RESEARCH ARTICLE

Physiologically-based pharmacokinetic modelling of infant exposure to efavirenz through breastfeeding [version 1; referees: 2 approved with reservations]

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Abstract

Background: Very little is known about the level of infant exposure to many drugs commonly used during breastfeeding. The aim of this study was to develop a physiologically-based pharmacokinetic (PBPK) model for predicting infant exposure to maternal efavirenz through breastmilk.

Methods: A breastfeeding PBPK model combining whole-body maternal and infant sub-models was constructed from drug-specific and system parameters affecting drug disposition using mathematical descriptions. The model was validated against published data on the pharmacokinetics of efavirenz in nursing mother-infant pairs. Further simulations were conducted to assess exposure in the context of the 400 mg reduced dose of efavirenz as well as best- and worst-case scenarios.

Results: The model adequately described efavirenz pharmacokinetics, with over 80% of observed data points (203 matched breast milk and plasma pairs) within the predictive interval. All parameters were within 2-fold difference of clinical data. Median (range) predicted versus observed breast milk AUC0-24, Cmax and Cmin at the standard 600 mg dose were 75.0 (18.5-324) versus 68.5 (26.3-257) µg.hr/mL, 4.56 (1.17-16.0) versus 5.39 (1.43-18.4) µg/mL, and 2.11 (0.38-12.3) versus 1.68 (0.316-9.57) µg/mL, respectively. Predicted plasma AUC0-24, Cmax and Cmin at 400 mg reduced dose were similar to clinical data from non-breastfeeding adults. Model-predicted infant plasma concentrations were similar to clinical data, 0.15 (0.026–0.78) µg/mL at the 400 mg maternal dose in pooled analysis, approximately 25% lower than simulated exposure at 600 mg. The maximum exposure index was observed in the youngest infants, 5.9% (2.2-20) at 400 mg and 8.7% (3.2-29) at 600 mg. Thirteen and 36% of 10 days-1 month old infants were predicted to have exposure index above the 10% recommended threshold at 400 mg and 600 mg maternal dose, respectively.

Conclusions: This application of PBPK modelling opens up opportunities for expanding our understanding of infant exposure to maternal drugs through breastfeeding.

Keywords
breastfeeding, PBPK modelling, efavirenz, infant
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Author roles: Olagunju A: Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Methodology, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; Rajoli RKR: Methodology; Atoyebi SA: Formal Analysis, Writing – Review & Editing; Khoo S: Resources, Supervision, Writing – Review & Editing; Owen A: Conceptualization, Resources, Supervision, Writing – Review & Editing; Siccardi M: Conceptualization, Methodology, Supervision, Writing – Review & Editing

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Introduction
The re-enactment of The Best Pharmaceuticals for Children Act (BPCA), The Pediatric Research Equity Act (PREA) in the United States, and The Paediatric Regulation in the European Union in 2007 were significant steps in paediatric health promotion. The BPCA and PREA were subsequently made permanent by the FDA Safety and Innovation Act in 2012, mandating necessary paediatric studies. Although these legal frameworks do not remove the ethical and logistical challenges of conducting research in paediatric patients, they reinforced that children should be protected through research, not from it, giving impetus to clinical studies in this population. For instance, a review of studies conducted under these changes and a breakdown of paediatric studies between September 27, 2007 and November 18, 2013 indicated that about 470 paediatric studies (involving more than 178,000 patients and 160 drugs) were completed under BPCA and PREA in the United States, reducing off-label paediatric drug use from over 80% to about 50%.

However, paediatric drug exposure is not limited to those administered for specific paediatric indications. More than 90% of nursing mothers take at least one drug in the early postnatal period, 17% up to 4 months after delivery, and 5% receive drugs for chronic conditions. For most drugs, the level of exposure of breastfed infants to maternal drugs through breast milk and the potential effects are unknown. At present, there is no legislation requiring drug companies to conduct clinical research in nursing mother-infant pairs to evaluate infant exposure through breast milk. Apparently to avoid legal liability, most drugs are labelled not to be used during lactation. However, this is not practical in many cases, especially for nursing mothers being treated for chronic conditions. For instance, under the current WHO guidelines HIV positive nursing mothers take antiretroviral drugs during breastfeeding for their own health and/or for prevention of mother-to-child transmission of HIV. Understandably, conducting clinical pharmacokinetics studies in nursing mother-infant pairs is fraught with ethical and logistical challenges.

Physiologically based pharmacokinetic (PBPK) models are increasingly being used in paediatric studies, with significant regulatory support. In fact, the US FDA Advisory Committee for Pharmaceutical Science and Clinical Pharmacology unanimously voted in support of modelling and simulation for paediatric drug development. Interestingly, the advances in PBPK modelling now allow for integration of compartments and parameters representing the anatomical and physiological features of a nursing woman (system parameters) with physicochemical, in vitro, preclinical, and clinical data (drug parameters) to generate predictions of drug-specific pharmacokinetics. In addition, system-specific parameters can be modified for extrapolations across different age groups. They also allow for integration of maternal and infant anatomy and physiology to simulate complex scenarios of infant exposure to substances through lactation. However, a cursory literature search indicates that the application of PBPK modelling in the study of infant exposure to xenobiotics through breast milk has largely been limited to environmental risk assessments. Only a single full article could be found on use of this approach to describe infant exposure to maternal therapeutic drugs through breast milk. The model was used to simulate morphine plasma concentrations in infants resulting from codeine use by mothers with fast, intermediate, or poor CYP2D6 metabolic capacity.

The aim of the present study was to develop a generic PBPK model to predict infant exposure to maternal drugs through breast milk. Published clinical data on infant exposure to the antiretroviral drug, efavirenz, at the standard 600 mg daily dose was used for model validation. Additional simulations were conducted to explore breast milk and plasma pharmacokinetics of efavirenz in mother-infant pairs at the 400 mg reduced dose recently approved by the WHO.

Methods
Model structure and parameterisation
The human breastfeeding model integrates a whole-body PBPK maternal model with a whole-body PBPK infant model (Figure 1). The maternal model was based on a previously validated adult model of orally administered efavirenz, an antiretroviral used to treat HIV infection, adapted for long-acting nanoformulations, with appropriate adjustments to exclude male-specific system parameters and an additional compartment introduced to represent the mammary gland. As previously described, individual organ weights and blood flows were predicted from anthropometric characteristics (age, height, weight, body mass index, and body surface area), based on values reported in a HIV positive breastfeeding cohort. The infant sub-model was scaled from maternal models for different age groups (10 days–1 month, 1–3 months, 3–6 months, and 6–12 months) to account for age-dependent anatomical and physiological changes in system parameters such as organ/tissue volumes and blood flows. Infants less than 10 days old were excluded because of residual intrauterine efavirenz exposure. Efavirenz-specific parameters included in the model have been presented in Table 1.

Modelling absorption, distribution, metabolism and elimination
A compartmental absorption and transit model incorporating both gastric emptying and small intestinal transit flow was used to describe drug absorption. Fraction of dose absorbed (F) was described using effective permeability (P eff) derived from Caco-2 permeability as previously described. Intestinal drug clearance (CL int) was calculated from CYP3A4 induction (Ind CYP3A4), intestinal CYP3A4 abundance (Ab CYP3A4), in vitro CYP3A4 intrinsic clearance (rCL int), and blood-to-plasma ratio (R) using equation (1). The fraction of drug escaping gut metabolism (F) was calculated using equation (2), where Q gut and f gut are intestinal blood flow and fraction unbound in the intestine, respectively.

\[ \text{CL}_{\text{int}} = \text{Ind}_{\text{CYP3A4}} \times \text{Ab}_{\text{CYP3A4}} \times (\text{CYP3A4 rCL}_{\text{int}}/R) \]  

\[ F_g = \frac{Q_{\text{gut}}}{(Q_{\text{gut}} + f_{\text{gut}} \times \text{CL}_{\text{gut}})} \]
Systemic circulation was defined as a function of the rate of blood flow to tissues (perfusion-limited) and by a mechanism based approach using tissue composition-based equations as previously described\(^{16,17}\).

The abundances of CYP450 enzymes in nursing mothers were based on reported \textit{in vivo} adult data\(^{18,19}\). CYP2B6 abundances for different infant age groups were based on data from human liver microsomal samples obtained from 102 infants previously reported by Croom \textit{et al}\(^{20}\). A plot of CYP2B6 expression in individual tissue samples from birth to 1 year was digitised using \textit{Plot Digitizer}. Samples with levels below the limit of detection (0.25 pmol/mg protein) were excluded\(^{20}\). The amount of microsomal protein per gram of liver (MPPGL),
intrinsic clearance (CL\textsubscript{int}), CYP2B6 induction (Ind\textsubscript{CYP2B6}), total intrinsic clearance (TCL\textsubscript{int}), total apparent clearance (CL\textsubscript{app}), systemic clearance (CL), and fraction escaping first-pass metabolism (F\textsubscript{h}) were calculated using equation (3) to equation (9) as previously described\textsuperscript{12}.

\[
\text{MPPGL} = 10^{(1.407 + 0.0158 \times \text{Age} - 0.00038 \times \text{Age}^2 + 0.000024 \times \text{Age}^3)} \quad (3)
\]

\[
\text{CL}_{\text{int}} = (\text{Ind} \times (r\text{CL}_{\text{int}}/R) \times \text{Ab}_{\text{CYP}} \times \text{MPPGL} \times \text{Wt}_{\text{liver}})) \quad (4)
\]

\[
\text{Ind}_{\text{CYP2B6}} = 1 + (\text{Ind}_{\text{max}} \times [\text{EFV}]_{\text{plasma}})/(\text{Ind}_{50} + [\text{EFV}]_{\text{plasma}}) \quad (5)
\]

\[
\text{TCL}_{\text{int}} = \text{CL}_{\text{int}} \times \text{Ab}_{\text{CYP}} \times \text{Wt}_{\text{liver}} \times \text{MPPGL} \quad (6)
\]

\[
\text{CL}_{\text{app}} = \sum_{u=1}^{n} \text{TCL}_{\text{int}} \quad (7)
\]

\[
\text{CL} = \frac{Q_h \times f_u \times \text{CL}_{\text{app}}}{Q_h + \text{CL}_{\text{app}} \times f_u} \quad (8)
\]

\[
F_h = 1 - \frac{\text{CL}}{Q_{\text{pv}}} \quad (9)
\]

\[
\text{Population variability}
\]

Variability in system and drug-specific parameters in both maternal model and the infant sub-model was introduced mainly through anthropometric characteristics as previously described. Variability in infant age was introduced using the MATLAB\textsuperscript{®} linspace function to generate equally spaced values within each group. Where physiological and anatomical data were used, MATLAB\textsuperscript{®} rule expressions, incorporating the mean, standard deviation, minimum and maximum parameter values, were used to introduce variability. Some of the parameters thus varied are absorption constants, microsomal protein per gram of liver and CYP450 enzymes abundance.

\[
\text{Modelling breastfeeding}
\]

Breastfeeding was described by oral dose of maternal breast milk twelve times a day, the concentration of efavirenz in breast milk ([EFV]\textsubscript{milk}) and the corresponding infant dose of efavirenz per feeding session (EFV Dose\textsubscript{milk}) were described using equation (10) and equation (11), respectively.

\[
[\text{EFV}]_{\text{milk}} = M/P_{\text{AUC}0-24} \times [\text{EFV}]_{\text{plasma}} \quad (10)
\]

\[
\text{Table 1. Drug-specific physicochemical properties and in vitro data for Efavirenz}\textsuperscript{12}.
\]

<table>
<thead>
<tr>
<th>Drug properties</th>
<th>Description</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW</td>
<td>Molecular weight</td>
<td>316</td>
</tr>
<tr>
<td>LogP</td>
<td>Octanol-water partition coefficient</td>
<td>4.60</td>
</tr>
<tr>
<td>pKa</td>
<td>Acid dissociation constant</td>
<td>10.2</td>
</tr>
<tr>
<td>R</td>
<td>Oral bioavailability</td>
<td>0.74</td>
</tr>
<tr>
<td>PSA</td>
<td>Polar surface area</td>
<td>38.33</td>
</tr>
<tr>
<td>HBD</td>
<td>Hydrogen bond donor</td>
<td>1</td>
</tr>
<tr>
<td>K (mg/mL)</td>
<td>Water solubility</td>
<td>0.00855</td>
</tr>
<tr>
<td>Fu</td>
<td>Fraction unbound in plasma</td>
<td>0.01</td>
</tr>
<tr>
<td>Vd (L/kg)</td>
<td>Volume of distribution at steady state</td>
<td>3.6</td>
</tr>
<tr>
<td>P\textsubscript{ef} (cm/s)</td>
<td>Effective permeability (Caco-2)</td>
<td>2.5 × 10\textsuperscript{-4}</td>
</tr>
<tr>
<td>CL_{int} (µL/min/pmol)</td>
<td>Intrinsic hepatic clearance by cytochrome P450 (CYP) enzymes</td>
<td></td>
</tr>
<tr>
<td>rCYP1A2 CL_{int}</td>
<td></td>
<td>0.008</td>
</tr>
<tr>
<td>rCYP2A6 CL_{int}</td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>rCYP2B6 CL_{int}</td>
<td></td>
<td>0.55</td>
</tr>
<tr>
<td>rCYP3A4 CL_{int}</td>
<td></td>
<td>0.007</td>
</tr>
<tr>
<td>rCYP3A5 CL_{int}</td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Ind_{CYP} (µM)</td>
<td>Hepatic CYPs induction</td>
<td></td>
</tr>
<tr>
<td>CYP2B6 Ind_{max}</td>
<td></td>
<td>5.76</td>
</tr>
<tr>
<td>CYP3A4 Ind_{max}</td>
<td></td>
<td>6.45</td>
</tr>
<tr>
<td>CYP2B6 Ind_{50}</td>
<td></td>
<td>0.82</td>
</tr>
<tr>
<td>CYP3A4 Ind_{50}</td>
<td></td>
<td>3.93</td>
</tr>
</tbody>
</table>
EFV Dose\textsubscript{milk} = V\textsubscript{milk} × [EFV]\textsubscript{milk} \quad (11)

Exposure Index = \frac{EFV \text{ Dose}\textsubscript{milk}}{EFV \text{ Dose}\textsubscript{therapeutic}} \quad (12)

where [EFV]\textsubscript{plasma} is simulated efavirenz concentration in plasma, 
M/P\textsubscript{0-24\text{AUC}} is the clinically observed milk-to-plasma AUC\textsubscript{0-24} ratio (median: 1.13; range: 0.50-1.93)\textsuperscript{11}, V\textsubscript{milk} is the volume of breast milk, and EFV Dose\textsubscript{therapeutic} is the recommended therapeutic dose of efavirenz for paediatrics, 10 mg/kg/day. In addition, two hypothetical milk-to-plasma ratios representing both ends of the observed range (0.5 and 2.0) were used to explore additional scenarios of infant exposure. Infant suckling rates from birth to 6 months of age were obtained from the literature\textsuperscript{21}. Suckling rate at 6 months was retained for older infants up to 12 months of age to reflect reduced breast milk intake following the introduction of alternative foods when exclusive breastfeeding ends at 6 months.

**Model simulation and evaluation**

The model was built and simulated using the SimBiology\textsuperscript{®} (version 5.1, MATLAB\textsuperscript{®} 2014b, MathWorks Inc., Natick, MA, USA). Virtual populations of nursing mothers-infant pairs (n = 100 per infant age group: 10 days–1 month, 1–3 months, 3–6 months, and 6–12 months) were simulated. Simulated mothers received the standard 600 mg dose of efavirenz once daily and the infants received no medication. All model simulations were run using female anatomical and physiological parameters to simulate efavirenz pharmacokinetics during lactation and breastfed infants were simulated as females because of the expected similarities between males and females at this early age. Selected physiological parameters are presented in Table 2. Additional simulations were conducted at the recently approved

<table>
<thead>
<tr>
<th>Table 2. Key simulated anatomical and physiological parameters (mean, SD) for infant sub-model.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organ Weights (kg)</strong></td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>10 days-1 month</td>
</tr>
<tr>
<td>Age (y)</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td><strong>Organ Weights (kg)</strong></td>
</tr>
<tr>
<td>Adipose</td>
</tr>
<tr>
<td>Blood</td>
</tr>
<tr>
<td>Bones</td>
</tr>
<tr>
<td>Brain</td>
</tr>
<tr>
<td>Heart</td>
</tr>
<tr>
<td>Intestines</td>
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<tr>
<td>Kidneys</td>
</tr>
<tr>
<td>Liver</td>
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<tr>
<td>Lungs</td>
</tr>
<tr>
<td>Muscle</td>
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<tr>
<td>Pancreas</td>
</tr>
<tr>
<td>Remaining</td>
</tr>
<tr>
<td>Skin</td>
</tr>
<tr>
<td>Spleen</td>
</tr>
<tr>
<td>Stomach</td>
</tr>
<tr>
<td>Thymus</td>
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<tr>
<td>Total weight</td>
</tr>
<tr>
<td><strong>Organ Blood Flows (L/h)</strong></td>
</tr>
<tr>
<td>Cardiac output</td>
</tr>
<tr>
<td>Adipose</td>
</tr>
<tr>
<td>Brain</td>
</tr>
<tr>
<td>Gonads</td>
</tr>
<tr>
<td>Gut</td>
</tr>
<tr>
<td>Hepatic artery</td>
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<tr>
<td>Hepatic vein</td>
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</tbody>
</table>
400 mg reduced daily dose to investigate efavirenz pharmacokinetics in breast milk and plasma of nursing mother-infant pairs if the alternative recommended dose is extended to nursing mothers.

The validity of model estimations was confirmed by comparison with reference values from the literature, with 2-fold difference set as acceptance criteria. For organ weights and blood flows, data from Coppoletta et al. and Pryce et al. were used. Pharmacokinetic parameters were evaluated at steady state and AUC<sub>0-24</sub> was calculated using the trapezoidal rule. The most comprehensive published clinical data of efavirenz pharmacokinetics in human breast milk and exposure of breastfed infants were used to validate predicted pharmacokinetic parameters.

**Results**

**Breastfed infant sub-model validation**

The validation of the adult model has been previously described. Key anatomical and physiological parameters predicted with the breastfed infant sub-model, including body weight, organ weights and blood flows, and CYP450 enzyme expressions, were within 50% difference of available data for all four age groups. For instance, predicted cardiac output calculated as a function of body weight was 44 L/h in 10 days-1 month and 91 L/h in 6-12 months infants, compared with the reference values of 36 L/h in new-borns and 72 L/h in 12 months old infants. Predicted infant body weights, organ weights, and blood flows calculated as fractions of cardiac output are presented in Table 2.

Model predictions for parameters relating to breastfed infants’ exposure to maternal efavirenz at the 600 mg dose also generally compared well with clinical data, except for the lower end of drug dose from breast milk which tended to be underestimated (39 and 47% of observed for average and maximum dose from milk, respectively). However, the resulting time-averaged plasma concentrations of efavirenz were within 2-fold difference of observed data, with average infant plasma concentration highest in the 10 days-1 month old at 0.27 (0.11–0.87) µg/mL, followed by 0.19 (0.055–0.89) µg/mL in 1–3 months old, 0.18 (0.041–0.67) µg/mL in 3–6 months old, and 0.15 (0.035–0.57) µg/mL in 6–12 months old infants (Table 4). This trend is comparable to the observed decrease from 0.19 µg/mL (0.52–0.71) in 9 days-3 months old, to 0.15 µg/mL (0.052–0.33) in > 3–6 months old, and 0.10 µg/mL (0.041–0.59) in > 6 months old infants in previously published clinical cohort.

Additionally, two different hypothetical scenarios of milk-to-plasma ratios representing approximately 50% and 200% of what has been reported were simulated to assess their implications for infant exposure. At the milk-to-plasma ratio of 0.5, median maximum infant exposure index (based on the recommended infant therapeutic dose of 10 mg/kg) was 2.78 (0.624–17.0) in pooled analysis of all four age groups compared with 6.35 (1.02–29.2) at the milk-to-plasma ratio of 1.13. The exposure index increased to 11.1 (2.49–59.7) at the hypothetical milk-to-plasma ratio of 2.0. The efavirenz concentration-time profiles in infant plasma for all four age groups at these milk-to-plasma ratios are presented in Figure 4.
Figure 2. Predicted (solid lines, mean; dotted lines, standard deviation) and observed (open circles) plasma efavirenz concentration-time profile in infants.

Figure 3. Comparison of predicted versus observed breast milk pharmacokinetic parameters of efavirenz and infant exposure indices. All predictions were within 2-fold difference (dotted lines) of the observed values (solid line).
Table 3. Predicted versus observed pharmacokinetic parameters of efavirenz in breast milk and plasma.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Model Predictions (600 mg daily dose)</th>
<th>Observed Data (600 mg daily dose)</th>
<th>Model Predictions (400 mg daily dose)</th>
<th>Observed Data (400 mg daily dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast milk AUC&lt;sub&gt;(0-24)&lt;/sub&gt; (µg.hr/mL)</td>
<td>75.0 (18.5-324)</td>
<td>68.5 (26.3-257)</td>
<td>52.7 (13.0-290)</td>
<td>-</td>
</tr>
<tr>
<td>Breast milk C&lt;sub&gt;max&lt;/sub&gt; (µg/mL)</td>
<td>4.56 (1.17-16.0)</td>
<td>5.39 (1.43-18.4)</td>
<td>3.16 (0.810-14.6)</td>
<td>-</td>
</tr>
<tr>
<td>Breast milk C&lt;sub&gt;min&lt;/sub&gt; (µg/mL)</td>
<td>2.11 (0.38-12.3)</td>
<td>1.68 (0.316-9.57)</td>
<td>1.51 (0.285-10.7)</td>
<td>-</td>
</tr>
<tr>
<td>Plasma AUC&lt;sub&gt;(0-24)&lt;/sub&gt; (µg.hr/mL)</td>
<td>67.2 (26.3-360)</td>
<td>60.7 (26.8-177)</td>
<td>46.9 (18.7-243)</td>
<td>49.9 (14.8-285)</td>
</tr>
<tr>
<td>Plasma C&lt;sub&gt;max&lt;/sub&gt; (µg/mL)</td>
<td>4.13 (2.08-17.7)</td>
<td>4.63 (2.05-9.76)</td>
<td>2.84 (1.44-12.0)</td>
<td>2.51 (0.95-12.2)</td>
</tr>
<tr>
<td>Plasma C&lt;sub&gt;min&lt;/sub&gt; (µg/mL)</td>
<td>1.95 (0.47-13.6)</td>
<td>2.03 (0.755-6.74)</td>
<td>1.40 (0.352-9.23)</td>
<td>1.46 (0.169-11.3)</td>
</tr>
</tbody>
</table>

Data are presented as median (range). Breast milk and maternal plasma data are from 400 virtual nursing mothers. Previously published data for the 600 mg standard dose involved 29 mothers (Ref. 11). Published data for the 400 mg reduced dose are from a cohort of non-breast feeding adults in the ENCORE1 trial (Ref. 26). Abbreviations: AUC<sub>(0-24)</sub>, area under the concentration-time curve during a 24-hour dosing interval; C<sub>max</sub>, maximum plasma concentration; C<sub>min</sub>, minimum plasma concentration.

Table 4. Indices of infant exposure to maternal efavirenz from breast milk at clinically observed milk-to-plasma ratio of 1.13 (0.50–1.93).

<table>
<thead>
<tr>
<th></th>
<th>400 mg</th>
<th>600 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Feeds Per Day</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

Exposure Indices at 10 Days-1 Month

<table>
<thead>
<tr>
<th></th>
<th>400 mg</th>
<th>600 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum efavirenz dose from milk (µg/kg/day)</td>
<td>593 (219 - 1980)</td>
<td>870 (317 - 2920)</td>
</tr>
<tr>
<td>Infant plasma efavirenz conc. (µg/mL)</td>
<td>0.21 (0.079 - 0.73)</td>
<td>0.27 (0.10 - 1.0)</td>
</tr>
<tr>
<td>Exposure Index (EI, %)*</td>
<td>5.9 (2.2-20)</td>
<td>8.7 (3.2-29)</td>
</tr>
<tr>
<td>Infants with EI Above 10.0 (%)</td>
<td>13</td>
<td>36</td>
</tr>
</tbody>
</table>

Exposure Indices at 1-3 Months

<table>
<thead>
<tr>
<th></th>
<th>400 mg</th>
<th>600 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum efavirenz dose from milk (µg/kg/day)</td>
<td>481 (167 - 1650)</td>
<td>702 (241 - 2430)</td>
</tr>
<tr>
<td>Infant plasma efavirenz conc. (µg/mL)</td>
<td>0.14 (0.042 - 0.65)</td>
<td>0.19 (0.055 - 0.87)</td>
</tr>
<tr>
<td>Exposure Index (EI, %)</td>
<td>4.8 (1.7-16)</td>
<td>7.0 (2.4-24)</td>
</tr>
<tr>
<td>Infants with EI Above 10.0 (%)</td>
<td>10</td>
<td>25</td>
</tr>
</tbody>
</table>

Exposure Indices at 3-6 Months

<table>
<thead>
<tr>
<th></th>
<th>400 mg</th>
<th>600 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum efavirenz dose from milk (µg/kg/day)</td>
<td>383 (95 - 1310)</td>
<td>558 (138 - 1940)</td>
</tr>
<tr>
<td>Infant plasma efavirenz conc. (µg/mL)</td>
<td>0.13 (0.031 - 0.78)</td>
<td>0.18 (0.041 - 0.67)</td>
</tr>
<tr>
<td>Exposure Index (EI, %)</td>
<td>3.8 (0.95-13)</td>
<td>5.6 (1.4-19)</td>
</tr>
<tr>
<td>Infants with EI Above 10.0 (%)</td>
<td>3</td>
<td>12</td>
</tr>
</tbody>
</table>

Exposure Indices at 6-12 Months

<table>
<thead>
<tr>
<th></th>
<th>400 mg</th>
<th>600 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum efavirenz dose from milk (µg/kg/day)</td>
<td>287 (70.8 - 961)</td>
<td>418 (102 - 1420)</td>
</tr>
<tr>
<td>Infant plasma efavirenz conc. (µg/mL)</td>
<td>0.11 (0.026 - 0.65)</td>
<td>0.15 (0.035 - 0.57)</td>
</tr>
<tr>
<td>Exposure Index (EI, %)</td>
<td>2.9 (0.71-9.6)</td>
<td>4.2 (1.0-14)</td>
</tr>
<tr>
<td>Infants with EI Above 10.0 (%)</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

Data are presented as median (range). Predicted infant plasma efavirenz concentrations (n = 100 per age group) did not change significantly during the dosing interval and average predicted values are presented.
Figure 4. Infant efavirenz concentration-time profiles at the observed and hypothetical milk-to-plasma ratios of 1.13, 0.5, and 0.2. Simulated concentration-time profiles were relatively flat in all age groups, reflecting the frequent doses received from breast milk. At the observed milk-to-plasma ratio of 1.13, the median (range) infant plasma concentration was highest in the 10 days–1 month old at 0.27 (0.11–0.87) µg/mL and lowest in 6–12 months old at 0.15 (0.035–0.57) µg/mL.
median (range) plasma efavirenz concentration averaged over the dosing interval for each infant were 0.104 (0.018–0.75) µg/mL at milk-to-plasma ratio of 0.5 and 0.30 (0.057–1.26) µg/mL at milk-to-plasma ratio of 2.0, compared with 0.19 (0.035–1.00) µg/mL at milk-to-plasma ratio of 1.13 and the previously reported 0.16 ng/mL (0.029–1.36)\textsuperscript{11}.

Breast milk pharmacokinetics and breastfed infants’ exposure in the context of 400 mg reduced dose of efavirenz

Further simulations were conducted for the four infant age groups at the observed milk-to-plasma ratio of 1.13 to explore the potential impact of reducing efavirenz dose to 400 mg on breast milk pharmacokinetics and plasma exposure in nursing mother-infant pairs. Breast milk C\textsubscript{min} and C\textsubscript{max} were below 1.0 µg/mL in 11.5 and 28% of simulated subjects, respectively, compared with 2.5 and 14.5% at the standard 600 mg dose. Plasma C\textsubscript{T1/2} and C\textsubscript{max} were below 1.0 µg/mL in 5 and 32% of simulated subjects, respectively, compared with 0 and 15% at the standard 600 mg dose. However, the number of subjects with C\textsubscript{max} above the 4.0 µg/mL toxicity threshold reduced from 50% at 600 mg to 24% at the reduced 400 mg daily dose. In pooled analysis, the resulting plasma concentration was 0.15 (0.026–0.78) µg/mL, approximately 25% lower than simulated exposure at 600 mg. The maximum exposure index was 4.29 (0.708–19.8), about 30% lower than at 600 mg and above 10% in 6.5% of simulated infants, compared with 18% of simulated infants at 600 mg. The indices of foetal exposure for the different age groups are presented on Table 4.

**Discussion**

PBPK modelling was applied for the prediction of breast milk and plasma pharmacokinetics of the antiretroviral drug efavirenz in nursing mother-infant pairs. The model integrates a previously validated whole-body oral adult PBPK model\textsuperscript{12} with a whole-body breastfed infant PBPK sub-model. System and drug-specific parameters for the infant sub-model were either obtained from the literature or scaled from the adult model, and variability was introduced to reflect in vivo observations. Breastfeeding was successfully described by repeated (2 hourly) ingestion of a volume of breast milk controlled by infant suckling rate\textsuperscript{15} Simulated breast milk and plasma pharmacokinetic parameters, as well as various measures of breastfed infants’ exposure, showed good agreement with observed data for the standard 600 mg daily dose of efavirenz\textsuperscript{11}, except for the lower end of infant plasma concentration range which tended to be underestimated. Plasma pharmacokinetic parameters in virtual subjects who received the reduced 400 mg dose were similar to those observed in adults who received the 400 mg in the ENCORE1 trial\textsuperscript{28}. About 5% of simulated subjects were predicted to have C\textsubscript{T1/2} below the recommended 1.0 µg/mL with the 400 mg dose, similar to the 4.7% observed in the trial.

PBPK models have been used to describe plasma and intracellular efavirenz pharmacokinetics following oral and intramuscular administrations, respectively\textsuperscript{12,27}. In addition to accurately predicting plasma pharmacokinetics as in previous models, breast milk pharmacokinetics predicted by the current model are very similar to observed clinical data\textsuperscript{11}. Willmann et al used similarly coupled PBPK models for mother-infant pairs to assess the risk of opioid poisoning to breast-fed neonates\textsuperscript{39}. Other previous applications in human lactation studies have been limited to environmental risk assessments where they are used to quantitatively describe the lactational transfer of inhaled contaminants\textsuperscript{30}, trichloroethylene and its metabolite\textsuperscript{31}, tetrachloroethylene and associated cancer risk for breast-fed infants\textsuperscript{32}, perchlorate and iodide including inhibition of iodide thyroidal uptake by perchlorate\textsuperscript{33}. An extensive review by Corley et al. describes the underlying assumptions, model structures, data and methods used in the development and validation of these early PBPK models\textsuperscript{34}. Similar models have been described for polychlorinated biphenyls\textsuperscript{35}, co-exposure to polychlorinated biphenyls and methyl mercury\textsuperscript{36}, persistent organic pollutants (including an initial infant body burden to represent intrauterine exposure)\textsuperscript{37}, manganese\textsuperscript{38}, and perfluoralkyl carboxylates and sulfonates\textsuperscript{39}. The use of a population pharmacokinetic modelling approach to predict infant exposure through breast milk has been reported for a number of drugs and was recently reviewed by Anderson et al.\textsuperscript{40} Examples include tramadol and its O-desmethyl metabolite\textsuperscript{41}, fluoxetine and its active metabolite norfluoxetine\textsuperscript{42}, nevirapine\textsuperscript{43}, and parecoxib and its active metabolite valdecoxib\textsuperscript{44}. However, a major advantage of the PBPK approach described here is that it does not require clinical pharmacokinetics data for model building unlike the population pharmacokinetics approach. Additionally, PBPK modelling offers higher fidelity to actual physiological conditions and can be used to simulate best- and worse-case scenarios once the requisite in vitro drug data have been integrated into with available physiological and anatomical data.

Replicating the ontogeny of drug metabolism enzymes is one of the major challenges in the development of paediatric PBPK model. Children often display developmentally unique differences in drug disposition compared to adults, making simple scaling using anthropometric characteristics unreliable. For instance, paediatric doses of efavirenz derived from adult dose using simple allometric scaling have been reported to result in sub-therapeutic and higher variability in plasma concentrations compared to adults\textsuperscript{39}. The CYP2B6 hepatic cytochrome P450 isoform accounts for over 90% of efavirenz metabolism. Polymorphisms in CYP2B6 gene is known to cause significant inter-individual variability in CYP2B6 enzyme expression and activity, resulting in variability in the metabolism of substrate drugs. We previously demonstrated that infant plasma efavirenz concentration resulting from breast milk exposure was influenced by both maternal and infant CYP2B6 genotypes\textsuperscript{11}. Therefore, we used paediatric CYP2B6 protein expression data available in the literature\textsuperscript{39}, and replicated in vivo variability using MATLAB rule expression that incorporated the mean, standard deviation, minimum and maximum values for each age stratum. Further variability in the resulting CYP2B6 intrinsic clearance was introduced through simple linear interpolation of age which modulates milligram of microsomal protein per gram of liver. In view of their minimal role in efavirenz metabolism, CYP2A6, CYP3A4, and CYP3A5 intrinsic clearances were scaled from adult values through
infant age, and variability in each age stratum was introduced by linear interpolation.

This approach adequately described infant plasma efavirenz concentrations resulting from breastfeeding in the presence of maternal efavirenz for the different age groups, demonstrating the reasonableness of age-related changes in model parameters as well as the associated scaling and variability. As with the clinical data, model predictions indicate that exposure to maternal efavirenz from breast milk resulted in measurable plasma concentrations in infants. The implications for possible development of drug resistance in infants who become infected call for further clinical investigation. The model can be used for other drug classes and therapeutic areas, provided the requisite drug-specific parameters are available or accurately predictable from other known parameters. The hypothetical milk-to-plasma ratios were included to illustrate the possibility of using this model to simulate best- and worse-case scenarios even where the milk-to-plasma ratio is unknown.

However, a number of limitations are identifiable in this model. First, the lack of milk-to-plasma ratio prediction component means that only hypothetical best- and worse-case scenarios can be predicted for drugs with no observed milk-to-plasma ratio. A number of models have appeared in the literature for predicting the milk-to-plasma ratio\(^{40,43}\). Unfortunately, their utility has been limited by lack of universal accuracy which may constitute additional source of uncertainty in this type of model. In addition, the present model did not consider the potential role of drug transporters in breast milk excretion because efavirenz is not a known substrate of any transporter in humans\(^{44,45}\). However, this can be incorporated for drugs with known active transport mechanisms in mammary gland as previously described for OATP1B1/1B3-mediated irbesartan hepatic uptake\(^{46}\). For instance, ABCG2 is known to be highly expressed in lactating human mammary gland\(^{47}\), involved in the secretion of its substrates into breast milk\(^{40,43}\), and can be affected by polymorphisms in ABCG2 gene\(^{48}\). Integrating such approaches with the current model can potentially extend its application to drugs with no available breast milk data and can be used as a tool in the drug development process. Lastly, the outputs of any model are only as reliable as the quality of input data.

In conclusion, the breastfeeding PBPK model described here opens up opportunities for expanding our understanding of infant exposure to maternal drugs through breast milk, including during the drug development process. Its application can help in bridging existing gaps and pave the way for evidence-based recommendations for drug use during lactation.

Data availability
The data underlying this study is available from Open Science Framework. Dataset 1: Physiologically-based pharmacokinetic modelling of infant exposure to efavirenz through breastfeeding.

http://doi.org/10.17605/OSF.IO/ZJTVS\(^{41}\).

Data is available under a CC0 1.0 Universal license.

Data used for validation was taken from Olagunji et al. https://doi.org/10.1093/cid/civ317\(^{41}\).

Maternal pharmacokinetics at 400mg were validated using the ENCORE1 results as presented by Dickinson et al. https://doi.org/10.1002/cpt.156\(^{46}\).

Competing interests
SK, A. Owen and MS have received research grants and/or travel bursaries from Merck, Bristol Myers and Squibb, GlaxoSmithKline, Pfizer, Abbott, ViV, Boehringer Ingelheim and Janssen Pharmaceuticals. The remaining authors have no competing interests to disclose.

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements
We would like to thank patients and staff at collaborating clinical sites in Benue State, Nigeria who participated in the clinical study that provided data for validating this model for their support. We also acknowledge Obafemi Awolowo University and the University of Liverpool for making the necessary resources available.

References


42. Begg EJ, Atkinson HC: Modelling of the passage of drugs into milk. Pharmaco/


Edmund V. Capparelli  
Division of Host-Microbe Systems and Therapeutics, University of California, San Diego, San Diego, CA, USA

The paper presents development of a PBPK model for EFV that integrates maternal PK and infant exposure via breastfeeding. It represents an extension of their prior work on PBPK modeling of ARVs and EFV penetration into breast milk. The most interesting aspect is the infant exposure. Overall it is a represents new information and an approach that would be of interested to readers.

Main comments:

While the author previous publication on the clinical trial of infant EFV exposure visa breast feeding1 demonstrated a pronounced effect of maternal/infant CYP2B6 genotype on infant exposure during breast feeding, it is not included in the PBPK model. Why not? A model that includes genotype specific CYP2B6 CLin in predicting exposure would provide much more interesting simulation and provide a more realistic expected distribution of infant EFV exposure via breast feeding.

Infant exposure following a 33% reduction in dosing using 400 vs 600mg of maternal dosing resulted a ~25% reduction. This probably doesn't warrant its current prominence in the abstract or paper unless the authors believe that this difference in infant levels is significantly different that the maternal dose reduction (i.e. 25% is different from 33%). A better case should be made that this is an important difference, then discussion on the cause of the difference is needed. Based on the overall model I suspect it is based on reduce autoinduction at the lower dose but other factors and assumptions may be involved.

Is there information that indicates CYP2B6 induction is quantitative similar in magnitude in newborns and adults and that the drivers for induction occurs at similar concentrations infants and adult? If there are no data supporting these assumptions, then they should be included in limitations. They are key to the “dose” effects observed. Similarly, the developmental model for CYP2B6 by Croom et al was performed in the absence of inducers or knowledge/impact of CYP2B6 genotype and should be discussed as limitation of the methods.

It appears it model assumed that absorption was similar in newborn as adults. If that is not the case it should be made clearer. Bioavailability of EFV appears to be lower in young infants and the approved doses are greater than the general pediatric dose of 10 mg/kg/d dose listed on page 6 and how the model addressed this is issue needs to be discussed.
In the methods of their prior CID paper, dried blood spots (DBS) and dried breast milk were used, yet EFV concentrations are referred to as being “plasma”. Was there a conversion between the two? How do the DBS and plasma concentration compare? Some studies have shown significant differences between simultaneous EFV DBS and plasma concentrations. Did the authors perform any PBPK simulations on whole blood/DBS concentrations and if so, how were the results affected?

Other comments:

A comparison of model infant predicted versus observed concentrations should be highlighted in the abstract with more details. The comparison data collected samples at 2 and 8 hours post dose (as described in the CID article) and age effects are the most interesting findings.

EFV is highly protein bound and plasma protein concentrations are lower in newborns/infants and can have different binding than older populations. How was this incorporated into the model?

The authors should consider a different first paragraph in the introduction. The emphasis on US regulatory developments in pediatrics has little relevance to the analysis as presented. If any regulatory or guidance documented are discussed, they should focus on the drugs in BM guidance and the difficulty in performing these studies as described, thus the value of PBPK simulation methods.

Consistent concentration units should be used there is a mix of uM and mcg/mL.

How do EFV dried milk spots concentrations compare to those from “fresh” breast milk? How does the BM fat content affect BM concentrations of a lipophilic drug EFV? Could some of the infant effect be driven by BM composition.

In the discussion first paragraph the sentence “except for the lower end of the infant plasma concentration range which tended to be underestimated” is confusing. What is meant by the “lower end”? Younger infants? Also figure 3 suggestions in all infants that simulated concentrations were greater than observed not under-estimated.

In the limitations, the emphasis of specific drug transporters, none previously shown to transport EFV, listed receives undue emphasis. The variability in BM fat content, lack of CYP2B6 polymorphisms potential age dependent protein binding, assumption out autoinduction and altered infant absorption are bigger potential limitations and should be discussed.

Figures 1 and 2 – why are arithmetic mean (+ SD) concentrations used when we know that there are significant polymorphisms in metabolism and they are not normally distributed. Medians and percentiles would be a more appropriate description distribution of the data.

Figure 3 – to present all of the comparisons the range of values encompasses over 1000-fold a log-log plot. While the log scale helps to allow integrating into a single figure, it compresses the figures and differences. It would be nice to see the simulated infant predicted vs observed in the different age groups as a separate figure with error bars. It would provide a better indication of the over prediction seen.

Figure 4 is difficult to interpret. While it is important to visually show the flatness of the infant conc vs time profile, the impact of M/P is near proportional to the infant concentrations. I suspect the minor deviations
from proportionality are due to predicted auto-induction being greater with higher M/P ratios. But the less than proportional increase with M/P ratios and even age feature are difficult to extract from the current figure. This figure could be a supplemental figure. A figure with comparing the concentration vs time profiles for maternal plasma/DBS, BM and infant plasma/DBS concentrations would be much more interesting.

Table 2 could be moved to a supplemental file.

References

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Referee Report 21 May 2018
doi:10.21956/aasopenres.13926.r26421

Jeffrey W. Fisher
National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR, USA

The use of the word drug in the text below refers to Efavirenz. This paper is well written and describes a PBPK model for lactation in an acceptable manner common in pharmacology. Since this is a relatively
new area for drugs, however, providing more modeling details than is customary, would be of great value. This computational effort is important for understanding lactational transfer of drugs and predicting levels of the drug in mother and infant.

The equation and model parameter values representing the mammary gland and milk compartment need to be shown in the paper. Include assumptions. Since this is new to your paper please include what you did. Is only the free concentration of the drug assumed to transfer to milk from plasma (I assume so)? Is there bi-directional transfer of the drug into and out of the milk compartment and plasma? Blood flow to mammary gland changes during lactation. The volume of maternal fat increases during pregnancy and decreases during lactation. This drug is lipophilic, thus fat is an important model parameter. Perhaps a sensitivity analysis would reveal this.

It is not clear if the maternal physiology initial conditions were those of a pregnant woman at birth? This would be the normal approach and then describe the changes in maternal physiology during lactation. Scaling of physiology for a neonate/infant less than 1-2 years of age is not recommended because of nonlinear growth not described by simple allometric functions. The authors are referred to Claassen et al. 2015. Current Pharmaceutical Design, 21, 5688-5698 for PBPK modeling of early life considerations for drugs.

To use a data set which contains mother-infant paired blood samples and breast milk samples is a wonderful situation to be in. It was unclear when samples of breast milk were taken relative to mother-infant blood samples and if mother-infant blood samples taken within a short period of time of each other? If so, plotting individual model predictions of mother’s blood and breast milk concentrations vs infant blood concentration would be worthwhile to understand how your model performs and gain insights into the nature of the mother-infant variability.

The breast milk and maternal plasma drug levels (bound plus free) track each other, except for some high levels in breast milk. This suggests fat:plasma partitioning of this drug is high and the drug quickly enters into milk (as shown in Fig. 1). The % fat in breast milk can be found in the literature, thus you can estimate a milk:plasma partition coefficient and predict milk levels of drug based on model predicted free concentration in plasma.

How do you predict the bound and free drug in infant plasma?

Is metabolism in the mother and infant based on model predicted free concentration of drug?

Is it expected that at birth the baby has a body burden of drug? If so, using cord blood values you could simulate the neonate to estimate the total drug burden, not just hair. Then your starting conditions would include this ‘background’ level of drug when lactational transfer starts. Including hair has successfully been included in PBPK models for metals (adults) if you are interested.


Unfortunately we do not have many measured blood flows in neonate/infant/child. Read Claassen et al. 2015 or other pediatric PBPK modeling papers for drugs. Your paper infers that blood flow rates to all the organs are known, which is not really true.
One other modeling lactation paper where mother-infant pair data exist with breast milk is found in Fisher et al. 2015 for the nutrient iodine (PLOS ONE | DOI:10.1371/journal.pone.0149300 March 1, 2016). Perhaps this may be of some value to you.

Are the high levels of drug in maternal plasma and breast milk correspond to the high levels in the nursing infant plasma 20+ hours after maternal dose of the drug? This is one reason to examine individual datasets for mother-infant plasma and breast milk.

Since you used Matlab consider publishing the code as a supplemental. This way what you did will be fully understood by modelers who write script.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests**: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.