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Internal exposure of young German adults to di(2-propylheptyl) phthalate (DPHP): Trends in 24-h urine samples from the German Environmental Specimen Bank 1999–2017

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A R T I C L E I N F O

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A B S T R A C T

Di(2-propylheptyl) phthalate (DPHP) is used as a substitute for high molecular weight phthalates like di(2-ethylhexyl) phthalate (DEHP) which were subjected to authorization under REACh in 2015. An earlier study on the time trend of exposure in human 24-h urine samples from the German Environmental Specimen Bank has revealed that metabolites of DPHP emerged in 2009 and 2012 (Schütze et al., 2015).

In order to better assess a potential trend and the present state of exposure to DPHP, we now measured 180 urine samples from the German Environmental Specimen Bank, 60 per year, collected in 2011, 2014 and 2017, randomized and blinded before analysis. Together with the previously analyzed samples, data for a total of 480 samples covering 19 years from 1999 to 2017 was thus generated. We were able to show that DPHP exposure of the studied population, university students from Münster (Northwestern Germany), has remained essentially constant since 2011, after a rapid increase starting around 2009. Even so, urinary metabolite concentrations were always in the low ppb or sub-ppb range, indicating that DPHP exposure of the general population is substantially lower than for other modern plasticizers, and far below levels currently regarded as critical.

DPHP is a plasticizer which is mostly used in non-sensitive applications with little probability of close contact to humans. Still, we observed how temporal trends of DPHP exposure largely follow trends of DPHP consumption in the Western European market. Our results hence demonstrate the potential of biomonitoring to sensitively detect the effects of industrial product strategy on the environment, even when biomarkers are present only at trace level.

1. Introduction

Di(2-propylheptyl) phthalate (DPHP, also known as bis(2-propylheptyl) phthalate) was developed as an alternative plasticizer to replace other phthalates such as di(2-ethylhexyl) phthalate (DEHP), di-*iso*-nonyl phthalate (DINP) and di-*iso*-decyl phthalate (DIDP). It is intended for use in polyvinyl chloride (PVC) and in vinyl chloride copolymers. Areas of application include tarpaulins, roofing membranes, wire and cable coatings, and automotive interiors. The Western European DPHP consumption has experienced significant growth since the beginning of the century, increasing from 29,000 metric tons per year (t/a) in 2003 to 170,000 t/a in 2014. In the past few years, however, consumption figures seem to have stabilized and only very moderate growth to around 179,000 t/a is forecasted until 2019 (Malveda et al., 2015).

In general, phthalates are susceptible to evaporation or leaching out of the polymeric end-use products in which they are contained. This is true for DPHP as well, though most probably to a lesser degree than for many other phthalates: DPHP is specifically marketed as a very durable plasticizer with low volatility (BASF, 2017; Perstorp, 2018). Nevertheless, the typical DPHP concentration in end-use products is as high as 30–60% (NICNAS, 2003). Hence, exposure of the general population to DPHP may be expected, for instance via plasticizer vapors released into car interiors, via dust abrasion from flexible PVC articles, or via skin contact with such products. Indeed, several studies have recently dealt with human biomonitoring of DPHP metabolites in urine or nails

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Abbreviations

BBzP	Butyl benzyl phthalate
cx-MPHxP	Mono(2-propyl-6-carboxyhexyl) phthalate
bw	body weight
DEHP	Di(2-ethylhexyl) phthalate
DiBP	Di-*iso*-butyl phthalate
DIDP	Di-*iso*-decyl phthalate
DINCH	Di-*iso*-nonyl cyclohexane-1,2-dicarboxylate
DINP	Di-*iso*-nonyl phthalate
DnBP	Di-*n*-butyl phthalate
DPHP	Di(2-propylheptyl) phthalate
ESB	Environmental Specimen Bank
HBM	Human biomonitoring
MPHP	Mono(2-propylheptyl) phthalate
OH-MPHP	Mono(2-propyl-6-hydroxyheptyl) phthalate
oxo-MPHP	Mono(2-propyl-6-oxoheptyl) phthalate
RfD	Reference dose
TDI	Tolerable daily intake

(Leng et al., 2014; Schütze et al., 2015; Giovanoulis et al., 2016; Alves et al., 2017) and with various pathways for exposure to DPHP (Giovanoulis et al., 2018).

As with other long-chain phthalate esters, metabolism of DPHP proceeds via hydrolysis to the monoester followed by oxidation of the remaining side chain (alcohol moiety) and glucuronidation of the products (Wittassek and Angerer, 2008; Leng et al., 2014; Klein et al., 2016). Further cleavage of the monoester to yield phthalic acid may also be expected for DPHP (see literature for similar phthalates, e. g. Albro et al., 1973; Thomas et al., 1984). However, phthalic acid is not relevant for analytical purposes since it lacks specificity – it is a potential metabolite of virtually any phthalate ester. Analytical DPHP biomonitoring methods mostly focus on the oxidized metabolites mono (2-propyl-6-hydroxyheptyl) phthalate (OH-MPHP), mono(2-propyl-6-carboxyhexyl) phthalate (cx-MPHxP) and mono(2-propyl-6-oxoheptyl) phthalate (oxo-MPHP). The primary metabolite mono(2-propylheptyl) phthalate (MPHP) has been examined by some researchers as well, but it was seldom found in urine samples (Wittassek and Angerer, 2008; Giovanoulis et al., 2016; Alves et al., 2017).

One of the challenging issues in DPHP bioanalysis is selectivity between DPHP and DIDP metabolites. In fact, DPHP and DIDP are isomers, and their metabolites produce isobaric ions in mass spectrometry. While technical grade DPHP itself is basically a mixture of 3 isomers, the main isomer accounting for approximately 81% of the product, DIDP is a much more complex mixture, as was discussed in detail elsewhere (EC, 2003; NICNAS, 2003; BfR, 2011). To solve this issue a gas chromatography – high resolution mass spectrometry (GC-HRMS) method for the selective determination of DPHP metabolites at trace level in human urine, separating them from DIDP markers, was developed by Gries et al. (2012).

The development of the analytical method and its application are part of a cooperation between the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) and the Verband der Chemischen Industrie e.V. (German Chemical Industry Association – VCI) (Kolossa-Gehring et al., 2017) aiming at the advancement of human biomonitoring. Its goal is to develop new HBM methods for chemicals which might expose the general population and/or have an impact on health. It started in 2010 and is managed by the German Environment Agency (UBA). Experts from scientific agencies in charge of chemical regulation, industry and science accompany the project in selecting substances and developing methods. DPHP has been one of the first substances within this BMU-VCI cooperation to go full circle from analytical method development and investigation of human metabolism to actual measurement in a population study.

The new method was subsequently applied by Schütze et al. (2015) for the analysis of 300 urine samples (24-h voids) from the German Environmental Specimen Bank (ESB). The ESB is, in addition to the German Environmental Survey GerES, one of the two Federal monitoring instruments in Germany to investigate the exposure of people living in Germany and its development over time (Kolossa-Gehring et al., 2012).

The study by Schütze et al. (2015) covered the years 1999, 2003, 2006, 2009, and 2012. DPHP metabolites were not detected in any of the samples up to 2006. Thereafter, an increasing trend was observed. Detection rates reached values of 3.3% of the samples in 2009 and 21.7% in 2012, mirroring increased DPHP use in the PVC industry. Most of those samples were positive for oxo-MPHP (up to 0.96 µg/L urine), and a few samples contained OH-MPHP at levels up to 0.64 µg/L urine. On the other hand, cx-MPHxP remained undetectable over the entire period. From these metabolite concentrations it was derived that the highest daily DPHP intake had amounted to 0.314 µg/kg bw for one of the urine donors. While this value remained far below the DPHP daily oral reference dose (RfD) of 0.1 mg/kg bw (Bhat et al., 2014) and the tolerable daily intake (TDI) of 0.2 mg/kg bw (UBA, 2015), the increasing detection rate of DPHP metabolites in the years 2009 and 2012 warranted additional investigations in order to conceive a clearer picture of DPHP exposure and internal body burden for the general German population.

Therefore, we have now analyzed a further 180 urine samples from the ESB, representing the years 2011, 2014, and 2017. Samples were selected and measured in the same way as for the previous studies (Schütze et al., 2015). Samples covering 2011 to 2017 are compared with those for the years 1999–2012 and conclusions are drawn regarding the current state of DPHP levels in the non-occupationally exposed general population. Additionally, the relevance of the different DPHP metabolites for human biomonitoring is re-assessed from an analytical chemistry point of view.

2. Material and methods

2.1. Analytical method

Quantitative determination of cx-MPHxP, OH-MPHP and oxo-MPHP in urine samples was performed according to published protocols (Gries et al., 2012; Schütze et al., 2015) and will be briefly summarized in the following. After adding deuterated internal standards (d_4-ring-deuterated cx-MPHxP, OH-MPHP and oxo-MPHP, IDM - Institut für Dünnschichttechnologie und Mikrosensorik e.V., Teltow, Germany), urine samples were enzymatically hydrolyzed in order to liberate glucuronidated DPHP metabolites. The samples were then extracted by liquid-liquid extraction and DPHP metabolites were derivatized to yield 1,1,1,3,3,3-hexafluoroisopropyl esters. After another extraction step, the resulting solutions were analyzed by capillary GC (7890A, Agilent, Waldbronn, Germany) coupled to HRMS (AutoSpec Premier, Micromass/Waters, Manchester, UK) in the negative chemical ionization mode. Separation was accomplished on a 60 m column with a medium polarity phase (phenylmethylpolysiloxane, Optima 17, 60 m, 0.25 mm, 0.25 µm, Macherey-Nagel, Düren, Germany). The limits of quantification (LOQ) were 0.15 µg/L for cx-MPHxP, 0.3 µg/L for OH-MPHP and 0.25 µg/L for oxo-MPHP (concentrations in urine). The reliability of quantitative data was validated by calculation of metabolite ratios in all samples with at least two DPHP metabolites above LOQ (in practice, this was only the case for samples containing OH-MPHP; these samples also exhibited oxo-MPHP values above the LOQ).

2.2. Study population

24-h urine samples were provided by the ESB. The ESB performs standardized sampling of 24-h urine, whole blood and blood plasma at four Universities in Germany on a yearly basis. The sampling procedure has been described before in detail (Lermen et al., 2014). Briefly, volunteers receive a container for collecting 24-h urine in combination with information material describing the standard collection procedure. The filled containers are subsequently collected at the mobile laboratory, where 24-h volume and urinary creatinine is measured. Aliquots are generated on site and stored over liquid nitrogen. The study protocol of the ESB has been reviewed and approved by the ethics committee of the Medical Association Westfalen-Lippe and the Medical Faculty of the University of Münster. Since 2012 it has been approved by the ethics committee of the Medical Association Saarland.

For this study cryo-preserved 24-h urine samples of 180 volunteers from Münster (Northwestern Germany) were provided for the years 2011, 2014 and 2017 (60 volunteers per year in equal sex ratio). A description of the sample composition in combination with the data from Schütze et al. (2015) is shown in Table 1.

Before delivery of the samples to Currenta, where all analytical work was performed, they were randomized and blinded by the ESB.

2.3. Statistical analysis

Microsoft Excel 2010 was employed for statistical analysis of urinary metabolite concentrations. All calculations were carried out as described previously (Schütze et al., 2015).

3. Results

DPHP metabolites were detected at or above LOQ in samples from all 3 years under scrutiny (2011, 2014, 2017). OH-MPHP and oxo-MPHP were quantifiable in 1.7%–3.3% and 15.0%–18.3% of the samples, respectively; whereas cx-MPHxP was consistently below the LOQ. The 95th percentiles for cx-MPHxP and OH-MPHP were below the LOQ for all years as well, while the 95th percentile for oxo-MPHP was always slightly above the LOQ. The maximum OH-MPHP value was recorded for one sample from the year 2014 (0.46 µg/L), while the concentration in a further sample was only marginally lower (0.45 µg/L, 2011). For oxo-MPHP, a maximum value of 1.45 µg/L was observed, and the second highest value was at 1.02 µg/L. Both of these samples were from 2017. A summary for the years 1999, 2003, 2006, 2009, 2011, 2012, 2014 and 2017 can be found in Table 2 (results for 1999–2009 and 2012 cited from Schütze et al., 2015).

It should be noted that while these quantitative results can be regarded as highly reliable for both cx-MPHxP and oxo-MPHP, the measurement uncertainty is significantly higher for OH-MPHP. In most samples, the determination of cx-MPHxP and oxo-MPHP was largely unaffected by any DIDP/DINP metabolite peaks. In those instances where significant amounts of isomeric DIDP/DINP metabolites did elute in the retention time range of cx-MPHxP and oxo-MPHP, chromatographic separation was adequate for confident identification and quantification of both cx-MPHxP and oxo-MPHP. The situation was, however, different for OH-MPHP. In general, a peak eluting just before OH-MPHP overlapped with the OH-MPHP signal, impeding accurate quantification at levels near the method LOQ. In most cases, a perpendicular drop integration was acceptable (at the expense of a higher measurement uncertainty, as pointed out above), but for 1 sample out of the total of 180, the DIDP/DINP background burden was so pronounced that meaningful results for OH-MPHP content could not be obtained. In view of these separation issues, the metabolite ratio between oxo-MPHP and OH-MPHP was calculated for those samples which were above LOQ for OH-MPHP, in order to examine the plausibility of the OH-MPHP results (see also “Relevance of individual DPHP metabolites for human biomonitoring” in the Discussion): the ratio oxo-MPHP/OH-MPHP (concentrations in µg/L) was in the range of 0.89–2.18 (average: 1.40, standard deviation: 0.48 or 34.5% relative; these figures include raw data from the paper by Schütze et al., 2015).

4. Discussion

4.1. Trends and current state of DPHP exposure

Although isolated samples in the present study exhibit higher oxo-MPHP concentrations than any previously measured ESB samples (Schütze et al., 2015), overall a further recent increase of DPHP body burdens in the study population cannot be deduced from our data. Detection rates for all metabolites are essentially similar for the years 2011, 2012, 2014, and 2017. The 95th percentiles and the maximum values also remain in the same orders of magnitude during these years. It seems a “plateau” of DPHP exposure has already been reached by now, after a relatively short period of increasing values around the year 2009, when DPHP metabolites first emerged in human urine samples from the ESB. This observation excellently matches DPHP market trends in Western Europe during those years. Between 2003 and 2007, consumption of this plasticizer grew rather moderately, before a slight decline up to 2009 during the economic crisis. A very sharp increase took place in 2010, followed by more modest growth in subsequent years and stabilization around 2013/2014 (data from Malveda et al., 2015, including mainly Germany, the Netherlands, Italy, the UK, France, Belgium, Sweden, Spain, and Denmark). Western European consumption of DPHP on the one hand and oxo-MPHP detection rates in urine on the other hand are graphically depicted in Fig. 1, illustrating

Table 1
Description of sample composition describing number of subjects, age, body mass index (BMI), urinary creatinine and 24-h urine volume.

Sampling year[a]	Subjects (male/female)	Age [years] AM (range)	BMI [kg/m^2] AM (range)	Urinary creatinine [mg/dL] AM (range)	24-h urine volume [mL] AM (range)
1999	60 (30/30)	24.3 (21–29)	22.9 (17.0–52.3)	104.86 (27.54–265.58)	1595 (550–4000)
2003	60 (30/30)	23.0 (20–28)	22.4 (17.6–34.3)	110.43 (23.00–341.00)	1757 (410–3500)
2006	60 (30/30)	24.0 (20–29)	22.1 (17.9–31.7)	88.92 (28.96–210.77)	1897 (719–4250)
2009	60 (30/30)	23.2 (20–28)	22.1 (17.9–34.1)	83.16 (27.06–218.81)	2074 (540–3660)
2011	60 (30/30)	23.3 (20–29)	22.9 (18.0–30.1)	86.85 (28.24–219.68)	1881 (393–2957)
2012	60 (30/30)	24.2 (20–30)	22.5 (18.3–30.5)	70.29 (20.45–218.09)	2108 (574–3027)
2014	60 (30/30)	23.0 (20–29)	21.9 (17.4–33.7)	70.76 (18.89–180.38)	1949 (264–3056)
2017	60 (30/30)	23.7 (20–29)	22.0 (17.4–33.7)	68.59 (17.81–164.77)	2157 (561–3211)
total	480	23.6 (20–30)	22.4 (17.0–52.3)	85.48 (17.81–341.00)	1927 (264–4250)
male	240	23.9 (20–30)	23.4 (17.5–52.3)	98.53 (18.89–341.00)	1973 (264–4000)
female	240	23.3 (20–30)	21.3 (17.0–34.3)	72.43 (17.81–223.67)	1881 (393–4250)

AM, arithmetic mean.
BMI, body mass index.
[a] Data for 1999, 2003, 2006, 2009 and 2012 cited from Schütze et al. (2015).

Table 2
DPHP metabolite concentrations in µg/L and detection rates ($n = 60$ samples each year, except for OH-MPHP which could not be analyzed in 1 sample from 2017, see text).

Year[a]	cx-MPHxP[b]				OH-MPHP[c]				oxo-MPHP[d]			
	% ≥ LOQ	P.50	P.95	Range	% ≥ LOQ	P.50	P.95	Range	% ≥ LOQ	P.50	P.95	Range
1999	0	< LOQ	< LOQ	< LOQ– < LOQ	0	< LOQ	< LOQ	< LOQ– < LOQ	0	< LOQ	< LOQ	< LOQ– < LOQ
2003	0	< LOQ	< LOQ	< LOQ– < LOQ	0	< LOQ	< LOQ	< LOQ– < LOQ	0	< LOQ	< LOQ	< LOQ– < LOQ
2006	0	< LOQ	< LOQ	< LOQ– < LOQ	0	< LOQ	< LOQ	< LOQ– < LOQ	0	< LOQ	< LOQ	< LOQ– < LOQ
2009	0	< LOQ	< LOQ	< LOQ– < LOQ	3.3	< LOQ	< LOQ	< LOQ–0.64	3.3	< LOQ	< LOQ	< LOQ–0.96
2011	0	< LOQ	< LOQ	< LOQ– < LOQ	1.7	< LOQ	< LOQ	< LOQ–0.45	18.3	< LOQ	0.47	< LOQ–0.69
2012	0	< LOQ	< LOQ	< LOQ– < LOQ	3.3	< LOQ	< LOQ	< LOQ–0.36	21.7	< LOQ	0.45	< LOQ–0.65
2014	0	< LOQ	< LOQ	< LOQ– < LOQ	3.3	< LOQ	< LOQ	< LOQ–0.46	15.0	< LOQ	0.40	< LOQ–0.81
2017	0	< LOQ	< LOQ	< LOQ– < LOQ	1.7	< LOQ	< LOQ	< LOQ–0.32	18.3	< LOQ	0.42	< LOQ–1.45

[a] Data for 1999, 2003, 2006, 2009 and 2012 cited from Schütze et al. (2015).

[b] LOQ for cx-MPHxP: 0.15 µg/L.

[c] LOQ for OH-MPHP: 0.3 µg/L.

[d] LOQ for oxo-MPHP: 0.25 µg/L.

the temporal, almost quantitative relation between these two parameters. The detection frequency of oxo-MPHP is clearly the best indicator of the general magnitude of DPHP exposure. OH-MPHP detection rate, OH-MPHP maximum values, oxo-MPHP maximum values and oxo-MPHP 95th percentiles (Table 2) also seem to reflect DPHP consumption, albeit with high uncertainty. In the case of OH-MPHP, these weaker effects are mainly caused by an increased measurement uncertainty, as stated in the Results section (a further discussion follows, see “Relevance of individual DPHP metabolites for human biomonitoring”). With a sample size of about 60 individuals per year, no uncertainty estimates at hand (e.g. confidence intervals), and at best one fifth of values equal to or larger than LOQ per year, statistical measures should be interpreted with caution. Nevertheless, maximum values are an indication for extremely highly exposed individuals; the 95th percentile is important because it suggests a threshold to high exposure. In addition, to obtain an improved understanding of the mediocre exposure trend, the median which is robust towards outliers should be also reported in future studies if possible. Yet, since metabolite levels were so low in the ESB urine samples, medians were always below the method LOQ, rendering this parameter useless for statistical analysis under these circumstances.

The exposure levels and frequencies we determined for this study population (students from the University of Münster in Northwestern Germany) is also in quite good agreement with biomonitoring results from other (smaller) populations both from Germany and Norway. Indeed, spot urine samples of 40 Currenta employees without occupational exposure to DPHP that were collected in May 2011 (Gries et al., 2012) showed similar metabolite concentrations: up to 0.93 µg/L for oxo-MPHP (detection rate 38%) and 0.51 µg/L for OH-MPHP (detection rate 8%). Significantly higher detection rates especially for OH-MPHP (39%; oxo-MPHP: 33%) and somewhat higher maximum concentrations (OH-MPHP: max. 3.81 µg/L, oxo-MPHP: max. 3.27 µg/L, cx-MPHxP: always below LOQ) were reported in a GerES V pre-study (n = 51; collected in 2013) by Leng and Gries (2017). This is in line with the observation of three-to five-fold higher exposure levels for plasticizers like phthalates in children compared to young adults (Becker et al., 2009). This relation indicates a higher risk for plasticizer exposures in early life. Giovanoulis et al. (2016) examined a Norwegian

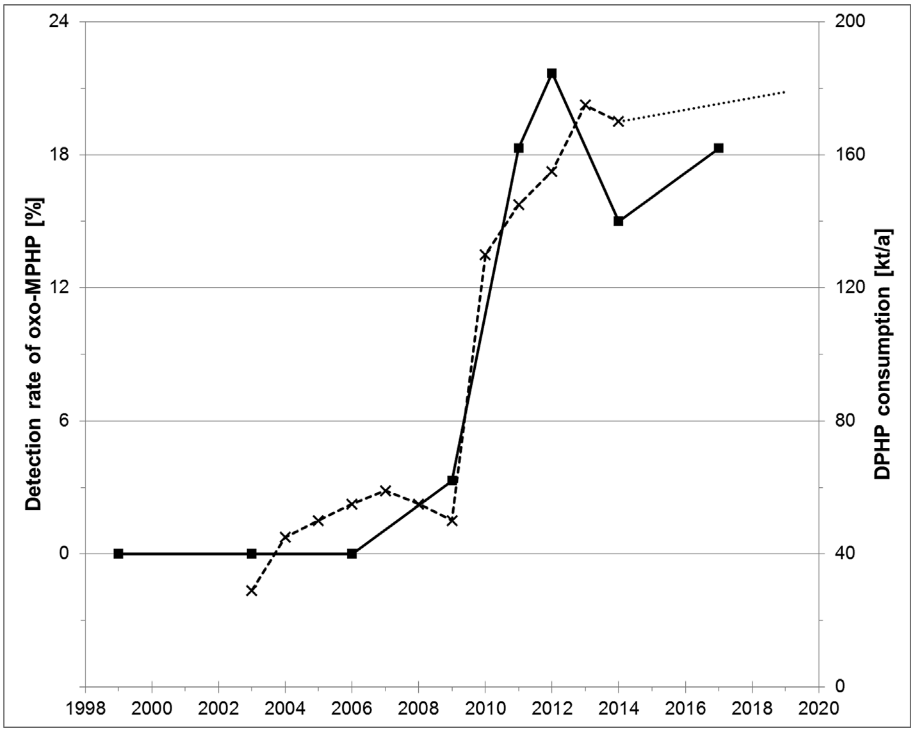

Fig. 1. Detection rate of oxo-MPHP in ESB urine samples (squares with solid line) and DPHP consumption in Western Europe (crosses with dashed line; after 2014: forecast, dotted line). Lines denote linear interpolation between data points. DPHP consumption prior to 2003 is unknown. DPHP consumption cited from Malveda et al. (2015) – note that DPHP consumption numbers from 2010 onwards are updated values, which explains the discrepancy for 2012 between this illustration and Fig. 1 in the paper by Schütze et al. (2015).

population and found the DPHP metabolite MPHP in 3% of afternoon urine spots and 37% of nail samples, while MPHP was not detected in any morning urine spots. Alves et al. (2017) detected neither MPHP nor OH-MPHP in urine or nail samples from Norway. While the latter two articles do indicate low abundances of DPHP metabolites in urine samples, possibly corroborating our results, comparability with our measurements is limited, mainly due to differences in analytical methodologies and way of sampling (e.g., LC-MS instead of GC-MS, MPHP/OH-MPHP instead of cx-MPHxP/OH-MPHP/oxo-MPHP, nails as an alternative body compartment and sample matrix).

Schütze et al. (2015) hypothesized that the relatively low DPHP body burdens inferred from urine analysis might reflect the distance of DPHP from consumers. This is confirmed by our current measurements. Other effects may also contribute to low DPHP body burdens. In particular, it is known that high molecular mass plasticizers migrate less than their low molecular weight counterparts (Eckert et al., 2016). Thus, leaching during contact with plasticized products might be less pronounced for DPHP than for other, smaller phthalates. Human Biomonitoring assessment values (HBM values) for DPHP were derived by the German Human Biomonitoring Commission (UBA, 2015; Apel et al., 2017) as sum of the metabolites OH-MPHP and oxo-MPHP and were set for children and adults (1 mg/L and 1.5 mg/L respectively). All in all, we may conclude that DPHP exposure of the population under scrutiny has not significantly increased during the second decade of the 21st century, that it remains far below critical levels, and that it is much lower than exposure to other modern plasticizers such as DINCH (see Schütze et al., 2015). Future human biomonitoring investigations might therefore focus on subpopulations that would be expected to be more extensively exposed to DPHP, or especially sensitive to potential yet unknown health hazards caused by DPHP.

Most importantly children and babies constitute a subpopulation which is particularly sensitive and, moreover, susceptible to additional exposure routes (Becker et al., 2009). Additionally, contact with DPHP may be expected, in particular, for persons who are occupationally exposed to this plasticizer. In the European Union, DPHP is not banned from use in toys and childcare articles, unlike a number of other phthalates (EP, 2006; ECHA, 2015), but manufacturers explicitly advise against using DPHP in such products. They recommend alternative, non-phthalate plasticizers for this kind of applications. In spite of these recommendations, DPHP has in the past been found in toys at levels between 10.1% and 48.2% by weight. A worst-case DPHP daily intake of up to 135 μg/kg bw was derived from migration experiments with these toys (BfR, 2011). This is in the range of published RfD and TDI values (Bhat et al., 2014; UBA, 2015), and several orders of magnitude higher than intakes calculated from the urine samples in our investigations (cf. Schütze et al., 2015). Taking this into account, human biomonitoring surveys involving toddlers might seem indicated to ascertain possible exposure levels. However, the BfR publication dates back to 2011 and its findings might not be up to date any more, due to shifts in the toy market. Indeed, a more recent survey (McCombie et al., 2017) has revealed that nowadays, DPHP is rarely used in toys, as it was found only in 4 out of 118 samples from 88 toys. This study was limited to toys from the Swiss market, but we may anticipate it is quite representative for many European countries, whereas conclusions for other parts of the world are perhaps less obvious.

4.2. Relevance of individual DPHP metabolites for human biomonitoring

From an analytical chemistry perspective, oxo-MPHP is certainly the most valuable diagnostic metabolite to detect low-level DPHP exposure via urine biomonitoring by GC-HRMS. As a matter of fact, Gries et al. (2012) already observed during method development that cx-MPHxP and oxo-MPHP were chromatographically well separated from any interfering DIDP or DINP metabolites. On the other hand, they reported that separation of OH-MPHP from such a DIDP/DINP background was only incomplete, possibly leading to interference by isobaric ions at the retention time of OH-MPHP. Even high resolution mass spectrometry cannot be expected to resolve this type of interferences.

The samples which we now analyzed fully confirmed these observations. The method LOQ of 0.3 μg/L for OH-MPHP is realistically attainable in real samples only as long as interference from DIDP/DINP metabolites remains relatively low. The value of OH-MPHP as a biomarker is further reduced by the fact that hardly any samples were positive for OH-MPHP while being negative for oxo-MPHP, which is entirely consistent with current knowledge about DPHP metabolism. The opposite situation, with oxo-MPHP above the LOQ but OH-MPHP below LOQ, arose frequently. Still, the selectivity problem encountered with OH-MPHP can be partly alleviated by calculation of the ratio between OH-MPHP and oxo-MPHP and comparison with data from metabolism studies. As stated in the Results section, the oxo-MPHP/OH-MPHP concentration ratio was in the range of 0.89–2.18 for those samples in which OH-MPHP was detectable. In a human metabolism study Leng et al. (2014) determined the molar urinary excretion fractions for five volunteers who had been orally dosed with DPHP. For a time frame of up to 48 h after ingestion of ring-deuterated DPHP, they reported the following molar fractions in % of oral dose (mean ± standard deviation): OH-MPHP-d_4 (326.4 g/mol), 10.70 ± 3.61; oxo-MPHP-d_4 (324.4 g/mol), 13.52 ± 4.04. Our oxo-/OH-MPHP ratios measured in the ESB samples are quite consistent with these metabolic data, considering that all our OH-MPHP values were only slightly above the LOQ, that substantial interference from DIDP/DINP metabolites did occur at this trace level, and that the available human metabolism data is rather limited (obtained from 5 male individuals only). Our OH-MPHP results are therefore plausible, even though more exact quantification might seem desirable in this case.

Finally, cx-MPHxP was not found in any of the ESB samples under investigation, neither in the present study nor in the previous work by Schütze et al. (2015). As cx-MPHxP is only a minor metabolite of DPHP (Wittassek and Angerer, 2008; Leng et al., 2014), and overall metabolite concentrations were low, this is very plausible.

Hence we may expect that monitoring of oxo-MPHP will usually suffice to confidently determine DPHP exposure in the general population. Nevertheless, at higher exposures closer to the TDI (and therefore more relevant from a toxicological point of view), OH-MPHP quantification will be much less hampered by DIDP/DINP backgrounds. It would thus constitute a perfectly adequate biomarker at such levels, for example near the HBM-I value of 1 mg/L morning urine for children or 1.5 mg/L morning urine for adults (sum of oxo-MPHP and OH-MPHP, see UBA, 2015). Such scenarios are perhaps conceivable for persons who are occupationally exposed to DPHP.

5. Conclusions

Our results enable us to answer open questions that arose in previous research (Schütze et al., 2015): We may now assume that DPHP intakes of the general German population did not further increase in the past few years. Because DPHP exposure continues to be considerably below critical levels such as HBM-I values, TDI and RfD, risk management steps are not of priority at the present time. However, toxicological data for DPHP is yet less complete than for many other plasticizers, and future, more thorough insights in this field could warrant a new hazard assessment. The results from HBM studies like GerES V (field work 2014 to 2017, results in preparation) including more vulnerable or more extensively exposed subpopulations such as small children will help elucidating higher exposed subgroups and exposure pathways.

While inter alia the phthalates DEHP, DnBP, DiBP, and BBzP are regulated through REACH and their levels in urine were shown to decline (Koch et al., 2017) over the past years, usage of substitutes like DINCH and DPHP has become more important. Monitoring of the development of the trends of exposure with these substitutes will continue to be of great importance for years to come. Especially the higher exposure levels found in children compared to adults require further human biomonitoring activities for plasticizers, including regulated compounds, their substitutes, and co-exposures.

The comparison of our human biomonitoring data with DPHP industrial consumption trends is of scientific interest. Depending on the choice of biomarker and its statistical parameters, qualitative or even nearly quantitative relation between amounts of DPHP consumption and the occurrence of the particular urinary biomarker was found, as discussed above. Similar correlations have been observed for other chemicals in the past, including plasticizers like DEHP (Helm, 2007) and di-*iso*-nonyl cyclohexane-1,2-dicarboxylate (DINCH; Schütze et al., 2014). The principal suitability of human biomonitoring to detect industrial product strategy and its influence on the environment is thus well-documented in the literature. Yet, human DPHP exposure is far lower than DEHP and DINCH exposures. Indeed, DEHP has been widely used for decades and is known to be ubiquitous. DINCH, on the other hand, is a much newer product, but since it is regarded as a safe substitute for some toxic phthalates, it is often used in sensitive applications, close to the consumers, such as food contact materials, medical devices or toys. Accordingly, daily intake values for DINCH and DEHP are typically 1 or 2 orders of magnitude higher than for DPHP (Helm, 2007; Schütze et al., 2014, 2015), which obviously also implicates higher DEHP/DINCH metabolite concentrations in urine, as compared with DPHP metabolites. Our results strikingly demonstrate how human biomonitoring can contribute to an understanding of human exposure to xenobiotics, even in scenarios where human contact with relevant products is scarce and body burden with these substances is accordingly low.

Conflicts of interest

None.

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