Development of a Novel Maternal-Fetal Physiologically Based Pharmacokinetic Model I: Insights into Factors that Determine Fetal Drug Exposure through Simulations and Sensitivity Analyses

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Received January 25, 2017; accepted May 25, 2017

ABSTRACT

Determining fetal drug exposure (except at the time of birth) is not possible for both logistical and ethical reasons. Therefore, we developed a novel maternal-fetal physiologically based pharmacokinetic (m-f-PBPK) model to predict fetal exposure to drugs and populated this model with gestational age–dependent changes in maternal-fetal physiology. Then, we used this m-f-PBPK to: 1) perform a series of sensitivity analyses to quantitatively demonstrate the impact of fetoplacental metabolism and placental transport on fetal drug exposure for various drug-dosing regimens administered to the mother; 2) predict the impact of gestational age on fetal drug exposure; and 3) demonstrate that a single umbilical venous (UV)/maternal plasma (MP) ratio (even after multiple-dose oral administration to steady state) does not necessarily reflect fetal drug exposure. In addition, we verified the implementation of this m-f-PBPK model by comparing the predicted UV/MP and fetal/MP AUC ratios with those predicted at steady state after an intravenous infusion. Our simulations yielded novel insights into the quantitative contribution of fetoplacental metabolism and/or placental transport on gestational age–dependent fetal drug exposure. Through sensitivity analyses, we demonstrated that the UV/MP ratio does not measure the extent of fetal drug exposure unless obtained at steady state after an intravenous infusion or when there is little or no fluctuation in MP drug concentrations after multiple-dose oral administration. The proposed m-f-PBPK model can be used to predict fetal exposure to drugs across gestational ages and therefore provide the necessary information to assess the risk of drug toxicity to the fetus.

Introduction

Fetal exposure to drugs has become increasingly common. This can be attributed to the rising use of therapeutic drugs among pregnant women (Mitchell et al., 2011) as pre-existing maternal conditions (e.g., epilepsy, asthma) or conditions that developed during pregnancy (e.g., gestational diabetes and hypertension) must be treated to ensure the health and welfare of the mother and therefore her fetus. Sometimes, it is the unborn child that is the target of the treatment [e.g., to prevent maternal-fetal human immunodeficiency virus (HIV) transmission] (McGowan and Shah, 2000). Consequently, the ability to quantitatively evaluate fetal exposure to drugs and risk of toxicity, not only at term but also earlier during pregnancy when the fetus is most vulnerable to teratogens, is needed.

Unlike the general population, fetal exposure to drugs ingested by the pregnant mother cannot be readily studied prior to birth for ethical and logistical reasons. Even at the time of birth, assessment of fetal exposure to drugs is limited to a single cord plasma concentration measurement and reported as the umbilical vein (UV)/maternal plasma (MP) drug concentration ratio. As shown here, in most clinical scenarios, this UV/MP ratio does not reflect the extent of fetal drug exposure relative to that in the mother. Nor does this ratio provide information on fetal drug exposure over time [i.e., fetal plasma area under the curve (AUCf)] or maximum fetal plasma drug concentration (Cmax,f) that often drives drug efficacy and/or toxicity in the fetus. Further, since cord blood sampling is limited to term, fetal drug exposure during early gestation remains unknown.

The current work was supported by National Institutes of Health National Institute on Drug Abuse [Grant P01DA032507]. Z.Z. was supported by the Office of Women’s Health, US Food and Drug Administration [ORISE Fellowship] for part of the submitted work. No other potential conflicts of interest relevant to this article are reported.

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https://doi.org/10.1124/dmd.117.075192.

This article has supplemental material available at dmd.aspetjournals.org.

ABBREVIATIONS: AUCf, fetal plasma area under the curve; AUCmp, maternal plasma area under the curve; AUCmp, maternal plasma area under the curve; Cmax,f, maximum fetal plasma drug concentration; ClD0, fetal metabolic clearance; ClMPI, placental apical uptake clearance; ClMPI, maternal systemic clearance; ClD0, placental metabolic clearance; ClMPI, placental apical efflux clearance; ClD0, transplacental passive diffusion clearance; ClD0, unbound transplacental passive diffusion clearance; C, concentration-time; Fabs, fraction absorbed; Fg, intestinal bioavailability; F/M, fetal/maternal plasma ratio; GA, gestational age; HIV, human immunodeficiency virus; k0, first-order absorption rate constant; m-f-PBPK, maternal-fetal physiologically based pharmacokinetic; MP, maternal pregnancy physiologically based pharmacokinetic; PBPK, physiologically based pharmacokinetic; P-gp, P-glycoprotein; PK, pharmacokinetics; P/M, placental/maternal plasma ratio; steady state, steady state after an intravenous infusion; UV, umbilical vein; Vss, volume of distribution at steady state.
unknown. To overcome these gaps in knowledge, mechanistic understanding of the determinants of fetal drug exposure and noninvasive approaches to predict fetal exposure across gestational ages (GAs), such as physiologically based pharmacokinetic (PBPK) modeling and simulation, are needed.

Although a number of attempts to develop fetal PBPK models have been made (Clewell et al., 2007; Yoon et al., 2011; Loccisano et al., 2013), upon closer examination, except for a recently published model (De Sousa Mendes et al., 2016, 2017), the majority of these models are not intended to predict fetal exposure to therapeutic agents prescribed to pregnant women. Their limitations include the following: 1) incomplete inclusion of pregnancy-caused changes in maternal and fetal physiology; 2) not accounting for the alterations in maternal drug disposition; and 3) exclusion of fetal body compartments important for the disposition of therapeutic drugs. Recently, our laboratory has successfully refined and verified a mechanistic maternal pregnancy PBPK model (m-PBPK) that can predict the maternal disposition of drugs cleared by one or more cytochrome P450 enzymes during pregnancy (Ke et al., 2012, 2013, 2014). However, this model contains only a lumped tissue compartment representing the placenta and fetus. Therefore, the goals of the current investigation were as follows: 1) to develop a maternal-fetal PBPK (m-f-PBPK) model by incorporating a physiologically relevant fetal-PBPK into our previously verified m-PBPK; 2) to quantitatively demonstrate the impact of fetoplacental metabolism and placental transport on fetal drug exposure; 3) to quantitatively predict the impact of GA on fetal drug exposure; and 4) to show that the UV/MP ratio after a single dose or after multiple dosing (even at steady state) does not necessarily represent the extent of fetal drug exposure.

Materials and Methods

Model Structure and General Assumptions. Briefly, an m-f-PBPK model was built using MATLAB (R2014b; MathWorks, Natick, MA) by adding the placenta, the amniotic fluid compartment, and fetal organs important in drug disposition (e.g., liver and kidney) and distribution (e.g., brain) (Fig. 1) to our verified m-PBPK model (Ke et al., 2012, 2013, 2014). The remaining fetal organs were lumped into a single compartment referred to as “rest of body.” Our model also accounted for the marked differences in fetal circulation compared with that in the mother (Polin et al., 2004). For instance, it is the venous blood that carries oxygenated blood (via the UV) from the placenta to the fetus. The majority of this flow bypasses the fetal liver through the ductus venosus before perfusing fetal tissues.

General model assumptions include the following:

- The bidirectional maternal-placental and fetal-placental unbound transplacental passive diffusion clearances across the placenta are equal and always present.
- For a given drug, the magnitude of CL_{PD,u} is directly proportional to the placenta villous surface area, which increases with GA.
- The UV plasma drug concentration represents the systemic fetal plasma venous drug concentration.
- Fetal renal clearance is negligible during the first 20 weeks of gestation (Polin et al., 2004). After week 20, it consists of only glomerular filtration clearance, which can be estimated from fetal plasma protein binding and inulin clearance estimated in preterm (weeks 23–40) and term neonates (within first 14 days of life).
- Compared with fetal swallowing, the movement of amniotic fluid between the amniotic sac and maternal circulation is negligible (Gilbert and Brace, 1993). Therefore, fluid transfer between these two compartments was considered to be zero.

Collection and Analyses of Fetal Physiologic Parameters. To populate the m-f-PBPK model with fetal physiologic parameters, a systematic literature search was carried out using PUBMED to obtain these parameters (Table 1). The search strategy was aimed to identify cohort studies whereby the parameters of interest was longitudinally examined during gestation. Data

![Fig. 1. Schematic diagram of the full m-f-PBPK model. Solid arrows indicate tissue blood flows, whereas dashed arrows indicate clearances. f/F, fetal; PD, passive diffusion; M, maternal; P, placental; A, amniotic fluid; met, metabolism; renal, renal excretion; reabsorp, amniotic fluid swallowing. CL_{PD,F} and CL_{PD,M} represent unidirectional transporter-mediated clearances.](image-url)
<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Formula</th>
<th>References</th>
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<tbody>
<tr>
<td>Maternal placental blood flow (l/h)</td>
<td>$1.71 + 0.207GA + 0.0841GA^2 - 0.0013GA^3$ ($R^2 = 0.991, RSE = 2.54, N = 5; GA: 0–40 weeks$)</td>
<td>(Abduljalil et al., 2012)</td>
</tr>
<tr>
<td>Fetal serum albumin (g/l)</td>
<td>$-31.7 + 4.35GA - 0.101GA^2 + 0.0009GA^3$ ($R^2 = 0.987, RSE = 1.44, N = 15; GA: 10–40 weeks$)</td>
<td>(Gitlin and Perricelli, 1970; Krauer et al., 1984; Moniz et al., 1985; Weiner et al., 1992)</td>
</tr>
<tr>
<td>Fetal serum α₁-acid glycoprotein (g/l)</td>
<td>$0.02e^{0.0016GA}$ ($R^2 = 0.519, RSE = 0.0669, N = 19; GA: 16–38 weeks$)</td>
<td>(Krauer et al., 1984)</td>
</tr>
<tr>
<td>Fetal brain volume (ml)</td>
<td>$36.0 - 7.53GA + 0.400GA^2$ ($R^2 = 0.998, RSE = 5.83, N = 15; GA: 12–41 weeks$)</td>
<td>(Gruenwald and Minh, 1961; Cussen et al., 1990; Hansen et al., 2003; Archie et al., 2006)</td>
</tr>
<tr>
<td>Fetal liver volume (ml)</td>
<td>$16.6 - 2.92GA + 0.143GA^2$ ($R^2 = 0.996, RSE = 2.93, N = 15; GA: 12–41 weeks$)</td>
<td>(Gruenwald and Minh, 1961; Cussen et al., 1990; Hansen et al., 2003; Archie et al., 2006)</td>
</tr>
<tr>
<td>Fetal stomach volume (ml)</td>
<td>$0.127e^{0.101GA}$ ($R^2 = 0.962, RSE = 0.184, N = 18; GA: 20–37 weeks$)</td>
<td>(Nagata et al., 1990)</td>
</tr>
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(continued)
| Parameter (units) | Formula | References | Graph
|------------------|---------|------------|-------|
| Fetal small intestine volume (ml) | \[0.0203e^{0.194GA}\] \((R^2 = 0.998, \text{RSE} = 0.0688, N = 36; \text{GA: 12–25 weeks})\) | (FitzSimmons et al., 1988; Nagata et al., 1990; Parulekar, 1991; Archie et al., 2006) | ![Graph 1](#)
| Fetal large intestine volume (ml) | \[0.078e^{0.169GA}\] \((R^2 = 0.866, \text{RSE} = 7.44, N = 44; \text{GA: 20–37 weeks})\) | (Rubesova et al., 2009) | ![Graph 2](#)
| Fetal total gut volume (ml) | \[-54.3 + 8.90GA - 0.479GA^2 + 0.0088GA^3\] \((R^2 = 0.998, \text{RSE} ≈ 0, N = 28; \text{GA: 13–37 weeks})\) | (Nagata et al., 1990; Parulekar, 1991; Archie et al., 2006; Rubesova et al., 2009) | ![Graph 3](#)
| Fetal kidney volume (ml) | \[2.37 + 0.619GA + 0.0335GA^2\] \((R^2 = 0.994, \text{RSE} = 0.994, N = 15; \text{GA: 14–41 weeks})\) | (Cussen et al., 1990; Hansen et al., 2003) | ![Graph 4](#)
| Fetal umbilical blood flow (l/h) | \[0.647 - 0.227GA + 0.0179GA^2\] \((R^2 = 0.9984, \text{RSE} = 0.235, N = 23; \text{GA: 18–40 weeks})\) | (Sutton et al., 1990; Lees et al., 1999; Tchirkov et al., 1998, 2002; Kiserud et al., 2000; Boito et al., 2002; Acharya et al., 2004; Flo et al., 2010) weighted by study size | ![Graph 5](#)
| Ductus venosus blood flow (l/h) | \[2.05 - 0.297GA + 0.0116GA^2\] \((R^2 = 1.00; \text{GA: 20–38 weeks})\) | (Bellotti et al., 2004; Kessler et al., 2008) | ![Graph 6](#)

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<tr>
<td>Fetal portal vein blood flow (l/h)</td>
<td>$0.714 + 0.0489GA + 0.0008GA^2$ ($R^2 = 1.00; GA: 20–38$ weeks)$^f$</td>
<td>(Bellotti et al., 2004; Haugen et al., 2004; Kessler et al., 2008)</td>
</tr>
<tr>
<td>Fetal brain blood flow (ml/min)</td>
<td>$5.56e^{0.0921GA}$ ($R^2 = 0.04, RSE = 10.8, N = 32; GA: 10–20$ weeks)$^f$</td>
<td>(Rudolph et al., 1971; Kenny et al., 1986)</td>
</tr>
<tr>
<td>Fetal kidney blood flow (ml/min)</td>
<td>$2.18e^{0.0865GA}$ ($R^2 = 0.707, RSE = 18.3, N = 66; GA: 10–41$ weeks)$^f$</td>
<td>(Rudolph et al., 1971; Kenny et al., 1986; Veille et al., 1993)</td>
</tr>
<tr>
<td>Fetal glomerular filtration clearance (l/h)$^f$</td>
<td>$0.00046e^{0.15GA}$ ($R^2 = 0.69, RSE = 0.03, N = 16; GA: 23–40$ weeks)</td>
<td>(Arant, 1978; Hansen et al., 1983; Coulthard, 1985; van den Anker et al., 1995)</td>
</tr>
<tr>
<td>Fetal gut blood flow (ml/min)</td>
<td>$1.67e^{0.124GA}$ ($R^2 = 0.999, RSE = 4.67, N = 32; GA: 10–20$ weeks)$^f$</td>
<td>(Rudolph et al., 1971; Veille et al., 1993; Kenny et al., 1986)</td>
</tr>
<tr>
<td>Fetal rest of body compartment volume (ml)$^f$</td>
<td>$290.0 - 62.5GA + 3.22GA^2$ ($R^2 = 0.998$)</td>
<td>(Abduljalil et al., 2012)</td>
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(continued)
from the control arm of case-control studies and healthy subjects of cross-sectional studies were considered for inclusion. Other inclusion criteria included the following: 1) human; 2) uncomplicated singleton pregnancies; and 3) otherwise uneventful pregnancies with conditions thought not to affect the parameter of interest (e.g., preterm birth data were used to estimate fetal renal function). If the data were not tabulated and only graphs were present, individual data points were digitized using Digitizer (a free tool available on http://www.mathworks.com/matlabcentral/). When multiple qualified studies were available for a physiologic parameter of interest, data were pooled, stratified by GA (measured in weeks from the first day of the last menstrual cycle), and summarized using the approach previously published (Abduljalil et al., 2012). For a given GA, the overall average values (overall mean across studies ± overall S.D.) are overlaid when applicable. Residual standard error (RSE) and N (the number of overall means across GAs) are also provided in the formula column when relevant.

### TABLE 1—Continued

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<thead>
<tr>
<th>Parameter (units)</th>
<th>Formula</th>
<th>References</th>
<th>Graph*</th>
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<tr>
<td>Syncytiotrophoblast volume (ml)</td>
<td>$-6.83 + 0.650GA + 0.037GA^2$ $(R^2 = 0.757, \text{ RSE} = 3.86, N = 6; \text{ GA: 10–41 weeks})$</td>
<td>(Mayhew et al., 2003)</td>
<td><img src="image" alt="Graph" /></td>
</tr>
<tr>
<td>Placental villous surface area ($m^2$)</td>
<td>$4.66 - 0.788GA + 0.0383GA^2 - 0.0004GA^3$ $(R^2 = 0.922, \text{ RSE} = 1.008, N = 23; \text{ GA: 12–41 weeks})$</td>
<td>(Wang and Zhao, 2010)</td>
<td><img src="image" alt="Graph" /></td>
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*In the graphs above, the x-axes are GA in weeks, whereas the y-axes show the GA-dependent trends of the respective parameters in units, as indicated in the first cell of each row. The GA-specific average values (overall mean across studies ± overall S.D.) are overlaid when applicable. Residual standard error (RSE) and N (the number of overall means across GAs) are also provided in the formula column when relevant.

**Average maternal placental blood flow values at various GAs and the resultant equation were those from the meta-analysis performed by Abduljalil et al. (2012).**

**Calculated using a reported fetal small intestine (SI) diameter formula (Parulekar 1991) and the reported fetal SI length (FitzSimmons, Chinn et al., 1988), assuming cylindrical SI and negligible gut wall thickness.**

**Fetal gut consists of fetal stomach, small intestine, and large intestine. Unfortunately, there are no reported data on fetal small intestine volume during the second half of gestation. Because the reported data on these three organs overlapped between week 20 and week 25, the small intestine volume percentage (SI%) of the total gut volume for only this range of gestation was fitted to various models. A linear model (SI% = 0.652 GA + 11.766) was best describing the GA dependency of SI% volume within this range, was used to estimate the SI% volume beyond week 25. Then, the total fetal gut volume after week 25 was calculated by dividing the sum of fetal stomach and large intestine volumes by their corresponding volume percentage (i.e., 1 – SI%).**

**Calculated as the difference between total liver venous blood flow and umbilical venous blood flow.**

**The average values computed from published GA-dependent formulae were used as individual measurements were not available.**

**Calculated as the product of fetal combined cardiac output (CCO) (Rudolph et al., 1971) and the percentage CCO [before week 20 (Kenny et al., 1986) and after week 20 (Veille et al., 1993)] in respective organs.**

**Because it is not possible to measure fetal glomerular filtration rate (GFR) or renal function in utero, inulin clearance measured in preterm and term newborns were collected as a surrogate for fetal GFR. It is worth pointing out that GFR value continues to increase after birth as a result of the drop in renal vascular resistance and increase in renal blood flow (Guignard et al., 1975). Consequently, only measurements taken within 7 days after birth were included in our literature search.**

**Fetal rest of body compartment was back-calculated as fetal weight [based on published fetal volume formula (Abduljalil et al., 2012) assuming a 1 mg/ml density throughout gestation] minus the sum of fetal organ volumes and fetal blood volume [based on the reported average feto-placental blood volume of 123 ml/kg fetal weight during the second and third trimesters (Pasmant et al., 2009)].**

Sensitivity Analyses to Identify Key Determinants of Fetal Drug Exposure. To identify the quantitative impact of key factors (e.g., fetal metabolic clearance) that influence maternal-placental/fetal plasma concentration-time (C-T) profiles of a drug at term (week 40), we conducted a series of simulations of two hypothetical drugs, X and Y (Table 2), using our newly developed m-fPBPK model. Drug X was designed to be a neutral compound (e.g., the HIV nucleoside drugs zidovudine and didanosine, which are predominately un-ionized at physiologic pH with intermediate permeability across the placenta and minimal plasma protein binding). Therefore, all the variables and parameters discussed here for drug X should be read as unbound values. A drug with these characteristics was chosen to quantitatively illustrate the impact of fetoplacental metabolism and placental transport on fetal exposure to drugs. Drug Y was designed to represent highly lipophilic, neutral drugs with high permeability across the biologic membrane. It was significantly bound to plasma albumin. Consequently, its maternal-fetal distributional equilibrium was affected by differences in maternal versus fetal plasma albumin concentrations. Relevant examples include protease inhibitors and many biopharmaceutics classification system class I and II drugs. These drugs are cleared by cytochrome P450 enzymes that have altered activity during pregnancy (Isoherranen and Thummel, 2013).

Additional assumptions made for the hypothetical drugs X and Y are as follows:

- Drug X is neutral, follows linear kinetics, and has negligible binding in the MP and fetal plasma and in the placenta. Therefore, all concentrations and clearances of drug X represent their corresponding unbound values.
- Drug Y is neutral, exhibits linear kinetics, and binds to plasma albumin. Its binding in the placenta tissue is the same as that in the MP.
- Maternal absorption of drug X or Y is first order and does not change during pregnancy (i.e., first-order absorption rate constant (kₐ), fraction
absorbed ($F_a$), and fraction escaping gut metabolism or intestinal bioavailability ($F_{exg}$).

- Maternal and fetal tissue-to-plasma partition coefficients are identical for both drugs and remain constant throughout pregnancy.
- Except where indicated, the fetal renal clearance of drug X or Y is negligible.
- Drug X or Y swallowed by the fetus (i.e., the amniotic fluid) is instantly and completely absorbed from the fetal intestine and is not metabolized there.

Although some of the above assumptions were made to simplify the simulations (e.g., neutrality thus zero ionization; unaltered maternal absorption during pregnancy), others were made (e.g., fetal renal secretion of drugs) because these values cannot be determined.

Except where indicated, the simulations were conducted using our m-f-PBPK model, where only one parameter was changed at a time (Table 5). Week 20 and week 40 were chosen, respectively, to represent our steady-state (inf) model (see Supplemental Material).

The above values for drug X and drug Y at term (week 40) were based on the reported didanosine and midazolam PK parameters in the literature, respectively. B/P, blood/plasma concentration ratio; CLiv, intravenous clearance; CLm0, renal clearance; fe, fraction renally eliminated; fm, fraction metabolized; fu,plasma, unbound fraction in the plasma.

### Results

#### Fetal Physiologic Parameters

The time-variant fetal physiologic parameters used to populate the m-f-PBPK model show that the GA-dependent changes in fetal physiologic parameters are pronounced (Table 1). For example, umbilical venous blood flow (i.e., fetal placental blood flow) increases by approximately 6.2-fold (from 3.3 to 20.2 l/h) from week 20 to week 40. Some fetal physiologic values change with GA in an opposite direction to the corresponding values in the mother. For example, the fetal plasma albumin concentrations increase with GA, whereas the reverse is true for the mother.

#### Impact of Maternal Metabolism and Placental Passive Drug Permeability on Fetal Exposure to Drug X or Y in the Absence of Placental/Fetal Metabolism or Placental Transport.

As expected, after continuous intravenous infusion of drug X or Y, both steady state (steady-state$^{\text{ent}}$) maternal venous plasma (MP) and fetal venous plasma (fetal plasma) concentrations and the time to reach steady state after an intravenous infusion (steady-state$^{\text{ent}}$) were inversely dependent on the maternal systemic clearance (CLm0) when $V_m$ was held constant (Fig. 2, a and b; Fig. 3, a and b). In contrast, for both drugs, the MP C-T profile, but not the fetal plasma C-T profile, was independent of the CLPD of the drug as were the maternal and fetal steady-state$^{\text{ent}}$ plasma concentrations (Fig. 4a; Fig. 5a). Although total fetal plasma steady-state$^{\text{ent}}$ concentrations of drug Y were consistently higher than those in the mother (Fig. 3, a and b; Fig. 5, a and b), for both drugs the corresponding unbound steady-state$^{\text{ent}}$ UV/MP ratio remained at unity irrespective of the magnitude of CLm0 or CLPD values (Fig. 2b; Fig. 4b for drug X; data not shown for drug Y). However, for both drugs, the time to reach fetal steady-state$^{\text{ent}}$ plasma concentration was prolonged with lower CLm0 or CLPD values (Fig. 2b; Fig. 3b; Fig. 4b; Fig. 5b). Of note, the simulated UV/MP ratio of drug X (which is not bound to plasma proteins) or Y (after correcting for plasma protein binding) matched those predicted by our steady-state$^{\text{ent}}$ model (see Supplemental Material).

### Summary of drug-specific parameters used in the simulations at term (week 40)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Drug X</th>
<th>Drug Y</th>
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<tbody>
<tr>
<td>Molecular weight</td>
<td>236.23$^a$</td>
<td>325.8$^b$</td>
</tr>
<tr>
<td>logP</td>
<td>0.05$^b$</td>
<td>3.13$^c$</td>
</tr>
<tr>
<td>B/P ratio</td>
<td>1.17$^d$</td>
<td>0.66$^d$</td>
</tr>
<tr>
<td>$V_m$ (l/kg)</td>
<td>0.79$^e$</td>
<td>1.1$^e$</td>
</tr>
<tr>
<td>$f_{plasma}$</td>
<td>0.09$^f$</td>
<td>0.032$^f$</td>
</tr>
<tr>
<td>$F_a$</td>
<td>1.0$^g$</td>
<td>0.88$^g$</td>
</tr>
<tr>
<td>$k_t$ (h$^{-1}$)</td>
<td>1.5$^h$</td>
<td>4.0$^h$</td>
</tr>
<tr>
<td>$CL_{PD}$ (l/h)</td>
<td>18.1$^i$</td>
<td>0.085$^i$</td>
</tr>
<tr>
<td>$CL_{PD}$ (l/h)</td>
<td>45.6$^j$</td>
<td>43.0$^j$</td>
</tr>
<tr>
<td>$CL_{PD0}$ (l/h)</td>
<td>1.80$^k$</td>
<td>22.7$^k$</td>
</tr>
<tr>
<td>$f_m$ and $f_e$</td>
<td>$f_m = 61%$, $f_e = 39%$$^d$</td>
<td>$f_m, f_e = 92%$, $f_e \approx 0%$$^d$</td>
</tr>
<tr>
<td>$CL_{PD}$ (l/h)</td>
<td>0.9$^m$</td>
<td>0$^m$</td>
</tr>
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* Literature value (Tuntland et al., 1999).
* Calculated from the reported blood/plasma didanosine AUC ratio (Barry et al., 1993).
* Predicted by Simcyp (version 14) based on literature value (Knupp et al., 1991). $V_m$ increased slightly from 0.68 l/kg at week 20 to 0.71 l/kg to week 40.
* Reported plasma binding of didanosine is <5%. Minimal binding of 1% was assumed for ease of data interpretation.
* Reported average didanosine absolute bioavailability is 23.5% (range 14–33% (77)) (Knupp et al., 1991). Animal studies indicate that didanosine is rapidly and completely absorbed. Therefore, Fa was assumed to be 1. The reported intravenous nonrenal clearance (~30 l/h) (Knupp et al., 1991) does not fully explain the first pass effect. $F_a$ of 0.78 was used to recover oral PK.
* Literature value (Velasque et al., 2007).
* Irreversible human fetal drug X clearance at term was calculated as the product of fetal didanosine clearance in the macaque fetus (dam weight normalized) (Tuntland et al., 1999) and the average term body weight of 85 kg in human pregnant women (Abduljalil et al., 2012). The reported steady-state fetal/dam didanosine plasma concentration ratio is ~0.5 (Tuntland et al., 1999). Therefore, placental passive diffusion clearance was estimated as 1.8 l/h (Supplemental eq. 2), assuming that the same F/M AUC ratio holds true for drug X in human maternal-fetal pairs. Therefore, Fa was assumed to be 1. The reported intravenous nonrenal clearance (~30 l/h) (Knupp et al., 1991) does not fully explain the first pass effect. $F_a$ of 0.78 was used to recover oral PK.

After a single oral dose of drug X or Y, fetal drug exposure (AUC) was inversely proportional to $CL_{PD0}$ (Fig. 2c; Fig. 3c) but independent of $CL_{PD}$ (Fig. 4c; Fig. 5c), although the fetal plasma time to reach maximum drug concentration ($t_{max}$) and $C_{max}$ were affected by both clearances. As expected, their UV/MP ratios varied over time until maternal-fetal distributional equilibrium was achieved (Fig. 2d; Fig. 3d; Fig. 4d; Fig. 5d). Interestingly, for both drugs, the deviation from unity became significantly dampened with decrease in $CL_{PD0}$ (Fig. 2d; Fig. 3d) or increase in $CL_{PD}$ (Fig. 4d; Fig. 5d).

### Impact of Dosing Interval ($\tau$) on UV/MP Ratio of Drug X or Y in the Absence of Placental/Fetal Metabolism or Placental Transport.

The time-variant fetal physiologic parameters used to populate the m-f-PBPK model show that the GA-dependent changes in fetal physiologic parameters are pronounced (Table 1). For example, umbilical venous blood flow (i.e., fetal placental blood flow) increases by approximately 6.2-fold (from 3.3 to 20.2 l/h) from week 20 to week 40. Some fetal physiologic values change with GA in an opposite direction to the corresponding values in the mother. For example, the fetal plasma albumin concentrations increase with GA, whereas the reverse is true for the mother.

#### Impact of Maternal Metabolism and Placental Passive Drug Permeability on Fetal Exposure to Drug X or Y in the Absence of Placental/Fetal Metabolism or Placental Transport.

As expected, after continuous intravenous infusion of drug X or Y, both steady state (steady-state$^{\text{ent}}$) maternal venous plasma (MP) and fetal venous plasma (fetal plasma) concentrations and the time to reach steady state after an intravenous infusion (steady-state$^{\text{ent}}$) were inversely dependent on the maternal systemic clearance (CLm0) when $V_m$ was held constant (Fig. 2, a and b; Fig. 3, a and b). In contrast, for both drugs, the MP C-T profile, but not the fetal plasma C-T profile, was independent of the CLPD of the drug as were the maternal and fetal steady-state$^{\text{ent}}$ plasma concentrations (Fig. 4a; Fig. 5a). Although total fetal plasma steady-state$^{\text{ent}}$ concentrations of drug Y were consistently higher than those in the mother (Fig. 3, a and b; Fig. 5, a and b), for both drugs the corresponding unbound steady-state$^{\text{ent}}$ UV/MP ratio remained at unity irrespective of the magnitude of CLm0 or CLPD values (Fig. 2b; Fig. 4b for drug X; data not shown for drug Y). However, for both drugs, the time to reach fetal steady-state$^{\text{ent}}$ plasma concentration was prolonged with lower CLm0 or CLPD values (Fig. 2b; Fig. 3b; Fig. 4b; Fig. 5b). Of note, the simulated UV/MP ratio of drug X (which is not bound to plasma proteins) or Y (after correcting for plasma protein binding) matched those predicted by our steady-state$^{\text{ent}}$ model (see Supplemental Material).
above scenarios, the observed distributional equilibrium UV/MP ratio of drug X and Y remained higher than the expected unbound steady-stateinf UV/MP ratio of 1.0, and the extent of this deviation was inversely related to \( \text{CL}_{\text{MP}} \) (Fig. 2, f and h; Fig. 3, f and h).

**Impact of Placental Drug Transport, Fetal Metabolism, and Placental Metabolism on Fetal Exposure to Drug X or Y.** Overall, variations in these pathways (Table 3) produced significant changes in fetal plasma C-T profiles of drug X without affecting maternal pharmacokinetics (PK) (data not shown). As expected, after a single oral dose of 400 mg of drug X, fetal plasma concentrations, \( C_{\text{max, f}} \), and placental AUC (AUCp) were identical (Fig. 6a2). In contrast, an increase of the same magnitude in fetal metabolism (CLf0) relative to CLm0 (Fig. 2, f and h; Fig. 3, f and h).

### Table 3

<table>
<thead>
<tr>
<th>Drug X</th>
<th>Dosing Regimen</th>
<th>( \tau )</th>
<th>( \text{CL}_{\text{m0}} )</th>
<th>( \text{CL}_{\text{p0}} )</th>
<th>( \text{CL}_{\text{f0}} )</th>
<th>( \text{CL}_{\text{MP}} )</th>
<th>( \text{CL}_{\text{PM}} )</th>
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<tbody>
<tr>
<td>Figure 2, a and b</td>
<td>Week 40, 16.7 mg/h continuous i.v. infusion</td>
<td>N/A</td>
<td>4.5 vs. 45</td>
<td>1.8</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Figure 2, c and d</td>
<td>Week 40, 400 mg single oral dose</td>
<td>N/A</td>
<td>4.5 vs. 45</td>
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<td>0</td>
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<td>Figure 2, e and f</td>
<td>Week 40, 133.3-mg multiple oral doses</td>
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<td>4.5 vs. 45</td>
<td>1.8</td>
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<tr>
<td>Figure 2, g and h</td>
<td>Week 40, 400-mg multiple oral doses</td>
<td>24 h</td>
<td>4.5 vs. 45</td>
<td>1.8</td>
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</tr>
<tr>
<td>Figure 4, a and b</td>
<td>Week 40, 16.7 mg/h continuous i.v. infusion</td>
<td>N/A</td>
<td>45</td>
<td>1.8 vs. 18</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Figure 4, c and d</td>
<td>Week 40, 400-mg single oral dose</td>
<td>24 h</td>
<td>45</td>
<td>1.8 vs. 18</td>
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<td>0</td>
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</table>

N/A, not applicable; week, gestational week.

**A PBPK Model of Factors Determining Fetal Drug Exposure**

![Figure 6](image_url)

**Figure 6c** 0 0, 0.18, 0.90, 1.8 0 0 0 0

**Figure 6d** 0 0, 0.18, 0.9, 1.8 0 0 0 0

**Figure 6e** 0 0, 0.18, 0.9, 1.8 0 0 0 0

**Figure 6f** 0 0, 0.18, 0.9, 1.8 0 0 0 0

**Figure 6g** 0 0, 0.18, 0.9, 1.8 0 0 0 0

**Figure 6h** 0 0, 0.18, 0.9, 1.8 0 0 0 0

**Figure 7** a3–e3 deviated from its respective steady-stateinf F/M drug concentration ratio and therefore did not represent the F/M AUC ratio.

Overall, after a single oral dose of drug Y, within the test range, variations in the same set of drug Y clearance pathways (Table 4) produced similar changes in fetal plasma drug concentrations (Fig. 7, a1–e1) without altering the MP C-T curve of drug Y (data not shown). As was the case with drug X, both P/M and F/M AUC ratios decreased as these clearance pathways became larger with the exception of CLPM, which was positively correlated with P/M and F/M ratios. After accounting for binding, the impact of fetal clearance was larger than that of placental clearance on F/M and P/M AUC ratios (Fig. 7, a2–e2). In addition, the predicted drug Y UV/MP ratio at distributional equilibrium (Fig. 7, a3–e3) was consistently higher than the expected steady-stateinf UV/MP ratios (Supplemental eq. 1).

**Impact of GA on Fetal Disposition of Drug X.** Maternal and fetal plasma C-T profiles after a single 400-mg oral dose of drug X at weeks 20 and 40 were simulated using the m-f-PBPK model under various scenarios outlined in Table 3. GA significantly altered fetal plasma C-T profile while minimally affecting maternal PK (Fig. 8). Furthermore, the impact of GA on fetal exposure to drug X depended on the clearance
mechanisms within the fetoplacental unit. In scenario 1 (Fig. 8, a and b), where drug X was assumed to passively diffuse across the placenta without placental drug transport or irreversible clearance in the fetoplacental unit (e.g., metabolism), despite a modest 16% decrease in Cmax,f, the F/M plasma AUC ratio remained at unity and did not change with advancing GA. In scenario 2 (Fig. 8, c and d), the addition of fetal metabolism produced significant reductions in both the Cmax,f and AUCf, resulting in F/M AUC ratio of 0.48 and 0.50 at weeks 20 and 40, respectively. In scenario 3 (Fig. 8, e and f), where P-gp–mediated CLPM was assumed to be 20% of CLPD with no fetal metabolism, advancing GA from week 20 to week 40, and the associated decrease in CLPM resulted in substantial changes in the shape of the fetal C-T curve as well as in increase in Cmax,f (by 118.5%) and fetal AUC (by 3.6-fold). Finally, in scenario 4 (Fig. 8, g and h), the combination of both fetal metabolism and placental efflux resulted in the lowest Cmax,f and AUCf at both GAs.

### Impact of GA on Fetal Disposition of Drug Y

Overall, GA had a marked effect on maternal-fetal plasma C-T curves of drug Y. In scenario 1, where neither fetoplacental metabolism nor placental drug transport was present and maternal hepatic unbound intrinsic clearance remained independent of GA, increasing GA resulted in slightly lower MP concentrations of drug Y (Fig. 9a), as evidenced by a 19% reduction in MP AUC from week 20 to week 40. Over the same period, fetal plasma AUC increased significantly by 67% (Fig. 9b). This decrease in

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<tr>
<th>Drug Y Dosing Regimen</th>
<th>GA (Week)</th>
<th>CLm0</th>
<th>CLPD</th>
<th>CLf0</th>
<th>CLPM, u</th>
<th>CLPM, u, t</th>
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<td>Figure 3, a and b</td>
<td>Week 40</td>
<td>0.63 mg/h continuous i.v. infusion</td>
<td>N/A</td>
<td>12 vs. 43</td>
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<td>Week 40</td>
<td>2.5-mg multiple oral doses</td>
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<td>12 vs. 43</td>
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CLm0, unbound maternal plasma clearance; CLPD, unbound placental drug transport; CLf0, unbound fetal metabolism; CLPM, unbound placental efflux clearance; CLPM, u, t, unbound placental uptake clearance; CLPM, u, b, unbound placental metabolism; N/A, not applicable; week, gestational week.

At week 20, baseline value CLm0 was set at 43 l/h based on the published midazolam clinical data at term (Ke et al., 2012), whereas CLPD was estimated through sensitivity analysis to match reported fetal UV plasma midazolam drug C-T profile (Zhang and Unadkat, 2017). At week 20, CLm0 was slightly decreased primarily resulting from more plasma albumin binding.

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a Denotes transplacental passive diffusion clearance after accounting for binding.
b Denotes efflux clearance mediated by P-gp located on the apical side of placenta; assumed to be 20% of CLPM at week 40.
c Back-extrapolated based on the assumed 5-fold decrease in placental P-gp expression and the reported 2.6-fold increase in the placenta volume (Abduljalil et al., 2012).
AUCm, in conjunction with the increase in AUCf, resulted in a pronounced ~100% increase in F/M AUC ratio from week 20 to 40. However, after correcting for plasma protein binding, unbound F/M AUC ratios remained unity at both GAs (Fig. 9c). In scenario 2 when a 100% induction in maternal CLint,hep at week 40 relative to that at week 20 was assumed, the resultant higher CLm0 gave rise to lower the maternal plasma AUC (AUCm) and thus a 34% decrease in term AUCf compared with scenario 1. Of note, F/M AUC ratios, both total and unbound, were identical to those in scenario 1 (Fig. 9f) with unbound F/M AUC ratios being unity. Like drug X, the introduction of CLPM (20% of CLPD at term) in scenario 3 produced markedly lower fetal plasma drug concentrations and hence much smaller AUCf at week 20 compared with week 40 (Fig. 9h). Unbound F/M AUC ratios reduced to lower-than-unity values (~0.2 and ~0.8 at week 20 and 40, respectively) (Fig. 9i). Last, in scenario 4, the combination of placental efflux and increasing CLm0 with GA resulted in a pronounced difference in maternal-fetal plasma drug C-T curves between week 20 and week 40. The increase in CLm0 from week 20 to week 40 in GA led to significantly lower maternal drug Y concentrations and a greater than 50% reduction in AUCm at week 40. When compared with scenario 3, this scenario had much lower Cmax,f and AUCf values at week 40 (Fig. 9, j and k), whereas the F/M AUC ratios were of the same magnitude (Fig. 9l).

Discussion

Here we present a novel maternal-fetal PBPK model that, to our knowledge, allows for the first time the capability to predict the fetal disposition of pharmaceutical drugs at various GAs. The model incorporated the unique fetal vascular physiology and allows future incorporation of placental transport and metabolism within the fetoplacental unit (in progress in our laboratory). This model has been verified by us, at term, where the predicted and observed maternal and fetal plasma concentrations of theophylline and
zidovudine were in a good agreement (Zhang and Unadkat, 2017). However, since such verification data can only be collected at term and does not speak to which factors might affect this ratio (after a single dose or at steady state), we have used this novel m-f-PBPK model to quantitatively demonstrate the factors that can affect this ratio and when this ratio can and cannot be used to estimate fetal exposure to drugs (Figs. 2–9). Although some of these results are obvious from fundamental pharmacokinetic principles, others are not. These unexpected results are highlighted below and summarized in Supplemental Table 2. Also, note that all fetoplacental clearance pathways below refer to clearance values after accounting for binding.

**When Does UV/MP Ratio Reflect Fetal Drug Exposure?**

Contrary to a widely held belief, the UV/MP ratio, even at distributional equilibrium, does not indicate fetal drug exposure relative to that in the mother (AUC_f/AUC_m) (Figs. 2–7). The only exception is when the drug is administered to steady state via an infusion or when MP concentrations, at steady state, do not fluctuate much after multiple oral administration situations that occur infrequently in the clinic. The same UV/MP ratio is often deemed to infer the mechanisms by which the drug crosses the placenta. A greater than unity ratio is often interpreted as accumulation of the drug in the fetal compartment (Else et al., 2011). In contrast, a ratio of less than unity is interpreted as low fetal exposure and is attributed to low maternal drug concentrations (Chappuy et al., 2004a,b), fetal metabolism (Ngan Kee et al., 2009), and/or placental efflux (Marzolini and Kim, 2005; Else et al., 2011). However, as shown by our simulations, such interpretations can be false. Although fetoplacental metabolism or placental transport processes cannot be discounted based on a single UV/MP ratio, such deviation from unity is more likely due to the time-dependent distributional kinetics of the drugs across the placenta.
In the absence of fetal/placental metabolism or placental transport, the unbound steady-state maternal UV/MP ratio and therefore the unbound F/M AUC ratio after single or multiple doses (assuming linearity) should be unity (Supplemental eq. 1). Indeed, it is (Figs. 2-7). In all other cases, the degree of deviation of this ratio from unity (sometimes by an order of magnitude) not only varied with time but also depended on the magnitude of CL_{PD} (Figs. 4 and 5) and CL_{m0} (Figs. 2 and 3). The extent of this deviation decreased when the fetal compartment was allowed to rapidly equilibrate with the maternal compartment. Even in these cases where no active placental uptake was invoked, the UV/MP ratio at distributional equilibrium still exceeded unity (Fig. 2d, placental uptake was invoked, the UV/MP ratio at distributional equilibrium still exceeded unity (Fig. 2d), but not the maternal (solid lines) drug X plasma C-T profile at week 40. (a) After infusion (16.7 mg/h, i.v.) at week 40, decreasing CL_{PD} from 18 l/h (red) to 1.8 l/h (blue) did not affect the maternal (the red and blue solid lines overlap) or fetal steady-state plasma concentrations of the drug. (a and b) The corresponding UV/MP ratios indicate that at steady-state these ratios do not change with alterations in CL_{PD} (18 l/h, red; 1.8 l/h, blue), but the time to reach the steady-state ratio was prolonged. (c) After a single oral dose (400 mg), increasing CL_{PD} from 1.8 l/h (blue) to 18 l/h (red) significantly modified the shape of fetal plasma C-T curves (dashed lines) but not MP drug concentrations (solid lines; blue and red lines overlap). (d) Corresponding changes in UV/MP ratio indicate that higher CL_{PD} values (red) not only shortened the time to reach distributional equilibrium but also reduced distributional equilibrium UV/MP ratio compared with lower CL_{PD} (blue). After multiple oral doses (400 mg; τ = 24 hours), alterations in CL_{PD} did not affect the MP drug X C-T curve but significantly changed the shape of fetal plasma drug concentrations and not by passive placental transfer and/or placental transport would be incorrect. Similarly, another frequently reported ratio, the amniotic fluid/UVD drug concentration ratio, varied with time and did not reflect fetal drug exposure (see Supplemental Material).

**Unbound F/M AUC Ratio Is Determined by the Magnitude of Placental Clearance Relative to Fetoplacental and/or Placental Transport Clearance.** Another common misconception about fetal drug exposure (i.e., AUC_{f}) is that AUC_{f} is mainly driven by MP drug concentrations and not by passive placental transfer and/or fetoplacental metabolism. The reasoning behind it is that fetal/placental clearances are thought to be minor compared with maternal clearance of the drug, due to the small size of the fetal liver (only about 9.5% of maternal liver weight at term (Abduljalil et al., 2012)) or the limited metabolic capacities of the placenta and the fetal liver. Therefore, it is often assumed that when active transport across the placenta is absent, the unbound F/M AUC ratio will approximate unity. Although MP drug concentrations do drive fetal plasma drug concentrations, the above reasoning about F/M AUC ratio is false because it fails to recognize an additional critical factor, CL_{PD}. It is the ratio of these two clearances (i.e., the magnitude of fetoplacental clearances relative to CL_{PD} and not relative to CL_{m0}) that determines the unbound F/M AUC ratio. These determinants of fetal drug exposure, however, have been largely overlooked by others (Hill and Abramson, 1988; Marzolini and Kim, 2005; Myllynen et al., 2007; Bernick and Kane, 2012) and are discussed below with examples (Figs. 6 and 7).
In essence, metabolism in the fetus magnifies the reduction in fetal simulations, due to fetal metabolism, the unbound steady-state ratio of drug concentrations between fetal (F) and maternal (M) compartments. (Fig. 6; Supplemental eqs. 1 and 2). Because any drug taken by mother has to first traverse the placenta to reach fetal circulation, the placenta essentially behaves like the reference point is clearance (CL) at the placental level. Consistent with our simulations, due to fetal metabolism, the unbound steady-state ratio of drug concentrations between fetal (F) and maternal (M) compartments remained lower CLPD values from 22.5 l/h (red) to 2.25 l/h (blue) did not affect the maternal (the red and blue solid lines overlap) or fetal steady-state ratio of drug concentrations between fetal (F) and maternal (M) compartments remained lower CLPD values from 22.5 l/h (red) to 2.25 l/h (blue) did not affect the maternal (the red and blue solid lines overlap) or fetal steady-state ratio of drug concentrations between fetal (F) and maternal (M) compartments. (Fig. 6, a, d, and e). In other words, fetal and maternal compartments rapidly equilibrate. Therefore, AUCF will approach or equal AUMC after single-dose or multiple-dose administration of the drug (assuming linearity) (Fig. 7b). In reality, for lipophilic drugs with limited fetoplacental metabolic capacities, a negligible effect on AUCF is expected when CLPD is much greater than fetal/placental metabolism and/or placental transport (e.g., lipophilic drugs such as drug Y), the placenta becomes “transparent.” In other words, the expression of drug efflux/influx occurs only in the placenta, which will significantly alter fetal drug exposure if their magnitude is significant relative to CLPD (Fig. 6). It is important to stress here that the reference point is CLPD and not CLmax. Consistent with our simulations, the unbound steady-state ratio of drug concentrations between fetal (F) and maternal (M) compartments varied. However, even for such drugs, in the presence of significant fetoplacental metabolism and/or placental transport relative to CLPD, fetal drug exposure will be significantly altered (e.g., remifentanil) (Egan, 1995; Coonen et al., 2010). Several lipophilic HIV protease inhibitors that are P-gp substrates (e.g., nindavir, ritonavir, and saquinavir) (Unadkat et al., 2004) have UV/MMP ratios that are considerably lower than unity (Marzolini and Kim, 2005). However, as pointed out earlier, these non–steady-state ratios should be interpreted with caution. Nevertheless, a role of placental P-gp efflux likely contributes to their low fetal exposure as these drugs are excellent substrates of P-gp (van der Sandt et al., 2001; McCormack and Best, 2014).

The Impact of GA on Fetal Drug Exposure. The expression of fetoplacental drug-metabolizing enzymes and placental transporters is known to change with GA (Myllynen et al., 2009). For this reason, fetal drug exposure (with no change in maternal dosing regimen) is likely to...
Fig. 6. Impact of changes in feto-placental metabolism and placental transport on fetal plasma and placental drug X concentration, P/M AUC, F/M AUC, and UV/MP ratios after a single 400-mg oral dose of drug X. Changes in feto-placental clearance pathways differentially impacted fetal exposure to drug X, P/M plasma AUC ratio (hatched bar), F/M plasma AUC ratio (solid bar), and the UV/MP ratio after a single 400-mg oral dose of drug X at week 40. The CLPD of drug X was held at 1.8 l/h. The clearance pathway variance is indicated at the extreme left of the first panel of each row. The indicated clearance was set at 0, 0.18, 0.9, or 1.8 l/h, respectively (0%, 10%, 50%, or 100% of CLPD; black, red, green, and blue lines, respectively). Other than CLMP (d1), increasing any of the indicated clearance pathways resulted in lower fetal plasma drug X concentrations (a1–c1 and e1). When CLf0 was present, the resultant F/M plasma AUC ratio was lower (b2 and c2) than when only CLP0 was present (a2). In all cases, the predicted UV/MP ratio at distributional equilibrium was significantly greater than its steady-statein value (Supplemental eq. 1). The UV/MP ratio at distributional equilibrium decreased with an increase in CLP0, CLf0, CLf0 plus CLP0, or CLPM (a3, b3, c3, and e3, respectively) and increased with an increase in CLMP (d3). See Table 3 for the clearance values used in these simulations.
change with GA (Figs. 8 and 9). But these changes are not intuitive. When drug X passively diffused across the placenta without being transported or metabolized in the fetoplacental unit (Fig. 8, a and b), the progression of gestation did not affect AUCf because the latter was solely driven by AUCm, which remained virtually the same from week 20 to week 40. In scenario 2, where only fetal metabolic clearance was present, AUCf was significantly reduced and appeared nearly equal between week 20 and week 40 (Fig. 8d). This is because the CLf0/CLPD ratio remained similar from week 20 to week 40 (0.53 and 0.50, respectively) due to the fact that fetal body volume (and therefore fetal metabolic clearance) and the placental surface area (and therefore CLPD) increased with GA in a nearly parallel fashion (Table 1). Placental P-gp...
Fig. 8. Impact of GA on maternal and fetal drug X plasma concentration and F/M AUC ratio after a single 400-mg oral dose of drug X at week 20 (red) or week 40 (blue). The effect of GA on fetal exposure to drug X varies with fetoplacental clearance mechanisms involved. Maternal (solid) and fetal (dashed) C-T profiles as well as F/M plasma AUC ratios were simulated after a single 400 mg oral dose of drug X at week 20 (red) and week 40 (blue) under different scenarios. In scenario 1, where no irreversible loss of drug X occurred in the fetoplacental unit, the advancement of GA did not significantly affect fetal-maternal drug disposition (a) or the F/M plasma AUC ratio of unity (b). In scenario 2, the addition of fetal metabolism (increased proportionally with the fetal body volume as GA increased) significantly reduced fetal plasma drug concentrations (c versus a) and resulted in an ~50% decrease in F/M AUC plasma ratio at both GAs (d). In scenario 3, placental P-gp efflux clearance decreased with GA (based on reported P-gp expression and changes in placental volume with GA). Consequently, fetal exposure to drug X increased with GA, reflected by higher fetal plasma drug concentrations (e) and increased F/M plasma AUC ratios (f). Finally, in scenario 4, where both fetal metabolism and placental efflux were present, a further reduction in fetal exposure was observed at both GAs (g and h). See Table 3 and Table 5 for the clearance values used in these simulations.
expression is significantly higher during early gestation versus term (Mathias et al., 2005). This gestational effect in P-gp expression resulted in lower fetal exposure to drug X at week 20, whereas at week 40, due to lower placental P-gp efflux clearance, fetal exposure to drug X increased by 1.9-fold (Fig. 8, e and f). When both clearance pathways were present, the change in placental P-gp expression was a major determinant of the GA effect on drug X AUC (Fig. 8, g and h).

Challenges for the Next Generation of Fetal PBPK Models. Like other fetal PBPK models, our m-f-PBPK model also has some limitations. The use of our model to predict fetal exposure to drugs prior to week 20 may be limited for the following reasons. First, data on many fetal physiologic parameters prior to week 20 (Table 1) are not available. Second, fetal skin is not completely keratinized and is highly permeable during the first half of gestation (Polin et al., 2004). Therefore, drugs that extensively partition into the skin/subcutaneous layer may readily cross into the amniotic fluid. Such movement of drugs could be incorporated into a future iteration of this model. In order for the model to be useful for drugs that are extensively metabolized by the fetus or transported/metabolized

Fig. 9. Impact of GA on maternal and fetal drug Y plasma concentration and F/M AUC ratio after a single 15-mg oral dose of drug Y at week 20 (red) and week 40 (blue). The effect of GA on fetal exposure to drug Y varies with the fetoplacental clearance mechanisms involved. Maternal (solid) and fetal (dashed) C-T profiles, maternal (solid) and fetal (hatched) plasma AUCs, as well as F/M total (solid) and unbound (hatched) AUC ratios were simulated after a single 15-mg oral dose of drug Y at week 20 (red) and week 40 (blue) under different scenarios. In scenario 1, where no irreversible loss of drug Y occurred in the fetoplacental unit under a constant maternal hepatic unbound intrinsic clearance (CL_{int,u,hep}), the advancement of GA resulted in an increased fetal plasma AUC despite the decrease in MP AUC (a and b) but did not affect the F/M plasma AUC unbound ratio of unity (c). In scenario 2, the assumed 100% higher CL_{int,u,hep} at week 40 compared with week 20 significantly decreased maternal-fetal plasma drug Y concentrations (hence, AUCs) at term (d versus a; e versus b), whereas F/M AUC plasma ratios at both GAs were identical to those in scenario 1 (f versus c). In scenario 3, placental P-gp–mediated efflux clearance decreased with GA. Consequently, fetal exposure to drug Y increased with GA, which is reflected by higher fetal plasma drug concentrations (g) and increased F/M plasma AUC ratios (i). Finally, in scenario 4, the increase in fetal exposure from week 20 to week 40 persisted in the presence of both a decrease in placental efflux and an increase in CL_{int,u,hep} with GA, but to a smaller extent compared with scenario 3 (k versus h). Note that F/M AUC plasma ratios at both GAs were identical to those in scenario 3 (l versus i). See Table 4 and Table 5 for the clearance values used in these simulations.
in the placenta, the model will need to be populated with GA-dependent changes in the expression of these enzymes and transporters. Such studies, using quantitative targeted proteomics, are in progress in our laboratory.

Currently, the lack of critical data hinders further development of fetal PBPK models. First, fetoplacental physiologic data across developmental stages are much needed, including the substantial changes in placental and fetal circulation and fetal organ sizes and composition. Obtaining such information will rely on the advancements in non-invasive physiologic measurement techniques and devices. Before such information becomes available, cross-species extrapolation through PBPK modeling and simulation can be conducted (Poet et al., 2010; Yoon et al., 2011). However, this approach will not work when there are significant interspecies differences in fetoplacental metabolism and placental transport. Another challenge lies in obtaining data for PBPK model validation. As detailed above, the UV/M ratio can change dramatically with time. Therefore, we propose that the maternal dosing regimen, the time after the last maternal dose when maternal and UV drug plasma concentrations are obtained, be recorded and the unbound drug concentration in these samples be measured.

In summary, through simulations we have shown that even when fetoplacental metabolic or placental transport clearance is small, it can significantly determine fetal drug exposure provided that the magnitude of these clearances is comparable to the CL_{p,uv} of the drug (likely for hydrophilic drugs). In addition, we have shown that the single time point UV/M ratio (except at steady state after an intravenous infusion or when maternal concentrations do not fluctuate much after multiple oral administration), routinely reported in the literature, cannot be used as an indicator of F/M plasma drug exposure ratio even at distributional equilibrium (after single or multiple doses). Therefore, one promising alternative is to dynamically estimate fetal drug exposure in humans at term and earlier in gestation through PBPK models such as the one presented here. However, prior to using the proposed model, it needs to be verified with fetal exposure data. In our companion article (Zhang and Unadkat, 2017), we describe such a verification using midazolam, zidovudine, and theophylline.

Acknowledgments
We thank Dr. Masoud Jamei for help and support on the model. We also thank Dr. Nina Isoherranen for valuable discussions.

Authorship Contributions
Participated in research design: Zhang and Unadkat.
Conducted experiments: Zhang and Imperial.
Contributed new reagents or analytic tool: Patilea-Vrana, Wedegadera, and Gaouha.
Performed data analysis: Zhang.
Wrote or contributed to the writing of the manuscript: Zhang and Unadkat.

References

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Drug Metabolism and Disposition

Supplementary Information

Development of A Novel Maternal-Fetal Physiologically Based Pharmacokinetic Model I: Insights into Factors that Determine Fetal Drug Exposure through Simulations and Sensitivity Analyses

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Department of Pharmaceutics, University of Washington, Seattle, WA (ZZ, MI, GP, and JU); Simcyp Limited (a Certara company), Sheffield, UK (JW and LG)

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University of California San Francisco, Department of Bioengineering and Therapeutic Sciences (MI, current affiliation)
Figure Legends

**Supplementary Figure S1:** A three compartment model representing the maternal (M), placental (P), and fetal (F) compartments: 0- irreversible elimination. The remaining abbreviations are as in Figure 1. For simplicity, excretion of drugs from fetus into the amniotic fluid was assumed to be negligible.

**Supplementary Figure S2:** Changes in fetal renal clearance (fCL\textsubscript{R}) or fetal amniotic fluid swallowing rate (fCL\textsubscript{reabsorp}) affected drug concentrations in fetal plasma and amniotic fluid differently. Following a single oral dose of 400 mg drug X at week 40, fCL\textsubscript{R} (0.196 L/h) or fCL\textsubscript{reabsorp} (0.024 L/h) of drug X was varied by 0.5-, 1-, and 5-fold (red, green, and blue, respectively). Fetal plasma drug X concentrations remained independent of changes in either fCL\textsubscript{R} (a) or in fCL\textsubscript{reabsorp} (b), except when fCL\textsubscript{R} was increased by 5-fold (but even in this case the AUC was not changed). In contrast, amniotic fluid drug X concentrations were quite sensitive to changes in these two pathways. Increasing fCL\textsubscript{R} resulted in higher amniotic fluid drug concentrations (c), whereas increasing fCL\textsubscript{reabsorp} reduced amniotic fluid drug X exposure (d). Increasing fCL\textsubscript{R} from 0.098 L/h to 0.981 L/h proportionally increased amniotic fluid drug X AUC (solid bars) while fetal plasma AUC (hatched bars) remained constant (e). Similarly, increasing the magnitude of fCL\textsubscript{reabsorp} from 0.01 L/h to 0.10 L/h proportionally decreased amniotic fluid AUC (solid bars) without affecting fetal plasma AUC (hatched bars)(f). Similar trends were observed with fetal:maternal (F:M) plasma AUC ratio (circles) and amniotic fluid:maternal plasma (AF:MP) AUC ratio (squares) (e and f, respectively). AF:MP and amniotic fluid:umbilical venous plasma (AF:UV) drug X concentration ratios also varied significantly when fCL\textsubscript{R} (g and i, respectively) or fCL\textsubscript{reabsorp} (h and j).
respectively) was altered. Increasing fCL\textsubscript{R} resulted in higher AF:MP and AF:UV ratios, while increasing fCL\textsubscript{reabsorp} reduced both ratios. See Table 2 for the clearance values used in these simulations.

**Supplementary Figure S3:** In this schematic, X\textsubscript{c} and X\textsubscript{p} denotes the amount of drug in the central compartment and the peripheral compartments, respectively; k\textsubscript{10} is the central compartment elimination rate constant, whereas k\textsubscript{12} and k\textsubscript{21} are the inter-compartmental distribution rate constants.
Supplementary Table 1

<table>
<thead>
<tr>
<th>Figure</th>
<th>Dosing regimen</th>
<th>CL_{m0} (L/h)</th>
<th>CL_{PD} (L/h)</th>
<th>CL_{MP} (L/h)</th>
<th>CL_{PM} (L/h)</th>
<th>CL_{p0} (L/h)</th>
<th>CL_{f0} (L/h)</th>
<th>fCL_{R} (L/h)</th>
<th>fCL_{reabsorp} (L/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure S2 a,c,e,g,i</td>
<td>400mg single oral dose of drug X at week 40</td>
<td>45</td>
<td>1.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.90</td>
<td>0.1,0.20,0.98</td>
<td>0.021</td>
</tr>
<tr>
<td>Figure S2 b,d,f,h,j</td>
<td>45</td>
<td>1.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.90</td>
<td>0.20</td>
<td>0.01,0.02,1.0</td>
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</tbody>
</table>

CL_{m0}, maternal systemic clearance; CL_{PD}, transplacental passive diffusion clearance; CL_{MP}, placental efflux clearance; CL_{PM}, placental apical uptake clearance; CL_{p0}, placental metabolic clearance; CL_{f0}, fetal metabolism; fCL_{R}, feal renal secretion clearance; fCL_{reabsorp}, fetal swallowing of amniotic fluid
## Supplementary Table 2

<table>
<thead>
<tr>
<th>Condition</th>
<th>Can the unbound UV:MP ratio be used to indicate the unbound F:M AUC ratio</th>
<th>The extent of deviation of the unbound UV:MP ratio from the predicted unbound F:M AUC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous IV infusion</td>
<td>Yes at steady-state</td>
<td>None at steady-state</td>
</tr>
<tr>
<td>Single dose (IV or oral)</td>
<td>No</td>
<td>↑ as drug’s $t_{1/2}$ gets shorter or the drug becomes more polar; ↓ with increasing $t_{1/2}$ or lipophilicity of the drug</td>
</tr>
<tr>
<td>Multiple doses when $\tau &lt;$ drug $t_{1/2}$</td>
<td>No</td>
<td>↑ as $\tau$ becomes shorter relative to drug $t_{1/2}$</td>
</tr>
<tr>
<td>Multiple doses when $\tau &gt;&gt;$ drug $t_{1/2}$</td>
<td>Yes</td>
<td>↓ as $\tau$ becomes longer relative to drug $t_{1/2}$</td>
</tr>
</tbody>
</table>

### Unbound F:M AUC ratio

<table>
<thead>
<tr>
<th>Condition</th>
<th>Predicted unbound F:M AUC ratio</th>
<th>The extent of the deviation of unbound F:M AUC ratio from unity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipophilic drugs with insignificant feto-placental metabolism or placental efflux</td>
<td>$\sim 1$</td>
<td>minimal</td>
</tr>
<tr>
<td>Lipophilic drugs with significant feto-placental metabolism or placental efflux</td>
<td>$&lt; 1$ or $&lt;&lt; 1$</td>
<td>↑ as feto-placental metabolism or placental efflux increases relative to $\text{CL}_{PD}$</td>
</tr>
<tr>
<td>Polar drugs with insignificant feto-placental metabolism or placental efflux</td>
<td>$\sim 1$</td>
<td>minimal</td>
</tr>
<tr>
<td>Polar drugs with significant feto-placental metabolism or placental efflux</td>
<td>$&lt;&lt; 1$ or $&lt;&lt;&lt; 1$</td>
<td>↑ as feto-placental metabolism or placental efflux becomes larger relative to $\text{CL}_{PD}$</td>
</tr>
<tr>
<td>Polar drugs transported into the fetus by placental uptake transporters</td>
<td>$&gt; 1$</td>
<td>↑ as placental uptake becomes larger relative to $\text{CL}_{PD}$</td>
</tr>
</tbody>
</table>
Supplementary Figures

Figure S1
Figure S2
Figure S3

\[ X_c \quad \xrightarrow{k_{12}} \quad X_p \]

\[ k_{21} \quad \xleftarrow{} \quad k_{10} \]
Supplementary Methods

Verification of model implementation using a steady-state infusion three compartment model

At steady-state attained by IV infusion of the drug to the mother (steady-state_{inf}), maternal-fetal plasma concentration of drugs can be described by a model consisting of maternal (M), placental (P), and fetal (F) compartments (steady-state_{inf} model; Figure S1) even for a drug that demonstrates multi-compartmental behavior. Moreover, at steady-state_{inf}, the fetal:maternal (F:M) plasma unbound drug concentration ratio (i.e. the $C_{f,ss,inf}/C_{m,ss,inf}$ ratio below) predicted by this steady-state_{inf} model should be equal to the F:M plasma unbound AUC ratio predicted by the m-f-PBPK model after a single dose of the drug or within a dosing interval at steady-state (assuming linear pharmacokinetics). Therefore, these $C_{f,ss,inf}/C_{m,ss,inf}$ ratios predicted by this steady-state_{inf} model were compared against F:M unbound plasma AUC predictions made by our m-f-PBPK model as a means to ensure the correct implementation of our m-f-PBPK model. Of note, the predicted F:M plasma drug concentration ratios and F:M AUC ratios of drug Y equal its corresponding unbound values (Figures 5-9)

For simplicity, all clearances should be read as their corresponding unbound values.

Besides the assumption that the bidirectional maternal-placental and placental-fetal passive diffusion unbound clearances (CL_{PD}) are equal and always present, this steady-state_{inf} model also assumes for the drug: (1) all clearances do not exceed their respective organ blood flows; (2) uptake and efflux transporters are located on the apical (maternal-facing) side of the placenta; (3) fetal renal excretion is negligible.
Using this steady-state$_{\text{inf}}$ model, the F:M plasma and placenta:maternal plasma (P:M) drug concentration ratios at steady-state$_{\text{inf}}$ can be described by the following equations (see below for derivation).

\[
\frac{C_{f,u,ss,\text{inf}}}{C_{m,u,ss,\text{inf}}} = \frac{CL_{PD} + CL_{MP}}{CL_{PD} + 2CL_{f0} + CL_{p0} + CL_{PM} + \left(\frac{CL_{p0} + CL_{PM}}{CL_{PD}}\right) \cdot CL_{f0}} \quad (\text{Eq. 1})
\]

\[
\frac{C_{p,u,ss,\text{inf}}}{C_{m,u,ss,\text{inf}}} = \frac{(CL_{PD} + CL_{MP}) \cdot (1 + \frac{CL_{f0}}{CL_{PD}})}{CL_{PD} + 2CL_{f0} + CL_{p0} + CL_{PM} + \left(\frac{CL_{p0} + CL_{PM}}{CL_{PD}}\right) \cdot CL_{f0}} \quad (\text{Eq. 2})
\]

where $C_{f,u,ss,\text{inf}}$, $C_{p,u,ss,\text{inf}}$ and $C_{m,u,ss,\text{inf}}$ are steady-state$_{\text{inf}}$ drug unbound concentrations in the fetal plasma, placenta, and maternal plasma, respectively; $CL_{PD}$ is the bidirectional passive diffusion clearance; $CL_{f0}$ and $CL_{m0}$ are the irreversible fetal and maternal metabolic clearances, respectively; $CL_{PM}$ and $CL_{MP}$ are the efflux and uptake transporter mediated clearances, respectively. Note, all clearances should be read as unbound clearances.

It is immediately evident that the $\frac{C_{f,u,ss,\text{inf}}}{C_{m,u,ss,\text{inf}}}$ ratio is independent of maternal clearance. When fetoplacental metabolism and placental transport are absent, this ratio reduces to unity. The differing impact of these individual clearance pathways on the $\frac{C_{f,u,ss,\text{inf}}}{C_{m,u,ss,\text{inf}}}$ ratio (and therefore, as indicated above, F:M plasma AUC ratio) is further discussed below.

**Derivation of steady-state$_{\text{inf}}$ P:M and F:M ratios**

Under steady-state$_{\text{inf}}$ when a drug is infused into the maternal compartment,
where \( C_{f,u,ss,inf} \) and \( C_{p,u,ss,inf} \) are drug unbound concentrations in the fetal plasma and placenta at steady-state after infusion (steady-state_{inf}), respectively, \( CL_{PD} \) is the bidirectional passive diffusion clearance, and \( CL_{f0} \) is the irreversible fetal clearance. It is worth keeping in mind that passive diffusion of the drug across the placenta will be present for all drugs and will depend on the drug’s lipophilicity.

Rearrangement of Eq. 3 gives:

\[
\frac{C_{f,u,ss,inf}}{C_{p,u,ss,inf}} = \frac{CL_{PD}}{CL_{PD} + CL_{f0}} \quad (Eq. 4)
\]

Similarly, for the placenta,

\[
CL_{PD}(C_{m,u,ss,inf} - C_{p,u,ss,inf}) + CL_{MP} \cdot C_{m,u,ss,inf} = CL_{PD}(C_{p,u,ss,inf} - C_{f,u,ss,inf}) + (CL_{p0} + CL_{PM}) \cdot C_{p,u,ss,inf}
\]

\[
(Eq. 5)
\]

where \( C_{m,u,ss,inf} \) is steady-state_{inf} drug unbound concentration in the maternal plasma; \( CL_{PM} \) and \( CL_{MP} \) are the non-saturated efflux and uptake placental transporter mediated clearances, respectively.

Rearranging and substituting Eq. 4 into Eq. 5 yields:

\[
\frac{C_{f,u,ss,inf}}{C_{m,u,ss,inf}} = \frac{CL_{PD} + CL_{MP}}{CL_{PD} + 2CL_{f0} + CL_{p0} + CL_{PM} + (CL_{p0} + CL_{PM}) \cdot CL_{f0}} \quad (Eq. 6)
\]

Substituting Eq. 4 into Eq. 6 and rearranging yields:
The impact of fetal/placental metabolism and placental transport on the steady-state fetal:maternal plasma drug concentration ratio is apparent with the following examples:

(1) When, except for CL\(_{PD}\), none of the above fetoplacental clearance pathways are present, Eq. 6 simplifies to Eq. 8:

\[
\frac{C_{f,ss,inf}}{C_{m,ss,inf}} = \frac{CL_{PD}}{CL_{PD} + CL_{f0}} = 1 \quad (\text{Eq. 8})
\]

In this case the placenta becomes “transparent”, meaning that the \(\frac{C_{f,ss,inf}}{C_{m,ss,inf}}\) ratio becomes unity.

(2) When only \(CL_{p0}\) and \(CL_{PD}\) are present, Eq. 6 simplifies to Eq. 9:

\[
\frac{C_{f,ss,inf}}{C_{m,ss,inf}} = \frac{CL_{PD}}{CL_{PD} + CL_{p0}} \quad (\text{Eq. 9})
\]

Now the \(\frac{C_{f,ss,inf}}{C_{m,ss,inf}}\) ratio is determined by the magnitude of \(CL_{PD}\) relative to \(CL_{p0}\).

For instance, when these two clearances are of the same magnitude, this ratio becomes 0.5.

(3) When only \(CL_{f0}\) and \(CL_{PD}\) are present, Eq. 6 simplifies to Eq. 10:

\[
\frac{C_{f,ss,inf}}{C_{m,ss,inf}} = \frac{CL_{PD}}{CL_{PD} + 2CL_{f0}} \quad (\text{Eq. 10})
\]
Now the \( \frac{C_{f,ss,inf}}{C_{m,ss,inf}} \) ratio is determined by the magnitude of \( CL_{PD} \) relative to \( CL_{f0} \).

Note that the \( CL_{f0} \) term has a coefficient of 2. Therefore, the introduction of fetal clearance results in a further decrease in the \( \frac{C_{f,ss,inf}}{C_{m,ss,inf}} \) ratio compared with the above case. For instance, when \( CL_{PD} \) and \( CL_{f0} \) are of the same magnitude, this ratio becomes 0.33.

(4) When both \( CL_{p0} \) and \( CL_{f0} \) as well as \( CL_{PD} \) are present, \textbf{Eq. 6} simplifies to \textbf{Eq. 11}:

\[
\frac{C_{f,ss,inf}}{C_{m,ss,inf}} = \frac{CL_{PD}}{CL_{PD} + 2CL_{f0} + CL_{p0} + CL_{p0} \cdot CL_{f0}} \quad (\text{Eq. 11})
\]

Now the \( \frac{C_{f,ss,inf}}{C_{m,ss,inf}} \) ratio becomes a composite function that is determined by the magnitude of \( CL_{f0} \) and \( CL_{p0} \) relative to \( CL_{PD} \). When \( CL_{f0} \) and \( CL_{p0} \) are of the same magnitude as \( CL_{PD} \), this ratio reduces to 0.2. Furthermore, when \( CL_{p0} < < CL_{PD} \), \textbf{Eq. 11} simplifies to \textbf{Eq. 10}, whereas when \( CL_{f0} < < CL_{PD} \), \textbf{Eq. 11} simplifies to \textbf{Eq. 9}.

(5) When only \( CL_{PM} \) and \( CL_{PD} \) are present, \textbf{Eq. 6} simplifies to \textbf{Eq. 12}:

\[
\frac{C_{f,ss,inf}}{C_{m,ss,inf}} = \frac{CL_{PD}}{CL_{PD} + CL_{PM}} \quad (\text{Eq. 12})
\]

Again, when placental efflux clearance is invoked the \( \frac{C_{f,ss,inf}}{C_{m,ss,inf}} \) ratio is determined by the magnitude of \( CL_{PD} \) relative to \( CL_{PM} \). For example, when these two clearances are of the same magnitude, the \( \frac{C_{f,ss,inf}}{C_{m,ss,inf}} \) ratio is 0.5.

(6) When only \( CL_{MP} \) and \( CL_{PD} \) are present, \textbf{Eq. 6} reduces to \textbf{Eq. 13}:
\[
\frac{C_{f,ss,inf}}{C_{m,ss,inf}} = \frac{CL_{PD} + CL_{MP}}{CL_{PD}} \quad \text{(Eq. 13)}
\]

Unlike the above cases, introduction of placental uptake clearance results in an increase in the \(\frac{C_{f,ss,inf}}{C_{m,ss,inf}}\) ratio. Nonetheless, this ratio is dependent on the magnitude of \(CL_{PD}\) relative to \(CL_{MP}\). For example, when these two clearances are of the same magnitude, the \(\frac{C_{f,ss,inf}}{C_{m,ss,inf}}\) ratio is 2.

Under various scenarios of our sensitivity analyses, the m-f-PBPK model simulated steady-state \(F:M\) and \(P:M\) ratios of drug X or Y were consistent with those predicted by the above maternal-placental-fetal three compartment model, confirming the correct implementation of our m-f-PBPK model. In addition, this exercise, as detailed above, yielded novel and surprising quantitative information on the impact of fetoplacental metabolism and placental transport on fetal exposure to drugs.

**Impact of fetal renal clearance and amniotic fluid fetal swallowing rate on amniotic fluid exposure to drug X**

The reported physiological value of fetal renal clearance (fCL\(_R\)) (Supplementary Table 1) and fetal amniotic fluid swallowing rate (fCL\(_\text{reabsorp}\)) (Pritchard, 1966) was varied by 0.5-, 1-, and 5-fold respectively. As anticipated, changes in neither pathway altered maternal PK (data not shown). Within the tested range, only a 5-fold increase in fCL\(_R\) modestly altered the shape of fetal C-T curve. When fCL\(_R\) (0.098L/h) was increased from to 0.196L/h or 0.981L/h, the corresponding \(C_{max,f}\) decreased by 2.6% and 16.4% respectively (Figure S2a) but the AUC\(_f\) remained unchanged (Figure S2e). In contrast, alterations in fCL\(_\text{reabsorp}\) did not affect \(C_{max,f}\) or AUC\(_f\) (Figure S2b and Figure S1f).
Increase in fCL\textsubscript{R} or fCL\textsubscript{reabsorp} significantly affected drug distribution into amniotic fluid in opposite direction. As fCL\textsubscript{R} increased from 0.098L/h to 0.196 L/h, the predicted amniotic fluid AUC (AUC\textsubscript{af}) increased from 13.4 mg·h/L to 26.8 mg·h/L (Figure S2c, e). When fCL\textsubscript{reabsorp} was increased from 0.01L/h to 0.02L/h the respective AUC\textsubscript{af} value was reduced from 53.4 mg·h/L to 26.7 mg·h/L (Figure S2d, f). Similar trends were observed with the amniotic fluid:maternal plasma (AF:MP) and amniotic fluid:UV (AF:UV) drug concentration ratios. Both ratios increased rapidly with time (Figure S2g-j). Moreover, after the establishment of fetal plasma-amniotic fluid distributional equilibrium, both ratios significantly deviated from the corresponding AF:MP and AF:UV AUC ratios.

See Table 2 for the clearance values used in these simulations.

**Supplementary Discussion**

Amniotic fluid drug concentration is often mistaken for an indicator of fetal drug exposure. For example, high amniotic fluid concentrations were associated with continuous fetal exposure to bupropion.(Fokina et al., 2016) But this is not the case. When a renally cleared drug enters the fetal circulation, it is excreted into amniotic fluid via fetal urination (fCL\textsubscript{R}) and then reabsorbed through fetal swallowing of amniotic fluid (fCL\textsubscript{reabsorp}; assuming instant and complete absorption) at late gestation.(Underwood et al., 2005) Therefore, high amniotic fluid drug concentrations do not necessarily result from high fetal exposure but rather is due to the much greater fCL\textsubscript{R} relative to fCL\textsubscript{reabsorp}. This disconnect between the amniotic fluid and fetal plasma drug concentrations is illustrated in **Supplementary Figure S2**. Amniotic fluid effectively acted as a “reservoir” for drug X because of its 9-fold higher fCL\textsubscript{R} (0.192 L/h) compared to fCL\textsubscript{reabsorp} (500mL/day). Drug X amniotic fluid drug concentrations, and therefore amniotic fluid drug AUC
(AUC$_{af}$), increased proportionally with fCL$_{R}$ but was inversely related to fCL$_{reabsorp}$ (Figure S2c-f). In contrast, AUC$_{f}$ remained independent of the variations in fCL$_{R}$ or fCL$_{reabsorp}$ (Figure S2e,f). This is because the amniotic fluid acts as a distribution compartment not a compartment where drug elimination takes place. Indeed, the observed AUC$_{af}$/AUC$_{m}$ of tenofovir, a placental P-gp substrate, is as high as 2.76, although tenofovir AUC$_{f}$/AUC$_{m}$ of 0.45 was observed in the same study.(De Sousa Mendes et al., 2016) Again, like the UV:MP ratio, AF:UV ratio does not reflect fetal drug exposure (Figure S2g-j).

**Theoretical discussion on the effect of CL$_{m0}$ and CL$_{PD}$ on fetal:maternal drug concentration ratio**

Drug distribution in the coupled maternal-fetal pair may be envisioned to be analogous to the distribution of a drug between a central compartment (mother) and a peripheral compartment(fetus). For a drug that demonstrates two compartment pharmacokinetics, the differential equations for the rate of change in the amount of drug in the central and peripheral body compartments based on the model presented in Supplementary Figure S3 are

\[
\frac{dX_c}{dt} = -(k_{i0} + k_{i2})X_c + k_{21}X_p \quad (1)
\]

and

\[
\frac{dX_p}{dt} = k_{i2}X_c - k_{21}X_p \quad (2)
\]

respectively.
where \( X_c \) and \( X_p \) are the amount of drug in the central compartment and the peripheral compartments, respectively, \( k_{10} \) is the central compartment elimination rate constant, and \( k_{12} \) and \( k_{21} \) are the inter-compartmental distribution rate constants.

Take the Laplace transform of both sides of (1)

\[
s \cdot \overline{X_c} - X_0 = -(k_{10} + k_{12}) \cdot \overline{X_c} + k_{21} \cdot \overline{X_p} (3)
\]

where \( X_0 \) is the dose available to the central compartment.

Similarly for (2)

\[
s \cdot \overline{X_p} = k_{12} \cdot \overline{X_c} - k_{21} \cdot \overline{X_p} (4)
\]

Rearrange (3) and (4) to get:

\[
\begin{cases}
(s + k_{10} + k_{12}) \cdot \overline{X_c} - k_{21} \cdot \overline{X_p} = X_0 \\
-k_{12} \cdot \overline{X_c} + (s + k_{21}) \cdot \overline{X_p} = 0
\end{cases}
\]

Solving Eq. 5 gives:

\[
X_c = X_0 \cdot \left( \frac{\lambda_1 - k_{21}}{\lambda_1 - \lambda_2} e^{-\lambda_1 t} + \frac{k_{21}}{\lambda_1 - \lambda_2} e^{-\lambda_2 t} \right)
\]

\[
X_p = X_0 \cdot \left( \frac{-k_{12}}{\lambda_1 - \lambda_2} e^{-\lambda_1 t} + \frac{k_{12}}{\lambda_1 - \lambda_2} e^{-\lambda_2 t} \right)
\]

where \( \lambda_1 \) and \( \lambda_2 \) are microconstants.(Gibaldi and Perrier, 1982)
Hence, \[
\frac{X_p}{X_c} = \frac{X_0 \cdot \left( -\frac{k_{12}}{\lambda_1 - \lambda_2} e^{-\lambda_1 t} + \frac{k_{12}}{\lambda_2 - \lambda_1} e^{-\lambda_2 t} \right)}{X_0 \cdot \left( \frac{\lambda_1 - k_{21}}{\lambda_1 - \lambda_2} e^{-\lambda_1 t} + \frac{k_{21} - \lambda_2}{\lambda_1 - \lambda_2} e^{-\lambda_2 t} \right)} \quad (7)
\]

Rearranging Eq. 7 yields:

\[
\frac{X_p}{X_c} = \frac{-k_{12} e^{-\lambda_1 t} + k_{12} e^{-\lambda_2 t}}{(\lambda_1 - k_{21}) e^{-\lambda_1 t} + (k_{21} - \lambda_2) e^{-\lambda_2 t}} \quad (8)
\]

In the post-distributive phase (i.e., as \( e^{-\lambda_1 t} \) approaches zero)

Eq. 8 reduces to:

\[
\frac{X_p}{X_c} = \frac{k_{12}}{k_{21} - \lambda_2} \quad (9)
\]

Substituting \( X_p \) and \( X_c \) with \( C_p V_p \) and \( C_c V_c \), respectively and rearranging yields:

\[
\frac{C_p}{C_c} = \frac{k_{12}}{k_{21} - \lambda_2} \cdot \frac{V_c}{V_p} \quad (10)
\]

where \( C_p \) and \( C_c \) are drug concentrations in the peripheral and central compartment, respectively, and \( V_p \) and \( V_c \) are the volume of distribution for the central and peripheral compartments, respectively.

\( \lambda_2 \) is a hybrid parameter \( \lambda_2 = \frac{1}{2} \left[ \frac{1}{2} \left( k_{12} + k_{21} + k_{10} \right) - \sqrt{(k_{12} + k_{21} + k_{10})^2 - 4k_{21}k_{10}} \right] \) that reflects the drug elimination from the body. It increases as \( k_{10} \) increases. (Gibaldi and
Therefore, increasing $k_{10}$ will result in higher $\frac{C_p}{C_c}$ and thereby elevating the peripheral (fetal):central (maternal) plasma drug concentration ratio.

On the other hand, for a passively diffused drug:

$$k_{12} = \frac{CL_{pd}}{V_c} \text{ and } k_{21} = \frac{CL_{pd}}{V_p}$$

where $CL_{pd}$ is the inter-compartmental passive diffusion clearance.

Substituting for $k_{12}$ and $k_{21}$ in Eq. 10 yields:

$$\frac{C_p}{C_c} = \frac{1}{1 - \frac{\lambda_2 V_p}{CL_{pd}}} \quad (11)$$

It is evident from Eq. 11 that increasing $CL_{pd}$ will reduce the peripheral:central (fetal:maternal) drug plasma concentration ratio.
References


Verification of a Maternal-Fetal Physiologically Based Pharmacokinetic Model for Passive Placental Permeability Drugs

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**Supplementary Figures**

**Figure S1**

- **a** shows the structure of the MATLAB PBPK model for the non-pregnant population.
- **b** is the schematic diagram of the maternal-fetal full PBPK model. Solid arrows indicate tissue blood flows and dashed arrows indicate clearances. CL: clearance; Prefixes: f-fetal; Subscripts: PD- passive diffusion, M- maternal, P- placenta, F- fetus, A- amniotic fluid, met- metabolism, renal-renal excretion, reabsorp- amniotic fluid swallowing. CL\(_{FP/PF}\) and CL\(_{MP/PM}\) represent the unidirectional placental transporter-mediated clearances between the fetus and the placenta and between the mother and the placenta.
Figure S2: Impact of varying the unbound transplacental passive diffusion clearance (CL\textsubscript{PD,u}) of midazolam, theophylline, and zidovudine on their maternal plasma (a, c, and e, respectively) and umbilical venous plasma (b, d, and e, respectively) concentration-time (C-T) profiles. Variations in CL\textsubscript{PD,u} resulted in virtually no changes in the predicted maternal plasma drug concentrations for all three drugs. In the lower CL\textsubscript{PD,u} range (<250 L/h), varying CL\textsubscript{PD,u} resulted in changes in umbilical venous plasma midazolam C-T curves. However, these changes diminished as CL\textsubscript{PD,u} was further increased. In contrast, within the tested range (50-1000L/h), changes in CL\textsubscript{PD,u} resulted in negligible changes in umbilical venous plasma C-T profiles of theophylline or zidovudine.
Table S1: Sensitivity analysis of midazolam transplacental passive diffusion clearance (CL_{PD,MDZ})

<table>
<thead>
<tr>
<th>time</th>
<th>Observed UV plasma conc.</th>
<th>CL_{PD} (L/h) ²</th>
<th>2.27</th>
<th>4.54</th>
<th>11.35</th>
<th>22.7</th>
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<td></td>
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<td>Pred PE</td>
<td>Pred PE</td>
<td>Pred PE</td>
<td>Pred PE</td>
<td>Pred PE</td>
<td></td>
</tr>
<tr>
<td>h</td>
<td>ng/mL</td>
<td>ng/mL %</td>
<td>ng/mL %</td>
<td>ng/mL %</td>
<td>ng/mL %</td>
<td>ng/mL %</td>
<td>ng/mL %</td>
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</tr>
<tr>
<td>0.33</td>
<td>6</td>
<td>0.72 -88.03</td>
<td>1.89 -68.42</td>
<td>4.87 -18.85</td>
<td>7.83 30.49</td>
<td>9.25 54.19</td>
<td>10.22 70.39</td>
<td></td>
</tr>
<tr>
<td>0.33</td>
<td>9</td>
<td>0.72 -92.02</td>
<td>1.89 -78.95</td>
<td>4.87 -45.90</td>
<td>7.83 -13</td>
<td>9.25 2.79</td>
<td>10.22 13.59</td>
<td></td>
</tr>
<tr>
<td>0.45</td>
<td>4</td>
<td>1.45 -63.63</td>
<td>3.45 -13.65</td>
<td>7.72 92.89</td>
<td>11.51 187.75</td>
<td>13.16 228.98</td>
<td>14.29 257.23</td>
<td></td>
</tr>
<tr>
<td>0.75</td>
<td>29</td>
<td>3.14 -89.16</td>
<td>6.29 -78.31</td>
<td>11.76 -59.45</td>
<td>15.98 -44.91</td>
<td>17.57 -39.42</td>
<td>18.63 -35.77</td>
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</tr>
<tr>
<td>0.83</td>
<td>7</td>
<td>3.51 -49.89</td>
<td>6.80 -2.83</td>
<td>12.34 76.29</td>
<td>16.46 135.16</td>
<td>17.96 156.61</td>
<td>18.95 170.73</td>
<td></td>
</tr>
<tr>
<td>0.83</td>
<td>19</td>
<td>3.51 -81.54</td>
<td>6.80 -64.20</td>
<td>12.34 -35.05</td>
<td>16.46 -13.36</td>
<td>17.96 -5.46</td>
<td>18.95 -0.26</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>4.15 -76.93</td>
<td>7.63 -57.61</td>
<td>13.12 -27.09</td>
<td>16.89 -6.14</td>
<td>18.16 0.91</td>
<td>18.97 5.40</td>
<td></td>
</tr>
</tbody>
</table>

Prediction precision 77.31 51.99 50.79 61.55 69.77 79.05
Prediction bias -77.31 -51.99 -2.45 39.43 56.94 68.76
Sum of squares 1215.41 839.68 426.04 327.92 346.53 376.57

* term midazolam unbound fraction increases from 0.3 to 0.454 as a result of hemodilution caused by pregnancy.
Supplementary Methods

Ordinary Differential Equations Defining the Mass Balance in the Fetal-PBPK

I. Symbols representing the variables and parameters used in the models

\( V \) volume (L)

\( C \) concentration (\( \mu \)M)

\( (t) \) gestational age-dependent

\( Q \) blood flow rate (L/h)

\( K_p \) tissue: plasma partition coefficient

\( B_P \) blood: plasma partition ratio

\( C_L \) clearance (L/h)

Subscriptions:

- subscription \( a \) arterial
- subscription \( F:M \) from fetal to maternal
- subscription \( M:F \) from maternal to fetal
- subscription \( A:M \) from amniotic fluid to maternal
- subscription \( M:A \) from maternal to amniotic fluid
- subscription \( af \) amniotic fluid
- subscription \( FP \) from fetal placental blood to placenta
- subscription \( PF \) from placental to fetal placental blood
subscription p    plasma
subscription PD    passive diffusion
subscription pl    placenta
subscription PM    from placental to maternal placental blood
subscription MP    from maternal placental blood to placenta
subscription PD    passive diffusion
subscription af    amniotic fluid
subscription gut    gut
subscription kid    kidneys
subscription liv    liver
subscription per    peripheral
subscription bra    brain
subscription pl    placenta

Superscriptions

superscription m    maternal
superscription f    fetal
superscription s    placental
II Equations

Maternal placental blood

The mass balance in the maternal placental blood compartment can be described using the following differential equation Eq. 1

\[
V_{p}^{m}(t) \frac{d[C_{pl}]^{m}}{dt} = Q_{pl}(t)(C_{a}^{m} - C_{pl}^{m}) \\
+ CL_{PM} \frac{fu_{pl}(t)}{BP_{pl}^{m}(t)} C_{pl}^{m} - CL_{MP} \frac{fu_{pl}(t)}{BP_{pl}^{m}(t)} C_{pl}^{m} \\
+ CL_{AM} \frac{fu_{af}(t)}{BP(t)} C_{af}^{m} - CL_{MA} \frac{fu_{pl}(t)}{BP(t)} C_{pl}^{m} \\
+ CL_{PDPM} \left( \frac{fu_{pl}^{s}(t)}{\beta_{pl}^{s}} C_{pl}^{s} - \frac{fu_{pl}^{m}(t)}{BP_{pl}^{m}(t)} \frac{1}{\alpha_{pl}^{m}} C_{pl}^{m} \right)
\]

where \( C_{a}^{m} \) is the arterial blood concentration in the mother.

Placenta

The mass balance in the placental membrane compartment can be described using the following differential equation Eq. 2

\[
V_{pl}^{s}(t) \frac{d[C_{pl}]^{s}}{dt} = CL_{PP} \frac{fu_{pl}^{s}(t)}{BP_{pl}^{s}(t)} C_{pl}^{s} - CL_{PP} fu_{pl}(t) C_{pl}^{s} \\
+ CL_{MP} \frac{fu_{pl}(t)}{BP_{pl}^{m}(t)} C_{pl}^{m} - CL_{SP} fu_{pl}(t) C_{pl}^{s} \\
+ CL_{PDPF} \left( \frac{fu_{pl}(t)}{BP_{pl}^{s}(t)} \frac{1}{\alpha_{pl}^{s}} C_{pl}^{s} - \frac{fu_{pl}^{s}(t)}{BP_{pl}^{s}(t)} \frac{1}{\beta_{pl}^{s}} C_{pl}^{s} \right) \\
+ CL_{PDPF} \left( \frac{fu_{pl}(t)}{BP_{pl}^{s}(t)} \frac{1}{\alpha_{pl}^{m}} C_{pl}^{m} - fu_{pl}(t) \frac{1}{\beta_{pl}^{s}} C_{pl}^{s} \right) \\
- CL_{p0} C_{pl}^{s}
\]

where \( CL_{p0} \) is the irreversible placental metabolism.
The fetal unit comprises of the fetal placental blood, amniotic fluid, the fetal circulatory system, fetal liver, fetal gut, fetal kidneys, fetal brain, and a lumped peripheral compartment representing the rest of the fetus.

The following equation is used to describe the concentration in the placenta/fetal compartment:

\[
V_{pl}^f(t) \frac{d[C_{pl}^f]}{dt} = Q_{pl}^f(t)(C_a^f - C_{pl}^f) + CL_{PF} f_{u_{pl}}^f(t)C_{pl}^f - CL_{FP} \frac{f_{u_{pl}}^f(t)}{BP^f(t)} C_{pl}^f \]  
\[
+ CL_{PF} f_{u_{pl}}^f(t) \left( \frac{1}{\beta_{pl}^s} C_{pl}^s - \frac{fu_{pl}^f(t)}{BP^f(t)} \alpha^f C_{pl}^f \right) \]  

\( (3) \)

Amniotic fluid

\[
V_{af}^f(t) \frac{d[C_{af}^f]}{dt} = CL_{MA} \frac{fu_{pl}^m(t)}{BP^m(t)} C_{pl}^m - CL_{AM} f_{u_{af}}^f(t)C_{af}^f \]  
\[
+ CL_{renal}^f(t) C_v^f - CL_{reabs}^f C_{af}^f \]  

\( (4) \)

where CL\(_{renal}^f\) is the renal clearance based on the fetal venous plasma concentration (please see the following Eq.5 for fetal kidneys and Eq. 11 for fetal central venous blood compartment).

Fetal kidneys

\[
V_{kid}^f(t) \frac{d[C_{kid}^f]}{dt} = Q_{kid}^f(t)(C_a^f - \frac{C_{kid}^f}{KP_{kid}^f(t) / BP^f(t)}) \]  

\( (5) \)
Note the renal excretion has been considered in the previous equation (4), as well as in equation (11), and not directly from this kidney compartment.

**Fetal guts**

The following equation is used to describe the concentration in the gut:

\[
V_{\text{gut}}^f \frac{d[C_{\text{gut}}^f]}{dt} = Q_{\text{gut}}^f(t)(C_a^f - \frac{C_{\text{gut}}^f}{Kp_{\text{gut}}^f(t)/BP^f(t)}) + CL_{\text{reabs}}^f C_{af}^f
\]  

(6)

**Fetal liver**

The fetal liver is a well-stirred perfusion-limited organ, consisting of the tissue and its capillary blood. On the basis of well-stirred model, the emergent concentration is the driving concentration for hepatic metabolism.

Therefore the following equation is used to describe the concentration of liver compartment:

\[
V_{\text{liv}}^f \frac{d[C_{\text{liv}}^f]}{dt} = Q_{\text{port, a}}^f(t)C_a^f + Q_{\text{port, y}}^f(t)C_{pl}^f + Q_{\text{gut}}^f(t)\frac{C_{\text{gut}}^f}{Kp_{\text{gut}}^f(t)/BP^f(t)}
\]

\[
- (Q_{\text{hep, ven}}^f(t) + Q_{\text{hep, art}}^f(t) + Q_{\text{gut}}^f(t))\frac{C_{\text{liv}}^f}{Kp_{\text{liv}}^f(t)/BP^f(t)}
\]

\[
- fu^f(t) \cdot CL_{f0}^f \cdot \frac{C_{\text{liv}}^f}{Kp_{\text{liv}}^f(t)/BP^f(t)}
\]

(7)
where CL_{f0} is the fetal hepatic metabolic clearance and subscription hep_ven and hep_art and gut refer to the hepatic blood supplied by umbilical vein (minus ductus venosus blood flow) and by the hepatic artery, respectively. The former is from the fetal placental blood compartment (Figure 1).

**Fetal peripheral compartment**

\[
V_{per}^f \frac{d[C_{per}^f]}{dt} = Q_{per}^f(t)(C_a^f - \frac{C_{per}^f}{Kp_{per}^f(t)BP^f(t)})
\]  

(8)

**Fetal brain**

\[
V_{bra}^f \frac{d[C_{bra}^f]}{dt} = Q_{bra}^f(t)(C_a^f - \frac{C_{bra}^f}{Kp_{bra}^f(t)BP^f(t)})
\]  

(9)

where subscription bra means brain compartment.

**Fetal central arterial blood compartment**

\[
V_a^f \frac{d[C_a^f]}{dt} = (Q_{pl}^f(t) + Q_{kid}^f(t) + Q_{gut}^f(t) + Q_{port, a}(t) + Q_{per}^f(t) + Q_{bra}^f(t))(C_v^f - C_a^f)
\]  

(10)
Fetal central venous blood compartment

\[ V_v^f(t) \frac{d[C_v^f]}{dt} = (Q_{pl}^f(t) - Q_{hep-ven}^f(t))C_{pl}^f \]

\[ - (Q_{pl}^f(t) + Q_{kid}^f(t) + Q_{gat}^f(t) + Q_{hep-arr}^f(t) + Q_{pro}^f(t) + Q_{bra}^f(t))C_v^f \]

\[ + Q_{kid}^f(t) \frac{C_{kid}^f}{Kp_{kid}^f(t)/BP_{kid}^f(t)} \]

\[ + Q_{per}^f(t) \frac{C_{per}^f}{Kp_{per}^f(t)/BP_{per}^f(t)} \]

\[ + Q_{bra}^f(t) \frac{C_{bra}^f}{Kp_{bra}^f(t)/BP_{bra}^f(t)} \]

\[ + (Q_{gut}^f(t) + Q_{port-a}^f(t) + Q_{port-v}^f(t)) \frac{C_{lv}^f}{Kp_{lv}^f(t)/BP_{lv}^f(t)} \]

\[ - \frac{CL_{renal}^f(t)}{BP_{renal}^f(t)} C_v^f \]

(11)