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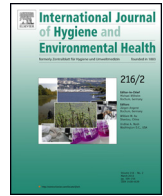
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## Human biomonitoring pilot study DEMOCOPHES in Germany: Contribution to a harmonized European approach



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### ABSTRACT

Human biomonitoring (HBM) is an effective tool to assess human exposure to environmental pollutants, but comparable HBM data in Europe are lacking. In order to expedite harmonization of HBM studies on a European scale, the twin projects COPHES (Consortium to Perform Human Biomonitoring on a European Scale) and DEMOCOPHES (Demonstration of a study to Coordinate and Perform Human Biomonitoring on a European Scale) were formed, comprising 35 partners from 27 European countries.

In COPHES a research scheme and guidelines were developed to exemplarily measure in a pilot study mercury in hair, cadmium, cotinine and several phthalate metabolites in urine of 6–11 year old children and their mothers in an urban and a rural region. Seventeen European countries simultaneously conducted this cross-sectional DEMOCOPHES feasibility study.

**Abbreviations:** AM, arithmetic mean; ATSDR, Agency for Toxic Substances and Disease Registry; BE, biomonitoring equivalent; CAPI, computer assisted personal interview; CI, confidence interval; COPHES, Consortium to Perform Human Biomonitoring on a European Scale; DEMOCOPHES, Demonstration of a Study to Coordinate and Perform Human Biomonitoring on a European Scale; DFG, German Research Foundation; EFSA, European Food Safety Authority; ESB, German Environmental Specimen Bank; ETS, environmental tobacco smoke; EQUAS, external quality assessment schemes; GerES, German Environmental Survey; G-EQUAS, German External Quality Assessment Scheme; GM, geometric mean; HBM, human biomonitoring; HBM-I, HBM-II value, human biomonitoring value I, human biomonitoring value II; ICI, interlaboratory comparison investigation; ICP-MS, inductively coupled plasma quadrupole mass spectrometry; IPA, Institute for Prevention and Occupational Medicine of the German Social Accident Insurance; IPASUM, Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine; JEFCA, Joint FAO/WHO Expert Committee on Food Additives; LOQ, limit of quantification; N, sample size; MAK-Commission, Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area; min., minimum value; max., maximum value; MRL, minimal risk level; PVC, poly-vinyl chloride; P10, P25, P50, P75, P90, P95, percentiles; QAU, COPHES Quality Assurance Unit; REACH, European chemicals legislation concerning the registration, evaluation, authorisation and restriction of chemicals (regulation EC 1907/2006); RfD, reference dose; SOP, standard operating procedure; TDI, tolerable daily intake; UBA, German Environment Agency; USEPA, United States Environmental Protection Agency.

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Cadmium  
Cotinine  
Phthalates

The German study population was taken in the city of Bochum and in the Higher Sauerland District, comprising 120 mother–child pairs. In the present paper features of the study implementation are presented. German exposure concentrations of the pollutants are reported and compared with European average concentrations from DEMOCOPHES and with those measured in the representative German Environmental Survey (GerES IV).

German DEMOCOPHES concentrations for mercury and cotinine were lower than the European average. However, 47% of the children were still exposed to environmental tobacco smoke (ETS) outside their home, which gives further potential for enhancing protection of children from ETS.

Compared with samples from the other European countries German participating children had lower concentrations of the phthalate metabolites MEP and of the sum of 3 DEHP-metabolites (MEHP, 5OH-MEHP and 5oxo-MEHP), about the same concentrations of the phthalate metabolites MBzP and MiBP and higher concentrations of the phthalate metabolite MnBP. 2.5% of the German children had concentrations of the sum of 4 DEHP-metabolites and 4.2% had concentrations of MnBP that exceeded health based guidance values, indicating reasons for concern.

Continuous HBM is necessary to track changes of pollutant exposure over time. Therefore Germany will continue to cooperate on the harmonisation of European human biomonitoring to support the chemicals regulation with the best possible exposure data to protect Europe's people against environmental health risks.

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## 1. Introduction

Human biomonitoring (HBM) focuses on measuring the uptake of chemicals by the human body and on assessing the internal exposure of humans. HBM considers all routes of uptake and all relevant sources, making it an ideal instrument for risk assessment and risk management and thus being a tool for scientists as well as for policy makers. In Germany, human exposure to chemicals is determined by HBM with two main instruments: the population representative German Environmental Survey (GerES) and the German Environmental Specimen Bank (ESB) (Kolossa-Gehring et al., 2012). In Europe, several other countries have also conducted HBM studies in the past (Castano et al., 2012; Cerna et al., 2012; Frery et al., 2012; Hohenblum et al., 2012; Schoeters et al., 2012). However, a comparison of the derived data is difficult because of different approaches in study methodology, analytical methods used and target populations investigated.

Since environmental chemicals are widespread throughout Europe and because HBM will enable the evaluation of the effectiveness of the European chemicals legislation REACH (European chemicals legislation concerning the registration, evaluation, authorisation and restriction of chemicals, EC 1907/2006), the European Commission called for the development of a coherent approach to HBM in Europe in close cooperation with the Member States. As a result, the twin projects COPHES (Consortium to Perform Human Biomonitoring on a European Scale) and DEMOCOPHES (Demonstration of a study to Coordinate and Perform Human Biomonitoring on a European Scale) were set up to build a sustainable framework and to demonstrate that HBM can be performed in a coherent and harmonized approach throughout Europe (Joas et al., 2012).

COPHES constituted 35 partners from 27 European countries (Joas et al., 2015). They developed a study design, a study protocol and guidelines and standard procedures for all aspects of a harmonized European pilot study: recruitment and fieldwork, specimen sampling, chemical analysis, data management, analysis and interpretation, ethics and communication (Becker et al., 2014; Casteleyn et al., 2015; Esteban et al., 2015; Exley et al., 2015; Fiddicke et al., 2015; Schindler et al., 2014). The members of COPHES decided to perform a cross sectional study and to measure mercury in hair, cadmium, cotinine and phthalate metabolites in urine of children aged 6–11 years and their mothers. The biomarkers of exposure were selected because of availability of analytical methods, information on toxicokinetics and health based guidance values, as well

as their potential to increase public and political awareness, and in order to gather experience with less common analytical procedures. Mercury, cadmium and cotinine were chosen because sufficient analytical experience and knowledge on toxicokinetics exist. Phthalate metabolites were included to take into account the increasing awareness as well as the level of analytical experience required for testing. The selected biomarkers can reliably be determined in hair and urine samples, which are relatively easy to sample and do not require highly trained technicians. The 17 European countries participating in the pilot study DEMOCOPHES were Belgium, Cyprus, Czech Republic, Denmark, Germany, Hungary, Ireland, Luxembourg, Poland, Portugal, Romania, Slovenia, Slovak Republic, Spain, Sweden, Switzerland, and United Kingdom.

Germany has long-lasting experience on HBM surveys. The German Environment Agency (UBA) participated in both the COPHES and DEMOCOPHES projects in order to make these efforts applicable throughout Europe, to transfer experience and standards to the European level and to contribute to build a European-wide HBM framework. In COPHES, UBA led the development of protocols and standard operating procedures (SOP) for participant recruitment and fieldwork (Becker et al., 2014) and UBA was responsible for the conduct of DEMOCOPHES in Germany. The establishment of a harmonized HBM in Europe is continued by the European Joint Programme “HBM4EU” launched by the European Union in the Framework Program for Research and Innovation, Horizon 2020, and coordinated by the UBA. The overarching goal of which is to generate knowledge, to inform the safe management of chemicals, and consequently protect human health in Europe. HBM data will build the basis to assess the risks from human exposure to chemicals and the resulting health impacts. By means of intensive communication with policy makers HBM4EU will promote the further development and design of new chemicals policies as well as the evaluation of existing measures. HBM4EU follows the key objectives to harmonize procedures for HBM across 26 countries, provide policy makers with comparable data on human internal exposure to chemicals and mixtures of chemicals in Europe, link data on internal exposure to chemicals to aggregate external exposure and identify exposure pathways and upstream sources, generate scientific evidence on the causal links between human exposure to chemicals and negative health outcomes, and adapt chemical risk assessment methodologies to use HBM data and account for the contribution of multiple external exposure pathways to the total body burden of various chemicals.

The present paper reports the results for the German DEMO-COPHES sample, the lessons learned from which will inform HBM4EU. The implementation of the study in Germany is described and the concentrations of the selected biomarkers of exposure are reported. These results are compared with the total European results (Den Hond et al., 2015) and with data obtained in the representative fourth German Environmental Survey (GerES IV) (Schulz et al., 2012a). Factors associated with exposure concentrations are presented. Particular attention is drawn on the proportion of participants exceeding health based guidance values and thus being potentially at risk for health impacts.

## 2. Material and methods

The German study protocol was elaborated based on the European consensus protocol of COPHES (Casteleyn et al., 2015). According to this common protocol each participating country recruited 120 mother-child pairs, half from an urban and half from a rural area, took scalp hair and morning urine samples and analyzed them for mercury (hair) and cadmium, cotinine and phthalate metabolites (urine). The mother and child pairs were included in the analysis if they gave informed consent, met the inclusion criteria, provided hair and morning urine samples and answered a questionnaire.

### 2.1. Study population and fieldwork

According to the COPHES guidelines for recruitment (Becker et al., 2014), the selection of the German participants followed a two-step procedure. First, the German federal state North Rhine-Westphalia was chosen as the study region. Secondly, population density served as a criterion to choose an urban and a rural sampling location. In North Rhine-Westphalia the city of Bochum was at the upper degree of urbanization and the Higher Sauerland District was at the lower degree of urbanization, so these two sites were chosen as the urban and the rural sampling location.

As children were the primary target group, the selection of participants focused on the child: children 6–11 years old were selected randomly via the respective inhabitant registries, a resident registration kept by the local public administration. The aim was to include 10 children of each age and of equal sex distribution in both the urban and the rural locations.

Besides the age of the child, further inclusion and exclusion criteria were: mother's age 45 years or younger; mother and child living in the same location for at least 5 years; only one child per mother eligible; only children who live at least 16 days per months with the mother; sufficient German language ability; not living in a hospital, institution or being homeless; and no metabolic disturbances or abnormal urine excretion.

Detailed study information, urine-vessels and instructions on how to collect the first morning urine sample were sent to the participating mother-child pairs by mail. During the home visit shortly afterwards, the interviewers received the morning urine samples from the mother and the child and took scalp hair samples from both. Additionally, the mother answered a face-to-face interview, including questions on socio-demography, nutrition and exposure related habits and behavior.

The Department of Hygiene, Social and Environmental Medicine, Ruhr-University Bochum, Germany, conducted the field work on behalf of the UBA. The interviewers participated in the COPHES training sessions on all aspects of fieldwork, which was mandatory for all participating countries in order to ensure the necessary harmonized performance of the study.

The study was approved by the ethics committee of the Faculty of Medicine of the Ruhr-University Bochum (No. 4040-11) and the data protection officer of the Ruhr-University Bochum.

Sampling took place between September and December 2011.

### 2.2. Biospecimen collection and handling

COPHES instructions for urine and hair sampling, storage and shipping were provided in COPHES deliverables and in the fieldwork training sessions. They can be found on the COPHES/DEMOCOPHES website (<http://www.eu-hbm.info/cophes/project-work-packages/trainings-agendas-and-presentations>).

In Germany, all first morning urine samples were collected in vessels that were prewashed with 2% nitric acid. After collecting the urine samples, the participants were asked to store the vessels refrigerated or in a cool place until they were handed over to the interviewers. The morning urine samples were transported refrigerated, aliquoted and stored at  $-18^{\circ}\text{C}$  until analysis.

Hair samples were cut close to the head surface using titanium scissors. Each hair sample was stored in a paper envelope, placed in a plastic bag at room temperature.

At the end of fieldwork, samples were arranged completely at random for chemical analysis. Subsequently the interviewers sent the urine samples frozen to the respective laboratories and delivered the hair samples to the analysing laboratory at room temperature.

### 2.3. Laboratory chemical analyses

Urine and hair samples were analyzed by laboratories, which used validated methods and participated in the interlaboratory comparison investigations (ICI) and external quality assessment schemes (EQUAS) guided and evaluated by the COPHES Quality Assurance Unit (QAU) (Esteban et al., 2015; Schindler et al., 2014) to ensure comparability between the measurements of the different European laboratories involved.

#### 2.3.1. Mercury

The analysis of mercury in the hair samples was performed by the Department of Hygiene, Social and Environmental Medicine, Ruhr-University Bochum. The 3 cm of hair which were closest to the scalp were analyzed with the recommended method of the respective COPHES deliverable, using the Direct-Mercury-Analyzer DMA-80 of MLS GmbH, Leutkirch, Germany. Hair samples were cut in pieces of 2–4 mm. About 3.5 mg were introduced directly into the analyzer without any pretreatment. They were dried, thermally decomposed, trapped on a gold amalgamator and quantitatively determined using atomic absorption spectrophotometry at 254 nm. The limit of quantification (LOQ) was  $0.003\ \mu\text{g/g}$ .

#### 2.3.2. Cadmium

The Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine (IPASUM), Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, quantified cadmium in the urine samples. Cadmium analysis was performed by using inductively coupled plasma quadrupole mass spectrometry (Quadrupole ICP-MS) with an ICP-MS of PerkinElmer, NexION 350D. This method was recommended by COPHES and evaluated by the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) of the German Research Foundation (DFG) and published by Wiley-VCH (Schramel et al., 1999) The LOQ was  $0.05\ \mu\text{g/L}$ .

**Table 1**  
Phthalate metabolites selected for European comparison in the DEMOCOPHES study.

Parent phthalate	Full name of parent phthalate	Primary metabolite	Secondary metabolite	Full name of metabolite
<b>Low molecular weight phthalates</b>				
DEP	Di-ethyl phthalate	MEP		Mono-ethyl phthalate
BBzP	Butyl benzyl phthalate	MBzP		Mono-benzyl phthalate
DnBP	Di- <i>n</i> -butyl phthalate	MnBP		Mono- <i>n</i> -butyl phthalate
DiBP	Di-isobutyl phthalate	MiBP		Mono-isobutyl phthalate
<b>High molecular weight phthalate</b>				
DEHP	Di-(2-ethyl-hexyl) phthalate	MEHP		Mono-(2-ethyl-hexyl) phthalate
			5OH-MEHP	Mono-(2-ethyl-5-hydroxy-hexyl) phthalate
			5oxo-MEHP	Mono-(2-ethyl-5-oxo-hexyl) phthalate
			5cx-MEPP	Mono-(2-ethyl-5-carboxy-pentyl) phthalate

### 2.3.3. Cotinine

The Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA) at the Ruhr-University Bochum carried out the analysis of cotinine in the urine samples. Two-dimensional liquid chromatography-mass spectrometry with isotope dilution quantification was applied as established by IPA. In short, urine samples were spiked with an internal standard solution of cotinine-d3 and chromatographically resolved in a two column assembly consisting of a Waters Oasis<sup>®</sup> HLB cartridge column (2.1 × 20 mm; 25 μm) and a Hypercarb (Thermo Scientific) (2.1 × 100 mm; 3 μm). Mass spectrometric detection and quantification was performed using a Waters Quattro Premier XE triple quadrupole mass spectrometer in positive ionization mode. The LOQ was 0.1 μg/L for cotinine.

### 2.3.4. Phthalates

IPA also analyzed phthalate metabolites in the urine samples. Based on the DFG method (Koch and Angerer, 2008), the phthalate metabolites were determined by multidimensional liquid chromatography with tandem mass spectrometric detection (Kasper-Sonnenberg et al., 2012; Koch et al., 2013). The limits of quantification ranged between 0.2 and 1.0 μg/L, depending on the respective metabolite. All metabolites selected for European evaluation by DEMOCOPHES and their acronyms are summarized in Table 1.

### 2.3.5. Creatinine

The quantification of creatinine in the urine samples was performed by IPASUM by the method of Jaffé (Blaszkiwicz and Liesenhoff-Henze, 2010).

## 2.4. Statistical analysis

Characteristics of the biomarker distributions (sample size (N), %> LOQ, minimum (min.), maximum (max.), arithmetic mean (AM) geometric mean (GM), confidence intervals (CI) and percentiles (P)) were calculated for mothers and children separately. These characteristics are summarized in the Supplementary Tables 1 and 2. For biomarkers in urine, volume based as well as creatinine adjusted concentrations are presented. Urine samples with a creatinine concentration lower than 300 mg/L or above 3000 mg/L were excluded from the analyses (WHO, 1996). Concentrations below the LOQ of the respective analytical method were assigned a value equal to half of the LOQ. Due to the skewed (approximately log-normal) distribution of the biomarker concentrations, GM is a parameter more suitable for assessment than AM. Although the European countries differ considerably in the number of inhabitants, 120 mother-child pairs from each country were included in the statistical calculations, except for Cyprus and Luxembourg, which contributed only half because of their very small populations. No further weighting of the country data was applied (Den Hond et al., 2015).

Differences between the German and the European GMs were assessed by looking at the overlaps of the CIs.

Specifically for each biomarker socio-demographic and behavioral factors as well as conditions of living which are suspected either by scientific knowledge or by biological plausibility to be associated with the biomarker concentration were analyzed in bivariate statistical analyses: differences of the GM of the respective subgroups were tested for significance via *t*-tests or one-way analyses of variance, such as differences between mothers and children, and differences by number of smokers in household. The 10–18 specific factors analyzed are listed by Den Hond et al., 2015; Supplemental Material.

Correlations between the values of each mother and her child were analyzed for each biomarker using Spearman rank correlation coefficients.

Statistical analyses were performed with the SPSS statistical package (version 20).

## 3. Results and discussion

### 3.1. Study population and fieldwork in Germany

The recruitment results are shown in Table 2. A total of 430 children in the urban region and 433 children in the rural region were invited to participate with their mothers in the study. Out of these, responses were received from 256 (urban) and 176 (rural) of the invited families, 88 (urban) and 99 (rural) of them expressed their willingness to participate. Twenty-eight (urban) and 39 (rural) mother-child pairs were excluded either because they met exclusion criteria or because of oversampling in individual one-year age groups of the children. The recruitment process finally resulted in the required number, age and sex distribution and in the characteristics of the participating mother-child pairs as shown in Table 3. Among the study population three mothers showed urinary creatinine levels below 300 mg/L while one mother had a creatinine level above 3000 mg/L. The urine analyses of these four mothers were excluded from the statistical analyses since their urine levels did not meet the inclusion criteria.

For all steps of the fieldwork COPHES had developed a study protocol in a systematic and transparent approach (Becker et al., 2014). Whenever necessary, the participating countries had the possibility to adapt certain study elements insofar this did not jeopardize the comparability of results. Adaptations of study elements in the German study conduct and observations are described below, adaptations by other countries are reported by Fiddicke et al. (2015):

- In Germany, the procedure of selection via inhabitant registries is well established and was applied successfully in several GerES (Schulz et al., 2007). Therefore, this approach was applied in the German DEMOCOPHES study. Although the participation rate with 14% was only moderate (Table 2), it yielded in the required number of participants and had the advantage that a strictly



**Table 2**  
Recruitment and participation in DEMOCOPHES in Germany.

Study area (sampling location)	Urban: City of Bochum North Rhine-Westphalia	Rural: Higher Sauerland District North Rhine-Westphalia
Population density	3900 inhabitants/km <sup>2</sup>	80 inhabitants/km <sup>2</sup>
Invited families	N = 430	N = 433
Families responding (by mail, telephone or personal contact)	60% (N = 256)	41% (N = 176)
Families willing to participate	21% (N = 88)	23% (N = 99)
Families excluded (exclusion criteria, oversampling)	7% (N = 28)	9% (N = 39)
Participating families	14% (N = 60)	14% (N = 60)

**Table 3**  
Characterization of the German DEMOCOPHES study population.

	Children	Mothers
N	120	120
Study area, N		
Urban	60	60
Rural	60	60
Sex, N		
Male	60	–
Female	60	120
Age, years		
Median (Min.–Max.)	8 (6–11)	40 (28–45)
Age group, N		
6–8 years	67 (56%)	–
9–11 years	53 (44%)	–
≤35 years	–	21 (18%)
36–40 years	–	43 (36%)
>40 years	–	56 (47%)
Urinary creatinine		
Concentration, mg/L, Median (min.–max.)	1119 (308–2668)	1070 (200–3162)
<300 mg/L (exclusion criterion), N	–	3
>3000 mg/L (exclusion criterion), N	–	1
Body Mass Index, kg/m <sup>2</sup>		
Median (Min.–Max.)	15.8 (11.8–26.5)	23.6 (18.3–50.8)
Smoking habits, N		
Daily smoker	0	21 (18%)
Occasional smoker	0	0
Former smoker	0	28 (23%)
Never smoker	120 (100%)	71 (59%)
Environmental tobacco smoke, N (former and never-smokers only)		
At home	10 (8%)	6 (6%)
Elsewhere	56 (47%)	66 (67%)
Fish consumption, N		
Several times per week	5 (4%)	9 (8%)
Once a week or less	115 (96%)	111 (93%)

random population sample could be chosen. Recruitment via schools as accomplished in 14 other DEMOCOPHES countries did not necessarily result in higher participation rates (Fiddicke et al., 2015).

- In Germany, several mothers willing to participate had to be excluded because of their age. A comparable rate of exclusion because of mother's age was also reported in other countries (Fiddicke et al., 2015). The mother's age limit as an inclusion/exclusion criterion should be reconsidered in future studies, when children aged 6–11 years and their mothers are targeted as the study population.
- With only three exceptions, home visits could be realized in the German study population. In contrast to visits at an examination center, home visits offer the opportunity to collect additional environmental samples in the residence for ambient exposure assessment. The high rate of successfully completed home visits implies that the extension of ambient exposure assessment can be integrated in the study design of future studies. Additional ambient exposure assessment was also applied successfully in several GerES since 1985 (Schulz et al., 2007, 2012a).

- The ethical approval by the Ruhr University, Bochum, required the non-responder procedure of the COPHES guidelines to be modified. First, the reply card had to be supplemented with the option to refuse study participation completely. Secondly, detailed information on the objective of non-responder questions had to be included in the study information.

Altogether the common COPHES study protocol for DEMOCOPHES could be applied in most parts using the preferred choice of options for recruitment and fieldwork (Becker et al., 2014). Only a few minor adaptations had to be made but no adaptations interfering with comparability were necessary.

### 3.2. Mercury in hair

The mercury content in the hair samples of the German and of all participants of the 17 European countries is shown in Table 4. Mercury was detected in the hair of all participating German children and their mothers. The concentrations ranged from 0.007 µg/g to 1.13 µg/g for the children and from 0.008 µg/g to 1.35 µg/g for the mothers (Supplementary Table 1). The GM of mercury concentrations was significantly higher in mothers (0.109 µg/g) than in children (0.055 µg/g). Compared with the GM of all 17 DEMOCOPHES countries, the German mercury concentrations were considerably lower, both for children and for mothers (GM 0.055 µg/g versus 0.145 µg/g for children and 0.109 µg/g versus 0.225 µg/g for mothers, Table 4). The individual mercury concentrations of the mothers and their children in Germany were considerably correlated ( $r = 0.425$ ,  $p < 0.001$ , Table 5). The associations of several factors with mercury concentration in hair were analyzed. Besides age, sex of the child, highest educational level of the family and area of residence, which were analyzed for each biomarker, additional factors were chosen which were suggested to be associated with mercury concentration in hair. No sex difference in the mercury concentration of the children's hair was observed. Only the frequency of fish consumption correlated with mercury concentration in hair, both for the mothers and for the children (Fig. 1). As mercury found in hair is typically organic, teeth with amalgam fillings, broken thermometers and energy saving lamps were not associated with the mercury concentrations in children's or mother's hair in this sample.

To interpret exposure levels, the hair mercury concentrations from this study are compared to existing health based guidance values. For mercury in hair several guidance values are available. None of the German children and mothers exceeded the provisional guidance value of 2.3 µg/g hair published by the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) (WHO, 2004). When compared to the value of 1.0 µg/g hair, which is derived from the reference dose set by the United States Environmental Protection Agency (USEPA) (NRC, 2000), one mother and one child (not related) exceeded this value, resulting in 0.8% for mothers and 0.8% for children, respectively (Table 7).

The German mercury results are in the range of most of the Northern and Central European countries (Den Hond et al., 2015). The widespread difference in mercury exposure among the European countries and the strong correlation of hair mercury

**Table 4**

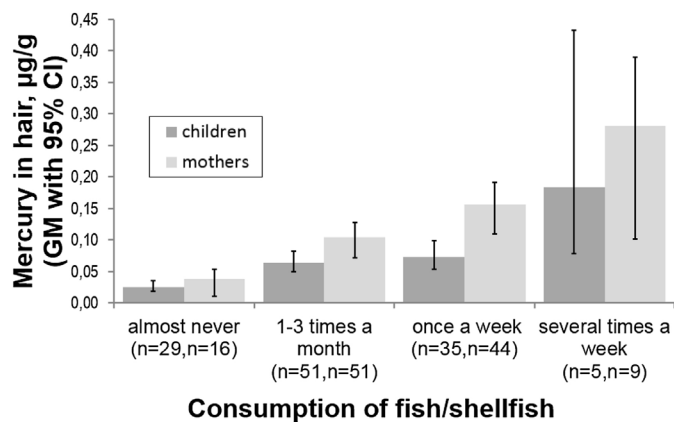
Frequency of quantification, geometric mean with 95% confidence interval (in parentheses) of mercury in hair and urinary cadmium and cotinine of children and their mothers in DEMOCOPHES in Germany and in all DEMOCOPHES countries.

	DEMOCOPHES in Germany			All DEMOCOPHES countries <sup>a</sup>		
		% >LOQ	GM (95% CI)		% >LOQ	GM (95% CI)
Mercury (µg/g hair)	Children (N = 120)	100	0.055 (0.046–0.066)	Children (N = 1836)	85.9	0.145 (0.139–0.151)
Mercury (µg/g hair)	Mothers (N = 120)	100	0.109 (0.091–0.132)	Mothers (N = 1.839)	90.5	0.225 (0.216–0.234)
Cadmium (µg/L urine)	Children (N = 119)	61	0.051 <sup>*</sup> (0.045–0.058)	Children (N = 1698)	70.1	0.071 (0.069–0.074)
Cadmium (µg/g creatinine)			0.046 <sup>*</sup> (0.042–0.052)			0.070 (0.067–0.072)
Cadmium (µg/L urine)	Mothers (N = 108)	98	0.199 <sup>*</sup> (0.178–0.223)	Mothers (N = 1685)	93.8	0.219 (0.211–0.228)
Cadmium (µg/g creatinine)			0.182 <sup>*</sup> (0.166–0.199)			0.196 (0.189–0.202)
Cotinine (µg/L urine)	Children (N = 120)	87	0.308 (0.244–0.387)	Children (N = 1818)	57.6	0.797 (0.759–0.837)
Cotinine (µg/g creatinine)			0.280 (0.222–0.353)			0.774 (0.736–0.815)
Cotinine (µg/L urine)	Mothers (N = 116)	86	0.916 (0.520–1.62)	Mothers (N = 1800)	62.4	2.75 (2.41–3.14)
Cotinine (µg/g creatinine)			0.813 (0.461–1.43)			2.45 (2.14–2.80)

N: sample size, LOQ: limit of quantification, GM: geometric mean, 95% CI: 95% confidence interval.

<sup>a</sup> data are from Den Hond et al. (2015), Supplemental Material.

<sup>\*</sup> German cadmium results, not included in the DEMOCOPHES European analysis.



**Fig. 1.** Mercury concentrations in hair of mothers and their children by fish and shellfish consumption in German DEMOCOPHES.

**Table 5**

Correlation of the biomarkers measured in mother-child pairs in DEMOCOPHES in Germany.

Biomarker	r <sub>s</sub>	P
Mercury	0.425	0.000
Cadmium	0.091	0.352
Cotinine	0.595	0.000
MEP	0.360	0.000
MBzP	0.524	0.000
MnBP	0.428	0.000
DiBP	0.400	0.000
DEHP (sum of MEHP + 5OH-MEHP + 5oxo-MEHP)	0.154	0.098
MEHP	0.256	0.006
5-OH-MEHP	0.159	0.088
5-oxo-MEHP	0.148	0.112

r<sub>s</sub> Spearman rank correlation, two-tailed test. Analyte µg/L urine. Samples with urinary creatinine concentrations below 300 mg/L or above 3000 mg/L were excluded.

concentrations between mothers and their children indicate a relationship with dietary habits and in particular with consumption of fish and other products from the aquatic environment (Castano et al., 2015; Den Hond et al., 2015).

Although mercury in hair was not measured in comparable studies in Germany, the results are in line with the determination of mercury in blood in the German Environmental Survey for Children (GerES IV). In GerES IV the highest mercury concentration in blood was 6.3 µg/L for children aged 6–14 (Becker et al., 2008) and 0.2% of the children exceeded the health based guidance value (HBM-I) of mercury of 5 µg/L blood (Schulz et al., 2012b; Apel et al., 2016).

The DEMOCOPHES data confirm the result of GerES IV that mercury exposure is not of great concern in Germany. Nevertheless as fish and other seafood products are important elements of a healthy diet and negative effects of even low mercury exposure levels have been postulated (Bellanger et al., 2013; Karagas et al., 2012; Valent et al., 2013), ongoing monitoring of the population for the current exposure to mercury is warranted.

Determination of mercury in hair is accepted as a reliable estimate of the internal dose (Harkins and Susten, 2003), it shows a direct correlation with blood levels and provides a good long-term marker of exposure to methyl mercury, but is not a marker of inorganic mercury exposure (UNEP/WHO, 2008). Factors like hair treatments, hair colour, ethnicity, age and external contamination may affect the incorporation of mercury in hair (Dakeishi et al., 2005; McDowell et al., 2004). In Germany there is only limited experience with mercury detection in scalp hair (Pesch et al., 2002). Determination of mercury in hair has never been applied in the GerES. This is due to the recommendation of the German Human Biomonitoring Commission to focus on blood and urine when measuring multiple substances in HBM studies as hair is only a reliable matrix for mercury and not for other metals (Bundesgesundheitsblatt, 2005). However measuring mercury in hair in the context of a harmonized study protocol, with the COPHES guidelines and with comparable and quality-assured analytical procedures has broadened Germany's experience and can be considered as an additional surveillance method for mercury in HBM studies, being less invasive than taking a blood sample.

### 3.3. Cadmium in urine

In the course of the study it became apparent that the ICP-MS-method is not completely specific for the quantification of cadmium in urine. The interferences of molybdenum oxide and tin have a substantial effect on cadmium quantification (Jarrett et al., 2008) and the influence of molybdenum oxide, especially, had been underestimated. Molybdenum oxide emerges from molybdenum content in urine and cannot be removed completely. Newer findings indicate that the molybdenum content in urine is generally high to such a degree as it should not be neglected. Former measurements of the German DEMOCOPHES samples excluded this fact and consequently were not in line with the rest of the DEMOCOPHES study. As COPHES QAU decided only to consider results of laboratories either being successful in two quality exercises with a required correction for molybdenum oxide or after reanalyzing the samples in another experienced laboratory, the German cadmium data were, therefore, not considered in the DEMOCOPHES European analysis. However after implementing a procedure that includes the correction of

molybdenum oxide as required by the COPHES QAU, the German laboratory undertook reanalysis of the DEMOCOPHES urine samples. The individual molybdenum contents were determined and the conversion of the molybdenum counts in cadmium counts was carried out on the basis of the measurement of a special molybdenum standard with known cadmium and molybdenum content. This additional signal, which was not considered in the former approach, was subtracted from the cadmium signal in the reanalysis. Concomitantly with the reanalysis the laboratory ensured the accuracy of the revised procedure by the successful participation in the German External Quality Assessment Scheme (G-EQUAS), an international proficiency test program for human biomonitoring parameters (Göen et al., 2012).

After reanalysis cadmium in urine was detected in 61% of the children and in 98% of the mothers (Table 4). The cadmium concentrations ranged from  $<0.05 \mu\text{g/L}$  to  $0.23 \mu\text{g/L}$  for children and from  $<0.05 \mu\text{g/L}$  to  $0.89 \mu\text{g/L}$  for mothers, being higher in mothers (GM  $0.199 \mu\text{g/L}$ ) than in children ( $0.051 \mu\text{g/L}$ ) (Supplementary Table 1). There was no correlation between the cadmium values of the children and their respective mothers ( $r=0.091$ , Table 5). None of the factors of exposure suspected to be associated with cadmium concentration in urine (for example age, smoking behavior, food consumption) was correlated with cadmium levels in urine, neither in the children nor in the mothers.

After applying the required molybdenum correction, the cadmium concentrations in Germany can now be compared to the other DEMOCOPHES countries. The participating German children had a slightly lower cadmium GM compared to the other DEMOCOPHES countries ( $0.051 \mu\text{g/L}$  versus  $0.071 \mu\text{g/L}$ ), the GM of the mothers did not differ (Table 4). The cadmium GM of the children was also slightly lower than the cadmium GM of  $0.068 \mu\text{g/L}$ , measured in 3–14 years old children in GerES IV (Becker et al., 2008). However, due to the COPHES selection criteria, the German DEMOCOPHES participants were not representative for the German population of this age. The current cadmium exposure of children aged 3–17 years in Germany will be monitored in a respective representative sample of the German population in the ongoing GerES V.

The German Human Biomonitoring Commission derived health based guidance values (HBM-I and HBM-II values) for cadmium in urine of  $0.5 \mu\text{g/L}$  for children and  $1.0 \mu\text{g/L}$  for adults (Schulz et al., 2012b; Apel et al., 2016). Biomonitoring equivalents (BE) were derived for cadmium in urine and blood (Hays et al., 2008). None of the German children or mothers exceeded the HBM-I or the BE values (Table 7).

### 3.4. Cotinine in urine

Cotinine in urine was detected in 87% of the children and in 86% of the mothers (Table 4). The maximum cotinine levels measured were  $22.4 \mu\text{g/L}$  for children and  $3420 \mu\text{g/L}$  for mothers (Supplementary Table 1). The GM was  $0.308 \mu\text{g/L}$  for children and  $0.916 \mu\text{g/L}$  for mothers, both were lower than the GM of the whole DEMOCOPHES sample ( $0.797 \mu\text{g/L}$  for children and  $2.75 \mu\text{g/L}$  for mothers, Table 4).

In GerES IV, a GM of  $1.3 \mu\text{g/L}$  was determined in a representative sample of 3–14 year old German children (Becker et al., 2008). Direct comparison however cannot be drawn as DEMOCOPHES was a non-representative sample and the age range differed in both study populations.

The wide range of cotinine concentrations observed in mothers can be explained by their smoking behavior. Mothers who were smokers had a GM of  $404 \mu\text{g/L}$ , whereas the GM of mothers who did not smoke was only  $0.3 \mu\text{g/L}$ . Of the mothers, 17.5% were daily smokers, but all children were never-smokers (Table 3). Cotinine concentrations correlated highly between the children and their

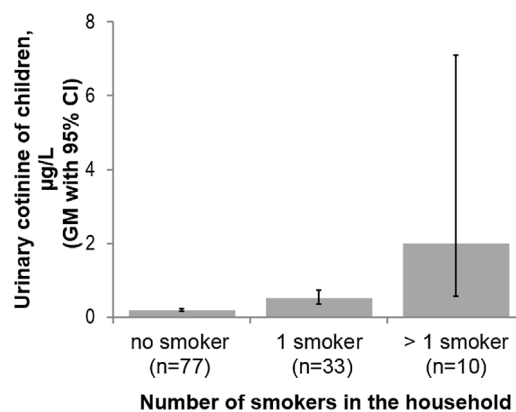


Fig. 2. Urinary cotinine concentrations in children by number of smokers in the household.

mothers ( $r=0.595$ ,  $p<0.001$ , Table 5). Moreover, as Fig. 2 shows, urinary cotinine in children increased with the number of smokers in the household.

It was reported that 8% of the children were exposed to environmental tobacco smoke (ETS) at home and 47% outside their home. Cotinine concentrations were significantly elevated in non-smoking mothers and children when exposed to ETS within the last 24 h.

The health impact of ETS on children is commonly recognized (CDC, 2006). There is no safe level of exposure to ETS regarding health impact on children and adults and there is also evidence that implementing smoke-free environments is the only effective way to protect the population from the harmful effects of exposure to ETS (WHO, 2007). Consequently, legislations on smoke-free environments were introduced in the European Union member countries in order to minimize or even prevent ETS (European Commission, 2013). Germany passed a smoke free law in 2007, but according to the Smoke Free Partnership it ranks as a country with limited protection against ETS (SFP, 2011). Since almost half of the children were exposed to ETS outside their home, the results of this study suggest that there is further potential for enhancing protection of children from ETS in Germany.

### 3.5. Phthalate metabolites in urine

The phthalate metabolites MEP, MBzP, MnBP and MiBP, 5OH-MEHP, 5oxo-MEHP and cx-MEPP were detected in all German children and their mothers, the primary DEHP-metabolite MEHP was detected in 93% of the children and in 85% of the mothers. Minimum and maximum values of the different phthalate metabolites are provided in the Supplementary Table 2. The German children had GMs for MBzP and MiBP and the mothers had GMs for MEP and MBzP which did not differ from those of all DEMOCOPHES countries. Compared to the whole DEMOCOPHES sample, the German children had lower GMs for MEP and the sum of 3 DEHP-metabolites (MEHP, 5OH-MEHP and 5oxo-MEHP) ( $22.7 \mu\text{g/L}$  versus  $34.4 \mu\text{g/L}$  and  $39.2 \mu\text{g/L}$  versus  $47.6 \mu\text{g/L}$ ) and a higher GM for MnBP ( $46.1 \mu\text{g/L}$  versus  $34.8 \mu\text{g/L}$ ), whereas the German mothers had lower GMs for MiBP and the sum of 3 DEHP-metabolites ( $25.1 \mu\text{g/L}$  versus  $30.1 \mu\text{g/L}$  and  $21.5 \mu\text{g/L}$  versus  $29.2 \mu\text{g/L}$ ) and also a higher GM for MnBP ( $31.5 \mu\text{g/L}$  versus  $23.9 \mu\text{g/L}$ ) (Table 6), hence showing some variation in the exposure pattern among the European countries.

Most measured phthalate metabolite concentrations of children and mothers were significantly correlated, only 5OH-MEHP and 5oxo-MEHP did not reach significance (Table 5). Compared to the mothers, children had 42–82% higher GMs of the urinary phthalate metabolites MBzP, MnBP, MiBP and DEHP and a 42% lower GM of



**Table 6**

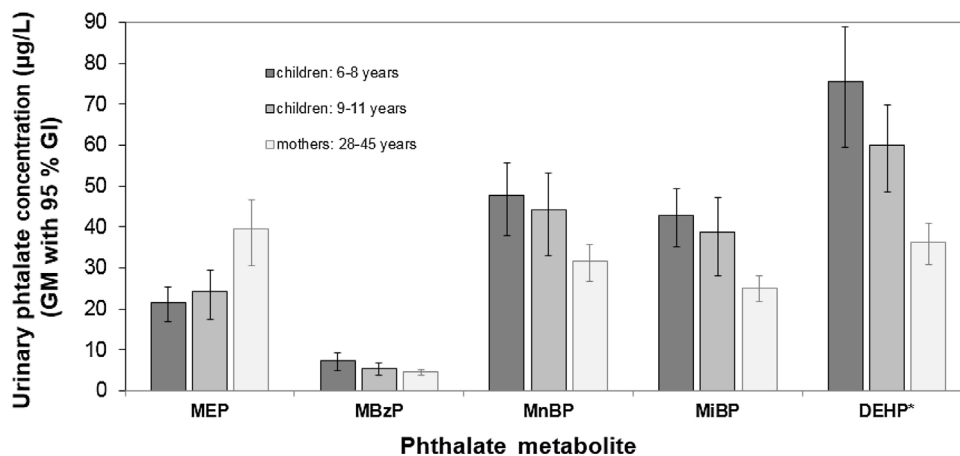
Frequency of quantification, geometric mean with 95% confidence interval (in parentheses) of phthalate metabolites of children and their mothers in DEMOCOPHES in Germany and in all DEMOCOPHES countries.

	DEMOCOPHES in Germany			All DEMOCOPHES countries <sup>a</sup>		
		% >LOQ	GM (95% CI)		% >LOQ	GM (95% CI)
MEP ( $\mu\text{g/L}$ urine)	Children (N = 120)	100	22.7 (19.5–26.4)	Children (N = 1816)	98.0	34.4 (32.8–36.0)
MEP ( $\mu\text{g/g}$ creatinine)			20.6 (18.1–23.5)			33.4 (31.9–34.9)
MEP ( $\mu\text{g/L}$ urine)	Mothers (N = 116)	100	39.4 (32.3–48.1)	Mothers (N = 1800)	95.2	48.2 (45.6–51.0)
MEP ( $\mu\text{g/L}$ creatinine)			35.0 (28.9–42.4)			42.9 (40.8–45.2)
MBzP ( $\mu\text{g/L}$ urine)	Children (N = 120)	100	6.47 (5.31–7.89)	Children (N = 1816)	95.2	7.15 (6.83–7.48)
MBzP ( $\mu\text{g/g}$ creatinine)			5.88 (4.94–7.00)			6.94 (6.66–7.24)
MBzP ( $\mu\text{g/L}$ urine)	Mothers (N = 116)	100	4.55 (3.83–5.40)	Mothers (N = 1800)	91.8	4.51 (4.31–4.72)
MBzP ( $\mu\text{g/L}$ creatinine)			4.03 (3.48–4.68)			4.02 (3.87–4.18)
MnBP ( $\mu\text{g/L}$ urine)	Children (N = 120)	100	46.1 (40.0–53.0)	Children (N = 1355)	99.9	34.8 (33.5–36.2)
MnBP ( $\mu\text{g/g}$ creatinine)			41.9 (37.4–46.9)			34.0 (32.8–35.2)
MnBP ( $\mu\text{g/L}$ urine)	Mothers (N = 116)	100	31.5 (27.4–36.1)	Mothers (N = 1347)	99.4	23.9 (23.0–24.9)
MnBP ( $\mu\text{g/L}$ creatinine)			27.9 (25.3–30.8)			21.5 (20.8–22.2)
MiBP ( $\mu\text{g/L}$ urine)	Children (N = 120)	100	40.9 (35.7–47.0)	Children (N = 1355)	99.8	45.4 (43.6–47.3)
MiBP ( $\mu\text{g/g}$ creatinine)			37.2 (33.2–41.8)			44.3 (42.7–46.0)
MiBP ( $\mu\text{g/L}$ urine)	Mothers (N = 116)	100	25.1 (22.1–28.4)	Mothers (N = 1347)	99.4	30.1 (28.9–31.4)
MiBP ( $\mu\text{g/L}$ creatinine)			22.2 (20.2–24.5)			27.0 (26.1–28.0)
DEHP <sup>b</sup> ( $\mu\text{g/L}$ urine)	Children (N = 120)	93–100	39.2 (34.3–44.7)	Children (N = 1816)	85.6	47.6 (46.0–49.3)
DEHP <sup>b</sup> ( $\mu\text{g/g}$ creatinine)			35.6 (31.7–40.0)			46.2 (44.9–47.7)
DEHP <sup>b</sup> ( $\mu\text{g/L}$ urine)	Mothers (N = 116)	85–100	21.5 (18.7–24.8)	Mothers (N = 1800)	81.6	29.2 (28.1–30.3)
DEHP <sup>b</sup> ( $\mu\text{g/L}$ creatinine)			19.1 (17.2–21.3)			26.0 (25.2–26.9)

Abbreviations: N: sample size, LOQ: limit of quantification, GM: geometric mean, 95% CI: 95% confidence interval.

<sup>a</sup> data are from Den Hond et al., 2015; Supplemental Material.

<sup>b</sup> The sum of the following 3 DEHP-metabolites were measured: MEHP + 5OH-MEHP + 5oxo-MEHP.



\*:  $\sum$  3 DEHP metabolites: MEHP + 5OH-MEHP + 5oxo-MEHP

**Fig. 3.** Urinary phthalate concentrations of younger (6–8 years) and older (9–11 years) children and of their mothers.

MEP. These relations were found for all DEMOCOPHES countries (Den Hond et al., 2015). Additionally, similar ratios were found with respect to younger and older children (Fig. 3): 6–8 year old children had 8–31% higher levels of MBzP, MnBP, MiBP and DEHP-metabolites and 11% lower levels of MEP than the children aged 9–11 years. Besides age, several factors such as use of personal care products, the consumption of cereals, hazelnut spread, ice cream and chewing gums, potential industrial phthalate contamination in the neighborhood, and poly-vinyl chloride (PVC) materials at home were positively correlated with phthalate concentrations in children and in mothers in all DEMOCOPHES countries (Den Hond et al., 2015) or in individual DEMOCOPHES participating countries (Cutanda et al., 2015). For the German DEMOCOPHES participants the use of fragrances and make-up were associated with higher MEP concentrations in mothers, the consumption of chewing gum with higher DEHP-metabolite concentrations in children and the

frequent consumption of cereals with elevated concentrations of MBzP in both, children and mothers. MBzP, MnBP and MiBP concentrations in children and MnBP and MiBP concentrations in mothers were higher for residents of the urban area than in residents of the rural area. Self-reported neighbourhood exposure to an industrial site with researcher deduced potential phthalate contamination was correlated with higher MnBP and DEHP-metabolite concentrations in children and with higher MnBP and MiBP concentrations in mothers. MBzP and DEHP-metabolite concentrations in children were associated with participant identified PVC materials in the home.

Comparison of the German data with representative GerES IV data of 3–14 year old German children analyzed for MBzP, MnBP, MiBP and DEHP-metabolites in urine (17.5  $\mu\text{g/L}$ , 95.6  $\mu\text{g/L}$ , 94.3  $\mu\text{g/L}$  and 91.3  $\mu\text{g/L}$ ) (Becker et al., 2009) revealed that the concentrations reported here amounted to only 37–52% of those

measured in GerES IV. Although the DEMOCOPHES and the GerES IV results cannot be compared directly because of the different age range, the lower concentrations found in DEMOCOPHES correspond to a decline of MBzP, MnBP and DEHP-metabolites observed in samples of the German Environmental Specimen Bank of students in the years 2002–2008 (medians declined from 7.8 µg/L to 3.8 µg/L for MBzP, from 65.4 µg/L to 19.6 µg/L for MnBP, and from 40.5 µg/L to 19.3 µg/L for DEHP-metabolites) (Göen et al., 2011). This may be a result of regulation on phthalates in consumer products (EC, 1999; EU, 2005).

Health based guidance values are available for MEP, MBzP, MnBP and for different combinations of DEHP metabolites (Aylward et al., 2009a,b; Schulz et al., 2012b; Apel et al., 2016). The proportion of children and mothers, exceeding either HBM-I values derived by the German Human Biomonitoring Commission (Schulz et al., 2012b; Apel et al., 2016) or BE values, derived by Aylward et al. (2009a,b), are shown in Table 7. Compared to the HBM-I value of 500 µg/L, 1.7% of the children had concentrations of 2 DEHP-metabolites (5OH-MEHP + 5oxo-MEHP) above the limit where adverse health effects cannot be excluded with sufficient certainty. Similarly, 2.5% of children exceeded the BE value of 4 metabolites of DEHP (MEHP + 5OH-MEHP + 5oxo-MEHP + cx-MEPP). Guidance values of MEP and MBzP were not exceeded. However, 4.2% of the children and 0.9% of the mothers exceeded the BE value for MnBP. These results show that even though average phthalate concentrations are lower in this study than in previous studies, a non-negligible proportion of children and mothers still exceed health based guidance values, indicating reasons for concern.

The results of DEMOCOPHES related to phthalates confirm that children are exposed concurrently to a mixture of phthalates. There is evidence, that phthalates can produce cumulative, additive adverse effects (Christiansen et al., 2009; Gray, 2008; Howdeshell et al., 2007). If cumulative exposure is taken into account, an even higher proportion of the population is expected to have exposure levels higher than health based guidance values. Therefore, evaluating the cumulative exposure to all endocrine active phthalates is needed (Lioy et al., 2015; NRC, 2008; Wittassek et al., 2011). Ongoing human biomonitoring of established, as well as of upcoming phthalates and their substitutes is needed to support actions to further reduce exposure in the general population and especially in high exposure groups.

#### 4. Conclusion

Germany conducted the human biomonitoring pilot study DEMOCOPHES as one of 17 European countries. The harmonized study protocol could be applied with only minor adaptations not interfering with comparability. However, the participation rate was fairly low and measures to improve it must be undertaken in future HBM studies.

Although the studied population samples were not representative for the respective countries, the harmonized study protocol allowed the collection of comparable HBM data in the different countries thus enabling elaboration of a broader European picture of the exposure to chemicals. Including males in the future will add to the picture. Compared to the European levels, Germany had lower levels for mercury and cotinine. This shows that European-wide actions only relying on German data could lead to actions that are not sufficiently protective for Europe.

Although there are regulations on phthalates in consumer products, health based guidance values for some phthalates were exceeded in German and in other European participants. These results indicate that human exposure to pollutants must continue to be monitored and reduced in Europe and in Germany.

**Table 7**  
German DEMOCOPHES participants exceeding health based guidance values.

Biomarker of exposure	Source of health-based guidance values:	Values <sup>a</sup> :			
		% exceeding health based guidance value			
		Children 6–11 years	GerES IV	Mothers 28–45 years	DEMOCOPHES
Mercury:	JEFCA-guideline based on USEPA reference dose	0.0%	–	0.0%	0.0%
Cadmium:	HBM-I value	0.8%	–	0.8%	0.8%
	BE based on ATSDR chronic MRL	0.0%	0.3%	0.0%	0.0%
DEP, measured as MEP; BBzP, measured as MBzP; DnBP, measured as MnBP;	BE based on USEPA subchronic RfD	0.0%	–	0.0%	0.0%
	BE based on USEPA subchronic RfD	0.0%	–	0.0%	0.0%
	BE based on EFSA subchronic TDI	0.0%	0.0%	0.0%	0.0%
	DEHP, measured as the sum of – 2 metabolites <sup>b</sup> :	4.2%	12.2%	0.9%	0.9%
– 3 metabolites <sup>c</sup> ; – 4 metabolites <sup>d</sup> :	HBM-I value	1.7%	1.4%	0.0%	0.0%
	BE based on USEPA chronic RfD	1.7%	6.3%	0.9%	0.9%
	BE based on USEPA chronic RfD	2.5%	6.5%	0.0%	0.0%

Abbreviations: GerES IV: German Environmental Survey IV (2003–2006), JEFCA: Joint FAO/WHO Expert Committee on Food Additives, USEPA: United States Environmental Protection Agency, HBM-I: human biomonitoring value I, BE: biomonitoring equivalent, ATSDR: Agency for Toxic Substances and Disease Registry, MRL: minimal risk level, RfD: reference dose, EFSA: European Food Safety Authority, TDI: tolerable daily intake.

<sup>a</sup> Health based guidance values for mercury: WHO Guideline, 2004 and NRC, 2000; cadmium: Schulz et al., 2012b and Hays et al., 2008; DEP, BBzP, DnBP: Aylward et al., 2009a; DEHP: Schulz et al., 2012b and Aylward et al., 2009b.

<sup>b</sup> 5OH-MEHP + 5oxo-MEHP.

<sup>c</sup> MEHP + 5OH-MEHP + 5oxo-MEHP.

<sup>d</sup> MEHP + 5OH-MEHP + 5oxo-MEHP + cx-MEPP.

Continuous HBM is necessary to track changes of pollutant exposure levels over time. In Germany, all chemicals measured in DEMOCOPHES were also determined in the population representative GerES and time trends are analysed in samples of the German Environmental Specimen Bank. The added value of participating in DEMOCOPHES was to increase our understanding of how exposure varies across Europe and to contribute to the development of a coherent and sustainable system of comparable HBM measurements for pollutant exposure estimation. Germany will continue to cooperate on the harmonisation of European human biomonitoring, currently through the HBM4EU Initiative, in order to support the EU chemicals regulation with the best possible exposure data and for the protection of all Europeans against environmental health risks.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ijheh.2017.01.012>.

## References

- Apel, P., Angerer, J., Wilhelm, M., Kolossa-Gehring, M., 2016. New HBM values for emerging substances, inventory of reference and HBM values in force, and working principles of the German human biomonitoring commission. *Int. J. Hyg. Environ. Health*, <http://dx.doi.org/10.1016/j.ijheh.2016.09.007>.
- Aylward, L.L., Hays, S.M., Gagne, M., Krishnan, K., 2009a. Derivation of biomonitoring equivalents for di-n-butyl phthalate (DBP), benzylbutyl phthalate (BzBP), and diethyl phthalate (DEP). *Regul. Toxicol. Pharmacol.: RTP* 55, 259–267.
- Aylward, L.L., Hays, S.M., Gagne, M., Krishnan, K., 2009b. Derivation of biomonitoring equivalents for di(2-ethylhexyl)phthalate (CAS No. 117-81-7). *Regul. Toxicol. Pharmacol.: RTP* 55, 249–258.
- Becker, K., Müssig-Zufika, M., Conrad, A., Lüdecke, A., Schulz, C., Seiwert, M., Kolossa-Gehring, M., 2008. *German Environmental Survey for Children 2003/2006 – GerES IV – Human Biomonitoring. Levels of Selected Substances in Blood and Urine of Children in Germany*. Federal Environment Agency (UBA), WaBoLu-Hefte 01/08.
- Becker, K., Göen, T., Seiwert, M., Conrad, A., Pick-Fuss, H., Müller, J., Wittassek, M., Schulz, C., Kolossa-Gehring, M., 2009. *GerES IV: phthalate metabolites and bisphenol A in urine of German children*. *Int. J. Hyg. Environ. Health* 212, 685–692.
- Becker, K., Seiwert, M., Casteleyn, L., Joas, R., Joas, A., Biot, P., Aerts, D., Castano, A., Esteban, M., Angerer, J., Koch, H.M., Schoeters, G., Den Hond, E., Sepai, O., Exley, K., Knudsen, L.E., Horvat, M., Bloemen, L., Kolossa-Gehring, M., consortium, D., 2014. *A systematic approach for designing a HBM pilot study for Europe*. *Int. J. Hyg. Environ. Health* 217, 312–322.
- Bellanger, M., Pichery, C., Aerts, D., Berglund, M., Castano, A., Cejchanova, M., Crettaz, P., Davidson, F., Esteban, M., Fischer, M.E., Gurzau, A.E., Halzlova, K., Katsonouri, A., Knudsen, L.E., Kolossa-Gehring, M., Koppen, G., Ligočka, D., Miklavcic, A., Reis, M.F., Rudnai, P., Tratnik, J.S., Weihe, P., Budtz-Jørgensen, E., Grandjean, P., Demo/Cophes, 2013. *Economic benefits of methylmercury exposure control in Europe: monetary value of neurotoxicity prevention*. *Environ. Health: A Glob. Access Sci. Source* 12, 3.
- Blaszkiewicz, M., Liesenhoff-Henze, K., 2010. Creatinine in urine. In: Angerer, J. (Ed.), *The MAK-Collection. Part IV: Biomonitoring Methods*, vol. 12. Wiley-VCH, Weinheim (p. 169).
- Bundesgesundheitsblatt, 2005. *Haaranalyse in der Umweltmedizin. Stellungnahme der Kommission Human-Biomonitoring des Umweltbundesamtes*. Bundesgesundheitsblatt-Gesundheitsforschung-Gesundheitsschutz 48, 246–250.
- CDC, 2006. *Surgeon General's Report – The Health Consequences of Involuntary Exposure to Tobacco*. CDC, Atlanta. Available at: [http://www.cdc.gov/tobacco/data\\_statistics/sgr/2006/pdfs/children-shs.pdf](http://www.cdc.gov/tobacco/data_statistics/sgr/2006/pdfs/children-shs.pdf).
- Castano, A., Sanchez-Rodriguez, J.E., Canas, A., Esteban, M., Navarro, C., Rodriguez-Garcia, A.C., Arribas, M., Diaz, G., Jimenez-Guerrero, J.A., 2012. *Mercury, lead and cadmium levels in the urine of 170 Spanish adults: a pilot human biomonitoring study*. *Int. J. Hyg. Environ. Health* 215, 191–195.
- Castano, A., Cutanda, F., Esteban, M., Part, P., Navarro, C., Gomez, S., Rosado, M., Lopez, A., Lopez, E., Exley, K., Schindler, B.K., Govarts, E., Casteleyn, L., Kolossa-Gehring, M., Fiddicke, U., Koch, H., Angerer, J., Den Hond, E., Schoeters, G., Sepai, O., Horvat, M., Knudsen, L.E., Aerts, D., Joas, A., Biot, P., Joas, R., Jimenez-Guerrero, J.A., Diaz, G., Pirard, C., Katsonouri, A., Cerna, M., Gutleb, A.C., Ligočka, D., Reis, M.F., Berglund, M., Lupsa, I.R., Halzlova, K., Charlier, C., Cullen, E., Hadjipanayis, A., Krskova, A., Jensen, J.F., Nielsen, J.K., Schwedler, G., Wilhelm, M., Rudnai, P., Kozepesy, S., Davidson, F., Fischer, M.E., Janasik, B., Namorado, S., Gurzau, A.E., Jajcay, M., Mazej, D., Tratnik, J.S., Larsson, K., Lehmann, A., Crettaz, P., Lavranos, G., Posada, M., 2015. *Fish consumption patterns and hair mercury levels in children and their mothers in 17 EU countries*. *Environ. Res.* 141, 58–68.
- Casteleyn, L., Dumez, B., Becker, K., Kolossa-Gehring, M., Den Hond, E., Schoeters, G., Castano, A., Koch, H.M., Angerer, J., Esteban, M., Exley, K., Sepai, O., Bloemen, L., Horvat, M., Knudsen, L.E., Joas, A., Joas, R., Biot, P., Koppen, G., Dewolf, M.C., Katsonouri, A., Hadjipanayis, A., Cerna, M., Krskova, A., Schwedler, G., Fiddicke, U., Nielsen, J.K., Jensen, J.F., Rudnai, P., Kozepesy, S., Mulcahy, M., Mannion, R., Gutleb, A.C., Fischer, M.E., Ligočka, D., Jakubowski, M., Reis, M.F., Namorado, S., Lupsa, I.R., Gurzau, A.E., Halzlova, K., Jajcay, M., Mazej, D., Tratnik Snoj, J., Posada, M., Lopez, E., Berglund, M., Larsson, K., Lehmann, A., Crettaz, P., Aerts, D., 2015. *A pilot study on the feasibility of European harmonized human biomonitoring: strategies towards a common approach, challenges and opportunities*. *Environ. Res.* 141, 3–14.
- Cerna, M., Krskova, A., Cejchanova, M., Spevackova, V., 2012. *Human biomonitoring in the Czech Republic: an overview*. *Int. J. Hyg. Environ. Health* 215, 109–119.
- Christiansen, S., Scholze, M., Dalgaard, M., Vinggaard, A.M., Axelstad, M., Kortenkamp, A., Hass, U., 2009. *Synergistic disruption of external male sex organ development by a mixture of four antiandrogens*. *Environ. Health Perspect.* 117, 1839–1846.
- Cutanda, F., Koch, H.M., Esteban, M., Sanchez, J., Angerer, J., Castano, A., 2015. *Urinary levels of eight phthalate metabolites and bisphenol A in mother-child pairs from two Spanish locations*. *Int. J. Hyg. Environ. Health* 218, 47–57.
- Dakeishi, M., NAKAI, K., Sakamoto, M., Iwata, T., Suzuki, K., Lui, X., Ohno, T., Kurosawa, T., Satoh, H., Murata, K., 2005. *Effects of hair treatment on hair mercury—the best biomarker of methylmercury exposure?* *Environ. Health Prev. Med.* 10, 208–212.
- Den Hond, E., Govarts, E., Willems, H., Smolders, R., Casteleyn, L., Kolossa-Gehring, M., Schwedler, G., Seiwert, M., Fiddicke, U., Castano, A., Esteban, M., Angerer, J., Koch, H.M., Schindler, B.K., Sepai, O., Exley, K., Bloemen, L., Horvat, M., Knudsen, L.E., Joas, A., Joas, R., Biot, P., Aerts, D., Koppen, G., Katsonouri, A., Hadjipanayis, A., Krskova, A., Maly, M., Morck, T.A., Rudnai, P., Kozepesy, S., Mulcahy, M., Mannion, R., Gutleb, A.C., Fischer, M.E., Ligočka, D., Jakubowski, M., Reis, M.F., Namorado, S., Gurzau, A.E., Lupsa, I.R., Halzlova, K., Jajcay, M., Mazej, D., Tratnik, J.S., Lopez, A., Lopez, E., Berglund, M., Larsson, K., Lehmann, A., Crettaz, P., Schoeters, G., 2015. *First steps toward harmonized human biomonitoring in Europe: demonstration project to perform human biomonitoring on a European scale*. *Environ. Health Perspect.* 123, 255–263.
- EC, 1999. *Commission Decision 1999/815/E of 7 December 1999 adopting measures prohibiting the placing on the market of toys and childcare articles intended to be placed in the mouth by children under three years of age made of soft PVC containing one or more of the substances di-iso-nonyl phthalate (DINP), di(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), di-iso-decyl phthalate (DIDP), di-n-octyl phthalate (DNOP), and butylbenzyl phthalate (BBP)*. Official Journal of the European Communities, L 315/46-L315/49 of 9.12.1999.
- EU, 2005. *Directive 2005/84/EC of the European Parliament and of the Council of 14 December 2005 amending for the 22nd time Council Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (phthalates in toys and childcare articles)*. *Off. J. Eur. Union*, L344/40-L344/43 of 27.12.2005.
- Esteban, M., Schindler, B.K., Jimenez, J.A., Koch, H.M., Angerer, J., Rosado, M., Gomez, S., Casteleyn, L., Kolossa-Gehring, M., Becker, K., Bloemen, L., Schoeters, G., Den Hond, E., Sepai, O., Exley, K., Horvat, M., Knudsen, L.E., Joas, A., Joas, R., Aerts, D., Biot, P., Borosova, D., Davidson, F., Dumitrascu, I., Fischer, M.E., Grandier, M., Janasik, B., Jones, K., Kasparova, L., Larssen, T., Naray, M., Nielsen, F., Hohenblum, P., Pinto, R., Pirard, C., Plateel, G., Tratnik, J.S., Wittsiepe, J., Castano, A., Laboratories, E.R., 2015. *Mercury analysis in hair: comparability and quality assessment within the transnational COPHES/DEMOCOPHES project*. *Environ. Res.* 141, 24–30.
- European Commission, 2013. *Report on the implementation of the Council Recommendation of 30 November 2009 on Smoke-free Environments (2009/C 296/02)*.
- Exley, K., Cano, N., Aerts, D., Biot, P., Casteleyn, L., Kolossa-Gehring, M., Schwedler, G., Castano, A., Angerer, J., Koch, H.M., Esteban, M., Schoeters, G., Den Hond, E.,



- Horvat, M., Bloemen, L., Knudsen, L.E., Joas, R., Joas, A., Dewolf, M.C., Van de Mierop, E., Katsonouri, A., Hadjipanayis, A., Cerna, M., Krskova, A., Becker, K., Fiddicke, U., Seiwert, M., Morck, T.A., Rudnai, P., Kozepesy, S., Cullen, E., Kellegher, A., Gutleb, A.C., Fischer, M.E., Ligočka, D., Kaminska, J., Namorado, S., Reis, M.F., Lupsa, I.R., Gurzau, A.E., Halzlova, K., Jajcaj, M., Mazej, D., Tratnik, J.S., Huetos, O., Lopez, A., Berglund, M., Larsson, K., Sepai, O., 2015. **Communication in a Human biomonitoring study: focus group work, public engagement and lessons learnt in 17 European countries.** *Environ. Res.* 141, 31–41.
- Fiddicke, U., Becker, K., Schwedler, G., Seiwert, M., Joas, R., Joas, A., Biot, P., Aerts, D., Casteleyn, L., Dumez, B., Castano, A., Esteban, M., Angerer, J., Koch, H.M., Schoeters, G., Den Hond, E., Sepai, O., Exley, K., Knudsen, L.E., Horvat, M., Bloemen, L., Katsonouri, A., Hadjipanayis, A., Cerna, M., Krskova, A., Jensen, J.F., Nielsen, J.K., Rudnai, P., Kozepesy, S., Gutleb, A.C., Fischer, M.E., Ligočka, D., Kaminska, J., Reis, M.F., Namorado, S., Lupsa, I.R., Gurzau, A.E., Halzlova, K., Mazej, D., Tratnik, J.S., Rivas, T.C., Gomez, S., Berglund, M., Larsson, K., Lehmann, A., Crettaz, P., Dewolf, M.C., Burns, D., Kellegher, A., Kolossa-Gehring, M., 2015. **Lessons learnt on recruitment and fieldwork from a pilot European human biomonitoring survey.** *Environ. Res.* 141, 15–23.
- Frery, N., Vandentorren, S., Etchevers, A., Fillol, C., 2012. **Highlights of recent studies and future plans for the French human biomonitoring (HBM) programme.** *Int. J. Hyg. Environ. Health* 215, 127–132.
- Göen, T., Dobler, L., Koschorreck, J., Müller, J., Wiesmuller, G.A., Drexler, H., Kolossa-Gehring, M., 2011. **Trends of the internal phthalate exposure of young adults in Germany—follow-up of a retrospective human biomonitoring study.** *Int. J. Hyg. Environ. Health* 215, 36–45.
- Göen, T., Schaller, K.H., Drexler, H., 2012. **External quality assessment of human biomonitoring in the range of environmental exposure levels.** *Int. J. Hyg. Environ. Health* 215, 229–232.
- Gray, L.E., 2008. U.S. Congress. House of Representatives. Committee on Energy and Commerce, Subcommittee on Commerce, Trade and Consumer Protection. **Hearing on Safety of Phthalates and Bisphenol-A in Everyday Consumer Products.** June 10, 2008. Wirtten testimony of Leon Earl Gray, Jr., Senior Reproductive Biologist and Toxicologist, U.S. EPA.
- Harkins, D.K., Susten, A.S., 2003. **Hair analysis: exploring the state of the science.** *Environ. Health Perspect.* 111, 576–578.
- Hays, S.M., Nordberg, M., Yager, J.W., Aylward, L.L., 2008. **Biomonitoring equivalents (BE) dossier for cadmium (Cd) (CAS No. 7440-43-9).** *Regul. Toxicol. Pharmacol.*: RTP 51, S49–56.
- Hohenblum, P., Steinbichl, P., Raffesberg, W., Weiss, S., Moche, W., Vallant, B., Scharf, S., Haluzá, D., Moshammer, H., Kundi, M., Piegler, B., Wallner, P., Huttrer, H.P., 2012. **Pollution gets personal! A first population-based human biomonitoring study in Austria.** *Int. J. Hyg. Environ. Health* 215, 176–179.
- Howdeshell, K.L., Furr, J., Lambright, C.R., Rider, C.V., Wilson, V.S., Gray Jr., L.E., 2007. **Cumulative effects of dibutyl phthalate and diethylhexyl phthalate on male rat reproductive tract development: altered fetal steroid hormones and genes.** *Toxicol. Sci.* 99, 190–202.
- Jarrett, J.M., Xiao, G., Caldwell, K.L., Henahan, D., Shakirova, G., Jones, R.L., 2008. **Eliminating molybdenum oxide interference in urine cadmium biomonitoring using ICP-DRS-MS.** *J. Anal. At. Spectrom.* 23, 962–967.
- Joas, R., Casteleyn, L., Biot, P., Kolossa-Gehring, M., Castano, A., Angerer, J., Schoeters, G., Sepai, O., Knudsen, L.E., Joas, A., Horvat, M., Bloemen, L., 2012. **Harmonized human biomonitoring in Europe: activities towards an EU HBM framework.** *Int. J. Hyg. Environ. Health* 215, 172–175.
- Joas, A., Knudsen, L.E., Kolossa-Gehring, M., Sepai, O., Casteleyn, L., Schoeters, G., Angerer, J., Castano, A., Aerts, D., Biot, P., Horvat, M., Bloemen, L., Reis, M.F., Lupsa, I.R., Katsonouri, A., Cerna, M., Berglund, M., Crettaz, P., Rudnai, P., Halzlova, K., Mulcahy, M., Gutleb, A.C., Fischer, M.E., Becker, G., Frery, N., Jensen, G., Van Vliet, L., Koch, H.M., Den Hond, E., Fiddicke, U., Esteban, M., Exley, K., Schwedler, G., Seiwert, M., Ligočka, D., Hohenblum, P., Kyrtopoulos, S., Botsivali, M., DeFelip, E., Guillou, C., Reniero, F., Grazuleviciene, R., Veidebaum, T., Morck, T.A., Nielsen, J.K., Jensen, J.F., Rivas, T.C., Sanchez, J., Koppen, G., Smolders, R., Kozepesy, S., Hadjipanayis, A., Krskova, A., Mannion, R., Jakubowski, M., Fucic, J.A., Pereira-Miguel, J., Gurzau, A.E., Jajcaj, M., Mazej, D., Tratnik, J.S., Lehmann, A., Larsson, K., Dumez, B., Joas, R., 2015. **Policy recommendations and cost implications for a more sustainable framework for European human biomonitoring surveys.** *Environ. Res.* 141, 42–57.
- Karagas, M.R., Choi, A.L., Oken, E., Horvat, M., Schoeny, R., Kamai, E., Cowell, W., Grandjean, P., Korrick, S., 2012. **Evidence on the human health effects of low-level methylmercury exposure.** *Environ. Health Perspect.* 120, 799–806.
- Kasper-Sonnenberg, M., Koch, H.M., Wittsiepe, J., Wilhelm, M., 2012. **Levels of phthalate metabolites in urine among mother-child-pairs—results from the Duisburg birth cohort study, Germany.** *Int. J. Hyg. Environ. Health* 215, 373–382.
- Koch, H.M., Angerer, J., 2008. **Di(2-ethylhexyl) phthalate (DEHP) metabolites (2-ethyl-5-hydroxyhexyl phthalate, 2-ethyl-5-oxohexyl phthalate, mono-(2-ethylhexyl)phthalate) in urine.** In: Angerer, J. (Ed.), *The MAK-Collection Part IV: Biomonitoring Methods.* Wiley-VCH, Weinheim (p. 73).
- Koch, H.M., Lorber, M., Christensen, K.L., Palmke, C., Koslitz, S., Brüning, T., 2013. **Identifying sources of phthalate exposure with human biomonitoring: results of a 48 h fasting study with urine collection and personal activity patterns.** *Int. J. Hyg. Environ. Health* 216, 672–681.
- Kolossa-Gehring, M., Becker, K., Conrad, A., Schröter-Kermani, C., Schulz, C., Seiwert, M., 2012. **Environmental surveys, specimen bank and health related environmental monitoring in Germany.** *Int. J. Hyg. Environ. Health* 215, 120–126.
- Lioy, P.J., Hauser, R., Gennings, C., Koch, H.M., Mirkes, P.E., Schwetz, B.A., Kortenkamp, A., 2015. **Assessment of phthalates/phthalate alternatives in children's toys and childcare articles: review of the report including conclusions and recommendation of the chronic hazard advisory panel of the consumer product safety commission.** *J. Expo. Sci. Environ. Epidemiol.* 25, 343–353.
- McDowell, M., Dillon, C.F., Osterloh, J., Bolger, M., Pellizzari, E., Fernando, R., Montes de Oca, R., Schober, S., Sinks, T., Jones, R.L., Mahaffey, K.R., 2004. **Hair mercury levels in U.S. children and women of childbearing age; reference range data from NHANES 1999–2000.** *Environ. Health Perspect.* 112, 1165–1171.
- NRC, 2000. **Toxicological Effects of Methylmercury.** National Academy Press, Washington, DC.
- NRC, 2008. **Committee on the Health Risks of Phthalates. Phthalates and Cumulative Risk Assessment: The Tasks Ahead.** National Academies Press, US Washington (DC), Available at: <http://www.ncbi.nlm.nih.gov/books/NBK215033>.
- Pesch, A., Wilhelm, M., Rostek, U., Schmitz, N., Weishoff-Houben, M., Ranft, U., Idel, H., 2002. **Mercury concentrations in urine, scalp hair, and saliva in children from Germany.** *J. Expo. Anal. Environ. Epidemiol.* 12, 252–258.
- SFP, 2011. **Smoke-free legislation in the EU.** Available at: <http://www.smokefreepartnership.eu/smoke-free-legislation-eu>.
- Schindler, B.K., Esteban, M., Koch, H.M., Castano, A., Koslitz, S., Canas, A., Casteleyn, L., Kolossa-Gehring, M., Schwedler, G., Schoeters, G., Hond, E.D., Sepai, O., Exley, K., Bloemen, L., Horvat, M., Knudsen, L.E., Joas, A., Joas, R., Biot, P., Aerts, D., Lopez, A., Huetos, O., Katsonouri, A., Maurer-Chronakis, K., Kasparova, L., Vrbik, K., Rudnai, P., Naray, M., Guignard, C., Fischer, M.E., Ligočka, D., Janasik, B., Reis, M.F., Namorado, S., Pop, C., Dumitrascu, I., Halzlova, K., Fabianova, E., Mazej, D., Tratnik, J.S., Berglund, M., Jonsson, B., Lehmann, A., Crettaz, P., Frederiksen, H., Nielsen, F., McGrath, H., Nesbitt, I., De Cremer, K., Vanermen, G., Koppen, G., Wilhelm, M., Becker, K., Angerer, J., 2014. **The European COPHES/DEMOCOPHES project: towards transnational comparability and reliability of human biomonitoring results.** *Int. J. Hyg. Environ. Health* 217, 653–661.
- Schoeters, G., Den Hond, E., Colles, A., Loots, I., Morrens, B., Keune, H., Bruckers, L., Nawrot, T., Sioen, I., De Coster, S., Van Larebeke, N., Nelen, V., Van de Mierop, E., Vrijens, J., Croes, K., Goeyens, K., Baeyens, W., 2012. **Concept of the Flemish human biomonitoring programme.** *Int. J. Hyg. Environ. Health* 215, 102–108.
- Schramel, P., Wendler, I., Dunemann, L., Fleischmann, M., Emmons, H., 1999. **Working group analyses in biological materials. antimony, lead, cadmium, platinum, mercury, tellurium, thallium, bismuth, tungsten, tin. determination in urine.** In: Angerer, J., Schaller, K., Greims, H. (Eds.), *Analysis of Hazardous Substances in Biological Materials*, vol. 6. Wiley-VCH, Weinheim, pp. 79–109.
- Schulz, C., Conrad, A., Becker, K., Kolossa-Gehring, M., Seiwert, M., Seifert, B., 2007. **Twenty years of the German Environmental Survey (GerES): human biomonitoring—temporal and spatial (West Germany/East Germany) differences in population exposure.** *Int. J. Hyg. Environ. Health* 210, 271–297.
- Schulz, C., Seiwert, M., Babisch, W., Becker, K., Conrad, A., Szewczyk, R., Kolossa-Gehring, M., 2012a. **Overview of the study design, participation and field work of the German Environmental Survey on Children 2003–2006 (GerES IV).** *Int. J. Hyg. Environ. Health* 215, 435–448.
- Schulz, C., Wilhelm, M., Heudorf, U., Kolossa-Gehring, M., 2012b. **Reprint of update of the reference and HBM values derived by the German human biomonitoring commission.** *Int. J. Hyg. Environ. Health* 215, 150–158.
- UNEP/WHO, 2008. **Guidance for Identifying Populations at Risk from Mercury Exposure.** United Nations Environment Program/World Health Organization, Geneva.
- Valent, F., Mariuz, M., Bin, M., Little, D., Mazej, D., Tognin, V., Tratnik, J., McAfee, A.J., Mulhern, M.S., Parpinel, M., Carrozzi, M., Horvat, M., Tamburlini, G., Barbone, F., 2013. **Associations of prenatal mercury exposure from maternal fish consumption and polyunsaturated fatty acids with child neurodevelopment: a prospective cohort study in Italy.** *J. Epidemiol. Jpn. Epidemiol. Assoc.* 23, 360–370.
- WHO, 1996. **Biological Monitoring of Chemicals Exposure in the Workplace—Guidelines.** Available at: [http://whqlibdoc.who.int/hq/1996/WHO\\_HPR\\_OCH\\_96.1.pdf](http://whqlibdoc.who.int/hq/1996/WHO_HPR_OCH_96.1.pdf).
- WHO, 2004. **Evaluation of Certain Food Additives and Contaminants.** WHO Technical Report Series 922. Sixty-First Meeting of the Joint FAO/WHO Expert Committee on Food Additives, Available at: [http://whqlibdoc.who.int/trs/WHO\\_TRS\\_922.pdf](http://whqlibdoc.who.int/trs/WHO_TRS_922.pdf).
- WHO, 2007. **Policy Recommendations on Protection from Exposure to Second-hand Tobacco Smoke.** Policy Recommendations, Available at: [http://www.who.int/tobacco/resources/publications/wntd/2007/policy\\_recommendations/en/](http://www.who.int/tobacco/resources/publications/wntd/2007/policy_recommendations/en/).
- Wittassek, M., Koch, H.M., Angerer, J., Brüning, T., 2011. **Assessing exposure to phthalates—the human biomonitoring approach.** *Mol. Nutr. Food Res.* 55, 7–31.