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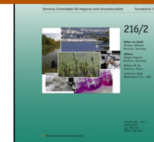

Phthalate metabolites in 24-h urine samples of the German Environmental Specimen Bank (ESB) from 1988 to 2015 and a comparison with US NHANES data from 1999 to 2012

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ABSTRACT

The German Environmental Specimen Bank (ESB) continuously collects 24-h urine samples since the early 1980s in Germany. In this study we analyzed 300 urine samples from the years 2007 to 2015 for 21 phthalate metabolites (representing exposure to 11 parent phthalates) and combined the data with two previous retrospective measurement campaigns (1988 to 2003 and 2002 to 2008). The combined dataset comprised 1162 24-h urine samples spanning the years 1988 to 2015. With this detailed set of human biomonitoring data we describe the time course of phthalate exposure in Germany over a time frame of 27 years. For the metabolites of the endocrine disrupting phthalates di(2-ethylhexyl) phthalate (DEHP), di-*n*-butyl phthalate (DnBP) and butylbenzyl phthalate (BBzP) we observed a roughly ten-fold decline in median metabolite levels from their peak levels in the late 1980s/early 1990s compared to most recent levels from 2015. Probably, bans (first enacted in 1999) and classifications/labelings (enacted in 2001 and 2004) in the European Union lead to this drop. A decline in di-isobutyl phthalate (DiBP) metabolite levels set in only quite recently, possibly due to its later classification as a reproductive toxicant in the EU in 2009. In a considerable number of samples collected before 2002 health based guidance values (BE, HBM I) have been exceeded for DnBP (27.2%) and DEHP (2.3%) but also in recent samples some individual exceedances can still be observed (DEHP 1.0%). A decrease in concentration for all low molecular weight phthalates, labelled or not, was seen in the most recent years of sampling. For the high molecular weight phthalates, DEHP seems to have been substituted in part by di-isononyl phthalate (DiNP), but DiNP metabolite levels have also been declining in the last years. Probably, non-phthalate alternatives increasingly take over for the phthalates in Germany. A comparison with NHANES (National Health and Nutrition Examination Survey) data from the United States covering the years 1999 to 2012 revealed both similarities and differences in phthalate exposure between Germany and the US. Exposure to critical phthalates has decreased in both countries with metabolite levels more and more aligning with each other, but high molecular weight phthalates substituting DEHP (such as DiNP) seem to become more important in the US than in Germany.

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1. Introduction

Phthalates are alkyl or aryl esters of phthalic acid (benzene-1,2-dicarboxylic acid). Depending on their alkyl chain length they are mainly used as solvents and formulating agents (low molecular weight (LMW) phthalates) or as plasticizers for polymers such as polyvinyl chloride (high molecular weight (HMW) phthalates) (Koch and Calafat, 2009). Some phthalates have been identified as endocrine disruptors in animal studies. Anti-androgenic properties have been linked to the reduction of fetal testicular testosterone production as one of the key events that lead to both structural and functional impairment of male reproduction and development (Furr et al., 2014). The endocrine disrupting potency leading to the multitude of effects termed the "phthalate syndrome" (Foster, 2006; Gray et al., 2006; National Research Council, 2008) seems to be restricted to phthalates with a backbone chain length between

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three and six carbon atoms and a total carbon count of the alkyl chain between four (di-iso- and di-*n*-butyl phthalate, DiBP and DnBP) and nine carbon atoms (di-isononyl phthalate, DiNP). Based upon the reduction of fetal testosterone production di-*n*-pentyl phthalate (DnPeP) has been reported to be eightfold more potent than di-(2-ethylhexyl) phthalate (DEHP) and the butyl phthalates while DiNP is threefold less potent (Furr et al., 2014; Hannas et al., 2011a, 2011b; Lioy et al., 2015). Because endocrine disrupting effects observed in animal studies are judged as relevant for humans, several phthalates have been classified as reproductive toxicants in Europe over the last 15 years and use restrictions and bans have been enacted (see Supplementary Table 1). Generally, since February 2015 (REACH sunset date), phthalates listed in Annex XIV of the REACH regulation (currently DiBP, DnBP, butyl benzyl phthalate (BBzP) and DEHP) may only be placed on the EU market or used in the EU if an authorization has been granted. Such (temporary) authorizations for specific applicants and specified uses have been granted so far only for the use of DEHP during the diffusion bonding and manufacture of aero engine fan blades (European Commission, 2014) or the use of DnBP as an absorption solvent in a closed system in the manufacture of maleic anhydride (European Commission, 2016).

First human biomonitoring studies published at the turn of the millennium revealed that the general population is ubiquitously and simultaneously exposed to several phthalates (Blount et al., 2000; Koch et al., 2003c). The number of urinary phthalate metabolites used as biomarkers of exposure has increased ever since and exposure to more than ten different phthalates can today be routinely determined using a set of about 20 urinary metabolites (Frederiksen et al., 2014; Kasper-Sonnenberg et al., 2012, 2014; Koch and Calafat, 2009; Silva et al., 2007). Phthalates are non-persistent chemicals, and the majority of a dose incorporated is excreted in urine within 24 h. Short chain, low molecular weight phthalates such as dimethyl phthalate (DMP), diethyl phthalate (DEP), DiBP and DnBP are predominantly excreted in urine as their simple monoesters after ester cleavage. With increasing chain lengths oxidative modification of the alkyl chain gains importance and high molecular weight phthalates such as DEHP, DiNP and di-isodecyl phthalate (DiDP) are predominantly excreted as side chain oxidized monoester metabolites (Anderson et al., 2001; Barr et al., 2003; Koch et al., 2004, 2012a; Koch and Angerer, 2007; Koch and Calafat, 2009; Wittassek et al., 2011).

In Germany, urinary phthalate metabolite measurements have been early on implemented in large scale population studies such as the German Environmental Survey on children (GerES IV) (Becker et al., 2004, 2009; Koch et al., 2007a; Schulz et al., 2012a; Wittassek et al., 2007a) and in biobanked 24-h urine samples from the German Environmental Specimen Bank (ESB) (Kolossa-Gehring et al., 2012). Using 24-h urine samples from ESB we already reported metabolite levels of five phthalates in samples collected between 1988 and 2003 (Wittassek et al., 2007b) and between 2002 and 2008 (Göen et al., 2011). The ESB is a monitoring instrument of the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety (BMUB), coordinated and maintained by the German Environment Agency (UBA). One aim of the ESB is to document and assess exposure trends via human biomonitoring. In this study we analyzed samples of the ESB collected between 2007 and 2015 extending the spectrum to 21 metabolites representing exposure to eleven phthalates. With the merged dataset we are now able to describe the time course of phthalate exposure over a time frame of 27 years, spanning from early years (pre 2000) with no regulatory measures in place to the year 2015, the REACH sunset date for the phthalates DiBP, DnBP, BBzP, and DEHP. Based upon commonly measured metabolites we are also able to compare our German ESB 24h-urine data with spot urine data from the US National Health and Nutrition Examination Survey (NHANES) covering the years 1999 to 2012.

2. Materials and methods

2.1. Subjects and urine specimens

For this study we analyzed phthalate metabolites in a subset of urine samples from the German Environmental Specimen Bank (ESB; www.umweltprobenbank.de). The ESB is a major component of the German environmental observation system and provides a scientific basis for decisions by the German Ministry for the Environment (BMUB). The long-term storage of environmental and human specimens is the main task of the ESB. Thus, these samples can be used to provide a continuous historical record of the state of the environment in Germany, even if certain environmental toxicants were not in the scientific focus or analytical methods did not exist at the time of collection. The ESB is coordinated and administered by the German Environment Agency (UBA) which is also responsible for scientific steering, central data maintenance and data assessment. A unique feature of the ESB is that it continuously collects 24-h urine samples in Germany since 1985 adhering to strict standard operating procedures. The general concept and sampling criteria of the ESB are described by Kolossa-Gehring et al. (Kolossa-Gehring et al., 2012; Kolossa-Gehring, 2012; Schröter-Kermani et al., 2016). All 24-h urine samples, after determination of their full volume, are aliquoted into polypropylene tubes and stored at temperatures below −150 °C (Lermen et al., 2014). The study protocol of sampling human specimens has been reviewed and approved by the ethics committees of the Medical Associations Saarland and Westfalen-Lippe and the Medical Faculty of the Westphalian Wilhelms-University Münster. All participants gave written informed consent on standardized forms approved by the same ethics committees.

In this study, chemical analyses of phthalate metabolites was performed in 300 urine samples collected in the years 2007, 2009, 2011, 2013 and 2015 (60 samples per year). All urine samples were mainly from students (age range 20–29years) enrolled at the University of Münster (Germany). For each year, volunteers were evenly distributed between 30 males and 30 females. All urine samples were blinded by the ESB before shipment to the analyzing laboratories at the IPA, Bochum, Germany. Samples were stored at −20 °C prior to sample preparation and analysis. This newly obtained dataset was then combined with the data from two previous measurement campaigns covering the years 1988 to 2003 (Wittassek et al., 2007a,b) and the years 2002 to 2008 (Göen et al., 2011). For harmonization, the age range of all datasets was restricted to study participants aged 20–29 years (the core population of the ESB), which resulted in the elimination of 11 out of the 240 participants from the second measurement campaign (2002–2008) that also included individuals 19 and 30 years of age. In all, phthalate HBM data from 1162 participants was included in this analysis. Samples from all three measurement campaigns have been collected at the same university in the city of Münster. Detailed sample information and anthropometric data for all study years are given in Table 1.

2.2. Chemical analysis

In the 24-h urine samples of the years 2007–2015 21 phthalate metabolites (representing exposure to 11 parent phthalates) were determined by on-line high performance liquid chromatography coupled to tandem mass spectrometry (HPLC–MS/MS) using internal isotope-labelled standards according to previously published methods (Kasper-Sonnenberg et al., 2012; Koch et al.,

Table 1
Anthropometric data of the study population (in bold: samples for first time analyzed in this study).

Year	Subject [number] (male/female)	Age [years] Mean (range)	24h-urine volume [mL] (range)	Urinary creatinine [g/L] (range)
1988	60 (30/30)	24.0 (21–29)	1520 (600–2800)	1.16 (0.20–2.23)
1989	60 (30/30)	24.1 (21–29)	1460 (500–2850	1.07 (0.49–2.79)
1991	60 (30/30)	24.5 (22–29)	1360 (400–2700)	1.30 (0.04–3.15)
1993	60 (30/30)	24.7 (20–29)	1660 (550–3100)	1.10 (0.26–2.63)
1996	146 (78/68)	24.2 (20–29)	1550 (290–2800)	1.11 (0.30–3.15)
1998	68 (38/30)	24.9 (20–29)	1570 (400–5000)	1.05 (0.22–2.57)
1999	60 (30/30)	24.0 (21–28)	1760 (700–4000)	1.01 (0.27–2.66)
2001	60 (30/30)	24.0 (20–29)	1750 (350–2800)	1.05 (0.30–2.43)
2002	57 (30/27)	23.0 (20–29)	1590 (650–2650)	1.13 (0.48–2.52)
2003	60 (30/30)	24.1 (20–28)	1750 (750–3650)	1.04 (0.28–2.95)
2004	60 (30/30)	24.2 (20–29)	1720 (460–3530)	1.11 (0.29–2.72)
2006	58 (29/29)	23.4 (20–28)	1980 (345–5030)	0.95 (0.29–2.52)
2007	**60 (30/30)**	**24.2 (20–29)**	**1880 (930–3760)**	**0.89 (0.24–2.17)**
2008	54 (29/25)	23.1 (20–29)	2140 (855–5460)	0.74 (0.15–1.73)
2009	**60 (30/30)**	**23.1 (20–28)**	**1890 (540–3660)**	**0.83 (0.27–2.19)**
2011	**60 (30/30)**	**23.2 (20–29)**	**1820 (390–3050)**	**0.89 (0.25–2.20)**
2013	**60 (30/30)**	**23.7 (20–29)**	**1890 (500–3190)**	**0.79 (0.29–1.71)**
2015	**60 (30/30)**	**23.2 (20–29)**	**1940 (270–4600)**	**0.72 (0.15–2.20)**
Total	1163	23.9 (20–29)	1730 (270–5460)	1.00 (0.15–3.15)
Female	569	23.5 (20–29)	1700 (270–5460)	0.85 (0.15–2.79)
Male	594	24.3 (20–29)	1750 (490–5040)	1.15 (0.20–3.15)

2003b, 2007b, 2012a, 2012b; Preuss et al., 2005). The naming of the parent phthalates and the respective monoester (primary metabolites) and oxidized metabolites (secondary metabolites) is shown in Table 2, including their limits of quantification (LOQ). In brief, for sample preparation, aliquots of 300 μl of the urine samples, calibration samples and quality control samples were added to 100 μl of ammonium acetate buffer (1 M; pH = 6.0–6.4), 6 μl β-Glucuronidase K12 from E.coli, arylsulfatase-free (Roche Diagnostics Mannheim, Germany; diluted 1:1 with ammonium acetate buffer) and 10 μl internal standard solution (of the isotope labelled analogues). Samples were incubated in a water bath for 2.5 h at 37 °C for enzymatic hydrolyses of the conjugates. After incubation, 10 μl of acetic acid were added to samples to adjust the pH value. Samples were frozen for at least 3 h at −18 °C to precipitate proteins. Thereafter, samples were thawed at room temperature and centrifuged for 10 min at 2,000*g* and the supernatant was transferred into a 1.8 ml glass vial for instrumental analysis.

For liquid chromatography 10 μl of the above solution were injected into an Agilent Technologies LC 1260 system (Agilent 1260 autosampler, two Agilent 1260 binary pumps). Sample clean-up and enrichment was performed on a Capcell Pak®C18-MG-II (10 × 4 mm, particle size 5 μm, Phenomenex) column. Chromatographic separation was performed on an Atlantis dC18 (2.1 × 150 mm; 3 μm; Waters). The solvents used were mixtures of LC–MS grade water and acetic acid; 99.95:0.05 (solvent A) and mixtures of acetonitrile and acetic acid; 99.95:0.05 (solvent B). The solvent gradients for the clean-up and analytical column are shown in Supplementary Table 2a and b. The 6-port valve was switched at 7 min to direct the analytical gradient in backflush mode through the clean-up column onto the analytical column. At 28 min the 6 port valve was switched back into starting position. The total run time for one sample was 34 min.

Mass spectrometric detection and quantification was performed on an AB Sciex 4500 triple quadrupole mass spectrometer used in negative ionization mode (ESI-). For each analyte two ion transitions were recorded. One was used for quantification (quantifier) and the other one for verification (qualifier). For the labelled internal standards one ion transition was recorded. The mass spectrometer was operated in scheduled multiple reaction monitoring (sMRM) mode. The respective ion transitions are given in Supplementary Table 3. The precision of the method was determined by analyzing a low and a high concentration control material pooled from native urine samples in each analytical batch. Metabolite concentrations and conjugation status thus represented the characteristics of general population urine samples. Precision data is presented in detail in Supplementary Table 4. Coefficients of variation were all below 16.1%. The accuracy of the method was determined by spiking standard solutions at two concentration levels (20 μg/L and 100 μg/L) to native urine samples. Relative recoveries for all metabolites were in the range between 86 and

Table 2
Parent phthalates, metabolites and the respective Limits of Quantification (LOQ).

	Parent phthalate	Primary metabolite	Secondary metabolite#	Full name of metabolite	LOQ (μg/L)
Low molecular weight (LMW) phthalates	Di-methyl phthalate (DMP)	MMP		Mono-methyl phthalate	1.0
	Diethyl phthalate (DEP)	MEP		Mono-ethyl phthalate	0.5
	Butylbenzyl phthalate (BBzP)	MBzP		Mono-benzyl phthalate	0.2
	Di-isobutylphthalate(DiBP)	MiBP		Mono-isobutyl phthalate	1.0
			2OH-MiBP	2OH-Mono-isobutylphthalate	0.25
	Di-*n*-butyl phthalate (DnBP)	MnBP		Mono-*n*-butyl phthalate	1.0
			3OH-MnBP	3OH-Mono-*n*-butyl phthalate	0.25
	Di-cyclo-hexyl phthalate (DCHP)	MCHP		Mono-cyclo-hexyl phthalate	0.2
	Di-*n*-pentylphthalate (DnPeP)	MnPeP		Mono-*n*-pentyl phthalate	0.2
High molecular weight (HMW) phthalates	Di(2-ethylhexyl) phthalate (DEHP)	MEHP		Mono(2-ethylhexyl) phthalate	0.5
			5OH-MEHP	Mono(2-ethyl-5-hydroxy-hexyl) phthalate	0.2
			5oxo-MEHP	Mono(2-ethyl-5-oxo-hexyl) phthalate	0.2
			5cx-MEPP	Mono(2-ethyl-5-carboxy-pentyl) phthalate	0.2
	Di-isononyl phthalate (DiNP)		OH-MiNP	7-OH-(Mono-methyl-octyl) phthalate	0.2
			oxo-MiNP	7-Oxo-(Mono-methyl-octyl) phthalate	0.2
			cx-MiNP	7-Carboxy-(mono-methyl-heptyl) phthalate	0.2
	Di-isodecyl phthalate (DiDP)and Di-Propyl-heptyl phthalate (DPHP)*1		OH-MiDP	6-OH-Mono-propyl-heptyl phthalate	0.2
			oxo-MiDP	6-Oxo-Mono-propyl-heptyl phthalate	0.2
			cx-MiDP	Mono(2,7-methyl-7-carboxy-heptyl) phthalate	0.2
	Di-*n*-octyl phthalate (DnOP)	MnOP		Mono-*n*-octyl phthalate	0.2
			MCPP*2	Mono-(3-carboxypropyl) phthalate	0.5

differing metabolite abbreviations used in the US NHANES are as follows: 5OH-MEHP: MEHHP; 5oxo-MEHP: MEOHP; 5cx-MEPP: MECPP; cx-MiNP: MCOP; cx-MiDP: MCNP.
*1 The analytical method applied cannot distinguish between DiDP and DPHP metabolites.
*2 Metabolite of several HMW and LMW phthalates (currently known: DnBP, DnPeP, DnOP, DiNP, DiDP). MCPP has also been abbreviated in previous studies with 3cx-MPP.

125%. The limits of quantification (LOQ) of the individual phthalate metabolites were derived based on a signal-to-noise ratio of 6 (see Table 1). Accuracy and international comparability of the analytical procedure was ensured and verified by the successful participation in the External Quality Assessment Scheme (EQUAS) of the European COPHES/DEMOCOPHES projects on human-biomonitoring (Den Hond et al., 2015; Schindler et al., 2014) and by obtaining certificates of the German External Quality Assessment Scheme for Biological Monitoring (G-EQUAS, www.g-equas.de) offered for the metabolites MnBP, MiBP, MBzP, MEHP, 5OH-MEHP, 5oxo-MEHP and 5cx-MEPP. The creatinine concentrations in urine were measured according to the Jaffé method.

2.3. Statistical analysis

Calculations and statistical analysis was performed using Microsoft® Excel 2010® and IBM® SPSS® Statistics 23. Descriptive statistical analyses (median, 95th percentile, range) were performed for metabolite concentrations expressed in μg/L urine and for creatinine adjusted concentrations in μg/g creatinine. Concentrations below the respective LOQ were set to LOQ/2 for data treatment. Box-plot visualization of the data was generated with OriginPro 9.1® from OriginLab Corporation.

3. Results and discussion

3.1. Phthalate metabolites in ESB (1988–2015)

Phthalate metabolite levels (in μg/L) in the 24-h urine samples of the ESB expressed as the median (50P), the 95th percentile (95P) and the maximum value are presented in Table 3a (metabolites of the LMW phthalates DMP, DEP, DiBP, DnBP and BBzP), Table 3b (metabolites of the HMW phthalates DEHP and DiNP) and Table 3c (metabolites of the HMW phthalates DiDP/DPHP and DnOP; and other metabolites: MCPP, MnPeP, MCHP). The tables include the results from the two previous measurement campaigns of 1988–2003 (Wittassek et al., 2007b) and 2002–2008 (Göen et al., 2011). The results of the current measurement campaign (2007–2015) are highlighted in bold. Results after creatinine correction (in μg/g creatinine) are reported in analogous fashion in the Supplementary Tables 5a–c. The metabolites MMP, MEP, 2OH-MiBP, 3OH-MnBP, OH-MiDP, oxo-MiDP, cx-MiDP, MnOP, MCPP, MnPeP and MCHP are for the first time reported in the current measurement campaign. Cx-MiNP has been included since the second campaign (2002–2008). The results from the current measurement campaign are in line with the results of the previous campaigns. All metabolites except MnPeP, MCHP and MnOP could be detected and quantified in the vast majority of samples analyzed. For MnPeP, MCHP and MnOP we recorded only sporadic detections at very low levels. Box-plots of the urinary concentrations (in μg/L) for the individual metabolites over the years investigated are depicted in Supplementary Figs. 1a–c. For those phthalates captured via more than one metabolite (DiBP, DnBP, DEHP, DiNP, DiDP) we observed strong correlations between the respective metabolites over the whole concentration range (data not shown), confirming the findings of various previous studies (Barr et al., 2003; Becker et al., 2004; Enke et al., 2013; Göen et al., 2011; Koch et al., 2003c, 2012b). For the low molecular weight phthalates, the simple monoesters are the major urinary metabolites, while for the high molecular weight phthalates side chain oxidized monoester metabolites are the major urinary metabolites. Because urinary excretion fractions differ in part considerably between the various phthalates and their metabolites, a direct comparison between urinary metabolite levels in terms of exposure to the respective parent phthalates is difficult. E.g. similar urinary metabolite levels of the simple monoesters MnBP and MEHP indicate to external DEHP exposures that are roughly 10-times higher than external DnBP exposure. Such exposure extrapolations need to take into account urinary metabolite conversion factors, daily urine volume and other anthropometric factors (David, 2000; Koch et al., 2003a; Kohn et al., 2000; Wittassek et al., 2007b) and will be performed in a sister publication (in preparation). However, with this caveat in mind, the urinary metabolite levels can be used to investigate the general time course of phthalate exposure over the years preserved in the ESB samples, to investigate for possible differences between the males and the females of the ESB population, to compare metabolite levels with biomarker derived health-based guidance values (such as the BE, biomonitoring equivalent; HBM I, human biomonitoring value I)

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Table 3
(a) Urinary metabolite levels (in µg/L) of the low molecular weight phthalates DMP, DEP, DiBP, DnBP and BBzP.

	DMP			DEP			DiBP						DnBP						BBzP		
	MMP			MEP			MiBP			2OH-MiBP			MnBP			3OH-MnBP			MBzP		
	50P	95P	Max	50P	95P	Max	50P	95P	Max	50P	95P	Max	50P	95P	Max	50P	95P	Max	50P	95P	Max
1988	–	–	–	–	–	–	29.3	108	318	–	–	–	178	724	882	–	–	–	7.8	22.8	203
1989	–	–	–	–	–	–	27.7	104	187	–	–	–	189	578	1480	–	–	–	8.7	35.6	79.1
1991	–	–	–	–	–	–	39.8	390	588	–	–	–	182	429	699	–	–	–	15.4	69.5	99.7
1993	–	–	–	–	–	–	29.8	89.1	209	–	–	–	164	1090	1970	–	–	–	7.2	48.0	86.0
1996	–	–	–	–	–	–	42.9	235	534	–	–	–	105	443	2090	–	–	–	8.4	126	687
1998	–	–	–	–	–	–	36.5	207	263	–	–	–	79.2	293	686	–	–	–	7.5	38.5	95.5
1999	–	–	–	–	–	–	32.1	133	216	–	–	–	71.7	362	800	–	–	–	5.2	87.2	289
2001	–	–	–	–	–	–	32.7	161	435	–	–	–	63.4	443	1780	–	–	–	5.2	23.8	39.8
2002	–	–	–	–	–	–	30.7	96.1	172	–	–	–	66.4	155	7490	–	–	–	8.0	30.1	58.0
2003	–	–	–	–	–	–	30.4	107	151	–	–	–	50.8	131	704	–	–	–	5.9	18.9	30.6
2004	–	–	–	–	–	–	25.4	64.6	100	–	–	–	38.5	101	2370	–	–	–	6.3	17.0	186
2006	–	–	–	–	–	–	31.8	142	317	–	–	–	29.3	105	250	–	–	–	3.6	15.6	31.8
2007	**8.0**	**102**	**147**	**53.6**	**400**	**1910**	**19.3**	**79.3**	**121**	**8.2**	**24.0**	**39.7**	**16.4**	**37.3**	**57.0**	**1.4**	**4.1**	**5.4**	**2.9**	**16.9**	**44.0**
2008	–	–	–	–	–	–	24.5	98.1	231	–	–	–	19.4	63.8	73.1	–	–	–	3.8	10.1	21.6
2009	**7.1**	**54.6**	**182**	**37.5**	**317**	**1160**	**19.7**	**79.4**	**132**	**5.9**	**27.3**	**60.5**	**16.3**	**48.9**	**72.6**	**1.4**	**3.7**	**7.6**	**3.2**	**16.0**	**37.7**
2011	**4.4**	**35.2**	**40.3**	**17.9**	**190**	**6080**	**20.3**	**50.8**	**133**	**5.8**	**22.0**	**36.5**	**14.8**	**27.3**	**43.3**	**1.5**	**4.0**	**5.8**	**3.3**	**13.2**	**66.5**
2013	**3.5**	**29.2**	**58.0**	**20.9**	**93.3**	**502**	**16.4**	**52.5**	**52.9**	**4.6**	**12.3**	**21.4**	**11.2**	**29.9**	**93.2**	**1.1**	**3.0**	**7.6**	**2.0**	**12.8**	**27.6**
2015	**2.8**	**14.3**	**22.1**	**13.5**	**71.2**	**468**	**9.8**	**33.4**	**670**	**2.8**	**11.9**	**203**	**8.0**	**21.3**	**42.0**	**0.8**	**2.9**	**4.7**	**1.2**	**13.7**	**134**

(b) Urinary metabolite levels (in µg/L) of the high molecular weight phthalates DEHP and DiNP.

	DEHP												DiNP								
	MEHP			5OH-MEHP			5oxo-MEHP			5cx-MEPP			OH-MiNP			oxo-MiNP			cx-MiNP		
	50P	95P	Max	50P	95P	Max	50P	95P	Max	50P	95P	Max	50P	95P	Max	50P	95P	Max	50P	95P	Max
1988	9.4	30.0	94.5	27.8	77.8	275	20.1	62.3	251	27.8	84.5	340	1.5	7.3	11.4	0.6	3.3	4.3	–	–	–
1989	9.7	32.1	129	28.2	60.8	275	22.0	46.2	196	39.0	75.0	282	1.8	13.7	53.4	0.7	3.6	32.5	–	–	–
1991	10.4	39.8	62.4	36.0	114.0	142	25.6	80.3	118	40.2	142.5	241	2.2	12.8	85.4	0.8	3.1	38.4	–	–	–
1993	8.4	34.4	88.0	26.0	85.5	133	20.1	62.2	109	30.3	108.3	159	1.8	10.0	12.3	0.7	4.8	9.0	–	–	–
1996	7.8	31.6	68.8	20.0	80.6	216	16.1	57.2	192	26.8	116.1	300	2.0	10.9	27.9	1.0	5.0	15.2	–	–	–
1998	6.7	29.6	64.3	17.0	56.2	165	14.3	44.8	181	21.6	70.5	181	2.1	37.0	59.3	1.1	24.2	50.6	–	–	–
1999	5.4	18.8	36.8	13.4	52.1	77.7	11.1	33.1	67.8	18.7	57.7	167	1.9	10.9	17.9	1.0	7.2	11.5	–	–	–
2001	6.4	29.3	48.0	16.4	59.1	113	14.0	43.0	109	23.0	69.2	185	2.1	13.6	57.7	1.1	5.3	63.8	–	–	–
2002	7.2	18.7	41.9	19.7	51.8	63.8	14.0	34.3	50.3	19.0	50.6	59.1	3.5	9.8	35.9	2.2	6.2	15.7	4.3	10.8	40.6
2003	4.6	23.2	63.9	13.4	37.6	83.2	12.2	34.3	57.9	17.5	54.5	129	2.3	9.8	29.0	1.6	5.9	19.1	–	–	–
2004	5.6	32.5	63.5	16.2	102.7	134	11.8	59.8	108	16.5	87.4	135	2.8	16.0	63.9	2.1	9.5	37.9	3.2	12.4	63.4
2006	4.1	11.1	16.2	13.2	29.4	38.9	8.3	25.2	33.1	13.5	30.7	36.7	3.2	20.2	24.7	2.2	14.4	24.9	4.0	28.2	40.4
2007	**3.6**	**23.0**	**58.0**	**10.8**	**41.5**	**192**	**6.3**	**22.0**	**106**	**12.7**	**46.6**	**270**	**2.2**	**10.4**	**30.4**	**1.0**	**4.4**	**10.2**	**2.0**	**8.0**	**19.2**
2008	3.3	12.3	15.9	9.3	22.3	28.5	5.9	14.1	15.3	9.7	23.6	26.1	3.6	17.6	35.9	2.2	12.6	27.2	3.5	25.1	41.8
2009	**3.4**	**10.8**	**33.1**	**8.8**	**34.8**	**244**	**5.1**	**17.5**	**101**	**8.9**	**38.8**	**121**	**2.4**	**12.2**	**39.1**	**1.2**	**7.9**	**21.0**	**2.8**	**11.9**	**73.3**
2011	**1.8**	**4.8**	**8.3**	**7.5**	**18.3**	**28.3**	**5.6**	**13.9**	**24.1**	**7.3**	**20.1**	**34.0**	**2.9**	**23.2**	**212**	**1.7**	**14.3**	**156**	**3.7**	**20.3**	**158**
2013	**1.4**	**4.4**	**9.7**	**5.7**	**14.8**	**21.5**	**3.9**	**11.4**	**17.1**	**5.9**	**14.8**	**20.9**	**2.5**	**8.2**	**38.7**	**1.1**	**4.0**	**26.6**	**2.3**	**9.6**	**44.0**
2015	**1.1**	**4.4**	**14.6**	**4.2**	**17.9**	**62.9**	**3.2**	**10.7**	**41.1**	**3.8**	**14.0**	**49.9**	**2.4**	**15.3**	**23.3**	**0.9**	**4.7**	**18.4**	**2.0**	**12.5**	**57.0**

(c) Urinary metabolite levels (in µg/L) of the high molecular weight phthalates DiDP/DPHP and DnOP; and other metabolites (MCPP, MnPeP, MCHP) for the first time reported in samples

	DiDP/DPHP									DnOP			various			DnPeP			DCHP		
	OH-MiDP			oxo-MiDP			cx-MiDP			MnOP			MCPP			MnPeP			MCHP		
	50P	95P	Max	50P	95P	Max	50P	95P	Max	50P	95P	Max	50P	95P	Max	50P	95P	Max	50P	95P	Max
2007	**0.7**	**3.9**	**23.3**	**0.3**	**1.7**	**12.9**	**0.5**	**3.2**	**13.0**	**<LOQ**	**<LOQ**	**1.0**	**0.6**	**3.5**	**7.8**	**<LOQ**	**<LOQ**	**0.2**	**<LOQ**	**<LOQ**	**0.2**
2009	**0.7**	**2.4**	**18.3**	**0.3**	**1.1**	**4.1**	**0.5**	**1.5**	**12.9**	**<LOQ**	**<LOQ**	**<LOQ**	**0.6**	**2.2**	**5.6**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**
2011	**1.0**	**5.9**	**47.7**	**0.2**	**1.5**	**10.5**	**0.6**	**2.7**	**24.1**	**<LOQ**	**<LOQ**	**<LOQ**	**0.7**	**2.8**	**22.5**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.4**
2013	**0.8**	**3.0**	**9.3**	**0.3**	**1.6**	**12.9**	**0.4**	**1.1**	**4.0**	**<LOQ**	**<LOQ**	**0.7**	**0.5**	**1.9**	**20.0**	**<LOQ**	**<LOQ**	**1.3**	**<LOQ**	**<LOQ**	**1.2**
2015	**0.8**	**3.4**	**5.2**	**0.3**	**0.8**	**2.1**	**0.4**	**1.5**	**5.1**	**<LOQ**	**<LOQ**	**<LOQ**	**0.3**	**2.9**	**23.3**	**<LOQ**	**<LOQ**	**0.7**	**<LOQ**	**<LOQ**	**0.9**

and to compare metabolite levels from this study with other population studies such as NHANES that also provide metabolite data over a longer time frame (1999–2012).

3.2. Time course of metabolite excretion in ESB (1988–2015)

In Fig. 1 we present the median urinary levels of major or representative metabolites of the LMW phthalates (Fig. 1A) and the HMW phthalates (Fig. 1B) for each year included in the three measurement campaigns of ESB. In earlier measurements from the ESB, MnBP (the major metabolite of DnBP) has been the dominant metabolite in the full spectrum of phthalate metabolites. Median levels were higher than 100 µg/L in the late 1980s and early 1990s but decreased to levels below 10 µg/L in 2015. This decrease in exposure seems to have been influenced by the provisional ban of DnBP in toys and childcare articles commencing in 1999 which was made permanent by an EU Directive in 2005 (2005/84/EC). Another decision considerably influencing the decrease in DnBP exposure seems to have been its classification as a reproductive toxicant in 2001 (see Supplementary Table 1) which also prohibited its use in cosmetics. The structural analogue of DnBP, the phthalate DiBP has not been prohibited for use in toys and childcare articles but has been shown to have a toxicological profile similar to DnBP in the meantime (Furr et al., 2014; Hannas et al., 2011b). Ini-

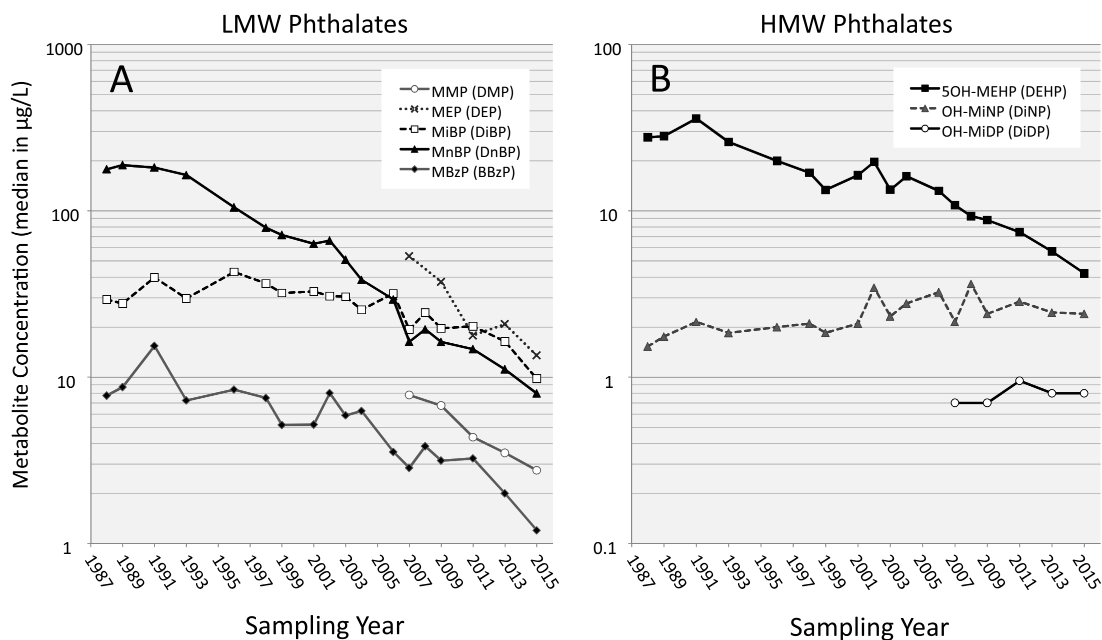

Fig. 1. Concentrations of key LMW and HMW phthalate metabolites (median, in µg/L) in the German ESB over the years 1988–2015.

tially, MiBP levels have been lower than MnBP levels, but contrary to MnBP MiBP metabolite levels remained rather constant until 2011 (between 20 and 40 µg/L), surpassing the metabolite levels of MnBP in 2006. In the last years, probably due to its classification as a reproductive toxicant in 2009, also for MiBP a decline in metabolite levels set in to median levels around 10 µg/L in 2015. For MBzP, metabolite levels have always been lowest in the metabolite spectrum of LMW phthalate metabolites. Metabolite levels dropped from values around 10 µg/L in the early 1990s to a median value of around 1 µg/L in 2015, paralleling the decline in MnBP levels. Similar to DnBP, BBzP has been banned in toys and childcare articles since 1999 and labelled as a reproductive toxicant in 2004. The metabolites MMP and MEP have only been included in the latest measurement campaign. The parent phthalates DMP and DEP are not restricted in Europe. However, also for these phthalates a considerable decline in metabolite levels (for MMP from around 8 µg/L in 2007 to 3 µg/L in 2015; for MEP from 54 µg/L in 2007 to 14 µg/L in 2015) is apparent, possibly due to the increasing demand for "phthalate-free" cosmetics.

The time course of metabolite excretion for the HMW phthalates DEHP, DiNP and DiDP/DPHP is illustrated in Fig. 1B by their hydroxylated metabolites 5OH-MEHP, OH-MiNP and OH-MiDP, respectively. For 5OH-MEHP, similar to the restricted/classified LMW phthalates, there is an apparent decline from median metabolite levels around 30 µg/L in the early 1990s to around 4 µg/L in 2015. DiNP (and DiDP) have been restricted in certain toys since 1999 but have not been labelled as reproductive toxicants. However, DiNP has been shown to possess some endocrine disrupting potential, around a factor of 3 weaker than DEHP (Furr et al., 2014; Hannas et al., 2011b). For the DiNP metabolite OH-MiNP some increase from 1.5 µg/L in 1988 to values above 3 µg/L between 2002 and 2008 seems to have turned to lower median values again (2.4 µg/L in 2015). OH-MiDP median values have only been analyzed in the latest measurement campaign, but are below 1 µg/L. OH-MiDP reflects exposure to DiDP and DPHP, because the applied HPLC method cannot distinguish between the specific metabolites of DPHP and the isomeric metabolite mixture of DiDP (Gries et al., 2012). Schütze et al. (2015) have recently shown, based upon samples from the ESB, that the DPHP specific metabolite OH-MPHP only contributes a negligible share to the isomeric OH-MiDP metabolite (Schütze et al., 2015). However, the same authors have also reported that the ESB samples indicate an increasing exposure to DPHP.

3.3. Male vs. female metabolite levels in ESB (2007–2015)

In Table 4 the median metabolite levels of the male and female subpopulation are depicted for the individual years of the recent measurement campaign and the recent measurement campaign as a whole (in bold). With some fluctuations between the years, viewed over all years, median values for males and females are remarkably similar and do not indicate to profound exposure differences between males and females. This is in general agreement with the previous ESB measurement campaigns. For the DEHP metabolites 5OH-MEHP and 5oxo-MEHP Göen et al. (2011) reported somewhat higher 5OH-MEHP levels and somewhat lower 5oxo-MEHP levels in males compared to the females, which is also confirmed in the present study. This observation might point to some gender dependent difference in oxidative DEHP metabolism.

Other studies, including studies from our group, have reported some exposure driven differences in phthalate levels between male and female populations and depending upon age and other factors, most probably caused by different lifestyle and nutritional pattern. These influencing factors are probably not captured by the rather homogeneous composition of our study population (young, healthy adults, 20–29 years). Therefore, we consider the ESB study population as a reliable source providing general time trends of phthalate exposure. However, specific sub-populations such as children or pregnant women might experience phthalate exposures that are higher (or lower) than reported for the ESB population.

3.4. Metabolite levels compared to biomarker derived health-based guidance values

The urinary metabolite levels determined in the 24 h urine samples of the ESB can be compared to health based guidance values (see Table 5). Such health based guidance values, called biomonitoring equivalents, BE, (Hays et al., 2007; Hays and Aylward, 2012) or human biomonitoring values, HBM values, from the German

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Table 4
Median metabolite levels (in µg/L) of the male (♂) and female (♀) population of the German ESB over the sampling years 2007–2015.

	2007		2009		2011		2013		2015		2007–2015	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
n	30	30	30	30	30	30	30	30	30	30	**150**	**150**
MMP	5.1	8.0	8.0	6.4	4.3	4.5	3.3	3.6	3.0	2.5	**4.2**	**4.5**
MEP	91.5	34.0	27.2	51.6	23.1	16.4	18.0	23.2	14.1	12.3	**23.9**	**23.8**
MiBP	23.1	16.7	21.7	19.0	17.4	26.1	17.0	13.7	10.0	9.1	**17.1**	**15.5**
2OH-MiBP	8.2	7.6	6.2	5.9	4.5	7.8	4.8	4.4	2.9	2.6	**4.7**	**5.2**
MnBP	18.8	14.8	13.9	17.4	11.0	20.5	10.5	11.6	8.0	7.8	**11.1**	**12.5**
3OH-MnBP	1.4	1.5	1.4	1.4	1.1	1.8	1.1	1.1	0.8	0.8	**1.3**	**1.2**
MBzP	3.6	2.6	3.0	3.3	2.6	3.9	2.2	1.9	1.5	1.1	**2.6**	**2.5**
MEHP	4.5	3.5	3.2	3.4	1.9	1.7	1.5	1.3	1.2	1.0	**2.3**	**2.0**
5OH-MEHP	11.6	10.4	8.5	9.2	6.9	8.9	6.2	5.0	4.5	4.2	**7.4**	**6.8**
5oxo-MEHP	6.2	6.3	4.6	5.6	4.7	6.5	3.7	4.7	2.8	3.3	**4.5**	**4.9**
5cx-MEPP	11.7	13.0	8.4	9.2	6.8	9.1	5.8	6.3	3.6	4.0	**6.9**	**7.3**
7OH-MiNP	2.5	1.5	2.5	2.4	2.3	3.1	2.2	2.8	2.6	2.3	**2.5**	**2.4**
7oxo-MiNP	1.1	0.9	1.2	1.2	1.4	2.0	1.2	1.1	0.8	1.0	**1.2**	**1.2**
7cx-MINP	2.2	1.6	2.8	2.9	3.6	3.9	2.0	2.5	2.3	1.9	**2.5**	**2.4**
OH-MiDP	0.9	0.7	0.8	0.7	1.0	0.9	0.9	0.6	0.6	0.8	**0.8**	**0.7**
oxo-MiDP	0.4	0.3	0.3	0.3	<LOQ	0.2	0.2	0.2	<LOQ	0.4	**0.3**	**0.3**
cx-MiDP	0.6	0.5	0.6	0.5	0.6	0.6	0.4	0.3	0.4	0.4	**0.5**	**0.5**
MCPP	0.7	0.6	0.6	0.7	0.6	0.8	<LOQ	0.5	<LOQ	<LOQ	**0.6**	**0.6**

Table 5
German ESB participants exceeding health based guidance values derived for urinary phthalate metabolites.

Phthalate	health-based guidance value			% exceeding health based guidance value		
	Type #	Biomarkers/metabolites	Value	ESB total (1985–2015)	ESB pre 2002 (1985–2001)	ESB (2007–2015)
DEP	BE	MEP	18,000 µg/L	0	0	0
BBzP	BE	MBzP	3800 µg/L	0	0	0
DnBP	BE	MnBP	200 µg/L	13.8	27.2	0
DEHP	HBM-I value	5OH-MEHP + 5oxo-MEHP	300 µg/L (women in childbearing age)	0.5	1.1	0
		5OH-MEHP + 5oxo-MEHP	750 µg/L (adult males)	0	0	0
	BE	MEHP + 5OH-MEHP + 5oxo-MEHP	260 µg/L	1.5	2.3	1.0
DiNP	BE	OH-MiNP + oxo-MiNP + cx-MiNP	1800 µg/L	0	0	0

Abbreviations: HBM-I value: human biomonitoring value I, BE: biomonitoring equivalent.
health based guidance values for DEP, BBzP, DnBP: Aylward et al., 2009a; DEHP: Schulz et al., 2012a,b and Aylward et al., 2009b; DiNP: Hays et al., 2011.

Human Biomonitoring Commission (Angerer et al., 2011; Schulz et al., 2012b) have been derived for several phthalates.

The derivation of these BE and HBM values has been based on relating a chronic exposure guidance value (such as the TDI, ADI or the RfD) to steady-state concentrations of phthalate metabolites in urine. While phthalate exposure is known to occur intermittently (Preau et al., 2010), and due to rapid elimination kinetics (Wittassek et al., 2011), urinary metabolite concentrations can vary substantially in spot urine samples. The 24 h urine samples of the ESB represent a good approximation of the mean (chronic) exposure over a period of one day. Thus, contrary to spot urine samples, where interpretation has to be performed with added caution in respect to BE or HBM values, we consider the 24 h urine samples of the ESB a highly robust measure of the mean daily exposure, even at the upper percentiles or maximum metabolite concentrations of the study population. Previous studies have shown that the mean (or median) concentrations of biomarkers determined in population studies from spot urine samples or 24 h urine samples are very comparable. However, high-end concentrations (95th percentiles and maxima) in a population of 24-h samples tend to be only half the high-end concentrations in a population of spot samples (Aylward et al., 2016; Christensen et al., 2012).

The BE guidance values for DEP, BBzP and DiNP have never been exceeded in any of the ESB samples. The maximum value observed for MEP (6080 µg/L) in 2011 is a factor of three below the respective BE value of 18,000 µg/L; the maximum value observed for MBzP (687 µg/L) in 1996 is a factor of five below the respective BE value of 3800 µg/L (Aylward et al., 2009b). DiNP metabolite levels were several orders of magnitude below the BE value. This BE value has been derived from the EFSA TDI (Spongiosis hepatitis) and does not take account of the endocrine disrupting potency of DiNP (Hays et al., 2011). For the DnBP metabolite MnBP around 14% of all ESB samples from 1988 to 2015 exceeded the BE value of 200 µg/L. However, these exceedances mainly occurred in urine samples collected before 2002. 27% of these samples from 1988 to 2001 exceeded the BE value. In none of the samples from the recent measurement campaign (2007–2015) the BE value has been exceeded. In 2015, the 95th percentile of urinary MnBP (21.3 µg/L, see Table 3a) has been a factor of 10 lower than the BE. Considering a similar toxicological profile of DiBP, but with no BE value derived so far, the 95th percentile of urinary MiBP (33.4 µg/L, see Table 3a) has also been considerably below the BE derived for MnBP. For the DEHP metabolites both HBM values and BE values have been derived, based on a slightly different set of metabolites, and based upon different toxicological endpoints (Aylward et al., 2009a; HBM Commission, 2007; Schulz et al., 2012b). In the ESB samples collected before 2002 2.3% of the study population exceeded the BE value and 1.1% of the women exceeded the HBM I value. While no exceedances of the HBM I value were observed for the samples from the recent measurement campaign (2007–2015), still 1.0% exceeded the BE value.

Currently, HBM and BE values do only evaluate exposures to single phthalates. Endocrine active phthalates, however, are known to act in a concerted, dose additive manner (Howdeshell et al.,

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2008b, 2008a, 2015; Lioy et al., 2015; National Research Council, 2008). As can be seen from this and other studies, the population is still continuously and simultaneously exposed to a wide range of different phthalates. A cumulative risk assessment based upon metabolite levels in the ESB, taking into account their common anti-androgenic potential, will therefore be performed in a subsequent study (publication in preparation).

3.5. Metabolite levels from ESB (1988–2015) compared to NHANES (1999–2012, 20 years and older)

We can compare metabolite levels and time courses of excretion of the metabolites over the years investigated in the German ESB with metabolite data published in the exposure reports of U.S. NHANES. Temporal trends for phthalate exposure in the US have already been reported by Zota et al. (Zota et al., 2014) encompassing NHANES data from 2001 to 2010. In the most recent exposure report (Fourth report, Updated Tables, February 2015, www.cdc.gov/exposurereport/; last accessed July 20, 2016) nationally representative biomonitoring data (for the non-institutionalized U.S. population) have been published for the phthalates starting from the sampling years 1999/2000 in 2-year intervals with the most recent set from 2011/2012. Contrary to the 24 h urine samples of ESB, NHANES is based on spot urine samples with sampling sessions in the morning, the afternoon, or the evening. For comparison with ESB we selected the reported median levels (in μg/L) of the NHANES population subset "20 years and older". The sample size of this subset ranged between 1461 and 1914 for each of the two-year NHANES cycles with a total sample size of 11,565.

In Fig. 2, concentrations of key metabolites (median, in μg/L) of eight phthalates (DMP, DEP, BBzP, DiBP, DnBP, DEHP, DiNP and DiDP) determined in both populations are depicted, separately for the respective study years. MCPP, a metabolite of several LMW and HMW phthalates, cannot be attributed to a single phthalate. On first sight one can see similarities and differences between ESB and NHANES, both in terms of urinary metabolite levels and time courses over the years investigated. As a common feature, the worldwide change in the phthalate market, with dramatic changes in phthalate production and uses, influenced by toxicological findings and national regulations and restrictions, seems to be reflected in the biomonitoring data (Bizzari et al., 2013; Lioy et al., 2014). However, also country specific production and use patterns seem to exist. For the LMW phthalate DMP, MMP metabolite levels indicate considerably lower exposures in the US (median levels for 2003/2004 and 2005/2006 were below the LOQ) compared to Germany. German levels, however decreased from 8 μg/L (in 2007) to 2.8 μg/L (in 2015). For DEP, MEP levels in the US steeply declined from 125 μg/L in 2003/2004 to around 30 μg/L in 2011/2012. German values were generally lower, also declining, but settling at concentrations around 20 μg/L. MBzP levels seemed to decline in both countries with only slightly higher levels in the US (metabolite levels in both countries in 2011/2012 around 4 μg/L). For the butyl phthalate isomers DiBP and DnBP some counter-rotating developments can be observed for MiBP. While MiBP levels were considerably higher for many years in Germany (around 30 μg/L until 2006), US levels were very low in 2001/2002 (2.4 μg/L) but tripled to levels around 7.5 μg/L in 2009/2010. In both countries, in recent years, levels seem to be declining. Historically, DnBP exposures seem to have been much higher in Germany (median values peaking almost 200 μg/L in the early 1990s) compared to the US, albeit the first direct comparison is only possible for the years 1999/2000 (Germany: 72 μg/L; US: 23 μg/L). Due to the rapid decline in Germany with stable values in the US until 2007/2008, recent metabolite levels are quite comparable (10–15 μg/L).

Based upon the carboxylated metabolites of the HMW phthalates DEHP, DiNP and DiDP (measured both in NHANES and ESB) again similarities and differences can be observed between Germany and the US. DEHP exposure has been dropping in both countries with cx-MEPP concentrations in the US always slightly higher than in Germany. However, historic German levels from the early 1990s were higher than US levels from 2001/2002. For DiNP, metabolite data is indicating to considerably different developments in Germany and the US. While metabolite levels have been rather comparable in 2005/2006 (around 4 μg/L) metabolite levels have been steeply increasing in the US to almost 20 μg/L in 2011/2012 with German levels constantly remaining below 4 μg/L, even dropping to 2 μg/L in 2015. For DiDP, no time trends are apparent for the US and Germany, but US metabolite levels are 5-times higher (2.5 μg/L vs. 0.5 μg/L). The somewhat higher exposure to HMW phthalates in the US (especially DiDP) might also be reflected in the 5-times higher levels of MCPP. In this respect, it has again to be pointed out, that ESB samples are collected as complete 24 h urine samples while NHANES samples are collected as spot urine samples. While there are indications that population data based on spot urine samples results in somewhat higher concentrations in the upper percentiles than population data based on 24 h urine samples, population means and population medians resulting from the two sampling regimes are very comparable (Aylward et al., 2016; Christensen et al., 2012). A more detailed comparison between NHANES and ESB data (not shown) indeed reveals that at comparable median concentrations, the 95th percentiles in NHANES are two- to three-times higher than in the ESB. In part, this observation can certainly be attributed to the different sampling regimes (spot vs. 24 h). Other reasons for the larger spread in NHANES compared to ESB might be that the study population in NHANES is much more heterogeneous in terms of age, ethnicity, geography, social background, lifestyle etc. than the rather homogeneous study population of the ESB at one sampling location.

4. Conclusions

With this study we provide valuable insights into the time course of phthalate exposure in Germany from 1988 to 2015. Exposure to most critical (endocrine active) phthalates has been considerably higher before the turn of the millennium but various regulatory measures enacted in Europe and changes in the phthalate market obviously have led to a decline in exposures. For phthalates regulated at a later stage (such as DiBP) also their decline set in at later years. In recent years, exposure to most of the phthalates, regulated or not (such as DMP and DEP), seems to be declining, probably because of a dwindling acceptance of phthalate-containing products and the rising importance of phthalate alternatives. For those phthalates used as plasticizers for polymers, the drop in DEHP exposure has only resulted in a moderate increase in exposure to DiNP, a possible substitute with no labelling requirements. In Germany, DEHP does not seem to be fully substituted by other HMW phthalates (e.g. DiNP and DiDP), but also by non-phthalate, non-aromatic plasticizers. We have previously reported, also based upon ESB samples, a profound increase in exposure to DINCH (1,2-Cyclohexane dicarboxylic acid diisononyl ester) (Schütze et al., 2012, 2014). In a pilot study we have also reported the widespread exposure to di-(2-ethylhexyl) terephthalate (DEHTP) in Germany (Lessmann et al., 2016a, 2016b). Currently, we assume these alternatives having a preferred toxicological profile over the regulated phthalates. Nevertheless, a continuous surveillance of these alternatives is advisable as it allows intervening with regulatory measures, if exposures are exceeding health benchmarks, or if new toxicological findings lead to a re-evaluation of these health benchmarks.

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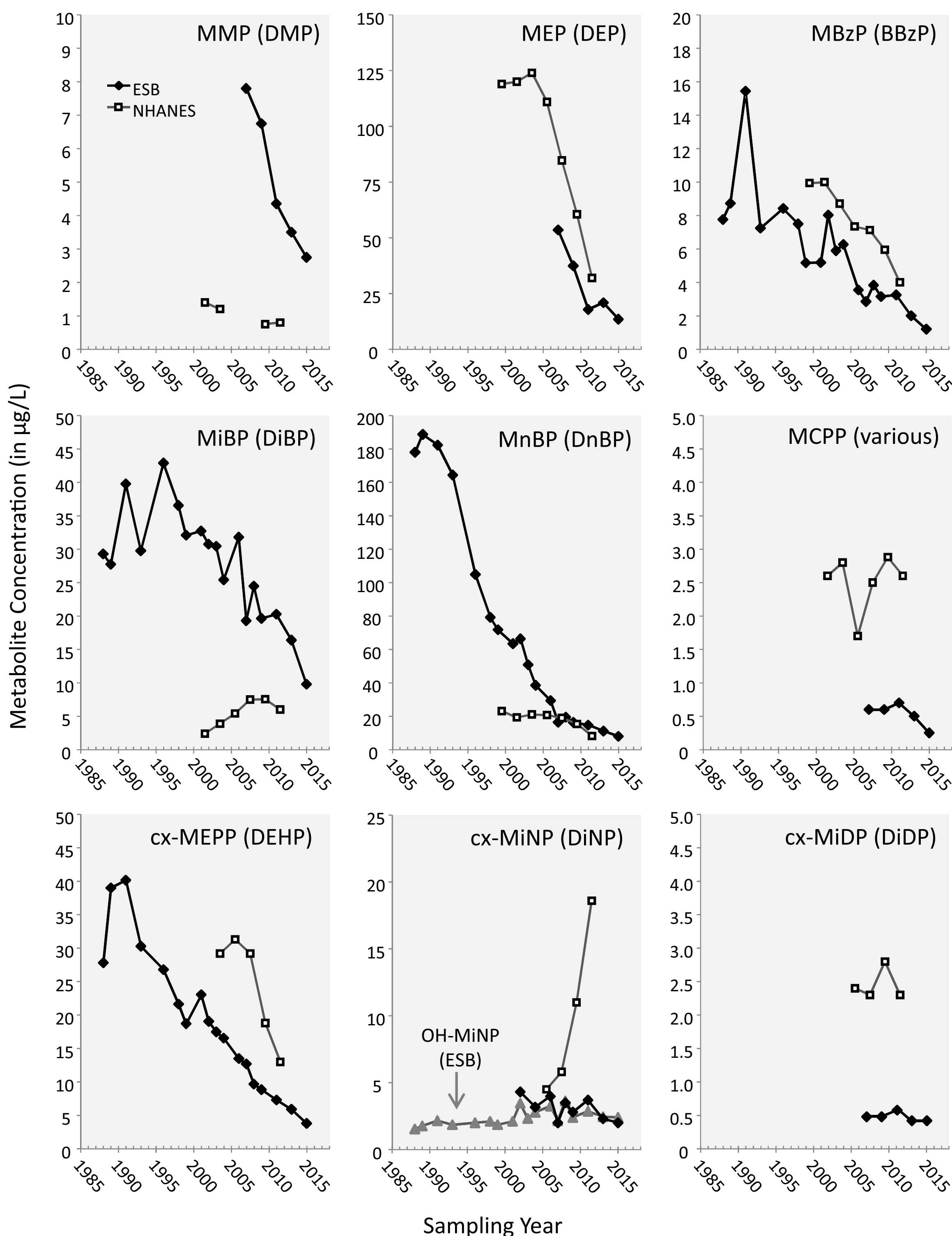

Fig. 2. Concentrations of key phthalate metabolites (median, in µg/L) in the German ESB (20–29 years) compared to US NHANES (20 years and older) over the different years of sampling (black diamonds for ESB, white boxes for NHANES).

A comparison with health based guidance values for individual phthalates showed, that ESB participants exceeded health benchmark values (BE, HBM I) for DEHP and DnBP mainly in the years before regulations set in. Still, 1% of the study population exceeded the BE of DEHP also in the most recent measurement campaign. Two aspects have to be kept in mind in regard to these findings: first, endocrine active phthalates are known to act in a dose additive manner, and, albeit exposures to individual phthalates are below respective health based guidance values, the cumulative phthalate exposure might still exceed levels indicating concern (Lioy et al., 2015; National Research Council, 2008). Second, the study population of the ESB cannot be regarded representative for the whole German population. Data from the German Environmental Survey (GerES) indicates that children can experience higher exposures to some phthalates than adults and younger children have higher exposures than older children or adolescents (Koch et al., 2007a;

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Wittassek et al., 2007a). Similarly, other sub-populations with specific food consumptions or life-style habits might also experience different (or higher) amplitudes of phthalate exposure.

A comparison of ESB with NHANES has shown that phthalate exposures in general are rapidly changing with similarities between countries but also country specific differences. A comparison of worldwide human biomonitoring data of phthalate exposure therefore has always to take into account the country of origin and the year the respective samples are collected. Even within Europe we can find in part considerable differences in phthalate exposure (Cerna et al., 2015; Den Hond et al., 2015). Although regulatory measures on phthalates are the same within the European Union, country specific characteristics in life-style, product use and/or food consumption can lead to differences in phthalate exposure.

The results from this study illustrate that human biomonitoring is an ideal tool to describe and evaluate phthalate exposure both in the past (documenting the effect and success of regulatory measures on the exposure) but also in regard to recently collected samples (documenting the current extent of exposure). Thus, human biomonitoring is able to profoundly support and complement the whole process in a chemical's evaluation from exposure assessment, via risk assessment to risk management.

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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.2016.11.003.

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