.187 (4.209, 12.27)	3.863 (2.266, 6.585)	0.557 0.260, 1.193)	6.219 (3.644, 10.61)	10.92 (6.401, 18.64)	14.92 (8.702, 25.57)	1.803 1.056, 3.080)	1.790 (1.048, 3.058)	2.819 (1.653, 4.809)	0.843 (0.489, 1.452)
DEHP	21.7 (19.0, 24.8)	20.4 (17.8, 23.3)	16.8 (13.9, 20.3)	37.3 (32.6, 42.6)	21.1 (18.5, 24.1)	24.0 (21.0, 27.4)	43.4 (38.0, 49.6)	34.0 (29.7, 38.8)	32.3 (29.7, 38.8)	15.9 (13.2, 19.3)	43.9 (38.4, 50.2)	37.2 (32.6, 42.5)	51.5 (45.0, 58.9)	28.4 (24.8, 32.4)	28.1 (24.6, 32.1)	39.4 (34.5, 45.0)	15.5 (13.5, 17.8)
MEP	37.1 (30.1, 45.8)	31.2 (25.2, 38.5)	87.7 (65.2, 117.9)	59.2 (48.0, 73.0)	38.5 (31.2, 47.6)	37.3 (30.2, 46.1)	160.0 (129.5, 197.6)	50.9 (41.2, 62.8)	55.2 (44.8, 68.1)	36.4 (27.0, 49.1)	42.5 (34.5, 52.5)	55.9 (45.3, 68.9)	44.2 (35.8, 54.7)	46.5 (37.7, 57.4)	46.8 (37.9, 57.8)	52.2 (42.3, 64.4)	27.4 (22.1, 33.9)
MBzP	6.5 (5.6, 7.7)	3.9 (3.3, 4.6)	2.4 (1.9, 3.0)	4.7 (4.0, 5.6)	4.5 (3.8, 5.2)	4.5 (3.8, 5.2)	8.5 (7.2, 9.9)	4.3 (3.7, 5.1)	3.4 (2.9, 4.0)	3.6 (2.8, 4.5)	4.5 (3.9, 5.3)	5.6 (4.8, 6.6)	2.5 (2.1, 2.9)	13.8 (11.8, 16.2)	4.4 (3.8, 5.2)	4.4 (3.8, 5.2)	1.7 (1.5, 2.0)
MnBP	30.5 (27.1, 34.4)	13.9 (12.3, 15.7)	16.1 (13.6, 19.1)	NA	29.7 (26.4, 33.5)	21.6 (19.2, 24.4)	30.8 (27.3, 34.7)	NA	20.2 (18.0, 22.8)	18.3 (15.4, 21.7)	48.2 (42.8, 54.4)	22.3 (19.8, 25.1)	27.1 (24.0, 30.6)	NA	23.8 (21.1, 26.8)	NA	13.1 (11.6, 14.9)
MiBP	38.6 (34.1, 43.6)	14.4 (12.7, 16.3)	43.7 (36.8, 52.0)	NA	24.6 (21.8, 27.8)	41.6 (36.7, 47.1)	37.0 (32.7, 41.8)	NA	26.5 (23.4, 29.9)	21.1 (17.7, 25.1)	53.6 (47.4, 60.6)	28.4 (25.1, 32.1)	34.7 (30.6, 39.2)	NA	34.9 (30.8, 39.4)	NA	17.6 (15.5, 19.9)

Figure 2.


Log2(Biomarker Country/Biomarker Overall mean)
2.5
2
1.5
1
0.5
0
0.5
-1
-1.5
-2
-2.5
COT
Cd
MiBP
MnBP
DEHP
MBzP
MEP
Hg
RO_C
RO_M
HU_M
HU_C
PL_C
CZ_C
PL_M
SK_C
DE_C
DE_M
CH_M
UK_M
CH_C
UK_C
LU_C
LU_M
SE_C
SE_M
DK_C
DK_M
BE_C
BE_M
SI_M
SI_C
IE_C
SK_M
CZ_M
IE_M
CY_C
CY_M
PT_C
PT_M
ES_M
ES_C