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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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ABSTRACT

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 over time 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.

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; cx-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; MEHP, mono-(2-ethylhexyl) phthalate; MPHP(-d4), non- or ring-deuterated mono-(2-propylheptyl) phthalate; MPHP, mono-(2-propylheptyl) phthalate; MPHP-d4, ring-deuterated mono-(2-propylheptyl) phthalate; NOAEL, no observed adverse effect level; OH-MPHP(-d4), non- or ring-deuterated mono-(2-propyl-6-hydroxyheptyl) phthalate; OH-MPHP, mono-(2-propyl-6-hydroxyheptyl) phthalate; OH-MPHP-d4, ring-deuterated mono-(2-propyl-6-hydroxyheptyl) 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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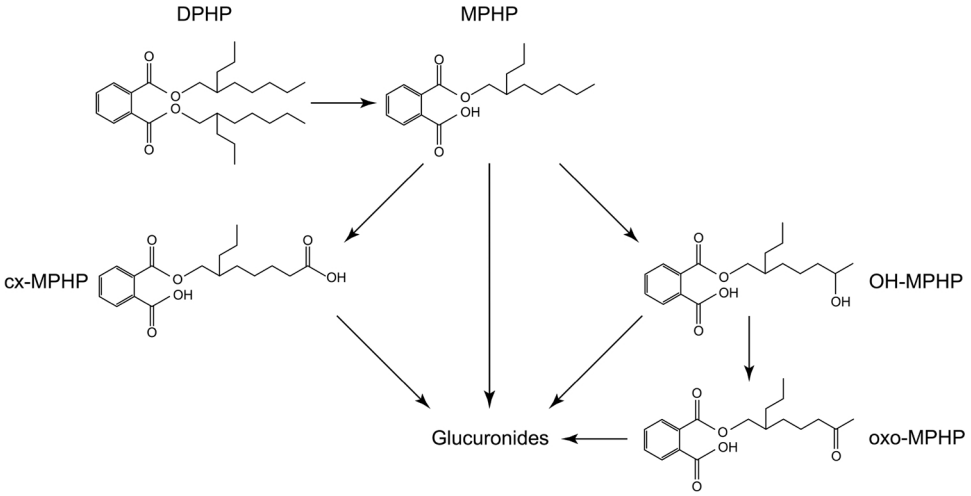

Fig. 1. Metabolic pathway of DPHP (Gries et al., 2012).

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 per 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 per day using a benchmark response level of 10% (10 mg/kg bw per day) 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.

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%, ^{13}C-NMR), MPHP (purity 90%, ^{13}C-NMR), and MPHP-d4 (two batches: purities 95%, GC analysis and 75%, ^{13}C-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 ^{1}H-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 a 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