Toxicology Letters xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Toxicology Letters

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

Urinary metabolite excretion after oral dosage of bis(2-propylheptyl) phthalate (DPHP) to five male volunteers – Characterization of suitable biomarkers for human biomonitoring

Gabriele Leng [a,*], Holger M. Koch [b], Wolfgang Gries [a], Andre Schütze [b], Angelika Langsch [c], Thomas Brüning [b], Rainer Otter [c]

[a] Currenta GmbH & Co. OHG, Institute of Biomonitoring, Leverkusen, Germany
[b] Institute for Prevention and Occupational Medicine – Institute of the Ruhr-Universität Bochum, Bochum, Germany
[c] BASF SE, Ludwigshafen, Germany

HIGHLIGHTS

- Urinary excretion of three specific, secondary, oxidized metabolites (oxo-MPHP, OH-MPHP and cx-MPHxP) of DPHP was monitored following oral uptake by five volunteers.
- Urinary elimination half-lives for these metabolites are between 6 and 8 h.
- 22.9% of the DPHP dose is excreted as one of the above three metabolites within 24 h, until 48 h post dose an additional 1–2% is excreted.
- Based upon molar excretion fractions the DPHP intake of the general public and of workers can be calculated from urinary metabolite levels.

ARTICLE INFO

Article history:
Received 18 March 2014
Received in revised form 30 May 2014
Accepted 23 June 2014
Available online xxx

Keywords:
Biomonitoring
Di(2-propylheptyl)phthalate
DPHP metabolites
Urinary excretion
Human volunteer study
HPLC–NESI–MS/MS

ABSTRACT

Di(2-propylheptyl) phthalate (DPHP), a high molecular weight phthalate, is primarily used as a plasticizer in polyvinyl chloride and vinyl chloride copolymers for technical applications, as a substitute for other phthalates currently being scrutinized because of endocrine disrupting effects.

We determined urinary excretion fractions of three specific DPHP metabolites (mono-2-(propyl-6-hydroxy-heptyl)-phthalate (OH-MPHP), mono-2-(propyl-6-oxoheptyl)-phthalate (oxo-MPHP) and mono-2-(propyl-6-carboxy-hexyl)-phthalate (cx-MPHxP)) after oral dosing of five volunteers with 50 mg labelled DPHP-d4 and subsequent urine sampling for 48 h. These excretion fractions are used to back calculate external intakes from metabolite measurements in spot urine analysis. Following enzymatic hydrolysis to cleave possible conjugates, we determined these urinary metabolites by HPLC–NESI–MS/MS with limits of quantification (LOQ) between 0.3 and 0.5 μg/l.

Maximum urinary concentrations were reached within 3–4 h post dose for all three metabolites; elimination half-lives were between 6 and 8 h. We identified oxo-MPHP as the major oxidized metabolite in urine representing 13.5 ± 4.0% of the DPHP dose as the mean of the five volunteers within 48 h post dose. 10.7 ± 3.6% of the dose was excreted as OH-DPHP and only 0.48 ± 0.13% as cx-MPHxP. Thus, within 48 h, 24.7 ± 7.6% of the DPHP dose was excreted as these three specific oxidized DPHP metabolites, with the bulk excreted within 24 h post dose (22.9 ± 7.3%).

These secondary, oxidized metabolites are suitable and specific biomarkers to determine DPHP exposure. In population studies, however, chromatographic separation of these metabolites from other isomeric di-isodecyl phthalate (DIDP) metabolites is warranted (preferably by GC–MS) in order to distinguish DPHP from general DIDP exposure. Palatinol®, Hexamoll® and DINCH® are registered trademarks of BASF SE, Germany.

* Corresponding author at: Currenta GmbH & Co. OHG, Institute of Biomonitoring, Chempark Leverkusen, Building L 9, 51368 Leverkusen, Germany. Tel.: +49 2143065679; fax: +49 2143021307.
E-mail address: gabriele.leng@currenta.de (G. Leng).

http://dx.doi.org/10.1016/j.toxlet.2014.06.035
0378-4274/

1. Introduction

Di(2-propylheptyl) phthalate (DPHP), CAS No. 53,306-54-0, a REACH (Regulation (EC) No. 1907/2006) registered high molecular weight phthalate, is primarily used as a plasticizer in polyvinyl-chloride and vinyl chloride copolymers for technical applications. DPHP, which is marketed under, e.g., the trade name "Palatinol® 10-P", is produced by esterification of phthalic anhydride with a C10 alcohol consisting of 90% 2-propyl-heptanol and 10% 2-propyl-4-methylhexanol or 2-propyl-5-methylhexanol. There are currently two different C10 phthalates on the market. DPHP and di-isodecyl phthalate (DIDP) as described with the CAS No. 68,515-49-1: 1,2-benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich. Another DIDP described by CAS No. 26,761-40-0 is no longer produced in Europe and is not REACH registered. Furthermore, there are two C9 phthalates (di-isononyl phthalates, DINPs) on the market: DINP1 (1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich, described with CAS No. 68,515-48-0 and DINP2 (di-isononyl phthalate) with CAS No. 28,553-12-0. While DINP2 solely consists of C9 isomers DINP1 contains up to 10% C10 isomers. Thus, the broad isomer distribution of DINP1 (including C10 moieties) can also interfere with the analytical detection of both DIDP and DPHP. The lack of sufficient analytical separation of DINP and DIDP resulted in a group-TDI by EFSA (EFSA, 2005) for food contact applications (Commission Regulation (EU) No. 10/2011).

The phthalates DINP, DPHP and DIDP are currently used as substitutes for di-(2-ethylhexyl) phthalate (DEHP) which is listed under REACH as a substance of very high concern (SVHC). Based on their low volatility and low vapor pressure, the C10 phthalates DPHP and DIDP are predominantly used in high temperature-resistant products such as electrical cables, carpet backing and car interiors, but they are also used for outdoor applications like roofing membranes or tarpaulins (European Commission, 2003; NICNAS, 2003, 2008; Wittassek, 2008). DPHP is currently not used in food contact. Because plasticizers are not chemically bound in PVC products and thus can migrate out of these products, exposure of humans and the environment is possible. Therefore, a collaborative project between the German Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety (BMUB) and the German Chemical Industry Association (VCI) evaluated a specific human biomonitoring method to determine exposure of the general population to DPHP using reliable and specific urinary biomarkers (Federal Ministry for the Environment, 2010). We recently developed such a method for DINCH® (di-isononyl-cyclohexane-1,2-dicarboxylate), a non-aromatic high molecular weight phthalate substitute mainly intended for sensitive applications such as toys, food contact materials and medical devices (Koch et al., 2013a,b; Schütze et al., 2012, 2014). For DPHP, however, exposure needs to be distinguishable from DIDP/DINP exposure. Previous exposure assessments based on human biomonitoring have reported the cumulative exposure (Kasper-Sonnenberg et al., 2012; Koch et al., 2009) to all phthalates containing C10 alkyl chains (DPHP, DINP, DIDP), because the complex isomeric composition of DINP/DIDP interfered with the selective detection of the DPHP specific 2-propyl-heptyl based side chain metabolites.

We used the method developed by Gries et al. (2012) to reliably detect and quantify DHPH metabolites in the presence of other DIDP/DINP metabolites. Wittassek and Angerer, 2008 showed that DPHP is metabolized similarly to DEHP (Koch et al., 2004), i.e., the monoester is formed by ester cleavage in a first step followed by extensive ω and ω-1 oxidation of the remaining single alkyl side chain. A metabolism scheme of DPHP is presented in Fig. 1. The secondary, oxidized metabolites are the predominant metabolites. The monoester MPHP is only a minor metabolite (<1% formed from the parent compound and excreted with urine), which is typical for all high molecular weight phthalates. The secondary metabolites have an added analytical benefit in that they are not subject to issues of sample contamination as described by Kato et al. (2004) and Schindler et al. (2014).

We investigated renal excretion and metabolic conversion of DPHP by measuring three oxidized metabolites of the propylheptyl side-chain, mono(propyl-6-oxo-heptyl) phthalate (oxo-MPHP), mono(propyl-6-hydroxyheptyl) phthalate (OH-MPHP) and mono (propyl-6-carboxyhexyl)- phthalate (cx-MPHxP) following oral

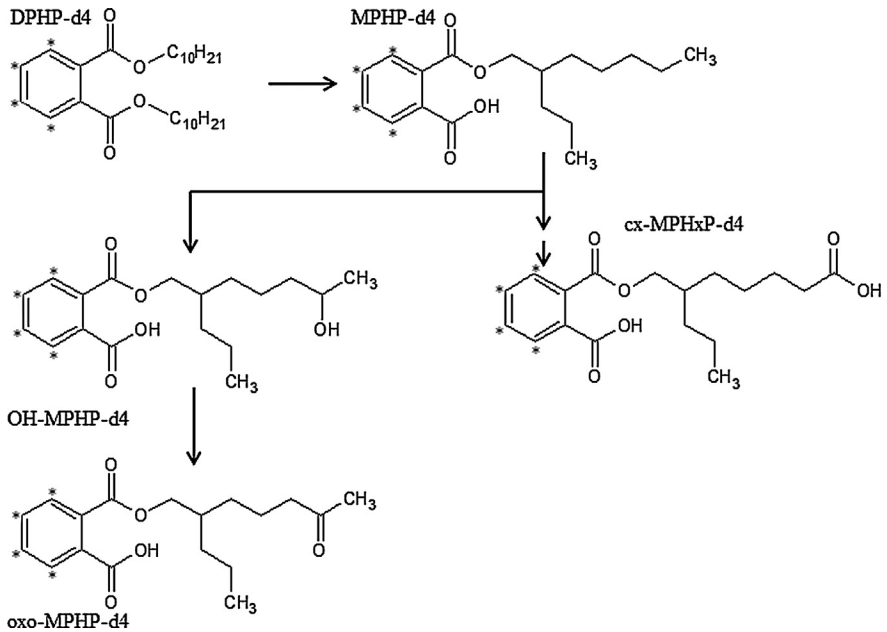

Fig. 1. Proposed human metabolism of DPHP, based upon its major 2-propylheptyl alkyl-chain isomer. The 2-propyl-heptyl side chain makes up about 90% the DPHP side chains; the remainder is made up of 2-propyl-4-methylhexyl and 2-propyl-5-methylhexyl side chains. The stars (*) depict the positions of the deuterium label.

Please cite this article in press as: Leng, G., et al., Urinary metabolite excretion after oral dosage of bis(2-propylheptyl) phthalate (DPHP) to five male volunteers – Characterization of suitable biomarkers for human biomonitoring, Toxicol. Lett. (2014), http://dx.doi.org/10.1016/j.toxlet.2014.06.035