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Evaluation of exposure to phthalate esters and DINCH in urine and nails from a Norwegian study population

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ABSTRACT

Phthalate esters (PEs) and 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH) used as additives in numerous consumer products are continuously released into the environment, leading to subsequent human exposure which might cause adverse health effects. The human biomonitoring approach allows the detection of PEs and DINCH in specific populations, by taking into account all possible routes of exposure (e.g. inhalation, transdermal and oral) and all relevant sources (e.g. air, dust, personal care products, diet). We have investigated the presence of nine PE and two DINCH metabolites and their exposure determinants in 61 adult residents of the Oslo area (Norway). Three urine spots and fingernails were collected from each participant according to established sampling protocols. Metabolite analysis was performed by LC-MS/MS. Metabolite levels in urine were used to back-calculate the total exposure to their corresponding parent compound. The primary monoesters, such as monomethyl phthalate (MMP, geometric mean 89.7 ng/g), monoethyl phthalate (MEP, 104.8 ng/g) and mono-n-butyl phthalate (MnBP, 89.3 ng/g) were observed in higher levels in nails, whereas the secondary bis(2-ethylhexyl) phthalate (DEHP) and DINCH oxidative metabolites were more abundant in urine (detection frequency 84–100%). The estimated daily intakes of PEs and DINCH for this Norwegian population did not exceed the established tolerable daily intake and reference doses, and the cumulative risk assessment for combined exposure to plasticizers with similar toxic endpoints indicated no health concerns for the selected population. We found a moderate positive correlation between MEP levels in 3 urine spots and nails (range: 0.56–0.68). Higher frequency of personal care products use was associated with greater MEP concentrations in both urine and nail samples. Increased age, smoking, wearing plastic gloves during house cleaning, consuming food with plastic packaging and eating with hands were associated with higher levels in urine and nails for some of the metabolites. In contrast, frequent hair and hand washing was associated with lower urinary levels of monoisobutyl phthalate (MiBP) and mono(2-ethyl-5-hydroxyhexyl) phthalate (5-OH-MEHP), respectively.

Abbreviations: PEs, phthalate esters; PCPs, personal-care products; DINCH, 1,2-cyclohexane dicarboxylic acid diisononyl ester; DEHP, bis(2-ethylhexyl) phthalate; DPHP, bis (2-propylheptyl) phthalate; DiNP, diisononyl phthalate; DiDP, diisodecyl phthalate; SI, supplementary information material; OH-MINCH, cyclohexane-1,2-dicarboxylic mono hydroxyisononyl ester; oxo-MINCH, cyclohexane-1,2- dicarboxylic mono oxoisononyl ester; cx-MINCH, cyclohexane-1,2-dicarboxylic mono carboxyisooctyl ester; DI, daily intake rate; A-TEAM, Advanced Tools for Exposure Assessment and Biomonitoring; MS, mass spectrometer; MRM, multiple reaction monitoring; DF, detection frequency; LOQ_m, method limit of quantification; ICC, intra-class correlation coefficient; CI, confidence interval; IQR, interquartile range; MW, molecular weight; HQ, hazard quotient; TDI, tolerable daily intake; RfD, reference dose; EFSA, European Food Safety Authority; USEPA, U.S. Environmental Protection Agency; HI, Hazard-Index; DiBP, diisobutyl phthalate; DnBP, di-n-butyl phthalate; BBzP, benzyl butyl phthalate; PVC, polyvinyl chloride; MEHP, mono(2-ethylhexyl) phthalate; MMP, monomethyl phthalate; MPHP, mono(2-propylheptyl) phthalate; 5-OH-MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; 5-oxo-MEHP, mono(2-ethyl-5-oxohexyl) phthalate; 5-cx-MEPP, mono(5-carboxy-2-ethylpentyl) phthalate; GM, geometric mean; MEP, monoethyl phthalate; MnBP, mono-n-butyl phthalate; MiBP, monoisobutyl phthalate; DEP, diethyl phthalate; NOAEL, Non-Observed-Adverse-Effect-Level; AFs, assessment factors; MBzP, monobenzyl phthalate; REACH, Registration Evaluation Authorization and Restriction of Chemicals

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1. Introduction

PEs are ubiquitous environmental contaminants due to their wide use in the manufacturing of polymeric materials and various consumer products. Low molecular weight PEs are used as industrial solvents, lubricants, and as components in PCPs and air fresheners (Dodson et al., 2012). High molecular weight PEs are commonly used as plasticizers, imparting better flexibility and durability in everyday consumer products, such as PVC flooring, adhesives, food packaging, clothing, toys, etc. (Hauser and Calafat, 2005).

Since 2002, the alternative plasticizer DINCH which was especially developed for applications with close human contact, replaced many of the higher molecular weight PEs in food packaging materials, medical devices, children items and toys, because it is less toxic due to the non-aromatic structure (Crespo et al., 2007; Biedermann-Brem et al., 2008; SCENIHR, 2015). The global production volumes of PEs can reach 10^6 t/year (Koch and Calafat, 2009), while in the European economic area, DEHP which has a production volume up to 10^5 t/year and DINCH with more than 10^4 t/year, are currently the most commonly used plasticizers (ECHA, 2016).

PEs and DINCH are released from the products by evaporation, migration, abrasion and diffusion, and the human exposure to these pollutants occurs mainly via ingestion (eg. food, hand to mouth contact, unintended dust ingestion and toddlers suckling on plastic materials), inhalation (eg. air and respiratory dust fraction) and transdermally (eg. direct contact with plastics, personal care products and dust) (Wormuth et al., 2006; Heudorf et al., 2007; Wittassek and Angerer, 2008; Koch et al., 2013a; Weschler et al., 2015). Although these chemicals are rapidly metabolized and excreted by humans mainly through urine, they also have a pseudo-persistent profile due to considerable continuous exposure (Mackay et al., 2014; Bui et al., 2016), which raises concern about the endocrine disruption potential and reproductive toxicity for humans (Sharpe, 2001; Duty et al., 2003; Sharpe and Irvine, 2004; Swan et al., 2005). The most vulnerable population groups are pregnant women and children due to their small body mass and high exposure of the embryo/fetus during intra-uterine life (National Research Council, 2008).

Once the parent compounds enter the human body, they are rapidly metabolized to hydrolytic monoesters (primary metabolites). In the case of high molecular weight phthalates, such as DEHP and DPHP (Table SI1), but also for DiNP and DiDP, the primary metabolites are further oxidized to secondary/oxidative metabolites (Koch et al., 2005; Koch and Angerer, 2007; Silva et al., 2007a; Gries et al., 2012; Leng et al., 2014). Monoesters and oxidative metabolites can be excreted in urine unchanged, or they can undergo phase II biotransformation to produce glucuronide conjugates which have higher water solubility than the phase I primary and secondary metabolites, facilitating excretion (Calafat et al., 2006). The ratio between free monoesters and glucuronide conjugates excretion varies among different PEs (Hauser and Calafat, 2005). Analogously to high molecular weight PEs, DINCH secondary oxidative metabolites, OH-MINCH, oxo-MINCH and cx-MINCH have been identified as suitable urinary biomarkers for assessing exposure to DINCH (Koch et al., 2013b).

Levels of PE metabolites in urine have been extensively explored (Silva et al., 2007b; Wittassek et al., 2011; Den Hond et al., 2015; Lioy et al., 2015). However, recently PE metabolites have been successfully quantified in other non-invasive matrices, such as nails, which indicates that a part of the PE metabolites might end up in nails, instead of being rapidly excreted through urine (Alves et al., 2016a; 2016b). The advantages of introducing nails in the field of human biomonitoring are cost reduction of sampling procedures, less storage, sample stability and possible simplification of the ethical approval and recruitment. Also, nails reflect a wider exposure window (weeks to months) than urine ($\leq$48 h) (Alves et al., 2014).

The aim of this study is to evaluate the human exposure to PEs and DINCH through determination of their metabolites in urine (where metabolism and excretion is well understood), and present the metabolite levels in nails. Three urine spot samples (within 24 h) and fingernails from both hands were collected from a Norwegian study population (N=61). We have assessed the correlations of compound concentrations between urine and nails, as well as the relationships with different sociodemographic and lifestyle characteristics. Finally, based upon the urinary levels, we calculate the DI and perform a cumulative risk assessment for accounting effects of combined chemical exposures. Overall, we aim to provide a comprehensive evaluation of the exposure for the included Norwegian population.

2. Materials and methods

2.1. Study population and sample collection

The present study is part of the "A-TEAM" project, where a well-characterized human cohort consisted on study population of 61 adults (age: 20–66; gender: 16 males and 45 females) living in Oslo area (Norway) is used, in order to enhance knowledge for a variety of aspects related to internal and external exposure to selected consumer chemicals. The sampling campaign was conducted during winter 2013–2014, where indoor environment, dietary and biological samples were collected from the participants and their households (Papadopoulou et al., 2016).

Briefly, all participants were asked not to cut their fingernails for 2–3 weeks prior to the sample collection, and they were advised to remove any nail polish, dirt, debris and artificial nails before clipping their fingernails. One composite sample (both hands) per participant was collected in a paper envelope between the two sampling days. During the 2-day-sampling, 3 urine spot samples (afternoon – day 1, morning – day 2 and afternoon – day 2) were collected by each participant in 500 mL high-density polyethylene (HDPE) bottles with screw caps and security lids. Before sampling, the bottles were rinsed with methanol. All samples were stored inside a freezer (-20 °C) until analysis. The sampling campaign was approved by the Regional Committees for Medical and Health Research Ethics in Norway (Case number 2013/1269), and all participants completed a written consent form prior to participation.

2.2. Chemical analysis

2.2.1. Extraction from urine and nails

All urine and nail samples were spiked with 5 ng of IS prior the extraction. The nails extraction protocol applied in our study was recently developed by Alves et al. (2016b; 2016c) using a low sample amount ($\simeq$30 mg) and described in detail in SI. The levels of PE and DINCH metabolites were expressed as ng/g nail.

At the same time, PE and DINCH metabolites were determined in three urinary spots (within 24 h) collected per participant using direct analysis as it is described by Servaes et al. (2013). In summary, the deconjugation of the PE and DINCH glucuronides was performed via enzymatic cleavage (E. coli K12), and at the end, an aliquot was taken and injected in LC-MS/MS. Creatinine content was measured in all urine spots via a creatinine (urinary) colorimetric assay kit. The levels were expressed as μg metabolite per g creatinine ($\mu g/g_{crea}$).

Information on chemicals used during analysis can be found in SI.