Toxicology Letters 259 (2016) 80–86

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Di-(2-propylheptyl) phthalate (DPHP) and its metabolites in blood of rats upon single oral administration of DPHP

D. Klein[a,b,*], W. Kessler[a], B. Semder[a], C. Pütz[a], J. Lichtmannegger[a], R. Otter[c], J.G. Filser[a]

[a] Institute of Molecular Toxicology and Pharmacology, Helmholtz Zentrum München, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany
[b] Institute for Toxicology and Environmental Hygiene, Technical University of Munich, Biedersteiner Str. 29, 80802 Munich, Germany
[c] BASF SE, E-CPI/R, Carl-Bosch-Str. 38, 67056 Ludwigshafen, Germany

ARTICLE INFO

Article history:
Received 26 February 2016
Received in revised form 12 July 2016
Accepted 21 July 2016
Available online 22 July 2016

Keywords:
Di-(2-propylheptyl) phthalate
Metabolites
Oral treatment
Rat
Blood

ABSTRACT

Di-(2-propylheptyl) phthalate (DPHP) does not act as a reproductive toxicant or endocrine disruptor in contrast to other phthalates. Considering adverse effects of phthalates to be linked to their metabolism, it was the aim of the present study to investigate in the rat the blood burden of DPHP and its metabolites as a basis for understanding the toxicological behavior of DPHP. Rats were administered single oral doses of DPHP of 0.7 and 100 mg/kg body weight. Concentration-time courses of DPHP and metabolites were monitored in blood. The areas under the concentration-time curves in blood (AUCs), normalized for the dose of DPHP, showed the following order: DPHP < mono-(2-propyl-6-oxoheptyl) phthalate < mono-(2-propyl-6-hydroxyheptyl) phthalate = mono-(2-propylheptyl) phthalate < mono-(2-propyl-6-carboxyhexyl) phthalate (cx-MPHP). Glucuronidation of the monoesters accounted for less than 5% of total compounds. The elimination half-lives of the compounds ranged from 2.3 h (DPHP) to 8.2 h (cx-MPHP). The normalized AUCs of the metabolites were lower at the high dose of DPHP than at the low one indicating saturation kinetics of intestinal DPHP hydrolysis. The absence of toxicity to reproduction of DPHP may be related to the comparatively low bioavailability of the parent compound and its metabolites.

1. Introduction

Di-(2-propylheptyl) phthalate (DPHP), CAS No. 53306-54-0, is a high molecular weight branched phthalate ester. The technical product is marketed amongst others as "Palatinol®10-P", which consists of about 99.5% phthalic ester. The alcohol moiety consists of 90% 2-propyl-heptanol and 10% 2-propyl-4-methylhexanol or 2-propyl-5-methylhexanol (Gries et al., 2012). The world-wide consumption of DPHP in 2012 was 208,000 metric tons (Schütze et al., 2015). DPHP is registered under REACH (Regulation (EC) No. 1907/2006) and intended as a plasticizer in PVC formulations. Commercial applications of DPHP include cables, carpet backing, car interiors, outdoor applications like pool liners, roofing membranes or tarpaulins, and also consumer products such as shoes and artificial leather (BASF, 2015; CPSC, 2011; NICNAS, 2003). Typical contents of the chemical in end-use products vary between 30 and 60% (w/w) (BfR, 2011; NICNAS, 2003). DPHP is used as a substitute for high molecular weight phthalates like di-isononyl phthalate (DINP) or di-isodecyl phthalate and for di-(2-ethylhexyl)

Abbreviations: AUC, concentration-time curve in blood calculated for $t \rightarrow \infty$; b. w., 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; DINP, di-isononyl 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; 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; $t_{1/2}$, half-life of the elimination phase.

* Corresponding author at: Institute of Molecular Toxicology and Pharmacology, Helmholtz Zentrum München, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany.
E-mail address: dominik.klein@helmholtz-muenchen.de (D. Klein).

http://dx.doi.org/10.1016/j.toxlet.2016.07.025
0378-4274/

phthalate (DEHP) which is under scrutiny due to toxicity to reproduction and endocrine activity.

Occupational exposure to DPHP may occur during production, packaging or cleaning of equipment (NICNAS, 2003). Recently it has been shown that also the general German population is exposed to DPHP due to its increased usage. The level of exposure for the general public ranges from 0.025 to 0.314 μg/kg b.w. per day (Schütze et al., 2015). Although DPHP is not intended for use in toys, food packaging or medical products (NICNAS, 2003), it has been detected in toys in contents of 10.1–48.2% (w/w) (BfR, 2011). The daily DPHP intake from such toys by children has been estimated to reach up to 135 μg/kg b.w. (BfR, 2011).

The metabolism of DPHP was studied in volunteers (Leng et al., 2014; Wittassek and Angerer, 2008). In analogy to other phthalates, the primary metabolite of DPHP is mono-(2-propyl-heptyl) phthalate (MPHP). MPHP is metabolized via omega and omega-1 oxidation, yielding mono-(2-propyl-6-carboxyhexyl) phthalate (cx-MPHP) and mono-(2-propyl-6-hydroxyheptyl) phthalate (OH-MPHP), respectively. The latter is further oxidized to mono-(2-propyl-6-oxoheptyl) phthalate (oxo-MPHP). All of these metabolites (Fig. 1) can be conjugated with glucuronic acid.

Toxicological data obtained in studies with rats suggest that DPHP is neither a reproductive toxicant nor an endocrine disruptor (BASF, 1995a, 2003, 2009; Furr et al., 2014). Following oral administration, increased liver weights and thyroid and pituitary effects are described, possibly in relation to a rat specific peroxisome proliferation (BASF, 1995b, 2009; Union Carbide, 1997, 1998). On the basis of the NOAEL of 40 mg/kg b.w. for subchronic toxicity in rats, an acceptable exposure for humans of 0.2 mg DPHP/kg b.w. per day has been derived (UBA, 2015). An oral reference dose of 0.1 mg/kg b.w. per day has been derived from the human equivalent 10% benchmark response level of 10 mg/kg b.w. per day for thyroid hypertrophy/hyperplasia in male adult rats (Bhat et al., 2014). Adverse effects of certain phthalates are related to metabolically formed monoesters (e.g. Foster et al., 1981; Oishi and Hiraga, 1980; Sjöberg et al., 1986). Regarding the species-specific burdens of the primary monoesters in venous blood, large species differences were identified in studies with DEHP (Kessler et al., 2004, 2012; Kurata et al., 2012; Rhodes et al., 1986). As a basis for a risk estimation of DPHP, it is therefore reasonable to compare the burdens of the metabolically formed monoesters in blood of rats and humans. So far, no such data is available. Therefore, we investigated in the present study concentration-time courses of DPHP and its metabolites in venous blood of rats. The animals were orally administered two doses of 0.7 and 100 mg DPHP/kg b.w. in order to cover a large dose range. The low dose equals that used in a comparative study conducted with humans (manuscript in preparation).

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.

Palatinol® 10-P (purity 98%, GC analysis), DPHP-d4 (two batches: one with a purity of 84% according to GC analysis, the other one with a purity of >95% according to ^{13}C NMR), MPHP (purity 90%, ^{13}C NMR), and MPHP-d4 (two batches: one with a purity of 95% according to GC analysis, the other one with a purity of 75% according to ^{13}C NMR) was supplied by BASF SE (Ludwigshafen, Germany). OH-MPHP, OH-MPHP-d4, oxo-MPHP, oxo-MPHP-d4, cx-MPHP, and cx-MPHP-d4 were gifts from the Institute for Biomonitoring, Currenta (Leverkusen, Germany) and were synthesized at the Institut für Dünnschichttechnologie (Teltow, Germany). The indicated purities of the compounds were ≥95% (determined by ^{1}H NMR). Acetonitrile (Promochem picograde) was purchased from LGC Standards (Wesel, Germany) and water (LCMS grade) from Fisher Scientific (Loughborough, United Kingdom). Heparin-Natrium 25,000 I.E. ratiopharm was from Ratiopharm (Ulm, Germany) and beta-glucuronidase (*E. coli* K12) from Roche Diagnostics (Mannheim, Germany). All other chemicals were purchased from Sigma-Aldrich (Steinheim, Germany) and were of highest purities available.

2.2. Animals and DPHP treatment

Male Wistar (Crl:WI(Han)) rats (250–280 g) were obtained from Charles River (Sulzfeld, Germany). This rat strain was also used in toxicity studies with DPHP (BASF 1995a, 1995b, 2003, 2009). The animals were housed in Macrolon® cages and provided with HEPA-filtered air in a TOP FLOW-IVC-system (Tecniplast, Buggugiate, Italy). The animal room was air-conditioned and the air was cleaned of particles by active charcoal filters. A constant light–dark cycle with light from 7:00 to 19:00 h was kept. Animals had free access to standard chow (Nr. 1324) from Altromin (Lage, Germany) and tap water.

DPHP was administered orally at a single dose of 100 or 0.7 mg/kg b.w. between 8 and 9 a.m. The experiments with 100 mg/kg DPHP were conducted prior to those with 0.7 mg/kg DPHP. For the high dose, DPHP (Palatinol® 10-P) was administered. For the low dose, Palatinol® 10-P could not be used because of background signals at the retention times of DPHP and its metabolites in blood of untreated rats (see 2.4 and the chromatograms (Supplementary Fig. 1) shown in the Supplementary material). Therefore, DPHP-d4 was used for the low dose. Both substances were given as aqueous emulsions in a saccharose solution (70% w/v) as vehicle. Emulsions of 10% (w/v) DPHP and 0.07% DPHP-d4 were prepared for the high and the low dose, respectively. The substances were administered by gavage using a graduated disposable syringe (1 ml) equipped with a stainless steel cannula. The animals received 1 ml emulsion per kg b.w. The actual dose was determined by reweighing the syringe and considering the purity of Palatinol® 10-P and that of the DPHP-d4 batch used. Immediately after sacrifice of the rats with carbon dioxide, blood samples of 6–7 ml were collected from the vena cava inferior using disposable syringes (10 ml) rinsed with heparin. For each DPHP dose, 3 experiments were performed with collection of blood at time points of 0, 0.25, 0.5, 0.75, 1.0, 2.0, 4.0, 6.0, 8.0, 10, and 24 h after DPHP treatment. For each experiment, one calibration was done using blood from control animals.

2.3. Sample collection and preparation

Blood was transferred in aliquots of 0.6 ml to 9 Eppendorff caps (1.5 ml) each containing 60 μl acetonitrile or an internal standard solution. For the animals dosed with 0.7 mg/kg DPHP-d4, the internal standard solution consisted of DPHP (550 nmol/l) and of MPHP, OH-MPHP, oxo-MPHP, and cx-MPHP (each: 220 nmol/l) dissolved in acetonitrile. For the animals dosed with 100 mg/kg DPHP, the internal standard solution consisted of DPHP-d4 (550 nmol/l) and of OH-MPHP-d4, oxo-MPHP-d4, and cx-MPHP-d4 (each: 220 nmol/l) dissolved in acetonitrile; MPHP-d4 was lacking in this solution because it was not available at the time when the experiments with 100 mg/kg DPHP were performed. For