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The Effect of Plasma Lipids on the Pharmacokinetics of Chlorpyrifos and the Impact on Interpretation of Blood Biomonitoring Data

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Lipophilic molecules, like chlorpyrifos (CPF), present a special problem for interpretation of biomonitoring data because both the environmental dose of CPF and the physiological (pregnancy, diet, etc.) or pathological levels of blood lipids will affect the concentrations of CPF measured in blood. The objective of this study was to investigate the distribution of CPF between plasma and tissues when lipid levels are altered in late pregnancy. CPF was sequestered more in the low-density lipid fraction of the blood during the late stages of gestation in the rat and returned to nonpregnant patterns in the dam after birth. Plasma partitioning of CPF increased with increases in plasma lipid levels and the increased partitioning of CPF into plasma lipids resulted in less CPF in other tissue compartments. Gavage dosing with corn oil also increased plasma lipids that led to a moderate increase of CPF partitioning into the plasma. To mechanistically investigate the potential pharmacokinetic effects of blood lipid changes, an existing CPF physiologically based pharmacokinetic/pharmacodynamic model for rats and humans was modified to account for altered lipid-tissue partition coefficients and for major physiological and biochemical changes of pregnancy. The model indicated that plasma CPF levels are expected to be proportional to the well-known changes in plasma lipids during gestation. There is a rapidly growing literature on the relationship of lipid profiles with different disease conditions and on birth outcomes. Increased blood concentrations of lipophilic chemicals like CPF may point to altered lipid status, as well as possibly higher levels of exposure. Thus, proper interpretation of blood biomonitoring data of lipophilic chemicals requires a careful consideration of blood lipids.

An understanding of the physiological factors that affect measurements of chemicals in blood is essential to improve the accuracy of pharmacokinetic models. Of particular interest are the substantial blood chemistry changes that occur in pregnant women and animals, especially in regard to blood lipid levels (LaBorde *et al*., 1999; Sattar *et al*., 1997; Saarelainen *et al*., 2006). The distribution of xenobiotic compounds among blood and tissues, as predicted by empirically derived partition coefficients (PC), is highly dependent on the lipophilicity of the compound and the lipid content of blood and tissues. The predicted distribution of drugs and other exogenous chemicals into tissues is also based on the ratio of chemical solubility between plasma and tissues (Poulin and Theil, 2000). PC are chemical constants, which describe distribution among interfacing phases (e.g., tissue and blood) at equilibrium. Processes such as these are essential determinants of pharmacokinetics and pharmacodynamics and are components of physiologically based pharmacokinetic (PBPK) models.

Previous studies have shown that lipid levels in blood or plasma can vary substantially. For example, oral and intragastric administration of vegetable oil has been shown to increase the concentration of triglycerides in the blood within 6 h in rats (Gershkovich and Hoffman, 2007; Ramirez, 1984; Ramirez and Friedman, 1983). Also, recent work in our laboratory has established that blood lipid levels can increase up to fourfold during pregnancy (McMullin *et al*., 2008). As a result, the distribution of the chemicals in plasma may be affected by these lipid changes, according to quantitative structure property relationships (QSPR) calculations. It is hypothesized that the pharmacokinetics of lipophilic compounds, like chlorpyrifos (CPF), will correlate with lipid levels (McMullin *et al*., 2008).

CPF, a lipophilic chemical with a log K_{ow} of ~5, has been shown to undergo gestational-related changes in maternal blood concentrations. In a study by Mattsson *et al*. (2000), pregnant Sprague-Dawley rats were gavage dosed with 0.3, 1, or 5 mg CPF/kg from gestational day (GD) 6 though postnatal day (PND) 10. CPF levels in dam blood of the high-dosed group just before birth (GD 20) were more than sevenfold greater than levels just after birth (109 ng/g on GD 20 vs. 15 ng/g on PNDs 1 and 5). The authors suggested that higher CPF levels just before birth may have been at least partly related to the lipophilicity of CPF and the hyperlipidemia that occurs during pregnancy. In this regard, the pharmacokinetics/pharmacodynamics of the lipophilic CPF will be intimately related to its blood and tissue solubility. This solubility, especially into adipose tissue, is expected to be driven primarily by partitioning into lipids, as discussed by numerous other researchers (Haddad *et al*., 2000; Phillips *et al*., 1989).

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Normal physiological variations present a considerable challenge to the researcher attempting to model the dosimetry of a given xenobiotic and to assess the importance of normal physiologically changes in an embryo, fetus or newborn. Therefore, a more accurate modeling of chemical exposure during gestation will require careful consideration of maternal blood chemistry and exposure. Most gestational models to date have made the assumption that tissue PCs in the pregnant dam are not different from the nonpregnant condition and do not change over the course of the pregnancy.

The primary objective of this study was to determine whether the partitioning of CPF between plasma and tissues changes when lipid levels are altered in late pregnancy. We evaluated changes in fat:plasma ratio ($P_{f:p}$) for CPF during gestation in the rat (GD 12, 15, and 18) as compared with nonpregnant rats. The $P_{f:p}$ of CPF was also determined for plasma from nonpregnant humans and from women in weeks 10 (first trimester), 25 (second trimester), and 37 (third trimester) of pregnancy. In addition, the effect of corn-oil–induced changes in lipid levels on the partitioning of CPF between plasma and tissues was also determined. Finally, because common tissue:blood PC QSPRs (Poulin and Theil, 2000) utilize both tissue-specific lipid composition and protein binding values, the percent of binding of CPF to plasma albumin protein, as a surrogate for non-lipoprotein binding, was determined.

These PCs were incorporated into a late gestational PBPK model that encompassed major physiological changes, such as blood flow and tissue volumes, occurring from GD 18 to birth in the rat. Specifically, this model isolated the effect of blood solubility changes on CPF distribution. Finally, the PBPK model was used to estimate the environmental exposures that resulted in the plasma CPF concentrations in late pregnancy of women as reported by Whyatt *et al*. (2005). These experiments and analyses provide data to model and understand the pharmacokinetics of CPF and possibly other highly lipophilic chemicals during pregnancy.

MATERIALS AND METHODS

In Vitro Partitioning of CPF into Oil and Plasma

Test Materials

For the *in vitro* partitioning studies with rat and human plasma, radiolabeled ^{14}C-CPF was obtained from Dow AgroSciences, LLC (Indianapolis, IN) with a specific activity of 28.9 mCi/mmol and a radiochemical purity of 97%. Pure All Natural Filipo Berio olive oil (Hackensack, NJ) was purchased from a local grocery store. Propylene carbonate (PrCarb) anhydrous and > 99% pure corn oil, were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO).

Animals

Both nonpregnant and gestating Crl:CD (Sprague-Dawley) female rats were purchased from Charles River Laboratories Inc. (Portage, MI). Jugular cannulated Crl:CD female rats were purchased from Taconic (Germantown, NY) for the plasma time-course studies. All rats were 11–13 weeks old at the time of blood collection. All animals (one per cage) were housed in stainless steel cages with wire-mesh floors suspended above catch pans. LabDiet Certified Rodent Diet feed (PMI Nutrition International, St Louis, MI) and municipal water was provided *ad libitum*. Blood was collected from CO_2/O_2-anesthetized rats via cardiac puncture. The blood was collected in syringes containing heparin and was centrifuged at 10,000 × g to remove red blood cells. For corn oil dosing, feed was restricted approximately 16 h prior to the administration of corn oil and was returned about 4-h postdosing. The oral dose of corn oil (Sigma, St Louis, MO) was administered at a standard concentration of approximately 5 g/kg body weight. In accordance with the U.S. Department of Agriculture animal welfare regulations, nine CFR, subchapter A, Parts 1–4, the animal care and use activities required for conduct of this study were reviewed and approved by the Institution Animal Care and Use Committee.

Human Samples

Individual human plasma from 10 nonpregnant females and 10 females from week 10, from week 25, and from week 37 of gestation (plus or minus a week) were purchased from Bioreclamation, Inc. (Hicksville, NY) containing the anticoagulant K3 ethylenediaminetetraacetic acid (EDTA). The samples were thawed once and pooled in-house before use in the *in vitro* study.

***In vitro* method for tissue partitioning.** The partitioning of radiolabeled CPF from PrCarb into fat and plasma were determined using a modified in vitro method that was outlined in Murphy *et al*. (1995). In summary, plasma was diluted with an equivalent weight of physiological saline. A vial containing 4 ml of saline-saturated propylene carbonate (SSPrCarb) and 230 ppb ^{14}C-CPF was then weighed. Either 2 ml of the diluted plasma or 1 ml of olive oil (a surrogate for fat; Poulin and Theil, 2000) was added to the vial and weighed again. The vials were shaken for 5 h at 37°C. The reference vials containing 4 ml of SSPrCarb with 230 ppb CPF and 2 ml of physiological saline were prepared in triplicate. At the appropriate sampling time, the treatment (containing the tissue) vial and the reference vial sat for 1–5 min at room temperature to allow for phase separation. A 100-µl aliquot was removed from the bottom PrCarb layer and the top tissue layer in the treatment vial and bottom PrCarb layer and the top saline layer in the reference vial. Each aliquot was weighed and the radioactivity in the top layers and the bottom layers were determined by liquid scintillation spectroscopy (LSS). The tissue:PrCarb ratio can be expressed as

$$\text{Tissue : PrCarb ratio} = \frac{C_T}{C_{PrCarb}} \quad (1)$$

where C_T is the amount in the top layer (dpm) per g tissue in the vial and the C_{PrCarb} is the amount in the bottom layer (per g SSPrCarb) in the vial. For tissues not diluted with physiological saline (e.g., oil or fat), the ratio of the tissue:PrCarb expressed in Equation 1 can be used. However, for tissues that have been diluted or homogenized with physiological saline, the C_T has to account for the dilution and the influence of any possible sequestration of CPF by saline. In order to calculate this factor, the partitioning of CPF in the saline layer is needed and can be expressed as

$$\lambda_S = \frac{C_S}{C_{PrCarb}} \quad (2)$$

where C_s is the amount in the top saline layer (dpm) per g of saline added to the reference vial. The modified C_T term (C_{TM}) is expressed as

$$A_{TM} = A_T - (C_{PrCarb} \times \lambda_S \times W_{SF}) \quad (3)$$

$$C_{TM} = \frac{A_{TM}}{W_T} \quad (4)$$

where A_{TM} is the actual amount partitioned by the tissue, A_T is the amount (dpm) measured in the top layer, C_{PrCarb} is the concentration measured in the bottom PrCarb layer (dpm/g PrCarb), W_{SF} is the weight of the saline fraction added to the tissue, and W_T is the weight of the tissue comprising the tissue/saline mixture (top layer). Alternatively, Equation 5 can be used to determine the Plasma:PrCarb ratio.

$$\text{Plasma : PrCarb ratio} = \frac{\{[(\text{dpm/g in top layer}) \times (\text{wt of top layer})] - [(\text{dpm/g in PrCarb layer}) \times (\lambda_S) \times (W_{SF})]\}/W_T}{C_{PrCarb}}$$

Each tissue condition was performed in triplicate. The $P_{t:p}$ for CPF was calculated as the tissue:PrCarb ratio divided by the plasma:PrCarb ratio.

Blood Clinical Chemistry

The plasma component parameters of cholesterol, phospholipids, and triglycerides from the corn-oil–dosed animals and the pooled gestational plasmas from rats and humans were measured using a Hitachi 912 Clinical Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). The phospholipid levels in plasma were measured with a commercially available *in vitro* assay from Wako Pure Chemical Industries (Wako Diagnostics, Richmond, VA). Total cholesterol and triglycerides were converted from mg/dl to mmol/l using the average molecular weights of 390 and 890 g/mol, respectively.

Blood Fractionation

Test Materials and Dose Solution Preparation

Radiolabeled CPF ([ring-4-^{3}H]chlorpyrifos) was obtained from Amersham Biosciences (now GE Healthcare, Piscataway, NJ) with a specific activity of 5 Ci/mmol and a radiochemical purity of 100% (determined via high-performance liquid chromatography). Two ethanol-based dose solutions of ^{3}H-CPF were prepared such that fortification of 2 ml of whole blood with 10 µl of dose solution would yield CPF concentrations of either 3 or 100 ng ^{3}H-CPF/ml of blood. The low-level dose solution was prepared at a concentration of 640 ng CPF/ml ethanol (and a ^{3}H activity level of 9 µCi/ml); the high-level dose solution was prepared at a concentration of 20,000 ng CPF/ml ethanol (and a ^{3}H activity level of 30 µCi/ml). The high-level solution was augmented with nonradiolabeled CPF obtained from Dow AgroSciences, LLC (assayed purity of 99.8%).

Fractionation of ^{3}H-CPF among blood fractions. Whole blood used for the fractionation experiments was obtained from adult female, fetuses, or neonates of CD rats (Crl:CD(SD)IGS BR). The adult female rats used were either nonpregnant, pregnant dams from gestation days (GD) 10, 15, 18, 20, or dams from PND 0 or 4. Blood was also collected from GD 18 and GD 20 fetuses, and from PND 0 and PND 4 neonates. Blood was collected in tubes containing EDTA which has been the anticoagulant used by other investigators in preparing plasma for fractionation of lipoproteins via ultracentrifugation (Mills *et al*., 1984; Rodriguez-Sureda *et al*., 2002).

Whole blood specimens were obtained from three adult rats comprising a particular gestation or lactation group. The blood from each individual rat was subdivided among four replicates, of ~2-ml volume, yielding twelve replicates per treatment (with "treatment" referring to blood from particular gestation or PND). Six of the twelve replicates of whole blood were fortified at 3 ng/ml ^{3}H-chlorpyrifos (^{3}H-CPF); the remaining six replicates were fortified at 100 ng ^{3}H-CPF/ml blood. Because of the limited amount of blood available from fetuses or neonates, it was not always possible to obtain a 2-ml volume per replicate or 12 replicates per treatment.

After fortification with ^{3}H-CPF, the blood samples were vortexed briefly (~15 s), then centrifuged for 15 min at ~1200 × g in a Sorvall GLC-2B centrifuge (Thermo Fischer Scientific, Inc., Waltham, MA) to sediment red blood cells (RBC) from plasma. A small aliquot from each plasma layer was sampled and counted by LSS to determine the percentage of spiked radioactivity associated with the plasma phase. The remaining plasma from each sample was transferred to a 4.7-ml OptiSeal ultracentrifuge tube (Beckman Coulter, Inc., Fullerton, CA). The RBCs were transferred to Combusto-cones (Packard Instrument Company, Meriden, CT) and oxidized on a Packard Model 387 Oximate tissue oxidizer. The oxidized samples were counted by LSS to determine the radioactivity associated with the RBCs. The plasma samples were adjusted to a density of 1.21 g/ml using a solution of NaBr and then centrifuged for 44 h at ~250,000 × g using a Beckman Optima LE-80K ultracentrifuge (Beckman Coulter, Inc., Fullerton, CA) employing techniques described by Rodriguez-Sureda *et al.* (2002). The plasma was adjusted to the density of 1.21 g/ml which is the density threshold separating low-density lipoproteins/intermediate-density lipoproteins/high-density lipoproteins (HDL) from very-high-density lipoproteins (Fig. 1) (Havel *et al*., 1955). Following ultracentrifugation, the layers of ultracentrifuged plasma attributed to low-density (< 1.21; top), infranatant (mid), and high-density (> 1.21; bottom) fractions were carefully decanted using procedures described by Mills *et al.* (1984) and transferred to scintillation vials for determination of ^{3}H activity by LSS.

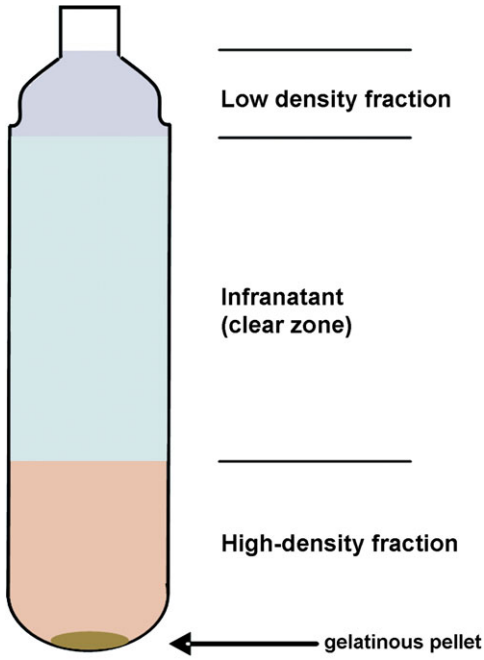

FIG. 1. Schematic diagram of the density layers formed during the fractionation of whole blood as outlined in the ''Materials and Methods.''

Protein Binding Determined by Equilibrium Dialysis

Test Materials

The specific activity of the ^{3}H-CPF used to prepare the 3 ng CPF/ml was 5 Ci/mmol (~31,700 DPM/ng), whereas the specific activity for the 100 ppb ^{3}H-CPF was 1.26 Ci/mmol (8000 dpm/ng).

Dialysis Assay

The two sides of glass dialysis cells were separated by a 12- to 14-kDa MWCO regenerated cellulose membrane. One side of each dialysis cell (treated side) was fortified to attain concentrations of either 3 or 100 ng CPF/ml in Saline Ringer bicarbonate buffer (3-ml volume) prepared with 20, 4, 0.4, or 0.04 mg/ml of human serum albumin or 20 and 0.04 mg/ml of rat serum albumin. On the other side of the cellulose membrane (Buffer side) was an equal volume of the buffer. Each condition was performed in triplicate. The dialysis cells were shaken gently at room temperature for approximately 24 h after the addition of CPF. After equilibration, two 100-µl aliquots were removed from each side of the dialysis cell. The total radioactivity in each aliquot was determined with a scintillation counter and the average of the two replicates was calculated. The fraction unbound (f_u) was determined using Equations 6 and 7 for each replicate.

$$f_b = \frac{[\text{Treated side(dpm)} - \text{Buffer side (dpm)}]}{[\text{Treated side(dpm)} + \text{Buffer side (dpm)}]} \quad (6)$$

$$f_u = 1 - f_b \quad (7)$$

PBPK Model

Model Structure

The foundation of the model is as described in (Timchalk and Poet, 2008; Timchalk *et al*., 2002a, 2003, 2005, 2007). This model integrates target tissue dosimetry, plasma protein binding of both CPF and oxon, and dynamic

response (i.e., esterase inhibition) describing uptake, metabolism, and disposition of CPF, CPF-oxon, and trichloropyridinol (TCP) and the associated cholinesterase (ChE) inhibition kinetics in blood and tissues following acute and chronic oral and dermal exposure, and has been improved and validated over several years (Timchalk and Poet, 2008; Timchalk *et al*., 2002a, 2003, 2005, 2007). The normal nonpregnant rat PBPK model accurately reflects most published CPF pharmacokinetic and pharmacodynamic data published thus far. For the assessment of the potential effects of blood lipid changes on dosimetry during pregnancy, the model was modified, with the addition of placenta, mammary, and fetal compartments. The late GD model was simplified from the full pregnancy model structures published by Clewell *et al*. (2003) for the rat and Gentry *et al*. (2002, 2003) for the human. For the CPF and CPF-oxon late GD model, the fetus is comprised of liver, blood, brain, and remaining body compartments. The placental compartment includes flow-limited kinetics as part of the dam, with a first-order transfer rate, between the placenta and fetal blood compartment for both CPF and CPF-oxon. The model includes CYP450 metabolism of CPF to the active oxon and to the inactive TCP, A-esterase (PON1) hydrolysis of oxon to TCP, and B-esterase mediated metabolism of the oxon to TCP in both the dam and fetal livers. TCP produced from the metabolism of CPF or CPF-oxon in both the maternal and fetal compartments was summed as a single compartment in the dam. The model parameters are listed in Tables 1 and 2.

Partition coefficients. In the previous model, mathematical algorithms were used to calculate tissue:blood partitioning coefficients (Poulin and Krishnan, 1995; Timchalk *et al*., 2002b). The algorithms incorporated equations describing lipid as well as water content for each tissue including plasma and red blood cells. In this current model, the experimentally determined PCs for CPF in plasma were measured using the modified method outlined by Murphy *et al*. (1995), described earlier in the Materials and Methods. The measured olive oil:plasma PC was calculated from the plasma:PrCarb and olive oil:PrCarb ratios (olive oil:PrCarb/plasma:PrCarb). The olive oil:plasma PC is considered an adequate surrogate for fat:plasma, as per Poulin and Theil (2000) and Murphy *et al*. (1995).

The theoretical tissue:blood PCs for a nonpregnant adult animal were determined using the QSPR outlined in Poulin and Krishnan (1995), with the substitution of $K_{oil:water}$ for $K_{octanol:water}$ as discussed in Poulin and Theil (2000) and are presented in Table 1. Gestational-related changes of CPF solubility in the blood were estimated semiempirically by adjusting the denominator of the Poulin and Krishnan QSPR (Poulin and Krishnan, 1995) and the QSPR for oil:water outlined in Bartels *et al*. (2008). To estimate the actual changes in PC values at GD 18 from nonpregnant animals, the nonpregnant values in Table 1 were adjusted by the same fold change as the *in vitro* experimentally derived nonpregnant and pregnant values at GD 18 (listed in Table 3). The PC values for CPF-oxon were estimated in the same manner and assuming the fold difference between nonpregnant and pregnant rats was similar to that of CPF. Finally, predictions of fat:blood PC values for gestational and corn-oil–dosed samples were calculated as discussed in Poulin and Krishnan (1995) and Poulin and Theil (2000) from the neutral and phospholipid concentrations, for comparison with the semi-empirically determined values and are presented in Table 3 and Figure 2D.

Validation of the Model against Available Data

Data to validate the fetal model, though limited, were used to demonstrate that the theories and concepts were logical and resulted in reasonable simulations. The data of Mattsson *et al*. (2000) were used to compare model output to the available data. Human data for CPF exposure and its potential effect on birth outcomes have been investigated in three studies, two in urban women and one in women living near an agricultural area (Bradman and Whyatt, 2005; Berkowitz *et al*., 2004; Eskenazi *et al*., 1999; Perera *et al*., 2003; Whyatt *et al*., 2002, 2004, 2005; Young *et al*., 2005). These studies have recently been reviewed by Needham (2005) and Zhao *et al*. (2005). In one of the studies conducted with women living in an urban environment (Perera *et al*., 2003; Whyatt *et al*., 2002, 2004), maternal blood was collected within 1 day postpartum and umbilical cord blood was collected at delivery. CPF was detected in 98% of the maternal and 94% of the cord plasma samples

TABLE 1
Rat PBPK Model Parameters

Parameter	NP rat	Late GD	Fetus	Source
Body weight (kg)	0.28	0.36	0.0045	Clewell *et al*., 2003
Percentage of body weight				
Blood	6	6	8.5	Gentry *et al*., 2003
Brain	1.2	1.2	15	Green *et al*., 1999
Liver	4	7	4	Clewell *et al*., 2003
Mammary	NA	4.4	NA	Fisher *et al*., 1989
Slowly perfused	70.8	66.4	NA	Calculated
Fetal body	NA	NA	72.5	Calculated
Flows (l/h/kg)				
Cardiac output	15	15	67	Clewell *et al*., 2003
Percentage of cardiac output				
Brain	3	3	3	Brown et al, 1997
Liver	25	25	4.5	Clewell *et al*., 2003
Mammary	NA	9	NA	Fisher *et al*., 1989
Placenta	NA	12.3	NA	Clewell *et al*., 2003
Fetal body	NA	NA	92.5	Calculated
Partition coefficients (CPF)[a]				
Brain:blood	16.5	12.5	12.5	
Diaphragm:blood	3.84	2.92	NA	
Fat:blood	250	190	NA	
Liver:blood	12.8	9.71	9.71	
Rapidly perfused:blood	7.67	5.83	NA	
Slowly perfused:blood	3.84	2.92	NA	
Mammary:blood	7.67	5.82	NA	Estimated = rapid
Placenta:blood	NA	5.82	NA	Estimated = rapid
Fetal body	NA	NA	2.92	Estimated = rapid
Partition coefficients (CPF-oxon)				
Brain:blood	5.64	4.28	4.28	
Diaphragm:blood	1.79	1.36	NA	
Fat:blood	75.2	57.1	NA	
Liver:blood	4.44	3.38	3.38	
Mammary:blood	2.96	2.25	NA	Estimated = rapid
Rapidly perfused:blood	2.96	2.25	NA	
Slowly perfused:blood	1.79	1.36	NA	
Placenta:blood	NA	2.25	NA	Estimated = rapid
Fetal body	NA	NA	2.25	Estimated = rapid
Metabolic rate constants				
CYP450:CPF → CPF-oxon				
KM (μM/l)	2.25	2.25	2.25	Optimized
VmaxC (μmol/h/kg)	14.3	10.7	5.4	Poet *et al*., 2003[a]
CYP450:CPF → TCP				
KM (μM/l)	3.5	3.5	3.5	Poet *et al*., 2003
VmaxC (μmol/h/kg)	60.9	45.7	22.9	Poet *et al*., 2003
A-est oxon → TCP (liver)				
KM (μM/l)	450	450	450	Optimized
VmaxC (μmol/h/kg)	38,002	38,002	380	Lassiter *et al*., 1999*
A-est oxon → TCP (blood)				
KM (μM/l)	450	450	450	Optimized
VmaxC (μmol/h/kg)	40,377	40,377	404	Lassiter *et al*., 1998*
Placenta → fetal transfer				
KfT (/h)	NA	0.5	NA	Optimized

Note. References are for pregnant and fetal models, parameters for the nonpregnant mode are from Brown *et al*. (1997) or Timchalk *et al*. (2002a, b).

[a]Metabolism rate constants estimated based on literature reports at 50% of adult activity for the fetus and 75% of nonpregnant activity for the dam. See text for more detail.

TABLE 2
Human PBPK Model Parameters

Parameter	Nonpreg	Late GD	Fetus	Source
Body weight (kg)	58	67.7	3.4	Mardones-Santander *et al*., 1998
Percentage of body weight				
Blood	6	6	8.5	Gentry *et al*., 2003
Brain	2	2	15	Roelfsema *et al*., 2004
Fat	21	26	NA	Young *et al*., 1997
Liver	3	4.6	4	Gentry *et al*., 2003
Mammary	0.5	1.7	NA	ICRP, 1992
Fetal body	NA	NA	72.5	Calculated
Flows (l/h/kg)				
Cardiac output	15	15	54	Gentry *et al*., 2003
Percentage of cardiac output				
Brain	11	11	11	Brown et al, 1997
Liver	23	23	4.5	Clewell *et al*., 2003
Mammary	NA	9	NA	Brown et al, 1997
Placenta	NA	12.3	NA	O'Flaherty *et al*., 1992
Fetal body	NA	NA	84.5	Calculated
Placenta → fetal transfer				
KfT (l/h)	NA	19	NA	Optimized

Note. References are for pregnant and fetal models, parameters for the nonpregnant model are from Brown *et al*. (1997) or Timchalk *et al*. (2002a, b).

with mean concentrations of 3.9 ± 4.8 and 3.7 ± 5.7 pg/g (~11 pmol/l), respectively. Because the raw data was not available from the Whyatt/Perara cohort, the equation they reported to derive umbilical cord CPF concentrations from the mother's blood samples (when the cord blood was not available) was used (Whyatt *et al*., 2004). This equation was used to predict concentrations from which the human placenta to fetal zero order transfer rate constant (KfT) was optimized.

Sensitivity Analysis

A sensitivity analysis was conducted in acslXtreme v2.4 (Aegis Technologies, Huntsville, AL) to identify the importance of parameters that affect estimates of rat blood CPF C_{max}. The analysis measured the change in model output corresponding to a 1% change in a given model parameter when all other parameters were held fixed for repeat oral exposure to 5 mg/kg CPF. A normalized sensitivity coefficient of 1 indicates that there is a one-to-one relationship between the fractional change in the parameter and model output; values close to zero indicate a small effect on model output. A positive value for the normalized sensitivity coefficient indicates that the output and the corresponding model parameter are directly related and a negative value indicates they are inversely related.

RESULTS

Partition Coefficient Determination of CPF with Lipid Altered Plasma

In vitro *partitioning of CPF into gestational rat and human plasma*. Previous reports have suggested that higher CPF levels just before birth may be partially attributable to the lipophilicity of CPF and the hyperlipidemia that occurs during pregnancy (Mattsson *et al*., 2000). To further investigate this claim the PC of CPF was measured with plasma collected from rats at GD 12, 15, and 18 and human plasma from pregnant females at first, second, and third trimesters. The measured plasma:PrCarb ratios and the olive oil: plasma ratios for CPF are listed in Table 3. Olive oil was determined as an acceptable alternative to fat (Poulin and Theil, 2000). As shown in Table 3, the plasma:PrCarb ratio increased by 29% on GD 18 plasma (rat), as compared with nonpregnant (NP), and 41% with third trimester plasma, as compared with nonpregnant women. Both show a corresponding decrease in the fat: plasma ratio. The

TABLE 3
The Average Measured Plasma:PrCarb Ratios, Olive Oil:Plasma Coefficients, Lipid Levels, and Predicted Fat:Blood Partition Coefficients for Rats and Humans throughout Gestation, and Rats Administered Corn Oil

	Measured		Lipid levels (mg/dl)					
	Plasma:PrCarb	Olive oil:plasma[a]	Cholesterol	Triglycerides	Total neutral lipids	Phospholipids	Total lipids	Calculated fat: blood PC[b]
Rat plasma								
Nonpregnant	0.017	129	68	162	230	153	383	209
GD 12	0.015	147	54	87	141	102	243	250
GD 15	0.017	129	48	106	154	100	254	244
GD 18	0.022	100	78	365	443	180	623	156
Human plasma								
Nonpregnant	0.017	129	159	124	283	182	465	178
Trimester	0.018	122	198	121	319	226	545	166
Trimester	0.021	105	255	229	484	247	731	135
Trimester	0.024	92	285	290	575	306	881	121
Rat plasma[c]								
Fasted rat	0.012	185	59.6	31.4	91	115	206	273
Corn-oil–dosed rat	0.014	152	53.4	111	165	119	283	237

[a]The oil:PrCarb partitioning ratio was 2.2. Olive oil was used as a substitute for fat.
[b]Fat:blood partition coefficient calculated with Poulin and Krishnan (1995) and Poulin and Theil (2000) QSPRs.
[c]Experimental values from plasma samples obtained 6 h after administration of corn oil.

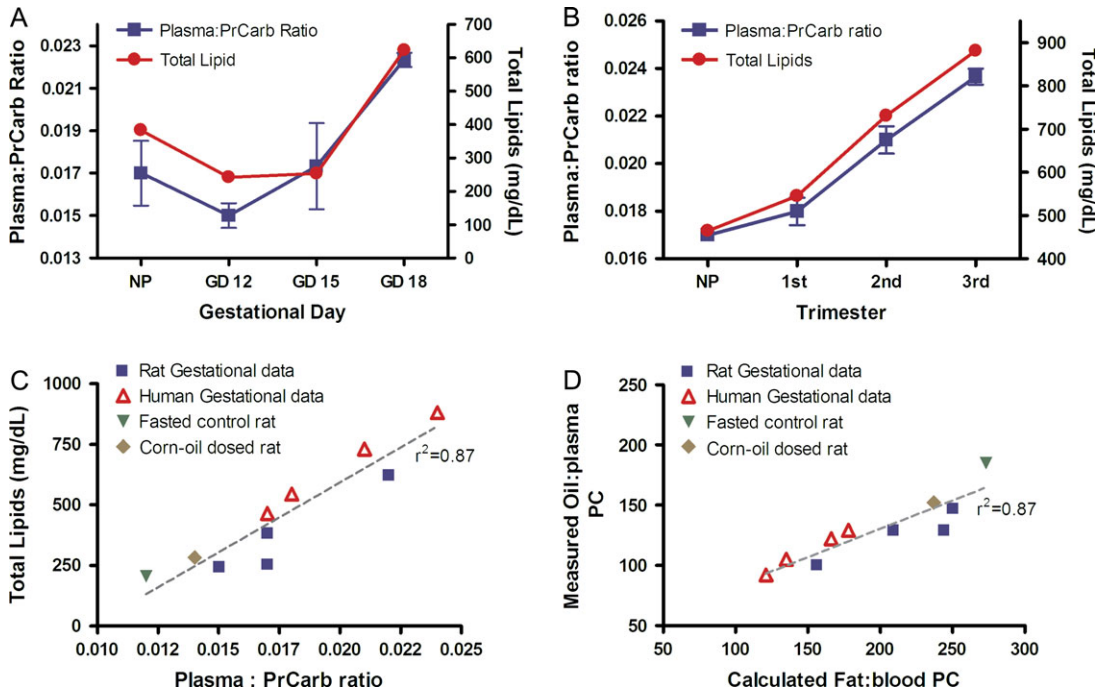

FIG. 2. The total lipid levels and measured CPF partition coefficients during gestation in rats (A) and humans (B). The red line and circles represent the total lipid in the pooled plasma samples ($n = 1$) and the blue lines and squares show the measured plasma:PrCarb ratio for CPF ($n = 3$); (C) overall correlation of plasma:PrCarb ratio versus total blood lipids; (D) correlation of measured oil:plasma PC versus predicted fat:blood PC.

levels of cholesterol, triglyceride, and phospholipids were also measured in each of the samples tested (Table 3). The lipids levels measured in rat and human plasma were consistent with published lipid levels (Johnson, 2004; McMullin *et al*., 2008).

The level of triglyceride increase at GD 18 in the rat was consistent with the levels reported previously (McMullin *et al*., 2008). The level of triglyceride increased 125%, whereas the plasma:PrCarb ratio only increased by 29% by GD 18 (Table 3). When the levels of cholesterol, triglycerides, and phospholipid levels were summed to generate the total lipid levels, the plasma:PrCarb ratio correlated well with the changes in total lipids for both rats and humans more closely than changes to plasma:PrCarb based solely on triglyceride changes (Fig. 2).

Fractionation of CPF-spiked dam and pup blood. To further investigate the role of blood lipids seen during gestation, rat blood was treated with either 3 or 100 ppb ^{3}H-CPF and fractionated by density using ultracentrifugation. Prior to ultracentrifugation, blood was separated into plasma and RBC by low-speed centrifugation in glass tubes. Good recoveries of ^{3}H-CPF were obtained in the initial low-speed centrifugation, with an overall recovery of 101 ± 7% of radioactivity recovered between the plasma and RBC. The distribution of ^{3}H-CPF between plasma and RBC changed during gestation, with a plasma/RBC distribution of CPF increasing from approximately 55/45 in nonpregnant rats to approximately 75/25 on GD 20 (Fig. 3). Ultracentrifugation of the plasma yielded an average recovery of 73 ± 4% radioactivity when summed among the individual fractions, with the loss likely attributed to absorption onto the plastic surfaces of the ultracentrifuge tubes. Fractionation of the plasma showed an increase in the amount of CPF sequestered into the low-density fraction of the plasma in later stages of pregnancy, as shown in Figure 3. The relative amount of CPF in the RBC and high-density fraction during late-stage gestation decreased. The amount of radioactivity associated with the relatively large volume of the infranatant and the small solid gelatinous pellet (~30 mg) that precipitated at the bottom of the ultracentrifuge tube never exceeded 5% of total recovered radioactivity (Figs. 1 and 3). At GD 21 (or PND 0) there was a sharp decrease in levels of CPF in the low-density fractions. The neutral lipids of triglyceride and cholesterol are components of the low-density fraction (Mills *et al*., 1984; Noto *et al*., 2006). McMullin *et al*. (2008) showed that during late-stage gestation, there was an increase in triglyceride levels, but little change in cholesterol in the blood of rats. At birth and during early postnatal period, the triglyceride levels return to nonpregnant levels which also correlate with a decrease in CPF sequestered in the low-density fraction of blood. The data at GD 0 represents a nonpregnant rat.

CPF partitioning in plasma from corn-oil–dosed rats. Because the level of triglycerides is one component that drives the partitioning of CPF into plasma and blood, the next step was to evaluate other laboratory-based sources of blood lipid changes on tissue:blood partitioning. Administration of vegetable (i.e., corn) oil has been reported to alter blood chemistry profiles. We therefore investigated the effect of dosing pure corn oil (predominately triglycerides) to a rat to determine the effect of

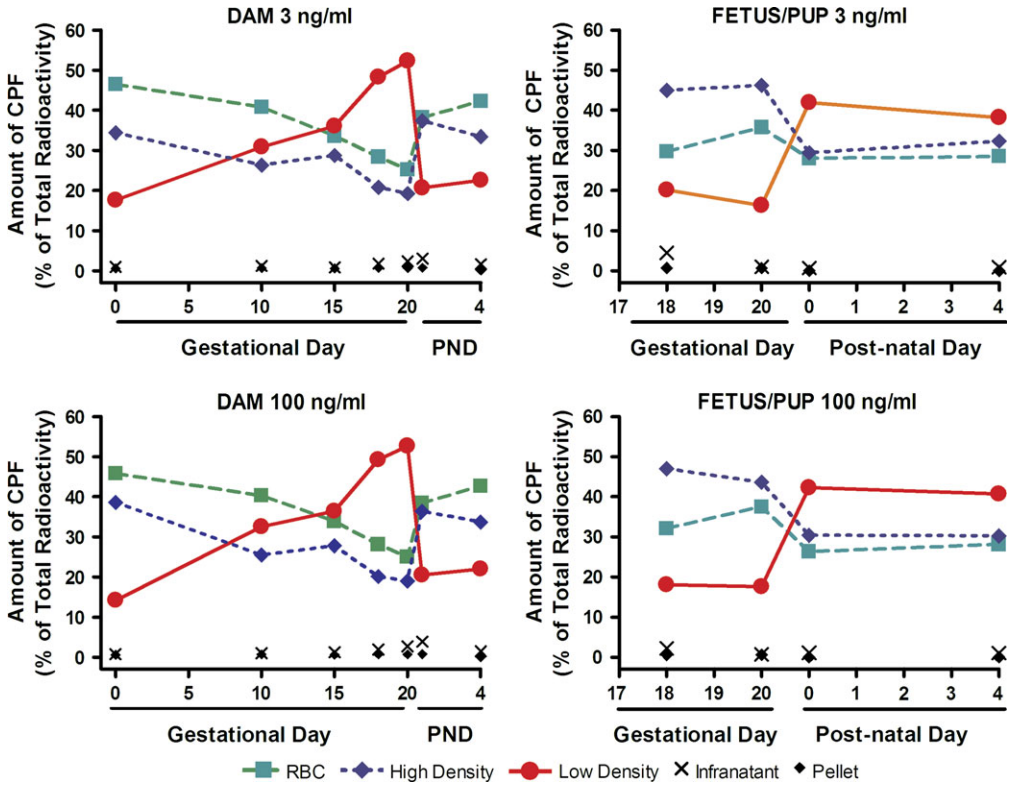

FIG. 3. The distribution of 3 and 100 ppb CPF into fractionated blood from dams, fetuses, and pups throughout late-stage gestation and early lactation. The red line (circles) shows the amount of CPF associated with the lipids of the low-density fraction. The blue diamonds (blue line) are the CPF amounts in the fraction containing high-density lipids, whereas the green squares (green line) shows the RBC fraction. The black diamonds and the crossed symbols show the CPF levels in the pellet and the infranatant, respectively.

partitioning of CPF. First, the effect of corn oil was determined on the cholesterol, triglyceride, and phospholipid levels in fasted rats dosed with corn oil compared with levels in fasted control rats (Table 3, Fig. 4). There were no substantial differences in the relative changes of cholesterol and phospholipid levels (Figs. 4A and 4C). However, the triglyceride levels increased up to threefold higher than control after dosing with corn oil (Fig. 4B). Because the partitioning of CPF correlates with total lipids, the data shown in Figures 4A-C were summed to determine the changes in total lipids 12 h after dosing (Fig. 4D). There was a 40% increase in total lipids at 6 hours after corn oil dosing (Fig. 4E). Therefore, three rats were dosed with corn oil and sacrificed at 6 hours and the partitioning of CPF into the 6-h plasma was measured as compared with control rats. The results are shown in Table 3 and Figure 4F. The measured plasma:PrCarb ratio from the corn-oil–dosed rats was moderately higher (17%) than the control animals (0.014 vs. 0.012) which results in lower fat:plasma coefficient after corn-oil dosing (Fig. 4F, Table 3). Although this change was not statistically significant, it was consistent with the results of the *in vitro* partitioning in gestational plasma, where a 63% increase in total lipids was associated with a corresponding statistically significant decrease in olive oil: plasma partitioning (Table 3). The changes in the plasma:PrCarb ratios with total lipids in this experiment are consistent with the gestational-based changes seen in the in vitro partitioning experiment. As shown in Figure 2C, the overall correlation between the datasets was high ($r^2 = 0.87$), indicating that the pregnancy-related changes in plasma partitioning is highly dependent on blood lipid levels. Comparison of the measured olive oil:plasma PC values to the predicted fat:blood PC was also done and presented in Figure 2D. These measured PC values from gestational or oil-dosed plasma samples correlated well with fat:blood partitioning predicted from the published QSPRs ($r^2 = 0.82$), showing the accuracy of the QSPR approach. The absolute PC values predicted from the QSPRs (Table 3) were also fairly consistent with the semiempirically determined ones (Table 1), with nonpregnant rat fat:blood ratios of 250 (predicted) comparable to nonpregnant fed or fasted rats (209–273). These results show that the semiempirical PC values determined from this study were appropriate for use in the gestational PBPK model.

Protein Binding of CPF to Rat and Human Serum Albumin

To evaluate the effect of protein binding factor into the estimation of the CPF PC, the change in the percent of unbound CPF with increasing concentration of human serum

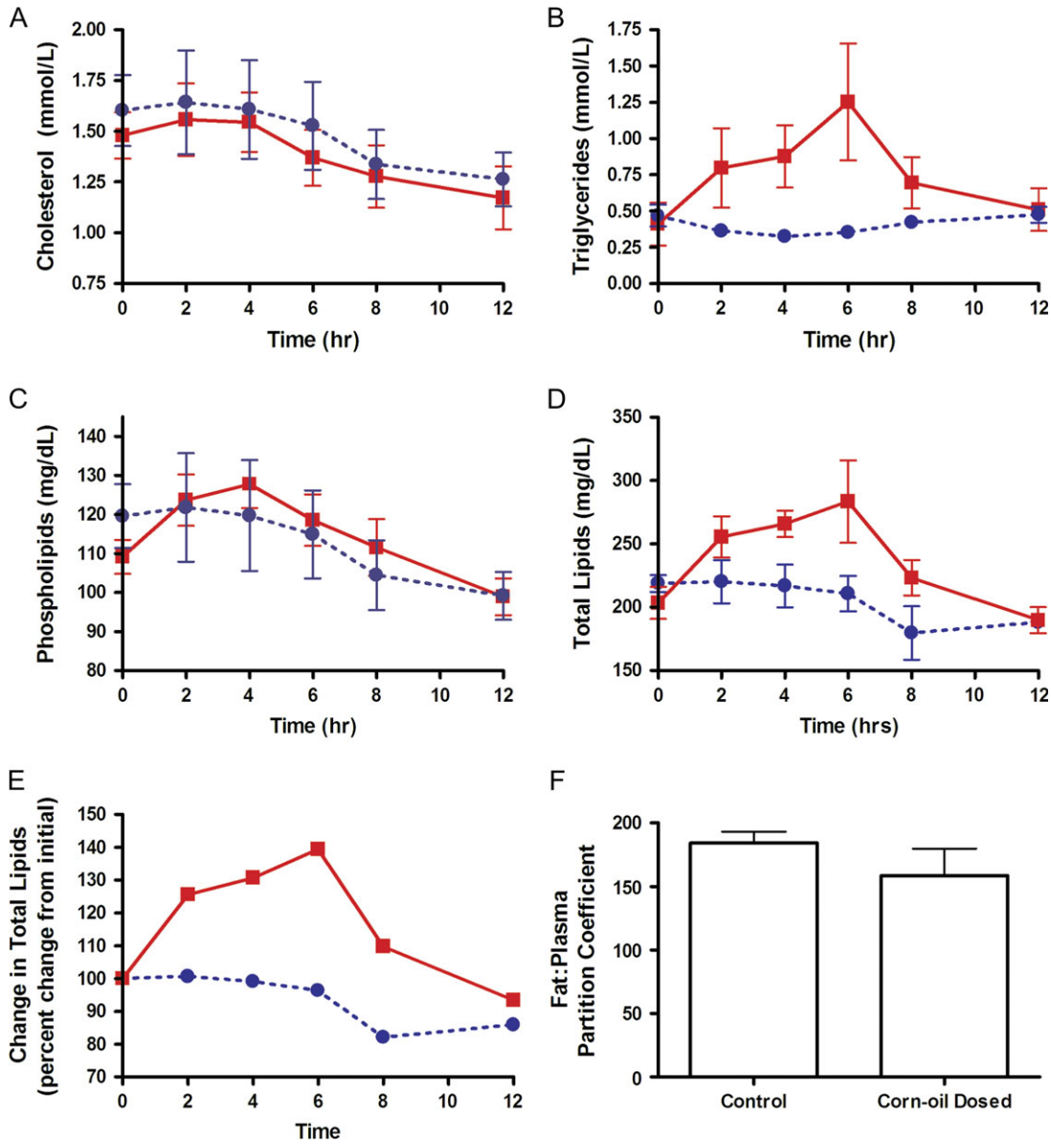

FIG. 4. The effect of corn oil on cholesterol (A), triglycerides (B), phospholipids (C), and total lipid levels (D, E) in plasma and CPF partition coefficients (F). The red squares and lines are the measured values from the corn-oil–dosed rats. The blue circles and lines are the control rats. The plasma:PrCarb ratios were determine using plasma collected at 6 h after dosing with corn oil. The values are the mean ± SD ($n = 4$).

albumin and rat serum albumin was measured (Fig. 5) at concentrations ranging from 0.04 to 20 mg/ml (reference values = 32 and 42 mg/ml in rat and human, respectively; Davies and Morris, 1993). Albumin was selected as a surrogate protein for the nonlipophilic blood proteins, due to its predominance (~50% of total blood protein) and commercial availability of rat and human sequences. The CPF was highly bound, with 2% of CPF unbound in the presence of 20 mg/ml rat or 20 mg/ml human serum albumin at both low (3 ng/ml) and high concentration (100 ng/ml) of CPF, indicating comparable binding between species. Attempts were made to measure the CPF-oxon binding to human serum albumin to aid in PBPK model development; however, there was considerable hydrolysis (> 85%) of CPF-oxon to TCP in the presence of the albumin (data not shown). This finding is consistent with previous reports of the rapid loss of the CPF-oxon in the presence of blood and serum albumin (Brzak *et al.*, 1998; Sogorb *et al.*, 2008).

CPF PBPK Model

In addition to the *in vitro* partitioning study, a late gestational PBPK/PD model to describe the dosimetry of CPF and its metabolites and the resulting changes in cholinesterase inhibition was developed to use as a tool to

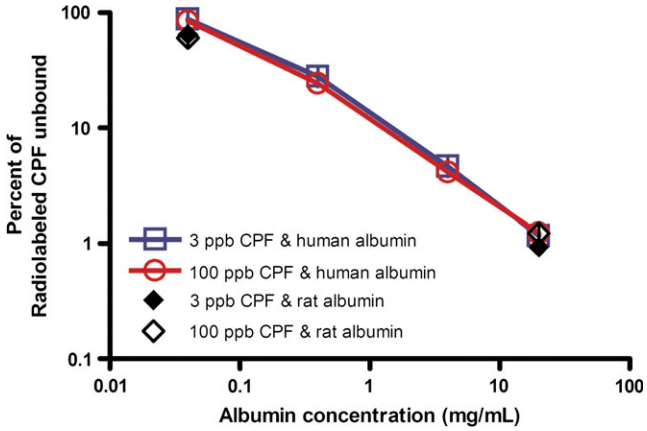

FIG. 5. The measured unbound fraction (f_u) of CPF with increasing concentrations of human and rat albumin. A 3 ppb (blue line, squares) and 100 ppb (red line, circles) concentration of CPF were evaluated with human serum albumin. The black symbols show the unbound fraction of 3 ppb (closed diamonds) and 100 ppb (open diamonds) of CPF with increasing concentrations of rat serum albumin.

test the hypothesis that lipid changes in the blood occurring during late pregnancy result in changes in CPF pharmacokinetics and pharmacodynamics in pregnant rodent dams and their fetuses. To test this hypothesis, only minimal changes in model parameters used in the original, nonpregnant model were made to describe the pregnant animals. These changes included static descriptions of major physiological changes occurring during late pregnancy, measured and estimated changes in enzyme activities, and an optimized maternal to fetal transfer rate constant (Table 1). With the exception of the fetal transfer rate constant, all other parameters were held constant and obtained from the previously published model (Timchalk *et al*., 2002b) through experimentation (Kousba *et al*., 2004, 2007; Lowe *et al*., 2006; Poet *et al*., 2003), or from the literature (see Tables 1 and 2). This approach with set parameters allowed us to investigate the effects of a blood partitioning in the dam/mother on both target tissue dosimetry and response to lipophilic compounds such as CPF. Although tissue volumes were changed to describe pregnancy (Clewell *et al*. 2003; Gentry *et al*. 2002, 2003). Other potential physiological changes suggested by this research, including potential differences in protein binding, as well as some physiological changes such as increased body water volume were not included.

Pregnant rat dosimetry. The model was used to compare the time course for blood CPF kinetics in the dam and fetus predicted with blood partitioning values determined for nonpregnant and late GD pregnant rats (Fig. 6). Application of this 1.3× higher blood partitioning result in a concomitant decrease in tissue partitioning and at the doses investigated (1–5 mg/kg/day), a linear increase in peak CPF concentrations in the blood. This apparent increase is blood CPF was

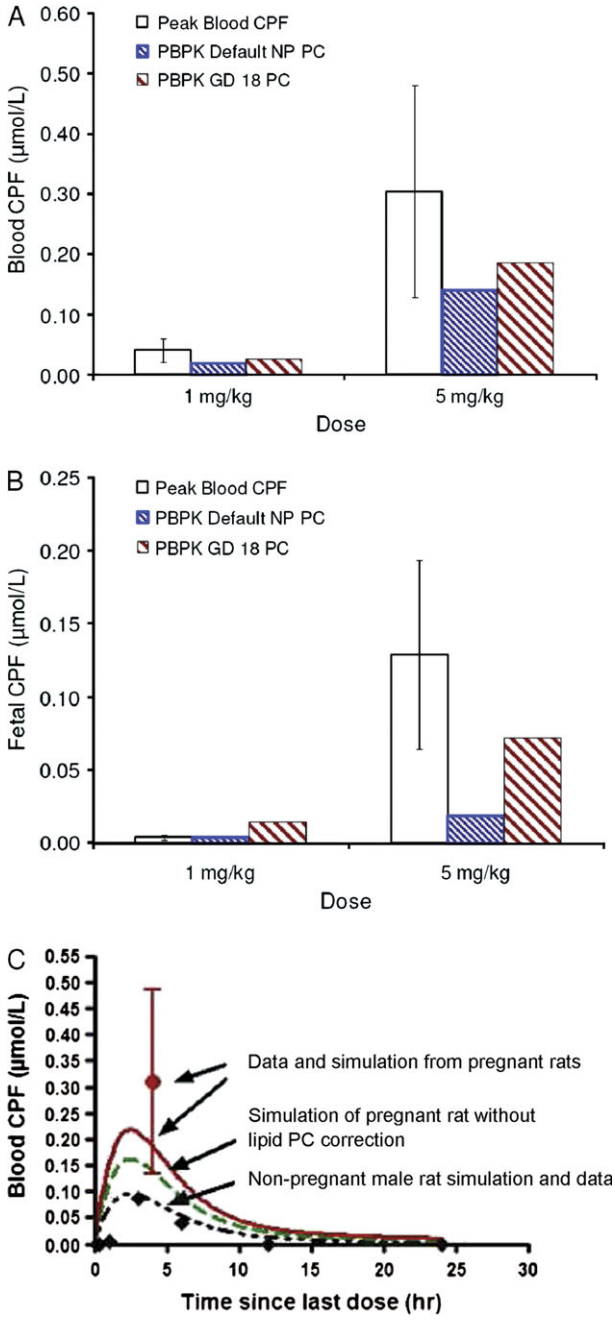

FIG. 6. Model predictions of dam (A) and fetal (B) CPF blood concentrations 4 h after oral dosing with 1 or 5 mg/kg CPF (~4 h) from Mattsson *et al*., 2000, mean and SD), and model predictions using the default, nonpregnant partitioning, or the change in predicted blood CPF resulting from a 1.3× increase in blood:buffer partitioning (). The model fits to the 5 mg/kg dose data are within, or below, the range of the actual data, but at the 1 mg/kg dose, the model predicts higher blood levels than were measured. (C) Output of the model vs. time showing the fit of the default NP model to the male rat data given in Mattsson *et al*. (2000) (dashed line, filled triangles), what the default pregnancy model predicts overtime (dash/dot line), and the pregnancy model with the addition of altered partitioning due to increased lipid levels (solid line, compare to filled circle). Note, no error bars shown for fetal concentrations, SD could not be determined from the data as provided by Mattsson *et al*. (2000).

consistent with the limited available data in pregnant rats. Applying the nonpregnant blood partitioning to the model, the predicted peak blood concentrations in the dam and fetus following a 5 mg/kg dose were approximately fourfold lower than the observed concentration (Mattsson *et al*., 2000), with the largest errors in the dam and fetal blood concentrations at 5 mg/kg/day. Applying a 1.3-fold higher blood partitioning from GD 18 results in a commensurate decrease in tissue: blood partitioning and leads to an increased predicted blood C_{max} from ~ 0.1 to ~ 0.14 μmol/l (Fig. 6A). Using the pregnancy-specific partitioning, the model fit both the 1 mg/kg/day and the 5 mg/kg/day data within a factor of 2–3, but because the model predicts a linear response in this range and the data is nonlinear, the model still under predicts the data from the high dose. In comparison, the model fits to the fetal blood concentration data from Mattsson *et al*. (2000) were substantially improved using the pregnancy-specific partitioning to describe the dam blood. As shown in Figure 6B, predicted 5 mg/kg/day fetal blood CPF concentrations, using the appropriate PCs, at 4 h after dosing, are 0.07 versus 0.02 μmol/l with the classic parameters. This value of 0.07 is quite consistent with the experimental value of 0.13 μmol/l. Overall, this updated model improves the prediction of both dam and fetal CPF blood levels within a factor of 2–3 at both experimental dose levels of 1 and 5 mg/kg/day.

Sensitivity Analysis

The goal of this analysis was to use the model as a tool to investigate some of the potential factors that may impact the pharmacokinetics in pregnancy, and in particular the maximum CPF blood concentration (C_{max}). The placental-fetal transfer rate had a strong effect on fetal blood levels, but because the dam blood levels are greater than fetal, it had almost no effect on maternal blood concentration (data not shown). Placental blood flow affected both dam and fetal blood concentrations equally and maximally shortly after dosing with a maximal coefficient of about 0.4.

The PCs describing the differential solubility of CPF between the tissue and blood are calculated by the following equation:

$$\mathrm{PC}_{\mathrm{tissue:blood}} = \frac{\mathrm{PC}_{\mathrm{tissue:buffer}}}{\mathrm{PC}_{\mathrm{blood:buffer}}}$$

where tissue can be any tissue compartment (e.g., fat). The sensitivity to tissue:blood partitioning was determined by observing the effect of changing the $\mathrm{PC}_{\mathrm{blood:\ buffer}}$ denominator on plasma CPF, therefore, the $\mathrm{PC}_{\mathrm{blood:\ buffer}}$ sensitivity represents a composite of the effects of all of the tissue:blood partitioning changes. The sensitivity coefficients were > 1 at peak (~3 h postdosing) blood CPF concentrations or when the fat greatly exceeded the blood CPF concentration (Fig. 7). At 5 mg/kg, blood CPF concentrations were less sensitive to hepatic V_{max} for the metabolism to TCPy or CPF-oxon.

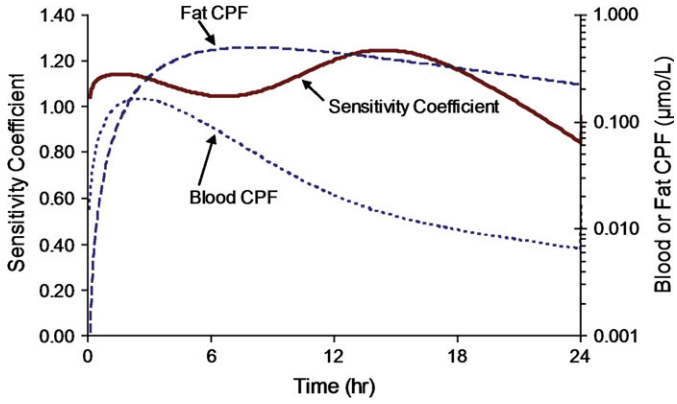

FIG. 7. Normalized sensitivity of plasma CPF concentration to the blood:buffer partition coefficient. The sensitivity of model output can be used to estimate the importance of that parameter. The model is highly sensitive to the blood: buffer partition coefficient. The blood and fat concentrations are shown for comparison (right axis). Early in the simulation, the sensitivity of the blood: buffer partitioning is likely to be driven by the blood concentration, later tissue concentrations become more important.

Human Late Gestational Model

Prior to using the model to estimate human exposure doses and the effects of pregnancy on maternal and fetal cholinesterase inhibition, the updated model parameters (see "Materials and Methods": *Partition Coefficients*) were applied and the model output was compared with the simulations from the original model (Timchalk *et al*., 2002b). The model fits to the human data were either not different or slightly improved over the 2002 publication (data not shown). The late GD pregnancy model was then used to evaluate the impact that changes in lipid partitioning, blood flow and tissue volume have on the concentrations of CPF in the blood of pregnant humans based on the data of Whyatt *et al*. (2002, 2004, 2005), where CPF was measured in the plasma and cord blood of urban mothers shortly after delivery. To conduct these estimations, it was assumed that the mothers were exposed to equal concentrations of CPF 3×/day for 10 days prior to parturition (to achieve periodicity) and blood partitioning was assumed to be equal to late gestation rat blood partitioning. The model was then used to estimate doses that would lead to the blood concentrations observed in the women.

Whyatt *et al*. (2005) measured umbilical cord blood as "close to delivery" as possible, and reported an average of 10.6 ± 16.3 pmol/l, in the 64% of cord blood samples in which CPF was detected. Unlike the rat at high doses, where the fetal blood CPF concentrations were approximately half of the dam blood concentrations, cord blood CPF was reported to be ~95% of the mothers' blood concentrations. Whyatt *et al*. (2005) reported using an equation to extrapolate from available cord blood data to estimate cord blood concentrations in missing samples. Using this equation, fetal cord blood concentrations are predicted to vary from ~25% at lower concentrations to 100% of the maternal concentrations. Extrapolating the

placenta to fetal transfer rate to use in the human model (Transfer rate = Constant × Body weight$^{0.25}$) resulted in an estimated transfer rate of 1.9/h. The application of this rate significantly underpredicted the fetal blood data available from Whyatt *et al.* (2002, 2005). Therefore, unlike the rat data, the transfer to the human fetus was dependent upon placental blood flow (see Fig. 8A). However, to maintain a consistent model structure, a transfer rate of 19/h, sufficiently high to not limit fetal blood concentration based on diffusion, was needed to fit the data. It should be noted, however, that the human studies compare maternal to cord blood, which is physiologically different than the rat fetal compartment.

The model predicted maternal and fetal blood CPF concentrations within the range of the mean maternal and cord blood concentrations reported by Whyatt *et al.* (2005) following three daily doses of 0.05 μg/kg, or 0.15 μg/kg/day (Fig. 8). Due to the higher blood partitioning in pregnant women, the model predicts a ~1.3-fold higher area under the blood concentration time curve (AUC) for CPF in exposed pregnant women in comparison to nonpregnant women (Fig. 8B).

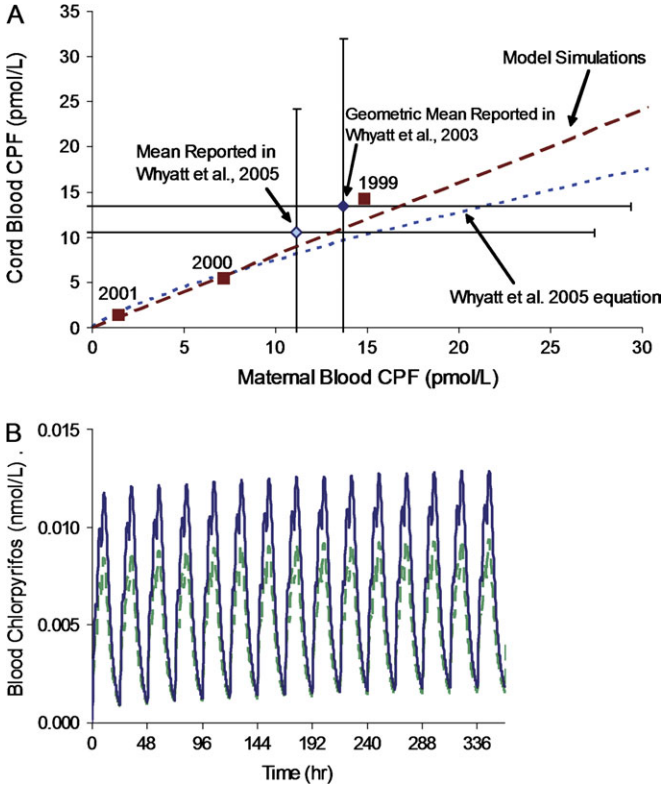

FIG. 8. (A) Simulated oral daily dosing to pregnant women to achieve the approximate blood CPF concentrations measured in the maternal (x-axis) and cord (y-axis) blood (Whyatt *et al.*, 2005) data points represent data from different sampling years, as indicated. It was assumed that three evenly spaced oral exposure episodes occurred/day. To obtain fetal concentrations as measured, the transfer to the fetus had to be described as blood flow limited. (B) Model output of a nonpregnant individual (dashed line) exposed to the same dose in (A) is shown for comparison. The solid line is the simulation for the same (pregnant) individual.

Because the current PBPK model still underestimates CPF blood concentrations in pregnant rats, it is possible the model over estimates the environmental CPF exposure in Whyatt *et al.* (2005). The current model estimates were comparable if somewhat higher than the estimates of environmental CPF exposures of adults (upper 95 percentile) by Barr *et al.* (2005), from CDC measurements of urinary TCP, was 0.036 μg/kg/day.

DISCUSSION

The distribution of drugs and other exogenous chemicals into tissues are heavily dependent on the ratio of solubility between plasma and tissues (Poulin and Theil, 2000). The pharmacokinetics of drugs and chemicals are clearly altered during pregnancy (Dawes and Chowienczyk, 2001; Little, 1999). Blood chemistry in pregnant rats and humans show a similar dynamic trend, such that plasma lipid content is fairly stable in early gestation (up to about 10 days in the rat and about 10 weeks in a human) and shows a consistent increase in later gestation (LaBorde *et al.*, 1999; McMullin *et al.*, 2008; Sattar *et al.*, 1997; Saarelainen *et al.*, 2006).

CPF is a widely used organophosphorus insecticide (Mattsson *et al.*, 2000). The possible relationship between elevated blood lipids during pregnancy and blood concentrations of CPF has been reported (Mattsson *et al.*, 2000). In this study by Mattsson *et al.* (2000), the authors suggested that higher CPF levels just before birth may have been at least partially related to the lipophilicity of CPF and the hyperlipidemia that occurs during pregnancy. Even though there is a three to fourfold increase in triglyceride levels during the late-stages of gestation (McMullin *et al.*, 2008), we report that the changes in partitioning of CPF into plasma trends more closely with overall lipid levels rather than the changes in the triglyceride levels (Fig. 2). This finding is consistent the in vitro blood fractionation work, in which the proportion of CPF associated with the lipid-containing fraction of whole blood increases through late gestation (Fig. 3). This finding also correlates with previous reports looking at pharmacokinetics of chemicals after high-fat meals (Phillips *et al.*, 1989; Sandanger *et al.*, 2003; Shayeganpour *et al.*, 2005). For instance, Phillips *et al.* (1989) reported a 22–29% increase in PCB, hexachlorobenzene, and DDE levels which correlated with total lipids levels in serum after feeding. Phillips *et al.* (1989) reported a 121% increase in triglyceride levels; however, when these authors normalized the increase chlorinated hydrocarbon levels to triglycerides, this correction overestimated hydrocarbon levels in the blood. Normalization to normal lipid levels gave a better estimation of hydrocarbon concentration in the blood. In addition, Gershkovich and Hoffman, (2007) show that the increases in triglycerides and lipid levels are responsible for drawing the lipophilic compound DDT from peripheral tissues into the blood compartment.

Our data also show that predosing with pure vegetable oil, a common dosing vehicle for lipophilic compounds, increases

total lipids ~ 70 mg/dl above control rats, due primarily to increased triglycerides (Figs. 4B, 4D, and 4E). Ramirez *et al.* (1984) and Gershkovich and Hoffman, (2007) also report increases in triglyceride levels following dosing with corn or peanut oil, with peak triglyceride levels occurring 4–6 h postdosing, and the increase above background found by Gershkovich (~120 mg/dl), similar to what was seen in the current study (Fig. 4). As seen in Figure 4F, there was a moderate decrease in fat:plasma partitioning of CPF in corn-oil–dosed rats compared with control rats. The absolute value of the plasma:PrCarb ratio from this experiment with plasma from fasted rats (0.012) was lower than the values of the plasma:PrCarb ratio (0.017) for nonfasted rats reported in Table 3 and Figure 2, consistent with the lower total lipid concentrations (206 vs. 383 mg/dl, respectively). Utilization of nonfasted animals would be expected to show a greater increase in lipid levels, as reported by Gershkovich and Hoffman, (2007) and therefore a greater concentration of CPF and a greater increase in the plasma:PrCarb ratio. Overall, our findings are consistent with other reports in the literature which show an increase in a lipophilic chemical concentration after an increase in lipid levels in the blood, but not with increased triglycerides (Phillips *et al.*, 1989; Sandanger *et al.*, 2003).

The QSPR explained in Poulin and Krishnan (1995) and Poulin and Theil (2000) have previously been popular in estimating PCs for a number of chemicals including CPF (Timchalk *et al.*, 2002b). Adjusting the Poulin and Theil QSPR with the twofold increase in triglyceride and cholesterol levels reported in McMullin *et al.* (2008) leads to a 35% difference in PC of fat: blood. This is consistent with the change seen in Table 3 and the fat: serum coefficients of the lipophilic polybrominated biphenyls in nonpregnant and pregnant women reported by Haddad *et al.* (2000) and Eyster *et al.* (1983). Whether such a change can be applied to all lipophilic chemicals ($\log K_{ow} > 4$) needs to be determined. Different lipophilic chemicals behave differently to changes in lipid levels (Gershkovich and Hoffman, 2007). The PCs calculated by Haddad *et al.* (2000) did overestimate the total partitioning (Lowe *et al.*, unpublished data).

In addition, the binding to albumin needs to be accounted for in estimating partitioning in blood and plasma. CPF is highly bound to serum albumin at physiological concentrations (Fig. 5). However, the gestational-based changes in plasma partitioning, as shown in Table 3, would also encompass pregnancy-related alternations in plasma protein levels, because actual plasma samples from pregnant rats and human donors were used for this experiment. Because the overall changes in plasma partitioning correlated well with total blood lipid levels (Fig. 2C), it appears that the lipid levels in the blood during gestation should be taken into account when understanding the pharmacokinetics of lipophilic compounds like CPF.

The partitioning values for erythrocytes and plasma, calculated as per the Poulin (Poulin and Krishnan, 1995; Poulin and Theil, 2000) publications were quite comparable for CPF (Peryth = 1.96, Pplasma = 1.94; data not shown). As a result, any changes in hematocrit would not be expected to alter tissue:blood ratios for this compound.

Although there are no studies showing concurrent dosing in pregnant and nonpregnant rats, several studies of gavage doses of CPF in either pregnant or nonpregnant rats indicate similar dose-responses for clinical signs and blood cholinesterase inhibition for doses lower than 1 mg/kg. The repeated-dose brain ChE no-observed-effect level appears to be about 1 mg/kg/day in both pregnant and nonpregnant rats. At higher doses there is a possibility of an $< 2\times$ increase in sensitivity in pregnant rats (Farag *et al.*, 2003; Hunter *et al.*, 1999; Lassiter *et al.*, 1999; Mattsson *et al.*, 2000; Maurissen *et al.*, 2000a,b; Nostrandt *et al.*, 1997; Ouelette *et al.*, 1983a,b; Rubin *et al.*, 1987; Zheng *et al.*, 2000). Because the model predicts CPF and the active oxon are partitioning less into tissues and remaining in the blood, the model estimates ~1% less inhibition in the brain following a 5 mg/kg dose in dams with the higher pregnancy-derived blood partitioning.

In pregnant animals exposed to CPF (Mattsson *et al.*, 2000), the fit of the model to the limited blood CPF data is improved by using the higher blood partitioning. This modeling effort emphasized blood chemistry changes, however many other parameters are also dynamic during pregnancy and the differences between predicted and observed may be due to other pregnancy-specific physiological changes. The transfer of CPF to the human fetus was optimized by inferring data from an equation derived from a regression analysis of data from urban women and cord blood soon after birth (Whyatt *et al.*, 2005). Because the PBPK model uses a first-order rate to describe the transfer between dam and fetus, the relationship between cord blood and dam blood is predicted to be linear. Using the equation described in Whyatt *et al.* (2004), the maternal-cord ratio would be expected to increase with maternal CPF concentration (see Fig. 8).

Just as in the rat, the PBPK model simulations of the AUCs for CPF in the human maternal blood as compared with a nonpregnant individual over the last 24 h of a repeated daily exposure indicate that the AUC of CPF will be higher in pregnant women than in nonpregnant individuals following the same exposure. The higher blood lipid content results in a sequestering of the CPF in the blood, however, this does not translate to higher tissue concentrations. This is in agreement with the suggestion by Zhao *et al.* (2005) that the exposure levels expected from these epidemiological studies are unlikely to be the cause of the alterations in birth weight and length among this urban cohort. Appropriate estimation of lipophilic chemical concentration in the blood, like CPF, is best done by correcting for blood lipid levels (Jaraczewska *et al.*, 2006; Johnson, 2004). Based on published reference data, average newborn blood lipids are fourfold lower than maternal lipid levels and will therefore have lower concentrations of lipophilic chemicals in their blood (Johnson, 2004; Sala *et al.*, 2001; Schecter *et al.*, 1998). The limited human data

for CPF (Whyatt *et al*., 2002, 2005), in which maternal and cord blood levels were reported approximately equal, is inconsistent with this rationale and required an unusually high transfer rate (as compared with rats) to the fetal compartment to fit the reported CPF neonatal blood concentration. Additional investigations on the diffusion transfer of CPF across the placenta will give greater insight into accurately estimating exposure levels in the fetus and newborn.

Birth outcome has also been correlated with maternal fatty acid status, and fatty acid status is related to the hyperlipidemia observed during pregnancy (Jensen, 2006; Velzing-Aarts *et al*., 2001). Because measurements of lipophilic chemicals in plasma or blood are affected by lipid profiles, this introduces a critical issue in biomonitoring studies. Higher levels of lipophilic chemicals in blood or plasma can be the result of higher levels of exposure and/or due to higher levels of blood lipids. That is, measurement of elevated but still very low levels of lipophilic chemicals in human blood may be a surrogate measure of elevated plasma lipids. The correlation of a health outcome to concentrations of lipophilic chemicals in blood may actually point to lipid profiles as the real endpoint of concern. As a consequence, it is not possible to interpret the meaning of slightly elevated levels of lipophilic chemicals without a thorough evaluation of people's lipid profiles.

This work was focused on understanding the implications of blood CPF partitioning changes during pregnancy and what impact these changes may have on dosimetry in both the pregnant mother and her developing fetus. Only a small number of researchers have investigated the link between blood lipid or lipoprotein and pharmacokinetics of drug (Bassissi *et al*., 2006; Ramaswamy *et al*., 1999; Wasan and Kwong, 1997). Biologically based models for pregnancy have been developed and do incorporate scaling equations to account for many physiological changes (reviewed in Corley *et al*., 2003). To date, however, blood lipid changes have not been considered in these models although it is well known that blood lipids strongly affect the dosimetry of lipophilic chemicals and drugs (Haddad *et al*., 2000; Needham, 2005; Procyshyn *et al*., 2003). Although lipid content in blood increases in both rats and humans during pregnancy, there are species-specific differences in lipid profiles (Ramaswamy *et al*., 1999). The current model applied measured rat blood partitioning to estimate human dosimetry. The current model, however, still under predicts the experimentally measured levels of CPF in blood of pregnant rats. In the future, it would be beneficial to investigate lipid profiles in rats and humans and determine the partitioning of CPF in the different lipid fractions to facilitate species extrapolations. In addition, because PON1 is associated with the HDL fraction of lipids, the profiles of HDL in the different species may be important. A more comprehensive understanding of species-specific lipoprotein concentration and profiles over the course of development will be necessary to understand and predict solubility in blood and tissues dose-response relationships in the fetus and mother.

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