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Comparison of aggregated exposure to di(2-ethylhexyl) phthalate from diet and personal care products with urinary concentrations of metabolites using a PBPK model – Results from the Norwegian biomonitoring study in EuroMix

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ABSTRACT

Phthalates are widely used as plasticisers in flexible plastics and containers for food and personal care products (PCPs) and contaminates foods and PCPs. A human biomonitoring (BM) study was performed to study exposure of chemicals from foods and PCPs. For two 24-h periods, adult volunteers (n = 144) in Norway kept diaries on food eaten and usage of PCPs, and collected 24-h urine. Aggregated exposure to di(2-ethylhexyl) phthalate (DEHP) from dietary and PCPs was estimated by Monte-Carlo simulation using Oracle Crystal Ball©. Simulated urinary concentrations using physiologically based pharmacokinetic (PBPK) models were compared with measured urinary metabolites of DEHP, mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP) and mono-2-ethyl 5-carboxypentyl phthalate (MECCP). DEHP exposure from food are approximately 10 times higher than exposure than from PCPs. The main contributors to dietary exposure are dairy, grain, fruits and vegetables, meat and fish. Body lotion contribute most to the exposure of DEHP from PCPs. Forward-dosimetry gives good convergence with 24-h urinary concentrations of simulated and measured BM data. The measured concentration of the MECCP metabolite correlated well with simulated high exposure, while the measured concentrations of MEHP, MEHHP and MEOHP partly overlapped with both simulated low, medium and high metabolite exposure.

1. Introduction

Phthalates are diesters of 1,2-benzenedicarboxylic acid (phthalic acid) and are a class of synthetic chemicals that are widely used as a plasticiser in flexible plastics and containers for food, personal care products (PCPs) and consumer products. Since phthalates are not covalently bound to plastic, they can leach into the environment, foods and PCPs. Phthalate metabolites were measured in the urine of participants in a Norwegian biomonitoring (BM) study from the H2020 project Euromix (Husøy et al., 2019), and metabolites of the phthalate di(2-ethylhexyl) phthalate (DEHP) were found in 88–100% of the urinary samples. The first step in the metabolism of dialkyl phthalates is hydrolysis to the corresponding monoester in the digestive tract. Phthalates having a long alkyl chain, such as DEHP, will be further oxidized on the alkyl chain in several steps. DEHP is converted to its primary monoester metabolite, mono(2-ethylhexyl)phthalate (MEHP),

Abbreviations: ADME, absorption, distribution, metabolism, and elimination; BM, biomonitoring; DBP, dibutyl phthalate; DEHP, di(2-ethylhexyl) phthalate; FFQ, food frequency questionnaire; KBS, food and nutrient calculation system; LOD, limits of detection; LOQ, limit of quantification; MCMHP, mono-2-carboxymethyl hexyl phthalate; MECCP, mono-2-ethyl 5-carboxypentyl phthalate; MEHHP, mono-2-ethyl-5-hydroxyhexyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MEOHP, mono-2-ethyl-5-oxohexyl phthalate; NIPH, Norwegian Institute of Public Health; PBPK, physiologically based pharmacokinetic; PCPs, personal care products

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which is further metabolized to hydroxyl-, oxo- and carboxy- metabolites (Silva et al., 2006). The elimination half-lives of the DEHP metabolites range from 5 to 24 h, with the carboxy- metabolites of DEHP having the longest half-lives (Koch et al., 2006).

Exposure to phthalates, including DEHP, has been associated with negative health effects such as reproductive and developmental toxicity. The European Chemicals Agency (ECHA) classified these chemicals as reproductive toxicants (Repr. 1B) (ECHA, 2017). A systematic review on epidemiology literature on the phthalates and reproductive effects confirmed that real-life human phthalate exposure, especially DEHP and dibutyl phthalate (DBP), may cause reproductive effects (Radke et al., 2018). However, it is suggested that effects on the immune system might be a more sensitive endpoint than reproductive toxicity. In rats exposed to DEHP, the effects on immune parameters occurred at lower doses than developmental effects (Tonk et al., 2012). Braun et al. (2013) showed an association between DEHP exposure and asthma and eczema when reviewing a number of epidemiological studies.

Physiologically based pharmacokinetic (PBPK) models that describe the absorption, distribution, metabolism, and elimination (ADME), are used to describe the fate and transport of a chemical in humans (Sharma et al., 2018a, b). PBPK models provide a sound scientific basis to extrapolate across species, routes of exposure and exposure scenarios and are used in risk assessments to support regulatory decision making (EFSA, 2015; Thiel et al., 2015). Sharma et al. (2018a) developed a PBPK model for DEHP and its major metabolites, mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP) and mono-2-ethyl 5-carboxypentyl phthalate (MECPP) upon oral dosing of DEHP.

This paper aims to compare measured concentrations of DEHP metabolites in urine from the particiants in the EuroMix BM study to individual-based simulated urinary levels of DEHP metabolites using the PBPK model from Sharma et al. (2018a), with input data provided by probabilistic exposure modelling of DEHP from foods and PCPss. Aggregated DEHP-exposure from foods and PCPs were estimated using individual-specific input data for food consumption and PCPs use from the EuroMix BM study. Existing data on DEHP concentrations in foods and PCPs from the literature were used, prioritising data from Norway over data from EU, and then worldwide when applicable.

2. Materials and methods

2.1. Study population, registration and sample collection

The human biomonitoring study (EuroMix study) was established at the Norwegian Institute of Public Health (NIPH) and is described elsewhere (Husøy et al., 2019). In brief, participants were recruited among employees from governmental institutes and authorities, and universities in the counties Oslo and Akershus in Norway between September 2016 and November 2017. The recording and sampling period consisted of two non-consecutive 24-h intervals, with 2–3 weeks between the two sampling periods. The study included 144 participants (44 males age 25–72 years old and 100 females age 24–72 years old) that completed day one, and 140 (43 males and 97 females) of the participants completed the full 2-day collections. During the two sampling periods, the participants were asked to fill in a food diary, a PCP diary, a food frequency questionnaire (FFQ) (only on the first day) and a questionnaire for socio-demographic and lifestyle characteristics (only first study day). The food diary comprised a 24-h weighed food record developed specifically for this project, and included recipes of mixed meals (if applicable). The recorded food consumption from the weighed diaries and FFQ were further registered and coded by a dietician into the food and nutrient calculation system (KBS) at the University of Oslo (UiO), which included standard recipes when specific recipes on the mixed meals were not reported. In the PCP diary, the participants recorded all PCPs used during the 24-h for both days, including the time of use and brand names of the products. Personal information, such as gender, education, age, height, weight, smoking habits, consumption of tap water, visits to swimming pool, skin types of hands (dry, normal, greasy) and number of child births were recorded in the personal questionnaire. In addition, certain health outcomes connected to allergy and asthma were reported.

During the two 24-h recording periods, the participant collected all urine voids in separate containers and marked the containers with time and date. The next day, the urine voids were collected and immediately stored individually, as well as pooled into 30 ml aliquots of urine representing three time periods (6:00–12:00, 12:00–18:00 and 18:00–6:00 the next day) and a pool representing the 24-h interval. The urinary pools of 30 ml in total were prepared from the samples for given time periods adjusted for volume, as described below.

Volume taken from individual sample for pooled sample (ml) = Volume of individual sample of urine (ml)/Total urine volume for time period (ml) x 30 ml.

Blood samples were taken at the end of the 24-h period. Out of 144 participants, 8 and 10 subjects did deliver incomplete 24-h urine collection, for day 1 and 2, respectively. Two and 3 participants did not agree to give blood samples on day 1 and 2, respectively.

The study was approved by the Regional Committees for Medical and Health Research Ethics (REK ID no 2015/1868) and all the participants provided their written informed consent. The demographic of the EuroMix BM study is given in Table 1. It should be noted that the EuroMix population is not representative for the general population in Norway, as the participants represent a relatively high educated and healthy part of the population (Husøy et al., 2019).

2.2. Quantification of phthalate metabolites in urine

Five metabolites of di(2-ethylhexyl) phthalate (DEHP), mono-2-

Table 1
Demographic characteristics of the participants in the EuroMix BM study.

Basic characteristics		Males (n = 44)	Females (n = 100)
Age (years, mean ± SD)		43.4 ± 11.7	42.2 ± 12.3
Weight (kg, mean ± SD)		82.0 ± 8.5	65.2 ± 8.9
Height (m, mean ± SD)		1.81 ± 0.06	1.68 ± 0.06
BMI (kg/m^2, mean ± SD)		25.0 ± 2.34	22.8 ± 3.78
Smoking status (n)	Non-smokers	26	64
	Previous smoking	11	24
	Occasional smokers	7	12
Education (n)	University/college up to 4 years	8	22
	University/college > 4 years	36	78
Women with children (n)	No children	–	45
	1 child	–	19
	2 children	–	26
	3-4 children	–	10

Table 2
Overview over the DEHP metabolites measured in urine.

Phthalate	Metabolite
Di(2-ethylhexyl) phthalate (DEHP)	Mono-2-ethylhexyl phthalate (MEHP) Mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP) Mono-2-ethyl-5-oxohexyl phthalate (MEOHP) Mono-2-ethyl 5-carboxypentyl phthalate (MECPP) Mono-2-carboxymethyl hexyl phthalate (MCMHP)

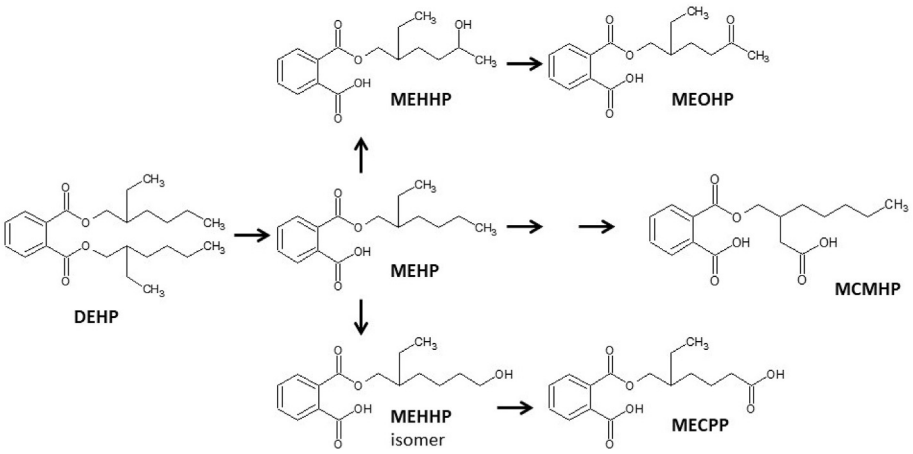

Fig. 1. The metabolic pathway of DEHP in humans, adapted from Ito et al. (2014).

ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), mono-2-ethyl 5-carboxypentyl phthalate (MECPP) and mono-2-carboxymethyl hexyl phthalate (MCMHP) were quantified in the three urine pools (Table 2 and Fig. 1). A 24-h concentration (μg/day) of each metabolite was estimated by adding the three concentrations of the time pools of urine, after multiplying the measured concentration (ng/ml) with urinary volumes (ml) (see below). The determination of phthalate metabolites in urine was performed using an on-line column switching liquid chromatography coupled to tandem mass spectrometry, as described previously (Sabaredzovic et al., 2015). In brief, labeled internal standard solution and enzyme solution to deconjugate glucoronidates (beta-glucuronidase in ammonium acetate buffer, pH 6.5) were added to the urine sample (300 μL). The samples were incubated for 1.5 h at 37 °C, after which 20% formic acid was added. The samples were centrifuged, and the supernatant was injected into the system. The limits of detection (LOD) were between 0.2 and 0.7 ng/mL (Table S1). The accuracy of the method ranged from 70% to 120%. In-house pooled urine samples and standard reference material from National Institute of Standards and Technology (NIST) were analysed along with the samples, and the precision was below 20% for the phthalate metabolites.

2.3. Scope of exposure modelling

The present study aims at estimating the non-dietary exposure of DEHP from PCPs and the exposure of DEHP from foods for the population in the EuroMix BM study. The frequency of PCPs use and the gram eaten of each food category are taken from the diaries in the Euromix study (Husøy et al., 2019).

The concentration levels of DEHP in different PCPs and foods were taken from the literature with a preference rule of concentrations as measured in Norway > Europe > rest of the world (Tables 3 and 4). To deal with variability and uncertainty of parameters used, estimation of the dermal and oral exposure was performed in a probabilistic way using Monte-Carlo simulation. This method is a common approach to incorporate variability and uncertainty of the parameters used in the estimation of human exposure (Mari et al., 2009; May et al., 2002; Rovira et al., 2016; Schuhmacher et al., 2001).

Tables 3 and 4 include the probabilistic distribution of parameters for the calculation of human exposure. Monte-Carlo simulation was carried out by Oracle Crystal Ball© software (Oracle Crystal Ball - Oracle 1–11.1.2.4.0). Exposures were calculated based on the propagation variable of variability and uncertainty given by each parameter probability function until 100,000 iterations.

2.4. Exposure modelling from diet

The exposure assessment of DEHP from the dietary intake for the EuroMix participants was calculated according to Eq. (1). The individual-based exposure on the survey day was modelled in 1000 Monte Carlo iterations as

$$Diet\ \ Exposure = \sum \frac{x \times C}{BW}\left[\frac{\mu g}{kg\ \ bw\ \ day}\right] \quad (1)$$

where C is the concentration of DEHP in foods (μg/g); x is the gram food eaten (g/day), and BW is the body weight (kg). Data used to assess the dietary exposure of DEHP is summarised in Table 3. All the food categories considered were also included in Table 3. The concentrations in food items that were below the LOD or limit of quantification (LOQ), as reported in the referred literature, was set to zero. The triangular distribution were selected as a distribution for the concentrations of DEHP in foods because of the limited concentration data from the literature.

2.5. Exposure modelling from PCP's

The exposure of DEHP through dermal contact for the EuroMix participants was calculated according to Eq. (2). The individual-based exposure on the survey day was modelled in 1000 Monte Carlo iterations as

$$Dermal\ \ exposure = \sum \frac{C \times PCP_{fr} \times PCP_a \times ABS \times R_f}{BW}\left[\frac{ng}{kg\ \ bw\ \ day}\right] \quad (2)$$

Table 3
Monte-Carlo parameters for the DEHP exposure from foods.

Parameter	Symbol	units	Distribution type	Distribution parameters	Reference
DEHP conc. in	C	–			
Grains and grain-based products		μg/g	T	43.0(18–61)	Sakhi et al. (2014)
Fruits and vegetables		μg/g	T	4.8(0.05–9.5)	Sakhi et al. (2014)
Meat and meat products		μg/g	T	0(0–64)	Sakhi et al. (2014)
Fish and other seafood		μg/g	T	0(0–35)	Sakhi et al. (2014)
Milk and dairy products		μg/g	T	126(19–173)	Sakhi et al. (2014)
Bottle Water		μg/g	T	0.1(0.07–0.18)	Santana et al. (2014)
Tap Water		μg/g	T	0.2(0.13–0.19)	Santana et al. (2014)
Consumption of foods	X				
		g/day	N	From diaries	Husøy et al. (2019)
Body weight	BW	kg	LN	65.2 ± 14.2	Husøy et al. (2019)

LN = Log-normal; T = Triangular; N = Normal distribution. Median, minimum, and maximum values were used for triangular distributions, and mean and standard deviation were used for log-normal distributions.

where C is the concentration of DEHP in PCPs (μg/g); PCP_{fr} is the frequency of application (application/day); PCP_a is the amount per application (g/application); ABS is the dermal absorption factor (non-dimensional); R_f is the retention factor for rinse-off products (non-dimensional), and BW is the body weight (kg). Data used to assess the dermal exposure of DEHP is summarised in Table 4. All PCPs considered are presented in Table 4.

2.6. Physiological based pharmacokinetic model

The previously developed PBPK model by Sharma et al. (2018a,b) was used for this study. The model is a flow limited model and comprises several compartments such as gut, liver, blood, fat, and gonads. The model describes the DEHP and its main metabolites such as MEHP, MEHHP, MECPP, MEOHP, and phthalic acid. The model does not include the DEHP metabolite MCMHP, as the kinetic parameters for this metabolite was not available. The details of model development and the description of the parameters can be found in Sharma et al. (2018a).

2.7. Forward modelling and comparison with urinary metabolites of DEHP

The estimated exposure from the individual diet as described in section 2.4 and 2.5 was used as an input for the forward PBPK modeling. The output of the model for each individual consisted of 15,000 iterations and each corresponded to the simulation of the model equations with parameters set defined by a random sample from the probability distributions (i.e. mean, standard deviation) of the exposure. The individual exposure estimates and the bodyweight from the EuroMix BM study were used to run the forward dosimetry PBPK model for each participant and the other model parameters were kept the same as in the model of Sharma et al. (2018a). The information from day 1 was used to fill in the missing information for day 2 for the participants

Table 4
Monte-Carlo parameters for the total dermal contribution to DEHP exposure.

Parameter	Symbol	units	Distribution type	Distribution parameters	Reference
DEHP conc. in	C	–			
Shower gel		μg/g	U	9.53-32.4	Guo et al. (2014)
Shampoo		μg/g	T	0.1(0–1.1)	Esteve et al. (2016)
Conditioner		μg/g	T	0.18(0–0.39)	Guo and Kannan (2013)
Deodorant		μg/g	T	4.98(0–65.3)	Guo and Kannan (2013)
Body lotion		μg/g	T	0.96(0–11.3)	Guo and Kannan (2013)
Anti-wrinkle		μg/g	T	0.4(0–2.45)	Guo and Kannan (2013)
Perfume		μg/g	T	15.00(7–130)	Wormuth et al. (2006)
Lipstick, lip-gloss		μg/g	T	1.79(0–6.45)	Guo and Kannan (2013)
Hair styling		μg/g	T	0.12(0–0.56)	Guo and Kannan (2013)
Eye makeup		μg/g	T	0.64(0–1.46)	Guo and Kannan (2013)
PCP frequency	PCP_{fr}				
All PCP's		Application/day	N	From diaries	Husøy et al. (2019)
PCP amount	PCP_a				
Shower gel		g/application	G	10.20 ± 8.0	Ficheux et al. (2016)
Shampoo		g/application	G	10.40 ± 7.6	Ficheux et al. (2016)
Conditioner		g/application	LN^g	10.0 ± 8.5	Ficheux et al. (2016)
Deodorant		g/application	LN^g	1.04 ± 0.8	Ficheux et al. (2016)
Body lotion		g/application	LN^g	9.55 ± 7.6	Ficheux et al. (2016)
Anti-wrinkle		g/application	LN^g	0.77 ± 0.66	Ficheux et al. (2016)
Perfume		g/application	LN^g	0.26 ± 0.18	Ficheux et al. (2016)
Lipstick, lip-gloss		g/application	LN^g	0.01 ± 0.01	Ficheux et al. (2016)
Hair styling		g/application	LN^g	3.59 ± 3.06	Ficheux et al. (2016)
Eye makeup		g/application	LN^g	0.01 ± 0.01	Ficheux et al. (2016)
Body weight	BW	Kg	LN	65.2 ± 14.2	Husøy et al. (2019)
Retention factor (rinse off)	R_f				
Shower gel		–	U	0.01	EFSA (2015)
Shampoo		–	U	0.01	EFSA (2015)
Conditioner		–	U	0.01	EFSA (2015)
Dermal absorption factor	ABS	–	U	0.1	EPA (2011)

LN = Log-normal; T = Triangular; U = Uniform; G = Gamma; N = Normal distribution. Mean, minimum, and maximum values were used for triangular distributions; Mean and standard deviation were used for log-normal distributions; Geometrical mean and geometrical standard deviation were used in log normal^g distributions; minimum and maximum values were used for uniform distributions, and location, scale and shape were used for gamma distribution.

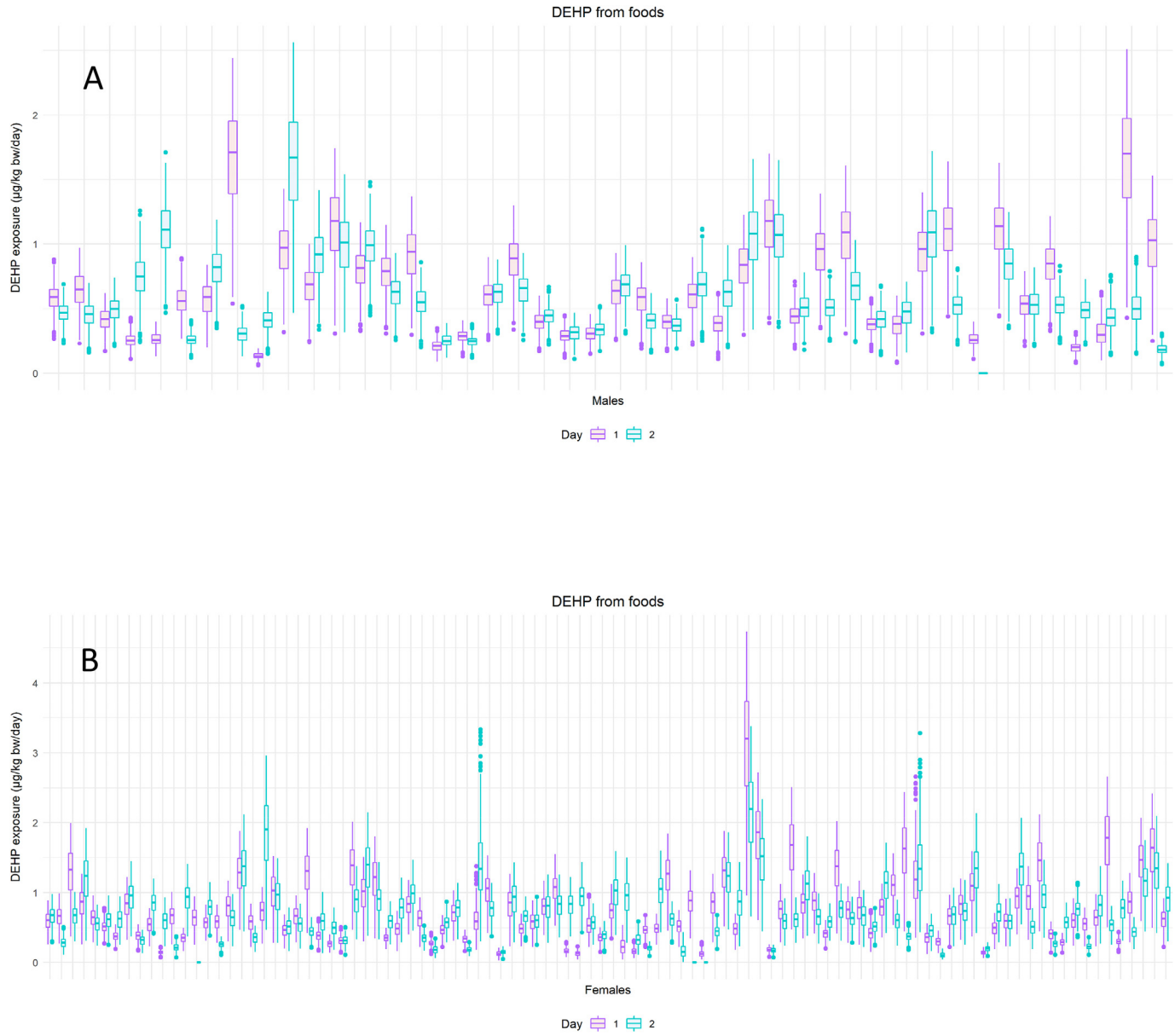

Fig. 2. Individual exposure to DEHP from foods for males (A, n = 44) and females (B, n = 100) from probabilistic exposure modelling for both study days as boxplot. Monte-Carlo simulation was performed for each individual with 1000 iterations.

that have not responded to the individual questionnaire and had missing urine samples. Summary of the modelled data for each individual was estimated as 2.5th percentile (low), 50th percentile (median) and 97.5th percentile (high).

2.8. Statistics

A two-way ANOVA test were used to compare the DEHP exposure from food and PCPs between males and females on day 1 and day 2, and to compare the urinary DEHP metabolite concentrations between males and females using R version 3.6.0.

3. Results

3.1. Exposure modelling from foods

The individual DEHP exposure from foods for both study days of the EuroMix BM study is shown in Fig. 2, and summarised in Table 5. The individual DEHP exposure from foods ranged from 0 to 2.56 µg/kg bw /day for males (Fig. 2A) and from 0 to 4.73 µg/kg bw/day for females (Fig. 2B). The mean DEHP exposure from foods were 0.66 ± 0.40 and 0.59 ± 0.33 µg/kg bw/day on study day 1 and 2 for males, and 0.73 ± 0.50 and 0.69 ± 0.44 µg/kg bw/day on study day 1 and 2 for females (Table 5). There was a statistically significant difference between the DEHP exposure from foods on day 1 and day 2 ($p < 0.001$), and between the DEHP exposure from foods for males and females ($p < 0.001$). The main contributors to the mean dietary DEHP exposure were milk and dairy products, with a percentage contribution of

Table 5
Exposure to DEHP (µg/kg bw/day) from foods for males and females on study day 1 and 2.

Gender	Day	Mean	SD	Min	P25	P50	P95	Max
Males	1	0.66	0.40	0.06	0.34	0.58	1.38	2.51
	2	0.59	0.33	0.00	0.38	0.52	1.23	2.56
Females	1	0.73	0.50	0.03	0.39	0.62	1.67	4.73
	2	0.69	0.44	0.00	0.39	0.64	1.52	3.38

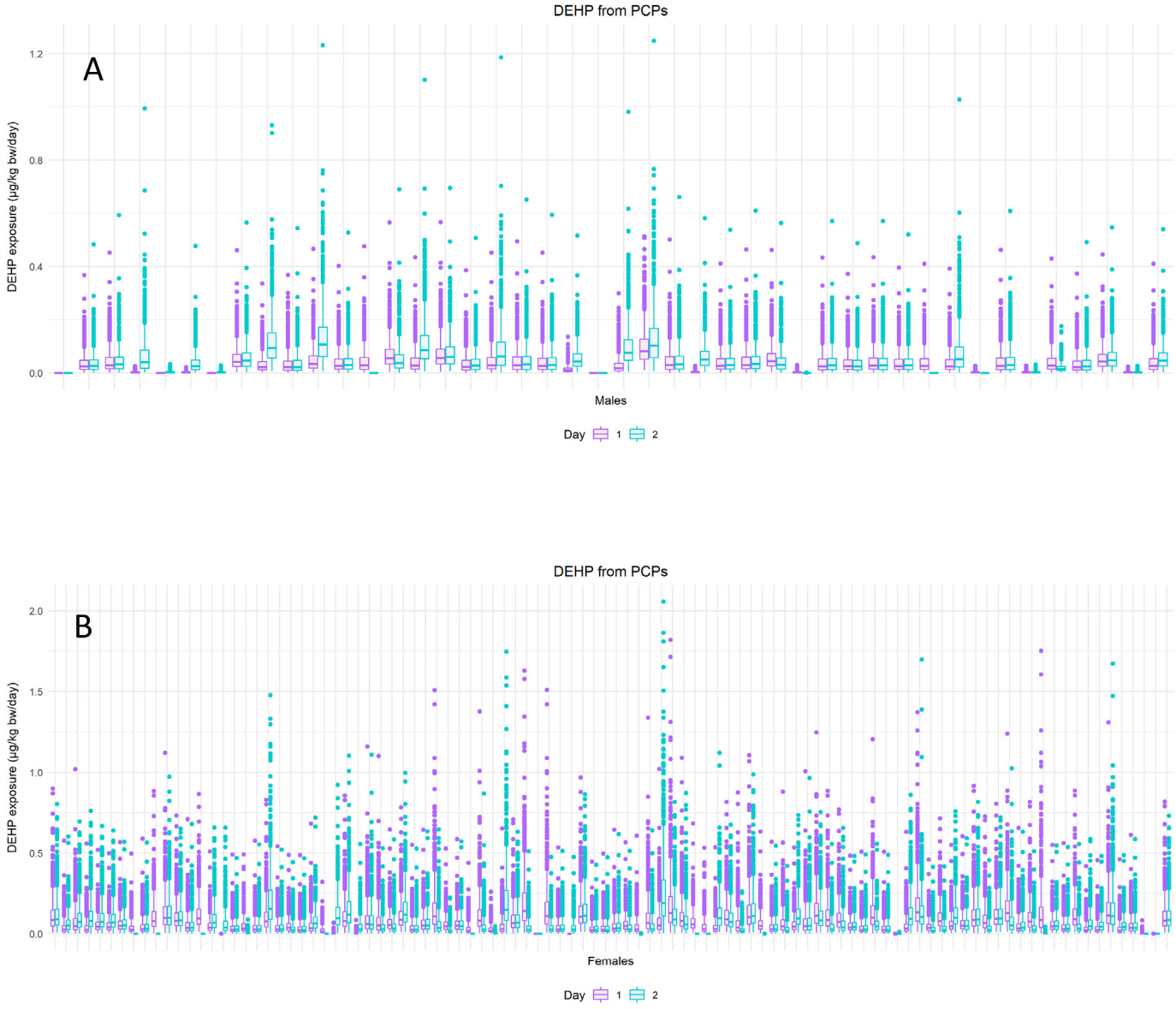

Fig. 3. Individual exposure to DEHP from PCPs for males (A, n = 43) and females (B, n = 97) from probabilistic exposure modelling for both study days as boxplot. Monte-Carlo simulation was performed for each individual with 1000 iterations.

69.3% and 62.8% of the total exposure for males and females respectively. Other major food groups contributing to DEHP exposure were grains and grain-based products > fruits and vegetables > meat > fish.

3.2. Exposure modelling from PCPs

The individual DEHP exposure from PCPs for both study days of the EuroMix BM study is shown in Fig. 3, and the DEHP exposure from PCPs are summarised in Table 6. The individual DEHP exposure from PCPs ranged from 0 to 1.25 µg/kg bw/day for males (Fig. 3A) and from 0 to 2.06 µg/kg bw/day for females (Fig. 3B). The mean DEHP exposure from PCPs where 0.034 ± 0.044 and 0.047 ± 0.066 µg/kg bw/day on study day 1 and 2 for males, respectively, and 0.073 ± 0.091 and 0.066 ± 0.091 µg/kg bw/day on study day 1 and 2 for females, respectively (Table 6). There was a statistically significant difference between DEHP exposure for males and females from PCPs ($P < 0.001$), and between DEHP exposure from PCPs on day 1 or day 2 ($p < 0.01$). The results show that diet was the major contributor to DEHP exposure for both males and females, with an external exposure approximately 10 times higher than for the exposure from PCPs. The major contribution to DEHP exposure from PCPs were anti-wrinkle cream > body lotion > perfume for females, and deodorant > perfume = body lotion > shower gel for males. Due to the low DEHP exposure from PCP, only exposure from diet was used in the forward modelling using PBPK.

Table 6
Exposure to DEHP (µg/kg bw) from PCPs for males and females on study day 1 and 2.

Gender	Day	Mean	SD	Min	P25	P50	P95	Max
Males	1	0.034	0.044	0	0.005	0.019	0.12	0.57
	2	0.047	0.066	0	0.006	0.026	0.17	1.25
Females	1	0.073	0.091	0	0.019	0.045	0.24	1.82
	2	0.066	0.091	0	0.012	0.037	0.23	2.06

3.3. DEHP metabolites in urine

Five different DEHP metabolites, MEHP, MEHHP, MEOHP, MECPP,

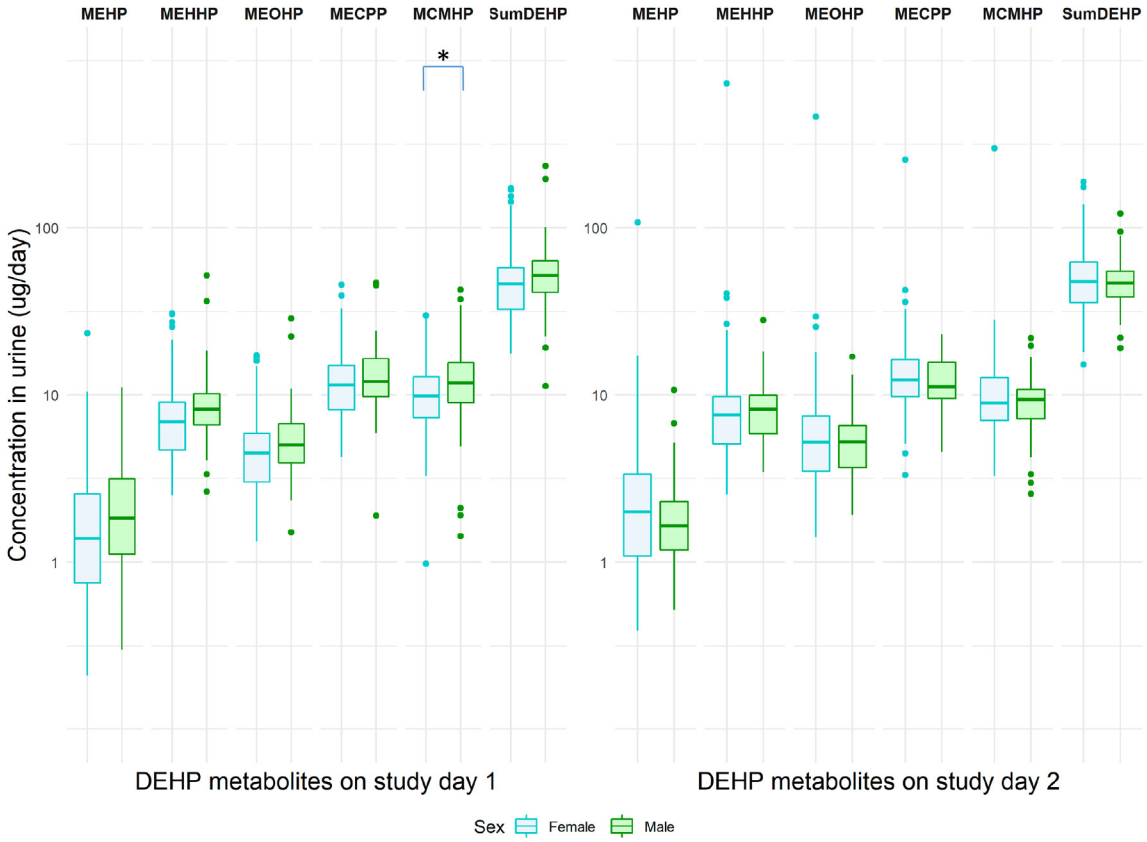

Fig. 4. Concentrations of DEHP metabolites in the 24 -hour urine on day 1 and day 2. DEHP expressed as the metabolites (μg) MEHP, MEHHP, MEOHP, MECPP, MCMHP and as a sum of the mol adjusted metabolites (SumDEHP). * Significant difference $P < 0.05$.

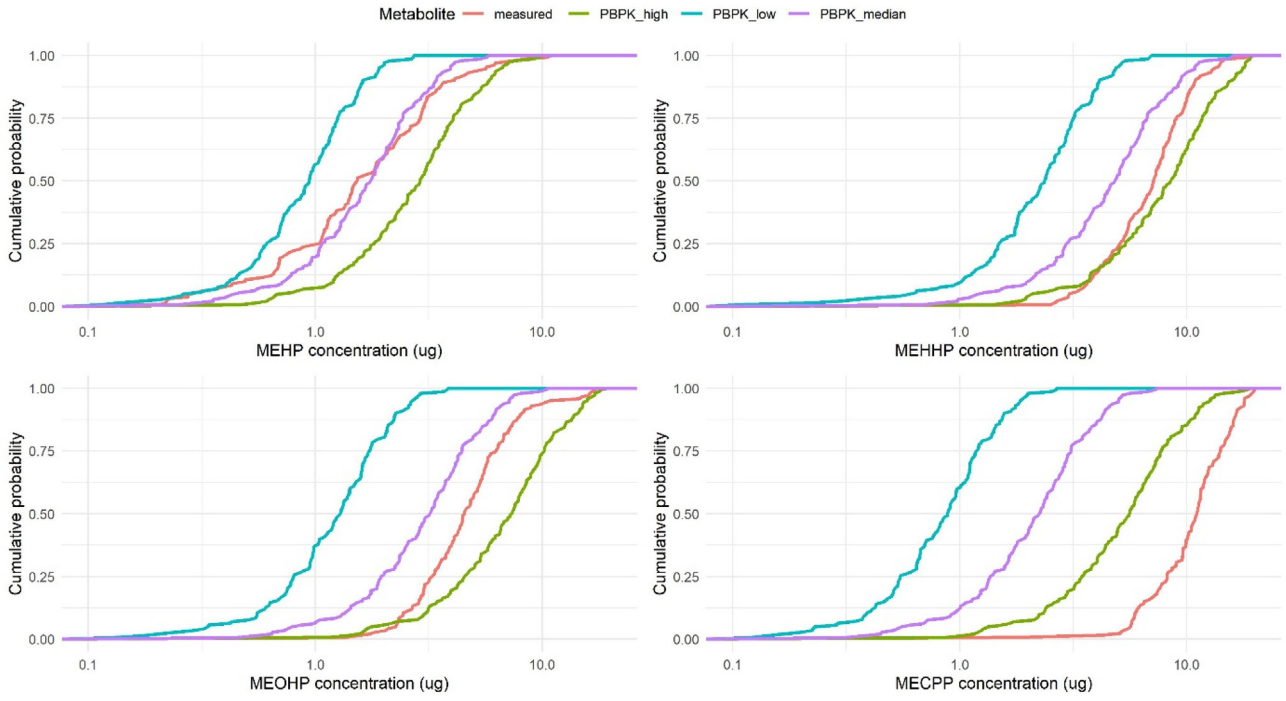

Fig. 5. Cumulative distribution function of modelled concentrations of DEHP metabolites (PBPK_low, PBPK_median, PBPK_high) and the measured DEHP metabolites for study day one.

and MCMHP, were measured in the urine of the participants from study day 1 and 2 (Fig. 4, Table S1). The highest metabolite concentrations were found for the metabolite MECPP, with a median concentration of 11.2–12.0 μg/day and 11.5–12.3 μg/day for males and females, respectively. This was followed by MCMHP > MEHHP > MEOHP > MEHP, which ranged from 1.8 to 11.7 μg/day and from 1.4 to 9.9 μg/day for males and female, respectively (Table S1). There was a significant difference in the measured MCMHP metabolite ($P = 0.041$) between sexes on study day one, but no significant differences were observed between the sexes and the measured DEHP metabolites for study day two.

3.4. Forward modelling using the PBPK model and correlation with BM concentrations in urine

The PBPK model was used to predict the cumulative amount of four metabolites namely MEHP, MEOHP, MEHHP and MECPP after an oral administration of estimated individual DEHP exposure from the diet (144 participants). The exposure estimate for each participant was normally distributed, and therefore the mean and SD were used in the modelling in the current study. Then the model was simulated for each participant and the prediction results were obtained by calculating the median (PBPK_median), and their two extremes correspond to 2.5 (PBPK_low) and 97.5 percentiles (PBPK_high) from the ensemble of 15,000 iterations. A primary validation of the exposure estimates using forward dosimetry PBPK model was performed by comparing the individual human biomonitoring urine data against the PBPK predicted urine data i.e. 2.5th percentile (PBPK_low), median (PBPK_median), and 97.5 percentiles (PBPK_high) for the exposure estimate for each individual. The results from the PBPK modeling showed good overall agreement between the cumulative distribution of 24-h urinary concentrations of simulated and measured BM data for the three metabolites MEHP, MEOHP and MEHHP for both study day one (Fig. 5) and study day two (Figure S1). The distribution of the simulated urinary concentrations for MECPP were below the measured urine concentration for both days, even for the 97.5 percentile (Fig. 5 and S1). The measured individual concentration of MEHP was more dispersed and overlapped with both simulated low, medium and high DEHP exposure for both study days, while for MEOHP and MEHHP the individual measurements overlap mostly with the simulated 50 and 97.5 percentiles (Fig. 6 and S2). For few individuals the measured MECPP metabolites overlap with the simulated 97.5 percentile, but for most individuals the simulated concentrations are underestimating the measured. The individual-based simulated DEHP metabolite concentrations are generally underestimated compared to the measured metabolite concentrations, with the exception for MEHP. MEHP from medium DEHP exposure (PBPK_median) under- or overestimate the measured urine concentrations with more than 3-fold for 24% and 22% of the individuals for study day 1 and 2, respectively. However, for MEHHP, MEOHP and MECPP the simulated urine concentrations from medium DEHP exposure are mainly underestimated. The simulated MEHHP and MEOHP concentration in urine underestimated the measured concentration with more than 3-fold for 21–23% of the individuals for both day 1 and 2, while only 1–2% of the individuals had an overestimation of more than 3-fold (Fig. 7). The largest underestimation was observed for the MECPP metabolite, where the simulated MECPP concentrations were underestimated with more than 3-fold for 77–80% of the individuals on both days, and none where overestimated with the same magnitude (Fig. 7). The results from the PBPK modelling shows that there are a reasonable agreement between individual-based simulated and measured urine concentrations for MEHP, MEHHP and MEOHP, while for the MECPP the simulated concentrations were underestimating the measured for most individuals.

4. Discussion

4.1. Exposure estimates from foods and PCPs

Probabilistic exposure estimates were performed for DEHP from foods and PCPs for the 144 participants in the EuroMix study. The exposure estimate ranged from 0.52 to 0.64 μg/mg bw/day for foods, and from 0.019 to 0.045 μg/kg bw/day for PCPs, including both study days. This is in reasonable agreement with the exposure estimates of 0.4 μg/kg bw/day in a study by Sakhi et al. (2014), using the National dietary survey Norkost 3 (Totland et al., 2012) and of 1.00 μg/kg bw/day for foods and 0.087 μg/kg bw/day for dermal contact reported for mothers in a Spanish mother and child cohort (Martinez et al., 2018). There was a significant difference between the exposure estimate of DEHP from foods between study day 1 and 2, both for males and for females, with a reduced DEHP exposure on day 2. The reason for this is not clear, but it might be that underreporting of food consumption is more frequent on the second day of the study than on the first day. The main contributors to the mean dietary DEHP exposure in the EuroMix BM study were milk and dairy products, followed by grains and grain-based products. This was in line with the reported contribution from grain and grain based products by Sakhi et al. (2014), but in contradiction to the determinants from foods (butter and oil) found for DEHP in urine for the Euromix study (Husøy et al., 2019). Milk and dairy, and grain and grain-based products have also been reported as an important source for DEHP in other studies (Schecter et al., 2013; Sui et al., 2014). The major contribution to DEHP exposure from PCPs were anti-wrinkle cream > body lotion > perfume for females, and deodorant > perfume = body lotion > shower gel for males. Body lotion were also reported to be a positive determinant for dermal exposure to DEHP in a study among Mexican women (Romero-Franco et al., 2011), but were reported to be negatively associated with DEHP exposure in female adults (Sakhi et al., 2017).

4.2. DEHP metabolites in the urine

Concentrations of DEHP metabolites in urine are considered to be the best biomarkers for exposure. Due to a short half-life and variable exposure, the metabolite concentrations for DEHP varies considerable for an individual within a day, and between individuals (Frederiksen et al., 2011). Therefore, 24-h urine are assumed to give a more accurate picture of daily exposure than spot urine. The results from the two days of 24-h urine showed similar DEHP metabolite concentration in the EuroMix study participants, giving confidence in our BM results of this population. The concentrations of DEHP metabolites in the urine are shown to decline 10-fold from its peak in the late 1980s (Koch et al., 2017). However, the detection rate for DEHP metabolites in the urine was 88–100% in the Euromix BM study participants (Husøy et al., 2019), which is in line with previous findings (Sakhi et al., 2017). DEHP comes from plastics through migration to foods and PCPs, and in a intervention study where the participants avoided foods that had been in contact with plastic for 3 days, the urinary levels was reduced with 50%. This indicates that foods in contact with plastics is one of the major sources of DEHP exposure (Ackerman et al., 2014).

4.3. Comparison between measured and modelled concentrations of DEHP metabolites

Simulated concentrations of the DEHP metabolites from low, median and high DEHP exposure (PBPK_low, PBPK_median and PBPK_high) were generally in good agreement with the measured concentrations when considering the overall exposure for day 1 (Figs. 5 and 6) and day 2 (Figure S1 and S2). However, the dispersed dots in Fig. 6, indicates that individual-based correlation between simulated DEHP metabolites in urine and measured concentrations was not as strong. However, the relationship is mostly within a 3-fold difference

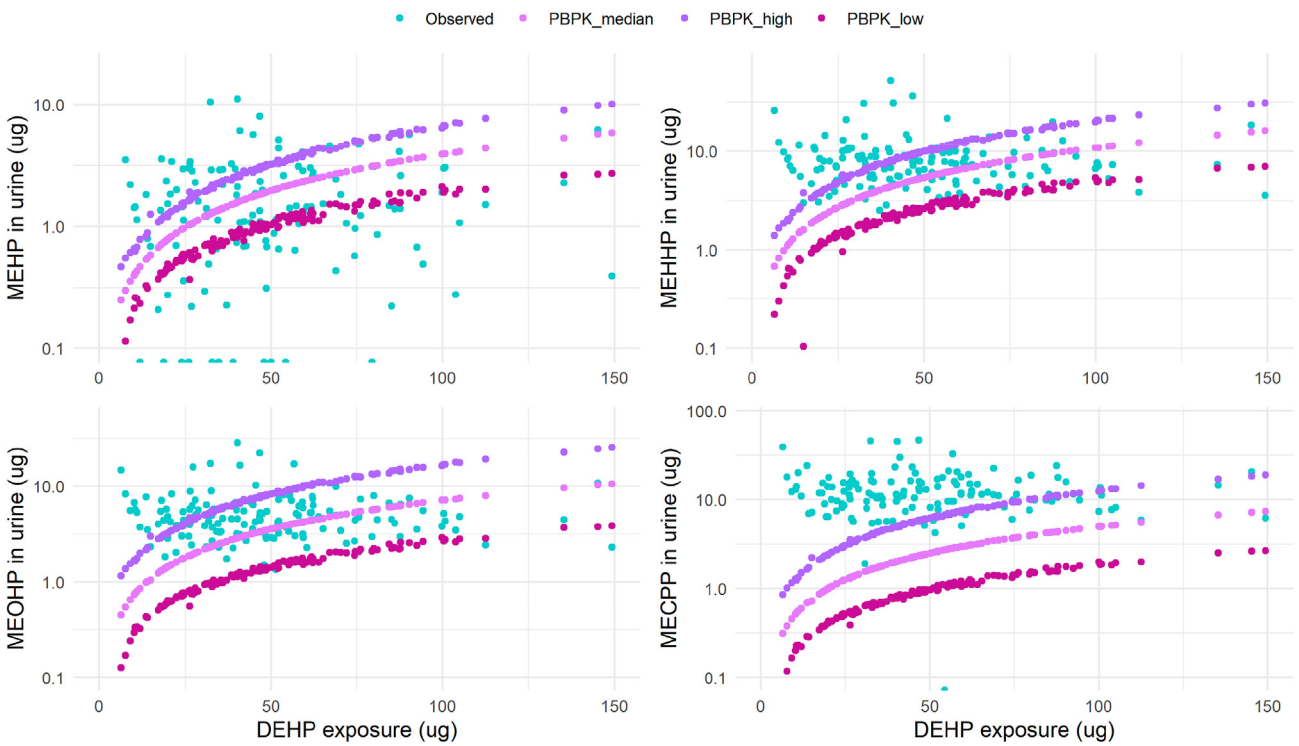

Fig. 6. Comparison of the distribution between the simulated and measured concentrations of the DEHP metabolites MEHP, MEOHP, MEHHP and MECCP in 24-h urine and the DEHP exposure from the diet for study day one.

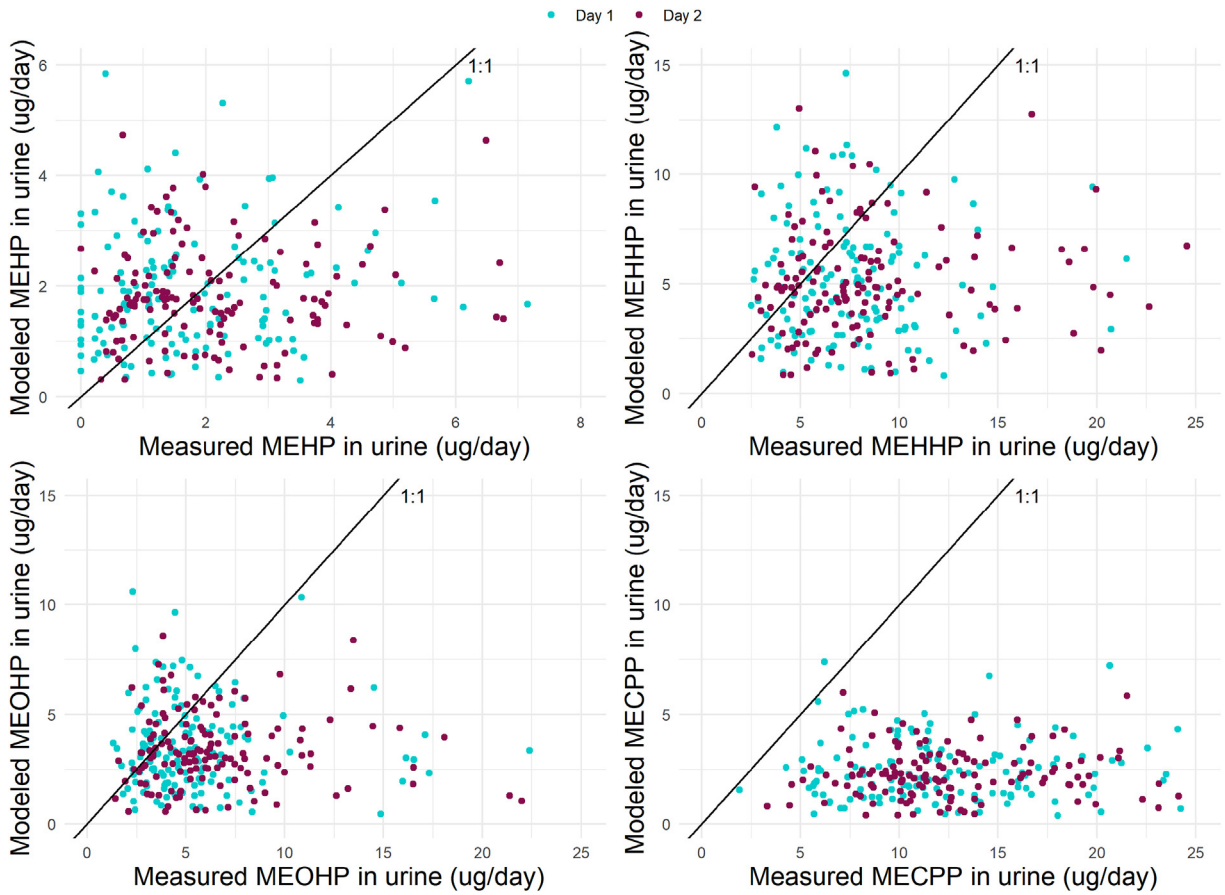

Fig. 7. Individual-based comparison of measured urinary DEHP metabolites (MEHP, MEHHP, MEOHP, MECPP) versus modelled metabolites in urine from medium DEHP exposure (PBPK_median) of day 1 (blue) and day two (purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

for the DEHP metabolites, with the exception of MECPP. The PBPK model was previously reported to perform well with the human data from Koch et al. (2004) and Koch et al. (2005), where humans were exposed to deuterium-labeled DEHP and metabolites were measured in serum and urine (Sharma et al., 2018a). However, the predicted 97.5th percentile with these human data seems to correlate well with the given dose, indicating an underestimation of the metabolites by the PBPK model also with these data. Moreau et al. (2017) performed a reverse dosimetry through a PBPK model for DEHP, which overestimated the exposure three-fold compared to the estimated exposure from the daily intake of DEHP. The main reason for the deviation between modelled and measured data for the EuroMix population are most likely due to the uncertainty in the exposure estimate due to limited DEHP concentration data in foods. Only dietary and dermal exposure were included in the exposure estimations. However, DEHP exposure through dust and inhalation is regarded as less important sources compared to diet for such a high molecular weight phthalate, while dermal exposure were found to be negligible (Giovanoulis et al., 2018). In general, the in the simulation of urinary metabolites for chemicals with relatively short half-life and potentially high variation in exposure within a day and between days, as for DEHP, is connected with uncertainty. This is especially evident when the simulated and measured metabolites are correlated for each individual. The simulated urinary metabolites is also highly affected by the lach of DEHP concentration data in foods.

4.4. Limitation in the modelling

The PBK model (Sharma et al., 2018a) used for this study has been validated with several independent data including human study data of Koch et al. (2005). Simulated urinary amount of DEHP metabolites (cumulative amount) shown a good compliance with the experimentally observed cumulative amount of Koch et al. (2005). Sharma et al. (2018a) model did not account for the MCMHP metabolite due to lack of in vitro metabolic data, considered to be another important metabolite for the biomonitoring study. Some of the uncertainty of the present study comes from the dosing estimates of the individual subjects derived from the concentration data and personal dairies. Another limitation of the current PBK model is poor metabolic kinetic data of some metabolites like MECPP, which is reflected in its higher predictive uncertainty. The observed biomonitoring MECPP metabolite data points are beyond or near the 97.5th of the modeling percentile. The underprediction of certain metabolites could be for either of two reasons. Firstly, experimentally derived metabolic kinetics are still data poor and these parameters have high sensitivity and is reflected in their predictivity. Secondly, the model also lacks some metabolites' tissue distribution and only include elimination of metabolites as directly proportional to their plasma clearance (Sharma et al., 2018a).

5. Conclusions

Verification of the exposure data using forward-dosimetry through the PBPK model give good convergence with 24-h urinary concentrations of simulated and measured BM data. The measured concentration of the MECCP metabolite seems to correlate with the simulated high exposure, while the measured concentrations of MEHP, MEHHP and MEOHP were more dispersed and partly overlapped with both simulated low, medium and high metabolite exposure.

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CRediT authorship contribution statement

T. Husøy: Conceptualization, Investigation, Formal analysis, Resources, Writing - original draft, Writing - review & editing, Project administration. **M.A. Martínez:** Formal analysis, Writing - original draft. **R.P. Sharma:** Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **V. Kumar:** Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **M. Andreassen:** Investigation, Writing - original draft. **A.K. Sakhi:** Methodology, Formal analysis, Writing - original draft. **C. Thomsen:** Methodology, Writing - original draft. **H. Dirven:** Conceptualization, Resources, Writing - original draft.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2020.111510.

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