The development of a pregnancy PBPK Model for Bisphenol A and its evaluation with the available biomonitoring data

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Abstract

Recent studies suggest universal fetal exposure to Bisphenol A (BPA) and its association with the adverse birth outcomes. Estimation of the fetal plasma BPA concentration from the maternal plasma BPA would be highly useful to predict its associated risk to this specific population. The objective of current work is to develop a pregnancy–physiologically based pharmacokinetic (P-PBPK) model to predict the toxicokinetic profile of BPA in the fetus during gestational growth, and to evaluate the developed model using biomonitoring data obtained from different pregnancy cohort studies. To achieve this objective, first, the adult PBPK model was developed and validated with the human BPA toxicokinetic data. This validated human PBPK model was extended to develop a P-PBPK model, which included the physiological changes during pregnancy and the fetus sub-model. The developed model would be able to predict the BPA pharmacokinetics (PKs) in both mother and fetus. Transplacental BPA kinetics parameters for this study were taken from the previous pregnant mice study. Both oral and dermal exposure routes were included into the model to simulate total BPA internal exposure. The impact of conjugation and deconjugation of the BPA and its metabolites on fetal PKs was investigated. The developed P-PBPK model was evaluated against the observed BPA concentrations in cord blood, fetus liver and amniotic fluid considering maternal blood concentration as an exposure source. A range of maternal exposure dose for the oral and dermal routes was estimated, so that simulation concentration matched the observed highest and lowest mother plasma concentration in different cohorts' studies. The developed model could be used to address the concerns regarding possible adverse health effects in the fetus being exposed to BPA and might be useful in identifying critical windows of exposure during pregnancy.

Key words: Bisphenol A; pregnancy-PBPK; fetal exposure; biomonitoring; window of exposure.

1. Introduction

BPA is produced at over 2 billion pounds/year and is found in wide variety of dietary and non-dietary products. The dietary sources include both canned and non-canned foods categories ranging from “meat and meat products”, “vegetables and vegetable products”, and other packaged foods, and food handling consumer products like baby bottles, beverage containers etc. (WHO, 2010; EFSA, 2015). The non-dietary sources include medical devices, dental sealants, dust, thermal papers, toys and cosmetics (Mendum et al., 2011; EFSA, 2015). Although ingestion of the BPA from food or water is the predominant route of exposure (Lorber et al., 2015), there are other non-dietary routes, which also equally contributes to the total BPA exposure, such as inhalation of free BPA (concentrations in indoor and outdoor air), indirect ingestion (dust, soil, and toys), and dermal route (contact with thermal papers and application of dental treatment) (Myridakis et al., 2016). Recently reported studies have found relatively more contribution of the dermal route to overall internal BPA concentration than the oral route’s exposure (Biedermann et al., 2010; Mielke et al., 2011). In addition, recent studies (De Coensel et al., 2009; Sungur et al., 2014) show that temperature has a major impact on the BPA migration level into water; an increase from 40 ºC to 60 ºC can lead to a 6 - 10 fold increase in the migration level.

BPA and its metabolites have been detected in maternal blood, amniotic fluid, follicular fluid, placental tissue, umbilical cord blood, urine and breast milk (Schönfelder et al., 2002; Ikezuki et al., 2002; Kuroda et al., 2003; Kuruto-Niwa et al., 2007; Lee et al., 2008; Zhang et al., 2011,2013; Cao et al., 2012; Nahar et al., 2013; Gerona et al., 2014; Teeguarden et al., 2016). In different rodents’ studies, it has been seen that low dose of bisphenol exposure during the gestational period has effects on the fertility, brain development, and the behavioural changes in their later life stages, signify BPA pleiotropic effects (Palanza et al., 2002; Cabaton et al., 2013; Snijder et al., 2013; Harley et al., 2013). Rubin and Soto, (2009) reviewed the prenatal BPA exposure and its effects on adipocytes differentiation, a major cause of obesity. U.S. Environmental Protection Agency (EPA) has declared the BPA as an endocrine-modifying chemical, which has been found to be reproductive, developmental, systemic toxicant, obesogenic and, weakly estrogenic (Moriyama et al., 2002; Rey et al., 2003; Patisaul et al., 2009; Xi et al., 2011; Wang et al., 2012; Vafeiadi et al., 2016; Sharma et al., 2016).

Adult human studies have reported that BPA has a very short half-life. It rapidly detoxifies to nontoxic conjugate substance such as BPA-glucuronide (BPAG) and BPA-sulfate (BPAS), collectively called as BPA conjugates (BPA-C), by glucuronidation

and sulfation metabolic process (Völkel et al., 2002; Teeguarden et al., 2015; Thayer et al., 2015). However, in the case of the specific populations such as developing fetus, growing infants, and young children, whose chemical metabolizing systems are underdeveloped, even moderate exposure can lead to higher internal concentration of BPA (Divakaran et al., 2014). Moreover, the reactivation of these conjugates (deconjugation),BPAG and BPAS, by the fetal tissue and the placenta has been reported(Ginsberg and Rice, 2009; Nahar et al., 2013),causing an increase in BPA internal exposure to the fetus. The recent human pharmacokinetics studies showed low amount of BPA plasma concentration even with the high oral dose, in contrast, exposure amount of BPA for the different cohorts are estimated to be very low against higher BPA plasma concentration obtained in biomonitoring studies (Völkel et al., 2005, 2002; Teeguarden et al., 2015; Thayer et al., 2015). Mielke and Gundert-Remy, (2009) compared the observed biomonitoring data of Schönfelder et al., (2002) study against the model predicted plasma concentrations of BPA using the simple kinetic approach and physiological based pharmacokinetic (PBPK) model, and found 3000 fold lower difference between the model prediction and the observed biomonitoring data. This wide discrepancy between the pharmacokinetic models' prediction and the biomonitoring data could be due to physiological variation, genetic polymorphisms among populations, exposure variation and exclusion of non-oral routes of exposure. However, the possible contamination during sample collection and analysis could be one reason for this discrepancy (Longnecker et al., 2013; Ye et al., 2013) but it is beyond the scope of this paper. Functional polymorphism in glucuronidation enzyme responsible for the BPA metabolisms has been reported by Trdan Lusin et al., (2012). It has been found that BPA after dermal exposure has a longer half -life of 8hr as it bypass the first pass metabolism, and attains the steady state in blood by the 4^{th} day, whereas single oral dose intake completely eliminates in 6-8hr and never reach steady state even with daily dosing (Biedermann et al., 2010; Mielke et al., 2011; Mielke and Gundert-Remy, 2012; Gundert-Remy et al., 2013).

Previously, adult human, rat and monkey PBPK models have been developed for the BPA and its conjugates (Shin et al., 2004; Edginton and Ritter, 2009; Fisher et al., 2011; Yang et al., 2015, 2013; Yang and Fisher, 2015). The pregnancy physiologically- based pharmacokinetic (P-PBPK) models have long been used to estimate the exposure of the chemical to the fetus (Corley et al., 2003). The P-PBPK model for mice was previously developed (Kawamoto et al., 2007), which showed the potential exposure of BPA to the fetus. However, a P-PBPK model for the human has not yet been developed. The pharmacokinetic data for chemicals are often limited in

specific populations of pregnant mother and fetus, due to the ethical and technical reason, which often lead to difficulties in building a kinetic model. However, the use of a physiological based pharmacokinetic model can simplify this complexity, based on its capability to predict the kinetics of chemical via a mechanistic understanding of its absorption, distribution, metabolisms, and elimination inside the body. The overall aim of this study was to improve the understanding of the chemical toxicokinetic relationship between the mother and the fetus by developing a P-PBPK model for the BPA and its conjugates. This would enable to predict the fetus plasma and organs BPA concentration by estimating the mother plasma BPA concentration and, thus helps in identifying the critical window(s) of exposure to the fetus during its gestational period of development. The conceptual model diagram is provided in Figure 1 showing the study design undertaken for this work. The P-PBPK model development has followed following phases: a) development and validation of the adult PBPK model, b) Extension of the developed adult PBPK model to a P-PBPK with the inclusions of dynamic physiological changes during the pregnancy and the prediction of chemical toxicokinetic profile in both mother and fetus compartment and c) evaluation of developed P-PBPK model against the biomonitoring data of available pregnant cohort population. An additional case study of this model has been recently published in Martínez et al., (2017), where simulation of prenatal BPA exposure via dietary intake of pregnant women recruited from Tarragona county was performed.

2. Methodology and parameterization

Development of the P-PBPK model retains entire feature that used to describes the adult BPA and BPA-C (BPAG and BPAS) kinetics like partition coefficient for the organs, fraction unbound, metabolism (Vmax and Km) and elimination (urinary elimination). The physiological changes that occur during pregnancy like changes in plasma volume, fat volume, amniotic fluid, placental and fetal growth are described as dynamic parameters that depend on the gestational period(Gentry et al., 2003; Abduljalil et al., 2012). Besides the oral mode of exposure, dermal mode of exposure was included in the development of the pregnancy-PBPK model. The oral exposure was divided into three equal doses and dermal as a single dose. Considering the gestational growth physiology in the case of the pregnant mother and fetus, the development of a P-PBPK model has been described in the following section. The models were coded in the R program (version 3.2.3), and model equations are provided in the supplementary material (Annex-I).

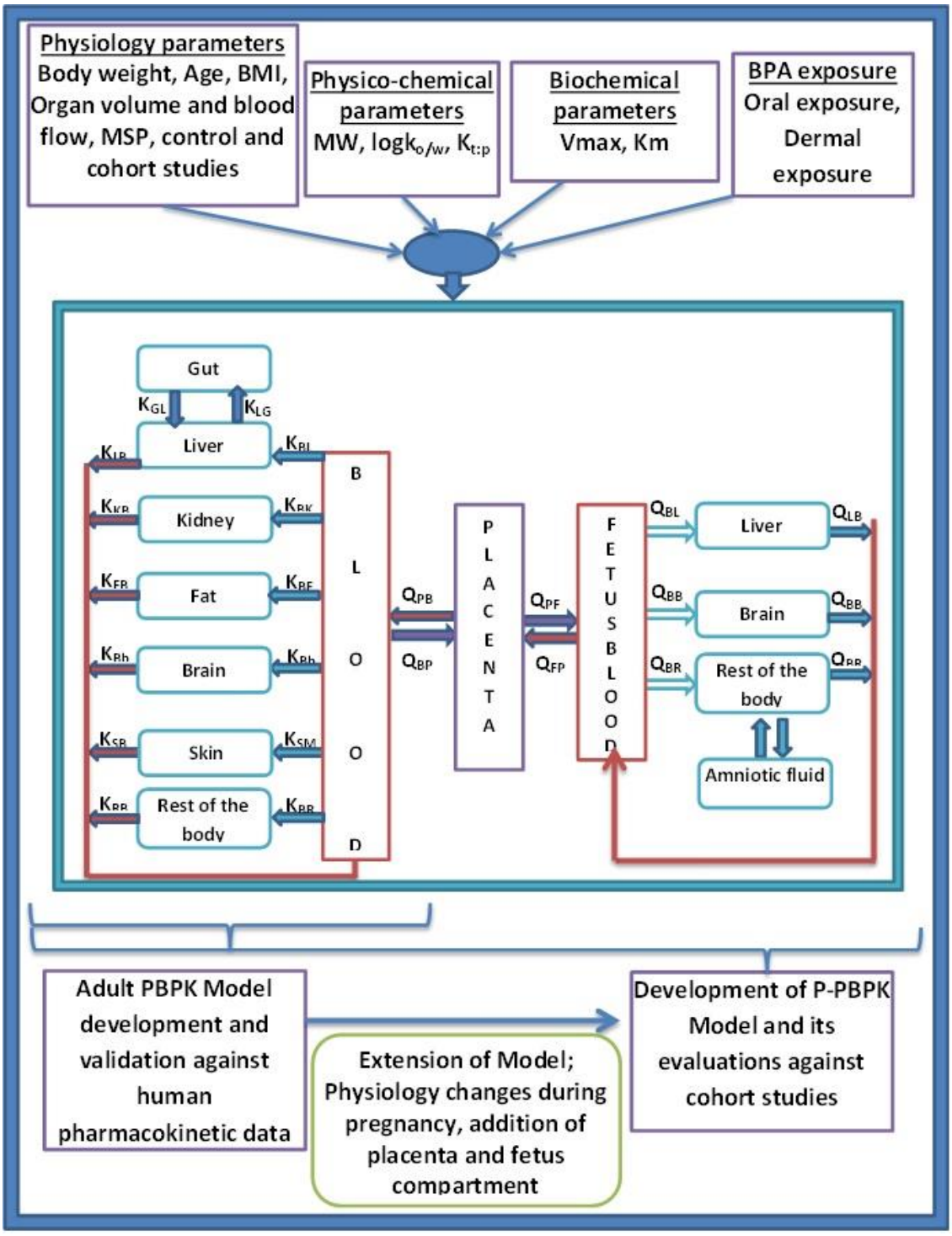


Figure 1. A conceptual model for the development of P-PBPK model. It involves the development of the adult PBPK model and extension of this model to the P-PBPK model with the addition of placenta and fetus sub-compartment. MW = molecular weight, BMI = basal metabolic index, MSP = microsomal protein, K = partition

coefficient and subscripts L = Liver, B= blood, b = brain, K= kidney, S= skin, R= rest organ, G= gut, Q = cardiac blood flow, P = placenta, F = fetus.

2.1. General pregnancy-PBPK Model structure

The basic structure of the P-PBPK model has been adapted from an adult model, which included plasma, liver, kidneys, fat, brain, skin and a rest of the body compartment for the remaining tissues. The placenta and the fetus compartments were added into the model. The fetus compartment is further extended to fetus sub-model considering liver, kidney, brain, and plasma as fetus sub-compartments. The fetus sub-model considered the fetus-specific metabolic processes and included important target organs for the prediction of internal target dosimetry. The physiological and metabolic parameters were applied for the fetus model as dynamic parameters of gestational period and chemical-specific parameters such as partition coefficient were kept similar to the adult human model in the case of both Mother and fetus organs.

The source of exposure to the fetus was via unbound concentration of the chemical in the mother placenta, assuming only the mother directly exposed to the chemical. The placental-fetal unit assumes a bidirectional transfer process describing BPA and BPA-G transfer between mother placenta to fetus plasma and vice versa. The transfer rate was assumed as a simple diffusion process. Transport of chemical from fetal plasma into the fetal compartments like liver, kidney, brain, and rest of the body was assumed to be simple diffusion described by partition coefficient (same as of mother tissue). The amniotic fluid compartment was included in the current P-PBPK model. Transfer rates between the amniotic fluid compartment and the fetal body were described as a simple diffusion process.

The elimination of BPA in the mother was assumed to be similar to adult human, which occurs via its rapid metabolism in the liver and intestine, subsequently excreted via urine. However, the clearance of BPA and its conjugates in the fetus was described with first order transfer rate from fetus plasma to mother plasma via the placenta.

2.2. Gestational growth Physiology Model

The dynamic physiological parameters for the pregnant mother that changes during the gestational period such as plasma volume, hematocrit percentage, the fetus and the placental growth were accounted for the development of P-PBPK model. The increase in maternal body weight was accounted by considering the dynamic growth of mother's

organ and fetus growth into the model. The volumes of liver, kidney, skin, brain, and gut of mother were calculated by taking constant fractions of the non-pregnant maternal body weight (Brown et al., 1997) provided in Table A.1. For the rest of the body compartment for pregnant mother and fetus was calculated by subtracting the sum of all organs volume from the total maternal and fetus body weight respectively. Additionally, increased the blood flow to the organs such as kidney, fat and placenta were considered to calculate the increase in maternal cardiac output (O'Flaherty et al., 1992; Gentry et al., 2003, 2002). All physiological parameters were considered as a function of gestational day and the model equations were adapted from different literature sources (O'Flaherty et al., 1992; Gentry et al., 2003, 2002; Abduljalil et al., 2012) and are provided in appendix-I.

The fetus model was sub compartmentalized into liver, plasma, brain, amniotic fluid and rest of the body. Fetal body and mother placental volume was modeled by using equation (1) and equation (2), respectively, described by Gentry et al., (2003). The quantity of amniotic fluid for the gestational day was calculated by applying polynomial equation (3), as described by Abduljalil et al., (2012). Fetal blood flow was defined as a function of fetal blood volume and is adapted from the Clewell et al., (1999). Fetus plasma blood flow to the individual organs was calculated using equation (5) that implies multiplication of the fetal cardiac output with a constant fraction of the fetal blood flows to those organs, which assumed to be same as mother, as described by Gentry et al., (2003). Blood plasma flow to the rest of body was derived by subtracting the sum of total blood plasma flow to the organ from the total fetal cardiac output. The dynamic growth of the fetus volume was calculated during its gestational growth using equation (1). The fetus growth data provided by Brown et al., (1997) and ICRP, (2002) were used to calculate the fetus organ weight as a constant fraction of its body weight which is dynamic parameter described in equation (1). Thus the fetus organ volume was estimated by multiplying fetal body volume with constant fraction value of the organs described in equation (4).

The fetus, placenta, and amniotic fluid growth kinetics were calculated by applying the following equations:

$$V\_fetus = (3.779 * exp(-16.081 * (exp(-5.67e-4 * (GD * 24)))) + 3.883 * exp(-140.178 * (exp(-7.01e-4 * (24 * GD)))) \quad \text{eq(1)}$$

$$V\_placenta = 0.85 * (\exp(-9.434 * \exp(-5.23\text{e}-4 * (\text{GD} * 24)))) \quad \text{eq(2)}$$

$$V\_amniotic\ fluid = 1.9648 * (GD/7) - 1.2056 * (GD/7)\text{^}2 + 0.2064 * (GD/7)\text{^}3 - 0.0061 * (GD/7)\text{^}4 + 0.00005 * (GD/7)\text{^}5$$

231

232 eq(3)

233 Where, V_fetus = volume of the fetus in L, GD = gestational day, V_placenta = volume
234 of the placenta in L, and V_amnitoic fluid = volume of the amniotic fluid in mL.

235 The organ volume of the fetus was scaled by using the following general equation:

236 $Vorgan_{fetus} = Forgan_{fetus} * V\_fetus$ eq(4)

237 Where, $Vorgan_{fetus}$ = the organ volume in L, $Forgan_{fetus}$ = constant fraction of organ
238 of fetus volume and V_fetus = total volume of the fetus

239 The blood flow to the fetus organ was calculated by using the following general
240 equation:

241 $Qorgan_{fetus} = FQorgan_{mother} * QCplasma_{fetus}$ eq(5)

242 Where, $Qorgan_{fetus}$ = the blood flow to organ in L, $FQorgan_{mother}$ = constant fraction
243 of the cardiac blood flows to the organs in mother, and $QCplasma_{fetus}$ = fetus cardiac
244 total blood flow.

245 All the physiological parameters are provided in the annex-A (Table A.1). The dynamic
246 growth pregnancy physiology equations are taken from previous studies (Gentry et al.,
247 2003; Abduljalil et al., 2012) summarised in Table 1.

248

249 Table 1. Parameterization of pregnant mother and fetus physiology

Mother Tissue volume	
Liver volume [b]	$\mathrm{V\_Liver} = \mathrm{F_{Liver}} * \mathrm{BWinit}$
Kidney Volume [b]	$\mathrm{V\_Kidney} = \mathrm{F_{kidney}} * \mathrm{BWinit}$
Gut volume[b]	$\mathrm{V\_Gut} = \mathrm{F_{Gut}} * \mathrm{BWinit}$
Brain Volume[b]	$\mathrm{V\_Brain} = \mathrm{F_{Brain}} * \mathrm{BWinit}$
Plasma volume [c]	$\mathrm{V_{Plasma}} = (2.50 - 0.0223\mathrm{GA} + 0.0042 * \mathrm{GA}^2 - 0.00007 * \mathrm{GA}^3) * \mathrm{BW}$
Initial fat volume[a]	$\mathrm{V\_Fat_{init}} = \mathrm{BWInit} * \mathrm{F_{fat}}$
Fat volume [a]	$\mathrm{V\_Fat} = \mathrm{BWInit} * (\mathrm{F_{fat}} + 0.09 * \mathrm{e}^{-12.90995862} * \mathrm{e}^{-0.000797} * \mathrm{GD} * 24)$
HCT[c]	$\mathrm{HCT} = 39.1 - 0.0544 * (\mathrm{GA} * 7) - 0.0021 * (\mathrm{GA} * 7)^2$
Placenta Volume[a]	$\mathrm{V\_placenta} = .85 * \left(\mathrm{e}^{-9.434 * \mathrm{e}^{-5.23\mathrm{E}-4 * \mathrm{GD} * 24}}\right)$

Increase in Body weight of pregnant women as due to change in fat, placenta, feus and amniotic fluid weight	$BW = BWinit + \left(V_{Fat} - V_{Fat_{init}} \right) + V\_placenta + V\_fetus + V\_Aminiotic\ fluid$
Fetus tissue volume	
Fetus volume [a]	$V\_fetus = 3.779 * \left(e^{-16.08 * e^{-5.67 * e^{-4} * GD * 24}}\right) + \left(e^{-140.78 * e^{-7.01 * e^{-4} * 24 * GD}}\right)$
Fetal plasma volume [b]	$V_{Plasma_{fetus}} = F_{Plasma_{fet}} * V_{fetus}$
Fetus liver volume [b]	$V\_liver\_fetus = F_{liverfet} * V\_fetus$
Fetus kidney volume [b]	$V\_kidney\_fetus = F_{kidneyfet} * V\_fetus$
Fetus brain volume [b]	$V\_brain\_fetus = F_{brainfet} * V\_fetus$
Amniotic fluid volume[c]	$V\_Aminiotic\ fluid = 0 + 1.9648 * GA - 1.2056 * GA^2 + 0.2064 * GA^3 - 0.0061 * GA^4 + 0.00005 * GA^5$
Fetus rest of body volume	$V\_restbody\_fetus = (0.92 * V\_fetus) - (V\_Plasma\_fetus + V\_liver\_fetus + V\_kidney\_fetus + V\_brain\_fetus)$
Blood flow to mother tissue (L/h)	
Initial cardiac output for blood [b]	$QC\_Blood\_init = QCC * BWinit^{(.75)}$
Adjust initial cardiac output for plasma flow [b]	$QC\_Plasma\_init = QC_{init} * (1 - HCT)$
Plasma flow to liver [b]	$Q\_Liver = F\_QLiver * QC\_Plasma\_init$
Plasma flow to gut [b]	$Q\_Gut = F\_QGut * QC\_Plasma\_init$
Initial flow to fat [b]	$Q\_Fat_{init} = F\_QFat * QC\_Plasma\_init$
Changing flow to the fat [a]	$Q\_Fat = Q\_Fat_{init} * \left(\frac{V_{Fat}}{V_{Fat_{init}}}\right)$
Blood flow to placenta[a]	$Q\_Placenta\_blood = 58.5 * V\_placenta$
Plasma flow to placenta	$Q\_Placenta = Q\_Placenta\_blood * (1 - HTC)$
Renal plasma flow[c]	$QKidney = 53 + 2.6616 * GA - 0.0389 * GA^2$

Cardiac output[b]	$QC = QC\_init + (Q\_Fat - Q\_(Fat\_init)) + (QKidney - QKidney\_int) + QPlacenta$
Blood flow to fetus (L/h)	
Cardiac output for fetus[b]	$QCblood\_fetus = FQfetus * V\_Plasma\_fetus$
Fetal cardiac output adjusted to plasma[b]	$Qplasma\_fetus = QC\_Blood\_fetus * (1 - HCT_{fetus})$
Fetal liver blood flow [b]	$QLiver\_fetus = FQLiver * QC\_Plasmafetus$
Fetal kidney blood flow [b]	$QKideny\_fetus = FQKidney * QC\_Plasmafetus$
Fetal brain blood flow [b]	$QBrain\_fetus = FQBrain * QC\_Plasmafetus$

a = (Gentry et al., 2002), **b** = standard scaling method for PBPK, **c** = (Abduljalil et al., 2012)
GD= Gestational day, GA = Gestational age in week

2.3. BPA Pharmacokinetics

The conceptual schema has been provided in the Figure 2 showing distribution of BPA and its metabolites in the body.

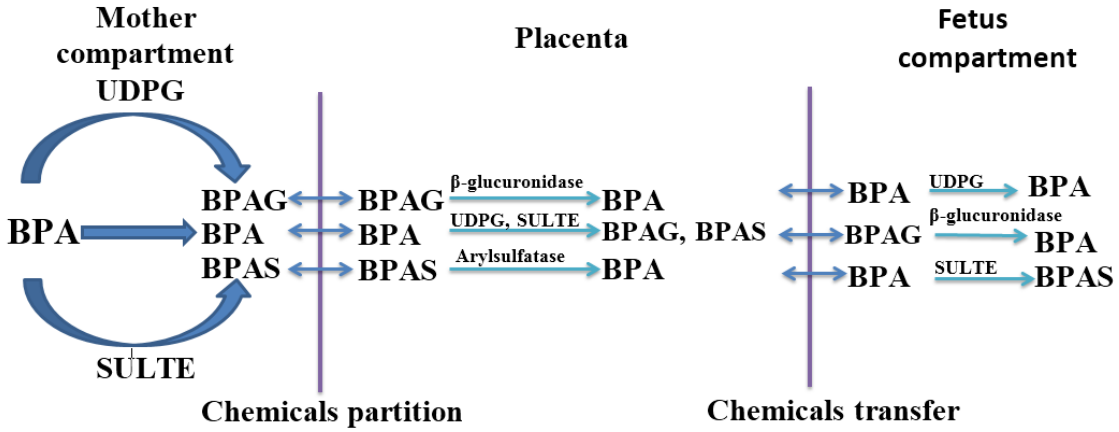


Figure 2. The pharmacokinetics of BPA and its conjugates in both mother and fetus. The placental-fetal unit assumes a bidirectional transfer process of BPA and BPA-C describing the distribution of BPA and its metabolites in mother and fetus body.

In the present P-PBPK model of BPA, physiological changes during pregnancy were included. Metabolism in pregnancy was introduced via scaling of the in-vitro Vmax for glucuronidation and sulfation, considering the pre-pregnancy body weight. The BPA metabolism data for the fetus was scaled using human in-vitro data and fetus microsomal protein content, and, growing fetus liver and body weight. Two metabolic kinetic parameters namely Vmax (maximum rate of reaction) and Km (affinity of the

substrate for the enzyme), for mother and fetus, is taken from in-vitro studies and has been scaled to in-vivo. The pharmacokinetic data are provided in the annex-A (Table A.2).

2.3.1. Oral uptake and gut metabolism: Generally, the oral ingestion of BPA through diet is considered as the major route of exposure (WHO, 2010). It is rapidly absorbed through the gut and maximum concentration in the blood achieves at 0.42-1 h. Studies have shown that oral bioavailability of BPA is very low, as it passes through first pass metabolism, in the intestine and liver, being completely absorbed from the gut (Völkel et al., 2005, 2002; Mielke and Gundert-Remy, 2012).

Both BPA and BPAG uptake from the gut to the system was described by first order reaction, considering gastric emptying delay for BPA arrival to the gut. The oral absorption rate of the BPA was optimized against the Yang el al. (2015) data. The data on uptake of BPAG from the intestine to the liver was taken from the previous study of Yang and Fisher, (2015).

Most of the oral administered BPA metabolizes into BPAG by intestinal UDPGT (Mazur et al., 2010; Trdan Lusin et al., 2012). The in-vitro in-vivo extrapolation (IVIVE) approach and saturation metabolism kinetic (eq. 6) were applied for describing BPA glucuronidation in the mother intestine (Cubitt et al., 2009; Yoon et al., 2014). The scaling of in-vitro Vmax parameter to in-vivo (IVIVE) was done applying equation (7) that used microsomal protein content per gram tissue and weight of tissue per kg body weight. For the scaling of Vmax, the amount of microsomal protein in the gut of 3mg/g (MPPGG) and the weight of human gut 30g/kg body weight was taken into account (Yang and Fisher, 2015)

The metabolism is described by using the following equation:

$$\frac{\boldsymbol{dAmet}}{\mathrm{dt}} = \frac{\mathrm{Vmax} * \mathrm{C_{organ}} * \mathrm{fu}}{\mathrm{Km} + \mathrm{Corgan} * \mathrm{fu}} \qquad \text{eq(6)}$$

Where, $\frac{dAmet}{dt}$ = the amount of metabolism produced with time, Vmax = the maximum metabolism rate, *Km* = the concentration of substrate required to attain 50 percent of its Vmax, C_{organ} = the concentration of substrate at target metabolism organ, and fu = fractional unbound.

Vmax was scaled to in-vivo per kg BW from in-vitro cell line studies by using the following method:

$$Vmax(intestine) = (Vmax_{invitro} * MPPGG * Vgut)/BW^{.75} \qquad \text{eq(7)}$$

Where, $Vmax_{invitro}$ = in-vitro value of metabolic capacity in per gram of microsomal protein (intestinal cell line) , $MPPGG$ = microsomal protein per gram of gut , Vgut = total gut weight in gram, and BW = whole body weight in kg.

2.3.2. Dermal absorption and metabolism: Recently published papers raised the issue of underestimation of BPA exposure via the dermal route given that BPA presence in materials that frequently comes in contact with the human skin (Biedermann et al., 2010; Lassen et al., 2011; Mendum et al., 2011). In-vitro viable skin culture model experiments showed that the skin has potential to absorb and metabolize BPA into BPAG and BPAS (Kaddar et al., 2008; Zalko et al., 2011a). Recently, Mielke et al., (2011) published internal dosimetry model of BPA compared oral route with 90 percent absorption rate, with dermal route considering different reported absorption rates such as 10 (EU, 2003), 13 (Morck et al., 2010), 46 (Zalko et al., 2011b), and 60 (Biedermann et al., 2010) and showed importance of the dermal absorption for the estimation of BPA internal exposure level.

In the present study, the dermal route of exposure was considered for the development of P-PBPK model. Considering the fact of wide variation of the absorption rate of BPA via skin, highest reported permeability coefficient (kas = 0.25 1/hr), data for the adult model provided by Mielke et al., (2011) was used to develop the P-PBPK model. The following equation (8) was applied for calculating skin absorption:

$$\frac{d}{dt}skin = Qskin * (Cplasma * fu - Cskin * \frac{fu}{K\_skin\_plasma}) + (Capp\_skin - cskin/K\_skin\_vehicle) * t\_df * kas * A/1000$$

eq(8)

Where, $Qskin$ = the cardiac blood flow to skin, $Cplasma$ = the plasma chemical concentration, fu = the fractional unbound, K_skin_plasma = the plasma skin partition coefficient, $Capp\_skin$ = the applied concentration of chemical to the skin surface, $Cskin$ = the concentration of chemical in the skin compartment, $K\_skin\_vehicle$ = the vehicle skin partition coefficient, t_df = the time delay factor for absorption to reach to plasma, A = skin surface area and kas = the permeability rate constant.

2.3.3. Metabolism in the adult liver: Phase-II glucuronidation reaction is a major pathway in human for chemicals or drugs detoxification. The resulting conjugates of glucuronic acid to the chemicals increase its hydrophilicity and are generally considered to be pharmacologically inactive (Sperker et al., 1997b). BPA undergoes rapid metabolism to form glucuronidation and sulfation conjugates in the liver by

uridine-diphospho-Glucoronide transferase (UDPGTs) and sulfotransferase (SULT)enzyme respectively (Kim et al., 2003; Hanioka et al., 2008, Hanioka et al., 2011). The reported values of Vmax and Km for glucuronidation from different in vitro studies show variability in glucuronidation (Elsby et al., 2001; Kuester and Sipes, 2007; Kurebayashi et al., 2010; Mazur et al., 2010; Trdan Lusin et al., 2012).

In the present study, the rate of reaction for both glucuronidation and sulfation for the PBPK model was derived by IVIVE scaling approach. The current hepatic in-vitro cell line data were used for deriving maximum reaction velocity (Coughlin et al., 2012) using equation (9) that accounts microsomal protein value (32mg/g of liver) and liver weight (2.6 percentage of BW). The metabolism was described based on Michaelis-Menten equations using equation (6) and implemented into the current PBPK model. The fraction unbound in the microsomes was not accounted for in the calculation of the in vivo values.

$$Vmax(liver) = (Vmax_{invitro} * MPPGL * Vliver)/BW^{.75} \quad \text{eq(9)}$$

Where, $Vmax_{invitro}$ = in-vitro value of metabolic capacity in per gram of microsomal protein (hepatic cell line), $MPPGL$ = the microsomal protein per gram of Liver, Vliver = the total liver weight in gram, and BW =the whole body weight in kg

2.3.4. BPA metabolism in the human fetal liver: Formation of the glucuronide conjugates involves following steps such as rate of supply of substrate (chemicals to be conjugate), the rate of formation and supply of the co-substrate i.e., glucuronic acid, and the expression and the specific activity of the enzyme responsible for glucuronidation i.e., uridine-diphospho-Glucoronide transferase (UDP-GTs). The concentrations (µmol/Kg wet weight) of UDPGLcUA were 59.4 ± 11.3 (fetal liver), 301 ± 119 (adult liver), 17.8 ± 1.8 (mid-term placenta) and 17.0 ± 1.7 (near term placenta) (Beach et al., 1978; Cappiello et al., 2000; Coughtrie et al., 1988; Kawade and Onishi, 1981). The above data shows that the UDPGLcUA is present in the human fetal liver at a 5-fold lower concentration than in the adult liver. Another study has shown that the activity of UDPGT was null at an early stage of the fetus, showing glucuronidation as a potential limiting factor in the human fetus (Strassburg et al., 2002). The expression of these two isoforms UGT2B15 and 2B7 are detectable in human fetal livers during the second trimester of pregnancy and has been stated to account for 18% of the values calculated in adults (Divakaran et al., 2014).

In the present study, the glucuronidation of BPA in the model was considered for the fetus. The scaling of Vmax in the case of the fetus liver has been done before by

Gentry et al., (2003). However, Gentry method considers the fixed value of Vmax and uses fetus enzyme activity as a fraction of the adult value for the scaling method. For this study, similar to adult's scaling, the metabolism in the fetus liver was directly scaled from the in vitro hepatocyte data, considering the developmental changes in the fetus. The reported microsomal protein content per gram of fetus liver at the age of 9-22 gestational week was 10-16 mg (Pelkonen, 1973) and 26 mg (Pelkonen et al., 1973) in two different studies and for the scaling purpose 26 mg/g liver was taken presumably a realistic value at near term of pregnancy, when fetal metabolic capacity is matured. The liver weight for the fetus was provided as a dynamic parameter, which was scaled by taking constant fraction value of liver from ICRP (2002) data, (provided in the annex Table A.1) and its multiplication with growing fetus body (dynamic equation as a function of the gestational day). The concentration of microsomal fraction content per gram liver was assumed to be constant throughout the gestational day. This approach represents an increase in liver enzyme activity with the increase in the fetus liver and body weight. Thus the Vmax value increases with gestational age. The Vmax, maximum velocity reaction for BPA in the fetal liver was derived by using following equation:

$$Vmax_{fetus} = (Vmax_{invitro} * MPPGL_{fetus} * Vliver_{fetus})/BW\_fetus^{.75} \quad \text{eq(10)}$$

Where, $Vmax_{fetus}$ = maximum metabolism rate of fetus liver, $Vmax_{invitro}$ = reported in-vitro metabolism rate, $MPPGL_{fetus}$ = microsomal protein per gram of fetus liver, and $Vliver_{fetus}$ = liver volume of fetus.

2.3.5. Deglucuronidation in fetus compartment: β-Glucuronidase is an enzyme, which deconjugates the glucuronide conjugate xenobiotics (Sperker et al., 1997a). There is evidence for a significant role of the β-Glucuronidase in the fetus, although the role has not been well understood so far in the fetus kinetic modeling. In the animal fetus development studies, it has been found that deglucoronidation activity is more than glucuronidation at the developmental stage (Mccance et al., 1949; Lucier and Sonawane, 1977). In contrast at near term, a fetus glucuronidation activity is higher than deconjugation (Corbel et al., 2015). Domoradzki et al., (2003) studies in the fetus rats at different gestational age showed deconjugation activity of 443 nmol/h/mgMSP at the age of 22 weeks showing the importance of deglucuronidation in the fetus. Moreover, glucuronide conjugate versus free BPA ratio in the placenta and fetus showed that β glucuronidase is present at high concentration in placenta and other various tissues in the fetus (Ginsberg and Rice, 2009).

2.4. Fetoplacental BPA kinetics: Placenta acts as a barrier against xenobiotics such as chemicals and drugs to protect the fetus from being exposed to them. Morck et al., (2010), in an ex vivo placental perfusion study showed that BPA can easily cross the human placenta. Further, Borrirukwisitsak et al., (2012) reported that due to its lipophilic nature, BPA can easily cross the placental barrier. The finding of free BPA in fetus plasma in human biomonitoring (Schönfelder et al., 2002; Ikezuki et al., 2002; Kuroda et al., 2003; Lee et al., 2008; Zhang et al., 2013), showed evidence of transfer of BPA through the placenta. In contrast, very low level of BPAG in the fetus was found (Muna et al., 2013; Gerona et al., 2014) assuming due to the deglucuronidation in both placenta and fetus liver (Nahar et al., 2013; Gerona et al., 2014). In fact, Nishikawa et al., (2010) uterine perfusion experiments showed that small amount of BPAG is transferred to the fetus across the placenta showing very low bidirectional transfer of BPAG

The mother plasma and placenta partition coefficient value for BPA and BPAG were taken from a previous study of Csanády et al. (2002) and Kawamoto et al. (2007) respectively. In this model distribution of sulfation conjugate of BPA (BPAS) to the fetus compartment was not considered due to lack of data in placental transfer. The transfer rate constants for BPAG in this model were taken from the pregnant mice PBPK model and scaled to fetal body weight (Kawamoto et al., 2007), as there is no available human data. Additionally, the glucuronidation of BPA in placenta was described, considering Vmax and Km value from an in-vitro hepatic cell line (Coughlin et al., 2012). The in-vivo Vmax for the placenta was calculated using placenta microsomal content i.e., 11.3 mg/g (McLaughlin et al., 2000), placenta volume and the body weight. The scaling of Vmax for placenta glucuronidation was done using following equations:

$$Vmax_{placenta} = (Vmax_{invitro} * (MPPGP) * V\_placenta)/BW^{.75} \qquad \textit{eq (11)}$$

Where, $V\_placenta$ is the volume of placenta and it is a dynamic parameter, which depends on the Gestational day can be seen in equation 2. $MPPGP$ is microsomal protein per gram of placenta.

2.5. Amniotic fluid BPA kinetics: The human biomonitoring data had reported the presence of BPA and BPAG concentration in amniotic fluid. The increase in free BPA concentration with the increase in the gestational period was observed, as from second trimester to the third trimester (Edlow et al., 2012). Ikezuki et al., (2002) reported the five-fold higher concentration of free BPA at an early stage of pregnancy in comparison to the late week of gestational. This phenomenon might be due to the low metabolic capacity of fetus organ as well as the low volume of amniotic fluid at an early stage of

pregnancy. Further, the activity of beta-glucuronidase measured in amniotic fluid at early stage found to be higher than the later week of gestation. Whereas, glucuronidase activity is found to be higher in the later week of gestation (Matysek, 1980; Fetus et al., 1993). The above finding of increased activity in glucuronidase at an early stage of pregnancy could be some of the possible reasons for the increased level of free BPA at the early gestational age.

2.6. Partition coefficient for pregnant mother and fetus organs

The partition coefficient (PC) for liver, fat, brain, and skin were taken from the study done by Fisher et al., (2011). The placental and kidney partition coefficient for BPA were taken from Csanády et al., (2002) and the BPAS was not distributed to fetus tissues. However, to measure BPAG concentration in the fetus plasma, BPAG was distributed to maternal placenta using placenta partition coefficient taken from the previous mice study (Kawamoto et al., 2007). For other fetus compartments, partition coefficients were kept similar to as mother's organs partition coefficients. The partition coefficients used in the P-PBPK model are provided in the annex (Table A.2).

2.7. Pregnancy cohort studies

For this study, we have used 5 different pregnancy cohort studies that measure the BPA concentration in different matrices. Subject characterics are provided in the Table A.3, which was used as an input variable for the case specific scenario. Summary of the biomonitoring data is provided in the annex (Table A.4). Schönfelder et al. (2002) studies included 37 samples of both mother and fetus plasma (umbilical cord) between the gestational age of 32 to 41 week. Pregnant women of age ranging from 22-44 years old were recruited from Berlin and samples were collected at Benjamin Franklin Medical Center. In another study by Aris (2014), which included 61 pregnant women recruited from the eastern township of Canada at delivery time and both mother plasma and fetal cord blood BPA was analyzed.

Zhang et al. (2011) study included each 21 samples of human placental and fetal liver at the gestational age of 12.3–20 weeks and 11.3-22, respectively. Samples were obtained after elective pregnancy termination during 1998–2006 in the Greater Montreal area of Quebec. In addition, Cao et al. (2012) study included a large number of placenta and liver samples from the same population i.e. 128 and 28, respectively. In addition, Schönfelder et al. (2002) also studied placenta BPA concentration at the delivery time. Ikezuki et al. (2002) studied includes Japan population of each 37 women with an early and late pregnancy, where 37 maternal (late pregnancy) and 32 umbilical cord blood samples were collected at full-term delivery. In addition, 32 and 38

amniotic fluids samples were collected at 15–18 weeks gestation (early pregnancy) and at full-term (late pregnancy), respectively.

3. Results

3.1. Simulation and Validation of adult human PBPK model

Validation of the developed adult PBPK model was performed by comparing the model predictions with plasma data obtained from the human study by Thayer et al., (2015) in which volunteers were orally administered 100 µg/kg BW dose of deuterated BPA. These predictions were performed by taking into account only female volunteers, and their individual BMI and body weight. The exposure dose was normalized according to body weight and the fat content of individual volunteers was calculated based on body weight and BMI of the respective subject. Out of 14 subjects (male and female), only 7 female subjects were considered from Thayer's study and simulated time-plasma BPA data profile were validated against their observed data. The total duration of simulation was 24 h. Figures 3A, 3B and 3C depict the concentration–time profiles after single oral dosing of adult females (n = 7) for BPA (d6-BPA), and observations made by Thayer et al. (2015).

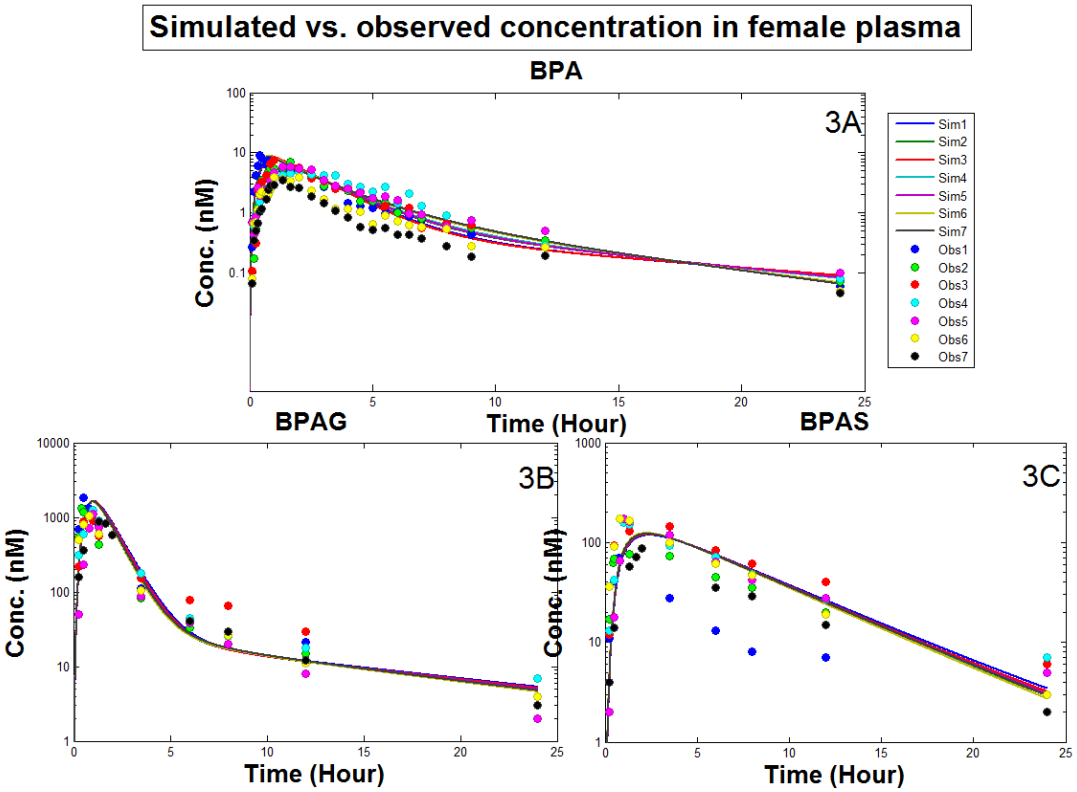


Figure 3. Concentrations–time profiles after oral dosing of adult females (n = 7) with 100 µg/kg of deuterated BPA (d6-BPA) (Thayer et al., 2015). A) Simulated individual (solid color lines) and observed individual plasma (dot points) d6-BPA concentrations; B) Simulated individual (solid color lines) and observed individual plasma (dot points) d6-BPAG concentrations; C) Simulated individual (solid color lines) and observed individual plasma (dot points) d6-BPAS concentrations. Simulations of individual patients were performed using individual body weights and their fat content while keeping other model parameters constant.

3.2. Simulation and evaluation of P-PBPK Model

Most of the reported human biomonitoring data for the fetus is for BPA and generally, BPAG and BPAS studies are under-reported (Ikezuki et al., 2002; Schönfelder et al., 2002; Kuroda et al., 2003; Lee et al., 2008; Zhang et al., 2013). Development of the present model includes BPAG and BPAS conjugates in the mother, whereas in the case of the fetus only BPAG has been accounted, which is the major metabolite produced in the mother. For this study, the distribution of BPA and BPAG from mother plasma to the placenta is described via partition coefficient. Following that transfer of both BPA and BPAG across the placenta was described as simple diffusion process between the placenta and fetus plasma. Human Biomonitoring data showed the presence of higher concentration of free BPA in the amniotic fluid in early pregnancy than compared to late pregnancy (Ikezuki et al., 2002; Edlow et al., 2012). The reason behind this difference could be the higher beta-glucuronidase activity in early and mid-gestational periods (Matysek, 1980). However, in the later week of gestation, as the fetus liver develops and matures that might increase the liver glucuronidation activity.

Though there is a lack of glucuronidase data specific to the fetus deconjugation, presuming deconjugation process as an important toxicokinetic process, in the present P-PBPK model it was taken into account for the fetus compartment. The assumption has made that deconjugation of the BPAG to BPA was based on first-order rate transfer constant. The half-life of the chemicals is used to establish the rate of deconjugation estimated to be 0.35 hr^{-1} ($k = 0.693/t_{1/2}$).The same value is used in the case of both placental and fetus deconjugation for simplification. A similar approach has been used in the previous study (Lorber et al., 2010) for transfer of one metabolite to another, but it should be considered as worst case scenario and it shows clearly there is a need for proper studies to parameterise this process. This steps would results in increased level of free BPA in the fetus plasma. To maintain the cyclic deconjugation and conjugation reaction into the model, the available free BPA

undergoes simultaneously for glucuronidation into the liver following distribution to the liver compartment to mimic the real biological phenomena.

The lack of validation of a model for the estimated exposure (for respective cohort) against biomonitoring data for cohorts via PBPK model has been observed in the previous study by Mielke and Gundert-Remy, (2009). Additionally, finding of differences in the biomonitoring data for free BPA concentration within the cohort and in between cohorts is observed in different biomonitoring studies (Ikezuki et al., 2002; Schönfelder et al., 2002; Kuroda et al., 2003; Lee et al., 2008; Zhang et al., 2013). Several possible reasons can be put forward to explain this inconsistency among which underestimation of exposure levels and not considering other routes of exposure than oral has been questioned by researchers (Mielke et al., 2011). The timing of sampling is one of the major concern that has not been accounted in biomonitoring data, which can be another source of variability in biomonitoring data due to fast absorption and elimination of BPA that never reach steady state concentration even with multiple doses. In targeted human kinetic studies (Völkel et al., 2002; Thayer et al., 2015), the observation of Cmax (maximum concentration) and elimination half-life within 1-3 hours of BPA exposure shows how crucial is the time of sampling. However variability due to the analytical method, contamination, source and route of exposure (EFSA, 2015; Longnecker et al., 2013; Ye et al., 2013), and importantly metabolic variation among population cannot be ruled out (Partosch et al., 2013; Nachman et al., 2014), which is beyond the scope of this manuscript.

Another complexity with the prediction of concentration for such chemicals might be due to their narrow time interval between the Cmax (the highest concentration) and Cmin (minimum concentration after exposure of chemical during 24 hr or before subsequent exposure of chemical) rising a question on observed biomonitoring data is because of high/low exposure or because of the schedule of sampling. Therefore evaluation of the developed model has two possibilities first; either by changing exposure dose for each biomonitoring study, second; by using two extreme exposure scenarios (low-high). In this study, it was assumed that sampled biomonitoring data can be from any point of the time-concentration profile and the exposure dose was estimated for the observed high and low mother plasma concentration. This assumption seems conservative, but for the current scenario, this might be the best solution, instead of estimating exposure for each biomonitoring study. Exposure dose for the biomonitoring data was estimated by taking the reference of a previous study (Mielke et al., 2011). In the present study, the oral exposure was divided into three

equal doses keeping dermal exposure as a single dose. Exposure dose for both the oral and dermal was estimated that matches the observed highest and lowest mother plasma concentration in different biomonitoring studies.This was done by simply applying trial and error method, a similar method was used before for other environment chemicals (Loccisano et al., 2013). Then the estimated dose was used for the simulation of a model that predicts the fetus plasma and organs concentrations at the different gestational period.

We have selected 5 different pregnancy cohort studies that measure the BPA concentration in different matrices.Two scenarios were selected for the simulation of PBPK model: one with the observed high mother plasma concentration population (Schönfelder et al., 2002), in turn dose of 44µg/kg/BW thrice in a day (TID) oral dose and 20µg/kg/BW single dermal exposure and other with the observed low mother plasma concentration (Ikezuki et al., 2002), in turn dose estimated to be 20µg/kg/BW (TID) oral dose and 9µg/kg/BW single dermal exposure.

Since the BPA has a very short half-life, even with well-distributed dosing schedule, the BPA plasma concentration shows sharp elimination curve profile and did not arrive at the steady state; a similar observation has been made by Mielke et al. (2011). In order to cover all the simulated data points considering essential for comparisons against the observed biomonitoring data points, which could be either result of random samples at any point of time not knowing the exact exposure time or exposure variability in sample subjects (VandeVoort et al., 2016). The model output data were summarized into boxplot for each gestational week, which included the range of value from higher to lower concentration.

The simulation was done for different matrices and results were presented in different Figures, a number from 4 to 7. Figure 4 & 5 shows the simulated results for mother and fetus BPA plasma concentration for the selected high and low dose exposure scenario respectively. Figure 6 shows the simulation results for the BPA concentration in liver and placenta during the mid-gestational week and the results were compared with the biomonitoring data obtained from Zhang et al. (2011) study. Figure 7 shows the BPA concentration in amniotic fluid. The amniotic fluid concentration of BPA by Ikezuki et al. (2002) was monitored at two stages, early and full term pregnancy. The low dose scenario was simulated for the Ikezuki et al. (2002) data on the concentration of BPA in mother and fetus plasma (Figure 5) and amniotic fluid concentration (Figure 7). The Figure 7 shows the predicted BPA concentration in amniotic fluid is well matched with the observed concentration. Moreover, the observed mother and fetus plasma

concentration (mean ± SD) by Ikezuki et al. (2002) is within the range of simulated low dose exposure scenario (Figure 8).

Figure 8 shows the predicted mean ± SD for the high and low dose scenario vs. observed mean ± SD of different cohort studies for the period during 32-40 week of gestation. Most of the observed mean concentration was covered by a simulated scenario in case of mother plasma given the large range between Cmax and Cmin. However, in the case of the fetus some observed mean values were not in the range, which could be due to the various factors such as; variability in the gender of fetus previously reported as significant, metabolic variability due to polymorphism (not considered in this study) and process of deglucuronidation, which need proper in-vitro investigation for parameterization.

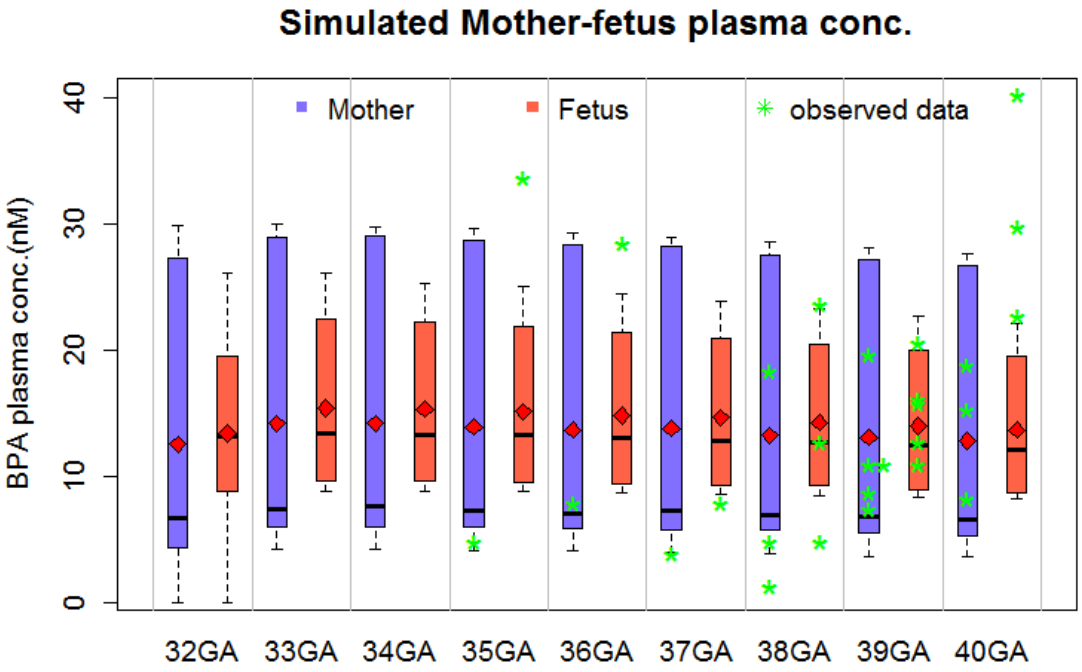
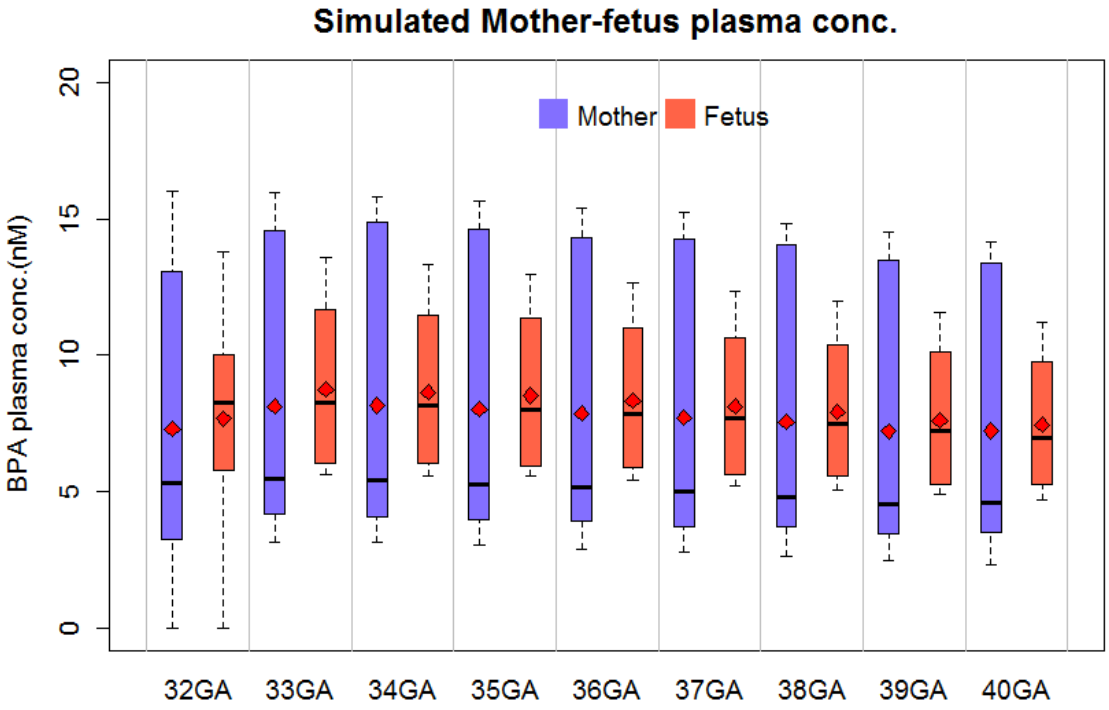


Figure 4. Observed vs predicted mother plasma and fetus plasma of volunteer participated in Schönfelder et al. (2002) study for 32 to 41week of GA; box plot containing mean (red diamond), median (horizontal line of boxplot), highest (upper bar of boxplot), lowest (lower bar of boxplot) value and observed value marked as green star.



618

619 Figure 5. Predicted mother plasma and fetus plasma for low dose scenario, estimated
620 from the Ikezuki et al. (2002) mother plasma concentration, for 32 to 41 week of GA;
621 box plot containing mean (red diamond), median (horizontal line of boxplot), highest
622 (upper bar of boxplot), and lowest (lower bar of boxplot) value.

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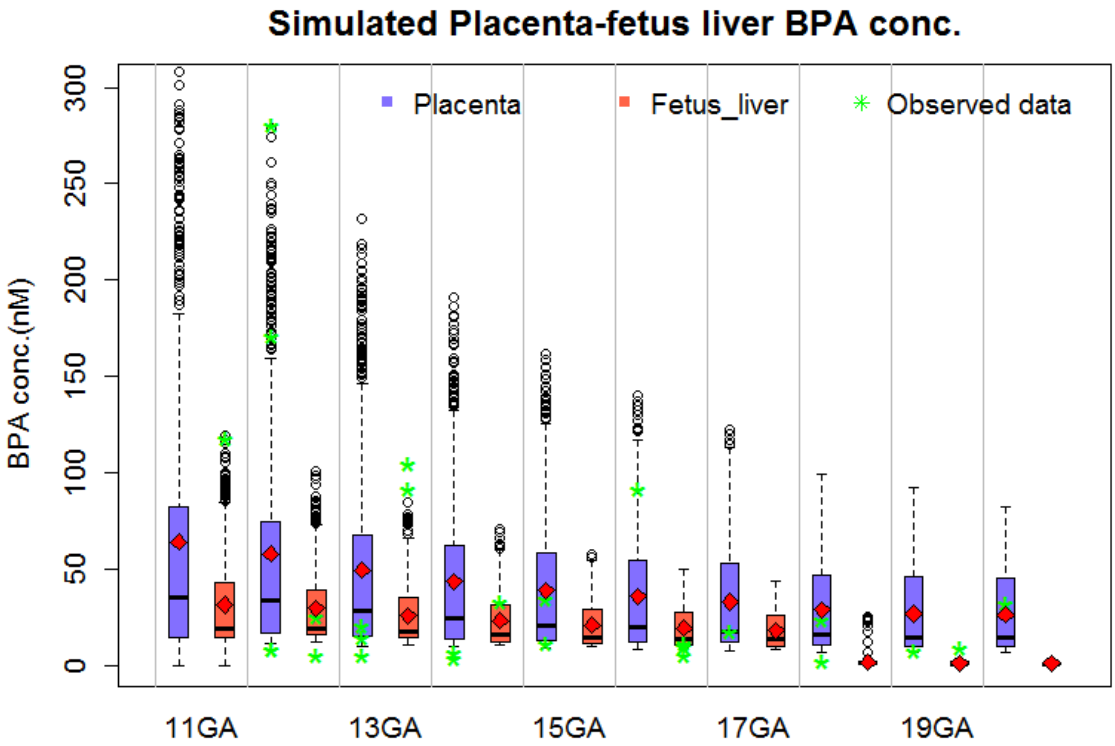
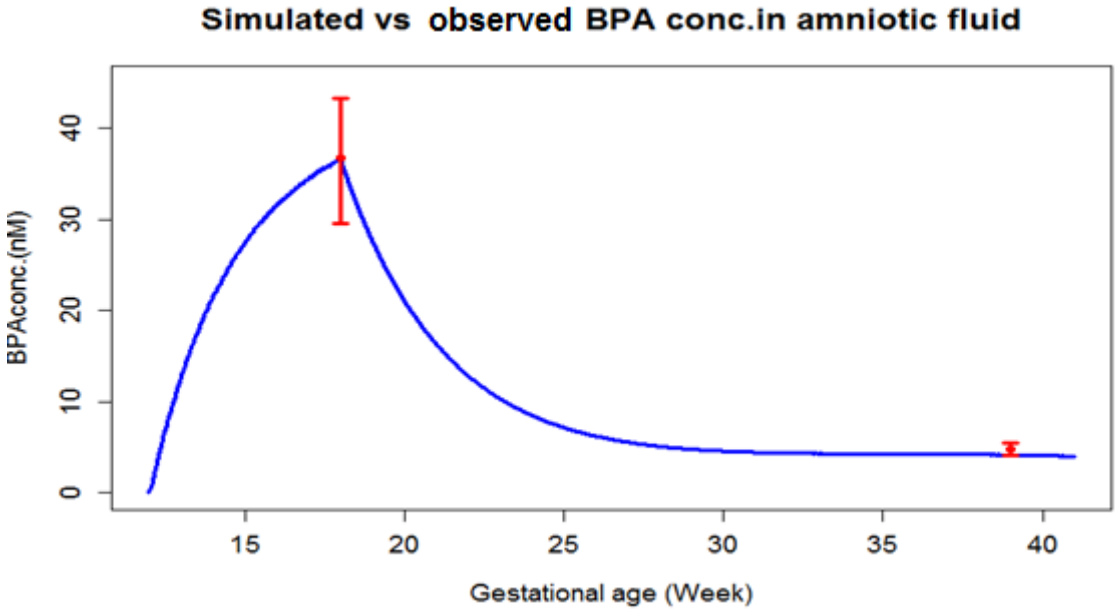


626 Figure 6. Observed vs predicted placenta and fetal liver for higher exposure scenario
627 for 11 to 22 week of GA; box plot containing mean (red diamond), median (horizontal
628 line of boxplot), highest (upper bar of boxplot), lowest (lower bar of boxplot) and
629 observed value (Zhang et al., 2011) marked as green star.



630

631 Figure 7. Simulated low dose exposure scenario for amniotic BPA concentration
632 starting from early mid-gestational to late gestational period (blue line curve) vs.
633 observed (mean ± SD) concentration in Ikezuki et al. (2002) studied during 15-18 and
634 32-40 weeks of pregnancy (red error bar).

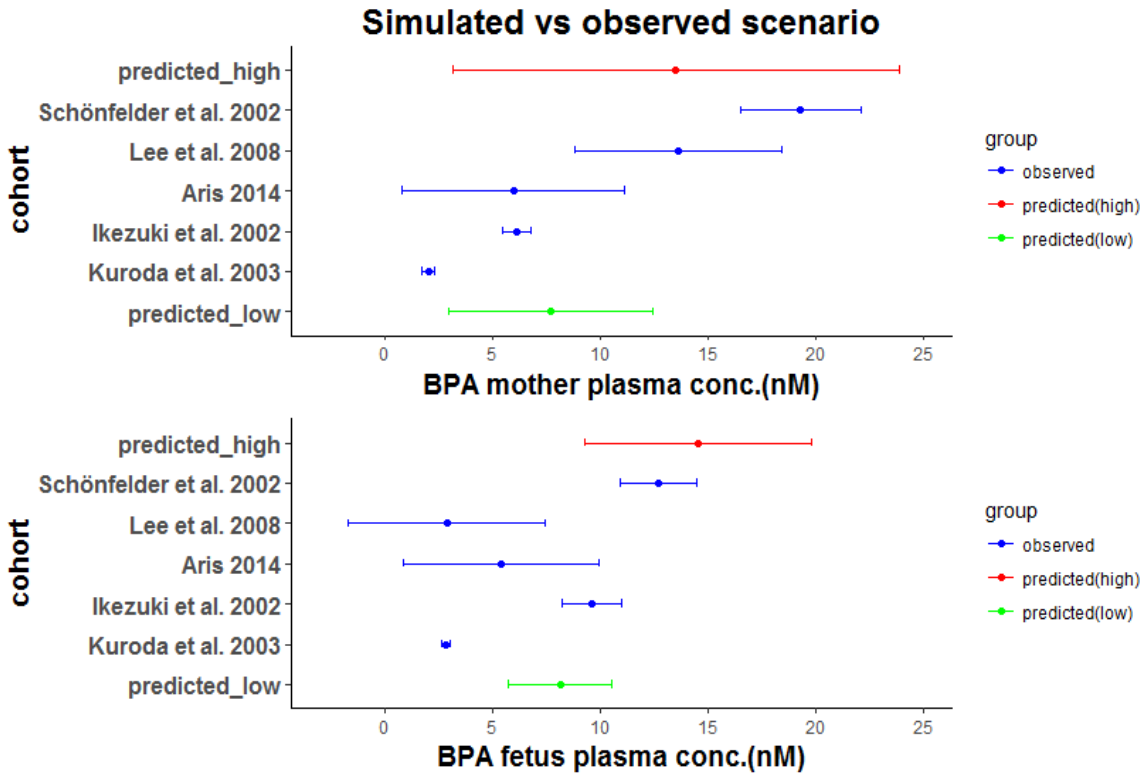


Figure 8. Simulated mean ± SD of BPA for two exposure scenario (high and low dose) for the period of 32-40 GA and the observed mean± SD of BPA in different studies for both mother and fetus BPA plasma concentration.

4. Discussions

The present study involved development and validation of the adult PBPK model and then an extension of this model to the pregnant mother to predict the toxicokinetic profile of BPA for both mother and fetus organs. Following the same parameterization of the previously developed model (Yang et al., 2015), in the present study, it was observed that results under predicts the free BPA and BPAS in plasma serum. The reason behind this could be the low absorption rate constant for free BPA, which leads to higher concentration available in the gut for the metabolism. The present adult model was slightly modified optimizing absorption rate constant and then the model was validated against the Thayer et al., (2015) human experimental data. For the validation of the adult model, only female subjects were taken into consideration and the simulation for the individual subjects was done considering their physiological parameters such as body weight and body mass index. The adult pharmacokinetic results have shown that BPA has very fast absorption and elimination process (Schönfelder et al., 2002) as it undergoes first pass metabolism and rapidly converted into more polar compounds (glucuronide conjugates). Due to high metabolic activity for

BPA, even higher or multiple doses has very less effect on time-concentration curve characteristic. However, variability in the BPA plasma concentration with respect to the time-concentration curve is much higher than inter-individual variation among subjects, showed plasma concentration is not only sensitive to dose but to time as well. The sudden drop in BPA concentration at peak is due to its higher metabolism rate, making a very sharp curve, which can be considered as benchmark characteristics of BPA. Even within a small fraction of the time, a large difference in BPA concentration was observed in this study. There were no significant changes in BPA plasma concentration observed among subjects, even individual fat content, calculated from body weight and BMI, has very little or no impact on plasma concentration. Although, some study has shown the genetic and gender variability in metabolism among the population (Hanioka et al., 2011). It has been reported that the concentration of BPA varies among different population cohorts such as male and female, pregnant and non-pregnant, adult, neonates, and children (Kim et al., 2003; Calafat et al., 2005; Vandenberg et al., 2010; Zhang et al., 2013; Aris, 2014). Polymorphism has been found to be one of the important factors in metabolic variability (Trdan Lusin et al., 2012). However, there are very few data available on functional polymorphism among the population causing metabolic differences in BPA metabolism. (Hanioka et al., 2011). In the present study, polymorphism variability has not been accounted, however, it cannot be ruled out. Further, the variation in biomonitoring data shows the need for considering different physiological states into the PBPK models. Some specific physiological parameter such as body weight, height, and dynamic physiological changes in the specific population such as pregnancy and fetus were accounted to capture variability. A number of P-PBPK models have been developed for various environmental chemicals in the past for the risk assessment application(O'Flaherty et al., 1992; Gentry et al., 2003, 2002; Loccisano et al., 2013) . Similar approach has been taken for the current P-PBPK model. However, in the current model approach, the model has included detailed chemical metabolism concept in both mother and fetus considering their dynamic growth parameters in order to mimic the real physiological process during gestational period.

The observed concentration in different cohorts during pregnancy was used for model evaluation. For instance, maternal blood concentration during pregnancy or at the delivery time was used for exposure estimation accounting both dermal and oral exposure. In the development of P-PBPK model, pregnancy growth dynamic equations were implemented into the model that mimics the physiology of pregnant mother, and the inclusion of the fetus compartment and its communication with the mother was

done via placenta blood flow. The metabolism of the BPA in placenta and fetus liver is found to be key parameters for the understanding of fetal exposure to parent BPA. The human hepatocyte in-vitro data was scaled to calculate the fetus liver metabolic activity. For the scaling of Vmax, the reported fetus microsomal protein content was used in place of adult microsomal content. The deglucuronidation process for the fetus liver and amniotic fluid was applied into plasma compartment for the simplification of the model. The P-PBPK model predictions were compared with different sets of the BPA biomonitoring data available in the literature. Simulation-matched study designs were used based on information in the original studies.

In order to predict the BPA concentration in fetus plasma for various population studies, observed maternal BPA plasma concentration during pregnancy was used for exposure estimation accounting both dermal and oral exposure. The predicted exposure concentrations for two scenarios (high and low mother plasma concentration considering Schönfelder et al., (2002) and Ikezuki et al. (2002) studies respectively), were chosen and seems to be significantly higher than the generally estimated exposure. A similar observation about predicted and observed concentrations of these two references (Schönfelder et al., (2002) and Ikezuki et al. (2002)) were made in previous studies (Mielke and Gundert-Remy, 2009; Mielke and Gundert-Remy, 2012). The exposure scenarios used in this study are: high dose scenario with 44µg/kg/BW thrice in a day (TID) oral dose and 20µg/kg/BW single dermal exposure and, low dose scenario with 20µg/kg/BW (TID) thrice in a day (TID) oral dose and 9µg/kg/BW single dermal exposure. A similar exposure dose was previously estimated by Mielke et al., (2011). However, in this study, the estimated dose is lower, given the fact that single oral dose was equally divided into three doses and lag time for dermal dose was included. The simulated results for mother and fetus plasma concentration for two exposure scenario showing median, mean, high and low value for each gestational week were presented in Figure 4 and 5. Most of the biomonitoring observed data are within the simulated results represented in Figure 8. Limited data availability for each gestational week is one of the limitations of the model validation. However, in some cases, fetus plasma of BPA was much higher (Figure 4), which might be explained by gender difference observed previously (Schönfelder et al., 2002), which was not included in the present model. Considering the mean value for each simulated week shown in Figure 4 and 5, fetus BPA mean concentration value is higher than the BPA in mother plasma, which could be explained by the fact that the elimination process in the fetus is not so effective and solely depends on diffusion of chemical back to mother plasma via placenta or to amniotic fluid. Additionally, the model predicted the Cmax

and Cmin relatively higher value for the mother plasma than the fetus plasma concentration.

Detailed biomonitoring sample of liver and placenta during 11 to 20 weeks of gestational has been reported (Zhang et al., 2011). It was observed that after the 17th week of gestational, free BPA concentration starts to decrease and appearance of BPAG in the liver, showing the development of the metabolic capacity of the fetus at this stage. To mimic this condition, metabolic activity in fetus liver and placenta was introduced at 17th gestational week. The simulated results for both fetus liver and placenta during mid-gestational were compared with the biomonitoring study of Zhang et al. (2011) (Figure 6). However, some observed data points were below the range of predicted value. An increase in metabolic capacity was observed with the increase in liver weight during the gestational development, which could explain the result of decreasing free BPA concentration.

The recent biomonitoring data by Aris, (2014) showed that BPA exposure to the fetus during the mid- gestational is very high ranging from LOD to 229 nM. This biomonitoring data shows that mid-gestational is a very critical window of exposure to the fetus. The developed P-PBPK model has also shown the higher BPA value during mid-gestational weeks compared to near term or at delivery. The reason of relatively higher exposure could be the fetus volume, which is very less at mid-gestational, and also the metabolic capacity, which is presumably active after the 18th week of gestational.

The pharmacokinetic differences for the fetus seem to be very dramatic as fetus metabolic capacity and organ physiology system are relatively immature at an early stage of fetal development. The faster chemical metabolism and elimination of the BPA by the maternal system ameliorate BPA kinetics in the fetus to a great degree. However, evidence of finding higher free BPA (Ikezuki et al., 2002; Schönfelder et al., 2002; Aris, 2014) in cord blood as compared to maternal blood in various populations indicates higher fetal exposure and sensitivity to BPA due to pharmacokinetic factors.

The simulation of the model for BPA concentration in amniotic fluid during mid-gestation (Figure 7) to near term showed the increasing concentration of the BPA with an increase in the gestational period. The BPA concentration increased until mid-gestational and then slowly started to decrease reaching to almost one and a half fold less than the observed mother plasma concentration. The predicted results are in agreement with observed data of Ikezuki et al., (2002), and have a linear relation with gestational time (less fluctuation in BPA concentration) suggesting amniotic fluid BPA

concentration as a good biomarker for identifying the critical window of exposure to the fetus. The prediction of the concentration of free BPA in amniotic fluid was slightly less than reported biomonitoring data observed in late gestational. This could be due to the prediction of slightly high amniotic fluid volume than normally observed in the late gestational period. Factors such as local deconjugation in placenta, the lipophilicity of chemical, relatively higher deconjugation than conjugation in the fetal compartment can affect the propensity for chemicals to reach a higher concentration in the fetal compartment (Nachman et al., 2014).

The developed P-PBPK model is in concordance with biomonitoring data and showed that BPA readily transferred to fetal serum and amniotic fluid after mother's exposure. Even, fast metabolism and rapid excretion of BPA and BPA-C are unable to prevent the BPA fetal exposure. The transfer rates of BPA from the placenta to the fetal compartment varied considerably. Deconjugation in placenta and fetus body is of major concern at early fetal life, where metabolism capacity is low, causing an increased level of unconjugated BPA in the fetus. Importantly, free BPA in the fetal compartment are more in steady state and persists even as the maternal level of BPA declines. The consideration of mechanistic approach such as dynamic growth parameters and their governing equations, and model structure could be useful for the development of P-PBPK model for different chemicals.

5. Conclusion

The present study proposed and prospectively developed a P-PBPK model for BPA that describes and predicts the fetus blood and tissues concentrations time profiles based on the mother's exposure scenario. Detail metabolic toxicokinetics in mother and fetus was reviewed and included in the proposed model. Glucuronidation and deglucuronidation in both mother and fetus liver and placenta are found to be an important mechanism that alters BPA toxicokinetic profile. For the development of the model, a two-stage approach was employed: first the development and validation of the adult PBPK model against the kinetic data from control human experimental study and second extension of the adult model to the P-PBPK model and further evaluation with the available BPA biomonitoring cohort studies. The prediction of higher concentration of BPA during the mid-gestational period in the amniotic fluid, placenta, and the fetus liver are in accordance with biomonitoring data, indicating mid-gestational period might be the critical window of exposure for the fetus. Due to the fast absorption and short half-life of BPA, it is showing extreme concentration variability with respect to time, which makes the task of prediction of biomonitoring data very difficult. This study

considered two extreme dose scenarios (min-max) for the simulation and in turn plotting of simulated data under the box plot to capture all the data set that allows comparing with biomonitoring data. It has an assumption that biomonitoring sample can be from any time point. However, in order to address the issue of temporal variation of short life chemical, there is a need to have very control case studies dealing with the timing of exposure (food intake) and schedule of sampling. In this study, there are several data gaps identified, which need to be addressed to improve the model. For example, kinetics of BPA glucuronidation/sulfation and deglucoronidation/desulfation at the fetus level, and placental BPA conjugation and deconjugation, and metabolic variation due to functional polymorphism among the different population, are some of the major concern.

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Figure Labels

Figure 1 Conceptual model for the development of P-PBPK model. It involves the development of adult PBPK model and extension of this model to P-PBPK model with the addition of placenta and fetus sub-compartment. **K = partition coefficient and subscripts L = Liver, B= blood, K= kidney, T= testis, S= skin, R= rest organ, G= gut.**

Figure 2. The pharmacokinetic of BPA and its conjugate in both mother and fetus. The placental-fetal unit assumes a bidirectional transfer process of BPA and BPA-C describing distribution of BPA and its metabolites in mother and fetus body.

Figure 3. Concentration–time profiles after oral dosing of adult females (n = 7) with 100 µg/kg of deuterated BPA (d6-BPA) (Thayer et al., 2015). A) Simulated individual (solid colour lines) and observed individual plasma (dot points) d6-BPA concentrations; B) Simulated individual (solid colour lines) and observed individual plasma (dot points) d6-BPAG concentrations; C) Simulated individual (solid colour lines) and observed individual plasma (dot points) d6-BPAS concentrations. Simulations of individual patients were performed using individual body weights and their fat content while keeping other model parameters constant.

Figure 4. Observed vs predicted mother plasma and fetus plasma of volunteer participated in Schönfelder et al. (2002) study for 32 to 41th week of GA; box plot containing mean (red diamond), median (horizontal line of boxplot), highest (upper bar of boxplot), lowest (lower bar of boxplot) value and observed value marked as black star.

Figure 5. Predicted mother plasma and fetus plasma for low dose scenario, estimated from the Ikezuki et al. (2002) mother plasma concentration, for 32 to 41th week of GA; box plot containing mean (red diamond), median (horizontal line of boxplot), highest (upper bar of boxplot), and lowest (lower bar of boxplot) value.

Figure 6. Observed vs predicted placenta and fetal liver for higher exposure scenario for 11 to 22nd week of GA; box plot containing mean (red diamond), median (horizontal line of boxplot), highest (upper bar of boxplot), lowest (lower bar of boxplot) and observed value (Zhang et al., 2011) marked as green star.

Figure 7. Simulated low dose exposure scenario for amniotic BPA concentration starting from early mid-gestational to late gestational period (blue line curve) vs. observed (mean ± SD) concentration in Ikezuki et al. (2002) studied during 15-18 and 32-40 weeks of pregnancy (red error bar).

Figure 8. Simulated mean ± SD of BPA for two exposure scenario (high and low dose) for the period of 32-40 GA and the observed mean± SD of BPA in different studies for both mother and fetus BPA plasma concentration.