Chapter 2

2A. Development of a human physiologically based pharmacokinetic (PBPK) model for phthalates and its metabolites: A bottom up modeling approach

Abstract: DEHP exposure to human comes from different sources such as food, diet, cosmetics, toys, medical products, and food wraps. Recently, DEHP and its metabolites were categorized as non-persistent endocrine disrupting compounds (EDCs) by the world health organization (WHO). Rat experimental studies have shown that phthalate and its metabolite(s) can cause hepatic, developmental and reproductive toxicity. In human, DEHP rapidly metabolizes into a toxic metabolite MEHP. This MEHP further metabolizes into the different chemical forms of 5OH-MEHP, 5oxo-MEHP, 5cx-MEPP and phthalic acid. A simple DEHP pharmacokinetics model has been developed, but with a limited number of metabolites. A chemical like DEHP which is extensively metabolized deserves a detail metabolic kinetics study. A physiologically based pharmacokinetics (PBPK) model of DEHP considering all the major metabolites in human, has not been developed yet. The objective of this study is to develop a detailed human PBPK model for DEHP and its major metabolites by using a bottom-up modelling approach with the integration of in vitro metabolic data. We will use an in-vitro-in-vivo extrapolation (IVIVE) and a quantitative structure-activity relationship (QSAR) method for the parameterization of the model. Monte Carlo simulations were performed to estimate the impact of parametric uncertainty on the model predictions. First, the model was calibrated using a control human kinetic study that represents the time course of DEHP metabolites concentrations in both the blood and the urine. Then, the model was evaluated against the published independent data on different dosing scenarios. The results of model predictions for the DEHP metabolites in both the blood and the urine were well within the range of experimentally observed data. The model also captured the time course profile of the observed data, attesting to the model's predictive power. The current developed PBPK model can further be used for the prediction of the time course of chemical concentrations for the different exposure scenarios not only in the blood and the urine but also in the other compartments. Moreover, this model can also be used to explore different biomonitoring studies with respect to human health risk assessment and might be useful for integrative toxicological studies aimed at improving exposure-target tissue dose–response relationship.

Keywords: DEHP; MEHP; Pharmacokinetics; PBPK; Human health Risk assessment; IVIVE; Endocrine disruptors; human biomonitoring
1. Introduction

Phthalates are ubiquitous environmental contaminants made up of dialkyl esters or alkyl and aryl esters of ortho-phthalic acid (1,2-dicarboxylic acid). Among Phthalates, Di-2-ethylhexyl phthalate (DEHP) is the most important because of its large and widespread uses in industries as a plasticizer. It is found in food, cosmetics, toys, medical products and food packaging, mostly used as a plasticizer. The total dietary intake (TDI) of 50 μg/kg BW/day limit has been set by the EFSA and the European chemical agency (ECHA) to assess the risk related to DEHP exposure (EFSA, 2015; ECHA, 2010). The total mean dietary intake of the DEHP in several cohorts studies estimated in the range of 0.42–11.67 μg/kg bw/day, which is far below the threshold set by the EFSA and the ECHA (Fromme et al., 2007; Dickson-Spillmann et al., 2009; Siøen et al., 2012; Heinemeyer et al., 2013; Martine et al., 2013; Martínez et al., 2017, 2018).

DEHP has a short half-life and it does not accumulate inside the body (Krotz et al., 2012). DEHP completely metabolizes into a toxic metabolite mono-(2-ethylhexyl) phthalate (MEHP). This MEHP further metabolizes into different chemical forms like 5-hydroxy MEHP, 2-ethyl-5-carboxypentyl phthalate (5-Cx MEPP) and phthalic acid. 5-oxo MEHP is another metabolite result of the 5-OH MEHP metabolism. Temporal variability in phthalate exposure from the different sources and their ability to generate several forms of metabolites can lead to a stable microenvironment exposure of phthalates to internal organs. The microenvironment exposure of the DEHP over a long period of time lead to a pseudo-steady state concentration (Meeker et al., 2009).

Currently, DEHP is of concern in its categorization as a non-persistent endocrine disruptor by the World Health Organization (WHO, 2010). Cobellis (2003) in his epidemiological study has shown the linkage between the exposure of DEHP and the prevalence of endometriosis in women. Other studies have also shown that environment relevant dose of phthalates alters estrous cycle, impaired oocyte maturation, decrease ovulation (Anas et al., 2003; Krisher, 2013; Hannon et al., 2014). DEHP and its toxic metabolite MEHP mainly alter the estrogen productions and its activity in granulosa cell, which are essential for the growth and secretion of the follicles, which might lead to infertility due to hypo-estrogenic, polycystic ovary and anovulatory cycles (Davis et al., 1994; Lovekamp-Swan and Davis, 2003). Many hypotheses of phthalates effect on male reproductive toxicities were proposed based on the animal studies, please refer to the given reference for more information (Richburg and Boekelheide, 1996; Richburg et al., 1999; Lee et al., 1999; Koji et al., 2001; Shelby, 2006; Sharma et al., 2017a). Several cohort studies have shown a correlation between the high levels of DEHP in urine with significant reduction in plasma testosterone concentrations (Duty et al., 2005; Pan et al., 2006).

Understanding the factors that govern the DEHP distribution and metabolisms within the quantitative framework of a physiologically based pharmacokinetic model is essential for better estimation of the physiological concentration of DEHP metabolites in the target tissues such as gonads. The Reliable Physiologically based Pharmacokinetic (PBPK)
model will be useful for establishing a suitable dose metric for targeted tissues (Fabrega et al., 2014), and exposure-dose-response relationship for the systems toxicology model (Sharma et al., 2017b, 2018). Since 1974, many pharmacokinetic analyses on DEHP and its metabolites have been conducted both in in-vitro and in-vivo (animal and humans) (Daniel and Bratt, 1974; Peck and Albro, 1982; Albro, 1986; Ito et al., 2005; Wittassek and Angerer, 2008; Choi et al., 2013). Several pharmacokinetic (PK) models have been developed accounting its major metabolites using simple compartmental approach (Koch et al., 2003, 2004, 2005, 2006; Lorber et al., 2010). Koch et al. (2003, 2004, 2005) experimentally examined several DEHP secondary metabolites concentration both in the blood and the urine describing their time course kinetics. A PK model developed by Lorber et al. (2010) has predicted the DEHP metabolites concentration both in the blood and urine which includes empirical fitting of the two key parameter against the experimental data namely; first is the fraction of chemicals available to pass through the metabolism; and the other is the rate of dissipation of these metabolites. However, It lacks the mechanistic metabolic kinetics (Michaelis-Menten reaction), considered the most important biotransformation process. Keys et al. (1999) and Cahill et al. (2003) developed a PBPK model of DEHP in both the rats and human, however, these models have not included all the metabolites and their kinetics, which might be due to insufficient data on the DEHP metabolic kinetics at that time. Recently, Choi et al. (2012) has reported on in vitro metabolic kinetics information on the DEHP and its metabolites both in the rat and human using hepatic cell line. To best of our knowledge, there is no published detailed target tissue dosimetry model (PBPK), which becomes essential for the chemical like DEHP that produces many metabolites (Daniel and Bratt, 1974; Ghosh et al., 2010). The purpose of this study is to develop a detailed PBPK model for the DEHP and its major metabolites for the adult human and its evaluation against the experimental data. A bottom-up modeling approach was used to develop the model. It includes the integration of in vitro metabolic and in silico data which uses IVIVE (in-vitro in-vivo extrapolation) and QSAR (Quantitative structure-activity relationship) tools. These tools led to creation of a PBPK model with minimal or no animal experiments, supporting the 3Rs strategies of minimizing the use of animal. An IVIVE tool has been successfully used in connection with a PBPK to derive the in-vivo kinetics from the in vitro studies using biologically appropriate scaling (Yoon et al., 2014; Martin et al., 2015). This work is part of two major EU projects, HEALS and EuroMix, where different aspects of in silico models and its applications in human biomonitoring are investigated (Martínez et al., 2017, 2018).

This article describes the physiologically based pharmacokinetic (PBPK) model that predicts the time variant concentrations of DEHP metabolites such as MEHP 5-OH MEHP, 5-cx MEPP, and 5-oxo MEHP in plasma upon oral dosing of DEHP. The model was used to simulate the cumulative amount of the DEHP metabolites in urine. The in vitro human gut and hepatocyte DEHP metabolic kinetics data were scaled and integrated into the model (Choi et al., 2013). Experimentally observed human DEHP metabolites concentration both in the plasma and the urine are used to calibrate the PBPK model. Further model was evaluated against the independent data on DEHP kinetics for different
dosing scenarios (Anderson et al., 2011). Prior mean parameter values were obtained from the published literature or derived from the in-vitro and in-silico experiments, whilst accounting for uncertainties in the range of ± 1 to ± 1.5 standard deviation. After sensitivity analysis the most uncertain parameter yet influential parameters were distributed statistically for Monte Carlo simulations.

2. Models and Methods

2.1 Overview of the modeling approach

The model was coded as a set of ordinary differential equations, written in the GNU MCSim modeling language and solved by numerical integration using the R “deSolve” package (Bois and Maszle, 1997). Model parameters values were derived from in vitro and in-vivo experiments reported in the literature or using the in-silico approach. Sensitivity analysis of the model was done using the mean value of the parameters. After sensitivity analysis, the most uncertain yet influential parameters were distributed statistically for Monte Carlo simulations to estimate the impact on model predictions of uncertainty in all of the selected parameters (Bois et al., 2010; Fábrega et al., 2016). Model equations are provided in Annex 2.

The exchange of the chemicals between blood and tissue in each organ is described by flow limited processes i.e. we implement a perfusion rate-limited PBPK model (not permeability limited). The model comprises several compartments i.e. gut, liver, blood, fat, gonad and a compartment representing the rest of the body (Fig. 1). The gonad compartment was included in the model for its later use in DEHP reproductive toxicity assessment. The only metabolite MEHP was distributed to the given compartments, while other metabolites were confined to the blood compartment presuming their volume of distribution is equivalent to the plasma volume. All physiological parameters such as blood flows and tissue volumes used in the model were obtained from the published literature and are provided in Table A.1 of Annex 2. The partition coefficients and fractional unbound were obtained from the in-silico approach or literature are provided in Table 1. The calibration of the model was carried out against the human pharmacokinetic experimental data on both the plasma and the urine level of DEHP metabolites reported in Koch et al. (2004, 2005). This involves the plasma concentration data during the first 8 h and the cumulative amount of metabolites in urine over 44 h following an oral dosing of 48.5 mg. Further evaluation of the developed PBPK model was done against the other independent pharmacokinetics study done by Anderson et al. (2011) for two different dosing scenarios. In this study, all major metabolites are considered namely; MEHP, 5-OH MEHP, 5-CX MEPP, 5-Oxo MEHP and phthalic acid. All the metabolic parameters were derived from in vitro cell line study are provided in Table 1.
2.2. Pharmacokinetics of DEHP and its Metabolites

The rate of metabolite formation is assumed to be equal to the rate of parent compound metabolism. DEHP metabolic pathway is provided in Fig. 2. DEHP metabolizes to MEHP, which metabolizes into different chemical forms such as 5-OH MEHP, 5cx-MEPP, and 2cx-MEPP. Among them, 5-OH MEHP further metabolizes into 5-Oxo MEHP. All the metabolites excrete via urine. Absorption of DEHP from the gut to the liver was described by partition coefficient. Both DEHP and MEHP distributed to compartments such as liver, fat, plasma and gonads. However, due to inadequate data on the partition coefficients for metabolites other than MEHP, their distribution limited to the plasma compartment. And the volume of distribution of these metabolites has set equal to the plasma volume.

Absorption

Koch et al. (2005) in his study reported that DEHP is completely absorbed from the gut and rapidly metabolized into the MEHP in the liver. The distribution of DEHP from the gut to the plasma is described by its partition coefficient between them. The partition coefficient (gut: plasma) was estimated using QSAR approach of Poulin and Krishnan tissue composition method (Poulin and Krishnan, 1996, 1995; Poulin and Theil, 2000). The MEHP uptake from the gut the liver was described by the first order rate constant (Adachi et al., 2015).
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Fig. 1. The figure represents a PBPK model for the DEHP and its metabolites. It includes mainly five compartments and clearance of chemical depends on both metabolism (mainly five metabolites) and urinary elimination. Following oral administration of DEHP (P), it readily metabolizes into MEHP (M1) and MEHP further metabolizes into 5-OH MEHP (M2), 5-cx MEPP (M3) and phthalic acid (M5). 5-OH MEHP (M2) is further metabolizing into 5-oxo MEHP (M4), for detail metabolic scheme refers to Fig. 2. The DEHP and MEHP are distributed to the given compartments. However other metabolites produced in guts and liver are transferred to blood compartments assuming their distribution in a single compartment. The metabolite phthalic acid (M5) was not utilized in this model for its further distribution to blood or its elimination (except for MEHP clearance, metabolic conversion to M5), as no data are available to calibrate its concentration in urine or blood.

Distribution

DEHP and MEHP was distributed to the several compartments using their partition coefficients estimated by in-silico or derived from the published literature and are provided in Table 2. DEHP partition coefficients were estimated using the QSAR approach based on tissue composition method (Poulin and Krishnan, 1996, 1995; Poulin...
and Theil, 2000). A log ko/w of 7.6 was used to estimate the tissue: plasma partition coefficients. The MEHP partition coefficient values which was experimentally determined through vial – equilibrium method by Keys et al. (2000) was used for the tissue distribution. Other metabolites distributions restricted to the blood compartment only, assuming their volume of distribution equivalent to the plasma volume. The metabolites formed in the liver transfer to the blood using first order uptake rate constants and these parameters were calibrated against the Koch et al. (2005) experimental data.

**Elimination**

Elimination of DEHP and its metabolites in urine was assumed to be directly proportional to its rate of clearance from the plasma. The model presumed that DEHP clearance solely depends on its metabolism into MEHP (Koch et al., 2004, 2005, 2006; Lorber et al., 2010).

The excretion rates for the MEHP and other metabolites were described by first-order rate equation. These excretion rates were obtained by using the relationship between the elimination rate constant and the chemical’s plasma half-life i.e. ratio of ln2 (0.693)/t1/2 (half-life). The mean half-lives for MEHP, 5-OH MEHP and 5-CX MEPP and 5-oxo MEHP was estimated by Lorber et al. (2010) was used for the model parameterization. These parameters values were used for the model simulation and calibration against the reported time course concentration of chemicals in the plasma and cumulative excretion profile in the urine reported (Koch et al., 2005). The elimination rate constant for MEHP was measured using half-life reported by Mittermeier et al. (2016).

2.3. **In vitro intestinal and Hepatocyte metabolic studies**

Metabolism of the DEHP both in the liver and gut to MEHP, 5-OH MEHP, 5oxo-MEHP, 5cx MEPP and phthalic acid was described by the Michaelis-Menten equation provided in Eq. (2). This equation includes two important parameters namely Vmax (maximum velocity of metabolic reaction) and Km (affinity i.e. concentration at which reactions occurs at the half maximal rate). The in vitro intestinal and hepatic metabolic rates for several DEHP metabolites were reported in Choi et al. (2012) where the author has described mainly five metabolites (MEHP, 5-OH MEHP, 5oxo-MEHP, 5cx MEPP and phthalic acid) kinetic both in the microsomal and cytosol fraction of the intestine and the liver. A high intrinsic clearance rate i.e. ratio between Vmax and Km for the metabolic conversion of DEHP to MEHP in the cytosolic fraction of intestine and liver was observed (Choi et al., 2012). However, intrinsic clearance for other metabolites in cytosolic fraction was reported to be insignificant. The in-vitro in-vivo extrapolation (IVIVE) method, which involves scaling of in vitro Vmax value to in vivo utilizes physiological specific parameters such as tissue-specific microsomal protein content or cytosol protein, specific tissue volume and, body weight (Yoon et al., 2014) was used to derive the metabolic parameters. The Eq. (1) describes the scaling approach which is used to derive the Vmax value as an input for the PBPK model. The Michaelis constant i.e. Km for the five metabolites in the gut and liver were set equal to the reported in-vitro cell line study provided in Table 1. The reported Vmax in-vitro values the maximum rate of reaction, were scaled to the whole body PBPK using Eq. (1). The reported quantity of MSP in the liver, and the gut is 52.5 mg/g liver and 20.6 mg/g intestine respectively (Cubitt et al., 2011). Mean value of 80.7 mg and 18 mg of cytosolic protein per gram of the liver and
the gut respectively are used for the IVIVE approach. In-vivo scaled Vmax values for each metabolite are provided in Table 2. The schema of metabolism is provided in Fig. 2.

\[
V_{\text{max}}(\text{intestine/liver}) = (V_{\text{max, in vitro, intestine/liver}} \times MPPGG/MPPGL/\text{CytosolPGG/CytosolPGL} \times V_{\text{gut/Vliver}})/BW^{75}
\]

Eq. (1)

Where,
Vmax is the maximum rate reactions value in the unit of µg/hr/kgBW^{75}; MPPGG is the microsomal protein per gram of gut; MPPGL is the microsomal protein per gram of liver; CytosolPGG is the cytosolic protein per gram of gut; CytosolPGL is the cytosolic protein per gram of liver;
Vgut and Vliver is the volume of gut and liver respectively

\[
\frac{dA_{\text{mets}}}{dt} = \frac{V_{\text{max}} \times C + f_u}{K_m + C \times f_u}
\]

Eq. (2)

Where,
\(C_t\) is the corresponding concentration in tissue and \(f_u\) is the fraction unbound constant.
\(V_{\text{max}}\) (µg/hr/whole body weight) is the maximum rate for the corresponding reactions;
\(K_m\) is the affinity constant concentration at which half of the \(V_{\text{max}}\) occurs.
\(\frac{dA_{\text{mets}}}{dt}\) is the rate of production of metabolites

**Metabolic pathway**

![Intestine/Liver](image)

Fig. 2. Represent the schematic metabolic pathway of DEHP in the human gut and liver. The productions of metabolites follow same structure in PBPK and were described using
Michaelis-Menten equation. The corresponding re1, re2, re3, re4, and re5 represent the Michaelis-Menten metabolic reaction used in the model represented in the Eq. (2).

2.4. In vivo Human Pharmacokinetics study

In-vivo pharmacokinetics of DEHP and its metabolites are well characterized in several studies (Koch et al., 2006, 2005, 2004; Anderson et al., 2011; Lorber et al., 2010). Koch et al. (2004, 2005) studies involved the self-dosing of 48.5 mg of D4-DEHP by volunteer (n = 1). The volunteer aged 61, 175 cm tall and weighing 75 kg. Plasma concentrations for MEHP, 5–OH MEHP, 5-oxo MEHP and 5-Cx MEPP were measured at 2,4, 6 and 8.3 h upon DEHP self-dosing. In the same study, urine samples were collected until 44 h and the cumulative amount of DEHP metabolites were reported. This study was accounted for the model calibration. Koch et al. (2005) monitored two metabolites namely 5-cx MEPP and 2cx MMHP in both plasma and urine. Koch et al. (2005) found 5–OH MEHP and 5-cx MEPP as major metabolites in the urine and observed no dose-dependency. The 5-cx MEPP metabolite was not included in the current model since there is no data on its metabolic kinetics (rate of production).

Anderson et al. (2011) analyzed DEHP pharmacokinetics in urine. For this analysis, two scenarios were considered: one at the high dose of 2.8 mg D4-DEHP and second at a low dose of 0.31 mg D4-DEHP. This pharmacokinetics study included 20 volunteers (10 males and 10 females) of following characteristics aged greater than 18 years, BMI between 19 and 32 kg/m2 and body weight greater than 60 kg. The cumulative amount of DEHP metabolites concentration in urine was reported as a percentage of mole dosing. The cumulative DEHP metabolites urine data were used for evaluation of the developed model keeping all the model's parameters same except subject body characteristics such as BW and BMI.

2.5. Sensitivity analysis

A Local sensitivity analysis was carried out for the PBPK model. The R package FME was used, which measures the alteration in model output for variable of interest by changing each parameter by 1 percentage up and down whilst keeping other ones constant. Detailed information about the functions of FME can be found in Soetaert and Petzoldt, (2010).

\[ S_{i,j} = \frac{\partial y_j}{\partial p_i} \cdot \frac{V_{pi}}{V_{yj}} \]

Where,

\( S_{i,j} \) is the sensitivity of parameter \( i \) for model variable \( j \) and is normalized and dimensionless. \( y_j \) is a model output variable (DEHP Metabolites time-plasma concentration profile), \( p_i \) is parameters involved in PBPK model, \( V_{pi} \) is the scaling of parameters \( p_i \) and \( V_{yj} \) is the scaling of variable \( y_j \).

These sensitivity functions collapsed into a summary of sensitivity values, which includes L1 norm, L2 norm, Mean, Min and Max. The magnitude of the time-averaged sensitivity values were used to rank the parameters.
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Where $L_1 = \frac{\sum |s_{ij}|}{n}$ and $L_2 = \sqrt{\frac{\sum (s_{ij})^2}{n}}$

2.6. Model parameters

Human physiological data, in vitro data and QSAR estimates, were used for the parameterization of the model. Only Pharmacokinetic specific parameters such as partition coefficients, metabolisms and elimination rate constant are selected for uncertainty analysis. Prior mean parameter values were obtained from in-silico, in-vitro and in-vivo experiments reported in the literature. The model parameters value is provided in Table 1. The model parameters are distributed lognormal in the range of $\pm 1$ to $\pm 1.5$ standard deviations accounting uncertainty on model predictions. Monte Carlo simulations were performed to estimate the uncertainty produced by sampling one random value (out of its assigned distribution) for each selected parameter. The model was then run and its outputs (predictions) recorded. These two steps were iterated 20,000 times, and the collected output values formed a random sample, for with we computed the mean, the SD, and any percentile of interest.

<table>
<thead>
<tr>
<th>Table 1. DEHP parameter values and statistical distributions</th>
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<tr>
<td>Parameters</td>
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<tr>
<td>------------</td>
</tr>
<tr>
<td>Molecular weight (DEHP)</td>
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<tr>
<td>Molecular weight (D4-MEHP)</td>
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<tr>
<td>Molecular weight (MEHP-OH)</td>
</tr>
<tr>
<td>Molecular weight (D4-5-oxo MEHP)</td>
</tr>
<tr>
<td>Molecular weight (D4-5-cx MEPP)</td>
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<tr>
<td>Octanol:water partition coefficient</td>
</tr>
</tbody>
</table>

Partition coefficients
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<table>
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<th>Structure</th>
<th>Parameter</th>
<th>Distribution</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gut/Plasma</td>
<td>$K_{\text{gut plasma}}$</td>
<td>$LN(12.86, 1.1)^b$</td>
<td>-</td>
</tr>
<tr>
<td>Liver/Plasma</td>
<td>$K_{\text{liver plasma}}$</td>
<td>$LN(10.16, 1.1)^b$</td>
<td>-</td>
</tr>
<tr>
<td>Gonads/Plasma</td>
<td>$K_{\text{gonads plasma}}$</td>
<td>$LN(6.5, 1.1)^b$</td>
<td>-</td>
</tr>
<tr>
<td>Fat/Plasma</td>
<td>$K_{\text{fat plasma}}$</td>
<td>$LN(188, 1.1)$</td>
<td>-</td>
</tr>
<tr>
<td>Rest of the body/Plasma</td>
<td>$K_{\text{rest body plasma}}$</td>
<td>$LN(6.24, 1.1)^b$</td>
<td>-</td>
</tr>
<tr>
<td>Liver/Plasma</td>
<td>$K_{\text{liver plasma M1}}$</td>
<td>$LN(1.7, 1.1)$</td>
<td>(Keys et al., 2000)</td>
</tr>
<tr>
<td>Gonads/Plasma</td>
<td>$K_{\text{gonads plasma M1}}$</td>
<td>$LN(0.6, 1.1)$</td>
<td>(Keys et al., 2000)</td>
</tr>
<tr>
<td>Fat/Plasma</td>
<td>$K_{\text{fat plasma M1}}$</td>
<td>$LN(0.12, 1.1)$</td>
<td>(Keys et al., 2000)</td>
</tr>
<tr>
<td>Rest of the body/Plasma</td>
<td>$K_{\text{rest body plasma M1}}$</td>
<td>$LN(0.38, 1.1)$</td>
<td>Set to slow perfused organ (muscle) (Keys et al., 1999)</td>
</tr>
<tr>
<td>Uptake rate of 5-OHMEHP to blood</td>
<td>$K_{M2}$</td>
<td>1/h</td>
<td>$LN(0.07, 1.5)$</td>
</tr>
<tr>
<td>Uptake rate of 5-oxo MEHP to the blood</td>
<td>$K_{M4}$</td>
<td>1/h</td>
<td>$LN(0.08, 1.5)$</td>
</tr>
</tbody>
</table>

### Absorption and elimination parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unbound fraction in plasma for MEHP</td>
<td>$f_{\text{up}}$</td>
<td>0.007</td>
</tr>
<tr>
<td>Oral absorption rate</td>
<td>$K_{\text{gut}}$</td>
<td>1/h</td>
</tr>
<tr>
<td>Elimination rate constant (M1)</td>
<td>KurineM1</td>
<td>1/h</td>
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<td>-------------------------------</td>
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<tr>
<td>Elimination rate constant (M2)</td>
<td>KurineM2</td>
<td>1/h</td>
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<tr>
<td>Elimination rate constant (M3)</td>
<td>KurineM3</td>
<td>1/h</td>
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<tr>
<td>Elimination rate constant (M4)</td>
<td>KurineM4</td>
<td>1/h</td>
</tr>
</tbody>
</table>

**Metabolic parameters for DEHP and its metabolites in gut**

<p>| DEHP to MEHP in intestinal MSP maximum reaction value | Vmaxgut M1 | ( \mu g/min/mg MSP ) | LN (0.11,1.1)^d | (Choi et al., 2013) |
| Conc. at half maximum value | KmgutM1 | ( \mu g/L ) | 6956 | (Choi et al., 2013) |
| DEHP to MEHP in gut cytosol maximum reaction value | Vmaxgut M1cyt_in vitro | ( \mu g/min/mg cytosol ) | LN (0.312, 1.1)^d | (Choi et al., 2013) |
| Conc. at half maximum value | Kmgut_cytM1 | ( \mu g/L ) | 7038 | (Choi et al., 2013) |
| MEHP to 5-OH MEHP maximum reaction value | Vmaxgut M2_invitro | ( \mu g/min/mg MSP ) | LN (0.0012, 1.1)^d | (Choi et al., 2013) |
| Conc. at half maximum value | KmgutM2 | ( \mu g/L ) | 22508 | (Choi et al., 2013) |
| MEHP to 5-carboxy MEPP maximum reaction value | Vmaxgut M3_invitro | ( \mu g/min/mg MSP ) | 0 | (Choi et al., 2013) |
| Conc. at half maximum value | KmgutM3 | ( \mu g/L ) | 0 | (Choi et al., 2013) |
| MEHP-OH to 5-oxo MEHP maximum reaction value | Vmaxgut M4_invitro | ( \mu g/min/mg MSP ) | LN (0.0012, 1.5)^d | (Choi et al., 2013) |</p>
<table>
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<tr>
<th>Metabolic parameters for DEHP and its metabolites in liver</th>
</tr>
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<tbody>
<tr>
<td><strong>DEHP to MEHP in liver MSP maximum reaction value</strong></td>
</tr>
<tr>
<td>V_{max}^{livM1}</td>
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<tr>
<td>Conc. at half maximum value KmlivM1</td>
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<tr>
<td><strong>DEHP to MEHP in liver cytosol maximum reaction value</strong></td>
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<tr>
<td>V_{max}^{livM1cyt} in vitro</td>
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<tr>
<td>Conc. at half maximum value Kmliv_cytM1</td>
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<tr>
<td><strong>MEHP to 5-OH MEHP maximum reaction value</strong></td>
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<tr>
<td>V_{max}^{livM2} in vitro</td>
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<tr>
<td>Conc. at half maximum value KmlivM2</td>
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<tr>
<td><strong>MEHP to 5-carboxy MEPP maximum reaction value</strong></td>
</tr>
<tr>
<td>V_{max}^{livM3} in vitro</td>
</tr>
<tr>
<td>Conc. at half maximum value KmlivM3</td>
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<tr>
<td><strong>MEHP-OH to 5-oxo MEHP maximum reaction value</strong></td>
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<tr>
<td>V_{max}^{livM4} in vitro</td>
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<tr>
<th>Conc. at half maximum value</th>
<th>KmlivM4</th>
<th>µg/L</th>
<th>23,117.7</th>
<th>(Choi et al., 2013)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEHP to phthalic acid maximum reaction value</td>
<td>Vmaxliv M5_invitro</td>
<td>µg/min/mg MSP</td>
<td>LN $(0.088, 1.1)^4$</td>
<td>(Choi et al., 2013)</td>
</tr>
<tr>
<td>Conc. at half maximum value</td>
<td>KmlivM5</td>
<td>µg/L</td>
<td>141315</td>
<td>(Choi et al., 2013)</td>
</tr>
</tbody>
</table>

a = value taken from PubChem  
b = partition coefficient calculated based on tissue composition method using (Poulin and Krishnan, 1996, 1995; Poulin and Theil, 2000)  
c = value is first estimated applying the following relationship i.e. elimination rate constant = 0.693/t1/2  
d = parameters value needs to scale to whole body weight prior to use in model

3. Results and Discussions

In this study, parameters such as partition coefficient, biochemical (metabolism), absorption, elimination as an input and target variables such as DEHP metabolites concentration as a model output, were considered to conduct sensitivity analysis and uncertainty analysis. The bottom-up approach was used to develop the PBPK model and all necessary parameters were derived from in-silico (QSAR), in vitro (metabolism) and published literature. The results are described and discussed in the following subsection.

3.1. Sensitivity analysis results

The local sensitivity analysis was carried out for all the kinetic parameters that were used in the development of the PBPK model. Human physiological parameters were included neither in the Monte Carlo model nor in the sensitivity analysis in view of their inherent variability. The sensitivity coefficient of parameters was estimated using the R FME package (Soetaert and Petzoldt, 2010) (described in Section 2.5), which uses the initial parameter value with allowable relative changes in that parameter, taking the parameters one by one. The results are provided in Table 2. It includes L1 and L2, norm, mean, minimum, maximum, and ranking. The table summarizes the statistics of the normalized and dimensionless parameter sensitivity results. The parameters were ranked based on the L1 value. A higher value of L1 signifies a higher sensitivity of the model output to changes in the parameter. The biochemical parameters such as $V_{\text{max}}$ and $K_{\text{m}}$ value have very close sensitivity coefficient. The mean sensitivity coefficient of $V_{\text{max}}$ has the negative effect and the $K_{\text{m}}$ has the positive effect on the model output. Hence in uncertainty analysis, instead of both $V_{\text{max}}$ and $K_{\text{m}}$, only $V_{\text{max}}$ was distributed statistically as result of sensitivity shows that they are highly correlated with each other. The $V_{\text{max}}$LiverM2 (metabolism of MEHP to MEHP–OH) is the most sensitive parameter (Rank 1) following partition coefficient of liver: plasma (Rank 3). The partition coefficient for the rest of the body and the metabolism of DEHP in the cytosol fraction of both gut and liver are under the rank of 10 which shows their high sensitivity compared to other parameters. The plots
for sensitive analysis output i.e. mean sensitivity coefficient are provided in Fig. A.1 (Annex 2). The summary statistics tables of parameters’ sensitivities for the output of DEHP metabolites concentration in plasma is provided in Tables A.5–A.8 (Annex 2).

<table>
<thead>
<tr>
<th>Table 2. Summary statistics of parameters’ sensitivities</th>
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<tr>
<td>Parameters</td>
</tr>
<tr>
<td>--------------------------------</td>
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<tr>
<td>$V_{max}$liverM2</td>
</tr>
<tr>
<td>$K_{ml}$liverM2</td>
</tr>
<tr>
<td>$K_{liver_plasma}$</td>
</tr>
<tr>
<td>$V_{max}$liverM4</td>
</tr>
<tr>
<td>$K_{ml}$liverM4</td>
</tr>
<tr>
<td>$K_{rest_body_plasma}$</td>
</tr>
<tr>
<td>$V_{max}$gut_cytM1</td>
</tr>
<tr>
<td>$K_{liver_plasmaM1}$</td>
</tr>
<tr>
<td>$V_{max}$liver_cytM1</td>
</tr>
<tr>
<td>$K_{ml}$liver_cytM1</td>
</tr>
<tr>
<td>$V_{max}$liverM3</td>
</tr>
<tr>
<td>$K_{ml}$liverM3</td>
</tr>
<tr>
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<td>$K_{T4}$</td>
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<td>$K_{urineM2}$</td>
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</tr>
<tr>
<td>$K_{urineM4}$</td>
</tr>
<tr>
<td>$K_{rest_body_plasmaM1}$</td>
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<table>
<thead>
<tr>
<th>VmaxliverM1</th>
<th>0.08</th>
<th>0.00</th>
<th>-0.08</th>
<th>-1.18</th>
<th>0.05</th>
<th>22</th>
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<tr>
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<td>25</td>
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<td>k_gonads_plasma</td>
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<td>VmaxgutM2</td>
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<td>0.00</td>
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<td>27</td>
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<tr>
<td>KmgutM2</td>
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<td>28</td>
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<td>Vplasmad</td>
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</tr>
<tr>
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<td>0.00</td>
<td>0.01</td>
<td>-0.02</td>
<td>0.66</td>
<td>34</td>
</tr>
<tr>
<td>VmaxgutM5</td>
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<td>0.00</td>
<td>0.00</td>
<td>-0.03</td>
<td>0.03</td>
<td>35</td>
</tr>
<tr>
<td>KmgutM5</td>
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<tr>
<td>VmaxgutM4</td>
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<td>0.00</td>
<td>-0.01</td>
<td>0.00</td>
<td>38</td>
</tr>
</tbody>
</table>

Table 2: Sensitivity results for both the rat and human PBPK model. It includes L1 and L2 norm, mean, minimum, maximum, and ranking. Ranking of parameter sensitivity coefficient was done based on L1 norm.

3.2. PBPK model calibration results and its evaluation with independent data

The time course of DEHP metabolites concentration in plasma and the cumulative amount in urine were predicted at the median, 2.5 and 97.5 percentiles and 20 random predictions. PBPK model has accounted the parameter statistical distribution followed by sampling one random value (out of its assigned distribution) and performing Monte Carlo simulation reflecting uncertainty in the model. The model does not include any variability factor related to physiological parameters. For the metabolic uncertainties, only Vmax values were statistically distributed but not Km considering that they are highly correlated with each other. Single oral dose of 48.5 mg DEHP as an input and the observed concentration of metabolites both in the blood and urine as an output were used to calibrate the model. Most of the parameters were derived via either from in-silico
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(estimation of the partition coefficient) (Poulin and Krishnan, 1996, 1995; Poulin and Theil, 2000) or from in vitro such as, partition coefficient determined (Keys et al., 2000) and in vitro metabolic data (human hepatocyte and intestinal cell line) (Choi et al., 2013). The parameters such as elimination rate constants for the metabolites are derived using a mathematical relationship described in models and methods section. The absorption rates of metabolites (mass transfer) from the gut to the liver were set as one (complete mass transfer) except MEHP whose absorption rate constant was derived from the literature (Adachi et al., 2015). The mass transfer rate of metabolites from the liver to the blood was calibrated against the observed data (Koch et al., 2005). The model was developed using the parameters derived from in-silico, in vitro data, and previously published literature, and certain default parameter values, which needed to be calibrate. Instead of optimizing all the parameters very specifically to get a point to point prediction against the observed data rather we statistically distributed all the parameters in a range of 1–1.5 ± SD (standard deviation) providing range of predictions. Then the model was verified against the blood and urine metabolites concentration data reported by Koch et al. (2005), so that observed data for all metabolites fall within the range (2.5th –97.5th) of model predictions. The predictions of the DEHP metabolites concentration in blood and urine included their metabolic kinetics both in the gut and the liver described by Michaelis Menten equation. And the parameters such as Vmax and Km were estimated in vitro by Choi et al. (2013) were scaled to the whole body (based on organ weight) and integrated into the model. Fig. 3(a–d) represents the PBPK model predictions for plasma concentrations of four DEHP metabolites. It can be observed that the model predictions agree quite closely to the observed data. The cumulative excretion of DEHP metabolites is also adequately predicted by the model represented in Fig. 4(a–d) and Table 2. The recently reported in vitro metabolism data shows that the production rate of MEHP from the DEHP is very high (Choi et al., 2013). A similar trend of the kinetic profile was also reported by Koch et al. (2005) where he observed very low or undetectable DEHP blood concentration. Given the above facts, the clearance of DEHP is presumed to completely depend on its metabolic conversion to MEHP. The Fig. 3(a) shows that predicted Cmax (highest chemical plasma concentration) of the MEHP is slightly lower than the observed data even at 97.5 percentile simulation. However, the time course trend of chemical concentrations in plasma is similar to the observed data points. In addition to that, post-Cmax, the predictability of the model are in close agreement with the observed points. The clearance of MEHP from the body includes both its metabolism and the urinary elimination.

Fig. 3(b) represents the model predictions for MEHP–OH concentrations in blood at 2.5, 50 (median) and 97.5th percentiles including 20 random simulations, and the observed data in green dots. The blood Cmax value for 5–OH MEHP is lower than MEHP and 5-Cx MEPP and more than its metabolite 5-oxo MEHP. The observed data points at the terminal elimination are predicted at the lower boundary of the model, where almost all chemicals are eliminated. All the observed blood data points are within the range of the model prediction (2.5, 50 and 97.5th percentiles). The observed production rate of 5–OH MEHP in gut and liver i.e. in vitro metabolism data (Vmax) is higher than the other metabolites (Choi et al., 2013). However, reported blood concentration by Koch et al. (2005) is less than 5-Cx MEPP, another metabolite. The reason for its lower blood plasma concentration is might be due to its higher volume of distribution than the other metabolites, the similar observation was noted previously by Lorber et al. (2010) during the calibration of the model. The other reasons might be its higher clearance to the urine.
and its further metabolism to 5-oxo MEHP. The production of 5–OH MEHP depends on the MEHP concentration in both the liver and the gut, and then its distribution to the blood. The transfer of 5–OH MEHP from the liver to blood was done using first order rate constant and is calibrated against the observed data. 5–OH MEHP clearance was done based on both its metabolism to the 5-oxo MEHP and the urinary elimination. The urinary elimination was described using first order using first order rate constant.

Similarly, PBPK model predictions for 5-cx MEPP plasma concentrations as shown in Fig. 3(c), which is the metabolite of MEHP, appears to be in close agreement with the observed data points. The volume of distribution (Vd) was confined to the plasma compartment volume since the distribution of the compound is unknown. The production of 5-cx MEPP metabolite from the MEHP in the gut was reported to be null in the in vitro experiment (Choi et al., 2013). So, the concentration of 5-oxo MEPP only depends on its production in the liver from the MEHP. Its clearance was described using first order rate constant from the blood to urine.

The model predictions for 5-oxo MEHP plasma concentrations as shown in Fig. 3(d), results from metabolism of 5–OH MEHP in both gut and liver, are in close agreements with the observed concentrations. All the observed data points are in compliance with the predicted range of percentile. Its production in gut and liver from the 5–OH MEHP is described using Michaelis-Menten reaction. Its volume of distribution is confined to a single compartment of plasma volume. The urinary elimination was described using first order elimination rate from the systemic circulation.
Fig. 3. PBPK model prediction of DEHP metabolites plasma concentrations upon 48.5 mg oral dosing in human. Red lines: median predictions; blue lines: 2.5 and 97.5 percentiles; gray lines: 20 random simulations. (a) Represents MEHP plasma concentration. (b) Represents 5-hyroxy MEHP plasma concentration. (c) Represents 5-carboxy MEPP plasma concentration. (d) Represents 5-oxo MEHP plasma concentration. The green dotes indicate the observed concentrations reported in (Lorber et al., 2010). Dose unit is converted to microgram prior to use as an input for the model.

The four metabolites’ blood concentrations are not only in close agreement with the observed data points but also captured the time course profile. The Fig. 4(a–d), presented PBPK prediction of the cumulative amount (μg) urinary excretion of four metabolites for 44h at median, 2.5 and 97.5 percentiles and for 20 random simulations. The simulated urinary amount of DEHP metabolites (cumulative amount) are also in compliance with the experimentally observed cumulative amount (Koch et al., 2005), results are provided in Table 2. It also summarizes the predicted vs observed metabolites elimination as a percent of applied dose in mole for three dosing scenarios based on Koch et al. (2005) study. The observed metabolites as a percentage of mole doses are within the range of predictions of the model not only for high dose (use for calibration) but also for other two independent dosing scenarios such as medium (2.15 mg) and low dose (0.35 mg).
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Fig. 4. PBPK model predictions of DEHP metabolites amount in urine following 48.5 mg oral dose. Red lines: median predictions; blue lines: 2.5 and 97.5 percentiles; gray lines: 20 random simulations. (a) Represents MEHP cumulative amount (μg) in urine. (b) Represents 5-hyroxy MEHP cumulative amount (μg) in urine. (c) Represents 5-carboxy MEPP cumulative amount (μg) in urine. (d) Represents 5-oxo MEHP cumulative amount (μg) in urine. Dose unit is converted to microgram prior to use as an input for the PBPK model.

<table>
<thead>
<tr>
<th>Study involved</th>
<th>Dose (μg)</th>
<th>MEHP</th>
<th>5OIH-MEHP</th>
<th>5cx-MEPP</th>
<th>5oxo-MEPP</th>
<th>Total dose in μg or percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koch et al., (2005)</td>
<td>48,500</td>
<td>2500</td>
<td>9000</td>
<td>7500</td>
<td>5000</td>
<td>23500</td>
</tr>
</tbody>
</table>

Table 3. Observed and PBPK predicted amount of DEHP (μg) metabolites in urine.
### Metabolites of the D4-DEHP Dose as percent of applied dose (mol)

<table>
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<tr>
<th></th>
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<tr>
<td></td>
<td>48,500</td>
<td>2,150</td>
<td>350</td>
<td></td>
<td>350</td>
<td></td>
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<tr>
<td>2.5th - 97.5th</td>
<td>48,500</td>
<td>48,500</td>
<td>2,150</td>
<td></td>
<td>350</td>
<td></td>
</tr>
<tr>
<td>(median)</td>
<td>1548.2-3122.7</td>
<td>4.4-8.9</td>
<td>4.3-8.7</td>
<td>6.2</td>
<td>4.3-8.7</td>
<td>6.2</td>
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<td></td>
<td>3988.6-10148</td>
<td>(6.4)</td>
<td>(6.2)</td>
<td>(14.6)</td>
<td>(6.2)</td>
<td>(14.5)</td>
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<tr>
<td></td>
<td>1585.4-7086</td>
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<td>8.9-23.3</td>
<td>(14.6)</td>
<td>8.8-23.2</td>
<td>(14.5)</td>
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<tr>
<td></td>
<td>1087-5497</td>
<td>15.0</td>
<td>19.0</td>
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<td></td>
<td>8209.2-25853.7</td>
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<td>20.52-66.3</td>
<td>(6.7)</td>
<td>20.5-66.2</td>
<td>(6.8)</td>
</tr>
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<tr>
<td></td>
<td>20.7</td>
<td>(39.44) %</td>
<td>(36.7) %</td>
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<td>20.7</td>
<td>(36.7) %</td>
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<td></td>
<td>14.6</td>
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<td></td>
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<td></td>
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</tbody>
</table>

*a = values are extracted from the graph presented in manuscript by Koch et al. (2005)*

Dose unit is converted to microgram prior to use as an input for the PBPK model.

Given that the model predictions fit the DEHP metabolites namely MEHP and other metabolites 5–OH MEHP, 5-ex MEPP and 5-oxo MEHP concentration in the blood and
urine upon 48.5 mg of a single oral dose of DEHP. The structure of the model and the model parameters remained unchanged from their calibrated values, and the predicted percentage mole elimination data for four metabolites in urine were compared with the data reported in Anderson et al. (2011) for the evaluation of model credibility. The study included 20 subjects, 10 male, and 10 female, and their overall mean body weight was 74.8 kg. The only additional change in the model is subject body weight. The present model does not include gender variability among 20 subjects, and the mean body weight was taken as an input for model simulation, as current model only accounted for the parametric uncertainty, not the variability. Two dosing scenarios namely high dose; a single oral dose of 2.8 mg DEHP and low dose; a single oral dose of 0.31 mg was used for the model simulations. The subject characteristic and dosing for respective studies are provided in Table A. (1–3). The predicted urinary data were converted into moles based on their molecular weight in order to standardize the exposure unit data. Then the relation; 

\(( \text{predicted amounts of metabolites in urine (moles)/amounts dose (moles)} \times 100)\), is used to calculate the percentage molar eliminations on moles basis (Anderson et al., 2011; Koch et al., 2005). The detailed summarized tables are provided in Tables A.5–A.7. The PBPK predicted a range of metabolites elimination as a percentage of doses in mole reflecting the uncertainty in the model. The model output was compared with the observed experimental data. Table 3 summarizes the predicted vs observed percentage amount elimination of metabolites. The experimentally observed cumulative amount of all metabolites in the urine is well within the range of PBPK simulation (Table 4).

### Table 4. Fraction excretion value (mole percentage) for observed and PBPK predicted of DEHP metabolites

<table>
<thead>
<tr>
<th>Metabolites of the D4-DEHP Dose (% mol elimination)</th>
<th>Study involved</th>
<th>Dose</th>
<th>MEHP</th>
<th>5OH-MEHP</th>
<th>5oxo-MEHP</th>
<th>Total molar elimination (%)</th>
</tr>
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<tr>
<td></td>
<td>Anderson et al., (2011)</td>
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<td>16.33</td>
<td>15.90</td>
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<td></td>
<td>Present study</td>
<td>310μg</td>
<td>4.3-8.7</td>
<td>8.8-22.9</td>
<td>4.3-18.5</td>
<td>3.0-15.2</td>
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<tr>
<td></td>
<td>2.5th - 97.5th (median)</td>
<td></td>
<td>(6.3)</td>
<td>(14.6)</td>
<td>(9.2)</td>
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<td></td>
<td>Anderson et al., (2011)</td>
<td>2800μg</td>
<td>5.67</td>
<td>14.86</td>
<td>11.97</td>
<td>10.00</td>
</tr>
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</table>
4. Conclusions and future work

The results showed that the current developed model can able to predict the plasma and the cumulative urine concentration of the DEHP metabolites for the different exposure scenario. The current model included four metabolites and the generation of metabolites was described mechanistically using integrated physiological parameters and Michaelis-Menten (M-M) parameters such as Vmax and Km derived from a human hepatic/intestine cell line. The sensitive analysis was done for all parameters and the metabolic parameters were found to be more sensitive than other parameters. Monte Carlo simulation was used accounting probabilistic information about pharmacokinetics parameters that estimated DEHP metabolites concentration in both the plasma and the urine at three percentile considering the uncertainty into the model. Some of the major strength of current predictive model over previously developed models for DEHP are: (1) it’s a detail PBPK model that predict the compound(s) or metabolite(s) concentration using the in vitro metabolism data with the application of IVIVE instead of using animal experimental data for its calibration or fitting, (2) production of metabolites was described using saturation kinetics (M-M equations) which retains its biological plausibility, (3) model can be individualized (personalized) for different populations by implementing the physiological variability into the model, (4) it can be used to predict the target tissue internal concentrations for further toxicodynamics study and human health risk assessments. The current developed model did not account for the 2-cx MEPP metabolite due to lack of in vitro metabolic data, considered to be another important metabolite for the biomonitoring study. The current PBPK model can be further extended for 2-cx MEPP, once the metabolic data are available. Detailed rat’s pharmacokinetic studies that include all metabolites could be very useful for further understanding metabolites tissue distribution. The current developed model can be applied in the biomonitoring and exposome studies for the human health risk assessment (Martinez et al., 2017, 2018). The developed model can be further extended for the development of an integrated PBPK/PD systems toxicology model (integrative systems toxicology) to establish the exposure-internal dose- response relationship (Sharma et al., 2017b)

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https://doi.org/10.1002/jat.1490


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