RESEARCH ARTICLE

Physiologically-based pharmacokinetic modelling of infant exposure to efavirenz through breastfeeding [version 1; referees: 1 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 worse-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 AUC_{0-24}, C_{max} and C_{min} 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 AUC_{0-24}, C_{max} and C_{min} 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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Referee Status: ?

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1 **Jeffrey W. Fisher**, US Food and Drug Administration, USA

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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[1]. 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[2,3]. 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[4]. 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[5–9]. 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[10].

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[11]. 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 intramuscular long-acting nanoformulations[12], with appropriate adjustments to exclude male-specific system parameters and an additional compartment introduced to represent the mammary gland. As previously described[13], 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[11]. 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[11,14]. Efavirenz-specific parameters included in the model have been are 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_a) was described using effective permeability (P_{eff}) derived from Caco-2 permeability as previously described[15].

Intestinal drug clearance (CL_{gut}) 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_g) was calculated using equation (2), where Q_{gut} and $f_{u,gut}$ are intestinal blood flow and fraction unbound in the intestine, respectively.

$$CL_{gut} = Ind_{CYP3A4} \times Ab_{CYP3A4} \times (CYP3A4\ rCL_{int}/R) \quad (1)$$

$$F_g = \frac{Q_{gut}}{(Q_{gut} + f_{u,gut} \times CL_{gut})} \quad (2)$$

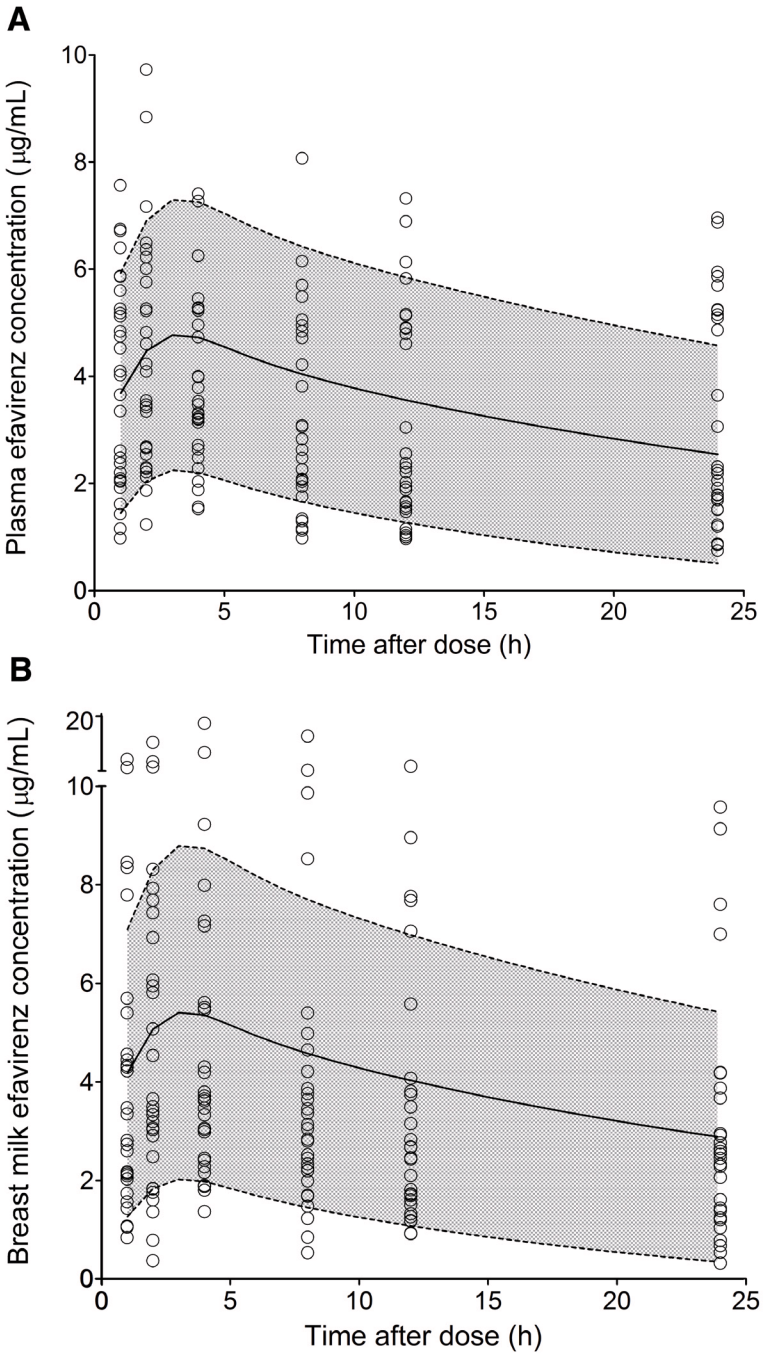

Figure 1. Predicted (solid lines, mean; dotted lines, standard deviation) and observed (open circles) plasma (**A**) and breast milk (**B**) efavirenz concentration-time profiles in women receiving 600 mg efavirenz. Over 80% of observed data points (n = 203 paired plasma and breast milk samples, from 29 subjects) were within the predictive interval.

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 *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 *et al*[20]. A plot of CYP2B6 expression in individual tissue samples from birth to 1 year was digitised using 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),

Table 1. Drug-specific physicochemical properties and *in vitro* data for Efavirenz[12].

Drug properties	Description	Values
MW	Molecular weight	316
LogP	Octanol-water partition coefficient	4.60
pKa	Acid dissociation constant	10.2
R	Oral bioavailability	0.74
PSA	Polar surface area	38.33
HBD	Hydrogen bond donor	1
K (mg/mL)	Water solubility	0.00855
Fu	Fraction unbound in plasma	0.01
Vd (L/kg)	Volume of distribution at steady state	3.6
P_{eff} (cm/s)	Effective permeability (Caco-2)	2.5×10^{-6}
CL_{int} (µL/min/pmol)	Intrinsic hepatic clearance by cytochrome P450 (CYP) enzymes	
rCYP1A2 CL_{int}		0.008
rCYP2A6 CL_{int}		0.05
rCYP2B6 CL_{int}		0.55
rCYP3A4 CL_{int}		0.007
rCYP3A5 CL_{int}		0.03
Ind_{CYP} (µM)	Hepatic CYPs induction	
CYP2B6 Ind_{max}		5.76
CYP3A4 Ind_{max}		6.45
CYP2B6 Ind_{50}		0.82
CYP3A4 Ind_{50}		3.93

intrinsic clearance (CL_{int}), CYP2B6 induction (Ind_{CYP2B6}), total intrinsic clearance (TCL_{int}), total apparent clearance (CL_{app}), systemic clearance (CL), and fraction escaping first-pass metabolism (F_h) were calculated using equation (3) to equation (9) as previously described[12].

$$MPPGL = 10^{(1.407+0.0158 \times Age-0.00038 \times Age^2 + 0.0000024 \times Age^3)} \quad (3)$$

$$CL_{int} = (Ind \times (rCL_{int}/R) \times Ab_{CYP} \times MMPGL \times Wt_{liver})) \quad (4)$$

$$Ind_{CYP2B6} = 1+(Indmax \times [EFV]_{plasma})/(Ind50+[EFV]_{plasma}) \quad (5)$$

$$TCL_{int} = CL_{int} \times Ab_{CYP} \times Wt_{liver} \times MPPGL \quad (6)$$

$$CL_{app} = \sum_{n=1}^{n} TCL_{int} \quad (7)$$

$$CL = \frac{Q_{hv} \times f_u \times CL_{app}}{Q_h + CL_{app} \times f_u} \quad (8)$$

$$F_h = 1 - CL/Q_{hv} \quad (9)$$

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® linspace function to generate equally spaced values within each group. Where physiological and anatomical data were used, MATLAB® 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.

Modelling breastfeeding

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

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

$$EFV\ Dose_{milk} = V_{milk} \times [EFV]_{milk} \quad (11)$$

$$Exposure\ Index = \frac{EFV\ Dose_{milk}}{EFV\ Dose_{therapeutic}} \quad (12)$$

where $[EFV]_{plasma}$ is simulated efavirenz concentration in plasma, $M/P_{AUC_{0-24}}$ is the clinically observed milk-to-plasma AUC_{0-24} ratio (median: 1.13; range: 0.50-1.93)[11], V_{milk} is the volume of breast milk, and EFV Dose(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[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® (version 5.1, MATLAB® 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 2. Key simulated anatomical and physiological parameters (mean, SD) for infant sub-model.

	10 days-1 month	1–3 months	3–6 months	6–12 months
Age (y)	0.06 (0.02)	0.18 (0.04)	0.38 (0.07)	0.80 (0.12)
Weight (kg)	3.89 (0.58)	4.81 (0.65)	6.40 (0.80)	8.65 (1.04)
Organ Weights (kg)				
Adipose	1.14 (0.57)	1.38 (0.56)	1.94 (0.61)	2.59 (0.75)
Blood	0.40 (0.00)	0.40 (0.00)	0.43 (0.01)	0.50 (0.03)
Bones	0.21 (0.04)	0.26 (0.06)	0.34 (0.07)	0.48 (0.11)
Brain[1]	0.40 (0.03)	0.46 (0.04)	0.58 (0.04)	0.82 (0.07)
Heart[1]	0.02 (0.00)	0.03 (0.01)	0.04 (0.01)	0.10 (0.03)
Intestines	0.06 (0.05)	0.08 (0.04)	0.09 (0.05)	0.12 (0.05)
Kidneys[1]	0.04 (0.00)	0.04 (0.00)	0.05 (0.00)	0.06 (0.00)
Liver[1]	0.18 (0.03)	0.22 (0.03)	0.27 (0.03)	0.34 (0.04)
Lungs[1]	0.06 (0.00)	0.07 (0.01)	0.08 (0.01)	0.10 (0.01)
Muscle	0.75 (0.46)	0.47 (0.38)	1.42 (0.73)	3.14 (0.95)
Pancreas[1]	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.02 (0.00)
Remaining	0.28 (0.03)	0.32 (0.04)	0.39 (0.04)	0.51 (0.06)
Skin	0.29 (0.06)	0.30 (0.05)	0.34 (0.06)	0.40 (0.05)
Spleen[1]	0.01 (0.00)	0.01 (0.00)	0.02 (0.00)	0.02 (0.00)
Stomach	0.10 (0.05)	0.10 (0.05)	0.11 (0.06)	0.11 (0.05)
Thymus[1]	0.01 (0.00)	0.02 (0.00)	0.02 (0.00)	0.02 (0.00)
Total weight	4.57 (0.10)	4.72 (0.12)	4.99 (0.14)	5.51 (0.18)
Organ Blood Flows (L/h)				
Cardiac output	44.66 (5.88)	53.96 (6.47)	69.60 (7.80)	91.05 (9.70)
Adipose	1.74 (0.23)	2.10 (0.25)	2.71 (0.30)	3.55 (0.38)
Brain	14.11 (1.86)	17.05 (2.04)	21.99 (2.46)	28.77 (3.07)
Gonads	0.22 (0.03)	0.27 (0.03)	0.35 (0.04)	0.46 (0.05)
Gut	3.89 (0.51)	4.69 (0.56)	6.06 (0.68)	7.92 (0.84)
Hepatic artery	2.90 (0.38)	3.51 (0.42)	4.52 (0.51)	5.92 (0.63)
Hepatic vein	3.90 (0.38)	4.51 (0.42)	5.52 (0.51)	6.92 (0.63)

	10 days-1 month	1–3 months	3–6 months	6–12 months
Kidneys	4.11 (0.54)	4.96 (0.60)	6.40 (0.72)	8.38 (0.89)
Lungs	0.54 (0.07)	0.65 (0.08)	0.84 (0.09)	1.09 (0.12)
Muscle	1.74 (0.23)	2.10 (0.25)	2.71 (0.30)	3.55 (0.38)
Pancreas	0.54 (0.07)	0.65 (0.08)	0.84 (0.09)	1.09 (0.12)
Portal vein	6.42 (0.58)	7.34 (0.64)	8.89 (0.77)	11.01 (0.96)
Rest of body	2.23 (0.29)	2.70 (0.32)	3.48 (0.39)	4.55 (0.49)
Skin	1.16 (0.15)	1.40 (0.17)	1.81 (0.20)	2.37 (0.25)
Spleen	0.89 (0.12)	1.08 (0.13)	1.39 (0.16)	1.82 (0.19)
Stomach	0.40 (0.05)	0.49 (0.06)	0.63 (0.07)	0.82 (0.09)

Reference values available from Pryce *et al.* and/or Coppoletta *et al.* [1]With the exception of pancreas in the 10 days-1 month stratum, infant organ weights and blood flows were within 50% fold difference of reference values.

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[22,23]. Pharmacokinetic parameters were evaluated at steady state and $AUC_{0\text{-}24}$ was calculated using the trapezoidal rule. The most comprehensive published clinical data of efavirenz pharmacokinetics in human breast milk and exposure of breastfed infants[11] were used to validate predicted pharmacokinetic parameters.

Results

Breastfed infant sub-model validation

The validation of the adult model has been previously described[12]. 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[24]. Predicted infant body weights, organ weights, and blood flows calculated as fractions of cardiac output are presented in Table 2[20,22,23].

Model-predicted breast milk and plasma pharmacokinetics of efavirenz in nursing mother-infant pairs

The adult model adequately described the plasma and breast milk pharmacokinetics of efavirenz, with over 80% of observed data points (203 matched breast milk and plasma pairs from 29 patients) falling within model predictive interval for both fluids (Figure 1, data plotted as mean ± SD, n = 400). The resulting plasma and breast milk pharmacokinetic parameters ($AUC_{0\text{-}24}$, C_{min}, and C_{max}) were within 2-fold of those observed in a cohort of postpartum women receiving 600 mg efavirenz as part of their antiretroviral regimen[11] (Figure 2). For instance, model-predicted versus observed median (range) breast milk $AUC_{0\text{–}24}$, C_{max} and C_{min} 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 (Table 3).

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[11], 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) 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 in our previously published clinical cohort[11].

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 (range) 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. The combined

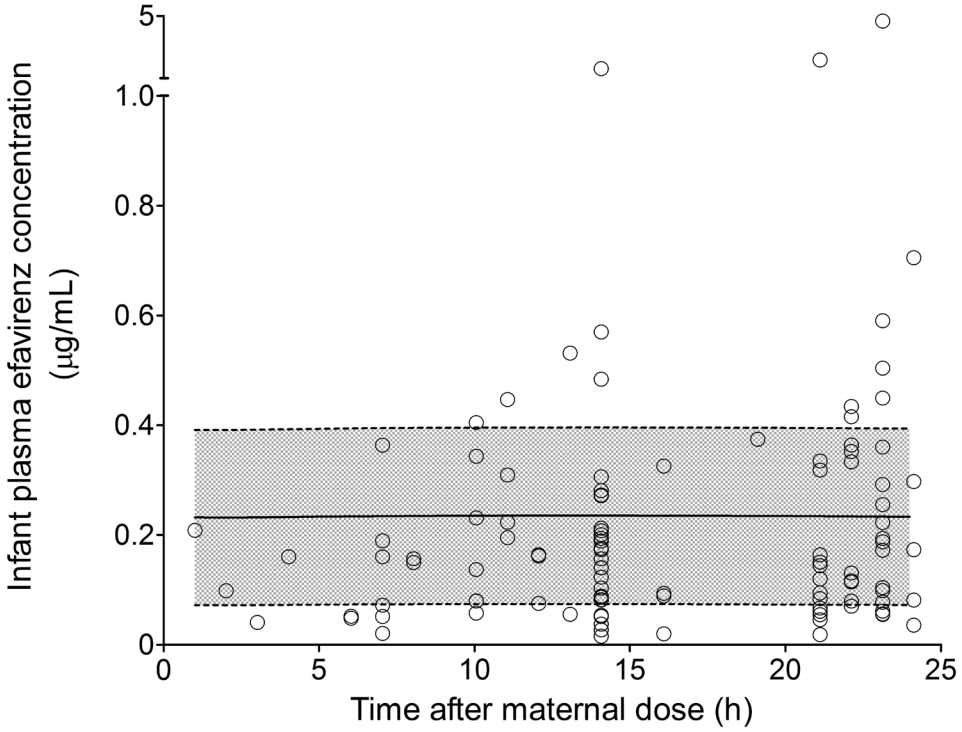

Figure 2. Predicted (solid lines, mean; dotted lines, standard deviation) and observed (open circles) plasma efavirenz concentration-time profile in infants.

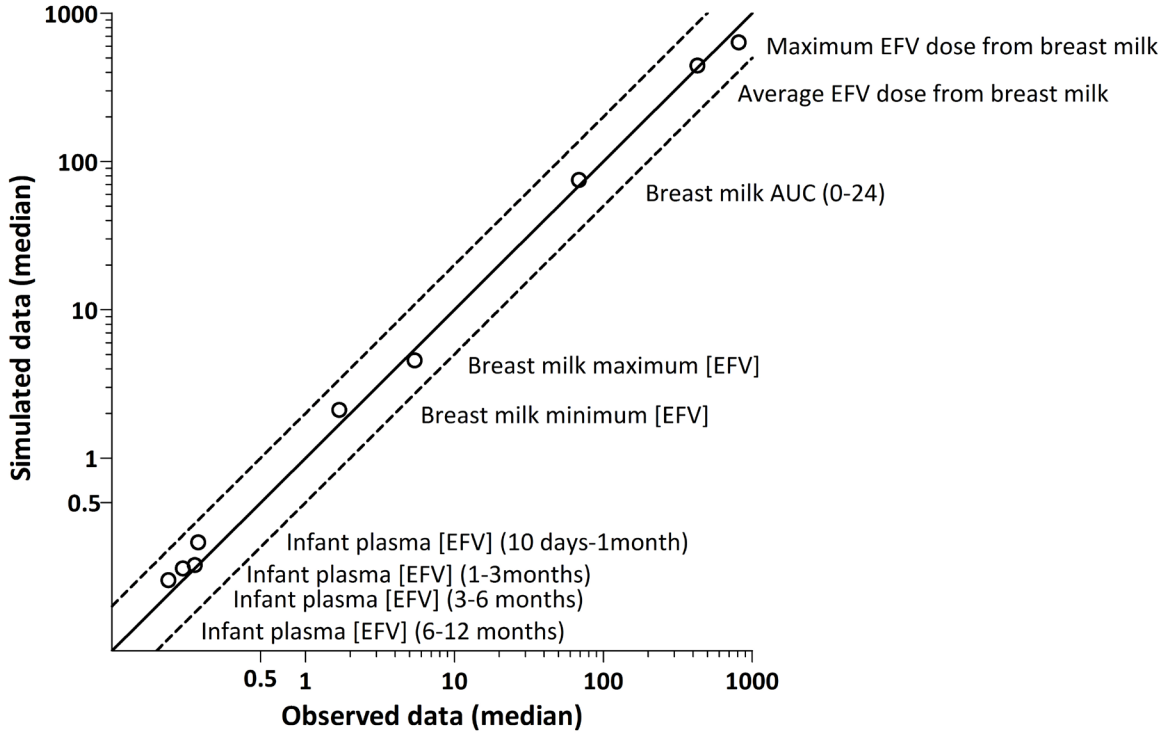

Figure 3. Comparison of predicted versus observed breast milk pharmacokinetic parameters of efavirenz and infant exposure indices. All predictions were within 2-fold difference (dotted lines) of the observed values (solid line).

Table 3. Predicted versus observed pharmacokinetic parameters of efavirenz in breast milk and plasma.

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

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: $AUC_{(0-24)}$, area under the concentration-time curve during a 24-hour dosing interval; C_{max}, maximum plasma concentration; C_{min}, 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).

	400 mg	600 mg
Number of Feeds Per Day	12	12
Exposure Indices at 10 Days-1 Month		
Maximum efavirenz dose from milk (µg/kg/day)	593 (219 - 1980)	870 (317 - 2920)
Infant plasma efavirenz conc. (µg/mL)	0.21 (0.079 - 0.73)	0.27 (0.10 - 1.0)
Exposure Index (EI, %)*	5.9 (2.2-20)	8.7 (3.2-29)
Infants with EI Above 10.0 (%)	13	36
Exposure Indices at 1-3 Months		
Maximum efavirenz dose from milk (µg/kg/day)	481 (167 - 1650)	702 (241 - 2430)
Infant plasma efavirenz conc. (µg/mL)	0.14 (0.042 - 0.65)	0.19 (0.055 - 0.87)
Exposure Index (EI, %)	4.8 (1.7-16)	7.0 (2.4-24)
Infants with EI Above 10.0 (%)	10	25
Exposure Indices at 3-6 Months		
Maximum efavirenz dose from milk (µg/kg/day)	383 (95 - 1310)	558 (138 - 1940)
Infant plasma efavirenz conc. (µg/mL)	0.13 (0.031 - 0.78)	0.18 (0.041 - 0.67)
Exposure Index (EI, %)	3.8 (0.95-13)	5.6 (1.4-19)
Infants with EI Above 10.0 (%)	3	12
Exposure Indices at 6-12 Months		
Maximum efavirenz dose from milk (µg/kg/day)	287 (70.8 - 961)	418 (102 - 1420)
Infant plasma efavirenz conc. (µg/mL)	0.11 (0.026 - 0.65)	0.15 (0.035 - 0.57)
Exposure Index (EI, %)	2.9 (0.71-9.6)	4.2 (1.0-14)
Infants with EI Above 10.0 (%)	0	5

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.

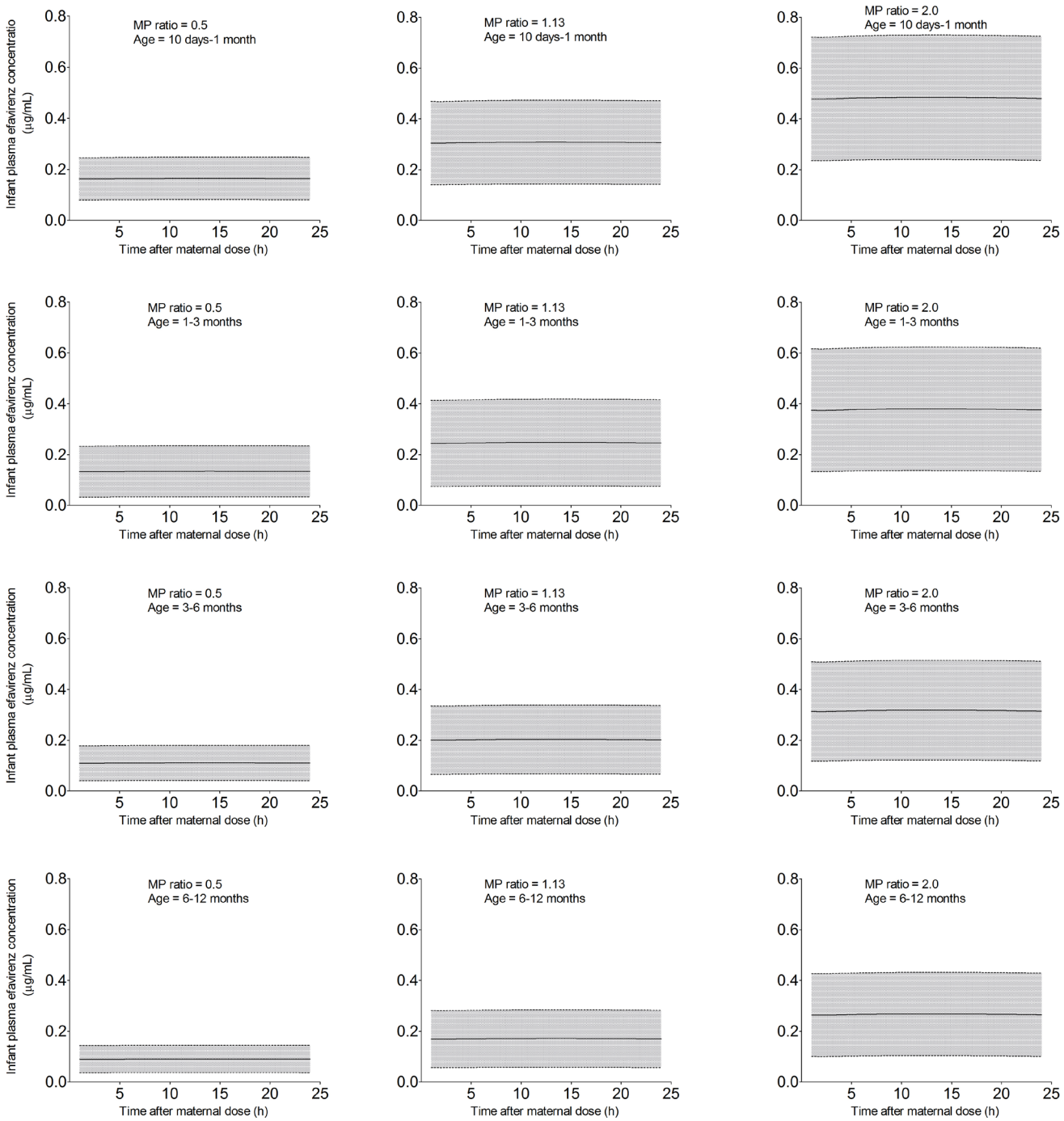

Figure 4. Infant efavirenz concentration-time profiles at the observed and hypothetical milk-to-plasma ratios of 1.13, 0.5, and 0.2. Simulated concentration-time profiles were relatively flat in all age groups, reflecting the frequent doses received from breast milk. At the observed milk-to-plasma ratio 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)[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_{12} and C_{min} 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_{12} and C_{min} 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_{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[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[25] 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[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[26]. About 5% of simulated subjects were predicted to have C_{12} 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[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[11]. Willmann *et al* used similarly coupled PBPK models for mother-infant pairs to assess the risk of opioid poisoning to breast-fed neonates[10]. 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[5], trichloroethylene and its metabolite[6], tetrachloroethylene and associated cancer risk for breast-fed infants[7,8], perchlorate and iodide including inhibition of iodide thyroidal uptake by perchlorate[9]. 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[28]. Similar models have been described for polychlorinated biphenyls[29], co-exposure to polychlorinated biphenyls and methyl mercury[30], persistent organic pollutants (including an initial infant body burden to represent intrauterine exposure)[31], manganese[25], and perfluoroalkyl carboxylates and sulfonates[32,33]. 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*.[34] Examples include tramadol and its O-desmethyl metabolite[35], fluoxetine and its active metabolite norfluoxetine[36], nevirapine[37], and parecoxib and its active metabolite valdecoxib[38]. 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[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[11]. Therefore, we used paediatric CYP2B6 protein expression data available in the literature[20], 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[48,49], and can be affected by polymorphisms in *ABCG2* gene[50]. 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[51].

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[11].

Maternal pharmacokinetics at 400mg were validated using the ENCORE1 results as presented by Dickinson *et al*. https://doi.org/10.1002/cpt.156[26].

Competing interests

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

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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.

You need to consider actual birth weights for this study if you did not, not published birth weights. A new infant growth paper contains growth equations for body weight and height: [Troutman JA, Sullivan MC, Carr GJ, Fisher J. Birth Defects Res. **2018** Mar 14. doi: 10.1002/bdr2.1214. [Epub ahead of print].

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.