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Di-iso-nonylphthalate (DINP) metabolites in human urine after a single oral dose of deuterium-labelled DINP

Holger M. Koch[a,b,*], Jürgen Angerer[b]

[a]*Berufsgenossenschaftliches Forschungsinstitut für Arbeitsmedizin (BGFA), Institute of the Ruhr-University Bochum, Bürkle-de-la-Camp Platz 1, D-44789 Bochum, Germany*
[b]*Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine, Friedrich-Alexander University Erlangen-Nuremberg, Schillerstrasse 25/29, D-91054 Erlangen, Germany*

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Abstract

Di-iso-nonylphthalate (DINP), a complex mixture of predominantly nine-carbon branched chain dialkyl phthalate isomers, has replaced di-(2-ethylhexyl)phthalate (DEHP) as the major plasticiser for polyvinylchloride (PVC) polymers. Similar to DEHP, DINP is a developmental and reproductive toxicant in rodents. This study for the first time describes human metabolism and elimination of DINP in a male volunteer after we applied a single oral DINP dose of 1.27 mg/kg body-weight. To avoid interference by omnipresent background exposure we used deuterium-labelled DINP. We investigated the urinary excretion of the simple monoester mono-iso-nonylphthalate (MINP) and oxidised isomers with hydroxy (OH-MINP), oxo (oxo-MINP) and carboxy (carboxy-MINP) functional groups. We used isomeric MINP and three specific oxidised isomer standards for quantification: mono-(4-methyl-7-hydroxy-octyl)phthalate (7OH-MMeOP), mono-(4-methyl-7-oxo-octyl)phthalate (7oxo-MMeOP) and mono-(4-methyl-7-carboxyheptyl)phthalate (7carboxy-MMeHP). These specific DINP metabolites are currently the only synthetic DINP metabolite standards available. Within 48 h we recovered 43.6% of the applied dose in urine as the above DINP metabolites, 20.2% as OH-MINP, 10.7% as carboxy-MINP, 10.6% as oxo-MINP and only 2.2% as MINP. Other oxidised DINP metabolites not determined in this study probably increase the share of the DINP dose excreted via urine. Elimination followed a multi-phase pattern, elimiation half-lives in the second phase (beginning 24 h post-dose) can only roughly be estimated to be 12 h for the OH- and oxo-MINP-metabolites and 18 h for carboxy-MINP metabolites. After 24 h, the carboxy-MINP metabolites replaced the OH-MINP metabolites as the major urinary metabolites. All oxidised DINP metabolites are suitable parameters for biomonitoring human DINP exposure.

Keywords: Human metabolism; Di-iso-nonylphthalate (DINP); Urine; Oxidised metabolites; Biomarkers of exposure

Introduction

Di-iso-nonylphthalate (DINP) has replaced di(2-ethylhexyl)phthalate (DEHP) as the major plasticiser for polyvinylchloride (PVC) polymers. Approximately 500,000 tonnes of DINP are annually being produced in Europe (AgPU, 2006). DINP is a mixture of esters of

*Corresponding author. Tel.: +49 234 3024647; fax: +49 234 302 4505.
E-mail address: koch@bgfa.ruhr-uni-bochum.de (H.M. Koch).

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phthalic acid with C8–C10 alkyl alcohols of different chain lengths and branching distributions. This is a major difference to DEHP which is a single chemical compound produced by esterifying 2-ethylhexanol with phthalic anhydride. In DINP 1 (CAS 68515-48-0) 75% of the ester chains are made up by C9-alcohols, the remaining 25% are made up by alcohols of other molecular weights (EC JRC, 2003). DINP 2 (CAS 28553-12-0) consists solely of C9 alcohols in the ester chain, however, of different branching distributions compared to DINP 1. Other types of DINP have been produced but are stated to have vanished from the market (EC JRC, 2003). The overall composition of the different DINP mixtures/types with their wide variety of isomers and homologues remains obscure. Only recently we identified the major alkyl side chain of both DINP 1 and DINP 2 to be the 4-methyloctyl chain with 20.7% in DINP 2 and 8.7% in DINP 1 (Koch et al., 2006c). DINP risk assessments in the USA and in Europe did not distinguish between the DINP types mainly because most of the underlying studies did not define the DINP-type investigated (EC JRC, 2003; Kavlock et al., 2002; EFSA, 2005).

Exposure to DINP can occur both through occupation and environment. Currently, exposure data, either by ambient monitoring or human biomonitoring is sparse (EC JRC, 2003; Kavlock et al., 2002; Wormuth et al., 2006). In human biological monitoring, by analysing specific metabolites in urine, we can determine the individual internal exposure to DINP covering all possible (known and unknown) sources of exposure (Koch et al., 2006c). Using specific metabolites there is no risk of external contamination through the omnipresence of DINP. When metabolite conversion factors (describing the quantity of renal elimination of specific metabolites in urine in relation to the oral dose) are known, one can estimate the external dose of this substance based on the urinary excretion of its metabolites. Therefore, knowledge of DINP's metabolism and elimination in humans has tremendous importance for human biomonitoring studies in general and for dose estimations with subsequent risk assessments in special. Dose estimations based on metabolite levels in individual urine samples and in urine samples of larger scale human biomonitoring studies have proven to be an excellent tool to determine the overall exposure to omnipresent environmental contaminants and thus have proven to be a valuable tool in the risk assessment of DEHP and other phthalates (Angerer and Weiss, 2002; CSTEE, 2004; Kavlock et al., 2006; David, 2000; Kohn et al., 2000; Koch et al., 2003a, b, 2006a, b; Marsee et al., 2006; Calafat et al., 2006; Angerer et al., 2006; Wittassek et al., 2006a, b). This is especially the case when ambient monitoring data is sparse, knowledge of sources of exposures are inconsistent or unknown and different sources contribute to exposures increments spanning several orders of magnitude (Paustenbach and Galbraith, 2006; Wormuth et al., 2006).

In rodents, DINP exhibits toxic effects on liver and kidney (EC JRC, 2003; Kavlock et al., 2002; EFSA, 2005; Kaufmann et al., 2002). Some of these effects are related to peroxisome proliferation which is judged to be of subordinate relevance for humans. For non-peroxisomal proliferation-related chronic hepatic and renal effects a non-observed adverse effect level (NOAEL) of 15 mg/kg body-weight/day has been derived (EFSA, 2005). Perinatal exposure to DINP also alters sexual differentiation in male rats, probably by inhibiting testicular testosterone synthesis. Effects like nipple retention and testis atrophy were comparable to effects caused by DEHP and di-*n*-butylphthalate (DnBP). However, no NOAEL has been derived for these effects yet (Gray et al., 2000).

McKee et al. (2002) have shown in rats, that approximately 50% of an oral DINP dose is excreted renally, mainly as ω-, ω-1- and β-oxidised metabolites of the monoester MINP (mono-iso-nonylphthalate). Quantitative aspects of the formation of these metabolites as well as their elimination kinetics have not been investigated. The proposed pathway of DINP metabolism from its major 4-methyloctyl side chain to its oxidised metabolites mono-(4-methyl-7-hydroxy-octyl)phthalate (7OH-MMeOP), mono-(4-methyl-7-oxo-octyl)phthalate (7oxo-MMeOP) and mono-(4-methyl-7-carboxyheptyl)phthalate (7carboxy-MMeHP) is shown in Fig. 1. We have these oxidised metabolites and the simple monoester MINP available as analytical reference standards both non-labelled and deuterium labelled. To our knowledge these are the first oxidised DINP metabolite standards available for biomonitoring purposes. We determined these metabolites in urine after a male human volunteer ingested a single dose of deuterium labelled DINP 2 applying a high-performance liquid chromatography tandem mass spectrometry (LC/LC–MS/MS) method (Koch et al., 2006c). Due to DINP's isomeric nature, a wide variety of isomeric metabolites is excreted in urine. Therefore, we quantified the sum of the simple MINP metabolites based on an isomeric MINP standard, the sum of the metabolites with a carboxylic acid functional group (carboxy-MINP) based on the specific standard 7carboxy-MMeHP, the sum of the metabolites with a hydroxy functional group (OH-MINP) based on the specific standard 7OH-MMeOP and the sum of the metabolites with an oxo functional group (oxo-MINP) based on the specific standard 7oxo-MMeOP, respectively (Koch et al., 2006c).

Materials and methods

Chemicals

Di-iso-nonylphthalate-3,4,5,6 D4 (D4-DINP) was custom synthesised from Dr. V. Belov of the Max