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RESEARCH ARTICLE

Pilot study testing a European human biomonitoring framework for biomarkers of chemical exposure in children and their mothers: experiences in the UK

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Abstract Exposure to a number of environmental chemicals in UK mothers and children has been assessed as part of the European biomonitoring pilot study, Demonstration of a Study to Coordinate and Perform Human Biomonitoring on a European Scale (DEMOCOPHES). For the European-funded project, 17 countries tested the biomonitoring guidelines and protocols developed by COPHES. The results from the pilot study in the UK are presented; 21 school children aged 6–11 years old and their mothers provided hair samples to measure mercury and urine samples, to measure cadmium, cotinine and several phthalate metabolites: mono(2-ethyl-5-hydroxyhexyl)phthalate (5OH-MEHP), mono(2-ethyl-5-oxohexyl)phthalate (5oxo-MEHP) and mono(2-ethylhexyl)phthalate (MEHP), mono-ethyl phthalate (MEP), mono-iso-butyl phthalate (MiBP), mono-benzyl phthalate (MBzP) and mono-n-butyl phthalate (MnBP). Questionnaire data was collected on environment, health and lifestyle. Mercury in hair was higher in children who reported frequent consumption of fish (geometric mean 0.35 μg/g) compared to those that ate fish less frequently (0.13 μg/g, p=0.002). Cadmium accumulates with age as demonstrated by higher levels of urinary cadmium in the mothers (geometric mean 0.24 μg/L) than in the children(0.14 μg/L). None of the mothers reported being regular smokers, and this was evident with extremely low levels of cotinine measured (maximum value 3.6 μg/L in mothers, 2.4 μg/L in children). Very low levels of the phthalate metabolites were also measured in both mothers and children (geometric means in mothers: 5OH-

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MEHP 8.6 μg/L, 5oxo-MEHP 5.1 μg/L, MEHP 1.2 μg/L, MEP 26.8 μg/L, MiBP 17.0 μg/L, MBzP 1.6 μg/L and MnBP 13.5 μg/L; and in children: 5OH-MEHP 18.4 μg/L, 5oxo-MEHP 11.4 μg/L, MEHP 1.4 μg/L, MEP 14.3 μg/L, MiBP 25.8 μg/L, MBzP 3.5 μg/L and MnBP 22.6 μg/L). All measured biomarker levels were similar to or below population-based reference values published by the US National Health and Nutrition Examination Survey (NHANES) and Germany's GerES surveys. No results were above available health guidance values and were of no concern with regards to health. The framework and techniques learnt here will assist with future work on biomonitoring in the UK.

Keywords DEMOCOPHES · Biomonitoring · Cotinine · Mercury · Phthalates · Cadmium · Environmental exposure

Introduction

In the UK, public health risk assessment of exposure to environmental chemicals uses environmental monitoring of air, water and land to compare with environmental standards and guidelines. This can be extrapolated to provide information on possible exposures of a population, but it does not necessarily reflect actual levels of chemical uptake. Human biomonitoring (HBM) provides a more direct measure of actual personal exposure to environmental chemicals, but interpretation can be limited due to a lack of UK population based reference levels to compare with. There are some health-based projects that have contributed to the understanding of environmental chemical exposures in the UK population, such as the Avon Longitudinal Study of Parents and Children (ALSPAC), which has published, for example, on lead and mercury exposures in pregnancy (Golding et al. 2013; Taylor et al. 2013). A study for the UK population by Bevan and colleagues collected data to develop reference ranges for a number of environmental chemicals in widespread use including benzene, cadmium, mercury and naphthalene (Bevan et al. 2013). However, the UK at present does not have a National HBM programme for chemical exposures, and ideally, future studies would have protocols that enable the work to be comparable with other biomonitoring programmes (Exley 2014).

A number of countries have long-term national biomonitoring programmes such as the National Health and Nutrition Examination Survey in the US (NHANES), the German Environmental Survey (GerES), the HBM project in the Czech Republic (CZ-HBM) and the Flemish Environment and Health Study (FLEHS). These programmes have established reference values, tracked trends of chemical exposures over time and determined regional differences in contaminant levels. The results from these studies can be used to inform policy needs, to evaluate effectiveness of policy measures and to determine whether environmental exposures lead to biological effects (Becker et al. 2007; Centers for Disease Control and Prevention 2014; Černá et al. 2012; Schoeters et al. 2012).

In 2003, the EU identified the importance of HBM but recognised that despite a number of European countries actively involved in HBM, there was a lack of comparable data and so it recommended that protocols be harmonised (Commission of the European Communities 2003). To enable the collection of comparable HBM data throughout Europe, a framework and protocols were developed by the 'Consortium to Perform Human Biomonitoring on a European Scale' (COPHES). COPHES was formed in 2009 with European scientists and stakeholders from 27 European countries and funded by the European Union (EU). In 2010, 'Demonstration of a Study to Coordinate and Perform Human Biomonitoring on a European Scale' (DEMOCOPHES) involving 21 European countries started work on a pilot study, funded by the EU and participating countries, to test in 17 countries the harmonised approach and protocols developed by COPHES (Joas et al. 2012).

The aim of DEMOCOPHES was for each participating country to test the protocols by recruiting 120 children and their mothers to provide urine and hair samples to measure exposure to cadmium, mercury, phthalates and environmental tobacco smoke and to complete an exposure questionnaire. Cadmium in urine, mercury in hair and exposure to environmental tobacco smoke, assessed by the measurement of cotinine, were chosen because they are of public health concern and would allow the testing of different biological samples and organic and inorganic substances for which health-based guidance values or reference values are available. Phthalates, which are used as plasticisers and solvents and are found in flooring and wall coverings, personal care products, medical devices and food contact materials, were also included (Angerer et al. 2011; Becker et al. 2009; Hauser and Calafat 2005). Population exposure to phthalates is ubiquitous, and the major pathway of exposure of the general population is via the diet (Angerer et al. 2011; Becker et al. 2009; Hauser and Calafat 2005). Exposure to phthalates is assessed by analysis of their urinary metabolites (Koch et al. 2003; Silva et al. 2004). The phthalates chosen by the consortium to be studied were di(2-ethylhexyl) phthalate, di-n-butyl phthalate (DnBP), di-iso-butyl phthalate (DiBP) and butyl benzyl phthalate (BBzP), which are classified as reprotoxic so their use is restricted according to Directive 2005/84/EC (European Commission 2005), and diethyl phthalate (DEP). The UK was one of the participating countries in the pilot study, and this paper discusses the results from the UK, which assessed the exposure to these chemicals in 21 children and their mothers.

Methods

The UK DEMOCOPHES pilot study protocol was based on the COPHES common European pilot study protocol with slight modifications (as listed below); discussion of the approach for designing the study has been published (Becker et al. 2013). The pilot study was cross-sectional in design and participating countries could recruit via population registries or by schools. In the UK, children (age group of 6 to 11 years) and accompanying mothers aged up to 50 years old (this is a slight modification to the COPHES protocol which recommended up to 45 years old) were recruited via schools and personal contacts around Stonehouse in Gloucestershire and Chilton in Oxfordshire. Twenty schools were approached for the study and three schools agreed to take part. Visits to these schools were arranged to talk to children and parents about the study, and information about the study was included in school newsletters. Invite letters were sent to the mothers of all eligible children (871 aged 6–11 years) through the school and/or email. Sampling ran from 30 January to 24 April 2012.

The inclusion criteria for the study were as follows: mother and child must have been living in the sampling area for the last 5 years. Only one child per mother (randomly selected) could be included in the study, the child must live most of the time (>16 days/month) with the mother and none of the participants should have metabolic disturbances or abnormal urine excretion. Participants were asked to bring a first-morning urine sample to an appointment at a local study centre, where they were asked to provide a hair sample and to answer a questionnaire of relevant exposure behaviours.

Information leaflets and consent forms were sent to participants in advance of the appointment along with urine sample containers (Starplex 80-mL polypropylene containers #3007-1) in order to provide first-morning samples at home on the day of the appointment. The urine samples were stored at 4 °C, before sending to the laboratory for aliquoting and analysis.

During the appointment at the study centre, the mother was asked to provide consent for themselves and their child to take part. Children were also given the opportunity to express their agreement to participate on an informed assent form, written in age-appropriate language. Then, the urine and hair samples of mother and child were collected, following the Standard Operating Procedure provided by the COPHES Quality Assurance Unit (Esteban et al. 2014). For the hair sampling, the hair was grasped from the middle of the back of the head and clipped out of the way. Several strands of hair were rolled up to form a lock and taped at distance from the root of about 5 to 6 cm, and the sample was cut using stainless steel scissor as close to the scalp as possible; for hair shorter than 3.5 cm, 5–10 strands of hair from different places on the back of the head were cut.

The questionnaire was performed as an interviewer-guided questionnaire with the mother. Developed by COPHES/DEMOCOPHES, it contained questions concerning residential environment and residence, nutrition, smoking behaviour, occupation and socio-demography; some questions on socio-demographic aspects were adapted according to UK classifications. Short additional questions for the hair and urine collections were asked, which covered height and weight, time of last void, whether they had consumed seafood in the last 48 h and whether they had had any recent hair treatments.

The study was approved by the London Riverside South West Research Ethics Committee (Reference 11/LO/1383) prior to commencement of the study. The ethics committee recommended slight modifications to the protocol. These were (1) a booklet for general practitioners to advise them of the study and provide information on the follow-up management of participants including whether repeat testing of the chemicals under study was required. (2) The results letter for individual results was amended to only be sent to those with high levels, above guidance values, stating what the known risks of those levels were and whether the levels correlated with any known outcomes. The letter also stated whether repeat testing was required. However, under the Environmental Information Regulations in the UK, if participants requested their individual results, they would be provided.

The storage and retention of personally identifiable data were carried out in accordance with the Data Protection Act, 1998. All personal identifiable information was entered onto a password-protected database, and participants were referred to with a personal identification number.

Sample handling

On receipt, samples were logged in Health and Safety Laboratory's (HSL) Biological Monitoring Database and assigned unique sample numbers. Sample volumes were measured, and samples were split into three containers and stored frozen (−20 °C) until analysis. Subsamples that were sent to other laboratories for analysis were sent frozen, by courier. The remaining UK samples are stored at HSL. The urine samples have been filtered to remove any cells, a requirement for storage in order to comply with UK tissue storage requirements, and are stored at −80 °C. The hair samples are stored at room temperature, out of direct sunlight.

Chemical analysis

Urine samples were measured for cadmium, phthalate metabolites (mono-ethyl phthalate (MEP), mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-oxo-hexyl)phthalate phthalate (5-oxo-MEHP), mono(2-ethyl-5-hydroxyhexyl)phthalate phthalate (5OH-MEHP), mono-isobutyl phthalate (MiBP), mono-n-butyl phthalate (MnBP) and

mono-benzyl phthalate (MBzP)), cotinine and creatinine to correct for urine dilution, and the hair samples were tested for mercury. Urinary creatinine concentrations were determined on all urine samples by the Jaffe alkaline picrate method (Cocker et al. 2011) on an ABX Pentra 400 (Horiba Diagnostics, Northampton, UK).

The analyses of mercury in hair, urinary cadmium and creatinine were carried out at the Health and Safety Laboratory in the UK. Mercury in hair was measured using a X7 Series 2 Thermo Fisher Scientific Inductively Coupled Plasma Mass Spectrometer (ICP-MS). The limit of quantification (LOQ) was 0.0225 μg/g. Cadmium in urine was analysed using a X7 Series 2 Thermo Fisher Scientific Inductively Coupled Plasma Mass Spectrometer (ICP-MS). The LOQ was 0.004 μg/L. Urinary phthalate metabolites were measured at the Flemish Institute of Technological Research (VITO) in Belgium using ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) (Waters Acquity UPLC-Waters Xevo TQ-S); the LOQ was 0.1–0.5 μg/L, depending on the analyte. Cotinine analysis was carried out at the Umweltbundesamt in Austria using LC-MS/MS; the LOQ was 1.2 μg/L.

The analyses were based on analytical standard operating procedures (SOPs) provided by the COPHES quality assurance unit (Angerer 2008; Blaszkewicz 2010; Müller 2005; Schramel 1999). The laboratories that analyzed the samples had successfully participated in the inter-laboratory comparability investigation (ICI), which was run to harmonise the analytical measures to improve the comparability of the analytical results, and the External Quality Assessment Scheme to improve the accuracy of the results (EQUAS), both of these were organised by COPHES/DEMOCOPHES (Schindler et al. 2014; Esteban et al. 2014).

Statistical analysis

Data was analysed with the SPSS statistical package (version 20). The mother and child pair counted as a case only if they gave informed consent, met the inclusion criteria, both provided a urine sample and a hair sample and answered the questionnaire. Values below the LOQ were replaced by ½ LOQ. If more than 50 % of the values for an analyte were below the LOQ, no means were calculated. The World Health Organization (WHO) recommends that urine samples with a creatinine concentration lower than 30 mg/dL or higher than 300 mg/dL be excluded from the analysis (WHO 1996); all the urine samples were between this range and so all were included in the analysis. For each biomarker, the data was stratified into relevant subgroups to study relevant confounders and investigate possible sources of exposure. Based on the numbers of participants within each subgroup, some analysis was only descriptive, and for the rest statistical analysis (*t* test or analysis of variance (ANOVA) after log transformation was possible. Themothers' data was compared with the children's data using the Spearman's rho or Pearson correlation coefficients, and all data was compared with HBM population based reference values and guidelines (where available) and other HBM studies.

Results

Of the 871 eligible child-mother pairs invited to take part in the study, just 22 replies were received, a 2.5 % response rate. Details of the pilot study population are presented in Table 1. Mercury was measured in hair samples, and cotinine, cadmium and phthalate metabolites were measured in urine samples from 21 children aged 6–11 years old and their mothers (one pair dropped out before sample collection).

Mercury in hair

Mercury levels in hair are shown in Table 2. Mercury was detected above the LOQ of 0.01 μg/g in all the study participants. Levels of mercury in hair was positively correlated in mothers and children; calculation of Spearman's rho correlation coefficient indicated a positive correlation of 0.68 (p= 0.001). The geometric mean level of mercury measured in the mothers' hair (0.16 μg/g) was lower than the level measured in the children's hair (0.19 μg/g), and the maximum value measured for mercury in hair was just 0.44 μg/g in the mothers compared to 1.18 μg/g in the children. Over a third (38 %) of children reported eating fish on a regular basis, and mercury levels were significantly higher (p=0.002) in these children who reported frequent fish consumption (0.35 μg/g) compared to those who ate fish less frequently (0.13 μg/g) and specifically sea fish consumption (0.37 and 0.16 μg/g respectively, p=0.03). Like the children, a third of mothers reported consuming fish on a regular basis, i.e. several times a week, but mercury levels were not significantly different to the mothers who reported eating fish less frequently. In addition to fish consumption, a number of other possible sources of mercury, including sources of other forms of mercury, were explored in the questionnaire, such as skin bleaching products and amalgam fillings. No participants reported using skin bleaching products, and there was no difference between participants with amalgam fillings compared to those without.

Urinary cotinine

Urinary cotinine provides a measure for recent exposure to nicotine as cotinine is an oxidised metabolite of nicotine and is excreted in the urine within 3–4 days of exposure (Benowitz 1996). There are no specific guidelines for cotinine levels, but persons with a value of more than 50 μg/g creatinine can be regarded as a smoker or a heavy exposed non-smoker (Riboli

Table 1 Details of the pilot study participants in the UK

	N	Categories	Children	Mothers
Age (years)	21	Median (p25 – p75)	9 (7–10)	43 (39–46)
		Min.-max.	6-11	32-50
		6-8 years n (%)	10 (47.6 %)	
		9-11 years n (%)	11 (52.4 %)	
Gender N (%)	21	Boy	8 (38.1 %)	
		Girl	13 (61.9 %)	
Body weight (kg)	21	Median (p25 – p75)	29 (26–35)	69 (62–81)
		Min.-max.	18-48	54-186
Height (cm)	21	Median (p25 – p75)	136 (127–145)	168 (161–172)
		Min.-max.	122-166	150-180
Body Mass Index (kg/m^2)	21	Median (p25 – p75)	15.8 (14.8 - 16.8)	26.14 (22.32 -31.60)
		Min.-max.	12.1- 21.4	19.96-62.15
Urinary creatinine (mg/L)	21	Median (p25 – p75)	898 (781– 1144)	1025 (836–1518)
		Min.-max.	489 - 1768	357 - 2085
Highest educational level of the family N (%)	21	Primary (ISCED 0–2)	———	0 (0.0 %)
		Secondary (ISCED 3–4)		3 (14.3 %)
		Tertiary (ISCED 5–6)		18 (85.7 %)
Smoking habits N (%)	21	Daily smoker	———	0 (0 %)
		Occasional smoker		1 (4.8 %)
		Former smoker		5 (23.8 %)
		Non-smoker		15 (71.4 %)
ETS at home N (%) (in former and non-smokers only)	20	Daily	1 (4.8 %)	1 (5.0 %)
		Less than daily	0 (0.0 %)	0 (0.0 %)
		Never	20 (95.2 %)	19 (95.0 %)
ETS elsewhere (in non-smokers only) N (%)	20	Yes	6 (28.6 %)	6 (30.0 %)
		No	15 (71.4 %)	14 (70.0 %)
ETS in last 24 hours (in non-smokers only) N (%)	20	Yes	1 (4.8 %)	2 (10.0 %)
		No	20 (95.2 %)	18 (90.0 %)
Use of personal care products N (%)	20	High	0 (0 %)	11(55.0 %)
		Moderate	8 (38.1 %)	9 (45.0 %)
		Low	13 (61.9 %)	0 (0.0 %)
Consumption of rice N (%)	21	At least once a week	3 (14.3 %)	2 (9.5 %)
		Less often	18 (85.7 %)	19 (90.5 %)
Consumption of fish (all types) N (%)	21	Several times per week	8 (38.1 %)	7 (33.3 %)
		Once a week or less	13 (61.9 %)	14 (66.7 %)
Amalgam teeth fillings N (%)	21	Yes	2 (9.5 %)	17 (81.0 %)
		No	19 (90.5 %)	4 (19.0 %)
PVC in house N (%)	20	Yes	———	9 (45.0 %)
		No		11 (55.0 %)

P25: 25th percentile; P75: 75th percentile; N: number; Min.: minimum; Max.: maximum. ISCED:International Standard Classification of Education; ETS: environmental tobacco smoke; 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 & creams, fragrances, deodorant, massage oil and nail polish; PVC: polyvinyl chloride.

et al. 1995). No participants had cotinine levels near or above 50 μg/g creatinine. In 90.5 % of mothers and 95.2 % of children, the cotinine level in urine was below the LOQ of 1.2 μg/L. The maximum value for cotinine recorded was 3.6 μg/L (4.1 μg/g creatinine) in the mothers and 2.4 μg/L (2.0 μg/g creatinine) in the children. These higher levels were in samples from participants who reported being exposed to tobacco smoke in the home. None of the mothers reported being regular smokers, and this was evident with extremely low levels of cotinine measured.

Table 2 Mercury in hair, urinary cadmium and urinary phthalate metabolites in the study participants

Biomarker of exposure	Number	Percent >LOQ	Units	Children				Mothers				Guidance values
				GM (95 % CI)	P90	P95	Max	GM (95 % CI)	P90	P95	Max	
Mercury in hair	21	100 %	μg/g	0.19 (0.14–0.27)	0.43	0.60	1.18	0.16 (0.12–0.22)	0.39	0.43	0.44	2.3 μg/g (JECFA 2006) 2 μg/g (UNEP/WHO 2008)
Urinary cadmium	21	100 %	μg/L μg/g creatinine	0.14 (0.12–0.17) 0.16 (0.14–0.18)	0.25 0.22	0.26 0.26	0.28 0.29	0.24 (0.19–0.31) 0.23 (0.19–0.29)	0.46 0.41	0.67 0.43	0.69 0.70	HBM I 0.5 μg/L, HBM II 1 μg/L (children and adolescents) (Schulz et al. 2011) HBM I 1.0 μg/L, HBM II 4.0 μg/L (adults) (Schulz et al. 2011)
Urinary MEP	21	100 %	μg/L μg/g creatinine	14.33 (9.05–22.69) 15.92 (10.12–25.04)	55.00 44.75	90.00 89.29	224.00 249.44	26.75 (18.31–39.81) 25.63 (16.40–40.07)	88.00 83.73	118.00 120.45	131.00 191.52	BE 18,000 μg/L (Aylward et al. 2009b)
Urinary DEHP metabolites												
MEHP	21	85.5 %	μg/L μg/g creatinine	1.42 (0.93–2.16) 1.58 (1.08–2.29)	3.40 3.27	7.50 9.96	9.00 12.52	1.16 (0.74–1.81) 1.11 (0.78–1.59)	4.00 3.52	5.10 4.08	8.50 5.85	
5-oxo-MEHP	21	100 %	μg/L μg/g creatinine	11.38 (9.26–14.00) 12.65 (10.33–15.48)	18.00 25.04	19.00 25.54	35.00 38.72	5.06 (3.72–6.88) 4.85 (3.76–6.25)	11.00 10.64	17.00 12.42	18.00 13.89	
5-OH-MEHP	21	100 %	μg/L μg/g creatinine	18.40 (14.87–22.75) 20.44 (16.61–25.14)	28.00 38.40	29.00 39.29	60.00 66.37	8.57 (6.36–11.55) 8.21 (6.43–10.48)	21.00 16.53	22.00 20.47	35.00 24.15	
Sum of 5-oxo-MEHP and 5-OH-MEHP	21	85.5 %	μg/L μg/g creatinine	29.78 (24.13–36.75) 33.09 (26.94–40.62)	46.00 63.44	48.00 64.83	95.00 105.09	13.63 (10.08–18.43) 13.06 (10.19–16.73)	31.00 27.17	39.00 32.89	53.00 61.50	HBM I 500 μg/L (children aged 6–13 years) HBM I 300 μg/L (women of reproductive age) (Schulz et al. 2011)
Sum of MEHP, 5-OH and 5-oxo	21	85.5 %	μg/L μg/g creatinine	31.20 (25.06–38.91) 34.67 (28.02–42.91)	49.40 66.71	55.50 74.79	104.00 117.61	14.79 (10.82–20.24) 14.75 (10.97–18.32)	36.00 30.69	44.10 36.97	61.50 43.89	BE 260 μg/L (Aylward et al. 2009a)
Urinary MiBP	21	100 %	μg/L μg/g creatinine	25.83 (18.70–35.69) 28.70 (22.27–36.98)	66.00 50.97	93.00 75.67	159.00 177.06	16.98 (11.56–24.94) 16.27 (11.86–22.86)	78.00 37.41	99.00 70.71	113.00 124.06	
Urinary MnBP	21	100 %	μg/L μg/g creatinine	22.64 (16.42–31.20) 25.15 (19.35–32.67)	65.00 53.28	69.00 66.41	70.00 72.38	13.46 (9.63–18.81) 12.90 (9.56–17.41)	37.00 30.81	46.00 32.44	47.00 38.85	

Table 2 (continued)

Biomarker of exposure	Number	Percent >LOQ	Units	Children				Mothers				Guidance values
				GM (95 % CI)	P90	P95	Max	GM (95 % CI)	P90	P95	Max	
Urinary MBzP	21	100 %	µg/L	3.46 (2.15–5.55)	8.80	11.00	114.00	1.63 (1.02–2.61)	5.20	6.20	7.50	BE 3800 µg/L (Aylward et al. 2009b)
			µg/g creatinine	3.84 (2.42–6.08)	7.38	21.61	92.76	1.56 (1.07–2.28)	4.52	4.82	5.13	

LOQ limit of quantification, *CI* confidence interval, *GM* geometric mean

Urinary cadmium

Cadmium accumulates in the kidneys and urinary cadmium reflects both recent and long-term exposure to cadmium (Agency for Toxic Substances and Disease Registry 2012). Geometric means and 90th and 95th percentiles are listed in Table 2. The maximum value for cadmium in the mothers was 0.69 µg/L (0.70 µg/g creatinine) and 0.28 µg/L (0.29 µg/g creatinine) in the children. The mothers' values were significantly higher than the children's values (p=0.004). There was no specific difference in cadmium levels in participants who reported being exposed to tobacco smoke in the home compared to participants who reported no exposure to tobacco smoke in the home. For children, a positive association was observed between urinary creatinine and urinary cadmium levels; those with higher urinary creatinine (1000–2000 mg/L) had significantly higher cadmium levels (p=0.001) than those with 300–1000 mg/L creatinine. All the participants' measured cadmium levels were below the German Commission on Human Biomonitoring (HBM) I values for both the children and mothers. These values correspond to the concentration of a substance in human biological material below which—according to the knowledge and judgement of the German Commission on Human Biomonitoring—adverse health effects are not expected (Schulz et al. 2007, 2011).

Urinary phthalate metabolites

Exposure to the phthalates DEP, DEHP, DiBP, DnBP and DBzP was assessed by the measurement of specific urinary metabolites, monoesters and oxidised metabolites (MEP, MEHP, 5-oxo-MEHP, 5-OH-MEHP, MiBP, MnBP, MBzP) which are short-lived and reflect recent exposure. The results for mothers and children are shown in Table 2. All the phthalate metabolite concentrations, except for MEP, were higher in the children compared to those in the mothers. The highest metabolite levels measured in the mothers were for MEP, geometric mean 26.8 µg/L, but they were not correlated with the children's levels. The lowest concentrations of metabolites for mothers were MEHP 1.16 µg/L (a weak correlation 0.43, p= 0.05) and MBzP 1.63 µg/L (not correlated with the children). MiBP was the highest metabolite level measured for children, geometric mean concentration 25.83 µg/L. In both mothers and children, the urinary levels of the DEHP metabolites MEHP, 5-oxo-MEHP and 5-OH-MEHP were highly correlated (Pearson correlation coefficient >0.70, p<0.01), and the secondary metabolites 5-oxo-MEHP and 5-OH-MEHP were higher than MEHP. All the participants' measured levels were below the HBM I values for the sum of the 5-oxo-MEHP and 5-OH-MEHP (Schulz et al. 2011).

Discussion

The UK DEMOCOPHES study recruited children (aged 6 to 11 years) and their accompanying mothers to measure exposure to cadmium, mercury, phthalates and cotinine, using human biomarker and questionnaire data. The main objective of the DEMOCOPHES pilot study was to test the feasibility of a harmonised EU HBM framework and harmonised study protocols developed by COPHES to generate comparable data.

For this pilot study, only 42 participants were recruited as the sampling process suffered from a poor response rate. A poor response rate can result in selection bias, for example, the education levels of the families who took part were mainly of tertiary (85.7 %) and higher secondary or post-secondary non-tertiary education level (14.3 %). The proportion of eligible participants who agree to enter the study (the response rate) influences the validity of the inference that the sample represents the population of interest.

People who are difficult to reach and those who refuse to participate once they have been contacted tend to be different from people who enrol. The main reason given by mothers for not participating was that they did not have the time to take part. Other reasons included a reluctance to travel to a study centre and children not wanting to provide a urine sample. Understanding the public's concerns with regards to taking part in a HBM study is useful and can be addressed in future studies when preparing the information material by highlighting and addressing the issues known to be of concern. Reasons for schools not taking part was often because they were busy or were involved in other projects. Contact with potential schools needs to occur much earlier in advance to engage the school community and so that the project can be included in the school curriculum. Lessons learnt from all the participating European countries with regards to communication in recruitment and dissemination of results are discussed in Exley et al. (2014).

In the UK, communication of the results was different to the recommended approach by COPHES which was to provide individual and aggregate results. The ethical committee in the UK favoured the clinical approach of only providing individual results when health-based guidance values and interventions are available. There is much discussion in the literature about the benefits of the two approaches to dissemination of results. A more open approach is favoured for transparency and would help with community engagement (Keune et al. 2008; Morello-Frosch et al. 2009; Exley et al. 2014). Participants may have felt that there was no benefit for them taking part in the study, and this may have contributed to the poor response rate.

Hair analysis is a useful way to determine exposure to mercury through fish consumption and represents exposure over the last 2–3 months (depending on the length of hair taken). There is a wide variation in mercury levels in hair in different countries (Miklavčič Višnjevec et al. 2014), and many published studies report mercury levels in hair of pregnant women, and/or on countries where fish is a large component of the population's diet. A summary of mercury levels measured in hair samples from children and females worldwide is shown in Table 3.

There are few population-based reference values for mercury in children's hair, and they cover different age ranges compared to our study (6–11 years old). In the DEMOCOPHES study, the Czech Republic had a lower level of mercury for children aged 6–11 years old compared to the UK. This highlights the usefulness of the DEMOCOPHES study where data are comparable. Switzerland, Denmark, Luxembourg, Sweden and Slovenia had similar levels in children compared to the UK whereas Portugal and Spain had higher levels, most likely due to their greater fish consumption (DEMOCOPHES 2013; Pirard et al. 2014; Cullen et al. 2014; Den Hond et al. 2015, Castaño et al. 2015).

In mothers, the 90th percentile (P90) was half that of the population reference values of the Flemish 2007–2008 biomonitoring study (Milieu Gezondheid 2010), and the US NHANES 1999–2000 study (McDowell et al. 2004), as shown in Table 3. Although these studies showed an increase in mercury in hair with age, the mean level of mercury measured in the mothers' hair was lower than the level measured in the children's hair; other studies have also reported no association with mercury levels in hair and age (Cordier et al. 1998; Li et al. 2008; Wranova et al. 2009). This may be due to different exposures to mercury, for example, it could have something to do with the different levels of contamination or the types of fish consumed by mothers compared to children in the UK. In children, a statistically significant increase in mercury in hair was associated with frequent consumption of fish (but not in the mothers). Anecdotal evidence suggests that in the UK, children are often given tuna to eat whereas mothers may choose a different type of fish, cod or plaice for example. In this study, data was collected on the frequency of eating all types of fish and then more specifically sea fish, shellfish and freshwater fish and other fish products. Collecting more detail on the type of fish consumed would be a useful addition to future studies of mercury to enhance exposure assessment. More specific questions such as how often the participants eat fish known to have higher levels of mercury could be included in future questionnaires; for example, shark, marlin, swordfish and tuna are known to contain higher levels of mercury than other fish (Davis et al. 2012).

Officially recognised guidelines for mercury in hair are not available so COPHES suggested using the value of 5 μg/g that served as a basis for the modelling of the commission (HBMC 1999), 1 μg/g defined as a reference dose by the US National Research Council (NRC 2000) or, the value used by DEMOCOPHES, 2.3 μg/g recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2006). More recently, the WHO proposed an initial screening level for further action of 2 μg/g hair (UNEP/WHO 2008). The

Table 3 Mercury levels measured in hair samples from children and females worldwide

Study population	Country/region	Period	Number	Mercury levels (μg/g)		Reference
Children						
1–5 years old	US	1999–2000	838	GM (min-max) P90	0.12 (0.10–0.12) 0.41	US NHANES study (McDowell et al. 2004)
6–11 years old	Belgium	2012	127	GM P75	0.2 0.3	(Pirard et al. 2014)
6–11 years old	Ireland	2012	120	GM (min-max) P90	0.097 (0.082–0.114) 0.352	(Cullen et al. 2014)
3–17 years old	France	2006–2007	1364	P95	1.20	(Fréry et al. 2012)
Average age 10.3±2.6 years old	Mexico	1994–1995, 1997–2001, 2001–2004	796	Mean (min-max) P90	0.56 (0.03–6.22) 1.12	(Basu et al. 2014)
14–16 years old	Belgium	2007–2008		P90	0.48	Flemish biomonitoring study (Milieu Gezondheid, 2010)
9–10 years old	Asahikawa, Japan	2008–2009	229	GM (min-max)	1.31 (0.31–3.96)	Ilmiawati et al. 2014
Females						
Mothers	Mexico	1994–1995, 1997–2001, 2001–2004	796	Mean (min-max) P90	0.53 (0.03–4.19) 1.02	(Basu et al. 2014)
Females	Belgium	2014	129	GM P75	0.38 0.6	(Pirard et al. 2014)
Females	Belgium	2007–2008		P90	0.9	Flemish biomonitoring study (Milieu Gezondheid, 2010)
Females	Ireland	2012	120	GM (min-max) P90	0.165 (0.137–0.198) 0.6	(Cullen et al. 2014)
Females	France	2006–2007	365	P95	1.74	(Fréry et al. 2012)
Females	US	1999–2000	1726	GM (min-max) P90	0.20 (0.16–0.24) 1.11	US NHANES study (McDowell et al. 2004)
Pregnant women frequent fish consumption	Seychelles			Mean	6.8	(National Research Council 2000)
Pregnant women frequent fish consumption	Faroe islands	1986–1987	1,019	GM (min-max)	4.17 (0.17–39.1)	(Grandjean et al. 2005)
Pregnant women, frequent fish consumption	Greece, Aegean Islands		219	GM (min-max)	1.36 (0.046–17.5)	(Gibičar et al. 2006)
Pregnant women	Mexico	1994–1995, 1997–2001, 2001–2004	371	Mean (min-max) P90	0.53 (0.03–4.19) 1.02	(Basu et al. 2014)
Pregnant and lactating women	Slovenia		574	Median (min-max)	0.297 (0.073–0.781)	(Miklavčič et al. 2011)

GM geometric mean, *P75* 75th percentile, *P90* 90th percentile, *P95* 95th percentile, *min* minimum, *max* maximum

mercury levels recorded in the participants' samples are much lower than these reference values, and they are in accordance with current thinking from the UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT). Based on the 2006 UK total diet study of metals and other elements, mercury was only detected in the offal, fish and other vegetable groups with fish the major contributor to the population's dietary exposure to mercury (25 %) (Rose et al. 2010). The COT concluded that the current dietary exposures to mercury in the UK are unlikely to be of toxicological concern (COT 2008).

A summary of cadmium levels measured in urine samples from children and adults worldwide are given in Table 4. The children's urinary cadmium results are similar to population-based reference values from the US NHANES study 2009–2010 (Centers for Disease Control and Prevention 2014). Higher cadmium levels were observed in the children who had higher levels of urinary creatinine, although the creatinine levels were within the range recommended by WHO (1996). It may not be appropriate to draw conclusions from this because of the small study number, but it has previously been identified that creatinine adjustment is not always the most

Table 4 Cadmium levels measured in urine samples from children and adults worldwide

Study population	Country/ region	Period	Number	Cadmium levels (μg/L)		Reference
Children						
6–11 years old	US	2009–2010	415	GM (95 % CI) P90 (95 % CI) P95 (95 % CI)	0.06 (0.05–0.06) 0.13 (0.12–0.16) 0.17 (0.14–0.23)	US NHANES Study (Centers for Disease Control and Prevention 2014)
6–11 years old	Belgium	2014	125	GM	0.04	(Pirard et al. 2014)
3–14 years old	Germany	2003–2006	1734	GM P95	0.08 0.22	(Schulz et al. 2009)
3–6 years old	Japan	2001–2004	255	GM	1.74	(Watanabe et al. 2013)
4–10 years old	Korea	2000	38	GM	1.33	(Moon et al. 2003)
5 years old	Bangladesh	2001–2003	320	GM P90	0.37 0.58	(Kippler et al. 2010)
6–9 years old						(Rodriguez-Barranco et al. 2014)
girls	Spain	2012	126	GM (95 % CI)	0.218 (0.17–0.26)	
boys			135		0.22 (0.19–0.26)	
Adults						
Females	US	2009–2010	1450	GM (95 % CI) P90 (95 % CI) P95 (95 % CI)	0.19 (0.17–0.21) 0.74 (0.62–0.88) 1.07 (0.91–1.31)	US NHANES study (Centers for Disease Control and Prevention 2014)
Females	Belgium	2014	125	GM P75	0.21 0.30	(Pirard et al. 2014)
Females	Bangladesh	2001–2003	444	GM P90	0.77 1.5	(Kippler et al.2010)
Females	France	2006–2007	1206	GM P95	0.31 0.93	(Fréry N 2012)
Females	Flanders, Belgium	2007–2009		P90	0.51	(Milieu Gezondheid 2010)
Adults never-smokers	Germany	1998	2106	GM P95	0.18 0.65	German Environmental Survey (Becker et al. 2003).
Adults	UK	2007	435	P95	0.90	(Bevan et al. 2013)
Adults	UK	2014	132	Median P95	0.13 0.52	(Morton et al. 2014)

GM geometric mean, *P75* 75th percentile, *P90* 90th percentile, *P95* 95th percentile, *95 CI* 95 % confidence interval, *min* minimum, *max* maximum

appropriate method for cadmium analysis (Barr et al. 2005; Pirard et al. 2014).

The levels of urinary cadmium in the mothers were low and comparable (within 1 μg/L) with the population-based reference values from a UK study (Bevan et al. 2013), the Flemish human biomonitoring program (Milieu Gezondheid 2010) and the US NHANES study 2009–2010 (Centers for Disease Control and Prevention 2014), and the German Environmental Survey value for non-smoking adults (Becker et al. 2003) shown in Table 4. The US reference value is slightly higher than the other reference values, but it does include both smoking and non-smoking women, whereas no mothers reported being smokers in our study as confirmed by the cotinine results.

Tobacco smoke is a known source of cadmium, and Bevan and colleagues noted little difference in levels of urinary cadmium between individuals who previously smoked compared to those who have never smoked (Bevan et al. 2013). None of the participants had high cotinine levels, which is a marker of recent exposure to tobacco smoke. For non-smokers, the main source of exposure to cadmium is through the diet (European Food Safety Authority 2009). In our pilot study, there was a significant difference in levels of cadmium in mothers who consumed rice at least once a week (p=0.04) compared to those who ate it less frequently, and the questionnaire data revealed that mothers with higher levels of cadmium in their urine samples reported to recently have consumed offal, a known source of cadmium exposure (Food Standards Agency 2009). The 2006 UK Total Diet Study recorded highest levels of cadmium in offal (0.08 mg/kg) and noted that food consumed in larger quantities makes the larger contribution to dietary exposure such as potatoes, cereals and bread (Food Standards Agency 2009). However, no children in our study reported eating offal more than once a month, and information on potatoes and bread consumption was not collected. For cereals, 90.5 % of the children reported eating cereals several times a week.

Urinary cotinine represents recent exposure, none of the mothers reported being regular smokers and this was evident with extremely low levels of cotinine measured. The low exposure to environmental tobacco smoke could be due to the

high socio-economic status of the study group (the mothers reported levels of education and income at the higher end of the scale) and/or due to the legislation to prohibit smoking in public places. There is less information available on levels of urinary cotinine in children. From this study, urinary cotinine in children from households with at least one smoker was found to be 2.0 μg/L, similar to that reported in the German Environmental Survey IV with data collected from 2003 to 2006 in which levels of cotinine in children from households with at least one smoker were found to be 2.6 μg/L, 4.6 μg/L with two smokers and 6.5 μg/L with three or more smokers (Conrad et al. 2010).

Very low levels of the phthalate metabolites of DEP, DEHP, DiBP, DnBP and BBzP were measured in both mothers and children. Average concentrations of all the metabolites, except for MEP, were higher in the children compared to those in the mothers, which has been shown in other similar studies (Frederiksen et al. 2013; Kasper-Sonnenberg et al. 2012; Silva et al. 2004). A possible explanation for this phenomenon is higher exposure in children due to more frequent hand-mouth contact or because of unique exposure patterns in children, for example, playing with toys or exposure via food (Angerer et al. 2011). In addition, others have reported exposures to phthalates via dust and the use of flooring or wall covering containing polyvinyl chloride (PVC), particularly in children (Ait Bamai et al. 2014; Carlstedt et al. 2013). For this study, there was no significant difference in phthalate levels in children living in homes with PVC flooring or wall coverings compared to those without PVC. Alternatively, the metabolism and excretion rate of these chemicals could be different in children compared to adults, so that similar exposure patterns can lead to higher levels in the body (Kasper-Sonnenberg et al. 2012), for example, it could indicate an enhanced oxidative metabolism in children compared to adults (Hauser and Calafat 2005).

For MEP (a metabolite of DEP), higher mean values were detected in urine of the mothers compared to the children. DEP is mainly used in cosmetics and personal care products (Heudorf et al. 2007), and the questionnaire data confirmed the assumption that the mothers used personal care products and cosmetics more frequently compared to their children. Although MEP was detected in the urine samples of the mothers, the geometric mean was almost fivefold lower than the US value for females, 2009–2010 (Centers for Disease Control and Prevention 2014).

MBzP and MnBP levels were lower than the relevant population-based reference values for Germany, GerES IV study 2003–2006 for children (Becker et al. 2009) and 2006–2008 for adults (Schulz et al. 2011) and US NHANES 2009–2010 (Centers for Disease Control and Prevention 2014). MiBP levels were almost double those of the US population reference value (P90) for both children (66.0 μg/L compared to 35.7 μg/L) and adult females (78.0 μg/L compared to 29.1 μg/L) (Centers for Disease Control and Prevention 2014) but lower than the GerES study 2003–2006 (P95) for children (93 μg/L compared to 300 μg/L, P95) and adults (99 μg/L compared to 140 μg/L) (Schulz et al. 2011). These different levels may reflect different patterns of usage of the parent phthalates in these countries. The variability may also be due to restriction of the use of phthalates and regulations that have come into place since the other studies were published. For example, the EU banned a number of phthalates from use in plastics to which infants may be exposed in 2005, and in 2008, the US enacted similar national legislation (Kamrin 2009).

The primary metabolite of DEHP is MEHP which was measured along with the secondary oxidised metabolites 5-oxo-MEHP and 5-OH-MEHP. The secondary metabolites were present at threefold to fourfold higher concentrations than MEHP (P95 5.10 μg/L MEHP compared to 17.00 μg/L 5-oxo-MEHP and 22.00 μg/L for 5-OH-MEHP), in line with previous studies, which have shown higher levels of the oxidised metabolites than MEHP (Becker et al. 2004; Fromme et al. 2007). The 95th percentile reference values for 5-oxo-MEHP and 5-OH-MEHP derived from the UK postal study for adults >18 years are two to four times higher than our results (Bevan et al. 2013). For our pilot study, first-morning urine samples were provided and phthalates have a short half-life (24 h) so only recent exposure is measured via a single urine sample, and other studies have reported day-to-day variation and intra-subject variability (Fromme et al. 2007; Kasper-Sonnenberg et al. 2012). For a more detailed look at phthalate exposures in the UK population, repeat sampling would help to characterise the variation.

Conclusions

The harmonised approach, protocols and the communication strategies developed by COPHES have been tested out in the UK as part of the European pilot study, DEMOCOPHES. In the UK, exposure to cadmium, mercury, phthalates and environmental tobacco smoke was assessed in 21 children and their mothers by sampling urine and/or hair. The small study number means that the results must be interpreted with caution; however, it is reassuring to note for the participants that no results were above available health guidance values and were of no concern with regards to health. All biomarker values were similar to or below population-based reference values published by the US NHANES and Germany's GerES surveys. This project has helped to develop and test a framework for the assessment of population exposure to environmental chemical pollutants using key model compounds. The difficulties experienced in recruitment and lessons learnt from the communication strategies have led to recommendations for future EU HBM work (Fiddicke et al. 2014; Exley et al.

2014). The framework and techniques learnt and developed here will contribute to future work on biomonitoring in the UK to determine population exposures to other environmental chemicals and will be enhanced by the ability to make international comparisons with Europe.

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