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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.

2.2.2. LC-MS conditions

The instrumental analysis was performed by Ultra Performance Liquid Chromatography (UPLC) coupled to Water Xevo TQ-S tandem mass spectrometer (MS, Waters, Milford, MA, USA) operating in negative electrospray ionization (ESI-). Chromatographic separation and conditions used were described by Servaes et al. (2013). The MS parameters were as follows: cone voltage varied between 4 to 44 V, depending on the analyte. The collision cell energy varied between 10 to 28 eV (Table SI2). The electrospray source block and the desolvation temperature were 120 °C and 350 °C, respectively. The argon collision gas flow was kept constant at 0.25 mL/min. The cone and desolvation nitrogen gas flow were set at 50 L/h and 800 L/h, respectively. The characteristic precursor and daughter ions that were selected for detection of PE and DINCH metabolites in MRM mode are presented in (Table SI2). The two resulting daughter ions were used for quantification (MRM 1) and confirmation (MRM 2).

2.3. Data and statistical analysis

All metabolite concentrations in the three urine spots and in nail samples were summarized by standard descriptive statistics. DFs were also reported for all measured metabolites. Concentrations of urinary phthalate metabolites were adjusted to creatinine content, in order to account for the urinary dilution (Blount et al., 2000). Further, statistical analyses were limited to metabolites with DF≥70%. Values below the LOQ_m were replaced with $LOQ_m/2$. All metabolites failed the Shapiro-Wilk test of normality and therefore the natural logarithm of the values was used in subsequent analyses.

We assessed the correlations between different PE metabolites in urine (average of three spots) by Spearman correlation coefficient, for non-normal distributed data. We evaluated the within-subject consistency of urinary PE metabolites in the three urine spots by calculating the ICCs at 95% CI using a one-way mixed model with random intercept. An ICC value near one indicates low within-subject variation and good reliability measurements of metabolites' concentrations. Spearman correlations between PE metabolites in three urine spots and nails were assessed.

The differences of the crude (non ln-transformed) PE metabolites measured in urine (average of three spots) and nails by categories of lifestyle and sociodemographic characteristics were reported (in medians; IQR) and evaluated, using the non-parametric Mann-Whitney test (for differences by two categories) or the Kruskal-Wallis test (for differences by three categories). In addition, the differences in PCPs use and consumed foods in plastic packaging by day were assessed by urine spot rather than for the average of the 3 urine samples, since relationships can vary according to the time of urine collection. The observed relationships were graphically presented in bar charts.

To explore the associations between all studied socio-demographic and lifestyle characteristics, and the individual urinary phthalate metabolites, we applied multivariate linear mixed models with a random intercept per participant and a fixed effect on all other characteristics. Similarly, multivariate linear regression models were used to study the association between different characteristics and concentrations of phthalate metabolites in nails. The relative change (and 95% CI) of individual phthalate metabolites per unit increase or per category of the different characteristics were calculated as: $(\exp\ \beta - 1) \times 100$, where β is the linear regression coefficient. Significant correlations, differences and associations were considered if $p < 0.05$, while for the identification of potential determinants of phthalate metabolite concentrations associations with $p < 0.2$ were also reported.

All statistical analyses were performed using STATA 14 (StataCorp, College Station, TX) and SPSS (SPSS for Windows, version 22.0, SPSS, Chicago, IL).

2.4. Daily intake estimates and cumulative risk assessment

The estimated DIs of PEs and DINCH were calculated based on the urinary creatinine corrected concentrations for each metabolite, the 24 h urinary creatinine excretion ($CE_{smoothed}$) and the existing 24 h human fractionary excretion factors (F_{UE}), which were derived after oral doses of the parent compounds (Supplementary information, Table SI1). The following two equations were used for the calculations: (Mage et al., 2004; Koch et al., 2003; Frederiksen et al., 2013)

$$CE_{smoothed}(g/day) = \mathrm{A} \times [(140 - age(year)] \times BW(kg)^{1.5} \times height(cm)^{0.5} \times 10^{-6} \quad (1)$$

$$DI\left(\frac{\mu g}{kg_{BW} \times day}\right) = \frac{\Sigma\left[\frac{UE_{metabolitecrea}(\mu g / g_{crea})}{MW_{metabolite}(\mu g / \mu mol)}\right] \times MW_{Phthalate}(\mu g/\mu mol) \times CE_{smoothed}(g/day)}{\Sigma F_{UE} \times BW(kg)} \quad (2)$$

where in Eq. (1), 140 and A (1.93 for males and 1.64 for females) are constants simplifying for the body surface area; the individual height (cm), body weight (Kg) and age for each participant, while in Eq. (2), UE is the concentration of the metabolite in urine (creatinine adjusted); $MW_{Phthalate}$ is the molar mass of the parent diester; $MW_{metabolite}$ is the molar mass of the corresponding metabolite.

The HQs calculated according to Eq. (3) allowed us to compare the estimated DIs based on urinary PE and DINCH concentrations for the Norwegian study population, with acceptable exposure levels in absence of significant risk for human health, expressed as TDI and RfD established by the EFSA or the USEPA.

$$HQ = \frac{DI}{TDI\ or\ RfD} \quad (3)$$

Moreover, cumulative risk assessment from combined chemical exposure doses was performed by using the HI, which is expressed by:

$$HI = \sum_{i=1}^{n} HQ_i \quad (4)$$

where n is the number of substances, and values below 1 are considered safe (Kortenkamp and Faust, 2010). Only the potential anti-androgenic chemicals, DiBP, DnBP, BBzP and DEHP, were included in the HI calculation.

2.5. Potential predictors of exposure

Information of socio-demographic and lifestyle characteristics that might affect the concentrations of PE and DINCH metabolites in adults was collected by the A-TEAM questionnaires. Participants were asked to report their gender, age, educational level, and smoking status (yes/no) by the time of their participation. In addition, they reported in questionnaires whether they are coloring their hair (yes/no), the average weekly frequency of washing their hair, the average daily frequency of washing their hands and eating with hands (e.g. eating sandwiches or other snacks without using cutlery), whether they are using gloves when cleaning their house (yes/no) and other questions regarding the materials on the

walls and floors of their house. In our study we were particularly focused on the questions regarding wall and flooring materials including PVC; hence we formed the variable "PVC in walls/floors" as an aggregate of vinyl or flooring in material in walls or floors of different rooms in the house. In the end of the 2-days sampling period, participants reported their use and frequency of use of a list of PCPs the last 24 h, including: hand soap, hand cream, shower soap or gel, lotion, face moisturizer, deodorant, perfume, nail polish, nail polish removal, shampoo, conditioner, hairspray, hair gel/wax, lipstick, foundation, mascara/eyeliner. The median number of total used PCPs was 6 (5th percentile: 3 products; 95th percentile: 10 products). The total number of individual PCPs and total times of PCPs applied in hands during the last 24 h were categorized in two groups (≤ 5 PCPs vs. >5 PCPs and <5 times/day vs. ≥ 5times/day) and used in further analyses. Additionally, during the 2-days sampling period participants were asked to report all the foods and drinks they have been consuming and also the packaging, cooking and serving materials (Papadopoulou et al., 2016). The total number of foods wrapped/enclosed in plastic packaging the two consecutive days and the average number were calculated and used in our analyses (<10 foods/day vs. ≥ 10 foods/day). We assumed that participants had the same daily routines over a long period of time, which allowed the correlation of short term reports (eg. use and frequency of PCPs, food behavior, etc.) with urine and nails, even though they represent different exposure periods.

3. Results and discussion

Data on PE and DINCH metabolites in urine spots samples (<24 h) and finger-nails from the Norwegian study population are presented in Table 1.

3.1. Urine

3.1.1. Exposure levels

Most of the urinary metabolites were frequently detected (DF $>85\%$) in almost all urine spots, except MEHP (DF of 20–28%), MMP (DF of 15–30%) and MPHP (DF of 0–3%). In fact, MEHP is usually one of the minor DEHP metabolites detected in urine (Preau et al., 2010; Dirtu et al., 2013; Langer et al., 2014; Valvi et al., 2015; Alves et al., 2016a; 2016b), due to its fast metabolism into secondary oxidative metabolites such as 5-OH-MEHP, 5-oxo-MEHP or 5-cx-MEPP which are considered more suitable biomarkers of DEHP (Preuss et al., 2005). Similarly, MPHP is also a minor DPHP metabolite according to Leng et al. (2014) (less than 1% is excreted in urine), however the presence of the other DPHP metabolites were not investigated. MMP is a metabolite of DMP that was seldom detected in urine of the Norwegian study population. This is in accordance with previous studies in Western populations where MMP is also one of the minor PE metabolites excreted in urine (Hogberg et al., 2008; Kasper-Sonnenberg et al., 2012). In contrast, studies in China have frequently detected MMP in much higher concentrations (Guo et al., 2011; Gao et al., 2016). DINCH secondary metabolites were in relatively low concentrations, however the high detection frequencies (DF $\geq 84\%$) reflect that these metabolites are most suitable biomarkers of DINCH exposure (Koch et al., 2013b).

3.1.2. Comparison with data on different population groups worldwide

The urinary levels for all PEs in the A-TEAM human cohort were comparable, but in most cases lower than in other biomonitoring studies around Europe, North America and Australia (Table SI3). Some of the lower molecular weight PE metabolites for instance, MEP, MnBP and MiBP were two to three fold lower in our study population when compared to other (Wittassek et al., 2007; Saravanabhavan et al., 2013; Larsson et al., 2014; Gomez Ramos et al., 2016), except in Belgium (Dewalque et al., 2014b) and Austria

Table 1
Descriptive statistics for PE and DINCH metabolites in three urinary spots (creatinine adjusted- μg/g creatinine) and nails (ng/g) of 61 Norwegian adults.

Metabolites	Spot 1 (Afternoon urine-Day 1) ($\mu g/g_{crea}$)		Spot 2 (Morning urine-Day 2) ($\mu g/g_{crea}$)		Spot 3 (Afternoon urine-Day 2) ($\mu g/g_{crea}$)		Nails[a] (ng/g)	
	DF (%)	GM (25th; 50th; 95th)	DF (%)	GM (25th; 50th; 95th)	DF (%)	GM (25th; 50th; 95th)	DF (%)	GM (25th; 50th; 95th)
MMP	30	0.05 (–; –; 5.3)	15	0.02 (–;–; 2.3)	25	0.04 (–; –; 2.9)	51	89.7 (45.7; 149.3; 814.1)
MEP	100	24.2 (5.7; 17.5; 250.5)	100	31.3 (10.6; 33.1; 309.9)	100	23.0 (7.7; 22.3; 189.3)	100	104.8 (38.9; 81.9; 1873.0)
MiBP	98	12.8 (7.2; 12.6; 50)	100	17.0 (11; 15.3; 49.9)	100	13.0 (7.7; 11.6; 47.4)	93	19.9 (16.9; 17.9; 69.8)
MnBP	98	13.4 (7.2; 11.4; 77.5)	95	15.5 (9.7; 13.3; 93.1)	80	9.0 (4.8; 9.1; 38.8)	100	89.3 (59.4; 56.0; 3272.3)
MBzP	100	3.5 (1.8; 3; 15.5)	100	4.2 (2.1; 4.4; 21.4)	100	3.3 (1.8; 3.3; 26.1)	92	2.6 (1.5; 1.7; 41.5)
MPHP	0	–	0	–	3	–	37	49.0 ($<LOQ_m$; 59.8; 775.9)
MEHP	20	– (–;–; 14.7)	23	– (–; –; 11.5)	28	– –; –; 17.9)	100	129.3 (62.3; 103.3; 682.5)
5-OH-MEHP	100	5.2 (2.8; 4.5; 24.1)	100	5.1 (2.9; 4.8; 20.3)	100	5.7 (3.2; 4.9; 30.6)	68	2.6 (0.65; 1.1; 854.6)
5-oxo-MEHP	100	5.3 (2.8; 4.7; 19.1)	98	4.5 (2.8; 5; 22.1)	100	5.4 (3; 4.8; 38)	29	0.21 ($<LOQ_m$; 0.72; 1.9)
OH-MINCH	85	0.32 (0.19; 0.5; 12)	90	0.30 (0.16; 0.39; 6.9)	84	0.20 (0.08; 0.24; 6.5)	19	0.10 (0.04; 0.25; 0.29)
cx-MINCH	85	0.29 (0.14; 0.43; 13.4)	85	0.23 (0.15; 0.41; 10.3)	85	0.24 (0.07; 0.43; 7.8)	25	0.15 ($<LOQ_m$; 0.84; 2.6)

DF-detection frequency (%); GM-geometric mean; 25th, 50th and 95th percentiles.
- Not calculated, levels were below method's limit of quantification ($<LOQ_m$) before the creatinine adjustment.
[a] Levels in nail samples for 59 participants.

(Hartmann et al., 2015) where the levels were comparable.

The GM levels for MMP in our study were clearly lower than in Belgian sub-populations (e.g. obese individuals) (Dirtu et al., 2013), and pooled urine samples in Australia (Gomez Ramos et al., 2016).

The high MEP concentrations in U.S.A., Australia and Greece (CDC, 2015; Myridakis et al., 2015; Gomez Ramos et al., 2016) compared to Norway (our data) might be due to the fact that DEP's presence in PCPs is not regulated. Actions to alert populations, such as the Campaign for Safe Cosmetics (www.safecosmetics.org) created since 2014, have led to DEP metabolite reduction over the last decade (Zota et al., 2014). The absence of DEP regulation in E. U. or U.S.A. is in contrast with the accomplished efficient implemented restriction of other PEs (e.g. DnBP, BBzP and DEHP) for the incorporation in cosmetics, toys or food contact materials (Directive, 2005/84/EC, 2007/19/EC; Ventrice et al., 2013). DEHP metabolites among the different population groups have been remarkable reduced comparing to >10 years ago (Becker et al., 2004; CDC, 2015).

The levels of OH- and oxo- MINCH metabolites for this Norwegian study population are comparable to the data obtained from American and German adults in 2012 (Silva et al., 2013; Schutze et al., 2014). In the same year, Fromme et al. (2016) observed the highest concentration values in children attending daycare centers, which indicates that the increasing production volumes of DINCH implies increased use in products, and thus it has a direct impact on human exposure. This suggests that the overall human exposure to substitute plasticizers should be more frequently and in depth investigated, and that studies should not only focus on monitoring the “classical” PE additives.

3.1.3. Daily intake estimates and cumulative risk assessment

The TDI (μg/kg body weight/day) established by EFSA and the RfDs used by USEPA are presented in Table 2. Additionally, because there is no established TDI for DMP, it was determined by the following equation:

$$\mathrm{TDI}=\frac{\mathrm{NOAEL}}{\mathrm{AFs}} \quad (5)$$

considering NOAEL of 750 mg/kg bw/day (Gray et al., 2000) and AFs of 2 for extrapolation from sub-chronic to chronic exposure, 4 for rats compared to humans, 2.5 for inter-species variation for remaining differences, 10 for intra-species variation for general population, and an additional 10-fold factor to protect sensitive human sub-populations (ECHA, 2012). The oral reference dose of 700 μg/kg bw/day for the non-phthalate alternative plasticizer DINCH was derived from Bhat et al. (2014) by using a human equivalent in rodents with a total uncertainty factor of 30. Also, the TDI for DnBP was used to calculate DI and HQ for DiBP rely on the suggestion that both isomeric forms of dibutyl phthalate (DBP) can contribute to adverse health effects (Borch et al., 2006; Frederiksen et al., 2013). The estimated daily intake rates (μg/kg bw/day) are presented in Table 2.

All estimated DIs were below the acceptable doses, while large differences between the median and 95th percentile for the DIs were observed for DEP and DINCH. Yet all the participants had low HQs for all chemicals (Table 2). The highest participant's HQ was 0.65 regarding the exposure to DnBP, but still it was below the value of 1 (Fig. 1a). The HI_{median} of 0.11 based on the TDI, and 0.04 based on RfD AA indicated a low anti-androgenic risk potential (Fig. 1), even when we performed the same cumulative risk assessment for the worst case scenario by using the max DI ($HI_{max}=0.78<1$).

The overall estimated exposure for women was twice that for men, 3.13 and 1.67 μg/kg b.w./day, respectively. In general, the low estimated DI for Norwegians, especially to more restricted chemicals with lower TDIs, such as DnBP, DiBP and DEHP is encouraging. The calculated DI in China (Guo et al., 2011) and Greece (Myridakis et al., 2015) were at least 3 times higher than in Norway (our study). Similar HI_{median} was found in Austria (i.e. 0.12) and USA (i.e. 0.14) (Christensen et al., 2014), while for the Belgian adults (Dewalque et al., 2014a), it was higher with some individuals exceeding the established limit. However, when TDI and RfD values are established, uncertainty factors are added thus meaning that exceeding a HI of 1 does not necessarily imply a risk. In principle, a $HI<1$ means no risk, providing that all substances with similar modes of action/end-points are considered. In the current cumulative risk assessment, the PEs considered are not the only chemicals with potential anti-androgenic or endocrine disruption properties, thus a more accurate HI should take into account the additional effects of other suspected chemicals (e.g. bisphenol A, parabens, triclosan, polychlorinated biphenyls, etc.), which exhibit similar adverse health effects (Maffini et al., 2006; Kortenkamp and Faust, 2010; Schug et al., 2011).

3.1.4. Relationships of PE and DINCH metabolites in urine

The concentrations of low molecular weight PEs (MEP, MiBP, MnBP and MBzP) in the morning urine spot were higher than in the two afternoon urine spots, though the difference was statistically significant only for MnBP and MiBP (p-value <0.05 for paired-samples by Wilcoxon Signed rank test, data not shown).

Strong correlations ($r=0.86$, $p<0.001$) were observed between DEHP oxidative metabolites (Table 3), which has been supported by several studies (Hines et al., 2009; Dewalque et al., 2014b; Alves et al., 2016b). Furthermore, the two oxidative DINCH metabolites (OH-MINCH and cx-MINCH) were strongly correlated ($r=0.82$, $p<0.001$), still OH-MINCH (ICC=0.47) had lower reproducibility

Table 2
Daily intake estimates (DIs) and hazard quotients (HQs) for risk assessment using a creatinine-based model calculation (values are presented as median; 95th percentile in μg/kg bw/day).

Chemical	DI	HQ	Established tolerable value		Reference
DMP	0.01; 0.18	1.0E−04; 5.0E−04	375*	RfD*	(Gray et al., 2000; ECHA, 2012)
DEP	0.89; 11.9	1.1E−03; 1.5E−02	800	RfD	(Brown et al., 1978; USEPA, 1987)
DiBP	0.52; 2.7	0.05; 0.27	10	TDI	(EFSA, 2005b;Borch et al., 2006)
		2.6E−03; 1.4E−02	200	RfD Anti-androgenic	
DnBP	0.39; 2.3	0.04; 0.23	10	TDI	
		3.9E−03; 2.3E−02	100	RfD Anti-androgenic	
BzBP	0.13; 0.56	3.0E−04; 1.1E−03	500	TDI	(EFSA, 2005c)
		6.0E−04; 2.8E−03	330	RfD Anti-androgenic	
DEHP	0.76; 3.3	0.02; 0.07	50	TDI	(EFSA, 2005a)
		0.03; 0.11	30	RfD Anti-androgenic	
DINCH	0.23; 4.0	3.0E−04; 5.8E−03	700	RfD	(Bhat et al., 2014)

TDI-tolerable daily intake; RfD-reference dose (μg/kg b.w./day).
*Calculated.

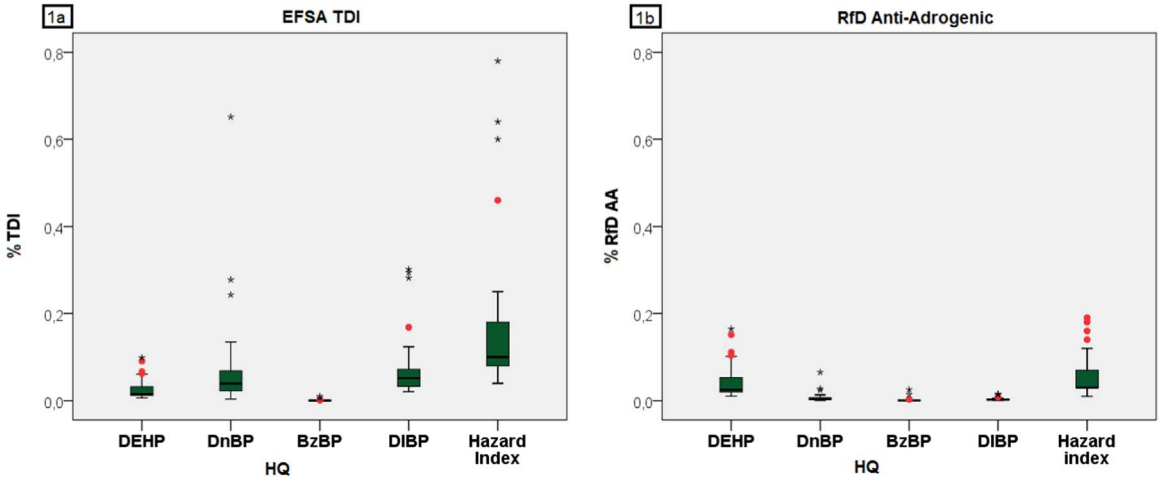

Fig. 1. Hazard index (HI) for the Norwegian human cohort based on TDI or RfD AA (EFSA, 2005a, 2005b, 2005c) for chemicals with similar adverse health effects.

Table 3
Correlations between mean PE and DINCH metabolites ($\mu g/g_{crea}$) of three urine spots and ICCs for creatinine-adjusted metabolites in all urine samples for 61 Norwegian adults.

3 urine spots	5-OH-MEHP	5-oxo-MEHP	MnBP	MBzP	MEP	MiBP	OH-MINCH	ICC[a]	95%CI
5-OH-MEHP								0.14	0.00,0.31
5-oxo-MEHP	**0.86****							0.14	0.00,0.31
MnBP	**0.52****	**0.54****						0.36	0.20,0.52
MBzP	0.41*	**0.49****	**0.55****					0.35	0.19,0.51
MEP	0.26*	0.23	**0.42****	0.19				0.68	0.56,0.78
MiBP	**0.55****	**0.54****	**0.59****	**0.47****	0.30*			0.34	0.19,0.51
OH-MINCH	0.40*	0.28*	**0.27****	0.32*	0.02	0.27*		0.47	0.32,0.62
cx-MINCH	0.33*	0.18	**0.29****	0.32*	-0.09	0.25*	**0.82****	0.66	0.53,0.76

[a] ICCs were calculated by linear mixed effect models using Ln-transformed creatinine-adjusted data. Values range from 0 (i.e. no reproducibility of the same measurement within a subject) to 1 (i.e. perfect reproducibility). Spearman coefficients are presented in Ln-transformed data.

* p-value < 0.05.

** p-value < 0.001.

between urinary measurement than cx-MINCH (ICC=0.66, Table 3).

Moderate to weak significant positive correlations were found between all PE metabolites, with MEP having the weakest correlation with other metabolites. On the other hand, MEP had the highest ICC among the selected PEs indicating high reproducibility between measurements in urine spots. MEP is mainly formed by a specific source of DEP exposure, since it is widely used in cosmetics (eg. perfumes, nail polish, body lotions, etc.), drug coatings, air refreshers and insecticides, whereas other PE additives are not so commonly used (Koo and Lee, 2004; Duty et al., 2005; Hauser and Calafat, 2005; Hubinger and Havery, 2006). This low intercorrelation between MEP and other PEs has been also confirmed in other studies (Frederiksen et al., 2010, 2011). Preau et al. (2010) also found low ICCs for 5-OH-MEHP (ICC=0.25) showing a high intra-individual variability, while for MEP (ICC=0.91) this effect was negligible.

Low ICCs were found for MnBP, MBzP, MiBP and DEHP metabolites, reflecting high within-person variation. Fromme et al. (2007) also measured the ICCs between the levels of ten PE metabolites detected in urine samples collected among 8 consecutive days. The ICCs adjusted to age, gender and creatinine content in urine ranged between 0.20 to 0.57 showing low reliability (high within-subject variability) of the repeated measurements over time. However, similar results were obtained for DEHP oxidative metabolites, still low ICCs (range of 0.13–0.22) were measured in 45 American women without finding any influential factor responsible for the high intra-individual variability (Peck et al., 2010).

3.1.5. *Potential predictors of exposure*

Participants who were smoking had higher DEHP oxidative metabolites and MBzP than non-smokers. In the multivariate models we found that smoking was related to a 72% and 77% increase of 5-oxo-MEHP and 5-OH-MEHP levels in urine respectively, compared to non-smokers. Smoking was also associated with higher urinary MBzP (i.e. more than two times in comparison with the non-smokers, Table 4), and it was positively though non-significantly related to urinary MnBP. To our knowledge, no association between smoking and concentration of PE metabolites in urine has been reported in other studies (Huang et al., 2007; Dewalque et al., 2014b; Geens et al., 2014; Larsson et al., 2014). However, the limited number of smokers (N=4) in our study does not allow us to have strong arguments for this association. At the same time, other co-factors, such as the hand to mouth contact during smoking or the contact with the material of cigarette filters might contribute and enhance this association.

The more frequent use of PCPs per day was positively associated with higher MEP concentrations in all urine samples (Fig. SI1), while in the multivariate models we found that the more frequent daily use (> 5 PCPs/day) was associated with an increase of 252% in urinary MEP (Table 4). The association of MEP with several PCPs (including the use of sunscreen, eye make-up, shampoo, and conditioner) has been highlighted by several authors (Larsson et al., 2014; Cavallari et al., 2015; Philippat et al., 2015), however this association was not always discriminated and significant when increasing the number of PCPs used.

In the univariate statistics, an increase of age was significantly associated ($p < 0.05$) to higher average concentrations of MnBP

Table 4
Multivariate linear regression mixed models of PE and DINCH urinary metabolites by lifestyle characteristics.

Percentage change (%) in urinary phthalate metabolites		(95%CI)
5-OH-MEHP		
Smoking		
No	Reference	
Yes	77.2	(5.3; 197.7)**
Washing hands		
≤ 8times/day	Reference	
> 8 times/day	−28.9	(−46.5; −5.6)**
Consumed foods with plastic packaging[a]		
< 10 foods/day	Reference	
≥ 10 foods/day	30.1	(−0.7; 70.4)*
5-oxo-MEHP		
Education		
≤ 12 years	Reference	
13–16 years	−38.9	(−69.7; 23.5)*
≥ 17 years	−26.5	(−62; 41.9)
Smoking		
No	Reference	
Yes	71.9	(−8.3; 222.8)*
PVC in walls/floors		
No	Reference	
Yes	−37.0	(−57.9; −5.7)**
MnBP		
Age (per year)	2.6	(0.7; 4.5)**
Smoking		
No	Reference	
Yes	70.2	(−15.9; 244.9)**
Eating with hands		
< 4 times/day	Reference	
≥ 4 times/day	33.4	(−10.2; 98.4)*
MBzP		
Age (per year)	1.3	(−0.5; 3.2)*
Education		
≤ 12 years	Reference	
13–16 years	−58.5	(−80.7; −10.5)**
≥ 17 years	−47.5	(−74.4; 7.8)*
Smoking		
No	Reference	
Yes	203.7	(52.8; 503.8)**
MEP		
Age (per year)	5.0	(2.2; 8.0)**
Education		
≤ 12 years	Reference	
13–16 years	−60.6	(−87.9; 28.5)*
≥ 17 years	−48.4	(−83; 56.1)
Eating with hands		
< 4 times/day	Reference	
≥ 4 times/day	71.8	(−5.2; 211.4)*
PCP in the last 24 h		
≤ 5 PCPs/day	Reference	
> 5 PCPs/day	251.5	(80.2; 585.5)**
MiBP		
Age (per year)	1.9	(0.6; 3.2)**
Wash hair		
< 4 times/week	Reference	
≥ 4 times/week	−25.0	(−43.4; −0.7)**
Wearing gloves when house cleaning		
No	Reference	
Yes	−26.5	(−47.1; 2.1)*
Consumed foods with plastic packaging[a]		
< 10 foods/day	Reference	
≥ 10 foods/day	23.1	(−4.1; 58.1)*
OH-MINCH		
Washing hands		
≤ 8times/day	Reference	
> 8 times/day	−67.2	(−89.7; 4.2)*
cx-MINCH		
Wearing gloves when house cleaning		
No	Reference	
Yes	253.2	(−34.7; 1810.6)*

[a] Average number of consumed foods packaged in plastic during the 2 days.
* p-value < 0.2.
** p-value < 0.05 of Wald test.

and MEP in urine (Table SI5). In multivariate analysis, every year increase in age was associated to greater urinary MiBP, MnBP and MEP levels with corresponding raises of 1.9%, 2.6% and 5%, respectively (Table 4). Cavallari et al. (2015) also reported higher urinary MEP levels for individuals between 40 and 60 years old, still no trend was clearly associated to aging.

By multivariate analysis we observed that middle (13–16 years) and high (≥ 17 years) education were associated with 59% and 48% reduction of MBzP urinary concentrations, respectively (Table 4). Low educated participants (≤ 12 years) had lower 5-oxo-MEHP and MEP in urine, although the associations did not reach a statistical significant level. In the univariate model, associations ($p < 0.2$) were found for the higher education level and decreased OH-MINCH and cx-MINCH levels. Still this association was not further evidenced in the multivariate analyses.

Washing the hands more frequently (> 8 times/day) reduced the DEHP and DINCH oxidative metabolites compared to less frequent hands washing (< 8 times/day, Table SI4), and especially for 5-OH-MEHP and OH-MINCH a contraction of 29% and 67%, respectively was further identified (Table 4).

Eating with hands (≥ 4 times/day), in univariable analysis, was positively though non-significantly ($p < 0.2$) associated to elevated MnBP and MEP urinary levels (Table SI4), and statistically associated ($p < 0.05$) with higher MiBP urinary levels. In the multivariate statistics, these associations remained for MEP and MnBP, where the increase in urine was 72% and 33% respectively (Table 4), while for MiBP no further association was observed.

Wearing gloves when cleaning the house could increase the levels of DINCH oxidative metabolites in urine (Table SI4), especially for cx-MINCH (increase of 250%, Table 4) and might decrease the MiBP (i.e. 27%).

In addition, participants who consumed ≥ 10 foods stored in plastic packaging during the two sampling days, had elevated levels of DEHP metabolites in the afternoon urine spots (Supplementary information, Fig. SI2), when comparing to those eating foods that were less frequently wrapped in plastic packaging. Nevertheless, none of these differences reached statistical significance. In the multivariate statistics, we also observed a trend of higher 5-OH-MEHP levels in urine due to high consumption of foods in plastics (> 10 food/day), though this association was not significant ($p < 0.2$, Table 4). In fact, DEHP has been considered a 'substance of very high concern' (SVHC) since 2008 under REACH, thus its incorporation in materials which contact directly with food is forbidden (Ventrice et al., 2013). Still, this association is possible to occur and it has been reported by other authors (Hauser and Calafat, 2005; Rudel et al., 2011), especially if plastic containers for food purposes are imported to EU from countries with less strict legislations. Therefore, the association with one of the major oxidative DEHP metabolites excreted via urine seems reasonable. In addition, in univariate and multivariate analysis we observed that consuming more foods in plastic packaging might also be associated with an increase of 23% of MiBP levels in urine (Table 4).

Living in a house with PVC in floors/walls was associated with a decrease of 37% of 5-oxo-MEHP levels in urine (Table 4). Previously, this unexpected association to 5-oxo-MEHP was not evidenced in the univariate model (Table SI4), but with MiBP instead, although it was not statistically significant ($p < 0.2$). In other studies, no significant correlation was observed between PVC in floors/walls at home and DEHP oxidative metabolites in urine, however the findings indicated significantly higher MBzP levels in mother-child couples (Larsson et al., 2014) and infants (Carlstedt et al., 2013).

Finally, higher frequency of washing the hair (≥ 4 times/week) was associated with decreasing MiBP urinary levels in 25% (Table 4). Previously in the univariate model, this association was

better associated with decreasing the OH-MINCH levels, but still this was not further confirmed for this metabolite in the multivariate statistics (Table 4). The increased MEP and MiBP levels were associated with coloring the hair, though not statistically significant ($p < 0.2$, Table SI4). After in the multivariate analysis, coloring the hair was not associated with any of the target metabolites.

3.2. Nails

3.2.1. Exposure levels

All selected analytes were detected in nails, with MEHP, MBzP, MiBP, MnBP and MEP being the most abundant metabolites (DF > 92%, Table 1). In particular, the hydrolytic DEHP monoester was present in all nail samples at 129.3 ng/g (GM value), while the oxidative metabolites 5-OH-MEHP and 5-oxo-MEHP had lower detection frequencies (71% and 29%, respectively) and concentrations (GM of 2.6 and 0.21 ng/g, respectively) than the precursor monoester. Our results agree with previous studies (Alves et al., 2016a; 2016b), suggesting that the part of MEHP (ca. 25%) which is not metabolized into oxidative metabolites and excreted in urine within 48 h (i.e. approximately 75% of DEHP is transformed and excreted into their metabolites within 48 h) can bioaccumulate in our body and be measured in nails (Koch et al., 2005). In blood, for 6 h after the DEHP exposure occurs, MEHP is the major metabolite formed with high levels, therefore it is possible to end up in nails (Kessler et al., 2012). Furthermore, the hydrolytic monoesters, such as MEHP, are prone to external contamination as they can be generated out from the hydrolysis of ubiquitous diesters (Wittassek and Angerer, 2008). Therefore it is possible to have hydrolysis of the parent compound (i.e. DEHP) directly on the nail plate. Nevertheless, more investigation is needed to clarify the mechanisms of incorporation/transfer of the metabolites into the nails, to study the potential differences in the accumulation rates by metabolite, among other factors that can influence and contribute for their (fast/slow) diffusion into the nail plate.

Similarly to DEHP oxidative metabolites, the presence of OH-MINCH and cx-MINCH in nails is low. In addition, Alves et al. (2016a) determined MEP (ranging from 15 to 977 ng/g), MnBP and MiBP (ranging from 39 to 814 ng/g) as the major metabolites in nails from Belgian adults, analogously to what was found in this study.

Although more research is needed, these results might indicate that there is an equilibrium between accumulation of a same metabolite in nails versus its excretion via urine which can be dependent of several intrinsic factors (e.g. age, gender, etc.), but also by the diffusion from blood (metabolism rate and transfer) to nails, among other. The fast excretion of the secondary oxidation metabolites of DEHP in all three urine spots and the higher DF of MEHP in nails is an example, suggesting that bioaccumulation versus excretion are accountable in different proportions depending on the metabolites formed (primary, oxidative secondary). To our knowledge, it is still not clear whether PEs are first transported to the nails and then metabolized or metabolized somewhere else in other body reservoirs (e.g. blood) and then transported to the nails. Therefore in order to understand how the PE metabolites are partially accumulated in nails, future studies on metabolism and exposure assessment in nails (and urine) are needed.

3.2.2. Relationships of PE metabolites in urine and nails

We found a strong positive significant correlation between MEP concentrations in nails and in all three urine spots (r=0.56–0.68, $p < 0.001$, Table SI5). A good correlation for MEP measured in fifteen collected urinary spots and in nails (r=0.73, $p < 0.05$), was also observed by Alves et al. (2016b), although this correlation was weak when only one urine spot was measured (Alves et al., 2016a). There was no correlation for MnBP, MiBP and MBzP between urine and nails, as well as no correlation was found for the DEHP metabolites between the two matrices.

Weaker but significant correlations were found between MnBP in nails and MBzP in afternoon urine spots (r=0.29 and r=0.31, $p < 0.05$, Table SI5). The same was observed between MEP afternoon urine spots and MnBP in nails (r=0.26–0.27, $p < 0.05$). The fact that only MEP was highly and consistently correlated between all urine spots and nails can be due to higher and constant human exposure to consumer products, where DEP is incorporated without any regulation for its applications as it exists for DnBP and BzBP. Furthermore, MEP is a specific metabolite of DEP, while MnBP can be generated both from DnBP and to a minor proportion also from BBzP.

Table 5
Multivariate linear regression models of PE metabolites in nails by lifestyle characteristics.

Percentage change (%) in phthalate metabolites in nails		(95% CI)
MEHP		
Age (per year)	2.1	(−0.9; 5.2)*
Consumed foods with plastic packaging[a]		
< 10 foods/day	Reference	
≥ 10 foods/day	−57.3	(−76.2; −23.3)**
MnBP		
Education		
≤ 12 years	Reference	
13–16 years	−82.5	(−97.2; 10.1)*
≥ 17 years	−43.4	(−89.5; 204.7)
Smoking		
No	Reference	
Yes	993.5	(116.6; 5429.6)**
Wearing gloves when house cleaning		
No	Reference	
Yes	121.9	(−27.2; 578.0)*
Consumed foods with plastic packaging[a]		
< 10 foods/day	Reference	
≥ 10 foods/day	−43.3	(−75.9; 33.8)*
MEP		
PCP in the last 24 h		
≤ 5 PCPs/day	Reference	
> 5 PCPs/day	186.0	(15.1; 609.9)**

[a] Average number of consumed foods packaged in plastic during the 2 days.
* p-value < 0.2.
** p-value < 0.05 of Wald test.

3.2.3. Potential predictors of exposure

In multivariate analysis, smoking was significantly associated with MnBP levels in nails (increase of 994%, Table 5). The results are consistent with those reported for urine (besides the low number of smokers in this study), where smoking seems to be an important factor on DBP exposure (also depending on age), although this factor is more significant when it is associated with the levels in nails instead.

In univariate analysis (Table SI6), participants who have been applying more frequently PCPs on their hands in the last 24 h demonstrated higher MnBP ($p < 0.2$) and MEP ($p < 0.05$) concentrations in their nails, which is probably indicating to external contamination. Female participants who have used more PCPs and/or colored their hair ($p < 0.05$) appeared to have higher MEP concentrations in nails (Table SI6). Using more than 5 PCPs/day was associated with a distinctly increased MEP concentration in nails after adjusting for all the other characteristics (i.e. 186%, Table 5). This association was also visible for urine, however once nails have a direct contact with the PCPs especially if they are applied in the hands, we assume that the PEs (diesters or metabolites) can be deposited directly in the nail plate. This makes the

quantitative exposure assessment and internal dose extrapolation difficult. Moreover, deposition to the nail bed (via dermal absorption and/or diffusion from blood) might occur through the metabolic transformation of diesters to monoesters (Lorber et al., 2010). Yet, the mechanisms of transfer from blood or other body compartments to nails, as well as their incorporation pathways into the nails are not yet explored, therefore this matter needs to be elucidated in order to understand how PEs can accumulate in nails.

Similarly, multivariable statistics disclosed the association ($p < 0.2$) between wearing gloves while cleaning the house and increasing MnBP levels in nails ($> 100\%$), where again direct contact of gloves and nails exists.

Other observations for nails were the increasing levels of MEHP per year (i.e. 2%, Table 5), and lower MnBP levels for people with lower education, although not significant.

In contrast to the observed trend of 30% increase in 5-OH-MEHP urinary levels related to higher consumption of foods in plastic packaging (> 10 foods/day), in nails this association was inverse by 57% decrease of MEHP concentrations (Tables 4 and 5). The significance of this finding needs further investigation. Differences between urine and nails might be explained by the different exposure windows they reflect, by differences in metabolic and kinetic behavior of the DEHP biomarkers in the different matrices, but also by possible external contamination influences on the biomarker MEHP.

4. Study limitations and future perspectives

A larger human study population (cohort) from different areas could be considered as more representative of the Norwegian population. Fingernails, but also toe nails would be relevant to analyze, once the levels and exposure are usually distinct. Moreover, fingernails are more exposed to external sources than toe nails, which may hamper the identification and understanding of internal versus external exposure. The sampling design should have included replicate nail samples over a longer period, in order to get a better understanding about the stability and reliability of the levels reported in different nail segments, and how nails can be considered good biomarkers of past exposure. This might also underline an exposure trend for a certain PE or DINCH.

There is a lack of bioaccessibility experiments in order to check possible hydrolysis which might occur directly on the nail plate. Further understanding is required about the metabolism in nails or in skin after the nail penetration, and the excretion against the bioaccumulation rate, which might be compound specific.

Another important point, could be the inclusion of other analytes in our analytical method such as DPHP, DiNP and DiDP oxidative metabolites, 5-cx-MEHP (for DEHP) and oxo-MINCH (for DINCH), since there are all good biomarkers of exposure in urine.

5. Conclusions

In our study, we observed a low exposure to PEs and DINCH for this Norwegian study population, based on estimated daily intakes using concentrations of their metabolites in urine. The cumulative risk assessment for combined plasticizer exposure, expressed as HQs and HI, was below established risk limits, even considering the worst case scenario (i.e. maximum values). Our findings suggest that, age, smoking, use of PCPs and many other every day habits, such as washing hands or eating food from plastic packages are possible contributors to plasticizer exposure, but still no firm conclusion can be drawn due to our small sample size.

The determined concentrations of PE metabolites in urine were in general lower than reported in other European studies, while concentrations of DINCH metabolites were comparable to reported levels from 2012, and higher than those reported before 2012. The low exposure to the critical PEs is in line with generally decreasing PE exposure due to regulatory restrictions and market changes (Goen et al., 2011; Zota et al., 2014). DINCH is a major substitute for critical PEs and that explains the increasing exposures to this plasticizer (Schutze et al., 2014). This development also illustrates the necessity of including new alternative plasticizers when developing methods to be used in biomonitoring studies (Lioy et al., 2014).

The applicability of using another non-invasive matrix, i.e. nails, and its comparison with three urine spot samples was assessed. MEP was the only metabolite where high correlations between urine and nails were observed. For the other PEs and DINCH, we observed no correlations, which warrant further investigation in terms of mechanisms of transfer and/or incorporation pathways of plasticizers into nails. Measurements of plasticizer metabolite levels in nails can only regarded as a first step in establishing this matrix as a future matrix for exposure assessment and risk characterization. Certainly, this matrix could extend the window of exposure measurement for short lived chemicals that are rapidly excreted in urine.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2016.07.025.

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