Physiologically based pharmacokinetic modeling in pregnancy: Model reproducibility and external validation

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NCT02793700. All patients provided written informed consent. The authors confirm that the PI for this study is Dr. David Haas and that he had direct clinical responsibility for patients.

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Abstract

Aim: Physiologically based pharmacokinetic (PBPK) models have been previously developed for betamethasone and buprenorphine for pregnant women. The goal of this work was to replicate and reassess these models using data from recently completed studies.

Method: Betamethasone and buprenorphine PBPK models were developed in Simcyp V19 based upon prior publications using V17 and V15. Ability to replicate models was verified by comparing predictions in V19 to those previously published. Once replication was verified, models were reassessed by comparing predictions to observed data from additional studies in pregnant women. Model performance was based upon visual inspection of concentration vs time profiles, and comparison of pharmacokinetic parameters. Models were deemed reproducible if parameter estimates were within 10% of previously reported values. External validations were considered acceptable if the predicted AUC and C\text{max} fell within 2-fold of the observed.

Results: The betamethasone model was successfully replicated using Simcyp V19, with ratios of reported (V17) to reproduced (V19) C\text{max} of 0.98-1.04 and AUC of 0.95-1.07. The model-predicted AUC ratios ranged from 0.98-1.79 compared to external data. The previously published buprenorphine PBPK model was not reproducible, as we predicted IV clearance of 70% that reported previously (both in Simcyp V15).

Conclusion: While high inter-study variability was observed in the newly available clinical data, the PBPK model sufficiently predicted changes in betamethasone exposure across gestation. Model reproducibility and reassessment with external data are important for the advancement of the discipline. PBPK modeling publications should contain sufficient detail and clarity to enable reproducibility.
What is already known about this subject:

- Physiologically based pharmacokinetic (PBPK) models are commonly utilized to predict changes in PK in special populations, such as pregnant women.
- Reproducibility and external validation are critical to fully verify a PBPK model and establish credibility of pharmacometrics as a discipline.
- Few studies have reported on reproducibility in PBPK modeling.

What this study adds:

- Two examples of PBPK model reproducibility are provided demonstrating that reproducible models can be employed to enhance further research while lack of reproducibility impedes expansion and engenders a lack of credibility in PBPK modeling and simulation.
- A PBPK model of betamethasone was reproduced and sufficiently described pharmacokinetics for two additional studies of betamethasone in pregnant women. However, a PBPK model for sublingual administration of buprenorphine was unable to be replicated, demonstrating importance of providing sufficient and clear details in publications.
- Recommendations are provided to enhance publication of reproducible PBPK models.
Introduction

While a large majority of pregnant women require medications during their pregnancy, [1, 2] they have historically been excluded from clinical studies. [3, 4] However, it is recognized that pregnancy leads to alterations in pharmacokinetics and pharmacodynamics of drugs. In addition to anatomical changes due to placentation and the developing fetus, pregnancy leads to a number of physiologic changes that can impact pharmacokinetics. Cardiac output increases, as does renal blood flow, leading to enhanced glomerular filtration. [5, 6] Plasma albumin and alpha-1 acid glycoprotein concentrations decrease, leading to increases in unbound fraction (fu) of some drugs. In general, hepatic enzymes, including CYP3A, CYP2D6, CYP2B6, and UGTs, are induced. [7-9] However, the activity of some drug metabolizing enzymes, such as CYP1A2, are decreased during pregnancy. [7] Several recent reviews provide detailed descriptions of physiologic changes during pregnancy that affect pharmacokinetics. [5, 6, 10, 11]

Recently, there have been efforts to increase clinical pharmacology studies in pregnant women, [3, 12, 13] particularly relating to pharmacokinetic changes. Physiologically based pharmacokinetic (PBPK) modeling has proven a valuable tool to enhance the utility of limited PK data collected during pregnancy to identify mechanisms of pharmacokinetic changes and to extrapolate PK across gestation. [14] A stepwise approach for PBPK model development in special populations is typically followed (Figure 1), in which the model is first developed and verified using data from studies in healthy volunteers or nonpregnant patients. Then, the population (system) parameters are altered to account for physiologic changes in pregnancy and predictions are compared to observed data collected from pregnant women. If indicated, sensitivity analyses may then be used to further inform system or drug parameters. Ideally, models should be externally validated for the population of interest, using pharmacokinetic data from studies other than those used in model development. However, as pharmacokinetic data available from pregnant women are limited, it is often difficult to obtain a separate set of data for model validation.
Model reproducibility is another important aspect of PBPK modeling. PBPK manuscripts should provide sufficient details to enable readers to recreate the model. Of note, this will vary significantly depending upon the substrate of interest, modeling software, and other project-oriented considerations such as data sharing requirements of funders and journals. The majority of PBPK models of pregnancy have been developed using Simcyp. [14] As updates are made to population parameters and structural aspects of Simcyp on an annual basis, it is also important to evaluate the effect of these changes on model predictions.

We have recently completed clinical pharmacokinetic studies in pregnant women on two drugs, betamethasone and buprenorphine. PBPK models for these drugs have been developed using previous versions of Simcyp and applied to pregnant women. [15, 16] The aim of this study was to reproduce these models, compare predictions generated by Simcyp V19 with those reported for Simcyp V15 and V17, and to externally verify the models using our observed plasma concentration data. We have also reviewed other reports of pregnancy PBPK models and propose recommended best practice approaches for reporting reproducible PBPK models.

**Methods**

PBPK models for betamethasone and buprenorphine developed in Simcyp V17 and V15, respectively, have been previously published. [15, 16] We extracted drug-specific parameters from these publications, input them into Simcyp V19, and compared our simulated output with that in the original publications as well as observed plasma concentrations obtained from studies recently completed by our group.

**Betamethasone**

The details of development and verification of the betamethasone full PBPK model for intravenous (IV), oral (PO), and intramuscular (IM) administration in healthy nonpregnant and pregnant subjects were reported by Ke and Milad. [15] Briefly, the authors described how the PBPK model was
established in Simcyp V17 by employing the physiochemical properties of betamethasone, including tissue to plasma partition coefficient (Kp) estimated using the Rodgers and Rowland method. [17]

Using a well-stirred liver model, CLint was back-calculated from the observed CLiv, [18] and assigned a fmCYP3A4 of 100% with a minor contribution of renal clearance (CLR = 0.49 L/h). IV and PO pregnancy models of betamethasone were built and verified based upon published plasma concentration versus time profiles from published data. The model also accounted for the different formulations available: betamethasone disodium phosphate and phosphate: acetate dual formulation. A custom “venous blood” dosing route was used to simulate the kinetics of betamethasone following IV and intramuscular (IM) administration to account for the conversion of the ester prodrug to betamethasone free base. A first-order process with different ka and Tlag was assumed for the input model of each one of the three administration routes evaluated. Sensitivity analysis was performed to establish the absorption parameters ka and Tlag for each administration route.

Default Simcyp demographic and physiological parameters for a North European Caucasian population of healthy adults were used to create a virtual population. For the pregnant population, the default Sim-Pregnancy file was used with a custom modification in CYP3A4 activity across gestation. We assumed the same CYP3A4 activity function used in the published PBPK model [15] instead of the current function implemented in Simcyp V19.

Using a best practices approach, the developed betamethasone drug file based on the nonpregnant population was unchanged and applied in the simulations for pregnant women in the model verification step. Concentration-time profiles using ten virtual trials with equal number of subjects to the corresponding clinical study were used for simulations. The trial design was based on five reported clinical studies [18-22] and matched the design used for PBPK model development and verification. [15] To confirm model reproducibility in Simcyp V19, we repeated the simulations conducted by Ke and Milad and compared our results to the observed and Simcyp V17 predicted data. Graphical data was extracted using WebPlotDigitizer version 4.4.
We further conducted an external validation of the pregnancy PBPK model for betamethasone by comparing predicted concentrations to those observed in our recently completed study of IM betamethasone phosphate:acetate (Celestone Soluspan) in pregnant women. Concentration-time profiles, using ten virtual trials of 35 pregnant women aged 20 to 44 years with a mean gestational age of 26, 30, or 34 weeks, were simulated under fasted conditions. The trial duration was 48 hours. Additionally, we also compared the model’s predicted concentrations to the observed concentrations in a recent clinical study, the b-Mhyalines trial published by Foissac et al., where pregnant women received two IM doses of 11.4 mg (equivalent dose as free base) betamethasone phosphate:acetate (Celestone Chronodese) 24 hours apart. [23] This prediction was based on ten virtual trials of 21 pregnant women aged 20 to 40 years with a mean gestational age of 30 weeks. The trial duration was 96 hours simulated under fasted conditions.

Pharmacokinetic studies of betamethasone were conducted at Indiana University in 242 singleton pregnant women ≥ 18 years of age who were at least 23 but less than 34 weeks of gestation and at imminent risk for preterm delivery. Exclusion criteria included known fetal anomaly, known placental abruption at the time of consent, hepatic or renal failure, or inability to provide consent. The study was approved by the Indiana University Investigational Review Board (IU IRB #1604640151) and registered on ClinicalTrials.gov (NCT02793700). Written informed consent was obtained from all participants. Women were recruited at either an initial or rescue course of the corticosteroid. Two 12 mg doses of betamethasone were given IM as betamethasone phosphate:acetate dual formulation (Celestone Soluspan, equivalent to 9.95 mg betamethasone free base) 24 hours apart. Blood was drawn prior to dosing and at the intervals 0.5-2 hours, 4-6 hours, 22-24 hours, and 48 hours after the first dose, and at delivery. Plasma samples were analyzed by a validated HPLC-MS/MS method. The linear range of detection was 0.1-100 ng/mL. Inter- and intra-day assay variability were <15%. AUC was calculated based upon population pharmacokinetic estimates. Full details of this analysis will be reported separately.
Buprenorphine

Development and verification of a full PBPK model for intravenous (IV) and sublingual (SL) administration of buprenorphine in healthy nonpregnant subjects was reported by Kalluri et al. and subsequently extended by Zhang et al. for application in healthy pregnant subjects [16, 24]. In the initial publication, Kalluri et al described the development of a buprenorphine PBPK model in Simcyp V15 based on buprenorphine physiochemical properties, extrapolating in vitro-in vivo (IVIVE) metabolic profiles, and estimating distribution parameters using the corrected Poulin and Theil method. The model was built and verified using published data from single and multiple dose IV and SL buprenorphine studies in opioid-dependent and non-opioid-dependent patients. We developed a buprenorphine drug file based on parameters provided in Tables 1-3 of Kalluri et al. [24] and, as reported by their publication, simulated a single 8 mg IV dose of buprenorphine in a healthy volunteer population. [25] We also assessed reproducibility of the SL absorption model for buprenorphine. As Simcyp does not include a buccal absorption model, Kalluri et al. had developed a customized SL administration route that included an inhalation route, oral absorption, and a depot release component to mimic overall SL absorption. The authors provided first-order absorption parameters for fa, Ka, Q_{gut}, and f_{u_{gut}} (Table 7 of Kalluri et al.) and details of dosing allocation (Table 6 of Kalluri et al.). As described in their publication, for an 8 mg SL dose, the nondepot component consists of 5 mg total which is then further broken down as 10% SL and 90% PO. This then leaves 3 mg of the dose in the “depot” component. Additionally, Zhang et al. report an additional parameter for lag time that was not included in the Kalluri et al. publication. As specified in Kalluri et al, the default Simcyp healthy volunteer patient population file was used to create all virtual populations. Demographic details (i.e. sex and age range) were adjusted to match the reported patient population. This buprenorphine PBPK model was subsequently applied to predict changes in buprenorphine PK in a pregnant population. [16]
Referencing these publications, we input buprenorphine-specific parameters into Simcyp V19 and simulated the IV [25] and SL [26] PK profiles in ten virtual trials of ten healthy subjects, matching age and sex to the clinical study, under fasting conditions. Due to potential differences between Simcyp versions, we also recreated the buprenorphine simulations in Simcyp V15.

Mirroring the betamethasone example above, we planned to externally validate the published pregnancy PBPK buprenorphine model by comparing predicted concentrations to those observed in our recently completed study of SL buprenorphine in pregnant women (IU IRB #1808181407). However, due to issues with model reproducibility (see Results), we were subsequently unable to complete this reassessment.

Data Analysis

To our knowledge, there are no set criteria for assessing model reproducibility. In theory, given the same input parameters, structural model, and starting seed, simulation outputs should be identical. However, as starting seeds are not reported for simulations, and to allow for updated population parameters and other changes between software versions, we judged a model to be reproducible if PK parameters (e.g. $C_{\text{max}}$, AUC, CL) were within 10% of the original report. There is no standard guidance that defines appropriate variance for the goodness of fit or validation criteria for model predictions. Most authors consider a 50% deviation as reasonable, considering the variability in drug physiological parameters and disposition across populations. [27] When reassessing a PBPK model based on an external validation dataset (i.e. data not included in initial manuscript), the more liberal 2-fold criteria was used to compare PK parameters.

Results

Betamethasone

Before evaluating the performance of the PBPK model to predict our in-house data from pregnant women, we ensured model reproducibility, using Simcyp V19, by confirming that betamethasone
disposition was adequately described in the nonpregnant population for the IV, PO, and IM administration routes. Simulations using Simcyp V19 showed comparable PK profiles with the observed data and reported predictions from Simcyp V17 as gauged by visual inspection (Figure 2) and comparison of $C_{\text{max}}$ and AUC values obtained in both nonpregnant and pregnant patients (Table 1). The simulated mean $C_{\text{max}}$ and AUC values were between 0.95-fold to 1.07-fold of the predictions by the previous Simcyp version. In agreement with the previous publication, the ratios of AUC and $C_{\text{max}}$ predicted by Simcyp V19 were within 25% of the respective observed values (Table 1). [15]

After confirming reproducibility of the published betamethasone model in Simcyp V19 (Supplementary File 1), we further reassessed the ability of the model to predict maternal betamethasone plasma concentrations using two additional studies in pregnant women. The b-Mhyalines trial population received betamethasone after 28 weeks gestation, with median maternal age of 31 (18 - 44) years, weight of 71 (53 - 23) kg. The study, which was conducted in France, did not provide details on race/ethnicity of the study population. The in-house clinical study provided a total of 1083 plasma concentrations above the lower limit of quantification (0.1 ng/mL) from 242 pregnant women. The majority of pregnant women in our study were Caucasian (45.0%) or African American (41.7%), with a median age of 27.7 (18 - 44) years, and a median weight of 85.7 (43.8 - 169) kg. The mean estimated gestational age (EGA) at first dose was 30.6 (22.9 - 33.9) weeks, and the mean EGA at delivery was 34.6 (24.1 - 41) weeks. The betamethasone PBPK model under-predicted AUC and $C_{\text{max}}$ for the b-Mhyalines study, but over-predicted AUC and $C_{\text{max}}$ compared to our observed data (Table 2). Figure 3 shows the predicted and observed plasma concentration versus time profiles at varying gestational ages compared to our in-house data (refer to Supplementary Files 2-4 for Simcyp output files respective to gestation ages 26, 30, and 34 weeks). Although the PBPK model accurately captured the decreased AUC observed with increasing gestational age, the predicted concentrations tended to be higher than those observed in our clinical study, with about 32% of observations falling outside of the 90% prediction interval. However, the mean predicted to
observed parameter ratios were within 2-fold for both studies, meeting our validation criteria for successful model reassessment (Table 2).

**Buprenorphine**

Similar to the betamethasone model, we assessed the buprenorphine PBPK model’s reproducibility by first confirming that buprenorphine disposition was adequately described in a nonpregnant population for both the IV and SL administration routes. The clearance of IV buprenorphine predicted by Simcyp V19 was 71% lower than the reported values obtained by Kalluri et al. in Simcyp V15 (37.5 L/h vs. 52.4 L/h, Table 3). Noting that this deviation could be caused by differences in the underlying default values between Simcyp versions, we attempted to replicate the model in Simcyp V15 as reported in the original publications (Supplementary File 5). However, this also resulted in underprediction of IV buprenorphine clearance compared to predicted clearances reported in Kalluri et al. (Table 3). In the Zhang et al. report, physiochemical and PK parameters are provided in Appendix Table 1A. [16] While in vitro Vmax, Km, and CLint values are identical to those reported in Table 4 of Kalluri et al., [24] the Zhang et al table does not include parameter values for ISEF or fu,mic. It should be noted that setting ISEF and fu,mic to the default value of 1 led to even lower predictions of clearance (10.4 L/h). Additionally, there was confusion with respect to distribution parameters. Table 2 of Kalluri et al. notes that the Kp for “Bone/additional” was optimized to 35 in order to estimate a predicted Vss of 2.48 L/kg. Initially, this was interpreted as requiring the “User-defined Additional Organ” option to be selected and setting both “Bone” and “Additional Organ” tissue:plasma partition coefficients to 35. However, this led to a predicted Vss of 2.86 L/kg. Removing the replacement organ and only changing bone Kp to 35, resulted in the reported Vss of 2.48 L/kg.

Despite this underprediction of IV clearance, as buprenorphine was administered SL in our pregnant opioid use disorder population, we investigated how Kalluri et al. implemented their custom SL dosing scheme, as the Simcyp software does not include a direct structural model for SL dosing.
However, it was unclear how the depot/non-depot and inhaled routes were implemented in Simcyp V19, as the custom dosing option under “Trial Design” allows for PO, IV, dermal, and inhaled dosing. “Depot” is not an input option under the absorption settings for either oral or inhaled dosing routes (see “Custom Dosing” tab in Supplementary File 6). Recognizing that changes in software may have influenced our interpretation of the methods, we attempted to recreate the custom SL dosing model in Simcyp V15 and V17. However, the interface for inputting custom dosing was similar between versions. Although we tried various combinations of inhaled and oral dosing, we were unable to accurately reproduce the SL buprenorphine absorption model as described by Kalluri et al.

Discussion

PBPK modeling is an important tool in expanding understanding of PK changes in special populations, such as pregnant women, where it is difficult or unethical to conduct clinical studies. However, the lack of clinical PK data in special populations often leads to limited ability to externally verify the PBPK model using an independent data set. This study aimed to reproduce and compare two reported pregnancy PBPK models using clinical data from recently completed studies. The PBPK model for betamethasone [15] was reproducible and reasonably predicted our observed data as well as that from another recent publication. [23] However, we were unable to reproduce the PBPK model for SL buprenorphine. [16, 24]

Ke and Milad verified the betamethasone pregnancy PBPK model using plasma concentrations of betamethasone in pregnant women (37 weeks mean gestational age) receiving a single IV dose of 10.6 mg of betamethasone disodium phosphate (equivalent to 8 mg of free betamethasone). [22] Clinically, however, pregnant women receive betamethasone injections for threatened preterm labor at less than 37 weeks gestation.[28] Additionally, the IM absorption profile was confirmed in nonpregnant subjects, but not in pregnant women. As betamethasone is most commonly administered IM during pregnancy, it is important to confirm that there are no significant differences in IM absorption in the pregnant population. Women in both the b-Mhyalines trial and our study
received two doses of betamethasone phosphate plus betamethasone acetate via IM administration 24 hours apart. While trial designs were similar between studies, the betamethasone formulations differed, with Celestone Soluspan (3 mg betamethasone sodium phosphate/3 mg betamethasone acetate) available in the US and Celestone Chronodose (3.9 mg betamethasone sodium phosphate/3 mg betamethasone acetate) used in Europe. In general, study populations were similar with respect to maternal and gestational ages and BMI, although our population contained African Americans and Hispanic women. Accounting for differences in formulation, the betamethasone pregnancy PBPK model underpredicted the $C_{\text{max}}$ and AUC of the b-Mhyalines trial [23] and overpredicted $C_{\text{max}}$ and AUC of our in-house data. However, predictions for both studies were within the 2-fold acceptance criteria. [27] As the Simcyp pregnancy population is based on Caucasian women, it is possible that differences in metabolism (e.g. CYP3A activity) may have contributed to the over-prediction of betamethasone exposure in our in-house study.

The between-study variability observed in the clinical studies of betamethasone is not entirely unexpected. As noted above, this may be due to population differences. Additionally, intrinsic variability in pharmacokinetics may not be adequately captured by the betamethasone PBPK model. The kinetics of betamethasone in healthy volunteers after IM administration are known to be highly variable, as demonstrated by a 6-fold difference in exposure across four studies, as reported by Ke and Milad. [15] The input values for the absorption rate constant (Ka) and lag time ($T_{\text{lag}}$) used for IM administration were optimized based on one study in nonpregnant subjects. [20] Another potential cause of variability is that each study used a different betamethasone phosphate/acetate formulation, with the Celestone Chronodose formulation used in Europe having a higher ratio of the faster-absorbing phosphate salt. Furthermore, differences in sample collection, including use of stabilizers and analytic methods may contribute to inter-study variability. Despite differences between predicted betamethasone exposure and that observed in our study, the PBPK model did successfully capture the effects of gestational age on betamethasone pharmacokinetics. As expected, the induced activity of CYP3A enzyme [7] leads to a higher betamethasone clearance and
lower AUC at 30 weeks gestation as compared to 26 weeks gestation. Additionally, changes in volume of distribution during pregnancy likely lead to the alterations observed in $C_{\text{max}}$.

While we successfully replicated the betamethasone model, we were unable to replicate the buprenorphine model. [16, 24] Three authors with varying levels of experience with PBPK modeling, independently developed drug models for buprenorphine based on the Kalluri and Zhang manuscripts. While parameter values for the IV model were easy to identify from various tables in the manuscripts, the resulting simulations predicted a significantly lower clearance compared to reports by the previous studies. Simcyp’s lack of a built-in model for SL absorption route required development of a combination dosing scheme. While the authors provided parameters specific to the SL model in addition to a table describing SL dosing allocation, the original publication lacked adequate details surrounding the SL custom dosing components relating to the simulation trial dosing design necessary to ensure model reproducibility. In brief, Kalluri et al. described their customized administration route as consisting of the following: an inhalation route to mimic the SL absorption, an oral absorption route to mimic the portion of the drug that is swallowed, and a depot release component to mimic the slow release of drug from the buccal tissue into the systemic circulation. This description, while arguably sound from a physiological standpoint, led to confusion when attempting to interpret how the “depot” compartment was implemented within Simcyp V19. Noting that there could have been a change in the user interface for dosing between V15 and V19, the authors wondered if V15 had included additional administration routes as V19 does not have a “depot” dosing compartment function. Additionally, when inputting the customized simulated trial dosing schedule, Simcyp V19 limits the number of administration routes to two instead of the three seemingly necessary for the published SL model. To rule out the above concerns, we also attempted to recreate the model in Simcyp V15 and V17, with no success. Ultimately, we found the description of the SL administration route was ambiguous, leaving room for interpretation by the reader. In fact, although Kalluri et al. provided drug input parameters, [24] additional details on the SL model
were presented in the group’s subsequent manuscript focused on applying the model to pregnancy. [16] For instance, the inclusion of $T_{lag}$ is only described in the second manuscript.

Our experiences highlight the importance of providing detailed descriptions of PBPK model structure and parameterization in publications. Details of the betamethasone PBPK model, including parameter values and assumptions made during model development, were sufficient for us to easily replicate in Simcyp. However, while the Kalluri et al. buprenorphine PBPK model manuscript contained the needed information to replicate the IV model (distribution, metabolism, and elimination parameters), the SL absorption model was not adequately described. Details regarding the complex absorption structure were spread across multiple sections of the manuscript (methods, results, and discussion) or only provided in a later publication. [16] Due to the ambiguity in the manuscripts with respect to construction of the SL absorption model, we attempted various permutations of inhaled/oral routes without success.

Reproducibility is a critical factor of scientific research, fostering advancement of knowledge. Yet, a survey completed by over 1500 scientists reported that more than 50% believe that reproducibility is a significant crisis. The same survey found that more than 70% of respondents stated they have tried and failed to reproduce published results. [29] While it would be expected that simulation models such as PBPK should be more reproducible that wet laboratory or clinical studies, only 4 of 12 published quantitative systems pharmacology models developed in R, Matlab, or PK-Sim/Mobi were functionally executable. [30] The Simcyp platform integrates a complex, structural PBPK and covariate model behind a user-friendly graphical interface in which the user inputs drug- and population-specific parameters. [31] Therefore, if one uses the same drug- and population-specific parameters in the same version of a software, there should be minimal variability between results. In fact, identical results should be obtained if the same initial seed values are used. [32]

Kirouac et al. note that results from QSP models should be reproducible based upon the publication and supporting documentation, without the need for a researcher to contact the author. [30] While
we report details of two examples exploring model reproducibility here, additional review of the literature of PBPK models in pregnancy revealed varying degrees of details available to support reproducibility efforts. We have also had previous experiences where models reported in the literature were not reproducible. We reviewed examples of pregnancy PBPK models developed in various software, including Simcyp, GastroPlus, PKSim, Matlab, and R. Advantages and disadvantages of open source versus commercial software are beyond the scope of this report. [33] For commercial software, publication content should be sufficient such that models can be adequately reproduced in the reported version of the software package used. For models developed in R, Matlab, or other software requiring compiling code, a working code file should be provided (with all necessary accessory files) as supplementary material in a linked open-access database (e.g. Github).

For PBPK models developed in software such as Simcyp or Gastroplus, it is critical that authors provide a clear description of each parameter and how/where to input values into the software. Authors should provide the comprehensive table of input parameters, including the Excel output files from Simcyp (such as Supplementary Files 1-6). We reviewed a variety of publications describing PBPK models of pregnancy. Most PBPK pregnancy models are developed in Simcyp and often include tables of drug-specific parameters (physio-chemical, absorption, distribution, and metabolism properties). As observed in our attempt to replicate the SL buprenorphine model, confusion arose when it was unclear how reported parameters were integrated into the software. Authors should use terminology consistent with that used in the software to avoid unnecessary confusion. For example, Kalluri et al describe in detail the SL buprenorphine dosing allocation between depot and nondepot components, but Simcyp does not appear to utilize the terms “depot” or “nondepot” when using a customized, oral dosing scheme. This inconsistency between author and software phrases ultimately resulted in our inability to confidently proceed with the SL buprenorphine PBPK model. We also noted that errors are more likely to occur when properties are calculated within the software. For instance, parameters in GastroPlus can be automatically generated based on in silico predictions from ADMET Predictor. We reviewed three published
pregnancy PBPK models built in GastroPlus, all of which had some missing parameter values (e.g., solubility and permeability). While all three publications included a table listing $K_p$ for different tissues, they did not state the method used to calculate $K_p$. Additionally, it is uncommon for authors to describe details of the study population, including changes made from default population settings, and clinical trial design. This is crucial, given disparities in outcomes often seen in drug responses based on participant characteristics. These details, as well as information relating to assumptions made during model development and limitations of the approach, are important aspects of model reporting, as outlined by FDA and EMA guidance documents. Table 4 provides a list of our recommendations for reporting reproducible PBPK models.

Journal editors and reviewers should also take responsibility for ensuring reproducibility. While reviewers may not have software access or technical expertise required to replicate models during the review process, at a minimum they should be asked to confirm that necessary information is available and clearly presented (e.g. model codes or output workspace files). And, as suggested by Kirouac et al., journals could develop teams with expertise and access to various softwares who could provide support reviewers relating to model testing for reproducibility.

**Conclusion**

This study, undertaken to externally validate PBPK models of betamethasone and buprenorphine in pregnant women, highlights the importance of model reproducibility. The betamethasone model was reproducible and predicted $C_{\text{max}}$ and AUC within two-fold for two separate studies. While we were able to reproduce components of the IV buprenorphine model, the SL absorption component was not reproducible. Hence, we were unable to validate the buprenorphine model’s ability to sufficiently capture our observed data from third trimester pregnant women. Moving forward, the pharmacometrics community and publishers should work together to enhance and potentially standardize PBPK model reproducibility.
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Data available on request from the authors.

The authors declare no conflicts of interest.

**Nomenclature of Targets and Ligands**

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 [39, 40]
References


Table 1. Mean predicted and observed $C_{\text{max}}$ and AUC of betamethasone in nonpregnant and pregnant subjects receiving intravenous, oral, or intramuscular doses of betamethasone.

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<th>Trial (Population; dose, route)</th>
<th>$C_{\text{max}}$ (ng/mL)</th>
<th>AUC (ng mL/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed [18]</td>
<td>101.0</td>
<td>771.7</td>
</tr>
<tr>
<td>Simcyp V19</td>
<td>108.9</td>
<td>856.6</td>
</tr>
<tr>
<td>Ratio V19/Obs</td>
<td>1.08</td>
<td>1.11</td>
</tr>
<tr>
<td>Ratio V17/V19</td>
<td>0.99</td>
<td>1.01</td>
</tr>
<tr>
<td>Observed [19]</td>
<td>24.1</td>
<td>231</td>
</tr>
<tr>
<td>Simcyp V17 [15]</td>
<td>24.7</td>
<td>175.2</td>
</tr>
<tr>
<td>Simcyp V19</td>
<td>24.7</td>
<td>188.2</td>
</tr>
<tr>
<td>Ratio V19/Obs</td>
<td>1.02</td>
<td>0.81</td>
</tr>
<tr>
<td>Ratio V17/V19</td>
<td>1.00</td>
<td>1.07</td>
</tr>
<tr>
<td>Observed [20]</td>
<td>25.8</td>
<td>542.2</td>
</tr>
<tr>
<td>Simcyp V17 [15]</td>
<td>27.2</td>
<td>588.4</td>
</tr>
<tr>
<td>Simcyp V19</td>
<td>26.8</td>
<td>577.3</td>
</tr>
<tr>
<td>Ratio V19/Obs</td>
<td>1.04</td>
<td>1.06</td>
</tr>
<tr>
<td>Ratio V17/V19</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>Observed [21]</td>
<td>157.0</td>
<td>1636</td>
</tr>
<tr>
<td>Simcyp V17 [15]</td>
<td>62.7</td>
<td>1196</td>
</tr>
<tr>
<td>Simcyp V19</td>
<td>65.3</td>
<td>1288</td>
</tr>
<tr>
<td>Ratio V19/Obs</td>
<td>0.42</td>
<td>0.76</td>
</tr>
<tr>
<td>Ratio V17/V19</td>
<td>1.04</td>
<td>1.07</td>
</tr>
<tr>
<td>Observed [22]</td>
<td>104.0</td>
<td>520.0</td>
</tr>
<tr>
<td>Simcyp V19</td>
<td>86.2</td>
<td>441.6</td>
</tr>
<tr>
<td>Ratio V19/Obs</td>
<td>0.83</td>
<td>0.85</td>
</tr>
<tr>
<td>Ratio V17/V19</td>
<td>-</td>
<td>0.95</td>
</tr>
</tbody>
</table>

AUC, area under the concentration-time curve from zero to infinity; $C_{\text{max}}$, peak plasma concentration.
Table 2. Mean predicted and observed C\textsubscript{max} and AUC of betamethasone in pregnant women at different gestational ages receiving 12 mg intramuscular dose of betamethasone phosphate:acetate.

<table>
<thead>
<tr>
<th>EGA</th>
<th>26\textsuperscript{a}</th>
<th>30\textsuperscript{a}</th>
<th>34\textsuperscript{a}</th>
<th>30\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK parameter</td>
<td>(C_{\text{max}}) (ng/mL)</td>
<td>(\text{AUC}) (ng/mL h)</td>
<td>(C_{\text{max}}) (ng/mL)</td>
<td>(\text{AUC}) (ng/mL h)</td>
</tr>
<tr>
<td>Simcyp V19 Predicted</td>
<td>46.59 (27.33 – 72.45)</td>
<td>645.54 (329.80 – 1156.67)</td>
<td>43.94 (25.82 – 68.24)</td>
<td>597.84 (309.26 – 1070.42)</td>
</tr>
<tr>
<td>Observed</td>
<td>28.46 ± 13.43</td>
<td>457.71 ± 146.66</td>
<td>23.38 ± 9.71</td>
<td>347.2 ± 193.79</td>
</tr>
<tr>
<td>Ratio V19/Obs</td>
<td>1.63</td>
<td>1.41</td>
<td>1.87</td>
<td>1.72</td>
</tr>
</tbody>
</table>

AUC, area under the concentration-time curve from zero to infinity; \(C_{\text{max}}\), peak plasma concentration; Obs., observed data; EGA, estimated gestational age.

Numbers in parenthesis represent the 5\textsuperscript{th}-95\textsuperscript{th} prediction interval.

\(\textsuperscript{a}\) In-house data; equivalent to 9.95 mg of betamethasone free base.

\(\textsuperscript{b}\) Foissac et al., equivalent to 11.4 mg of betamethasone free base.

\(\textsuperscript{c}\) Area under the curve from 0 to the time of delivery (simulations based on median time to delivery of 2.6 days)
Table 3. Mean predicted and observed AUC and clearance following an 8 mg intravenous dose of buprenorphine in nonpregnant subjects.

<table>
<thead>
<tr>
<th>PK parameter</th>
<th>AUC (ng mL/h)</th>
<th>CL (L/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed [25]</td>
<td>153.3</td>
<td>52.4</td>
</tr>
<tr>
<td>Simcyp V15 [24]</td>
<td>151 (82.1 – 269)</td>
<td>53.1 (29.8 – 97.4)</td>
</tr>
<tr>
<td>Simcyp V19 (current study)</td>
<td>217 (211 – 220)</td>
<td>37.5 (36.4 – 38.0)</td>
</tr>
<tr>
<td>Ratio V19/V15</td>
<td>1.42</td>
<td>0.71</td>
</tr>
<tr>
<td>Simcyp V15 (current study)</td>
<td>218 (213 – 222)</td>
<td>37.1 (36.1 – 37.7)</td>
</tr>
<tr>
<td>Ratio V15 current/V15 [24]</td>
<td>1.44</td>
<td>0.7</td>
</tr>
</tbody>
</table>

AUC, area under concentration-time curve from zero to infinity; CL, intravenous clearance
Numbers in parenthesis are the predicted 5th - 95th prediction interval.
Table 4. Recommendations for Publication of Reproducible PBPK Models

- Specify name(s) and version(s) of software and packages used.
- Provide model code and/or file(s) containing saved workspace (e.g. Simcyp wksz) as supporting information or through a publically available repository.
- Include details of all system parameters, or for commercial software, clearly describe the population used and any changes made to the default population when applicable.
- List all drug parameter values, including the variability assigned and source (reference) or calculation method, if determined in silico, in a single table.
- Include detailed description(s) of simulated trial design, including number of patients and trials simulated, patient demographics, dosing regimen, and length of study for all simulated studies.
- Discuss, in adequate detail, assumptions made during model development, as well as limitations specific to the model.
Figure 1. Best practices approach for pregnancy PBPK model development includes use of physicochemical, in vitro, and clinical data to inform drug parameters to predict concentrations in a healthy adult population using a learn and confirm paradigm (indicated by dashed arrows), if needed. Verification should include external validation of the model using data not implemented during model development. Once drug parameters are verified in a healthy adult population, system (population) parameters are adjusted to a special population of interest (e.g. pregnant women). Model-predicted plasma concentrations in pregnant population are compared to available literature data also in pregnant population. If necessary, adjustments in drug parameters (e.g. accounting for placental transport/metabolism) are made based on a learn-confirm paradigm (denoted by dashed arrow). After verification of model performance based on available literature data (if possible), the model can be used to simulate the new study and predictions can be compared to observed data.
Figure 2. Comparison of predicted betamethasone concentration-time curves using Simcyp V17 and V19. (A) 8 mg IV in healthy volunteers [18], (B) 2 mg PO in healthy volunteers [19], (C) 6 mg IM in healthy volunteers [20], (D) 12 mg IM in healthy volunteers [21], (E) 8 mg IV in pregnant women [22].

In all figures, the dashed red lines represent the mean predicted concentration data using Simcyp V17 and the solid black line is the mean predicted concentration data using Simcyp V19.
Figure 3. Mean predicted (solid black line) versus observed (open symbols) betamethasone concentrations in pregnant women administered two intramuscular doses of 12 mg betamethasone phosphate:acetate (in-house data). Predicted concentrations are based on (A) estimated gestational age (EGA) 26 weeks, (B) EGA 30 weeks, and (C) EGA 34 weeks. Grey dashed lines represent the 90% prediction interval.