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Exposure to phthalates in 5–6 years old primary school starters in Germany—A human biomonitoring study and a cumulative risk assessment

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ABSTRACT

We determined the internal exposure of 111 German primary school starters by analyzing urinary metabolites of six phthalates: butyl benzyl phthalate (BBzP), di-iso-butyl phthalate (DiBP), di-n-butyl phthalate (DnBP), di (2-ethylhexyl) phthalate (DEHP), di-iso-nonyl phthalate (DiNP) and di-iso-decylphthalate (DiDP). From the urinary metabolite levels, we calculated daily intakes and related these values to Tolerable Daily Intake (TDI) values. By introducing the concept of a relative cumulative Tolerable Daily Intake (TDI_{cum}) value, we tried to account for the cumulative exposure to several of the above-mentioned phthalates. The TDI_{cum} was derived as follows: the daily intake (DI) calculated from the metabolite level was divided by the TDI for each phthalate; this ratio was multiplied by 100% indicating the TDI percentage for which the DI accounted. Finally the % TDIs of the different phthalates were totalled to get the TDI_{cum}. A TDI_{cum} above 100% is a potential cause for concern. We confirmed the ubiquitous exposure of the children to all phthalates investigated. Exposures were within range of levels previously reported for GerES, albeit slightly lower. Regarding daily intakes, two children exceeded the TDI for DnBP, whereas one child closely approached the TDI for DEHP. 24% of the children exceeded the TDI_{cum} for the three most critical phthalates: DEHP, DnBP and DiBP. Furthermore, 54% of the children had total exposures that used up more than 50% the TDI_{cum}. Therefore, the overall exposure to a number of phthalates, and the knowledge that these phthalates (and other anti-androgens) act in a dose-additive manner, urgently warrants a cumulative risk assessment approach.

Introduction

Phthalates are widely used as plasticizers in polymers, primarily in polyvinyl chloride (PVC), in polyvinyl acetate (PVA) emulsions, nitrile rubbers and as gelling additives for nitrocellulose, cellulose ether, and polyacrylate and polyacetate dispersions. Phthalates are also used as multipurpose industrial solvents and lubricants, and are therefore utilized in many consumer products including building materials, household furnishings, toys, cosmetics and body care products, pharmaceuticals, nutritional supplements, medical devices, packaging, adhesives, clothing, automobiles, cleaning agents and insecticides (Wittassek et al., 2011; Koch and Calafat, 2009; NRC, 2008).

Several phthalates are endocrine disruptors and act on male reproductive development. Examples of such phthalates with endocrine disrupting/modulating potency in animal studies include: butyl benzyl phthalate (BBzP), di-iso-butyl phthalate (DiBP), di-n-butyl phthalate (DnBP), di (2-ethylhexyl) phthalate (DEHP) and di-iso-nonyl phthalate (DiNP) (Borch et al., 2006; Gray et al., 2000; Howdeshell et al., 2007). Phthalates suppress the endogenous production of testosterone and also influence insulin-like factor 3 production (Sharpe and Irvine, 2004; Wilson et al., 2004). This androgen insufficiency can lead to functional and structural impairment of reproduction and development, and manifests itself as malformations of the epididymis and the external genitalia (hypospadias), undescended testicles (cryptorchidism), impaired spermatogenesis, a delayed onset of puberty and a general reduction of male fertility (NRC, 2008; Foster, 2006; Gray et al., 2000; Noriega et al., 2009). It can also lead to signs of feminization (retention of nipples/areolae in male rodents) and demasculinization (reduced anogenital distance) (Foster, 2006; Lee et al., 2004; Tyl et al., 2004). This group of symptoms in experimental animals is called “phthalate syndrome”, which shows many similarities with the human testicular dysgenesis syndrome (TDS), a syndrome of increasingly common developmental disorders observed in humans (Sharpe and Skakkebaek, 2003). The pathways of androgen action are similar in both experimental animals and humans, suggesting that phthalates cause comparable adverse effects on

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reproduction and development in humans as they do in experimental animals. In recent years, several epidemiological studies have suggested that environmental exposure to a number of phthalates may be associated with adverse reproductive outcomes, like alterations in semen parameters (Duty et al., 2003a, 2004; Hauser et al., 2006), DNA damage in sperm (Duty et al., 2003b; Hauser et al., 2007), reduced reproductive hormone levels in adult men (Duty et al., 2005), decreased anogenital distance in male infants (Swan et al., 2005; Swan, 2008), abdominal obesity and insulin resistance (Hatch et al., 2008, 2010; Stahlhut et al., 2007), conduct or attention-deficit hyperactivity disorders (Engel et al., 2010; Kim et al., 2009) or a less male-typical behaviour in young boys (Swan et al., 2010). The relationship reported between phthalates and adverse outcomes in human offspring seem plausible; however, additional research is needed to confirm these findings.

In rodents, the most critical window of exposure is at the fetal stage during the androgen- (testosterone) regulated sexual differentiation (gestational days 15–17); however, some sexual development (e.g. testis descent) also occurs in the postnatal life. Thus, the most sensitive time for the rat is the prenatal period, although the prepubertal and pubertal rats are also sensitive. In humans, this most critical period seen in rats would correspond to fetal exposures during the end of the first trimester of pregnancy; however, one has to assume that later in life, all androgen/testosterone regulated mechanisms can also be influenced. Therefore, the focus of exposure assessment to phthalates is both on pregnancy and childhood/adolescence. Furthermore, phthalates have been shown to act in a dose additive manner. This warrants both a cumulative exposure and risk assessment (Howdeshell et al., 2008; Noriega et al., 2009; Rider et al., 2009, 2010; Wilson et al., 2008).

Because phthalates are not chemically bound to the polymer they can leach or outgas into the surrounding media including air, foodstuff and many other matrices. Contaminated foodstuff is considered to be a major source of exposure for some phthalates. Also, consumer products can lead to exposures through direct contact and use, by leaching into other products, or through general environmental contamination. Therefore, all routes of exposure are of relevance to humans (ingestion, inhalation, and dermal exposure). The widespread exposure of the general population to several phthalates (at the same time) has been shown in an increasing number of human biomonitoring studies in Europe, the US and Asia (Koch and Calafat, 2009; Wittassek et al., 2011).

Human biomonitoring is an important tool in exposure assessment. This is especially relevant for phthalates. Human biomonitoring determines the internal exposure to phthalates by measuring specific metabolites in urine. These metabolites are not prone to external contamination, a problem normally present when analyzing the parent phthalates in air, foodstuff or dust. Furthermore, these urinary metabolites represent an integral measure of exposure from all possible sources and routes. This way, human biomonitoring can also estimate exposures to phthalates from sources that are difficult to evaluate quantitatively, e.g. exposures due to leaching from toys by mouthing or chewing, or from sources that are altogether unknown. Therefore, especially in the case of phthalates, human biomonitoring has opened a new and alternative approach to the integral exposure and risk assessment of phthalates (Koch and Calafat, 2009; Wittassek et al., 2011).

Knowledge on metabolism and elimination of phthalates and thus the selection of appropriate biomarkers is essential to perform a valid human biomonitoring study. In a first rapid step, the phthalate diester is cleaved into the respective hydrolytic monoester. Secondly, the alkyl chain of the resulting hydrolytic monoester can be modified by various oxidation reactions. The extent of oxidative modification increases upon the alkyl chain length of the phthalate monoester. Low molecular weight phthalates (e.g. DnBP and DiBP) are mostly metabolized to their hydrolytic monoesters, whereas high molecular weight phthalates (e.g. DEHP, DiNP) are extensively transformed to oxidative metabolites (Barr et al., 2003; Kato et al., 2007; Koch et al., 2005; Koch and Angerer, 2007; Silva et al., 2006, 2007; Wittassek et al., 2011). As biomarkers of exposure, we use one of the most advanced set of phthalate metabolites currently available, including four monoester and ten oxidised monoester metabolites.

Table 1
Classification and labeling of the phthalates investigated in this study (Annex 1, Council Directive 67/548/EU) and restriction of uses in children's toys and child care articles (Directive 2005/84/EU).

Phthalate	Year	Reproduction	Development	Use restrictions in toys
DiBP	2009[a]	Cat. 3 (R 62)	Cat. 2 (R 61)	–
DnBP	2001[b]	Cat. 3 (R 62)	Cat. 2 (R 61)	X
BBzP	2004[c]	Cat. 3 (R 62)	Cat. 2 (R 61)	X
DEHP	2001[b]	Cat. 2 (R 60)	Cat. 2 (R 61)	X
DiNP	–	–	–	X
DiDP	–	–	–	X

R 60: may impair fertility; R 61: may cause harm to the unborn child; R 62: may possibly impair fertility.
[a] Directive 2009/2/EU.
[b] Directive 2001/59/EU.
[c] Directive 2004/73/EU.

We quantitatively describe the internal exposure of primary school starters in Germany to six phthalates deemed most critical in terms of reproductive and developmental toxicity (see Table 1). In Europe, the phthalates covered in this study are either restricted in all toys, child care articles or cosmetics (DEHP, BBzP, DiBP, DnBP) based on their classification as reproductive toxicants (Category 2) by the EU, or restricted in certain toys and childcare articles (DiNP, di-iso-decylphthalate (DiDP)) based on the precautionary principle (see EU Directives 2005/84/EC; 2004/93/EC; 2009/2/EC).

Materials and methods

Study subjects and sampling

In Germany, before admission to primary school, all children of the respective age group (between 5 and 6 years old) are subjected to a general health and developmental check up. This mandatory examination is performed by the local health authority with the aim to test the general readiness of the child to begin the first stage of compulsory education. The examination is conducted by a trained physician from the local health authority and mandated by German respective state law. Within the frame of this check up, we obtained a spot urine sample from the child for this study. We informed the parents about the aim of this study and obtained written consents.

Between February 26, 2007, and March 15, 2007, we acquired spot urine samples from 111 healthy primary school starters at the City of Frankfurt on the Main. On site sample and data collection was performed by physicians and trained staff of the pediatric division of the Public Health Department of the City of Frankfurt/Main. The study population was made up of 48 girls and 63 boys. The children were between 5.6 and 6.7 years old. Urine samples were immediately frozen at −18 °C until chemical analyses.

Urine analyses

Urine samples were analysed according to methods published previously (Koch et al., 2003, 2007b; Preuss et al., 2005) using 1 mL aliquots. In short, after enzymatic hydrolysis of the conjugates with arylsulfatase-free ß-glucuronidase (from *Escherichia coli* K12), the samples were analysed with multidimensional liquid chromatography tandem mass spectrometry (LC/LC–MS/MS). Phthalate

metabolites were stripped in an on-line arrangement from the urinary matrix on a restricted access material (LiChrospher® ADS-8, Merck, Darmstadt) precolumn, transferred in backflush-mode and chromatographically resolved on a reversed-phase analytical column HPLC (Luna Phenyl-Hexyl, Phenomenex). Detection was performed in negative ionisation mode and quantification was performed by isotope dilution with the respective deuterium labeled standard substances.

Metabolites analysed were: BBzP metabolite: monobenzyl phthalate (MBzP), DiBP metabolite: mono-iso-butyl phthalate (MiBP), DnBP metabolite: mono-n-butyl phthalate (MnBP), DEHP metabolites: mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (5OH-MEHP), mono(2-ethyl-5-oxohexyl) phthalate (5oxo-MEHP), mono(2-ethyl-5-carboxypentyl) phthalate (5cx-MEPP), mono[-2(carboxymethyl)hexyl] phthalate (2cx-MMHP), DiNP metabolites: mono-(4-methyl-7-hydroxy-octyl)phthalate (OH-MiNP), mono-(4-methyl-7-oxo-octyl)phthalate (oxo-MiNP), mono-(4-methyl-7-carboxyheptyl)phthalate (carboxy-MiNP), DiDP metabolites: mono-(2-propyl-6hydroxy-heptyl) phthalate (OH-MiDP), mono-(2-propyl-6oxoheptyl) phthalate (oxo-MiDP), mono-(2,7-methyl-7-carboxyheptyl) phthalate (carboxy-MiDP). For structures, see Koch and Calafat (2009). The determination of 2cx-MMHP has to be regarded as semi-quantitative because no standard substance was available and quantitation was performed in analogy to 5cx-MEPP (Koch et al., 2005; Preuss et al., 2005). Internal quality control was performed using self-prepared quality control urines. Variations of the measured concentrations of these quality controls were between 7% and 15% depending on the metabolite. The LOQ (limit of quantification) was 0.25 μg/L for all metabolites except MnBP and MiBP, for which it was 0.5 μg/L. Urinary creatinine concentrations were determined according to Larsen (1972).

Daily intake calculation

For the calculation of the daily intake of the individual phthalates, we utilized the results of urinary phthalate metabolite levels in the spot urine samples and individual data of each child regarding age, body weight and body height. We evaluated the daily intake of each parent phthalate separately for each child. For this purpose, we applied a children-specific creatinine based calculation model published previously (Koch et al., 2007a; Wittassek et al., 2007):

$$\mathrm{DI}\,(\mu\mathrm{g/kg_{body\ weight}/day}) = \frac{\mathrm{UE_{sum}}[\mu\mathrm{mol/g_{crea}}] \times \mathrm{CE_{smoothed}}[\mathrm{g/day}]}{F_{\mathrm{UE}} \times \mathrm{bw}[\mathrm{kg}]} \times \mathrm{MW_{Phthalate}}[\mu\mathrm{g/\mu mol}]$$

$\mathrm{UE_{sum}}$ is the molar urinary excretion sum of the measured urinary phthalate metabolites. For example, DEHP is represented by the sum of the urinary levels of the five metabolites MEHP, 5OH-MEHP, 5oxo-MEHP, 5cx-MEPP and 2cx-MMHP, expressed in micromole per gram creatinine. The smoothed creatinine excretion rates $\mathrm{CE_{smoothed}}$ are body height and gender based reference values for urinary creatinine excretion for healthy, Caucasian, 3–18 years old children in gram creatinine per day (Remer et al., 2002). The great variability in creatinine excretion among children is essential for a valid estimation and must be considered because creatinine excretion is primarily dependent on muscle mass, which decreases with decreasing age and body height in children (Barr et al., 2005; Heudorf and Angerer, 2001). We included all urinary values for calculation, even if the creatinine concentration was below 0.3 mg/L, a cut-off value indicating urine "too dilute" to be considered for further analyses in adult populations or occupational settings (Barr et al., 2005; UBA, 2005; WHO, 1996). The molar fraction, F_{UE} describes the molar ratio between the excreted amounts of the specific metabolites of each phthalate in relation to the oral intake of the parent phthalate. For example, a value of 0.669 for DEHP and its five metabolites means that within 24 h after oral intake of DEHP, 66.9% of the dose is excreted as one of the five metabolites in urine (Koch et al., 2005). Urinary excretion factors for the other phthalates have been published (Koch and Calafat, 2009; Wittassek et al., 2011). Finally, we normalized the daily intake of each phthalate to the body weight (bw) for each child.

Cumulative TDI (cumulative risk assessment)

To incorporate the contribution of the cumulative exposure into the cumulative risk assessment, we introduced the concept of a relative cumulative Tolerable Daily Intake ($\mathrm{TDI_{cum}}$). In a very simplistic approach, the daily intake (DI) calculated from the metabolite level was divided by the TDI for each phthalate; this ratio was multiplied by 100% indicating the TDI percentage for which the DI accounted. Finally the % TDIs of the different phthalates were totalled to get the $\mathrm{TDI_{cum}}$. A $\mathrm{TDI_{cum}}$ above 100% indicates that the total daily phthalate intake surpassed tolerable levels and therefore is a potential cause for concern.

$$\mathrm{TDI_{cum}}[\%] = \sum_{i=1}^{n} \frac{\mathrm{DI}_i(\mu\mathrm{g/kg\ bw/day})}{\mathrm{TDI}_i(\mu\mathrm{g/kg\ bw/day})} \times 100$$

TDI_i is the tolerable daily intake for each specific phthalate, DI_i is the daily intake (exposure level) of the phthalate (as calculated from our HBM data), n is the number of phthalates in the approach. For this approach, we restricted our analyses to phthalates with TDI values (DEHP: 50 μg/kg bw/day, DnBP: 10 μg/kg bw/day) or putative TDI values (DiBP: set to 10 μg/kg bw/day in analogy to DnBP) based on the same endpoints (anti-androgenicity). This $\mathrm{TDI_{cum}}$ approach closely resembles the Hazard Index (HI) that is a regulatory approach to a cumulative risk assessment based on the concept of dose-addition (Teuschler and Hertzberg, 1995; Kortenkamp and Faust, 2010).

Statistical analysis

Descriptive statistics for the study population were performed with IBM SPSS 19.

Results

The median creatinine concentration of the study population was 0.66 mg/L (mean: 0.70 mg/L; 95th percentile: 1.29 mg/L), which is low compared to adult populations. Ten out of the 111 children had creatinine values below 0.3 mg/L. However, all urine samples were included in the statistical analyses, because creatinine values below 0.3 mg/L in children do not necessarily indicate excessive dilution but are indicative of lower muscle mass compared to adults. For children, a smoothed creatinine value (Remer et al., 2002) adjusts for typical creatinine excretion (including the age, height and weight of the child as key parameters) in the latter calculation of the daily phthalate intake.

Descriptive statistics for the urinary metabolite levels (in μg/L) are summarized in Table 2a. Metabolites of DnBP, DiBP, and DEHP were detectable in every urine sample measured. Additionally, metabolites of DiNP, DiDP, and BBzP were detectable in 99%, 94%, and 86% of the urine samples. With respect to the dibutyl phthalates, it is apparent that the DiBP metabolite, MiBP is excreted at higher concentrations (median: 42.8 μg/L, 95th percentile: 215 μg/L) compared to the DnBP metabolite, MnBP (median: 36.8 μg/L, 95th percentile: 156 μg/L). Although DEHP metabolites still play a major role in terms of internal exposure (sum of 5 metabolites, median: 76.9 μg/L; 95th percentile:

Table 2a
Phthalate metabolites levels (in μg/L) in spot urine samples of 111 children, between 5.6 and 6.7 years old (48 girls and 63 boys).

Phthalate	Metabolite	% >LOQ	GM	Mean (95% conf. interval)	Median	P95	Range
DnBP	MnBP	100	32.6	51.1 (41.8–61.8)	36.8	156	1.7–341
DiBP	MiBP	100	45.5	78.1 (58.6–97.6)	42.8	215	2.0–871
BBzP	MBzP	86	5.3	20.1 (11.7–28.6)	7.2	93.7	<LOQ–298
DEHP	5OH-MEHP	100	17.8	28.8 (22.0–35.6)	17.4	86.1	1.3–311
	5oxo-MEHP	100	14.7	24.1 (18.9–29.3)	15.1	71.0	0.6–214
	5cx-MEPP	100	30.5	43.4 (34.3–52.6)	28.4	101	2.7–441
	2cx-MMHP	100	11.5	17.7 (12.9–22.5)	11.3	46.1	0.4–238
	MEHP	96	4.5	8.5 (5.3–11.8)	4.7	21.1	<LOQ–172
DiNP	OH-MiNP	96	5.4	10.0 (7.8–12.2)	7.0	25.5	<LOQ–83.3
	oxo-MiNP	78	2.3	5.2 (4.0–6.4)	4.2	12.5	<LOQ–51.6
	cx-MiNP	99	12.3	19.5 (14.9–24.0)	13.1	45.5	<LOQ–168
DiDP	OH-MiDP	60	0.5	1.2 (0.9–1.5)	0.4	4.9	<LOQ–9.8
	oxo-MiDP	30	<LOQ	0.3 (<LOQ–4.1)	<LOQ	1.2	<LOQ–3.4
	cx-MiDP	94	1.2	1.8 (1.4–2.2)	1.3	4.5	<LOQ–16.0

GM: geometric mean.
95P: 95th percentile.

325 μg/L), metabolites of DiNP (sum of 3 metabolites, median: 24.3 μg/L; 95th percentile: 83.5 μg/L) and DiDP (sum of 3 metabolites, median: 1.8 μg/L; 95th percentile: 29.2 μg/L) significantly add to the body burden. Descriptive statistics for the urinary metabolite levels (in μg/g crea) are summarized in Table 2b. Creatinine-adjusted values need to be interpreted with caution. A comparison with other creatinine-adjusted values from children of other age groups or groups with other age distributions is difficult, because creatinine in children is more a function of the stage of the physical development rather than urine dilution.

We investigated the internal phthalate exposure separately for boys and girls but found no significant differences (data not shown). We also compared the results of this study with the results of previous studies on internal phthalate exposures of children, such as pilot study (2001–2002) and main study (2003–2006) of the German Environmental Survey on children (GerES IV), and the children subpopulation of the most recent National Health and Nutrition Examination Survey (NHANES), see Table 3. Although this comparison spans several sampling years (from 2001 to 2007), differs with respect to the age of the children (from 3 to 14 years), sample size (111 vs. 342 children) and number of metabolites investigated, some important findings can be drawn from this comparison. As expected, the pattern of exposure to DiBP and BBzP are different in the US population compared to the German population. MiBP seems to be of lesser importance in the US than in Germany, contrary to observations made for BBzP. Comparing the results of this study with the results from GerES IV (pilot study and main study) we observe a very similar pattern of exposure, with generally lower internal exposures in our study. Currently, we can only speculate that this difference might be due to the difference in sampling years (2001–2002, 2003–2006 vs. 2007, indicating a trend to lower exposures for these phthalates), or due to the short sampling window of this study (February to March vs. all over the year) or the specific location (Frankfurt vs. GerES population).

From the urinary metabolite levels, we calculated the daily intake of each phthalate for each child, see Table 4. Calculations were performed for only 108 of the 111 children, because data on either body weight or height was missing for three children. Again, as explained above, no specimens were excluded because of creatinine values lower than 0.3 mg/L. The highest daily intakes were found for DEHP (median 4.5 μg/kg bw/day; 95th percentile 18.0 μg/kg bw/day). Daily intakes for DnBP, DiBP und DiNP were lower, but within the same range (median between 1.9 and 2.4 μg/kg bw/day; 95th percentiles between 6.4 and 11.0 μg/kg bw/day). Daily intakes for BBzP und DiDP were considerably lower (median ca. 0.3 μg kg bw/day; 95th percentile between 1.2 and 2.6 μg kg bw/day).

The daily intake values calculated in this study are in very good accordance with daily intake values calculated from data from the pilot phase of GerES IV (Koch et al., 2007a; Wittassek et al., 2007). The difference in the daily intake of DnBP (a factor of 2) might be explained by the substitution of DnBP with DiBP that took place in the years between the two studies, an idea supported by our observation that DiBP intake in our study accounts for the drop in DnBP intake. In two of the 108 children, the exposure to DnBP exceeded the Tolerable Daily Intake (TDI) derived by the European Food

Table 2b
Phthalate metabolites levels (in μg/g creatinine) in spot urine samples of 111 children, between 5.6 and 6.7 years old (48 girls and 63 boys).

Phthalate	Metabolite	% >LOQ	GM	Mean (95% conf. interval)	Median	P95	Range
DnBP	MnBP	100	53.6	73.7 (61.9–85.5)	57.3	183	4.2–325
DiBP	MiBP	100	74.9	122 (88.9–156)	65.1	336	8.4–1405
BBzP	MBzP	86	8.7	26.0 (16.5–35.4)	10.1	88.1	<LOQ–344
DEHP	5OH-MEHP	100	29.2	41.2 (32.8–49.6)	28.0	104	4.5–290
	5oxo-MEHP	100	24.3	34.2 (27.8–40.6)	24.3	97.2	3.7–205
	5cx-MEPP	100	50.3	64.8 (54.0–75.6)	46.9	158	9.2–394
	2cx-MMHP	100	19.0	27.2 (20.9–33.5)	18.6	69.3	4.0–247
	MEHP	96	7.3	11.5 (8.4–14.6)	7.8	25.8	<LOQ–153
DiNP	OH-MiNP	96	9.0	15.0 (12.1–17.9)	10.8	41.9	<LOQ–115
	oxo-MiNP	78	3.8	8.1 (6.3–9.9)	5.8	27.6	<LOQ–60.0
	cx-MiNP	99	20.2	31.1 (23.8–38.5)	19.7	91.6	<LOQ–287
DiDP	OH-MiDP	60	0.8	1.6 (1.2–2.1)	0.6	5.7	<LOQ–12.9
	oxo-MiDP	30	<LOQ	0.5 (0.4–0.6)	<LOQ	1.6	<LOQ–4.5
	cx-MiDP	94	1.9	2.7 (2.2–3.2)	2.2	7.0	<LOQ–20.0

GM: geometric mean.
95P: 95th percentile.

Table 3
Phthalate metabolites levels from this study (in μg/L), compared to other human biomonitoring studies on children. NHANES data taken from CDC (2009); GerES IV pilot data taken from Becker et al. (2004); GerES IV data taken from Becker et al. (2009).

Phthalate	Study	NHANES		GerES IV pilot		GerES IV		This study	
	Year	2003/2004		2001–2002		2003–2006		2007	
	Age	6–11 years		3–14 years		3–5 years		5–6 years	
	Size	$n = 342$		$n = 254$		$n = 137$		$n = 111$	
	Metabolite	Median	P95	Median	P95	Median	P95	Median	P95
DnBP	MnBP	36.7	191	166	624	100	364	36.8	156
DiBP	MiBP	7.0	40.6	–	–	97.8	317	42.8	215
BBzP	MBzP	35.0	255	18.8	123	19.7	73.2	7.2	93.7
DEHP	5cx-MEPP	51.6	391	–	–	68.2	277	28.4	101
	5OH-MEHP	36.5	318	52.1	188	51.7	201	17.4	86.1
	5oxo-MEHP	25.8	197	41.4	139	37.9	146	15.1	71.0
	2cx-MMHP	–	–	–	–	22.8	79	11.3	46.1
	MEHP	2.7	27.6	7.2	29.7	4.6	27.5	4.7	21.1
DiNP	OH-MiNP	–	–	–	–	12.8	59.4	7.0	25.5
	oxo-MiNP	–	–	–	–	6.1	31.1	4.2	12.5
	cx-MiNP	–	–	–	–	18.2	76.4	13.1	45.5
DiDP	OH-MiDP	–	–	–	–	–	–	0.39	4.9
	oxo-MiDP	–	–	–	–	–	–	0.13	1.2
	cx-MiDP	–	–	–	–	–	–	1.3	4.5

Table 4
Daily phthalate intake (in μg/kg body-weight/day) calculated for each child and each phthalate separately. Daily intakes from this study are compared to daily intakes calculated from the GerES IV pilot study (Koch et al., 2007a; Wittassek et al., 2007) and tolerably daily intake values derived by EFSA (2005a, 2005b, 2005c, 2005d, 2005e).

Study	This study			GerES IV pilot			TDI [μg/kg/day]
Size (age)	108 (5–6)			239 (2–14)			EFSA
Year	2007			2001/2002			
Phthalate	Median	P95	Max	Median	P95	Max	
DnBP	1.9	6.4	11.2	4.1	14.9	76.4	10
DiBP	2.1	11.0	59.4	–	–	–	–
BBzP	0.3	2.6	10.4	0.4	2.6	13.9	500
DEHP	4.5	18.0	44.5	4.3	15.2	140	50
DiNP	2.4	9.5	31.2	–	–	–	150
DiDP	0.3	1.2	2.2	–	–	–	150

Safety Authority (EFSA) of 10 μg/kg bw/day. TDI values for other phthalates were not exceeded. The maximum exposure of one child to DEHP (44.5 μg/kg bw/day) came close to the European TDI of 50 μg/kg bw/day. Regarding DEHP, the Reference Dose (RfD) of the US EPA is 20 μg/kg bw/day. Five children (out of the 108) exceeded this RfD. A TDI value is not yet established for DiBP. Assuming that DiBP and DnBP were of similar efficacies (Howdeshell et al., 2008), seven children would have exceeded a putative TDI for DiBP of 10 μg/kg bw/day.

For DEHP, in the median 8.9% and in the 95th percentile, 36% of the TDI was used up by the children; for DnBP 19.1% and 63.9%, respectively; and for DiBP, 21.1% and 109%, respectively. Considering only the two phthalates, DnBP and DEHP (for which official TDI values are available in the EU) in the median 28.3% of the TDI_{cum} would have been used up, at the 95th percentile 102%. Seven out of the 108 children would have exceeded the TDI_{cum} for these two phthalates. Taken the data for all three phthalates together (DnBP, DEHP and DiBP), in the median 56.2% of the TDI_{cum} would have been used up, at the 95th percentile 183%. 26 out of the 108 children would have exceeded this relative TDI_{cum} for the three phthalates in question. This approach is illustrated in Fig. 1 and has to be seen as a first crude approach to determine the cumulative exposure and additive toxicity of phthalates. Once reliable TDI values and/or TDI values based on the same or similar toxicological end point are available, other endocrine active phthalates (like DiNP or BBzP), or other environmental contaminants (with anti-androgenic properties, like the fungicides vinclozoline, procymidone or prochloraz), can be included in calculating such a relative cumulative TDI.

Discussion

Data on phthalate exposure in young children are limited. In addition to pregnant women, young children, especially boys, belong to a subpopulation that should be regarded as the most susceptible to the adverse effects of phthalate exposure. In the present

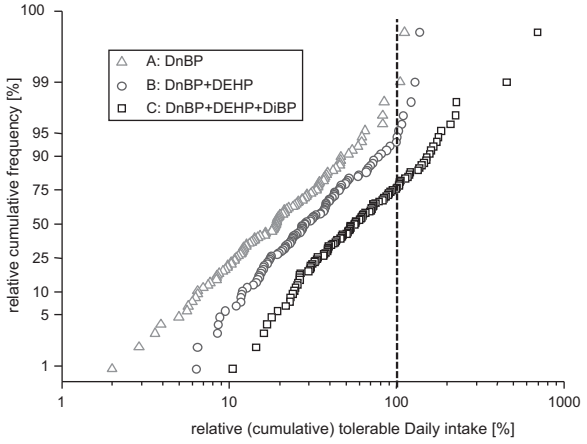

Fig. 1. Children exceeding the TDI for DnBP (scenario A) respectively the relative cumulative DI for the phthalates DnBP and DEHP (scenario B) and for the phthalates DnBP, DiBP and DEHP (scenario C). The dotted 100%-line illustrates the TDI respectively the relative cumulative TDI (TDI_{cum}) for the different scenarios. Two of the 108 children exceed the TDI for DnBP, 7 children exceed the TDI_{cum} for DnBP and DEHP and 26 children exceed the TDI_{cum} for DnBP, DEHP and DiBP.

study, we confirmed ubiquitous phthalate exposure in the selected study population. Almost every child was exposed to all six phthalates investigated. Furthermore, for the first time, we could prove the widespread exposure of children to DiDP. DiDP belongs to the group of phthalates that is strictly regulated with regard to its use in children's toys and child care articles. Highest internal exposures were measured for the phthalates DEHP, DiBP, DnBP and DiNP. Although the population of 5–6 years old primary school starters from the area of Frankfurt on Main cannot be regarded as representative for Germany, it provides an estimate of the current internal phthalate exposure situation of a not too small but well defined local study population without indications of special circumstances in terms of phthalate exposure.

Comparing the data of our study with data from populations originating in the USA, we found a different pattern of exposure to DiBP and BBzP. MiBP seems to be of lesser importance in the USA compared to Germany, a situation that is reversed for BBzP. These findings were previously confirmed in other studies, mainly in adult populations, which compared results from both countries (Koch and Calafat, 2009; Wittassek et al., 2011).

The exposure pattern shown here was very similar to GerES IV urine samples from the years 2001 to 2002 (pilot study) and from 2003 to 2006 (main study), with generally lower exposures in our study population, especially for DnBP. These findings seem to confirm a recent trend which suggests that DnBP exposure is declining whereas DiBP exposure is increasing. Similar findings can be noted for the high molecular weight phthalates DEHP, DiNP and DiDP. Although DEHP metabolites still play a major role in terms of internal exposure, metabolites of DiNP and DiDP significantly add to the body burden. Thus, this study confirms the finding of GerES and other population-based studies that the population is ubiquitously exposed to phthalates and high molecular weight phthalates, such as DiNP and DiDP have to be further investigated as major contributors of internal phthalate exposure, both in children and adults (Calafat et al., 2011; Koch and Calafat, 2009). The present study also shows that oxidised metabolites are ideal biomarkers of exposure for these high molecular weight phthalates.

Why exposures to most of the phthalates measured in this study are lower compared to GerES (2001/2 and 2003–2006) remains unclear. Most likely, the current study reflects the ongoing trend to substitute some of the phthalates deemed most critical with other phthalates or non-phthalate plasticisers deemed less critical, or that are not labeled as reproductive toxicants, or not restricted in use resulting in a reduction of exposure to the phthalates investigated in this study (Wittassek et al., 2007, 2011; Koch and Calafat, 2009).

Daily intake calculations based on human biomonitoring are currently regarded as the most reliable approach to quantify the overall exposure to a single phthalate and the sum of the most important phthalates (Wormuth et al., 2006; Koch and Calafat, 2009; Wittassek et al., 2011). Human biomonitoring determines the internal exposure to phthalates by measuring specific metabolites in urine. These metabolites are not prone to external contamination (a continuous problem when measuring the parent phthalates in ambient media). Furthermore, the urinary metabolites represent an integral measure of exposure from all possible sources and routes (inhalation, dermal, oral), known or unknown. Therefore, especially in the case of phthalates, human biomonitoring provides a new and alternative measure to determine integral exposure to phthalates and perform an integral and cumulative risk assessment of phthalates.

From the urinary metabolite levels, we calculated the daily intake of each phthalate for each child. The highest daily intakes were found for DEHP. Daily intakes for DnBP, DiBP und DiNP were lower. Daily intakes of BBzP and DiDP were considerably lower compared to the above four phthalates. The daily intake values calculated in this study are in very good accordance with daily intake values calculated from data of the pilot phase of GerES IV (Koch et al., 2007a; Wittassek et al., 2007). The decrease in the daily intake of DnBP might be explained by the substitution of DnBP with DiBP that took place in the years between the two studies. Consequently, it can be assumed that DiBP intake in our study accounts for the drop in DnBP intake.

The metabolite excretion factors (F_{UE}) used in this study to extrapolate from urinary metabolite levels to daily intakes have been generated in studies in adult humans, which at times arose from only one Caucasian male, 63 years old (Koch et al., 2005), or between six and seven volunteers (Anderson et al., 2001). F_{UE} values for children will probably never be generated because of ethical concerns. It is known that children exhibit a slightly different metabolite pattern compared to adults, i.e. oxidized metabolites were excreted in higher ratios compared to the simple monoester (Becker et al., 2004). However, the F_{UE} for DEHP, for example, reflects both the monoester and oxidized metabolites. For DEHP, the F_{UE} value used is 0.669 and is comprised by the 5 major DEHP metabolites. If children excreted a higher amount of these metabolites in urine, e.g. 80% instead of 66.9% we would overestimate the children's DEHP intake by 20%. If the children excreted only 50% of the DEHP dose as these metabolites in urine, we would underestimate the children's DEHP intake by 25%.

The mode of action of phthalates is well understood today, and there is general consensus among toxicologists that approaches need to be developed to evaluate the cumulative exposure to all of the endocrine active (anti-androgenic) phthalates. Dose addition models yielded results that concurred with actual dosing experiments of several phthalates simultaneously (Christiansen et al., 2009; Rider et al., 2008, 2009, 2010; Howdeshell et al., 2008). Taking into account these latest toxicological findings on phthalates, a consideration of each TDI for each individual phthalate would be misleading in terms of the overall tolerable phthalate intake. The ubiquitous and simultaneous exposure to a number of endocrine active phthalates and the knowledge that these phthalates act in a dose-additive manner warrants a cumulative risk assessment approach (Rider et al., 2010). The following steps provide an example of our basic cumulative risk assessment for phthalates: we calculated the daily intakes for each phthalate studied; related these intakes to the tolerable daily intake (TDI) values derived by EFSA; normalized each TDI to 100% (because the current TDIs derived by EFSA differ between the phthalates); added up the percentages of the daily intakes calculated in relation to the respective TDIs; and checked whether the cumulative TDI (at 100%) was exceeded. Despite its simplistic approach, the method may be used in further studies when new or more reliable TDI data are available. We found that TDI values can be exceeded (2 out of the 108 children) already for a single phthalate (DnBP). Using the cumulative approach which totalled the exposures to the three phthalates, DnBP, DEHP and DiBP, a TDI_{cum} exceedance was found in 25% of the study population. These findings warrant immediate further investigation. Future studies should include exposure to other endocrine active phthalates like DiNP and BBzP, as soon as TDIs are available that are based on the same end-point or mode of action and therefore are applicable for dose addition approaches. Currently the TDI for DiNP is based on liver toxicity and therefore cannot be implemented in our cumulative TDI approach. Also, other known endocrine disrupters/anti-androgens might need to be included in future cumulative exposure assessments, such as possible substitutes of the phthalates or other environmental chemicals of anti androgenic potential (NRC, 2008; Kortenkamp and Faust, 2010; Rider et al., 2010).

Conclusion

The extent of exposure of the general population (including children) to phthalates has been documented over the last decade in numerous publications, both in the USA and Europe. The calculation of daily intakes based on Human Biomonitoring data, and comparing these daily intakes to Tolerable Daily Intakes (TDIs) and Reference Doses (RfDs), respectively, has also been previously performed by us and others and is a well accepted approach. Our goal is to proceed in this direction, by considering cumulative exposures, even if significant knowledge gaps currently exist, including limited amounts of appropriate in vivo or human toxicity data on a large number of suspected endocrine disrupters and their cumulative action. The NRC (NRC, 2008) described the pressing need to evaluate the cumulative exposure to phthalates (and other endocrine disrupters), but did not take the final step to actually perform such an evaluation. We chose a simplistic approach to assess the potential for health effects by comparing the calculated daily intakes to TDIs and introducing the concept of the TDI_{cum} as a cumulative health benchmark for some phthalates (and possibly other anti-androgenic environmental chemicals with the same mode of action). We are well aware that our TDI_{cum} approach, as well as the Hazard Index (HI) approach by Kortenkamp and Faust (2010), serves only as an example to point out the extent of a cumulative exposure to chemicals with similar modes of action. Nonetheless, we have shown for the three phthalates – as Kortenkamp and Faust have shown for some phthalates including other environmental chemicals – that the cumulative exposure to manmade anti-androgenic chemicals exceeds acceptable and tolerable levels, respectively, in the upper exposure percentiles of the general population, including children. The relevance of these findings has to be evaluated in more detail, but steps to further reduce exposure to phthalates and other (endocrine active) environmental contaminants are required.

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