Single ingestion of di-(2-propylheptyl) phthalate (DPHP) by male volunteers: DPHP in blood and its metabolites in blood and urine

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1. Introduction

Di-(2-propylheptyl) phthalate (DPHP) is used as a plasticizer for polyvinyl chloride products. A tolerable daily intake of DPHP of 0.2 mg/kg body weight has been derived from rat data. Because toxicokinetic data of DPHP in humans were not available, it was the aim of the present work to monitor DPHP and selected metabolites in blood and urine of 6 male volunteers following ingestion of a single DPHP dose (0.7 mg/kg body weight). Concentration-time courses in blood were obtained up to 24 h for DPHP, mono-(2-propylheptyl) phthalate (MPHP), mono-(2-propyl-6-hydroxyheptyl) phthalate (OH-MPHP), and mono-(2-propyl-6-oxoheptyl) phthalate (oxo-MPHP); amounts excreted in urine were determined up to 46 h for MPHP, OH-MPHP, oxo-MPHP, and mono-(2-propyl-6-carboxyhexyl) phthalate (cx-MPHP). All curves were characterized by an invasion and an elimination phase the kinetic parameters of which were determined together with the areas under the concentration-time curves in blood (AUCs). AUCs were: DPHP > MPHP > oxo-MPHP > OH-MPHP. The amounts excreted in urine were: oxo-MPHP > OH-MPHP > > cx-MPHP > MPHP. The AUCs of MPHP, oxo-MPHP, or OH-MPHP could be estimated well from the cumulative amounts of urinary OH-MPHP or oxo-MPHP excreted within 22 h after DPHP intake. Not considering possible differences in species-sensitivity towards unconjugated DPHP metabolites, it was concluded from a comparison of their AUCs in DPHP-exposed humans with corresponding earlier data in rats that there is no increased risk of adverse effects associated with the internal exposure of unconjugated DPHP metabolites in humans as compared to rats when receiving the same dose of DPHP per kg body weight.

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ABSTRACT

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1. Introduction

Di-(2-propylheptyl) phthalate (DPHP), CAS No. 53306-54-0, marketed under the trade name "Palatinol® 10-P" among others, is a high molecular weight branched phthalate ester which is used as a plasticizer for polyvinyl chloride (PVC) products. Commercial applications include cables, car interiors, carpet backing, pool liners, roofing membranes or tarpaulins, and consumer products such as shoes and artificial leather (BASF, 2015; CPSC, 2011; NICNAS, 2003). Typical contents of DPHP in end-use products vary between 30 and 60% (w/w) (BfR, 2011; NICNAS, 2003). It was found in toys (10.1–48.2% (w/w); BfR, 2011), food packaging, and medical products (NICNAS, 2003). DPHP, like other plasticizers, is not chemically bound in PVC products so it can be released into the environment. Urine samples of the German

Abbreviations: AUC, area under the concentration-time curve in blood; bw, body weight; cx-MPHP(-d4), non- or ring-deuterated mono-(2-propyl-6-carboxyhexyl) phthalate; cx-MPHP, mono-(2-propyl-6-carboxyhexyl) phthalate; cr-MPHP-d4, ring-deuterated mono-(2-propyl-6-carboxyhexyl) phthalate; DEHP, di-(2-ethylhexyl) phthalate; DPHP(-d4), non- or ring-deuterated di-(2-propylheptyl) phthalate; DPHP, di-(2-propylheptyl) phthalate; DPHP-d4, ring-deuterated di-(2-propylheptyl) phthalate; MEMP, mono-(2-ethylhexyl) phthalate; MHPH (-d4), non- or ring-deuterated mono-(2-propylheptyl) phthalate; MHPH, mono-(2-propylheptyl) phthalate; MHPH-d4, ring-deuterated mono-(2-propylheptyl) phthalate; NOAEL, no observed adverse effect level; OH-MPHP(-d4), non- or ring-deuterated mono-(2-propyl-6-hydroxyhexyl) phthalate; OH-MPHP, mono-(2-propyl-6-hydroxyhexyl) phthalate; OH-MPHP-d4, ring-deuterated mono-(2-propyl-6-hydroxyhexyl) phthalate; oxo-MPHP(-d4), non- or ring-deuterated mono-(2-propyl-6-oxoheptyl) phthalate; oxo-MPHP, mono-(2-propyl-6-oxoheptyl) phthalate; oxo-MPHP-d4, ring-deuterated mono-(2-propyl-6-oxoheptyl) phthalate; PVC, polyvinyl chloride; T_{max}, time point of a maximum concentration

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Environmental Specimen Bank collected from male and female volunteers (age: 20–30 years) between 1999 and 2012 revealed an increasing DPHP exposure of the general German population (Schütze et al., 2015).

Urinary excretion of DPHP metabolites (Fig. 1) was investigated in volunteers following single ingestion of DPHP. Mono-(2-propyl-6-hydroxyheptyl) phthalate (OH-MPHP) and mono-(2-propyl-6-oxoheptyl) phthalate (oxo-MPHP) were the major metabolites, mono-(2-propylheptyl) phthalate (MPHP) and mono-(2-propyl-6-carboxyhexyl) phthalate (cx-MPHP) were quantitatively of minor relevance (Leng et al., 2014; Wittassek and Angerer, 2008). The relationship between an excreted oxidized metabolite (oxo-MPHP) and the oral dose ingested (Leng et al., 2014) was used when estimating from urinary excretion data a maximum daily intake of 0.32 μg DPHP/kg body weight (bw) for the general German population (Schütze et al., 2015).

Toxicological data of DPHP in humans are not available. In Wistar (Crl:WI(Han)) rats, it was neither a reproductive toxicant nor an endocrine disruptor (BASF, 1995a, 2003, 2009; Furr et al., 2014), unlike some other phthalates. Oral administration of DPHP to rats resulted in increased weights of liver and kidney, peroxisome proliferation in the liver, vacuolation of the adrenal zona glomerulosa, eosinophilia in the proximal tubulus of the kidney, and thyroid hypertrophy/hyperplasia as well as increased basophilic cells of the pituitary gland. A rat-specific peroxisome proliferation was discussed to be related to these effects (BASF, 1995b, 2009; Bhat et al., 2014; Union Carbide, 1997, 1998). It is not known whether the findings resulted from the parent compound or its metabolites. Based on the no-observed-adverse-effect level (NOAEL) of 40 mg/kg bw for subchronic toxicity in rats, a tolerable daily intake for humans of 0.2 mg DPHP/kg bw was derived by UBA (2015) being 625 times higher than the daily intake estimated by Schütze et al. (2015). Bhat et al. (2014) calculated an oral reference dose of 0.1 mg/kg bw for thyroid hypertrophy/hyperplasia in male adult rats. Both derivations followed a generic approach and took into account the increased sensitivity of the rodent thyroid gland as compared to human thyroid gland. Possible species differences in the internal exposures of DPHP and its metabolites were unknown. In order to fill this gap, we recently determined concentrations of DPHP and its metabolites in blood of male Wistar (Crl:WI(Han)) rats following oral administration of single DPHP doses of 0.7 and 100 mg/kg bw (Klein et al., 2016). The aim of the present work was to monitor corresponding concentrations in blood of volunteers over time following ingestion of a single DPHP dose (0.7 mg/kg bw). Another goal was to establish a correlation between DPHP or its metabolites in blood and metabolites of DPHP in urine.

Fig. 1. Metabolic pathway of DPHP (Gries et al., 2012).

2. Materials and methods

2.1. Chemicals

Standards of DPHP and its metabolites were used as non-deuterated or as ring-deuterated compounds. In the following, non-deuterated compounds are named DPHP, MPHP, OH-MPHP, oxo-MPHP, cx-MPHP and ring-deuterated compounds are named DPHP-d4, MPHP-d4, OH-MPHP-d4, oxo-MPHP-d4, cx-MPHP-d4. If it is not distinguished between non- and ring-deuterated compounds, the abbreviations are DPHP(-d4), MPHP(-d4), OH-MPHP(-d4), oxo-MPHP(-d4), and cx-MPHP(-d4), respectively.

DPHP (Palatinol®10-P, purity 98%, GC analysis), DPHP-d4 (two batches: purities 84%, GC analysis and > 95%, 13C-NMR), MPHP (purity 90%, 13C-NMR), and MPHP-d4 (two batches: purities 95%, GC analysis and 75%, 13C-NMR) were supplied by BASF SE (Ludwigshafen, Germany). OH-MPHP(-d4), oxo-MPHP(-d4), and cx-MPHP(-d4) were gifts from the Institute of Biomonitoring, Currenta GmbH & Co. OHG (Leverkusen, Germany) and were synthesized at the Institut für Dünnschichttechnologie e.V. (Teltow, Germany). The purity of these compounds was ≥95% as determined by 1H-NMR.

Acetonitrile for blood analysis (Promochem picograde) and for urine analysis (supra solv) was purchased from LGC Standards (Wesel, Germany) and from Merck (Darmstadt, Germany), respectively. Water for blood analysis (LCMS grade) and for urine analysis was from Fisher Scientific (Loughborough, United Kingdom) and from Millipore water cleaning system (Milli-Q, Merck, Darmstadt, Germany), respectively. Heparin-Natrium 25,000 I.E. was from Ratiopharm (Ulm, Germany), beta-glucuronidase (E. coli K12) from Roche Diagnostics (Mannheim, Germany), glacial acetic acid (p.a.) and hydrochloric acid 37% (p.a.) from Merck (Darmstadt, Germany), ammonium acetate (p.a.) from Fluka (Taufkirchen, Germany), and formic acid (99%, ULC/MS) from Biosolve B.V. (Valkenswaard, The Netherlands). All other chemicals were purchased from Sigma-Aldrich (Steinheim, Germany) and were of highest purities available.

2.2. Experimental design

Six healthy male adult volunteers (Table 1) gave written informed consent to participate in the study which was reviewed (project number 5913/13) by the Ethics Commission of the Faculty of Medicine of the Technical University of Munich (Munich, Germany). The volunteers had breakfast between 45 and 140 min before DPHP(-d4) ingestion in order to stimulate intestinal lipase secretion. DPHP(-d4) was ingested as a single dose of 738 ± 56 μg/kg bw (1.65 ± 0.13 μmol/kg bw) at 9:00...
The calibration curves were linear in the concentration range tested with a purity of 7% (w/v) in an aqueous saccharose solution (70% w/v) considering the purities of DPHP and DPHP-d4, respectively. The volunteers took 0.01 ml emulsion/kg bw from a graduated disposable syringe (1 ml). The exact dose was determined by weighing and the syringe. Blood samples of 10 ml were taken from the forearm vein via an indwelling catheter using heparinized disposable syringes 30 min before and 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, and 10 h after DPHP-d4 ingestion. The last blood sample was collected directly from the forearm vein after 24 h. Total urine was collected using screw-capped polypropylene bottles immediately before DPHP-d4 ingestion and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10, 12, 14, 18, 22, 26, 30, 34, 38, 42, and 46 h thereafter. Urine samples collected during nighttime were stored at −25 °C. For analysis, the samples were thawed in a refrigerated room at 4 °C. Aliquots of 20 ml were transferred into 50 ml Falcon tubes and sent on ice within one day to the Institute of Biomonitoring, Currenta GmbH & Co. OHG (Leverkusen, Germany).

2.4. Analysis of urine samples

2.4.1. Sample preparation

Preparation and analysis of urine samples were performed at the Institute of Biomonitoring, Currenta GmbH & Co. OHG. Creatinine was measured by the Jaffé reaction (Taussky, 1954). For the analysis of DPHP metabolites, aliquots of urine (100 μl) were transferred into 2-mL autosampler vials. Then, 5 μl of beta-glucuronidase, 10 μl of the internal standard solution (1 mg/l of each deuterated or non-deuterated metabolite in acetonitrile), and 1 ml ammonium acetate buffer (1 mol/l, pH 6.5) were added. The sample vials were incubated at 37 °C overnight. Thereafter, samples were centrifuged at 22000 g for 10 min at 10 °C and the supernatants were subjected to LC-MS/MS analysis.

2.4.2. LC-MS/MS analysis

Chromatographic separation was performed on a Waters Acquity UPLC System (Waters, Eschborn, Germany) equipped with a column-switching device. Five μl of the sample was injected onto a SPE column (Waters XBridge C8 direct connect, 2.1 mm × 30 mm, 10 μm; Waters, Eschborn, Germany) followed by sample enrichment and cleanup for 0.5 min. Thereafter, the analytes were transferred for 3 min via back flush from the SPE column onto the chromatographic column (Agilent Zorbax Eclipse plus C8, 2.1 mm × 100 mm, 1.8 μm; Agilent, Waldbronn, Germany). The transfer process was stopped after 3.5 min and the SPE column was disconnected. The time courses of the gradients are given in Supplementary Table 1. Mass spectrometric detection and quantification were carried out on a Waters Xevo TQs triple quadrupole (Waters, Eschborn, Germany) with negative electrospray ionization in the multiple reaction monitoring (MRM) mode and with nitrogen as desolvation and argon as collision gas. The ionization conditions are given in Supplementary Table 2.

2.4.3. Calibration and quantification

Calibration was carried out by spiking 100 μl of water with MPHP (-d4), OH-MPHP-d4, oxo-MPHP-d4, and cx-MPHP-d4 at final concentrations ranging from 0.05 μg/l to 2000 μg/l. All calibration samples were analyzed in exactly the same way as the urine samples. Calibrations were linear at analyte concentrations of between 0.1 and 1000 μg/l; quantification of between 1000 and 2000 μg/l was done by fitting a quadratic curve (r > 0.99 for each analyte). Samples with concentrations above the calibration range were analyzed again after sample dilution with water. The detection limit of all analytes was 100 μg/l for MPHP(-d4), 150 μg/l for OH-MPHP(-d4), 200 μg/l for oxo-MPHP(-d4), and 100 μg/l for cx-MPHP(-d4).

Table 1

Volunteer-specific data.

| Volunteer | Body weight (kg) | Age (y) | DPHP(-d4) dose (μg/kg bw) | Breakfast | Lunch |
|-----------|-----------------|--------|--------------------------|-----------|-------|
|           |                 |        |                          | Time      | Food  |
| Vol. 1    | 83              | 52     | 717                      | 08:15 a.m.| Salami sandwich, prezel |
| Vol. 2    | 75              | 64     | 639                      | 08:15 a.m.| Cheese sandwich         |
| Vol. 3    | 76              | 56     | 781                      | 08:15 a.m.| Cheese sandwich, prezel |
| Vol. 4    | 74              | 30     | 783                      | 06:40 a.m.| Bacon and eggs          |
| Vol. 5    | 90              | 35     | 775                      | 08:10 a.m.| Egg sandwich            |
| Vol. 6    | 108             | 53     | 733                      | 08:05 a.m.| Cheese sandwich         |

* Voluntees 1 and 2 ingested DPHP-d4, volunteers 3–6 ingested DPHP; time of intake: 09:00 a.m.
0.1 μg/L.

2.4.4. Method validation

Samples of human urine were spiked with DPHP metabolites covering concentrations of 1, 10, and 100 μg/l for MPHP(-d4) and 1, 10, 100, and 1000 μg/l for OH-MPHP(-d4), oxo-MPHP(-d4), and cx-MPHP (-d4). The samples were analyzed after the internal exposure to the data representing the invasion phase.

2.5. Kinetic analysis

Mean concentration-time curves of DPHP(-d4) and its metabolites in blood as well as of total metabolites in urine of the volunteers were obtained by fitting the data with a biexponential function (1) y = C1 * e^-αt + C2 * e^-βt using Prism 6 for Mac (GraphPad Software, La Jolla, USA). Kinetic parameters were obtained by first fitting the elimination phase (C2 * e^-βt). For the metabolites, it was taking into account that (2) C1 = -C2. These parameters were included into the biexponential function that was then fitted to the data representing the invasion phase in order to obtain the values of the parameter α. The technique is similar to the “curve stripping” procedure in which the exponential phases are extracted one by one, starting with the flattest slope of a semi-logarithmic plot. The time course of DPHP showed a lag phase of 2 h. This phase was fitted by the function (3) y = C * e^-t using Prism 6 for Mac (GraphPad Software, La Jolla, USA).

Half-lives of the invasion and the elimination phases are ln2/α and ln2/β, respectively. Areas under the concentration-time curves (AUC) in blood of the individual subjects were calculated up to 24 h (AUC0-24) using the trapezoidal rule. Mean AUCs up to infinity (AUC0-∞) were calculated using the parameters of the fitted curves.

3. Results

3.1. DPHP and its metabolites in blood

Concentration-time courses of DPHP(-d4) and its metabolites in blood of the individual subjects after single ingestion of DPHP(-d4) are shown in Fig. 2. Since the monoesters undergo glucuronidation, their concentration-time courses are given for both the free and the total (sum of free and glucuronidated) compounds. The concentrations of all compounds displayed various individual peaks diverging up to one order of magnitude. There was a volunteer-specific pattern concerning the maximum value ± SD of 6.1 ± 3.4%. The most abundant metabolites were OH-MPHP (-d4) and oxo-MPHP(-d4) about 7% of the corresponding maximum value. Half-lives of the invasion phase were between 1.1 h for MPHP(-d4) and 3.1 h for oxo-MPHP(-d4). Half-lives of the elimination phase were similar among the compounds varying between 4.1 and 4.6 h (Table 2).

The AUC is a common measure reflecting the internal exposure to a chemical. Table 3 compares the AUCs of DPHP(-d4) and its metabolites normalized for the dose of DPHP(-d4) per kg bw. Large interindividual differences in the AUC0-24 were found for all substances; they were most pronounced for the diester with a factor of 32. Interindividual AUC0-24 of the metabolites differed up to 10-fold (oxo-MPHP(-d4)). It is noteworthy that the metabolites appeared in the blood always earlier than the parent diester that displayed a lag phase of about 2 h.

In order to enable a general kinetic interpretation, curves were fitted to the concentration-time data of the compounds determined in all volunteers. The concentration-time curves could be described by functions with two exponential terms, that of the diester by an additional exponential term describing the lag-phase (Fig. 3). The parameters of the functions are summarized in Table 2. Maximum fitted concentrations (nmol/l blood) amounted to 116 for DPHP(-d4), 105 for MPHP(-d4), 35 for OH-MPHP(-d4), and 49 for oxo-MPHP(-d4) and were reached after 6, 3.0, 4.9, and 5.3 h, respectively. At 24 h after dosage, concentrations of DPHP(-d4), MPHP(-d4), OH-MPHP(-d4), and oxo-MPHP(-d4) had declined to 10%, 4%, 12%, and 14% of their maximum concentrations, respectively. Half-lives of total and free compounds were identical at both the invasion and the elimination phases. Half-lives of the invasion phase were between 1.1 h for MPHP(-d4) and 3.1 h for oxo-MPHP(-d4). Half-lives of the elimination phase were similar among the compounds varying between 4.1 and 4.6 h (Table 2).

The urinary excretion of DPHP(-d4) metabolites was related to the amount of creatinine in the same sample. The creatinine value (mean ± SD) from all volunteers and all time points was 1.9 ± 0.7 g/day being within the normal range (1.0–2.5 g/day; Manski, 2017). The urinary excretion of DPHP(-d4) intake, fitted contents of MPHP(-d4), OH-MPHP(-d4), and oxo-MPHP(-d4) were about 1% and that of cx-MPHP(-d4) about 7% of the corresponding maximum value. Half-lives of the invasion phase were similar among the metabolites and ranged between 1.6 and 2.4 h (Table 2). Half-lives of the elimination phase were almost identical for MPHP(-d4), OH-MPHP(-d4), and oxo-MPHP(-d4) with values of slightly more than 5 h; cx-MPHP(-d4) was eliminated considerably slower (half-life: 8.7 h, Table 2).

Cumulative excretion of the metabolites of DPHP(-d4) over time in relation to the dose of DPHP(-d4) are summarized in Table 4. Total urinary excretion was calculated as the sum of all metabolites. After 22 h, total excretion was 90 ± 6% of that after 46 h. At the latter time point, total excretion differed between 1.93% (volunteer 4) and 10.5% (volunteer 1) of the ingested dose of DPHP(-d4) with a mean value ± SD of 6.1 ± 3.4%. The most abundant metabolites were OH-MPHP(-d4) and oxo-MPHP(-d4) contributing to 37% and 60% (mean values) of the summed amounts in urine, respectively. MHPH(-d4) and cx-MPHP(-d4) were the metabolites excreted at the lowest amounts, accounting in sum for less than 4% of the total amount excreted. Considering that only a small portion of the oral dose of DPHP(-d4) was excreted via urine, it is most probable that excretion via the feces represents the major elimination way. The conclusion is supported by findings on the structural homologue di-(2-ethylhexyl) phthalate (DEHP) in marmosets in which up to 66% of the 14C-labeled oral dose was recovered from the feces (Kurata et al., 2012).
Fig. 2. Concentration-time courses of DPHP(-d4) and its metabolites in venous blood of male volunteers upon single oral intake of DPHP(-d4) (ingested dose: 738 ± 56 μg/kg bw). Volunteers 1 and 2 ingested DPHP-d4, volunteers 3–6 ingested DPHP. Free compounds: unconjugated metabolites; total compounds: sum of free and glucuronidated metabolites. Symbols: measured data (mean values of 3 determinations); lines: connections between the mean values of the measured data.
3.3. Correlation between DPHP and its metabolites in blood and metabolites in urine

The correlation of AUCs of DPHP(-d4), MPHP(-d4), OH-MPHP(-d4), or oxo-MPHP(-d4) in blood with the amounts of OH-MPHP(-d4) or oxo-MPHP(-d4) excreted within 22 h in the urine of the volunteers were evaluated by linear regression analysis in order to investigate whether metabolites in urine could be used to predict AUCs of DPHP and metabolites in blood. The 22-h time point was preferred over the 46-h one because, in general, a one-day collection period is more...
convenient than a longer one. Urinary MPHP(-d4) and cx-MPHP(-d4) were not considered because their excretion amounted each to less than 2% of the total amount excreted. Fig. 5 shows that urinary OH-MPH(-d4) correlated well with the AUCs of MPHP(-d4), OH-MPH(-d4), and oxo-MPH(-d4) in blood with values of $r^2$ of 0.9001, 0.8528, and 0.8721, respectively. The correlations based on urinary oxo-MPH(-d4) correlated well with the AUCs of MPHP(-d4), OH-MPH(-d4), and MPHP(-d4) in blood with values of $r^2$ of 0.9001, 0.8528, and 0.8721, respectively.

Table 3

| Parameter | AUC, total compound (nmol*h/l per μmol DPHP(-d4)/kg bw) | AUC, free compound (% of total compound) |
|-----------|--------------------------------------------------------|----------------------------------------|
| Individual AUC$_{0-24}$ | DPHP(-d4) | MPHP(-d4) | OH-MPH(-d4) | oxo-MPH(-d4) | DPHP(-d4) | OH-MPH(-d4) | oxo-MPH(-d4) |
| Volunteer 1 | 3029 | 1346 | 564 | 960 | 54 | 13 | 4.7 |
| Volunteer 2 | 397 | 539 | 231 | 128 | 73 | 11 | 2.7 |
| Volunteer 3 | 739 | 437 | 172 | 199 | 66 | 12 | 3.9 |
| Volunteer 4 | 96 | 160 | 123 | 97 | 55 | 27 | 4.5 |
| Volunteer 5 | 663 | 938 | 422 | 525 | 63 | 27 | 6.1 |
| Volunteer 6 | 839 | 439 | 164 | 285 | 55 | 24 | 3.9 |
| Mean AUC$_{0-24} ± SD$ | 961 ± 1048 | 643 ± 426 | 279 ± 175 | 366 ± 329 | 61 ± 7.7 | 22 ± 7.6 | 4.3 ± 1.1 |
| Mean AUC$_{0-24}$ | 844 | 618 | 291 | 440 | 65 | 22 | 5.1 |

$^a$ Volunteers 1 and 2 ingested DPHP-d4, volunteers 3–6 ingested DPHP.

$^b$ Sum of free and glucuronidated compounds.

$^c$ Calculated by using the trapezoidal rule.

$^d$ Calculated by using the curve parameters given in Table 2 and the mean dose of 1.65 μmol DPHP/kg bw.

4. Discussion

4.1. DPHP and its metabolites in blood

Previous studies suggested the gastrointestinal tract to be an important site of presystemic hydrolytic metabolism of orally administered phthalates in laboratory animals (DEHP: Albro and Thomas, 1973; Albro et al., 1982; Kessler et al., 2004; DPHP: Klein et al., 2016) and in humans (DEHP: Kessler et al., 2012). The produced ionic monooesters are taken up via the portal blood. Concerning the absorption of the lipophilic and non-ionic diesters in humans, the lymph seems to play an important role (discussed for DEHP in Kessler et al., 2012). According to the present results, the same deduction can be done for DPHP and MPHP. The early occurrence of MPHP in blood of the volunteers likely results from lipase-catalyzed hydrolysis of the diester in the stomach and the duodenum followed by rapid absorption of the monooester. The lag phase observed for DPHP and the longer half-life of the invasion phase compared to that of MPHP agree with the uptake into the lymph and with the slow lymph flow into the thoracic duct (Lindena et al., 1986).

The concentration-time course of MPHP in the systemic blood depends on the gastrointestinal absorption of MPHP, on the formation of MPHP from systemic DPHP, and on the elimination of MPHP by metabolism and excretion. The maximum concentration of MPHP in blood occurred earlier than that of DPHP, which is consistent with the interpretation that systemic MPHP is initially governed mainly by its formation in stomach and gut. The longer half-lives of the invasion phases of OH-MPH and oxo-MPH if compared to that of MPHP suggest that they are formed primarily from systemic MPHP and that first-pass metabolism of MPHP is quantitatively of minor relevance. The almost identical elimination half-lives of all of the compounds imply that elimination kinetics of the DPHP metabolites is determined by the metabolic elimination of systemic DPHP. Glucuronidation of the metabolites obviously does not influence their elimination as is substantiated by the parallel concentration-time courses of total and free metabolites.

Concerning systemic exposures, DPHP showed the largest
interindividual variation among all compounds, possibly resulting predominantly from differences in intestinal absorption. Despite the interindividual variations, the summed AUCs of OH- and oxo-MPHP were always similar to the AUC of MPHP. This hints to an almost exclusive metabolism of systemic MPHP via omega-1 oxidation, taking into account that MPHP and cx-MPHP are virtually not excreted in the urine of volunteers who ingested DPHP (MPHP: this study; Wittassek and Angerer, 2008; cx-MPHP: this study; Leng et al., 2014; Wittassek et al., 2008). The extent of glucuronidation of each metabolite was similar among all volunteers in spite of the comparatively large inter-individual variations in the AUCs by a factor of between about 5 (OH-MPHP) and 10 (MPHP and oxo-MPHP). It can be concluded that glucuronidation was not saturated.

The only other phthalate for which kinetic data were published in human blood is DEHP (Fromme et al., 2012; Kessler et al., 2012). Both DPHP and DEHP are absorbed in a similar way from the gut and have principally the same metabolic pathways. Half-lives of the elimination phase of the parent compounds and of their metabolites are similar, too.

![Fig. 4. Excretion of DPHP(-d4) metabolites (total compounds) in urine of volunteers upon single oral intake of DPHP(-d4) (ingested dose: 738 ± 56 μg/kg bw). Symbols: mean values ± SD (SD not given if below the lowest value on the y-axis) of the 6 volunteers. Lines: curve fits to the mean values. The parameters of the exponential functions describing the curves are given in Table 2.](image)

| Parameter | MPHP(-d4) | OH-MPHP(-d4) | oxo-MPHP(-d4) | cx-MPHP(-d4) | Total excretiona |
|-----------|-----------|--------------|---------------|--------------|-----------------|
| Time after intake | 22 h | 46 h | 22 h | 46 h | 22 h | 46 h | 22 h | 46 h | 22 h | 46 h |
| Volunteer 1 | 0.172 | 0.180 | 3.98 | 4.35 | 5.29 | 5.78 | 0.179 | 0.215 | 9.59 | 10.50 |
| Volunteer 2 | 0.055 | 0.071 | 1.15 | 1.53 | 2.15 | 2.64 | 0.050 | 0.072 | 3.40 | 4.31 |
| Volunteer 3 | 0.050 | 0.054 | 1.38 | 1.50 | 2.11 | 2.23 | 0.062 | 0.076 | 3.59 | 3.86 |
| Volunteer 4 | 0.021 | 0.023 | 0.68 | 0.72 | 1.08 | 1.14 | 0.040 | 0.044 | 1.81 | 1.93 |
| Volunteer 5 | 0.070 | 0.074 | 2.91 | 3.27 | 5.23 | 5.89 | 0.141 | 0.172 | 8.34 | 9.40 |
| Volunteer 6 | 0.092 | 0.097 | 2.10 | 2.24 | 3.84 | 4.08 | 0.100 | 0.118 | 6.13 | 6.52 |
| Mean | 0.077 | 0.083 | 2.03 | 2.27 | 3.28 | 3.63 | 0.095 | 0.116 | 5.48 | 6.09 |
| SD | 0.052 | 0.054 | 1.23 | 1.33 | 1.77 | 1.95 | 0.055 | 0.066 | 3.06 | 3.35 |

a Volunteers 1 and 2 ingested DPHP-d4, volunteers 3–6 ingested DPHP.
b Sum of all four metabolites.
Klein et al., 2016), the animal species in which the systemic toxicity of DPHP was investigated (see Introduction). At the same single oral dose (Anderson et al., 2011; Fromme et al., 2012; Koch et al., 2005). As was shown in blood and urine of volunteers after DEHP ingestion the lower internal exposure of MPHP as compared to MEHP. Unlike those of DEHP (Fromme et al., 2012), which is in agreement with lower presystemic lipolytic activity towards DPHP as compared to free or total MEHP becomes evident (calculation for free monoesters: ([AUC$_{0-24}$ of total MPHP \* 0.61/AUC$_{0-24}$ of DPHP] / [AUC$_{0-24}$ of free MEHP/AUC$_{0-24}$ of DEHP]); data for DEHP and MEHP from Table 3 in Kessler et al. (2012), data for DPHP and MPHP from Table 3 of the present work). The comparison of the free primary monoesters is of particular interest because free MEHP has been attributed to DEHP-dependent adverse effects (summarized in Gentry et al., 2011). The difference in the internal exposure of the monoesters might result from a lower intestinal absorption of MPHP as compared to MEHP or from a lower presystemic lipolytic activity towards DPHP as compared to MEHP. The AUCs of the secondary DPHP metabolites were also lower than those of DPHP (Fromme et al., 2012), which is in agreement with the lower internal exposure of MPHP as compared to MEHP. Unlike MPHP, MEHP is metabolized considerably via side-chain carboxylation as was shown in blood and urine of volunteers after DEHP ingestion (Anderson et al., 2011; Fromme et al., 2012; Koch et al., 2005).

Kinetische der DPHP in human kann mit dem in rats (Klein et al., 2016), the animal species in which the systemic toxicity of DPHP was investigated (see Introduction). At the same single oral dose of DPHP (normalized to 1 µmol DPHP/kg bw), its mean AUC$_{0-∞}$ in human blood was 844 mmol·h/l blood (Table 3). In rat blood the AUC$_{0-∞}$ of DPHP was too low to be quantified (≤13 mmol·h/l blood, calculated using the parameters of the curve given in Fig. 2B of Klein et al. (2012) and considering the oral dose of 0.7 mg DPHP/kg bw, i.e.,1.56 µmol/kg bw). The AUC$_{0-∞}$ of total metabolites were 3.2-fold (MPHP), 1.6-fold (OH-MPHP), and 4.4-fold (oxo-MPHP) higher in blood of humans than in blood of rats (calculated using data from Table 3, in the present work and Table 2 in Klein et al., 2016). In humans, the AUCs of the DPHP metabolites are determined most likely to a large extent by the metabolism of systemic DPHP whereas in rats the AUCs of the metabolites seem to be determined almost exclusively by intestinally formed MPHP that is absorbed via the portal vein. A major species difference in the kinetics of DPHP upon oral intake is obviously related to a lower intestinal hydrolysis and higher absorption in humans as compared to rats. The same explanation for corresponding findings on the kinetics of DEHP in both species was given in Kessler et al. (2012). Species differences in the AUCs of free DPHP metabolites also result from their degrees of glucuronidation. In rats, AUCs of free metabolites contributed to ≥95% of total metabolites (Klein et al., 2016); in humans, they decreased with the oxidation state of the metabolites from 65% for MPHP to about 5% for o xo-MPHP (mean values of AUC$_{0-∞}$). As a consequence, AUCs of free OH-MPHP and free o xo-MPHP were 2.8-fold and 4.4-fold lower, respectively, in human than in rat blood in spite of the higher AUCs of total metabolites in human blood. The AUC of free MPHP was 2.2-fold higher in human blood than in rat blood. Interestingly, Kessler et al. (2012) found the same species difference for free MEHP.

4.2. DPHP metabolites in urine

The excretion of DPHP metabolites in the urine of volunteers upon single oral ingestion of DPHP was also investigated in two earlier studies. Wittassek and Angerer (2008) determined metabolites in total urine of a single male volunteer up to 61 h after the uptake of DPHP. No information was given on the dose of DPHP, on details concerning the oral administration, and on the time course of metabolite excretion. Leng et al. (2014) investigated in 5 male volunteers the time dependence of the urinary excretion of secondary metabolites up to 48 h after ingestion of a DPHP dose of between 0.54 and 0.66 mg/kg bw. An ethanolic solution of the substance was mixed in a coffee or tea containing waffe cup with a chocolate surface that was consumed during breakfast. The kinetics of the metabolites in urine turned out to be similar as in our study as substantiated by comparing mean values of $T_{\text{max}}$ (Leng et al., 2014: 3.54–4.05 h; this study: 3.9–5.4 h) and of elimination half-lives (Leng et al., 2014: 6.51–8.16 h; this study: 5.2–8.7 h). In the two earlier studies and in the present one, OH-MPHP and oxo-MPHP were the predominant metabolites. However, the total amounts of metabolites excreted (given as mean molar fractions of the dose of DPHP) were distinctly higher in the earlier studies (Wittassek and Angerer, 2008: 34% after 61 h; Leng et al., 2014: 24.7% after 48 h) than in the present one (6.1% after 46 h). A dose-dependent effect can be excluded since the doses used in the present study and in that of Leng et al. (2014) were almost identical. The quantitative differences in the excretion of metabolites could hint to differences in the bioavailability of DPHP and MPHP resulting from presystemic processes. Hydrolysis of
DPHP and absorption of DPHP and MPHP in the digestive tract could be influenced by the dose vehicle, the fat content of the breakfast, and the time span between breakfast and DPHP intake. Lingual lipase, although contributing only little to the preduodenal lipase activity (Kulkarni and Mattes, 2014; Feher, 2017), might contribute to the hydrolysis of DPHP. Formation and absorption of MPHP in the mouth of those volunteers who ate the DPHP containing waffle cup during breakfast (Leng et al., 2014), could have been a cause for the increased urinary metabolite excretion as compared to the present study in which a bolus dose was swallowed immediately. On the other hand, no larger differences were seen between a bolus intake of the DPHP homologue DEHP (Kessler et al., 2012) and its intake via a piece of bread eaten during breakfast (Anderson et al., 2011). Urinary excretion of DEHP metabolites were quantitatively similar in the two volunteer studies in spite of the different methods of oral DEHP dosing.

4.3. Correlation between DPHP and its metabolites in blood and metabolites in urine

Schütze et al. (2015) quantified OH-MPHP and oxo-MPHP in urine samples of the German Environmental Specimen Bank that were collected between 1999 and 2012. The authors compared the data with the increasing DPHP consumption during this time span. Both metabolites were detected only in the samples from 2009 and 2012. The consumption of DPHP was reflected by an increasing detection frequency of oxo-MPHP but not of OH-MPHP. Consequently, urinary oxo-MPHP was considered as the most conclusive parameter for DPHP exposure. According to the present study, both urinary OH-MPHP and oxo-MPHP are suitable for estimating the AUCs of DPHP metabolites in blood, with urinary OH-MPHP being the more accurate one. However, data on urinary OH-MPHP might not be available because the amounts of OH-MPHP are 1.6fold less than those of oxo-MPHP at the same AUCs of metabolites in blood (see Fig. 5 and Table 4).

5. Conclusions

The following conclusions are drawn from the data presented.

- Experimentally determined concentrations of DPHP metabolites in blood of DPHP-exposed volunteers together with their urinary excretion data enabled to estimate the internal exposure to DPHP metabolites from urinary data upon oral exposure. Without knowledge of the correlation, reliable information on the internal exposure to DPHP cannot be obtained from urinary data solely as exemplified for MPHP being a major metabolite in blood but only a minor one in urine.

- The comparison of the per dose and kg bw normalized AUCs in blood (a measure of the internal exposure) of free metabolites of DPHP in DPHP-exposed humans with corresponding data in DPHP-exposed rats revealed only small differences between both species. Not taking into account possible differences in species-sensitivity, it can be concluded from the comparison of the AUCs that there is no increased risk of adverse effects associated with the internal exposure of free DPHP metabolites in humans as compared to rats when receiving the same dose of DPHP (up to 0.7 mg at least) per kg bw.

Transparency document

The Transparency document associated with this article can be found in the online version.

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Appendix A. Supplementary data

Supplementary material related to this article can be found in the online version, at doi: https://doi.org/10.1016/j.jxlet.2018.05.010.

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