Environment International 159 (2022) 107046

Contents lists available at ScienceDirect

Environment International

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

Assessment of susceptibility to phthalate and DINCH exposure through CYP and UGT single nucleotide polymorphisms

Anja Stajnko [a,*], Agneta Annika Runkel [a,b], Tina Kosjek [a,b], Janja Snoj Tratnik [a], Darja Mazej [a], Ingrid Falnoga [a], Milena Horvat [a,b]

[a] Jožef Stefan Institute, Department of Environmental Sciences, Ljubljana, Slovenia
[b] Jožef Stefan International Postgraduate School, Ljubljana, Slovenia

ARTICLE INFO

Handling Editor: Shoji F. Nakayama

Keywords:
Phthalate
DINCH
Single nucleotide polymorphism
Cytochrome P450 enzyme
UDP-glucuronosyltransferase
CYP2C9

ABSTRACT

Single nucleotide polymorphisms (SNPs) of cytochrome P450 (CYPs) and UDP-glucuronosyltransferase (UGTs) genes have been proposed to influence phthalates and 1,2-*cyclo*-hexanedicarboxylic acid diisononyl ester (DINCH) biotransformation but have not been investigated on a populational level.

We investigated the role of SNPs in *CYP2C9*, *CYP2C19, CYP2D6*, *UGT2B15,* and *UGT1A7* genes in the biotransformation of phthalates (DEHP, DEP, DiBP, DnBP, BBzP, DiNP, DidP) and DINCH by determining their urine metabolites.

From the Slovenian study population of 274 men and 289 lactating primiparous women we obtained data on phthalate and DINCH urine metabolite levels (MEHP, 5OH-MEHP, 5oxo-MEHP, 5cx-MEPP, MEP, MiBP, MnBP, MBzP, cx-MINP, OH-MiDP, MCHP, MnPeP, MnOP, 5OH-MINCH, 5oxo-MINCH), SNP genotypes (rs1057910 = *CYP2C9*3*, rs1799853 = *CYP2C9*2*, rs4244285 = *CYP2C19*2*, rs12248560 = *CYP2C19*17*, rs3892097 = *CYP2D6*4*, rs1902023 = *UGT2B15*2*, and rs11692021 = *UGT1A7*3*) and questionnaires. Associations of SNPs with levels of metabolites and their ratios were assessed by multiple linear regression and ordinary logistic regression analyses.

Significant associations were observed for *CYP2C9*2*, *CYP2C9*3*, *CYP2C19*17*, and *UGT1A7*3* SNPs. The most pronounced was the influence of *CYP2C9*2* and *3 on the reduced DEHP biotransformation, with lower levels of metabolites and their ratios in men and women. In contrast, carriers of *CYP2C19*17* showed higher urine levels of DEHP metabolites in both genders, and in women also in higher DiNP, DiDP, and DINCH metabolite levels. The presence of *UGT1A7*3* was associated with increased metabolite levels of DINCH in men and of DiBP and DBzP in women. Statistical models explained up to 27% of variability in metabolite levels or their ratios.

Our observations confirm the effect of *CYP2C9*2* and *3* SNPs towards reduced DEHP biotransformation. We show that *CYP2C9*2, CYP2C9*3, CYP2C19*17,* and *UGT1A7*3* SNPs might represent biomarkers of susceptibility or resilience in phthalates and DINCH exposure that have been so far unrecognised.

1. Introduction

Phthalates (PHs) are diesters of phthalic acid commonly classified as high molecular weight (HMW: 7–13 carbon atoms) or low molecular weight (LMW: 3–6 carbon atoms) PHs. HMW PHs are primarily used in plastics, while LMW PHs are additives in solvents and personal care products from where they migrate into the environment (Wittassek et al., 2011; Berger et al., 2019; Wang et al., 2019). The general population is frequently exposed via ingestion, inhalation, and dermal absorption (Benjamin et al., 2017; Wang et al., 2019). Exposure to PHs has been associated with various health issues, (Benjamin et al., 2017; Wang et al., 2019; Giuliani et al., 2020), therefore, the presumably less toxic substitute plasticizer 1,2-*cyclo*-hexanedicarboxylic acid diisononyl ester (Hexamoll® DINCH) has been introduced (Bui et al., 2016; Wang et al., 2019). DINCH and PHs are listed as a priority substance group within the pan-European HBM4EU project (Schoeters and Lange, 2020).

In the human body, PHs and DINCH undergo compound-dependent biotransformation in the liver and partly in the gut. Firstly, they are

* Corresponding author.
E-mail address: anja.stajnko@ijs.si (A. Stajnko).

https://doi.org/10.1016/j.envint.2021.107046
Received 2 August 2021; Received in revised form 8 December 2021; Accepted 9 December 2021

hydrolysed by esterase or lipases into the corresponding monoesters (phase I biotransformation) followed by oxidation of the monoester side chain by the cytochrome P450 enzymes (CYPs; phase I biotransformation) resulting into secondary metabolites – mainly with hydroxy, oxo, and carboxy functional groups. Most of the metabolites further undergo conjugation (phase II biotransformation), which is catalysed mainly by UDP-glucuronyl transferases (UGTs), forming hydrophilic conjugates that are easily excreted in within 48 h in urine (major pathway) or faeces (minor pathway) (Frederiksen et al., 2007; Benjamin et al., 2017; Lyche, 2017; Domínguez-Romero and Scheringer, 2019).

Accordingly, exposure to PHs and DINCH is assessed by the measurement of the primary and secondary metabolites in the urine; mostly without distinguishing between conjugated and non-conjugated forms. The patterns of urine metabolites show inter-individual variability, which can be attributed to the exposure as well as to differences in physiology; the latter influencing enzyme activity and, consequently, biotransformation capacity (Frederiksen et al., 2007; Yaghjyan et al., 2016; ATSDR, 2019; Domínguez-Romero and Scheringer, 2019). Important contributors to the inter-individual variability might also be single nucleotide polymorphisms (SNPs) in genes coding for the main metabolizing enzymes, such as CYPs and UGTs (Choi et al., 2012, 2013; Stein et al., 2013; Ito et al., 2014; Yaghjyan et al., 2016; Hanioka et al., 2017; ATSDR, 2019). This can consequently result in higher susceptibility towards the toxic effects of PHs and DINCH on human health. However, information on specific genes or enzyme isoforms involved in PH or DINCH biotransformation is scarce. The most extensively studied is the biotransformation of HMW diethylhexyl phthalate (DEHP) in animals and humans (ATSDR, 2019); as shown in Fig. 1.

Choi et al. (2012) assessed DEHP biotransformation by subcellular fractions of various human tissues and human CYPs. They identified CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, and CYP3A7 as the major CYP isoforms producing hydroxy, oxo, and carboxy secondary metabolites (5OH-MEHP, 5oxo-MEHP, and 5cx-MEPP, respectively). Moreover, the in vitro assessed effect of different CYP2C9 enzyme subisoforms defined by the SNPs rs1799853 (*CYP2C9*2*) and rs1057910 (*CYP2C9*3*) indicated reduced enzyme activity (Choi et al., 2012, 2013). Also, the rs743572 polymorphism in *CYP17A1* has been suggested to influence the effect of PHs exposure (DEP, DEHP, DnBP) on the development of leiomyoma (Huang et al., 2014). Although, in *CYP2C19* several functional SNPs have been reported – with rs4244285 (*CYP2C19*2*) and rs12248560 (*CYP2C19*17*) being the most common among Caucasians (Rosemary and Adithan, 2007; Hirota et al., 2013; Hiratsuka, 2016) – none have been yet investigated in the relation to PHs or DINCH biotransformation. Furthermore, around 85% of the applied DEHP dose is excreted in urine in the form of glucuronidated metabolites (Koch et al., 2006; Frederiksen et al., 2007; ATSDR, 2019). According to an in vitro study by Hanioka et al. (2017), the main UGT enzyme isoforms involved in MEHP glucuronidation in isolated human liver and intestine microsomes were UGT2B7, UGT1A9, and UGT1A7. Although, various SNPs resulting in induction or suppression of various UGT enzymes have been determined (Guillemette, 2003; UGT Nomenclature Commitee, 2005; Hanioka et al., 2017), their association with biotransformation and negative health effects of PHs or DINCH have been poorly investigated. The assessment of rs4148323 (*UGT1A1**6), rs7439366 (*UGT2B7**2), and rs1902023 (*UGT2B15*2*) polymorphisms has revealed a significant association of the latter two with total serum PHs (undefined) levels in patients with polycystic ovary syndrome (Luo et al., 2020).

The investigation of CYP and UGT polymorphisms' influence on urine levels of PHs or DINCH metabolites on a population level has been proposed (Choi et al., 2012; Ito et al., 2014; Yaghjyan et al., 2016; Hanioka et al., 2017) but, to our knowledge never performed. Therefore, the purpose of the present study was to test the possible role of selected SNPs in three CYP (*CYP2C9*, *CYP2C19*, *CYP2D6*) and two UGT *(UGT2B15*, and *UGT1A7)* genes in the biotransformation of PHs (DEHP, DEP, DiBP, DnBP, BBzP, DiNP, and DiDP) and DINCH in the Slovenian population of men and lactating women.

2. Material and methods

2.1. Study population

In this study, a subset of unrelated subjects was selected from a wider Slovenian Human Biomonitoring programme carried out between 2008 and 2014. In the original programme, 1084 participants – consisting of men and lactating *primiparous* women – were recruited from across Slovenia, with the aim to estimate trace elements' levels and persistent organic pollutants in a childbearing population and to estimate babies' exposure via maternal milk. Due to the existence of archived samples, levels of PHs and DINCH metabolites in urine were obtained in 2019–2020 for 603 participants, and Runkel et al. (2022) describe their exposure assessment in detail. Among these, genetic material was

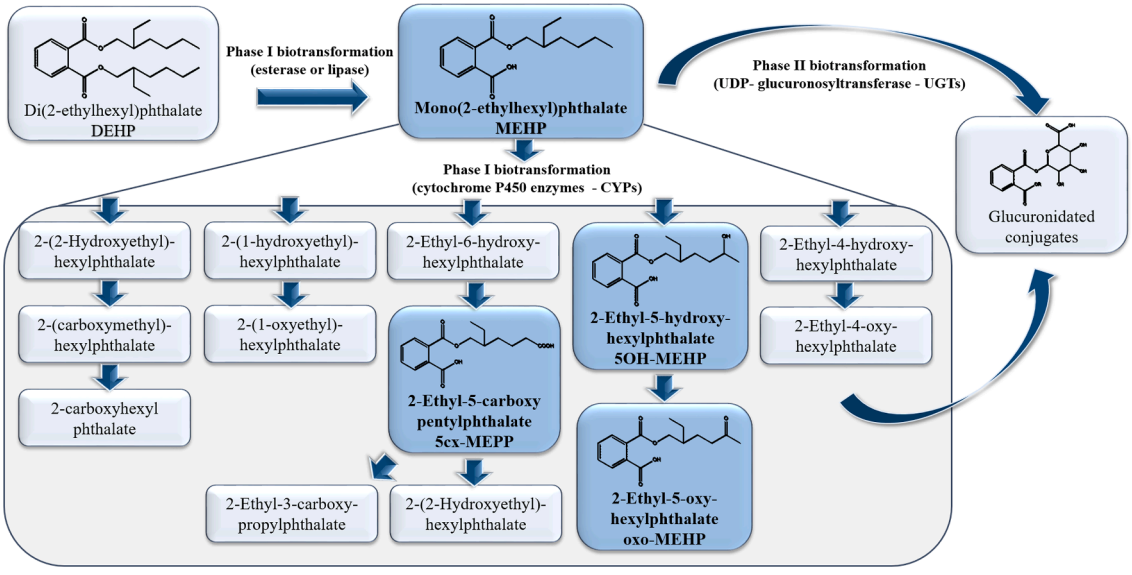

Fig. 1. Biotransformation of DEHP (adapted with permission from Koch et al., 2005). Highlighted are the major metabolites, which are determined in the present study.

obtained for 572 participants (298 lactating *primiparous* women and 274 men), who's data was used in order to test the present study's aim.

Stajnko et al. (2017) and Snoj Tratnik et al. (2019) describe the detailed recruitment and sampling procedures. Briefly, all participants provided a random spot urine sample and a sample of whole blood, and they completed questionnaires covering their general characteristics, socio-economic status, life-style, and dietary habits. The samples were aliquoted and stored at −20 °C prior to analyses. All participants signed an informed consent form, and the study protocol was approved by the Republic of Slovenia National Medical Ethics Committee, with numbers of accordance 42/12/07, 53/07/09 and 70/02/11. To be able to use biobanked samples, we obtained additional ethical approval (number of accordance 0120-431/2018/4), and all participants provided informed written consent.

2.2. Analyses of PHs and DINCH metabolites

The spot urine samples were sent to the VITO NV laboratory in Belgium for analysis of 13 PHs primary and secondary metabolites (MEP, MBzP, MiBP, MnBP, MCHP, MnPeP, MEHP, 5OH-MEHP, 5oxo-MEHP, 5cx-MEPP, MnOP, cx-MiNP, and OH-MiDP) and 2 secondary metabolites of DINCH (OH-MINCH and oxo-MINCH) (Table 1). The C or D labelled standards of phthalate or DINCH metabolites were supplied by Cambridge Isotope Laboratories (Andover, USA). VITO has proved its excellence by successfully participating in the ICI-EQUAS rounds (inter-laboratory comparison investigation) organised within the European project HBM4EU (Elbers and Mol, 2019). The laboratory measured the total content (conjugated and free form) of urinary PH and DINCH metabolites.

A brief description of the measurement process is as follows. First, β-glucuronidase in an ammonium acetate buffer solution was added to 1 mL of each sample and analysed using ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) after direct injection. Separation was achieved on an Acquity UPLC BEH PHENYL 1.7 μm, 2.1 × 100 mm column with mobile phase A: water + 0.1% acetic acid and B: acetonitrile (ACN) + 0.1% acetic acid and a total run time of 8 min. The analysis was performed in negative ionization mode with MRM detection. Contamination control was assured via the inclusion of procedural blanks and parallels as well as spiked quality control samples in water and urine. One independent quality control sample (G-EQUAS) was included to assure the repeatability and reproducibility of the results. Only linear weighted (1/X) calibration curves with squared regression coefficients >0.995 and residuals <10% were accepted. The inter-day repeatability for all metabolites was <10%. A deviation of 20% in recovery of quality control samples was considered acceptable and was obtained for all analytes. The method trueness was assessed via spiking experiments to overcome the lack of certified reference materials. The relative recovery (%) calculated as the ratio between experimentally observed concentrations and nominal concentrations was taken as an approximation of trueness and ranged between 80% and 120%.

The obtained LOQs were as follows: 0.1 ng/mL for 5oxo-MEHP, 5OH-MEHP, 5cx-MEPP, cx-MiNP, MnOP, MnPeP, OH-MiDP, OH-MINCH, and oxo-MINCH; 0.2 ng/mL for MBzP and MCHP; 0.5 ng/mL for MEP, MiBP, and MnBP; and 0.8 ng/mL for MEHP.

All results were adjusted to specific gravity (SG) to overcome the effects of urinary dilution. SG was measured on a PAL-10 S refractometer, closely following the method for SG correction described by Suwazono et al. (2005).

2.3. Analyses of selenium in blood

Aliquot of venous blood (0.3 mL) was analysed for selenium (Se) by Octopole Reaction System (ORS) Inductively Coupled Plasma Mass Spectrometry (ICP-MS; 7500ce, Agilent Technologies) equipped with an ASX-510 autosampler (Cetac). The LOD was 8 ng/g. The procedure was

Table 1
Primary and secondary metabolites of PHs and DINCH measured in the present study.

			Metabolites measured in the present study:	
Parent compound	**LMW**	**HMW**	**Primary metabolite**	**Secondary metabolites**
DEHP Di(2-ethylhexyl) phthalate		X	MEHP Mono(2-ethylhexyl) phthalate	5OH-MEHP (MEHHP[a]) Mono(2-ethyl-5-hydroxyhexyl) phthalate 5oxo-MEHP (MEOHP[a]) Mono(2-ethyl-5-oxyhexyl) phthalate 5cx-MEPP (MECPP[a]) Mono(2-ehtyl-5carboxypentyl) phthalate
DEP Di-ethyl phthalate	X		MEP Mono-ethyl phthalate	
DiBP Di-isobutyl phthalate	X		MiBP mono-isobutyl phthalate	
DnBP Di-n-butyl phthalate	X		MnBP mono-n-butyl phthalate	
BBzP Benzyl butyl phthalate	X		MBzP Mono-benzyl phthalate	
DiNP Diisononyl phthalate		X		cx-MiNP Monocarboxy-isononyl phthalate
DiDP Diisodecyl phthalate		X		OH-MiDP Monohydroxy-isodecyl phthalate
DCHP Dicyclohexyl phthalate	X		MCHP* Mono-cyclohexyl phthalate	
DnPeP Di-n-pentyl phthalate	X		MnPeP* Mono-n-pentyl phthalate	
DnOP Di-n-octyl phthalate		X	MnOP* Mono-n-octyl phthalate	
DINCH Di-(isononyl)-cyclohexane-1,2-dicarboxylate		X		OH-MINCH (MHNCH[a]) Cyclohexane-1,2-dicarboxylic acid-mono (hydroxyl-isononyl) oxo-MINCH (MONCH[a]) Cyclohexane-1,2-dicarboxylic acid-mono (oxo-isononyl)

* Could not be detected in >95% of samples and were as such excluded from the statistical analysis in the present study; LMW: low molecular weight compound, HMW: high molecular weight compound.

[a] Alternative commonly used metabolite nomenclature.

previously described by (Miklavčič et al., 2013).

2.4. Genotyping of selected SNPs

2.4.1. SNP nomenclature and selection

Within clinical studies, where CYPs and UGTs are mostly studied, the most widely adopted SNP nomenclature is the "star" (*) nomenclature, with *1 mainly defining reference or wild-type (fully functional) allele, while further numbers (e.g. *2, *3, *4, …) correspond to variant alleles of different SNPs (https://www.pharmvar.org/; https://www.pharmacogenomics.pha.ulaval.ca/ugt-alleles-nomenclature). To be consistent and transparent with the current literature, in this manuscript, we follow the recommendations by Kalman et al. (2016) and in case of each tested SNP reported both rs ID from dbSNP and its corresponding * allele nomenclature, as presented in Table 2 (e.g. *CYP2C9* SNP rs1057910: A > C allele change, resulting genotypes AA, AC, and CC; corresponding * nomenclature is *1 > *3 and *1/*1, *1/*3, and *3/*3).

Specific genes and corresponding SNPs were selected based on the following criteria:

(i) literature data on specific genes predominantly involved in the biotransformation of PHs – mainly DEHP – (Choi et al., 2012; Hanioka et al., 2017), and the reported functional influence of SNP on enzyme activity (Guillemette, 2003; UGT Nomenclature Commitee, 2005; Di et al., 2009; Hanioka et al., 2011; Choi et al., 2012, 2013; Cao et al., 2019; PharmVar: Pharmacogene Variation Consortium, 2021);
(ii) reported SNP's minor allele frequency (MAF) of ≥7% for the European population;
(iii) the availability of pre-designed hydrolysis probe assays (https://www.thermofisher.com/order/genome-database/).

Seven functional SNPs: rs1799853 (*CYP2C9*2*), rs1057910 (*CYP2C9*3*), rs4244285 (*CYP2C19*2*), rs12248560 (*CYP2C19*17*), rs38920979 (*CYP2D6*4*), rs1902023 (*UGT2B15*2*), and rs11692021 (*UGT1A7*3*) were selected. Their general information is presented in Table 2.

2.4.2. DNA isolation and genotyping

Genomic DNA was isolated from archived venous whole blood (0.5 mL) using the FlexiGene® DNA kit (Qiagen, Germany) following the manufacturer's instructions. The quality and quantity of DNA were evaluated by UV–VIS spectrophotometer NanoDrop 2000c (ThermoFisher Scientific, USA). DNA isolates were stored at −80 °C prior to genotyping.

Selected SNPs were genotyped using pre-designed TaqMan SNP Genotyping Assays (Applied Biosystems, USA; Table 2). The 5 μL reaction consisted of 2.5 μL of FastStart Essential DNA Probes Master (Roche, Germany), 1.875 μL of ultrapure nuclease-free water (Life Technologies, USA), 0.125 μL of 44X TaqMan probe/primer mix, and 0.5 μL of genomic DNA. LightCycler® 480 Instrument II and LightCycler480® Software version 1.5.1 (Roche, Germany) were used for the amplification and fluorescence detection. PCR cycling included the following steps: pre-PCR step (1 cycle: 50 °C for 2 min), activation step (1 cycle: 95 °C for 10 min), annealing and amplification step (50 cycles: 95 °C for 15 s and 61 °C for 1 min), and post-PCR step (1 cycle: 40 °C for 30 s). For each SNP, a subset of randomly selected samples was repeated as a control (~30%).

2.5. Statistical analyses

All statistical analyses were performed separately for the groups of men and lactating women, due to significant physiological differences between men and women that are intensified by the temporary physiological state (i.e. lactation) of the participating women (Waxman and Holloway, 2009; Moya et al., 2014). Descriptive statistics were used to assess general characteristics of the study population (age, BMI, education and smoking), levels of metabolites in urine (exposure biomarkers), and genotype and allele frequency distribution of SNPs. The descriptive statistics of metabolite levels are presented unadjusted and with SG adjusted data, while all further statistics were performed using only SG adjusted data.

To assess the efficiency of DEHP and DINCH oxidative biotransformation, the following metabolite ratios were calculated: 5OH-MEHP/MEHP, 5oxo-MEHP/MEHP, 5cx-MEPP/MEHP, and 5oxo-MEHP/5OH-MEHP for DEHP and oxo-MINCH/OH-MINCH for DINCH.

Statistical differences between groups were assessed using the Mann-Whitney *U* test, the Kruskal Wallis test with Dunn post hoc test, or Pearson's chi-squared test. The associations of seven SNPs with PHs and DINCH urine metabolite levels and their ratios were tested by multiple linear regression analyses (MLR) with levels of metabolites or ratio values as the dependent variable and SNPs as the independent variable. Each association of SNP with urinary metabolite level and ratio was tested in a separate model with adjustments for age, BMI, education, year of sampling, blood selenium, and smoking. The latter was, due to there being only one female smoker, tested in men only. The confounders were chosen based on their previously reported association with PHs or DINCH biotransformation, their influences on enzyme activity and their general physiological relevance (Bui et al., 2016; Yaghjyan et al., 2016; Klomp et al., 2020; Runkel et al., 2020). Selenium in whole blood was added as a rough estimate of selenium nutritional status, which can influence cytochrome P450 enzyme's activity (Burk, 1983; Jiang et al., 2020) and possibly, consequently, the biotransformation of PHs and DINCH. Moreover, in the case of DEHP metabolites, the models were additionally adjusted by the 5cx-MEPP/5OH-MEHP ratio as a rough approximation of the time between DEHP exposure

Table 2
Information on studied SNPs.

Gene	dbSNP ID	Variant allele nomenclature[a]	Chr/ location	nt change	Amino acid change	MAF EU	TaqMan assay ID	Reported effect on enzyme activity[b]
CYP2C9	rs1057910	*CYP2C9*3*	10/exon	A > C	Ile > Leu	7	C_27104892_10	C or *3: reduced
	rs1799853	*CYP2C9*2*	10/exon	C > T	Arg > Cys	12	C_25625805_10	T or *2: reduced
CYP2C19	rs4244285	*CYP2C19*2*	10/exon	G > A	Pro > Pro	15	C_25986767_70	A or *2: reduced
	rs12248560	*CYP2C19*17*	10/ promoter	C > T		22	C___469857_10	T or *17: increased
CYP2D6	rs3892097	*CYP2D6*4*	22/intron	C > T		19	C_27102431_D0	T or *4: reduced
UGT2B15	rs1902023	*UGT2B15*2*	4/exon	T > G	Asp > Tyr	52	C_27028164_10	A or *2: inconsistent
UGT1A7	rs11692021	*UGT1A7*3*	2/exon	T > C	Trp > Arg	36	C___287260_10	C or *3: reduced

Chr: chromosome; nt: nucleotide, MAF EU: minor allele frequency in populations with European ancestry (NCBI, 2021).

[a] "star" (*) nomenclature for the variant alleles based on the nomenclature consortium (https://www.pharmvar.org/, https://www.pharmacogenomics.pha.ulaval.ca/ugt-alleles-nomenclature).

[b] Based on the measured enzyme activity in various in vitro and pharmacogenomical studies (https://www.pharmvar.org/, https://www.pharmacogenomics.pha.ulaval.ca/ugt-alleles-nomenclature; Guillemette et al., 2000).

and urine spot sampling. This is based on the longer estimated elimination half-life of 5cx-MEPP (12–15 h) compared with 5OH-MEHP (~10 h) (Lorber et al., 2011; Meeker et al., 2012). As hypothesized by Lorber et al. (2011) "initially after exposure, the ratio of 5cx-MEPP to 5OH-MEHP in a spot urine sample is <1.0, but after 10 h or so, this ratio exceeds 1.0". Furthermore, to assess the MLR performance, diagnostic analyses were carried out to test for linearity, normality, homoscedasticity and multicollinearity.

The available personalized questionnaire data unfortunately do not include information on possible exposure, such as the use of personal care products or food packaging within the last days prior to sampling. This might, however, influence the assessment of the SNPs role in PHs or DINCH biotransformation. Therefore, to test for possible influences of such un-identified individual exposure sources (i.e. outliers), beside the Cook's distance test in MLR, the models described above were additionally tested with ordinal logistic regression analyses (OLR).For this purpose, the levels of each metabolite or their ratios were split into quartiles (1st: ≤25th perc.; 2nd: >25th perc. and ≤50th perc.; 3rd: >50th perc. and ≤75th perc.; and 4th: >75th perc.), and then used as categorical dependent variables. With this approach – commonly used in the epidemiological studies – we believe that the effect of outliers on the tested associations is reduced.

In the case of CYP enzymes – mainly responsible for reactions resulting in secondary metabolites – the corresponding SNPs were tested for associations with urine levels and/or ratios of secondary metabolites of DEHP, DiNP, DiDP, and DINCH. SNPs in UGTs were tested for associations with urine levels of all metabolites. Moreover, due to the sufficient number of subjects in certain SNP groups, namely, rs1902023 (*UGT2B15*2*), rs11692021 (*UGT1A7*3*), and rs12248560 (*CYP2C19*17*), analyses were performed based on allele (e.g. **2(*1/*2+*2/*2)* vs **1/*1*) and genotype stratification (e.g. **2/*2* vs. **1/*2* vs. **1/*1*), while in the case of other SNPs, we used only stratification by alleles.

The level of statistical significance (p-value) was set to ≤0.05. Values below the LOQ were substituted with a value of LOQ/2, and when appropriate, non-normally distributed data was log transformed to approximate normal distribution. Statistical analyses and visualisations of the results were carried out in statistical software R version 3.6.0 with RStudio version 1.2.1335 using the packages ggplot2 (Wicklam, 2016), stargazer (Hlavac, 2018), stats (R Core Team, 2019), and MASS (Venables and Ripley, 2002) and in OriginPro® version 2020b (OriginLab Corporation, USA).

3. Results and discussion

To date, the literature widely focusses on the effect of CYP and UGT SNPs on the biotransformation of pharmaceuticals, while studies on PHs and DINCH mostly investigate distribution, levels of exposure and its health outcomes. Their gene-environment interaction on a populational level has previously been pointed out, but no studies examining this issue exist to date. Therefore, in the present study, SNPs in the genes of *CYP2C9*, *CYP2C19*, *CYP2D6*, *UGT2B15*, and *UGT1A7* were tested for possible associations with PHs and DINCH metabolite levels and their ratios. For six tested SNP variant alleles, it was reported that they have an inhibitory effect, and for one (rs12248560 or *CYP2C19*17*), a stimulating effect on enzyme activity (Table 2). Here we observe similar trends regarding their impact on metabolite levels and/or ratios.

3.1. Study population and biomarkers of exposure and biotransformation

The participants' samples were obtained between 2008 and 2014, with the highest acquisition in the years 2012 and 2013 (71% and 61% for women and men, respectively). Thus, all subjects were recruited prior to the EU-wide restriction of DiBP, DEHP, BBzP, and DMP in 2015 (Tranfo et al., 2018). The general characteristics of the studied men and lactating women are presented in Table 3. Among the women and men, with average ages of 29 and 31, respectively, we noticed that 54% and 38% of women and men, respectively, hold at least a university degree. As such, the education level of the present population is skewed towards higher levels compared to the average education level of the Slovenian population (SURS, 2017).

Table 3
General characteristics of the studied population.

	Lactating women	Men
N (%)	289 (51)	274 (49)
Age (years)	29 ± 4	31 ± 6
Weight (kg)	65 ± 11	84 ± 13
Height (cm)	168 ± 6	180 ± 7
BMI (kg/m^2)	23 ± 4	26 ± 4
Current smokers (N (%))	1 (0.3)	13 (2)
Education (N (%))		
<University	129 (46)	162 (61)
≥University	153 (54)	102 (38)
Year of sampling N		
2008	10	10
2009	41	22
2010	10	16
2011	13	6
2012	140	87
2013	65	79
2014	8	49
Selenium in blood (ng/g)	96 (54–176)	117 (77–226)

Age, weight, height, and BMI are presented as arithmetic mean ± SD, and Selenium in blood as GM (min–max)

Concentration levels of metabolites of seven phthalates (DEHP, DEP, DiBP, DnBP, BBzP, DiNP, DiDP) and DINCH are presented in Table 4, while primary metabolites of DCHP, DnPeP, and DnOP could not be detected in >95% of samples and were thus excluded from the statistical analysis. The numbers of samples < LOQ ranged between 0% and 21% and between 0% and 38% for all other metabolites in men and women, respectively. In both men and women, the highest concentrations found were for MEP, followed by MiBP, MnBP, 5cx-MEPP, 5OH-MEHP, and 5oxo-MEHP, whereas the lowest concentrations were obtained for oxo-MINCH, followed by OH-MiDP and OH-MINCH. Despite different time frames of sampling, similar trends have been observed in other studies (Wang et al., 2019). Women had significantly lower concentrations of all metabolites compared with men ($p < 0.001$). This trend was not generally observed in our study from 2011, in which non-lactating women ($n = 155$) and men ($n = 177$) of similar ages from Slovenian urban and rural areas, with similar exposure levels among men and higher among women, were involved (Runkel et al., 2020). Therefore, the observed sex differences in the present study could be at least partially related to the generally upregulated drug biotransformation in women during pregnancy, which remains elevated after birth, or to the altered life style during lactation (Meeker et al., 2012; Moya et al., 2014; Zhao et al., 2018; Domínguez-Romero and Scheringer, 2019). This observation can be further supported by the DEHP metabolite ratios (5OH-MEHP/MEHP, 5oxo-MEHP/MEHP, 5cx-MEPP/MEHP, and 5oxo-MEHP/5OH-MEHP), which were significantly higher in women than in men (Table 4), indicating a higher DEHP biotransformation efficiency in women (if biotransformation is not concentration dependent).

The ratios' ranking order of 5cx-MEPP/MEHP > 5OH-MEHP/MEHP > 5oxo-MEHP/MEHP is the same in men and women. This aligns with the literature stating that the majority of MEHP is further metabolized to secondary metabolites (Frederiksen et al., 2007). The lower ratio between the oxo and hydroxy (oxo/OH) metabolites of DEHP and DINCH indicates a higher proportion of the OH metabolite as compared with oxo, which agrees with the current state of knowledge (Bolt et al., 2004; Koch et al., 2005, 2017; Völkel et al., 2016; Schütze et al., 2017).

Table 4
Descriptive statistics of primary and/or secondary PHs and DINCH metabolite levels in urine and their ratios in lactating women and men.[a]

		LACTATING WOMEN								MEN								p-Value*
		% <LOQ	N	GM	Min	P25	P50	P75	Max	% <LOQ	N	GM	Min	P25	P50	P75	Max	
Parent compound	**Metabolite levels**	**Unadjusted data (μg/L)**																
DEHP	MEHP	24.6	289	1.67	0.40	0.81	1.89	3.40	35.1	4.4	274	3.96	0.40	2.23	4.18	6.93	127	<0.001
	5OH-MEHP	0.3	289	4.95	0.05	2.21	5.25	10.3	77.1	0.0	270	8.18	0.48	4.29	8.63	15.3	161	<0.001
	5oxo-MEHP	0.7	289	3.32	0.05	1.54	3.61	6.99	66.7	0.8	266	4.66	0.05	2.62	4.99	8.67	101	<0.001
	5cx-MEPP	0.7	289	6.62	0.05	3.20	6.89	13.6	152	0.0	271	8.40	0.78	4.57	8.91	15.4	134	0.006
DEP	MEP	0.4	273	24.2	0.25	10.68	21.4	52.7	2354	0.4	226	54.1	0.25	17.2	48.7	159	4926	<0.001
DiBP	MiBP	1.7	288	16.7	0.25	8.08	17.6	35.8	524	1.1	268	23.3	0.25	11.8	25.1	47.1	371	<0.001
DnBP	MnBP	0.0	289	9.69	0.69	4.50	10.7	20.3	200	0.8	259	14.2	0.25	8.07	14.8	27.0	345	<0.001
BBzP	MBzP	11.8	288	1.89	0.10	0.97	2.23	4.96	109	4.0	274	3.18	0.10	1.63	3.28	6.27	317	<0.001
DiNP	cx-MiNP	0.7	289	1.71	0.05	0.89	1.70	3.28	105	0.0	273	2.65	0.20	1.61	2.65	4.38	107	<0.001
DiDP	OH-MiDP	22.8	289	0.32	0.05	0.17	0.37	0.70	107	7.3	274	0.73	0.05	0.41	0.73	1.45	33.9	<0.001
DINCH	OH-MINCH	21.1	289	0.56	0.05	0.19	0.47	1.51	220	9.6	271	0.97	0.05	0.34	0.83	2.27	268	<0.001
	oxo-MINCH	37.8	289	0.27	0.05	0.05	0.24	0.77	93.0	21.0	272	0.49	0.05	0.20	0.46	1.19	87.3	<0.001
		SG adjusted data (μg/L SG)																
DEHP	MEHP		287	1.95	0.25	1.16	2.01	3.20	33.0		271	4.45	0.50	2.87	4.50	7.57	87.8	<0.001
	5OH-MEHP		287	5.79	0.20	3.42	5.46	9.02	50.7		267	9.22	0.85	5.88	8.88	14.4	118	<0.001
	5oxo-MEHP		287	3.87	0.20	2.27	3.75	6.22	34.8		263	5.29	0.08	3.57	5.37	8.48	73.5	<0.001
	5cx-MEPP		287	7.71	0.12	4.68	7.00	12.2	96.1		269	9.44	1.46	5.69	9.41	14.5	115	<0.001
DEP	MEP		271	29.5	1.00	13.9	27.7	55.7	1046		224	65.3	1.25	22.3	55.9	161	5185	<0.001
DiBP	MiBP		286	19.7	0.33	12.3	19.6	32.4	262		265	26.2	0.42	15.5	25.8	42.7	374	<0.001
DnBP	MnBP		287	11.4	1.04	6.89	11.2	18.1	104		256	16.3	0.42	10.0	15.1	26.9	276	<0.001
BBzP	MBzP		286	2.21	0.09	1.44	2.65	4.28	65.4		271	3.57	0.10	2.17	3.78	6.09	264	<0.001
DiNP	cx-MiNP		287	1.99	0.15	1.22	1.87	2.79	43.6		270	3.00	0.54	1.74	2.90	4.57	97.1	<0.001
DiDP	OH-MiDP		287	0.37	0.04	0.20	0.37	0.64	71.2		271	0.83	0.04	0.48	0.79	1.39	86.0	<0.001
DINCH	OH-MINCH		287	0.66	0.04	0.20	0.50	1.25	292		268	1.12	0.04	0.49	0.91	2.16	244	<0.001
	oxo-MINCH		287	0.32	0.03	0.10	0.23	0.64	83.2		269	0.57	0.04	0.25	0.49	1.02	79.4	<0.001
	Ratios																	
DEHP	5OH-MEHP/MEHP		289	2.95	0.13	1.98	2.97	4.62	21.3		270	2.09	0.28	1.40	2.07	3.00	21.2	<0.001
	5oxo-MEHP/MEHP		289	1.98	0.13	1.30	1.97	3.12	15.6		266	1.20	0.13	0.83	1.20	1.73	13.1	<0.001
	5cx-MEPP/MEHP		289	3.94	0.13	2.49	4.01	6.18	33.4		271	2.14	0.29	1.29	2.23	3.21	21.2	<0.001
	5oxo-MEHP/5OH-MEHP		289	0.67	0.09	0.61	0.67	0.74	1.00		264	0.58	0.10	0.53	0.59	0.66	0.85	<0.001
	5cx-MEPP/5OH-MEHP		289	1.34	0.06	1.13	1.37	1.63	12.2		267	1.03	0.22	0.83	1.02	1.27	2.55	<0.001
DINCH	oxo-MINCH/OH-MINCH		289	0.49	0.09	0.36	0.49	0.81	4.02		270	0.50	0.14	0.40	0.51	0.66	3.50	0.649

* Difference between men and lactating women tested by Mann-Whitney *U* test.
[a] The exposure data (metabolites levels) for the wider set of participants (including those without available genetic material; n = 304 women and 299 men) is presented by Runkel et al. (2022).

Table 5
Genotype and allele frequencies of studied SNPs (N (%)).

Gene	SNP ID	Genotype	All	Lactating women	Men	HWE p-value	% of genotyped individuals
CYP2C9	rs1057910	AA or *1/*1	578 (85)	253 (88)	225 (83)	0.859	99.6
	or	AC or *1/*3	80 (14)	35 (12)	45 (17)		
	*CYP2C9*3*	CC or *3/* 3	3 (0.5)	1 (0.4)	2 (0.7)		
		MAF %	8	6	9		
	rs1799853	CC or *1/*1	433 (77)	225 (78)	208 (77)	0.100	99.5
	or	CT or *1/*2	114 (21)	57 (20)	57 (21)		
	*CYP2C9*2*	TT or *2/*2	13 (2)	7 (2)	6 (2)		
		MAF %	13	12	13		
CYP2C19	rs4244285	GG or *1/*1	436 (78)	227 (79)	209 (77)	0.525	99.5
	or	GA or *1/*2	118 (21)	59 (20)	59 (22)		
	*CYP2C19*2*	AA or *2/*2	6 (1)	3 (1)	3 (1)		
		MAF %	12	11	12		
	rs12248560 or	CC or *1/*1	297 (54)	149 (53)	148 (55)	0.188	97.9
	*CYP2C19*17*	CT or *1/*17	204 (37)	106 (38)	98 (36)		
		TT or *17/*17	50 (9)	26 (9)	24 (9)		
		MAF %	28	28	27		
CYP2D6	rs3892097	CC or *1/*1	374 (70)	195 (71)	179 (68)		
	or	CT or *1/*4	146 (27)	70 (26)	76 (29)		
	*CYP2D6*4*	TT or *4/*4	18 (3)	9 (3)	9 (3)	0.223	99.1
		MAF %	17	16	18		
UGT2B15	rs1902023	CC or *1/*1	139 (25)	74 (26)	65 (24)	0.997	99.6
	or	CA or *1/*2	280 (50)	147 (51)	133 (49)		
	*UGT2B15*2*	AA or *2/*2	142 (25)	66 (23)	76 (28)		
		MAF %	50	49	52		
UGT1A7	rs11692021 or	TT or *1/*1	193 (35)	100 (35)	93 (34)		
	*UGT1A7*3*	TC or *1/*3	282 (51)	150 (52)	132 (49)		
		CC or *3/*3	83 (15)	38 (13)	45 (17)	0.997	99.6
		MAF %	40	39	41		

HWE: Hardy-Weinberg Equilibrium; MAF: minor allele frequency.

3.2. Allele frequencies of the studied SNPs in the Slovenian population

Table 5 presents the distribution of genotypes and alleles for all selected SNPs of the whole study population and separately for men and lactating women. Each SNP was successfully genotyped in at least 95.6% of the study population and was in accordance with the Hardy-Weinberg equilibrium. The minor allele frequencies (MAF) were between 8% and 50% (for rs1057910 (*CYP2C9*3*) and rs1902023 (*UGT2B15*2*), respectively) and were, in the case of each SNP, similar to those reported for populations with European ancestry (Table 2). Moreover, there were no significant differences in the MAFs of SNPs between men and women.

3.3. SNPs influence on metabolite levels and biotransformation

The estimation coefficients for specific SNP genotypes and/or alleles from MLR analyses (confirmed also in OLR) are summarised in Fig. 2 (CYPs) and Fig. 3 (UGTs). In general, associations with p-values $\leq$0.05 or $\leq$0.1 – based on both MLR and OLR – are discussed. The supplements present additional summary statistics (Supplementary material: subgroup comparisons for urine levels (Tables SP1–SP5) and for metabolite ratios (Tables SP6 and SP7), and results of the regression models (Tables SP8–SP10)).

3.3.1. Cytochrome P450 enzymes – CYPs

An in vitro study on human and rat tissues identified isoforms ranked as CYP2C9 > CYP2C19 > CYP2D6 as the most efficient among the six major isoforms mainly responsible for the production of DEHP secondary metabolites (Choi et al., 2012). *CYP2C9*, CYP2*C19,* and *CYP2D6* are highly polymorphic with several functional SNPs, resulting in enzyme isoforms with decreased or increased activity when compared with their respective wild-type enzymes (Pelkonen et al., 2008; Hiratsuka, 2016; PharmVar: Pharmacogene Variation Consortium, 2021).

*3.3.1.1. CYP2C9 SNPs: rs1799853 (CYP2C9*2), rs1057910 (CYP2C*3).* In the present study, the results indicated a reduced oxidative biotransformation of DEHP in both lactating women and, even more evidently, in men carriers of the variant alleles of both SNPs (Fig. 2; Tables SP1, SP6, and SP8).

The presence of the **rs1799853 (*CYP2C9*2*) variant allele** was associated with lower urine levels of all three DEHP secondary metabolites in men, and in a lower 5cx-MEPP/MEHP ratio in men and women. Differences between variant allele carriers and non-carriers were as follows: 5OH-MEHP (P50: 7.35 vs. 8.99 μg/L SG; coef:-0.26), 5oxo-MEHP (P50: 4.20 vs 5.65 μg/L SG; coef:-0.28), and 5cx-MEPP (P50: 7.07 vs 10.3 μg/L SG; coef:-0.35) in men, and 5cx-MEPP/MEHP in men (P50: 1.73 vs 3.57, coef: −0.24) and in women (coef: −0.23; P50: 3.57 vs. 4.15).

Similarly, the presence of the **rs1057910 (*CYP2C9*3*) variant allele** resulted in three lower DEHP metabolite ratios in male carriers and in lower levels of 5cx-MEPP and 5cx-MEPP/MEHP ratios in female carriers. Results for carriers versus non-carriers were as follows: 5oxo-MEHP/MEHP (P50: 1.04 vs. 1.24; coef: −0.19), 5cx-MEPP/MEHP (P50: 1.46 vs. 2.33; coef: −0.38) and 5oxo-MEHP/5OH-MEHP (P50: 0.45 vs. 0.61; coef: −0.13) in men, and 5cx-MEPP (P50: 6.18 vs 7.38 μg/L SG; coef: −0.16) and 5cx-MEPP/MEHP (P50: 3.22 vs 6.18; coef: −0.46) in women.

Our results are aligned with in vitro studies by Choi et al. (2012, 2013), that report negative effects of these variant alleles on the catalytic activity of the enzyme, resulting in a lower production of DEHP-derived secondary metabolites. Moreover, the presumed negative impact of both SNPs was most noticeable on the production of 5cx-MEPP in both men and women (Fig. 2). Similar observations were also reported by Choi et al. (2012, 2013), who found that rs1057910 (*CYP2C9*3*) resulted in minor production of OH- and oxo-MEHP and a complete loss of 5cx-MEPP formation. Additionally, 5cx-MEPP also shows higher binding affinity compared with other metabolites (Choi et al., 2013), which could highlight the influence of genetic variations on its production.

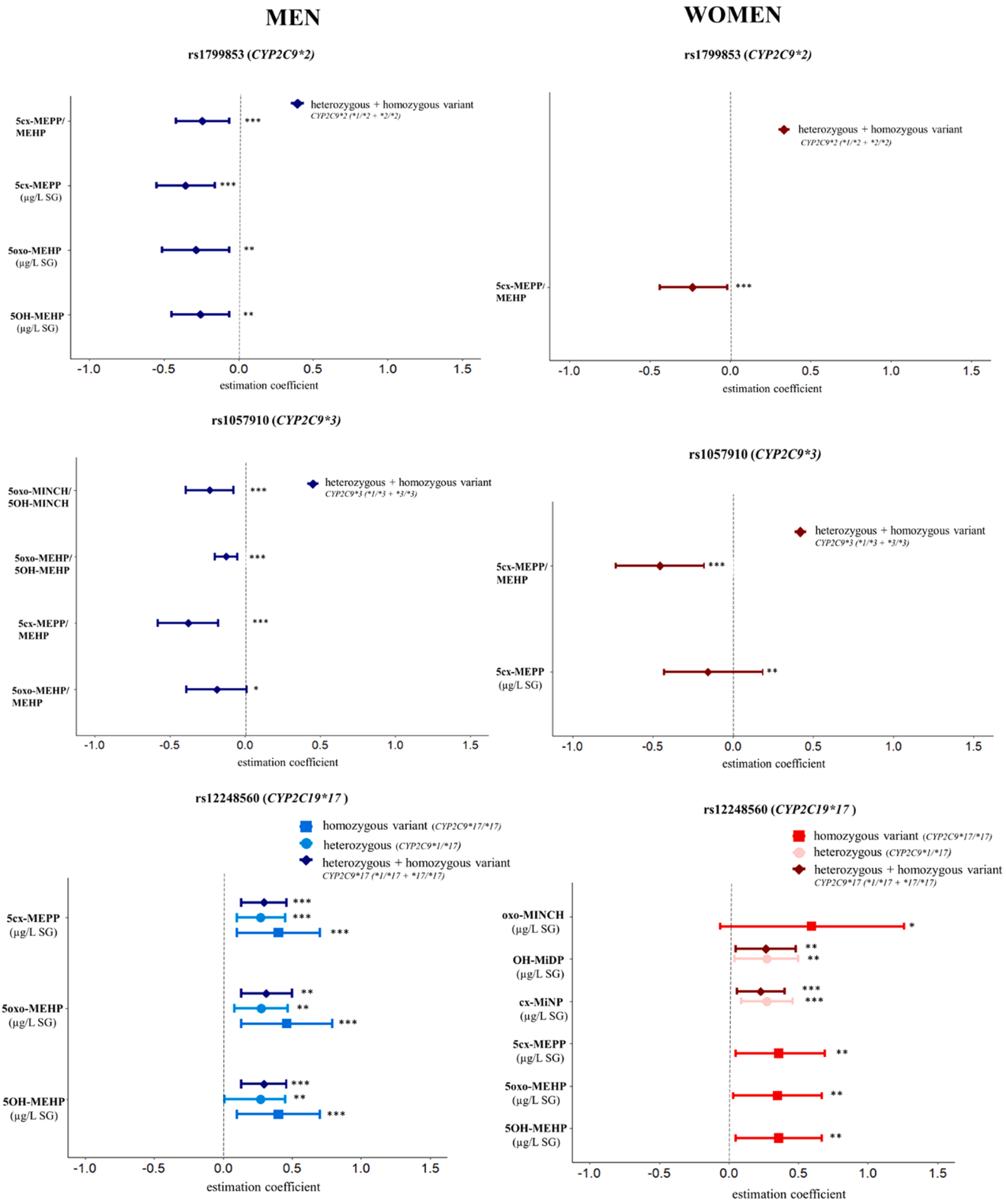

Fig. 2. Associations with p-values ≤0.05 or ≤0.1 for rs1799853 (*CYP2C9*2*), rs1057910 (*CYP2C9*3*), and rs12248560 (*CYP2C19*17*) SNPs with DEHP, DiDP, DiNP, and/or DINCH metabolite levels and/or ratios in men (left; blue) and lactating women (right; red). Presented are estimation coefficients of MLR analyses with a 95% confidence interval for heterozygote + variant homozygote, for heterozygote, and/or for homozygous variant when compared with homozygous wild-type (*1/*1); (***p < 0.01, **p < 0.05, *p < 0.1).

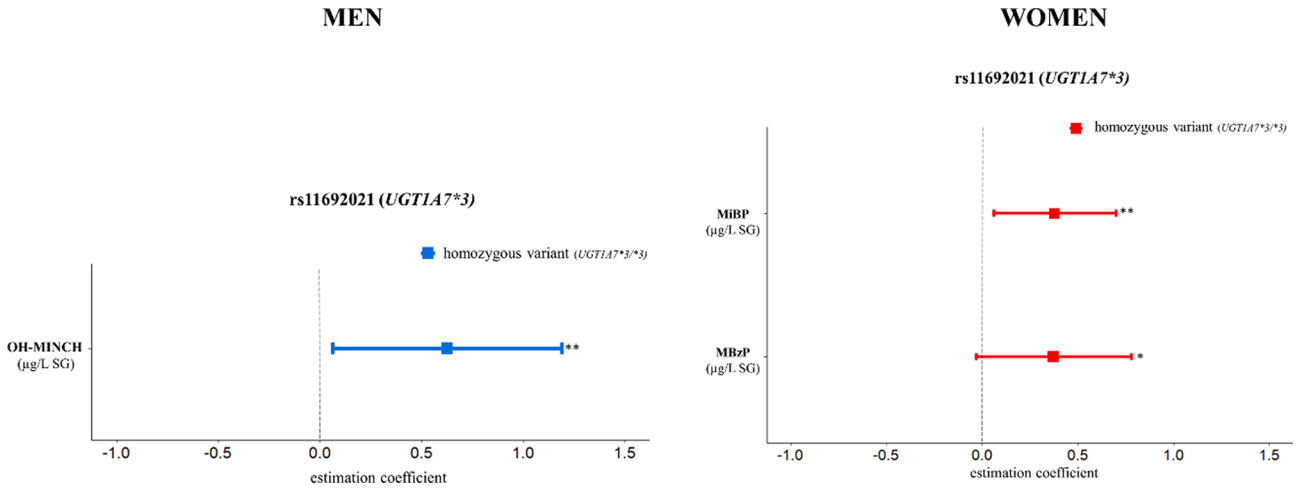

Fig. 3. Associations with p-values ≤0.05 or ≤0.1 for rs11692021 (*UGT1A7*3*) with DiBP, BBzP, or DINCH metabolite levels in men (left; blue) and lactating women (right; red). Presented are estimation coefficients of MLR analyses with a 95% confidence interval for homozygous variant compared with homozygous wild-type (*1/*1); (***p < 0.01, **p < 0.05, *p < 0.1).

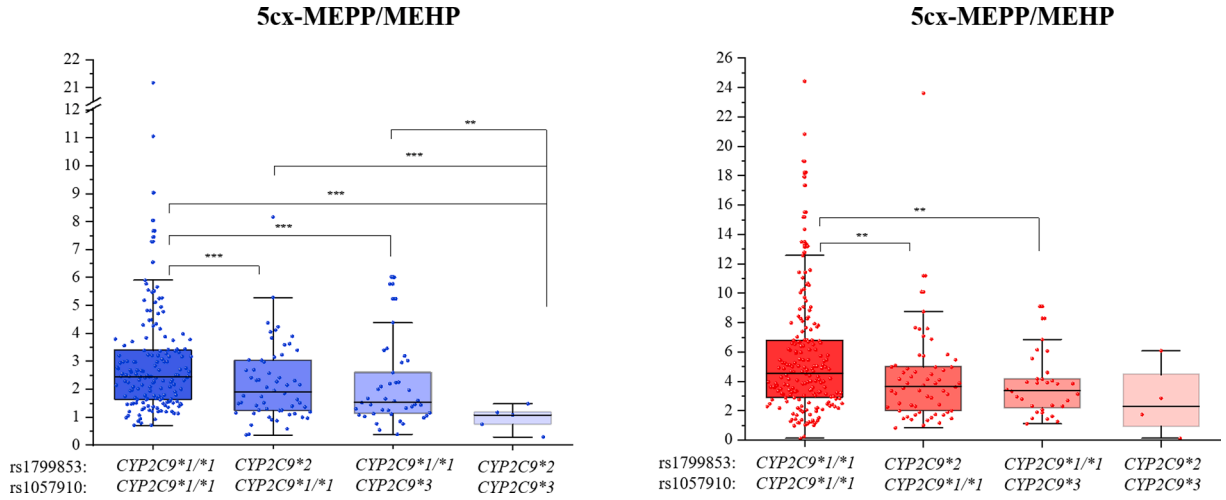

Fig. 4. 5cx-MEPP/MEHP ratio in men (left; blue) and women (right; red) based on the combination of the presence or absence of rs1799853 (*CYP2C9*2*) and/or rs1057910 (*CYP2C9*3*) variant alleles (***p < 0.01; **p < 0.05; *1/*1 represents wild-type genotype).

The association (linkage disequilibrium) between the variant alleles of rs1799853 (*CYP2C9*2*) and rs1057910 (*CYP2C9*3*) is very low. In our study population, only nine individuals (four women and five men) were identified as carriers of both variant alleles. However, as expected, those individuals show an even greater reduction in DEHP biotransformation, with the production of 5cx-MEPP being lower for 50% or more compared with wild-type carriers, as presented in Fig. 4. Furthermore, comparing both variant alleles, generally a slightly higher impact on DEHP biotransformation was observed in the case of rs1057910 (*CYPC9*3*) then rs1799853 (CYP2C9*2) (Fig. 2, Table SP1, SP6, and SP8), most evidently based on the 5cx-MEPP/MEHP ratio (Fig. 4). This could be explained by the rs1057910 (*CYPC9*3*) location that affects the catalytic unit of the enzyme influencing substrate recognition, which could lead to a higher reduction in activity than the rs1799853 (*CYP2C9*2)* variant allele (Rosemary and Adithan, 2007; Wang et al., 2009; Hirota et al., 2013).

Altogether, individuals with the presence of at least one of the discussed variant alleles – and especially those with the presence of both – might be more susceptible to the toxic effects of DEHP due to the reduced biotransformation of bioactive primary metabolite MEHP into less toxic secondary metabolites with better water solubility and faster excretion (Fig. 1) (Frederiksen et al., 2007; Yaghjyan et al., 2016). Moreover, % of MEHP (calculated based on the sum of all measured DEHP metabolites) – which is considered a possible indicator of susceptibility to PH exposure (Meeker et al., 2012) – was significantly higher for variant allele carriers (in the case of both SNPs) than for non-carriers (P50 MEHP% in carriers was for ~14% higher than in non-carriers; coef.: 0.11–0.22; data not presented).

For the other two HMW PHs evaluated in the present study – which also undergo oxidative biotransformation (DiNP and DiDP) –, we did not observe any associations of either variant allele with urine levels of their corresponding secondary metabolites (cx-MiNP and OH-MiDP, respectively). On the contrary, for DINCH, we observed a slight, although significant, reduction in the oxo-MINCH/OH-MINCH ratio in men for rs1057910 (*CYP2C9*3*) variant allele carriers compared with non-carriers (P50: 0.40 versus 0.5; coef: −0.24) (Fig. 2, Tables SP6 and SP9). As seen in the case of DEHP, the effect of variant alleles can be reflected by metabolite ratios and/or urinary levels; however, in our opinion, ratios better reflect biotransformation or enzyme activity than urinary levels alone do. From this viewpoint, examining additional primary or secondary metabolites of DiNP, DiDP, DiBP, DnBP, and DINCH, allowing for the assessment of their respective ratios, could improve the assessment of *CYP2C9* SNPs with those chemicals.

*3.3.1.2. CYP2C19 SNPs: rs4244285 (CYP2C19*2), rs12248560 (CYP2C19*17).* The **rs4244285 (*CYP2C19*2*) variant allele** results in an aberrant splicing site and, consequently, an altered mRNA reading frame, leading to reduced metabolic activity (Hirota et al., 2013). However, in the present study, we did not observe any significant associations of the SNP with PHs or DINCH metabolite urine levels or their ratios in men or women (Tables SP3, SP6 and SP8). To eliminate possible confounding by the influence of *CYP2C9* SNP variant alleles, the associations of rs4244285 (*CYP2C19*2*) were investigated only in wild-type homozygotes for both *CYP2C9* SNPs (n = 166 men and 193 women). However, results did not yield any significant associations (data not presented).

By contrast, the **rs12248560 (*CYP2C19*17*) variant allele,** located in the promotor region, leads to induced enzyme expression and, presumably, enhanced activity (Rosemary & Adithan 2007; Hirota et al., 2013). Indeed, in the present study, its presence was associated with higher levels of HMW PH and DINCH secondary metabolites in urine (Fig. 2 and Tables SP2 and SP8). Among men, carriers of at least one variant allele had significantly higher urine levels of all three DEHP secondary metabolites. Among women, the trends were similar but significant only in the case of variant allele homozygotes (*CYPC2C19*17/*17*).

The differences between male variant allele carriers and non-carriers were as follows: 5OH-MEHP (P50: 9.02 vs 8.84 μg/L SG; coef: 0.30), 5oxo-MEHP (P50: 5.88 vs. 4.91 μg/L SG; coef: 0.31), and 5cx-MEPP (P50: 10.4 vs. 8.42 μg/L SG; coef: 0.30); for variant homozygotes (*C19*17/*17)* the differences were even higher (coef: 0.40–0.46).

The differences between female variant homozygous carriers and wild-type carriers were as follows: 5OH-MEHP (P50: 6.63 vs 5.35 μg/L SG; coef: 0.36), 5oxo-MEHP (P50: 4.77 vs. 3.53 μg/L SG; coef: 0.35), and 5cx-MEPP (P50: 9.23 vs 6.67 μg/L SG; coef: 0.36). Furthermore, *C19*17/*17* women also had significantly higher levels of oxo-MINCH (P50: 0.45 vs. 0.20 μg/L SG; coef: 0.60), however, model performance did not pass the diagnostic analyses (Table SP8).

With respect to other PHs, the presence of the rs12248560 *(CYP2C19*17)* variant allele among women was significantly associated with slightly higher levels of cx-MiNP (P50: 1.97 vs. 1.65 μg/L SG; coef: 0.23) and OH-MiDP (P50: 0.40 vs. 0.32 μg/L SG; coef: 0.27).

Unlike CYP2C9, in the case of CYP2C19 no significant or consistent influence of the SNP on metabolite ratios was observed (Table SP8). Similarly, the *CYP2C19*17* allele was previously associated with faster clearance of certain drugs (e.g. escitalopram, sertraline) from patients' serum but did not show a significant effect on pharmacokinetic parameters when compared with the wild-type enzyme (Li-Wan-Po et al., 2010; Hirota et al., 2013). Therefore, for a better interpretation of the role of *CYP2C19*17* in PHs and DINCH biotransformation, further studies are needed.

*3.3.1.3. CYP2D6 SNP: rs39892097 (CYP2D6*4).* Finally, in the case of ***CYP2D6***, its most common SNP in Caucasians is **rs3892097 (*CYP2D6*4*)**. The presence of its variant allele was previously reported to result in reduced enzyme activity or, in the case of homozygous carriers, in an inactive enzyme (Ingelman-Sundberg, 2005; He et al., 2015).

The presence of the rs3892097 (*CYP2D6*4*) variant allele in the present study showed a tendency towards lower urine levels of PHs and DINCH metabolites and some of their ratios compared with wild-type carriers, but none of the associations were significant (Table SP3, SP6, and SP8). However, when considering only homozygous variant allele carriers (*CYP2D6*4/*4*), men indeed expressed significantly lower urine levels of all measured DEHP, DiNP and DiDP secondary metabolites compared with *CYP2D6*1/*4* and *CYP2D6*1/*1* carriers, while among women, such associations were not observed (data not shown). The low number of homozygous variant allele carriers (*CYP2D6*4 /*4*; 9 men and 9 women; Table 5) did not present sufficient statistical power to test associations in models; therefore, such associations should be further studied on a larger population size. Nevertheless, our observed results are in line with the statement by He et al. (2015) that homozygous variant allele carriers are most commonly associated with the phenotype of poor metabolizers.

3.3.2. UDP-glucuronosyl transferases – UGTs

According to previous studies, that have measured free and conjugated forms of PHs and DINCH primary and secondary metabolites in human urine, the majority of metabolites studied in the present work (Table 1) are excreted predominantly in glucuronidated form (>70%); only MEP and cx-MINP metabolites of DEP and DiNP, respectively, are excreted mainly in free form (Silva et al., 2003, 2013; Frederiksen et al., 2007; Seckin et al., 2009; Saravanabhavan and Murray, 2012).

*3.3.2.1. UGT2B15 SNP: rs1902023 (UGT2B15*2).* In the present study, we did not observe any significant associations between the SNP **rs1902023 (*UGT2B15*2*)** variant allele and urine levels or ratios of pH and DINCH metabolites, regardless of sex (Tables SP4, SP7, and SP9). For DEHP such results are in line with the in vitro study reporting negligible activity of the UGT2B15 recombinant enzyme in the glucuronidation of MEHP (Hanioka et al., 2017). Moreover, the influence of rs1902023 on its enzyme activity has been reported inconsistently; it has been associated with with a higher clearance of total (undefined) PHs in the serum of homozygous variant allele carriers (Luo et al., 2020) and by contrast, with decreased glucuronidation capacity for some anxiolytic pharmaceuticals (e.g. oxazepam, lorazepam) in kidney cells (HK293) (Guillemette, 2003; Clarke and Jones, 2009) and bisphenol A (Hanioka et al., 2011).

*3.3.2.2. UGT1A7 SNP: rs11692921 (UGT1A7*3).* **UGT1A7** is an extrahepatic enzyme expressed mainly in the small intestines (also in oesophagus, stomach, lungs and pancreas), and its common SNP **rs11692021 (*UGT1A7*3*)** was reported to lead to reduced activity (Guillemette et al., 2000; Miners et al., 2002; Guillemette, 2003; Clarke and Jones, 2009). However, in the present study, carriers of the rs11692021 *CYP1A7*3* variant allele showed a tendency towards higher excretion of all DEHP metabolites when compared with wild-type carriers (Table SP5), but no associations were statistically significant (Table SP9). Moreover, homozygous variant allele carriers (*UGT1A7*3/*3*) compared to wild-type carriers show significantly higher urine levels of MBzP (P50: 3.87 vs 2.26 μg/L SG; coef: 0.37) and MiBP (P50: 28.8 vs. 16.3 μg/L SG; coef: 0.38) among women, and among men higher urine levels of OH-MINCH (P50: 1.14 vs. 0.88 μg/L SG; coef: 0.63) (Fig. 3, Tables SP5 and SP9).

Interpreting the observed higher levels of metabolites in the urine of individuals with expected reduced UGT1A7 activity is challenging, but it could be related to either interaction with other undefined UGTs or their induced activity in the liver; glucuronidation can be compensated across UGT isoforms (Gao et al., 2021). For instance, UGT2B7 and UGT1A9 enzymes in the liver have shown the highest activity towards MEHP glucuronidation (Hanioka et al., 2016). However, their polymorphisms rs7439366 (*UGT2B7*2*) and rs72551330 (*UGT1A9*3*), respectively, unfortunately were not investigated in the present study due to their low occurrence in European populations or the unavailability of TaqMan genotyping assays (ThermoFisher Scientific, 2021). The role of their polymorphisms in PHs and DINCH biotransformation should be investigated in the future using a larger population size and alternative genotyping methods.

3.4. Evaluation of statistical models, predictors and study limitations

In the present study, predictors used in multiple linear regression models explained 1–27% variability of pH and DINCH metabolite levels in urine or their ratios – the highest for DEHP secondary metabolites levels (5OH-MEHP, 5oxo-MEHP, and 5cx-MEHP) – and only 2–11%

variability of DEHP and DINCH metabolite ratios (Table SP10). Among the predictors used, a significant influence was observed for year of sampling, age, current smoking in men, and, in the case of DEHP metabolites, the 5cx-MEPP/5OH-MEHP ratio (Table SP10). The model results suggest that urinary PHs metabolite concentrations decreased significantly over the sampling period from 2008 to 2014. As studies observed these compounds to be stable in urine at −70 °C for several years (Silva et al., 2008; Samandar et al., 2009), these trends can be attributed rather to the general utilization patterns of PHs in the European market(Tranfo et al., 2018; Wang et al., 2019) than to compound degradation. The obtained results for age and BMI are inconsistent, which agrees with the literature, in which to date, the effect of neither could be determined with certainty (Goodman et al., 2014; Chiang et al., 2016; Koch et al., 2017). The negative associations of current smoking among men with urinary metabolite levels (mainly DEHP-derived) is in need of re-evaluation on a population with a larger number of active smokers. The inclusion of 5cx-MEPP/5OH-MEHP as a model predictor indicated its potential to approximate for the time of DEHP exposure. According to the hypothesis by Lorber et al. (2011) the ratios in the present study with geometric means ≥1 in women and men (Table 4), indicate that exposure in general occurred 10 h or more before the spot urine sampling. However, one should keep in mind that this ratio might also be influenced by the differences in metabolism (Meeker et al., 2012), therefore, its use should be confirmed in future studies including also data on DEHP exposure. Selenium in blood was positively associated with DINCH metabolite levels in women, while no significant influence was observed for PH metabolite levels in either men or women; the observed selenium levels in our study population are within the reference range for adult populations (58–243 ng/mL) (Roberts et al., 2012).

Low coverage of the variability in the models could be explained by the missing information on individual's exposure (e.g. usage of personal care products or food packaging within the last days prior to sampling) as well as introduced uncertainty by the use of random spot urine samples, both of which represent a limitation of the present study. Therefore, in future studies, first morning urine samples should be obtained to limit the variation in time between exposure and measurement (Bastiaensen et al., 2020). As the number of measurable PH and DINCH primary and secondary metabolites in urine is continuously increasing (Schütze et al., 2017; Wang et al., 2019), more of them should be included in our future studies to more adequately estimate the influence of studied SNPs on PHs and DINCH biotransformation. Furthermore, especially in the case of UGT SNPs, the information on percentage of glucuronide-conjugated versus un-conjugated (free) forms of metabolites would be of great importance, as it would give more relevant insight into the biotransformation II pathway.

4. Conclusions

The present study investigated, for the first time, the possible influence of SNPs in *CYP2C9, CYP2C19, CYP2D6*, *UGT2B15* and *UGT1A7* genes on the biotransformation of phthalates and DINCH using human biomonitoring data on men and lactating women.

Our results confirm the previously only in vitro observed influence of rs1799853 (*CYP2C9*2*) and rs1057910 (*CYP2C9*3*) on the reduced biotransformation of DEHP and suggest a negative influence of rs1057910 (*CYP2C9*3*) on DINCH biotransformation. The latter was observed only in men. Moreover, rs12248560 (*CYP2C19*17*) was associated with a higher excretion of secondary metabolites of DEHP (men and women), DiNP and DiDP (women), while the rs11692021 (*UGT1A7*2*) resulted in higher urine levels of BBzP, DiBP (women) and DINCH metabolites (men).

Although most of the variance in phthalates and DINCH metabolites urinary levels and ratios remains unexplained, we demonstrate that the above-mentioned SNPs could represent important biomarkers of susceptibility to phthalates and DINCH exposure that have been so far unrecognised.

As genes studied in the present study were selected based on the DEHP biotransformation, in future, more attention should be directed into the identification of possible specific CYP and UGT isoforms and their SNPs, which are the most active in the biotransformation of DINCH and other phthalates.

Funding

This study is part of the NEUROSOME project (H2020-MSCA-ITN-2017 SEP-210411486), which was funded by the Horizon 2020 Research and Innovation programme under the Marie Skłodowska-Curie grant agreement no. 766251. Additionally, the present study was financed by the Slovenian Research Agency and the Chemicals Office of the Republic of Slovenia through the National Human Biomonitoring Programme (Contracts numbers: C2715-07Y000042, C2715-11-000005, C2715-18-634803, and C2715-19-634801), Jožef Stefan Institute programme group P1-0143 (Slovenia) and national project "Neurophysiological dysfunctions caused by low-level exposure to selected environmental pollutants in susceptible population" (NEURODYS; Contracts number: J7-9400).

CRediT authorship contribution statement

Anja Stajnko: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Agneta Annika Runkel:** Conceptualization, Investigation, Writing – review & editing, Resources. **Tina Kosjek:** Writing – review & editing. **Janja Snoj Tratnik:** Writing – review & editing. **Darja Mazej:** Writing – review & editing. **Ingrid Falnoga:** Writing – review & editing, Resources. **Milena Horvat:** Supervision, Project administration, Funding acquisition, Writing – review & editing, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to the NEUROSOME project and Lijana Kononenko for supporting the study. We thank all participants of the study, the personnel of the Regional Health Centres, who helped with recruitment and collection of biological samples, and Marta Jagodic Hudobivnik for her help in sample preparation.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.107046.

References

ATSDR, 2019. Toxicological Profile for Di(2-Ethylhexyl)Phthalate (DEHP) Draft for Public Comment. December. Available at: www.regulations.gov.

Bastiaensen, M., Malarvannan, G., Gys, C., Ait Bamai, Y.u., Araki, A., Covaci, A., 2020. Between- and within-individual variability of urinary phthalate and alternative plasticizer metabolites in spot, morning void and 24-h pooled urine samples. Environ. Res. 191, 110248. https://doi.org/10.1016/j.envres.2020.110248.

Benjamin, S., Masai, E., Kamimura, N., Takahashi, K., Anderson, R.C., Faisal, P.A., 2017. Phthalates impact human health: Epidemiological evidences and plausible mechanism of action. J. Hazard. Mater. 340, 360–383. https://doi.org/10.1016/j.jhazmat.2017.06.036.

Berger, K., Kogut, K.R., Bradman, A., She, J., Gavin, Q., Zahedi, R., Parra, K.L., Harley, K. G., 2019. Personal care product use as a predictor of urinary concentrations of certain phthalates, parabens, and phenols in the HERMOSA study. J. Expos. Sci. Environ. Epidemiol. 29 (1), 21–32. https://doi.org/10.1038/s41370-017-0003-z.

Bolt, H.M., Angerer, J., Koch, H.M., 2004. Di(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. Arch. Toxicol. 78 (3), 123–130. https://doi.org/10.1007/s00204-003-0522-3.

Bui, T.T., Giovanoulis, G., Cousins, A.P., Magnér, J., Cousins, I.T., de Wit, C.A., 2016. Human exposure, hazard and risk of alternative plasticizers to phthalate esters. Sci. Total Environ. 541, 451–467. https://doi.org/10.1016/j.scitotenv.2015.09.036.

Burk, R.F., 1983. Biological Activity of Selenium. Annu. Rev. Nutr. 3 (1), 53–70. https://doi.org/10.1146/annurev.nu.03.070183.000413.

Cao, X., Durairaj, P., Yang, F., Bureik, M., 2019. A comprehensive overview of common polymorphic variants that cause missense mutations in human CYPs and UGTs. Biomed. Pharmacother. 111, 983–992. https://doi.org/10.1016/j.biopha.2019.01.024.

Chiang, H.-C., Kuo, Y.-T., Shen, C.-C., Lin, Y.-H., Wang, S.-L., Tsou, T.-C., 2016. Mono(2-ethylhexyl)phthalate accumulation disturbs energy metabolism of fat cells. Arch. Toxicol. 90 (3), 589–601. https://doi.org/10.1007/s00204-014-1446-9.

Choi, K., Joo, H., Campbell, J.L., Andersen, M.E., Clewell, H.J., 2013. In vitro intestinal and hepatic metabolism of Di(2-ethylhexyl) phthalate (DEHP) in human and rat. Toxicol. Vitr. 27 (5), 1451–1457. https://doi.org/10.1016/j.tiv.2013.03.012.

Choi, K., Joo, H., Campbell, J.L., Clewell, R.A., Andersen, M.E., Clewell, H.J., 2012. In vitro metabolism of di(2-ethylhexyl) phthalate (DEHP) by various tissues and cytochrome P450s of human and rat. Toxicol. Vitr. 26 (2), 315–322. https://doi.org/10.1016/j.tiv.2011.12.002.

Clarke, S.E., Jones, B.C., 2009. UDP-Glucuronosyltransferases. In: Swarbrick, J. (Ed.), European Journal of Pharmaceutics and Biopharmaceutics. Informa Healthcare USA, Inc, USA, pp. 87–113. https://doi.org/10.1016/j.ejpb.2008.10.010.

Di, Y.M., Chan, E., Wei, M.Q., Liu, J.P., Zhou, S.F., 2009. Prediction of deleterious non-synonymous single-nucleotide polymorphisms of human uridine diphosphate glucuronosyltransferase genes. AAPS J. 11, 469–480. https://doi.org/10.1208/s12248-009-9126-z.

Domínguez-Romero, E., Scheringer, M., 2019. A review of phthalate pharmacokinetics in human and rat: what factors drive phthalate distribution and partitioning? Drug Metab. Rev. 51 (3), 314–329. https://doi.org/10.1080/03602532.2019.1620762.

Elbers, I., Mol, H., 2019. ICI/EQUAS REPORT; Phthalates/round_02 (2018)- Phthalate biomarkers in urine. Wageningen.

Frederiksen, H., Skakkebæk, N.E., Andersson, A.M., 2007. Metabolism of phthalates in humans. Mol. Nutr. Food Res. 51, 899–911. https://doi.org/10.1002/mnfr.200600243.

Gao, S., Bell, E.C., Zhang, Y., Liang, D., 2021. Racial Disparity in Drug Disposition in the Digestive Tract. Int. J. Mol. Sci. 22, 1038. https://doi.org/10.3390/ijms22031038.

Giuliani, A., Zuccarini, M., Cichelli, A., Khan, H., Reale, M., et al., 2020. Critical review on the presence of phthalates in food and evidence of their biological impact. Int. J. Environ. Res. Public Health 17 (16), 5655. https://doi.org/10.3390/ijerph17165655.

Goodman, M., LaKind, J.S., Mattison, D.R., 2014. Do phthalates act as obesogens in humans? A systematic review of the epidemiological literature. Crit. Rev. Toxicol. 44, 151–175. https://doi.org/10.3109/10408444.2013.860076.

Guillemette, C., 2003. Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. Pharmacogenomics J. 3, 136–158. https://doi.org/10.1038/sj.tpj.6500171.

Guillemette, C., Ritter, J.K., Auyeung, D.J., Kessler, F.K., Housman, D.E., 2000. Structural heterogeneity at the UDP-glucuronosyltransferase 1 locus: functional consequences of three novel missense mutations in the human UGT1A7 gene. Pharmacogenetics 10, 629–644. https://doi.org/10.1097/00008571-200010000-00006.

Hanioka, N., Kinashi, Y., Tanaka-Kagawa, T., Isobe, T., Jinno, H., 2017. Glucuronidation of mono(2-ethylhexyl) phthalate in humans: roles of hepatic and intestinal UDP-glucuronosyltransferases. Arch. Toxicol. 91, 689–698. https://doi.org/10.1007/s00204-016-1708-9.

Hanioka, N., Oka, H., Nagaoka, K., Ikushiro, S., Narimatsu, S., 2011. Effect of UDP-glucuronosyltransferase 2B15 polymorphism on bisphenol A glucuronidation. Arch. Toxicol. 85, 1373–1381. https://doi.org/10.1007/s00204-011-0690-5.

He, Z.-X., Chen, X.-W., Zhou, Z.-W., Zhou, S.-F., 2015. Impact of physiological, pathological and environmental factors on the expression and activity of human cytochrome P450 2D6 and implications in precision medicine. Drug Metab. Rev. 47, 470–519. https://doi.org/10.3109/03602532.2015.1101131.

Hiratsuka, M., 2016. Genetic polymorphisms and in vitro functional characterization of CYP2C8, CYP2C9, and CYP2C19 allelic variants. Biol. Pharm. Bull. 39, 1748–1759. https://doi.org/10.1248/bpb.b16-00605.

Hirota, T., Eguchi, S., Ieiri, I., 2013. Impact of Genetic Polymorphisms in CYP2C9 and CYP2C19 on the Pharmacokinetics of Clinically Used Drugs. Drug Metab. Pharmacokinet. 28, 28–37. https://doi.org/10.2133/dmpk.DMPK-12-RV-085.

Hlavac, M., 2018. Stargazer: Well-Formatted Regression and Summary Statistics Tables. Available at: https://cran.r-project.org/package=stargazer%0A%0A.

Huang, P.C., Li, W.F., Liao, P.C., Sun, C.W., Tsai, E.M., Wang, S.L., 2014. Risk for estrogen-dependent diseases in relation to phthalate exposure and polymorphisms of CYP17A1 and estrogen receptor genes. Environ. Sci. Pollut. Res. 21, 13964–13973. https://doi.org/10.1007/s11356-014-3260-6.

Ingelman-Sundberg, M., 2005. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): Clinical consequences, evolutionary aspects and functional diversity. Pharmacogenomics J. 5, 6–13. https://doi.org/10.1038/sj.tpj.6500285.

Ito, Y., Kamijima, M., Hasegawa, C., Tagawa, M., Kawai, T., Miyake, M., et al., 2014. Species and inter-individual differences in metabolic capacity of di(2-ethylhexyl) phthalate (DEHP) between human and mouse livers. Environ. Health Prev. Med. 19, 117–125. https://doi.org/10.1007/s12199-013-0362-6.

Jiang, Z., Gu, L., Liang, X., Cao, B., Zhang, J., Guo, X., 2020. The Effect of Selenium on CYP450 Isoform Activity and Expression in Pigs. Biol. Trace Elem. Res. 196, 454–462. https://doi.org/10.1007/s12011-019-01945-7.

Kalman, L., Agúndez, J., Appell, M.L., Black, J., Bell, G., Boukouvala, S., et al., 2016. Pharmacogenetic allele nomenclature: International workgroup recommendations for test result reporting. Clin. Pharmacol. Ther. 99, 172–185. https://doi.org/10.1002/cpt.280.

Klomp, S.D., Manson, M.L., Guchelaar, H.-J., Swen, J.J., 2020. Phenoconversion of Cytochrome P450 Metabolism: A Systematic Review. J. Clin. Med. 9, 2890. https://doi.org/10.3390/jcm9092890.

Koch, H.M., Bolt, H.M., Preuss, R., Angerer, J., 2005. New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. Arch. Toxicol. 79, 367–376. https://doi.org/10.1007/s00204-004-0642-4.

Koch, H.M., Preuss, R., Angerer, J., Foster, P., Sharpe, R., Toppari, J., 2006. Di(2-ethylhexyl)phthalate (DEHP): Human metabolism and internal exposure - An update and latest results. Int. J. Androl. 29, 155–165. https://doi.org/10.1111/j.1365-2605.2005.00607.x.

Koch, H.M., Rüther, M., Schütze, A., Conrad, A., Pälmke, C., Apel, P., et al., 2017. Phthalate metabolites in 24-h urine samples of the German Environmental Specimen Bank (ESB) from 1988 to 2015 and a comparison with US NHANES data from 1999 to 2012. Int. J. Hyg. Environ. Health 220, 130–141. https://doi.org/10.1016/j.ijheh.2016.11.003.

Li-Wan-Po, A., Girard, T., Farndon, P., Cooley, C., Lithgow, J., 2010. Pharmacogenetics of CYP2C19: functional and clinical implications of a new variant CYP2C19*17. Br. J. Clin. Pharmacol. 69, 222–230. https://doi.org/10.1111/j.1365-2125.2009.03578.x.

Lorber, M., Koch, H.M., Angerer, J., 2011. A critical evaluation of the creatinine correction approach: Can it underestimate intakes of phthalates? A case study with di-2-ethylhexyl phthalate. J. Expo. Sci. Environ. Epidemiol. 21, 576–586. https://doi.org/10.1038/jes.2010.43.

Luo, Y., Nie, Y., Tang, L., Xu, C.C., Xu, L., 2020. The correlation between UDP-glucuronosyltransferase polymorphisms and environmental endocrine disruptors levels in polycystic ovary syndrome patients. Medicine (Baltimore) 99, e19444. https://doi.org/10.1097/MD.0000000000019444.

Lyche, J.L., 2017. Phthalates. In: Gupta, C.R. (Ed.), Reproductive and Developmental Toxicology. Academic Press, London, UK, pp. 829–856. https://doi.org/10.1016/B978-0-12-804239-7.00044-5.

Meeker, J.D., Calafat, A.M., Hauser, R., 2012. Urinary phthalate metabolites and their biotransformation products: Predictors and temporal variability among men and women. J. Expo. Sci. Environ. Epidemiol. 22, 376–385. https://doi.org/10.1038/jes.2012.7.

Miklavčič, A., Casetta, A., Snoj Tratnik, J., Mazej, D., Krsnik, M., Mariuz, M., et al., 2013. Mercury, arsenic and selenium exposure levels in relation to fish consumption in the Mediterranean area. Environ. Res. 120, 7–17. https://doi.org/10.1016/j.envres.2012.08.010.

Miners, J.O., McKinnon, R.A., Mackenzie, P.I., 2002. Genetic polymorphisms of UDP-glucuronosyltransferases and their functional significance. Toxicology 181–182, 453–456. https://doi.org/10.1016/S0300-483X(02)00449-3.

Moya, J., Phillips, L., Sanford, J., Wooton, M., Gregg, A., Schuda, L., 2014. A review of physiological and behavioral changes during pregnancy and lactation: Potential exposure factors and data gaps. J. Expo. Sci. Environ. Epidemiol. 24, 449–458. https://doi.org/10.1038/jes.2013.92.

NCBI, 2021. dbSNP: short genetic varations. Available at: https://www.ncbi.nlm.nih.gov/snp/ [Accessed March 10, 2021].

Pelkonen, O., Turpeinen, MiiaPelkonen, O., Turpeinen, M., Hakkola, J., Honkakoski, P., Hukkanen, J., Raunio, H., 2008. Inhibition and induction of human cytochrome P450 enzymes: current status. Arch. Toxicol. 82, 667–715. https://doi.org/10.1007/s00204-008-0332-8.

PharmVar: Pharmacogene Variation Consortium, 2021. CYP enzyme family nomenclature. Available at: https://www.pharmvar.org/genes [Accessed December 11, 2021].

R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.

Roberts, W., McMillin, G.A., Burtis, C.A., 2012. Reference Information for the Clinical Laboratory. In: Tietz Texbook of Clinical Chemistry and Molecular Diagnostics. Elsevier Life Science, USA, pp. 2131–2188.

Rosemary, J., Adithan, C., 2007. The Pharmacogenetics of CYP2C9 and CYP2C19: Ethnic Variation and Clinical Significance. Curr. Clin. Pharmacol. 2, 93–109. https://doi.org/10.2174/157488407779422302.

Runkel, A.A., Mazej, D., Snoj Tratnik, J., Tkalec, Ž., Kosjek, T., Horvat, M., 2022. Exposure of men and lactating women to environmental phenols, phthalates, and DINCH. Chemosphere 286, 131858. https://doi.org/10.1016/j.chemosphere.2021.131858.

Runkel, A.A., Snoj-Tratnik, J., Mazej, D., Horvat, M., 2020. Urinary phthalate concentrations in the Slovenian population: An attempt to exposure assessment of family units. Environ. Res. 186, 109548 https://doi.org/10.1016/j.envres.2020.109548.

Samandar, E., Silva, M.J., Reidy, J.A., Needham, L.L., Calafat, A.M., 2009. Temporal stability of eight phthalate metabolites and their glucuronide conjugates in human urine. Environ. Res. 109, 641–646. https://doi.org/10.1016/j.envres.2009.02.004.

Saravanabhavan, G., Murray, J., 2012. Human biological monitoring of diisononyl phthalate and diisodecyl phthalate: A review. J. Environ. Public Health 2012. https://doi.org/10.1155/2012/810501.

Schoeters, G., Lange, R., 2020. Scoping documents for the second round priority substances. Deliverable Report D4.6. https://www.hbm4eu.eu/wp-content/uploads/2017/03/HBM4EU_D4.6_Scoping_Documents_2nd_priority_substances_v2.0.pdf.

Schütze, A., Otter, R., Modick, H., Langsch, A., Brüning, T., Koch, H.M., 2017. Additional oxidized and alkyl chain breakdown metabolites of the plasticizer DINCH in urine

after oral dosage to human volunteers. Arch. Toxicol. 91, 179–188. https://doi.org/10.1007/s00204-016-1688-9.
Seckin, E., Fromme, H., Völkel, W., 2009. Determination of total and free mono-n-butyl phthalate in human urine samples after medication of a di-n-butyl phthalate containing capsule. Toxicol. Lett. 188, 33–37. https://doi.org/10.1016/j.toxlet.2009.03.002.
Silva, M.J., Barr, D.B., Reidy, J.A., Kato, K., Malek, N.A., Hodge, C.C., et al., 2003. Glucuronidation patterns of common urinary and serum monoester phthalate metabolites. Arch. Toxicol. 77, 561–567. https://doi.org/10.1007/s00204-003-0486-3.
Silva, M.J., Jia, T., Samandar, E., Preau, J.L., Calafat, A.M., 2013. Environmental exposure to the plasticizer 1,2-cyclohexane dicarboxylic acid, diisononyl ester (DINCH) in US adults (2000–2012). Environ. Res. 126, 159–163. https://doi.org/10.1016/j.envres.2013.05.007.
Silva, M.J., Preau, J.L., Needham, L.L., Calafat, A.M., 2008. Cross validation and ruggedness testing of analytical methods used for the quantification of urinary phthalate metabolites. J. Chromatogr. B 873, 180–186. https://doi.org/10.1016/j.jchromb.2008.08.017.
Snoj Tratnik, J., Falnoga, I., Mazej, D., Kocman, D., Fajon, V., Jagodic, M., et al., 2019. Results of the first national human biomonitoring in Slovenia: Trace elements in men and lactating women, predictors of exposure and reference values. Int. J. Hyg. Environ. Health 222, 563–582. https://doi.org/10.1016/j.ijheh.2019.02.008.
Stajnko, A., Falnoga, I., Tratnik, J.S., Mazej, D., Jagodic, M., Krsnik, M., et al., 2017. Low cadmium exposure in males and lactating females–estimation of biomarkers. Environ. Res. 152, 109–119. https://doi.org/10.1016/j.envres.2016.09.025.
Stein, T.P., Schluter, M.D., Steer, R.A., Ming, X., 2013. Autism and Phthalate Metabolite Glucuronidation. J. Autism Dev. Disord. 43, 2677–2685. https://doi.org/10.1007/s10803-013-1822-y.
SURS, 2017. Socioeconomic characteristics of population. Available at: https://www.stat.si/StatWeb/en/News/Index/7128 [Accessed October 22, 2021].
Suwazono, Y., Akesson, A., Alfvén, T., Järup, L., Vahter, M., 2005. Creatinine versus specific gravity-adjusted urinary cadmium concentrations. Biomarkers 10, 117–126.
SURS, 2017. Socioeconomic characteristics of population. Available at: https://www.stat.si/StatWeb/en/News/Index/7128 [Accessed October 22, 2021].
Tranfo, G., Caporossi, L., Pigini, D., Capanna, S., Papaleo, B., Paci, E., 2018. Temporal Trends of Urinary Phthalate Concentrations in Two Populations: Effects of REACH Authorization after Five Years. Int. J. Environ. Res. Public Health 15, 1950. https://doi.org/10.3390/ijerph15091950.
UGT Nomenclature Committee, 2005. UGT Alleles Nomenclature Home Page. Available at: http://www.ugtalleles.ulaval.ca.
Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S. Fourth Edition.
Völkel, W., Kiranoglu, M., Dettbarn, G., John, A., Jessel, S., Seidel, A., et al., 2016. Urinary toxicokinetics of di-(isononyl)-cyclohexane-1,2-dicarboxylate (DINCH®) in humans following single oral administration. Toxicol. Lett. 248, 16–24. https://doi.org/10.1016/j.toxlet.2016.02.010.
Wang, B., Wang, J., Huang, S.-Q., Su, H.-H., Zhou, S.-F., 2009. Genetic Polymorphism of the Human Cytochrome P450 2C9 Gene and Its Clinical Significance. Curr. Drug Metab. 10, 781–834. https://doi.org/10.2174/138920009789895480.
Wang, Y., Zhu, H., Kannan, K., 2019. A Review of Biomonitoring of Phthalate Exposures. Toxics 7, 21. https://doi.org/10.3390/toxics7020021.
Waxman, D.J., Holloway, M.G., 2009. Sex Differences in the Expression of Hepatic Drug Metabolizing Enzymes. Mol. Pharmacol. 76, 215–228. https://doi.org/10.1124/mol.109.056705.
Wicklam, H., 2016. ggplot2: Elegant Graphics for Data Analysis.
Wittassek, M., Koch, H.M., Angere, J., Brüning, T., 2011. Assessing exposure to phthalates – the human biomonitoring approach. Mol. Nutr. Food Res. 55, 7–31. https://doi.org/10.1002/mnfr.201000121.
Yaghjyan, L., Carlsson, N.P., Ghita, G.L., Chang, S.-H., 2016. Associations of individual characteristics and lifestyle factors with metabolism of di-2-ethylhexyl phthalate in NHANES 2001–2012. Environ. Res. 149, 23–31. https://doi.org/10.1016/j.envres.2016.05.002.
Zhao, H., Li, J., Zhou, Y., Zhu, L., Zheng, Y., Xia, W., et al., 2018. Investigation on Metabolism of Di(2-Ethylhexyl) Phthalate in Different Trimesters of Pregnant Women. Environ. Sci. Technol. 52, 12851–12858. https://doi.org/10.1021/acs.est.8b04519.